Effect of ruminally protected arginine and lysine supplementation on serum amino acids, performance, and carcass traits of feedlot steers[1]

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ABSTRACT: One hundred twenty Angus × Simmental steers [322 ± 4.8 kg initial body weight (BW)] were blocked by BW and randomly allocated to 4 treatments arranged as a 2 × 2 factorial to evaluate the effects of supplemental arginine (none or 63 g/d of a 15.6% metabolizable arginine), supplemental lysine (none or 40 g/d of a 25% metabolizable lysine), and their interaction on performance and carcass composition of feedlot steers during a 170-d feeding period. The basal diet [dry matter (DM) basis] contained 52% dry-rolled corn, 22% dried distillers grains with solubles, 20% corn silage, and 6% vitamin-mineral supplement. Lysine balance was estimated to be −10.3 to −10.8 g for diets that did not contain supplemental lysine, and arginine supply was estimated to be +9.7 g for diets that did not contain supplemental arginine during period 1 (days 0 to 87). Lysine and arginine supplies met or exceeded requirements in period 2 (days 88 to 170). Rumen-protected arginine and lysine were top dressed daily until slaughter at a common BW (622 ± 5.5 kg). Data were analyzed using the MIXED procedure of SAS. Body weight, average daily gain, and DM intake were not affected ($P \geq 0.14$) by arginine or lysine supplementation. However, lysine increased gain:feed ($P = 0.05$) during period 1. Lysine decreased serum urea nitrogen ($P = 0.03$) on day 87, increased ($P = 0.01$) longissimus muscle (LM) area, decreased ($P \leq 0.01$) fat thickness and yield grade, and tended ($P = 0.06$) to increase moisture content of LM steaks. There tended to be an interaction for moisture content of steaks ($P = 0.09$), where arginine supplementation increased moisture content to a greater extent in steaks from cattle supplemented with lysine compared with steaks from cattle not fed supplemental lysine. Arginine tended to increase the proportion of Choice grade carcasses ($P = 0.09$) but did not change lipid content of steaks ($P = 0.59$). Arginine tended to decrease serum glutamate ($P = 0.09$) and lysine ($P = 0.07$) after 87 d of feeding. In conclusion, supplemental rumen-protected arginine and lysine did not improve performance, but lysine can increase carcass muscle and leanness, and although arginine did not increase lipid content of steaks, it may favorably shift carcasses to a greater quality grade.

Key words: arginine, beef feedlot, growth, lysine, meat quality

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INTRODUCTION

The need for metabolizable protein (MP) in order to maximize lean tissue deposition in growing cattle often exceeds their supply from dietary protein (Xue et al., 2011). Metabolizable protein in cattle is derived from microbial proteins synthesized in the rumen and from protein that

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escapes microbial fermentation (NASEM, 2016). Responses to postruminal infusions of amino acids indicate that microbial protein does not supply animals with adequate lysine (Chalupa and Chandler, 1975; Richardson and Hatfield, 1978). In diets with large proportions of corn, lysine is the first limiting amino acid for growing beef cattle because corn is low in lysine (Klemesrud et al., 2000b). In fact, supplemental rumen-protected lysine has increased the average daily gain and efficiency of cattle fed corn and corn by-product diets (Klemesrud et al., 2000b; Xue et al., 2011), but not in cattle fed diets containing soybean meal (Hussein and Berger, 1995), which is a better source of lysine.

Arginine is one of the most versatile amino acids; it is a precursor for synthesis of urea, nitric oxide (NO), and polyamines and it regulates important metabolic pathways that are critical for health, growth, reproduction, and homeostasis of animals (Morris, 2009). Despite the fact that ruminants can synthesize arginine, it is normally considered to be essential, because de novo synthesis is not sufficient to meet requirements, particularly during the early stages of growth or for increased levels of production (NRC, 2001). Dietary arginine has been demonstrated to increase intramuscular lipid content and decrease body fat in pigs (Tan et al., 2009, 2011) and to increase expression of genes that are key regulators of intramuscular fat deposition in beef cattle (Choi et al., 2014). Furthermore, arginine supplementation stimulated protein synthesis and increased muscle protein in pigs (Kim et al., 2004; Yao et al., 2008; Tan et al., 2009).

The hypothesis of this study was that supplementing feedlot cattle fed all corn product diets with rumen-protected arginine and/or lysine will improve performance and carcass quality compared with steers not supplemented with rumen-protected arginine or lysine. Therefore, the objective of this study was to evaluate serum amino acid concentration and subsequent effects on performance and carcass quality of feedlot cattle fed corn product-based diets supplemented with rumen protected arginine and/or lysine.

MATERIALS AND METHODS

This study was performed at the Purdue University Animal Sciences Research and Education Center (ASREC) in West Lafayette, IN. All procedures involving animal care and management were approved by the Purdue University Animal Care and Use Committee and were in accordance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Animals and Diets

One hundred twenty Angus × Simmental steers [initial body weight (BW) = 322 ± 4.8 kg] were blocked by BW (heavy and light) and breed composition, and allocated to 4 dietary treatments arranged as a 2 × 2 factorial. Thirty steers were fed each dietary treatment and steers were housed in pens of 6 (5 pens per dietary treatments). Pens (6.1 × 3.3 m) were located in a curtain-sided, slatted-floor finishing barn, and provided 30 cm of bunk space per animal. The basal diet was formulated to meet or exceed NASEM (2016) requirements for protein, energy, vitamins, and minerals and contained [dry matter (DM) basis] 52% dry-rolled corn, 22% dried distillers grains with solubles (DDGS), 20% corn silage, and 6% of a mineral and vitamin supplement (Table 1). Treatments were 1) a control diet with no arginine or lysine supplementation (CON), 2) rumen-protected arginine (Ajinomoto Heartland, Chicago, IL) top-dressed at 63 g/steer daily (ARG), 3) rumen-protected lysine (AjiPro-L, Ajinomoto Heartland) top-dressed at 40 g/steer daily (LYS), and 4) a diet with both rumen protected arginine and lysine top-dressed at

Table 1. Basal diet composition[1]

Dry rolled corn	52.0
Dried distillers grains with solubles	22.0
Corn silage	20.0
Vitamin/mineral supplement[2]	6.0
Nutrient composition	
NEm, Mcal/kg	1.98
NEg, Mcal/kg	1.35
NDF, %	21.37
Calcium, %	1.15
Phosphorus, %	0.39
Magnesium, %	0.18
Potassium, %	0.82
Sulfur, %	0.24

[1]Treatments were 1) a diet with no arginine or lysine supplementation (CON), 2) rumen-protected arginine (16% metabolizable arginine, Ajinomoto Heartland, Chicago, IL) top-dressed at 63 g/steer daily (ARG), 3) rumen-protected lysine (25% metabolizable lysine, AjiPro-L, Ajinomoto Heartland) top-dressed at 40 g/steer daily (LYS), and 4) a diet with both arginine and lysine top-dressed at 63 and 40 g/steer daily, respectively (ARG + LYS).

[2]Vitamin/mineral pre-mix contained (DM basis): 18.25% Ca, 0.44% Mg, 1.32% K, 0.18% S, 3.43 ppm Co, 183.33 ppm Cu, 9.66 ppm I, 522.90 ppm Fe, 440.41 ppm Mn, 4.48 ppm Se, 563.91 ppm Zn, 42.19 IU/g vitamin A, 4.98 IU/g vitamin D, 0.155 IU/g vitamin E, 413.6 ppm monensin (Rumensin 80, Elanco Animal Health, Indianapolis, IN).

63 and 40 g/steer daily, respectively (ARG + LYS). The metabolizable lysine content of the protected lysine was 25% and has been validated in previous studies (40% lysine, Whitehouse et al., 2017; 62.5% ruminal escape, Miura et al., 2017). The metabolizable arginine content of the protected arginine product was estimated to be 15.6% based on an arginine content of 25% and a ruminal escape value of 62.5% provided by the manufacturer. Protected arginine from this manufacturer has been analyzed previously (Meyer et al., 2018). Protein balance and amino acid content of the diets (Table 2) were analyzed using Beef Cattle Nutrient Requirements Model version 1.0.37.10 (BCNRM; 2016).

Daily feed deliveries were adjusted using the South Dakota State University 4-point bunk scoring system (Pritchard, 1993) during the experimental period (days 0 to 170) to allow for ad libitum feed intake with little or no accumulation of unconsumed feed. Feed delivery was recorded daily for each pen and any feed refusals were weighed, recorded, and discarded daily. Feed samples were collected every other week and dried in a forced air oven at 60 °C for 72 h. Dried feed samples were composited equally by weight, and a subsample was submitted to Cumberland Valley Analytical Services (Waynesboro, PA) for analysis of crude protein (CP; micro-Kjeldahl N × 6.25; method 960.52; AOAC, 2006) and minerals (Ca, P, Mg, K, S; method 968.08; AOAC, 2006). Neutral detergent fiber (NDF) was determined based on the procedure of AOAC (2006, method 2002.04) using heat-stable α-amylase (Termamyl 120 L, Type L, Novozymes A/S) and sodium sulfite, and acid detergent fiber (ADF) was determined based on AOAC (2006, method 973.18) with modifications to each procedure for use in an ANKOM Fiber Analyzer (ANKOM Technology Corp., Fairport, NY). As-fed formulations were adjusted for DM content accordingly every other week.

Steers were weighed monthly during the experiment to monitor average daily gain (ADG) and on 2 consecutive days at the onset of the experiment and prior to slaughter to determine initial and final BW, respectively. Individual body weights were measured prior to feeding. Average daily gain, DMI, and

Table 2. Protein balance[1] of diets during period 1 (days 0 to 87) and period 2 (days 88 to 170)

	Treatment[2]			
	CON	ARG	LYS	LYS + ARG
Crude protein, %	12.4	12.5	12.5	12.6
RDP[3], % of crude protein	47.4	47.0	47.0	46.6
RUP[4], % of crude protein	52.6	53.0	53.0	53.4
Metabolizable protein and amino acid balance				
Period 1 (Day 0 to 87)				
Metabolizable protein supply, g	873.9	907.3	890.6	877.9
Metabolizable protein balance, g	60.6	71.7	69.8	72.0
Arginine supply, g	36.6	47.6	36.8	45.9
Arginine balance, g	9.7	20.0	9.7	19.3
Lysine supply, g	41.8	42.7	52.2	51.0
Lysine balance, g	−10.3	−10.8	−0.3	−0.5
Supplied Lysine:Methionine	2.6	2.5	3.2	3.2
Period 2 (Day 88 to 170)				
Metabolizable protein supply, g	1012.4	990.4	964.5	970.2
Metabolizable protein balance, g	265.8	257.0	244.4	250.1
Arginine supply, g	42.3	51.0	39.9	49.7
Arginine balance, g	17.7	26.8	16.1	25.9
Lysine supply, g	48.2	46.6	55.7	55.3
Lysine balance, g	0.5	−0.3	9.6	9.2
Supplied Lysine:Methionine	2.5	2.5	3.2	3.1

[1]Calculated using the Beef Cattle Nutrient Requirements Model (BCNRM) of NASEM (2016) using period 1 and 2 initial and final body weights as well as average period dry matter intake from the study.

[2]Treatments were 1) a diet with no arginine or lysine supplementation (CON), 2) rumen-protected arginine (16% metabolizable arginine, Ajinomoto Heartland, Chicago, IL) top-dressed at 63 g/steer daily (ARG), 3) rumen-protected lysine (25% metabolizable lysine, AjiPro-L, Ajinomoto Heartland) top-dressed at 40 g/steer daily (LYS), and 4) a diet with both arginine and lysine top-dressed at 63 and 40 g/steer daily, respectively (ARG + LYS).

[3]Rumen degradable protein.

[4]Rumen undegradable protein.

gain:feed were calculated from days 0 to 87 (period 1), days 88 to slaughter (period 2), and days 0 to slaughter (overall). Scales (Tru-Test XR3000; Tru-Test Inc., Mineral Wells, TX) weighed to the nearest 0.9 kg (<453.6 kg) or 2.3 kg (>453.6 kg) and were checked for accuracy at each weigh date. Initial and final body weights from periods 1 and 2 as well as average dry matter intake for periods 1 and 2 were used in the BCNRM model to estimate protein and amino acid balance (Table 2).

Steers were vaccinated against Bovine Rhinotracheitis, Bovine Viral Diarrhea, Parainfluenza-3, and Bovine Respiratory Syncytial Virus (Bovi-Shield GOLD FP 5; Zoetis Animal Health, Florham Park, NJ), against *Haemophilus somnus*, *Pasteurella,* and *Clostridium* (Vision-7 Somnus; Merck Animal Health, Summit, NJ), and treated with an anthelmintic (Valbazen; Zoetis Animal Health) for internal and external parasites at the beginning of the study. Steers were implanted with Synovex-ONE Feedlot (200 mg trenbolone acetate and 28 mg estradiol benzoate; provided courtesy of Zoetis Animal Health) at the start of the study. Heavier pens of steers were weighed every other week as pen body weights approached 548 kg. Pens of steers that achieved an average body weight of approximately 548 kg were fed 300 mg of Optaflexx (ractopamine hydrochloride; provided courtesy of Elanco, Greenfield, IN) daily during the last 42 d before slaughter.

Blood Analyses

Blood samples were collected from the jugular vein of all steers at the beginning of the study (day 0), on day 87, and at slaughter (day 170) for analysis of serum urea nitrogen (SUN), and on day 87 and the day prior to slaughter from 4 steers per pen for analysis of amino acids. Blood samples were collected in BD Vacutainer serum tubes (Becton Drive, Franklin Lakes, NJ) and kept at room temperature for 30 to 60 min before centrifugation at 1,250 × *g* for 20 min at 4 °C. Serum was separated and stored at −20 °C until analysis of SUN and amino acids. The SUN was analyzed spectrophotometrically using a commercial diacetylmonoxime kit (Stanbio Urea Nitrogen Procedure No. 0580, Stanbio Laboratory, Boerne, TX) read at 530 nm in a Tecan Spark 10M multimode microplate reader (Tecan Trading AG, Mannedorf, Switzerland). Amino acids were analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) using an Agilent 1200 Series Rapid Resolution liquid chromatography system coupled to an Agilent 6460 triple quadrupole QQQ mass spectrometer (Agilent Technologies, Santa Clara, CA). The mass spectrometer used multiple reaction monitoring to analyze the amino acids.

Carcass Data Collection

Steers were transported 400 km to a USDA-inspected commercial packing facility (Tyson Foods, Joslin, IL) and were slaughtered at 3 different time points (156, 170, and 191 d) according to when 42 d of Optaflexx feeding was achieved (average pen BW of 622 ± 5.5 kg). Final body weights were not pencil shrunk. Hot carcass weight (HCW) was determined immediately after evisceration. After carcasses were chilled for 24 h, the following measurements were obtained by qualified personnel: subcutaneous fat thickness between the 12th and 13th ribs, longissimus muscle (LM) area by direct grid reading of the LM between the 12th and 13th ribs, kidney, pelvic and heart fat as a percentage of HCW, as well as marbling score, and USDA quality and yield grades. A subset of steers (2 per pen; 40 total) were selected based on average BW of the pen for collection of the *Longissimus lumborum* (LL). *Longissimus lumborum* muscle samples were collected caudal from the last rib on the right side of each carcass. Muscle samples were transported on ice to the meat laboratory at Purdue University, where they were cut into 2.54-cm thick steaks, untrimmed, and vacuum packaged. Steaks were aged for 14 d before freezing (−20 °C) and subsequent analysis of chemical composition. For proximate analysis of the LL samples, external fat was removed, homogenized, and proximate analyses were conducted in triplicate. Moisture was determined by forced-air oven drying at 100 °C for at least 24 h (Method 950.46; AOAC, 2006). Total nitrogen was determined using a nitrogen analyzer (Leco FP-2000; Leco Corp., St. Joseph, MI) and percent protein was calculated by multiplying total percent nitrogen by a factor of 6.25 (AOAC, 2006; method 992.15). Total ash was determined by ashing the sample at 600 °C in a muffle furnace for 8 h (Method 920.153; AOAC, 2006). Total fat was determined by the soxhelt method according to AOAC (2006, method 991.36).

Statistical Analysis

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), and the pen was considered the experimental unit. Performance for

periods was analyzed using repeated measures and the model included the random effects of the block and pen, the fixed effects of LYS, ARG, and day, as well as the LYS × day, ARG × day, and LYS × ARG × day interactions. The covariance structure that yielded the smallest Bayesian information criterion was used. Overall performance, carcass characteristics, and steak chemical composition were analyzed using MIXED procedure of SAS as a complete randomized block design and the model included the random effects of block and pen, and the fixed effect of LYS, ARG, and the LYS × ARG interactions. Treatment comparisons were made using Fisher's-protected least significant difference and the least square means statement was used to calculate adjusted means. The SLICE function of SAS was used to determine simple effects within time. Differences were considered statistically significant when $P \leq 0.05$, whereas $0.05 < P \leq 0.10$ was identified as a tendency.

RESULTS

Based on the BCNRM, the diets that did not contain arginine had a 9.7-g arginine supply balance during period 1 that supplemental arginine increased to 19.3 to 20 g. During period 2, the diets that did not contain arginine had a 16.1- to 17.7-g arginine supply balance that reached 25.9 to 26.8 g after arginine supplementation. The diets that did not contain lysine had a −10.8- and −10.3-g lysine supply balance during period 1 that supplemental lysine nearly eliminated (−0.3- and −0.5-g lysine supply balance for lysine supplemented cattle). Lysine:methione was 2.6 and 2.5 for CON and ARG treatments and was 3.2 and 3.2 for LYS and LYS + ARG treatments during period 1. During period 2, the diets that did not contain lysine had a 0.5- and −0.3-g lysine supply balance that achieved a balance of 9.6 and 9.2 g after lysine supplementation. Lysine:methione was 2.5 and 2.5 for CON and ARG treatments and was 3.2 and 3.1 for LYS and LYS + ARG treatments during period 1.

Two steers were removed from ARG and one steer was removed from LYS for reasons unrelated to treatments, changing the pen number from 6 animals per pen to 5 to 6 animals per pen. There were no interactions ($P \geq 0.13$) between ARG and LYS for any performance measure (Table 3). Body weight, ADG, and DMI were not affected ($P \geq 0.14$) by ARG or LYS during period 1, period 2, or overall. Supplemental-protected lysine increased gain:feed ($P = 0.03$) compared with no lysine during period 1, but not during period 2 or overall ($P \geq 0.21$).

Table 3. Effect of supplementing ruminally protected lysine (LYS), arginine (ARG), or their combination on performance of feedlot steers

	Treatment[1,2]					*P*-value		
Item	CON	ARG	LYS	LYS +ARG	SEM[3]	ARG	LYS	LYS × ARG
Body weight, kg								
Day 0	322.7	322.2	323.1	322.4	6.75	0.92	0.97	0.99
Day 87	481.1	484.2	491.4	485.5	6.75	0.83	0.40	0.75
Day 170	622.8	625.7	622.8	619.7	6.75	0.99	0.65	0.94
Average daily gain, kg/d								
Days 0 to 87	1.82	1.86	1.93	1.87	0.062	0.88	0.31	0.63
Days 88 to 170	1.66	1.68	1.66	1.55	0.062	0.44	0.27	0.41
Days 0 to 170	1.73	1.77	1.81	1.72	0.052	0.52	0.74	0.13
Dry matter intake, kg/d								
Days 0 to 87	9.1	9.4	9.2	9.0	0.36	0.96	0.63	0.80
Days 88 to 170	10.6	10.3	10.0	10.0	0.36	0.77	0.14	0.45
Days 0 to 170	9.8	9.8	9.7	9.5	0.42	0.73	0.49	0.71
Gain:feed, kg/kg								
Days 0 to 87	0.199	0.198	0.209	0.210	0.004	0.99	0.05	0.28
Days 88 to 170	0.157	0.168	0.168	0.156	0.004	0.91	0.86	0.26
Days 0 to 170	0.181	0.181	0.178	0.184	0.005	0.91	0.21	0.42
Days on Feed	175	174	168	175	3.7	0.67	0.73	0.58

[1]Treatments were 1) a diet with no arginine or lysine supplementation (CON), 2) rumen-protected arginine (16% metabolizable arginine, Ajinomoto Heartland, Chicago, IL) top-dressed at 63 g/steer daily (ARG), 3) rumen-protected lysine (25% metabolizable lysine, AjiPro-L, Ajinomoto Heartland) top-dressed at 40 g/steer daily (LYS), and 4) a diet with both arginine and lysine top-dressed at 63 and 40 g/steer daily, respectively (ARG + LYS).

[2]Five pens/treatment; 5 to 6 steers/pen.

[3]Standard error of the mean.

Supplemental-protected arginine did not influence gain:feed at any time ($P \geq 0.91$).

There were no interactions ($P \geq 0.17$) between ARG and LYS for SUN at any time point (Table 4). Supplemental-protected lysine did not affect SUN on day 0 or at slaughter ($P \geq 0.92$); however, SUN was decreased in steers fed lysine on day 87 ($P = 0.03$) compared with steers not fed lysine. Supplemental-protected arginine did not affect serum urea nitrogen (SUN) at any time point during the study ($P \geq 0.70$)

Serum amino acid concentrations are presented in Table 5 (day 87) and Table 6 (slaughter). Serum concentration of lysine was not affected by lysine supplementation ($P \geq 0.66$) and serum concentration of arginine was not affected by arginine supplementation ($P \geq 0.34$). Protected arginine supplementation tended to decrease lysine and glutamate concentrations in serum ($P \leq 0.10$) on day 87. On day 87, there tended to be an interaction ($P = 0.07$) for serum threonine, where supplemental arginine decreased serum threonine in diets with no supplemental lysine but increased serum threonine in diets that contained LYS. On day 170, steers fed LYS had decreased serum concentrations of asparagine compared with steers not fed supplemental lysine ($P = 0.03$). On day 170, there tended to be an interaction ($P = 0.07$) for serum asparagine, where supplemental arginine increased serum asparagine in cattle not fed supplemental lysine but increased serum aparagine to a lesser extent in cattle fed supplemental lysine. Supplemental-protected lysine or arginine did not affect serum concentrations of any other amino acid or amino acid class and no other interactions occurred on day 87 or at slaughter ($P \geq 0.12$).

There were no interactions ($P \geq 0.13$) between supplemental arginine and supplemental lysine for any carcass parameter (Table 7). Steers fed supplemental lysine produced carcasses that had decreased fat thickness ($P = 0.01$), increased LM area ($P = 0.01$), and decreased yield grades ($P < 0.01$) compared with steers not fed supplemental lysine. Supplemental-protected lysine did not affect ($P \geq 0.17$) hot carcass weight, dressing percentage, kidney, pelvic, and heart fat percentage, marbling score, or quality grade distribution. Steers fed supplemental arginine tended ($P = 0.09$) to produce a greater percentage of Choice average carcasses, although marbling score did not differ because of supplemental arginine ($P = 0.99$). Supplemental-protected arginine did not influence any other carcass parameter ($P \geq 0.17$).

Steers fed supplemental lysine produced *L. dorsi* steaks that tended ($P = 0.06$) to have increased moisture content compared with steers not fed supplemental lysine (Table 8). Supplemental protected lysine did not affect ($P \geq 0.23$) protein, lipid, or ash content of steaks. Supplemental-protected arginine did not influence proximate composition of steaks, including lipid content ($P \geq 0.11$). There tended to be an interaction for moisture content of steaks ($P = 0.09$), where supplemental arginine increased moisture content to a greater extent in steaks from cattle fed supplemental lysine compared with steaks from cattle not fed supplemental lysine. No other interactions among proximate composition measures were observed ($P \geq 0.13$).

DISCUSSION

The Cornell Net Carbohydrate and Protein System (CNCPS v6.5) model estimates whole animal arginine content at 6.75 g/100 g of protein (Van Amburgh et al., 2015), whereas the Beef Cattle Nutrient Requirements Model (BCNRM) estimates whole animal arginine content at 3.3 g/100 g of protein (NASEM, 2016). The difference in

Table 4. Effect of supplementing ruminally protected lysine (LYS), arginine (ARG), or their combination on serum urea nitrogen of feedlot steers

	Treatment[2,3]					*P*-value		
SUN[1], mg/dL	CON	ARG	LYS	LYS + ARG	SEM[4]	ARG	LYS	LYS × ARG
Day 0	8.40	8.70	8.53	8.67	0.583	0.70	0.93	0.98
Day 87	10.77	10.81	9.67	9.28	0.583	0.77	0.03	0.17
Day 170	10.05	9.35	9.45	9.84	0.583	0.78	0.92	0.81

[1]Serum urea nitrogen.

[2]Treatments were 1) a diet with no arginine or lysine supplementation (CON), 2) rumen-protected arginine (16% metabolizable arginine, Ajinomoto Heartland, Chicago, IL) top-dressed at 63 g/steer daily (ARG), 3) rumen-protected lysine (25% metabolizable lysine, AjiPro-L, Ajinomoto Heartland) top-dressed at 40 g/steer daily (LYS), and 4) a diet with both arginine and lysine top-dressed at 63 and 40 g/steer daily, respectively (ARG + LYS).

[3]Five pens/treatment; 5 to 6 steers/pen.

[4]Standard error of the mean.

Table 5. Effect of supplementing ruminally protected lysine (LYS), arginine (ARG), or their combination on serum amino acid concentration of feedlot steers at 87 d

	Treatment[1,2]					*P*-value		
Amino acids, mg/mL	CON	ARG	LYS	LYS +ARG	SEM[3]	ARG	LYS	LYS × ARG
Alanine	30.43	32.05	32.61	29.74	1.183	0.60	0.96	0.29
Arginine	58.36	56.59	57.14	53.69	2.717	0.34	0.45	0.66
Asparagine	0.51	0.42	0.42	0.42	0.054	0.43	0.37	0.52
Aspartate	2.95	2.51	4.95	2.90	0.863	0.16	0.17	0.20
Cystine	6.34	6.37	6.08	6.99	0.782	0.55	0.82	0.87
Glutamine	13.22	12.65	12.36	12.28	0.683	0.65	0.38	0.77
Glutamate	8.81	5.83	8.17	6.52	0.755	0.09	0.18	0.19
Glycine	14.41	13.65	14.07	14.98	0.996	0.94	0.62	0.81
Histidine	18.06	18.66	17.64	17.15	0.833	0.94	0.25	0.62
Isoleucine	16.10	14.98	15.65	15.58	0.725	0.42	0.92	0.75
Leucine	39.76	39.69	39.62	39.53	2.175	0.97	0.95	0.99
Lysine	15.05	13.66	17.19	12.88	1.519	0.07	0.66	0.22
Methionine	4.53	4.60	4.77	4.44	0.234	0.58	0.85	0.79
Phenylalanine	19.55	18.87	20.38	19.04	1.176	0.40	0.67	0.80
Proline	18.78	20.07	21.31	19.10	1.019	0.88	0.30	0.18
Serine	12.52	12.52	11.48	12.46	0.770	0.53	0.48	0.73
Threonine	2.05	1.84	1.64	1.99	0.115	0.55	0.27	0.07
Tryptophan	11.90	12.13	13.61	12.87	0.807	0.76	0.14	0.45
Tyrosine	40.92	37.59	38.89	37.33	1.967	0.22	0.57	0.56
Valine	67.59	70.42	67.04	71.07	3.711	0.36	0.99	0.83

[1]Treatments were 1) a diet with no arginine or lysine supplementation (CON), 2) rumen-protected arginine (16% metabolizable arginine, Ajinomoto Heartland, Chicago, IL) top-dressed at 63 g/steer daily (ARG), 3) rumen-protected lysine (25% metabolizable lysine, AjiPro-L, Ajinomoto Heartland) top-dressed at 40 g/steer daily (LYS), and 4) a diet with both arginine and lysine top-dressed at 63 and 40 g/steer daily, respectively (ARG + LYS).

[2]Five pens/treatment; 4 steers/pen sampled.

[3]Standard error of the mean.

accounting between the BCNRM and CNCPS models arises from the fact that, as a semiessential amino acid, the BCNRM model assumes that animals are capable of synthesizing 50% of arginine needs, thus decreasing the requirement by 50%. In contrast, the CNCPS model assumes that the diet must supply all of the animal's arginine needs. Estimates of whole animal lysine content are 6.26 and 6.40 g/100 g of CP for the CNCPS and BCNRM models, respectively.

As cattle increase in body weight and degree of fattening, protein deposition slows, and metabolizable protein requirements decrease (Owens et al., 2014; NASEM, 2016). The gain:feed response to supplemental lysine in period 1 but no performance response in period 2 is likely because protein deposition and MP requirements were greater in the faster growing animals (earlier) compared with the slower growing animals (later). Although supplemental lysine did not increase ADG during period 1, the increase in gain:feed in period 1 as a result of supplemental lysine appears to be more a result of a change in ADG rather than DMI. Because estimates of lysine requirements decreased and supply (DMI) increased for steers in period 2 compared with period 1, the lysine-estimated requirement was met for steers that were not supplemented with supplemental lysine in period 2; thus, it is not surprising that performance was not affected by supplemental lysine during period 2. Decreased SUN on day 87 but not at slaughter for steers fed supplemental lysine compared with steers fed no supplemental lysine also suggests that added protected lysine was effectively utilized for tissue growth early on. Increased N utilization during the first 87 d led to more accretion of protein as evidenced by increased LM area and carcass leanness. A decrease in SUN in steers fed supplemental lysine also suggests that other amino acids in circulation were more effectively used for tissue deposition of protein rather than excretion as urea. Previous studies in ruminants (Ponnampalam et al., 2005; Xue et al., 2011; Batista et al., 2016) have similarly reported that increased postruminal supply of lysine can stimulate protein anabolism in tissues, decreasing SUN load through effective regulation of the urea cycle.

Supplemental lysine, however, did not alter serum amino acids on day 87 or 170. Serum amino

Table 6. Effect of supplementing ruminally protected lysine (LYS), arginine (ARG), or their combination on serum amino acid concentration of feedlot steers at slaughter

	Treatment[1,2]					*P*-value		
Amino acids, mg/mL	CON	ARG	LYS	LYS + ARG	SEM[3]	ARG	LYS	LYS × ARG
Alanine	22.10	22.40	20.33	20.70	1.183	0.78	0.15	0.53
Arginine	61.02	56.72	54.58	57.28	2.717	0.77	0.29	0.42
Asparagine	0.96	1.05	0.84	0.92	0.054	0.12	0.03	0.07
Aspartate	2.00	2.22	2.94	1.81	0.863	0.60	0.76	0.80
Cystine	5.70	7.80	6.70	6.42	0.782	0.25	0.81	0.31
Glutamine	18.52	19.16	18.32	17.75	0.683	0.96	0.26	0.57
Glutamate	3.19	3.24	3.28	3.25	0.755	0.99	0.95	0.99
Glycine	15.21	14.03	13.79	14.06	0.996	0.65	0.49	0.75
Histidine	19.01	19.12	18.22	18.00	0.833	0.95	0.28	0.72
Isoleucine	14.57	14.98	13.52	14.34	0.725	0.40	0.25	0.55
Leucine	35.05	35.88	32.77	32.93	2.175	0.82	0.24	0.68
Lysine	17.15	18.40	17.43	16.29	1.519	0.97	0.55	0.80
Methionine	3.88	3.96	3.86	3.66	0.234	0.79	0.51	0.83
Phenylalanine	22.35	21.97	19.90	21.52	1.176	0.61	0.23	0.48
Proline	14.21	15.31	14.20	14.76	1.019	0.42	0.78	0.85
Serine	12.44	12.29	10.53	11.80	0.770	0.47	0.13	0.30
Threonine	2.28	2.26	2.19	2.25	0.115	0.86	0.67	0.95
Tryptophan	13.42	12.43	12.40	12.41	0.807	0.55	0.52	0.76
Tyrosine	26.47	29.11	26.40	23.66	1.967	0.98	0.17	0.30
Valine	66.80	70.85	66.86	65.25	3.711	0.74	0.46	0.74

[1]Treatments were 1) a diet with no arginine or lysine supplementation (CON), 2) rumen-protected arginine (16% metabolizable arginine, Ajinomoto Heartland, Chicago, IL) top-dressed at 63 g/steer daily (ARG), 3) rumen-protected lysine (25% metabolizable lysine, AjiPro-L, Ajinomoto Heartland) top-dressed at 40 g/steer daily (LYS), and 4) a diet with both arginine and lysine top-dressed at 63 and 40 g/steer daily, respectively (ARG + LYS).

[2]Five pens/treatment; 4 steers/pen sampled.

[3]Standard error of the mean.

Table 7. Effect of supplementing ruminally protected lysine (LYS), arginine (ARG), or their combination on carcass characteristics of feedlot steers

	Treatment[1,2]					*P*-value		
Item	CON	ARG	LYS	LYS + ARG	SEM[4]	ARG	LYS	LYS × ARG
Hot carcass weight, kg	388.0	390.0	389.3	387.9	3.40	0.92	0.91	0.62
Dressing, %	62.1	62.1	62.3	62.4	0.22	0.88	0.25	0.92
Fat thickness, cm	1.41	1.38	1.21	1.26	0.050	0.84	0.01	0.48
Longissimus muscle area, cm^2	90.0	90.3	93.8	92.7	0.92	0.68	0.01	0.48
Kidney, pelvic, heart fat, %	2.0	1.9	1.9	1.9	0.17	0.64	0.17	0.29
Yield grade	3.1	3.0	2.7	2.8	0.07	0.63	<0.01	0.43
Marbling score[3]	414	419	419	414	23.1	0.99	0.99	0.84
Quality grade distribution								
Select, %	0.0	0.0	0.0	3.3	0.02	0.34	0.34	0.34
$Choice^-$, %	52.1	45.1	64.7	38.6	0.12	0.17	0.80	0.42
Choice average, %	34.4	41.0	14.6	44.2	0.10	0.09	0.42	0.27
$Choice^+$, %	3.4	6.8	7.4	10.2	0.04	0.47	0.40	0.94
Prime, %	10.2	7.1	13.4	3.7	0.06	0.30	0.99	0.59

[1]Treatments were 1) a diet with no arginine or lysine supplementation (CON), 2) rumen-protected arginine (16% metabolizable arginine, Ajinomoto Heartland, Chicago, IL) top-dressed at 63 g/steer daily (ARG), 3) rumen-protected lysine (25% metabolizable lysine, AjiPro-L, Ajinomoto Heartland) top-dressed at 40 g/steer daily (LYS), and 4) a diet with both arginine and lysine top-dressed at 63 and 40 g/steer daily, respectively (ARG + LYS).

[2]Five pens/treatment; 5 to 6 steers/pen.

[3]Practically devoid = 100 to 199; slight = 200 to 299; small = 300 to 399; modest = 400 to 499; moderate = 500 to 599; slightly abundant = 600 to 699.

[4]Standard error of the mean.

Table 8. Effect of supplementing ruminally protected lysine (LYS), arginine (ARG), or their combination on chemical composition (g/100g) of meat

	Treatment[1,2]					*P*-value		
Item, g/100 g	CON	ARG	LYS	LYS +ARG	SEM[3]	ARG	LYS	LYS × ARG
Moisture	70.0	70.3	70.5	71.6	0.43	0.11	0.06	0.09
Protein	23.0	22.7	22.9	22.4	0.32	0.27	0.51	0.63
Lipids	5.8	5.8	5.4	4.8	0.55	0.59	0.23	0.58
Ash	1.2	1.2	1.2	1.2	0.03	0.18	0.16	0.13

[1]Treatments were 1) a diet with no arginine or lysine supplementation (CON), 2) rumen-protected arginine (16% metabolizable arginine, Ajinomoto Heartland, Chicago, IL) top-dressed at 63 g/steer daily (ARG), 3) rumen-protected lysine (25% metabolizable lysine, AjiPro-L, Ajinomoto Heartland) top-dressed at 40 g/steer daily (LYS), and 4) a diet with both arginine and lysine top-dressed at 63 and 40 g/steer daily, respectively (ARG + LYS).

[2]Five pens/treatment; 2 steers/pen sampled.

[3]Standard error of the mean.

acid profiles can be influenced by different factors, which make it difficult to interpret the net result. An increase in serum concentration of any essential amino acid in response to its supplementation generally signifies that its supply exceeds the capacity for protein synthesis, as dictated by the first-limiting amino acid (Bergen, 1979). In contrast, a decrease in serum concentrations of other amino acids, when a first-limiting amino acid is provided, indicates greater utilization for anabolic purposes, because supplementation of the limiting amino acid should eliminate previous restrictions that the basal diet may have imposed on protein synthesis (Wessels et al., 1997). Many amino acids were lesser in steers supplemented with lysine, but only serum concentrations of asparagine at slaughter were significantly decreased as a result of supplemental lysine. Decreased serum amino acids in steers fed supplemental protected lysine is consistent with increased carcass leanness and indicates that these amino acids may have been used for anabolic purposes.

Previous literature in regard to the effect of rumen protected amino acid supplementation on performance has demonstrated that supplemental-protected lysine produces a beneficial response when lysine content of primarily forage diets is not adequate. Klemesrud et al. (2000a) demonstrated that steers fed a 44% sorghum silage, 44% corn cob, and 4.15% corn gluten meal diet with incremental amounts of rumen-protected lysine had greater ADG compared with steers supplemented with urea. In diets with 45% wet corn gluten feed, 42.5% corn, 5% corn silage, and 5% alfalfa, Klemesrud et al. (2000b) observed an increase in ADG and gain:feed with up to 2.6 g/d of supplemental rumen-protected lysine. Furthermore, Xue et al. (2011) demonstrated that rumen-protected lysine did not alter DMI of a 50% corn stalklage, 34% ground corn, 10.9% brewers grain diet, but increased ADG and feed efficiency, with a maximum value achieved at 10 g/d of ruminally protected lysine supplementation. On the other hand, Lancaster et al. (2016) did not observe any effects of supplemental ruminal–protected lysine on total serum amino acids or growth performance when feedlot cattle were fed 45% corn, 20% DDGS, 20% corn silage diets that exceeded crude protein requirements. Hussein and Berger (1995) reported that in cattle fed diets containing soybean meal, which is a good source of lysine, supplemental lysine did not improve performance.

The increase in LM area and improvement in carcass leanness caused by supplemental lysine in the present study suggests that added lysine supported increased protein accretion and muscle synthesis. Burris et al. (1976) observed a linear increase in N retention in growing steers consuming corn-based diets that were abomasally infused with 12, 24, and 36 g of lysine. Furthermore, Oke et al. (1986) reported a 3.7% increase in N retention in lambs fed rumen-protected lysine compared with lambs not supplemented with rumen protected amino acids. Szabó et al. (2001) and Bidner et al. (2004) reported that pigs supplemented with lysine had increased loin eye area and decreased fat tissue compared with pigs not supplemented with lysine. In contrast, Klemesrud et al. (2000b) did not observe a difference in yield grade or quality grade when steers were supplemented with incremental amounts of rumen-protected lysine and Lancaster et al. (2016) did not find an effect of supplemental-protected lysine on any carcass parameter in steers. In diets supplemented with soybean meal, rumen-protected lysine did not affect carcass characteristics (Hussein and Berger, 1995). The diets in these studies likely provided a sufficient amount of lysine as to not see a response.

Meyer et al. (2018) observed that a similar rumen-protected arginine product (62.3% arginine) from the same manufacturer was 26% to 31% protected from ruminal fermentation. Even though ruminal protection of arginine in the current study may have been lower than predicted, arginine was not deficient in any diet in the current study; thus, it was not limiting growth and a growth response to supplementation was not detected. The fact that supplemental arginine did not alter performance in period 1 also indicates that the BCNRM model may predict dietary arginine needs for growing feedlot cattle more accurately than the CNCPS model. Previous studies have demonstrated that abomasal infusion of arginine increased growth hormone secretion and improved N metabolism in beef heifers (Davenport et al., 1990) as well as increased serum insulin-like growth factor and tended to improve average daily gain in lambs (Davenport et al., 1995). Arginine is thought to regulate the partitioning of dietary energy in favor of muscle protein accretion (Wu et al., 2007), and Yao et al. (2008) observed that arginine supplementation in pigs enhanced the activation of protein synthesis in skeletal muscle and increased ADG. Tan et al. (2009) also observed that supplementing arginine to swine diets increased ADG and increased gain:feed by 6.8%. However, Ma et al. (2010) observed no influence of arginine on performance when pigs were fed a corn- and soybean meal-based diet supplemented with 0%, 0.5%, or 1% L-arginine. Likewise, Madeira et al. (2014) reported that dietary supplementation of pigs with 1% of L-arginine during the grower-finisher phase did not affect performance.

A tendency for decreased lysine in serum on day 87 in the current study in response to supplemental arginine indicates that excess arginine may have decreased lysine absorption from the small intestine. Arginine and lysine share common chemical properties and are absorbed in the small intestine by the same transporters; thus, greater supplies of arginine in the small intestine could potentially compete with lysine for transport and cause a decreased supply in the serum (Batista et al., 2016). However, Abe et al. (1998) reported that an antagonism between arginine and lysine did not occur in calves that were administered excess lysine. A tendency for decreased serum glutamate on day 87 in response to supplemental arginine might reflect greater utilization of glutamate for urea production. Arginine activates N-acetylglutamate synthase, the first and rate limiting enzyme of the urea cycle that converts glutamate to N-acetylglutamate (Meijer et al., 1990).

Arginine promotes intramuscular lipid synthesis by upregulating the expression of key lipogenesis genes in the muscle, such as fatty acid synthase, stearoyl coA desaturase, and lipoprotein lipase (Tan et al., 2011; Choi et al., 2014). In addition, Chung et al. (2006) reported that arginine increases the differentiation of bovine pre-adipocytes into adipocytes by increasing peroxisome proliferator-activated receptor gamma, a crucial transcription factor controlling adipogenesis in intramuscular adipose tissue (Ladeira et al., 2018). Our study suggests that there was an upward shift in quality grade distribution as a result of supplemental arginine; but, no change in marbling score or lipid composition of steaks indicates that there was little effect of supplemental arginine on intramuscular fat deposition. Madeira et al. (2014) similarly reported that arginine supplementation did not increase intramuscular fat in boars. However, supplementation of arginine to pigs in other studies has improved meat quality by increasing lipid content of muscle (Tan et al., 2009; Choi et al., 2014; Ma et al., 2015; Hu et al., 2017).

In conclusion, supplemental rumen-protected lysine and arginine may not improve growth; however, supplemental rumen-protected lysine increased gain:feed during the first 87 d and increased LM area, carcass leanness, and N utilization, indicating that use of amino acids for anabolic purposes was increased. Supplemental rumen-protected arginine may favorably shift carcasses to a greater quality grade; but, no change in marbling score or lipid composition of steaks indicates that there was little effect of supplemental arginine on intramuscular fat deposition.

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