Effects of rumen-protected arginine supplementation and arginine-HCl injection on site and extent of digestion and small intestinal amino acid disappearance in forage-fed steers[1]

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ABSTRACT: Four ruminally and intestinally cannulated steers were used in a 4 × 4 Latin square to evaluate effects of rumen-protected Arg supplementation or intravenous Arg injection on small intestinal delivery of AA, site and extent of digestion, and ruminal fermentation. Steers were fed grass hay (7.2% CP, 67.6% NDF, 0.29% Arg) for ad libitum intake with no additional Arg (CON), 54-mg L-Arg/kg BW injected intravenously (Arg- INJ), 180-mg rumen-protected L-Arg/kg BW daily (Arg-RP180), or 360-mg rumen-protected L-Arg/kg BW daily (Arg-RP360). Half of each treatment dose was administered twice daily. Each period had a 7-d washout of hay only followed by a 14-d treatment and collection period. Ruminal disappearance (%) of Arg was greater ($P < 0.001$) for both Arg-RP treatments than CON and Arg-INJ, although the amount of Arg disappearing was greatest in Arg-RP360, followed by Arg-RP180, and least in CON and Arg-INJ ($P < 0.001$). Duodenal flow and small intestinal disappearance (g/d) of Arg was greatest in Arg-RP360, followed by Arg-RP180, and least in CON and Arg-INJ ($P < 0.004$). Ileal flow of Arg was greatest in Arg-RP360, intermediate in Arg-RP180, and least in CON ($P = 0.01$) because the proportional small intestinal disappearance of Arg was not different ($P = 0.96$). Steers fed Arg-RP360 had greater ($P = 0.01$) ileal flow of Orn and tended to have greater ($P = 0.09$) ileal flow of Glu than all other treatments. There were no differences in hay or total DMI, microbial efficiency, or OM, NDF, or ADF digestibility ($P \geq 0.10$). Total N intake and duodenal N flow were greater in Arg-RP360 than all other treatments ($P \leq 0.02$). Total tract N digestibility was greatest in Arg-RP360, followed by Arg-RP180, and least in CON and Arg-INJ ($P = 0.003$). Ruminal ammonia was greater ($P = 0.004$) in Arg-RP360 compared with CON and Arg-INJ and greater ($P = 0.06$) in Arg-RP180 than CON. There was no effect of treatment ($P \geq 0.37$) on total VFA, acetate, propionate, or butyrate concentrations. Results indicate that feeding rumen-protected Arg increases small intestinal Arg flow with minimal effects on ruminal fermentation and total tract digestibility of OM and fiber.

Key words: amino acids, arginine, digestion, fermentation, rumen-protected amino acids

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INTRODUCTION

Arginine is the precursor for many important compounds in the body, including nitric oxide and polyamines (Wu and Morris, 1998). Nitric oxide is involved in blood flow regulation by causing vasodilation, stimulating angiogenesis, and increasing vascular permeability (Martín et al., 2001; Roy et al., 2006). It is possible that effects of nitric oxide on blood flow regulation may have positive systemic effects on nutrient uptake, embryonic and fetal development, lactation, and ovarian function. Additionally, polyamines are important during embryonic and fetal development (Lefèvre et al., 2011). Data in both nonruminant and sheep models suggest that Arg supplementation may increase embryonic or fetal survival (Mateo et al., 2007; Luther et al., 2008; Lassala et al., 2011) and prevent intrauterine growth restriction (Vosatka et al., 1998; Lassala et al., 2010). Furthermore, data from ruminants suggest that increased milk production (Chew et al., 1984), as well as altered tissue-specific blood flow (Maltby et al., 2005; Luther et al., 2008) and endocrine regulation (Davenport et al., 1990a, 1990b; Recabarren et al., 1996; Ragland-Gray et al., 1997), may result from Arg supplementation. Taken together, these data indicate that targeted Arg supplementation could enhance productivity of reproducing beef females receiving suboptimal nutrition from poor quality forage.

Before supplementation of Arg to forage-fed cattle is possible, the efficacy of rumen-protected Arg and its effects on ruminal metabolism and site and extent of digestion need to be further delineated. The specific objectives of this study were to investigate the effects of injecting Arg-HCl intravenously or feeding rumen-protected Arg on small intestinal delivery of AA, site and extent of digestion, and ruminal fermentation in steers fed a forage diet. We hypothesized that feeding rumen-protected Arg would increase small intestinal delivery of Arg without affecting nutrient digestibility or ruminal fermentation.

MATERIALS AND METHODS

Animals and Diets

All procedures were approved by the North Dakota State University Animal Care and Use Committee. Four ruminally, duodenally, and ileally cannulated (open T-type intestinal cannulae) Holstein steers (initial BW = 418 ± 9 kg [SEM]) were used in a 4 × 4 Latin square design. Steers received one of four treatments in each experimental period: 1) ad libitum intake of grass hay (90.5% OM, 7.2% CP, 0.29% Arg, 67.6% NDF, 43.0% ADF, 0.55% Ca, 0.22% P, DM basis; 77.5% DM; **CON**), 2) CON with 54 mg L-Arg-/kg BW injected daily (**Arg-INJ**), 3) CON with 180-mg rumen-protected L-Arg/kg BW supplemented daily (**Arg-RP180**), and 4) CON with 360-mg rumen-protected L-Arg/kg BW supplemented daily (**Arg-RP360**). Half of each treatment dose was administered twice daily.

The Arg-INJ treatment was included in this study because of its use in research investigating Arg administration to reproducing female ruminants (Wu et al., 2007; Luther et al., 2008; Lassala et al., 2010, 2011). This was of interest to verify that injection of Arg-HCl would not affect digestion or absorption of Arg in the gastrointestinal tract, as well as to realize additional objectives of the study not included in the current paper. The Arg-INJ treatment was administered as L-Arg-HCl (Ajinomoto, Inc., Tokyo, Japan) dissolved in saline (0.35 g Arg-HCl/mL saline), adjusted to pH 7.0 with 1 *M* NaOH, and filtered through a 0.22-µm cellulose acetate filter (Corning, Inc., Corning, NY) into sterile glass bottles with sterile rubber caps. The 27 mg L-Arg/kg BW dose (given twice daily) was chosen based on previous studies in sheep in which this dose increased circulating Arg for 4 h post-injection (Wu et al., 2007; Luther et al., 2008).

Rumen-protected Arg (62.3% Arg, 16.5% N, DM basis, 96.3% DM) used in this study was based on U.S. Patent Application Ser. No. 61/321,604. This method for the controlled release of Arg-HCl has a core containing Arg-HCl coated by a double fatty layer. The inner layer that is in contact with the core is at least partially constituted by one or more free fatty acids, and the outer layer is at least partially constituted of a mixture of glycerides and fatty acids. The Arg-RP180 treatment was estimated to provide similar Arg delivery to circulation as Arg-INJ, based on an estimated ruminal protection of 50% provided by the manufacturer and small intestinal catabolism of 40% (Wu, 1998).

Each period lasted 21 d, which was divided into a 7-d washout period of hay only (days-6 to 0) and a 14-d treatment period when steers received their respective treatments (days 1 to 14). Steers were housed in a climate-controlled facility in individual pens (3.0 × 3.7 m) from days-6 to 7 and in individual tie-stall stanchions (1.0 × 2.2 m) from days 8 to 14, with ad libitum access to fresh water every day and trace-mineralized salt from days-6 to 7 of each period.

Steers were fed chopped grass hay (10.2 cm) for ad libitum intake in approximately equal amounts (not differing by >10%) at 0700 and 1900 h daily. Daily hay allowance was based on 110% of the average hay intake during the previous week to ensure ad libitum intake. Orts were collected and weighed daily before the morning feeding (0630 h) to determine daily as fed intake. During the treatment period, rumen-protected Arg was dosed intraruminally via the ruminal cannulae immediately before feeding hay. Although it is possible that ruminal dosing of rumen-protected Arg could lead to greater ruminal protection than would feeding of the product due to less mastication, the product was ruminally dosed to ensure that all product entered the rumen for each animal. Additionally, intravenous jugular injections of either Arg-HCl (Arg-INJ treatment) or saline (all other treatments, based on mL/kg BW dose of Arg-HCl) were administered using sterile 60-mL syringes and 18-gauge needles immediately before each feeding. Each injection took 5 to 10 s to deliver. Two-day BW (before morning feeding) was measured at the beginning of each period to determine necessary dosage of each Arg treatment and saline injection. During the entire study, steers gained minimal BW (10.1 ± 8.6 kg BW change).

Sample Collection

Hay and ort samples were collected daily and composited within steer for each week of each period. These were stored at −20°C until DM and nutrient analysis. Total fecal collections were obtained using stainless steel pans located behind the tie-stall stanchions from days 8 to 14. Drains are positioned in these tie-stall facilities to allow for urine to drain away from feces, and fecal matter was scraped from rubber mats under the hind legs at least every 4 h into the fecal pans (directly behind steers). Feces were weighed, mixed, subsampled (10% of total wet weight), and composited within each steer for each sampling period. The composited fecal collections were stored at 4°C, then each composite sample was mixed in a rotary mixer (Model H-600; Hobart Manufacturing Co., Troy, OH), and subsampled for DM and nutrient analysis.

Chromic oxide (8 g in a gelatin capsule; 16 g chromic oxide per day) was used as an indigestible flow marker and dosed into the ruminal cannula at 0700 and 1900 h daily beginning 5 d prior to intestinal digesta sampling and continuing through the collection period (days 5 to 14 of each period; Gilbery et al., 2006; Reed et al., 2007). Duodenal and ileal digesta (approximately 200 mL) were sampled 12 times from days 10 through 13 so that every other hour in a 24-h period was represented (0800, 1400, and 2000 h on day 10; 0200, 1000, 1600, and 2200 h on day 11; 0400, 1200, and 1800 h on day 12, and 0000 and 0600 h on day 13). Approximately 100 g from each sample were composited within steer for each period and stored at −20°C until DM and nutrient analysis.

At 0500 h on day 13, steers were dosed intraruminally with 200 mL of Co-EDTA (1.7 g Co), which served as a fluid dilution marker (Udén et al., 1980). At this time (just before dosing with Co-EDTA) and 0, 2, 4, 6, 8, 10, and 12 h post-feeding, 200 mL of ruminal fluid were collected using a suction strainer. Immediately following collection, ruminal pH was determined using a combination electrode pH meter (model 2000 pH/temperature meter, VWR Scientific Products, West Chester, PA). After this, each sample was acidified with 1 mL of 6.0 *M* HCl/100 mL ruminal fluid and stored at −20°C until analysis of ammonia, VFA, and Co.

On day 14 of each period, ruminal evacuations were performed before the evening feeding (1800 h) to determine ruminal fill. Ruminal contents were removed, weighed, mixed, sampled, and replaced before the 1900-h feeding. Samples (1,250 g; wet basis) were dried in a forced-air oven (55°C) and stored for analysis of DM. A 4-kg sample of ruminal contents was also sampled, treated with 2 L of a formalin-saline solution (3.7% formaldehyde and 0.9% NaCl), and stored at −20°C for isolation of bacterial cells (Zinn and Owens, 1986).

Nutrient and Laboratory Analyses

Hay, ort, and fecal samples were dried in a forced-air oven (55°C; The Grieve Corporation, Round Lake, IL) for 48 h and then ground through a Wiley Mill (Thomas Hill and Sons, Philadelphia, PA) to pass through a 2-mm screen. Ground diet and ort samples were analyzed for DM, ash, and N (procedure numbers 930.15, 942.05, and 984.13; AOAC, 1990) and sequentially analyzed for NDF (without sodium sulfite; with amylase; and without ash correction) and ADF (ANKOM Fiber Analyzer Model 200, Fairport, NY). Amino acid concentration of hay was determined by the University of Missouri Agricultural Experiment Station Chemical Laboratory using AOAC procedure number 982.30 E (2005). This included performic acid oxidation prior to acid hydrolysis for methionine analysis. Rumen-protected Arg was also analyzed

for N based upon these methods, and Arg concentration was provided by the manufacturer.

Duodenal and ileal samples were lyophilized (Virtis Genesis 25LL; The Virtis Company, Inc., Gardiner, NY) before being ground to 1 mm using a Wiley Mill. Ground fecal, duodenal, and ileal samples were analyzed for DM, ash, and N as described above, and for Cr concentration (prepared according to Williams et al., 1962) via atomic absorption spectroscopy with an air-plus-acetylene flame (Model 3030B, PerkinElmer, Inc., Wellesley, MA). Composite duodenal and ileal samples were also analyzed for AA concentration by the University of Missouri Agricultural Experiment Station Chemical Laboratory, as described above.

Ruminal fluid samples were thawed and centrifuged at 20,000 × *g* for 20 min at 4°C. The resulting supernatant was analyzed for concentration of Co using atomic absorption spectroscopy, VFA (Goetsch and Galyean, 1983) by GLC (Hewlett Packard 5890A Series II GC; Wilmington, DE), and ammonia by colorimetric spectroscopy (Broderick and Kang, 1980). The natural log of Co concentration was regressed on sampling time to determine fluid dilution rate.

Ruminal contents from the evacuations were analyzed for DM as described above. Contents stabilized with formalin were blended for 1 min on high speed (Model 37BL19 CB6; Waring Products, New Hartford, CT). The mixed contents were then strained through four layers of cheesecloth, and the liquid portion was centrifuged in 250-mL bottles at 500 × *g* for 20 min at 4°C. The supernatant was centrifuged again at 500 × *g* for 20 min at 4°C, and bacteria were separated from the resulting supernatant by centrifugation at 30,000 × *g* for 20 min at 4°C. Duodenal samples and isolated ruminal bacteria were analyzed for DM, ash, N, and purines (Zinn and Owens, 1986).

Calculations to determine nutrient digestibility and flow were performed as described by Cline et al. (2009) using total fecal collections, as described above. Fecal recovery of Cr based on total fecal collection was 97.6 ± 1.9% [mean ± SEM]; thus, nutrient flows were not adjusted for Cr recovery.

Statistical Analysis

Data were analyzed as a 4 × 4 Latin square (Cochran and Cox, 1957) using the mixed model procedure of SAS version 9.1 (SAS Institute, Inc., Cary, NC). The general model included random effects of steer and sampling period and the fixed effect of treatment. Ruminal pH, ammonia concentration, and VFA concentrations over time were analyzed as repeated measures using the best fit covariance structure [compound symmetry, autoregressive, heterogeneous compound symmetry, or heterogeneous autoregressive; determined using fit statistics] within the 4 × 4 Latin square, with sampling time as the repeated effect and steer within treatment as the subject. Means were separated using least significant difference if the overall treatment is $P < 0.10$, and considered significant if $P \leq 0.05$ or tendencies if $0.05 < P < 0.10$.

RESULTS

Amino Acid Intake, Disappearance, and Flow

Total Arg intake (Table 1) followed Arg intake from rumen-protected Arg, as designed, and was greatest for Arg-RP360 ($P < 0.001$), followed by Arg-RP180 ($P < 0.001$), and least for CON and Arg-INJ ($P < 0.001$). Ruminal disappearance of Arg as a percent of intake was greater ($P < 0.001$) for Arg-RP180 and Arg-RP360 (71.4 and 76.1 ± 5.2%, respectively) compared with CON and Arg-INJ (8.7 and 18.7 ± 5.2%, respectively). The amount of Arg (g/d) disappearing in the rumen was greatest for Arg-RP360 ($P < 0.001$; Table 1), followed by Arg-RP180 ($P < 0.001$), and least for CON and Arg-INJ ($P < 0.001$). Despite this, duodenal Arg flow (Table 1) was greatest for steers fed Arg-RP360 ($P < 0.001$), followed by Arg-RP180 ($P < 0.003$), and least for CON and Arg-INJ ($P < 0.003$). Steers fed Arg-RP360 also had greater ($P < 0.03$) ileal Arg flow (Table 1) compared with all other treatments. Additionally, Arg-RP180 tended to have greater ($P = 0.09$) ileal Arg flow than CON, but not Arg-INJ ($P = 0.16$). Although there was no treatment effect ($P = 0.96$) on small intestinal disappearance of Arg as a percent entering the duodenum (50.3, 48.6, 51.5, and 51.6 ± 4.6% for CON, Arg-INJ, Arg-RP180, and Arg-RP360, respectively), small intestinal disappearance of Arg in g/d was greatest for steers fed Arg-RP360 ($P < 0.02$), followed by Arg-RP180 ($P < 0.04$), and least for CON and Arg-INJ ($P < 0.04$).

Intake of Arg, Orn, Pro, Glu, Lys, His, and Met from hay (g/d) was not different ($P > 0.25$) among treatments, as expected. Ruminal disappearance (g/d, Table 1; and %, data not shown) of Orn, Pro, Glu, Lys, His, and Met was not affected ($P > 0.24$) by treatment. There were no differences ($P \geq 0.23$)

Table 1. Effects of rumen-protected L-Arg supplementation or injected L-Arg-HCl on Arg and selected AA intake, flow, and disappearance in forage-fed steers

	Treatments[a]					
Item	CON	Arg-INJ	Arg-RP180	Arg-RP360	SEM	*P*-value
AA intake, g/d						
Arg intake, g/d	24.4[b]	24.3[b]	98.7[c]	178.1[d]	5.9	<0.001
From rumen-protected Arg	0.0[b]	0.0[b]	76.1[c]	150.6[d]	0.6	<0.001
From hay	24.4	24.3	22.5	27.5	5.7	0.34
Orn	0.465	0.475	0.429	0.531	0.085	0.33
Pro	30.1	30.6	27.7	34.1	5.3	0.34
Glu	55.5	55.8	51.1	62.4	10.8	0.35
Lys	25.9	26.1	23.6	28.6	5.0	0.26
His	8.16	8.20	7.54	9.19	1.70	0.34
Met	7.65	7.62	7.06	8.66	1.77	0.34
Ruminal disappearance, g/d						
Arginine	4.2[b]	6.5[b]	68.7[c]	135.4[d]	3.5	<0.001
Ornithine	−0.828	−0.683	−0.876	−1.303	0.231	0.25
Proline	9.47	9.73	6.86	10.50	3.58	0.46
Glutamate	6.25	7.77	3.53	6.90	6.89	0.69
Lysine	−4.25	−2.88	−5.23	−4.61	3.09	0.46
Histidine	0.162	0.494	−0.056	0.515	1.047	0.76
Methionine	0.386	0.792	0.116	0.648	1.020	0.51
Duodenal flow, g/d						
Arg	19.4[b]	18.0[b]	29.2[c]	43.1[d]	3.4	<0.001
Orn	1.24	1.18	1.25	1.83	0.26	0.23
Pro	20.5	20.9	20.7	23.6	2.5	0.32
Glu	48.6	48.5	47.0	55.4	5.3	0.33
Lys	29.8	29.2	28.5	33.2	3.2	0.40
His	7.86	7.78	7.46	8.67	0.85	0.46
Met	7.17	6.88	6.85	8.00	0.91	0.40
Ileal flow, g/d						
Arg	9.07[b]	9.84[b,c]	13.58[c]	21.06[d]	2.13	0.01
Orn	0.709[b]	0.797[b]	0.793[b]	1.121[c]	0.094	0.01
Pro	13.3	14.6	15.0	18.1	1.9	0.17
Glu	26.7[b]	29.5[b]	30.5[b]	38.5[c]	4.1	0.09
Lys	11.9	13.8	14.5	17.8	2.0	0.15
His	4.58	5.23	5.66	7.09	0.89	0.15
Met	3.14	3.51	3.69	4.74	0.56	0.12
Small intestinal disappearance, g/d						
Arginine	9.58[b]	8.92[b]	14.86[c]	21.72[d]	1.80	0.003
Ornithine	0.543	0.384	0.468	0.730	0.227	0.66
Proline	6.96	6.44	5.45	5.47	1.97	0.48
Glutamate	21.1	19.4	15.7	16.7	4.0	0.44
Lysine	17.6	15.7	13.7	15.2	2.3	0.29
Histidine	3.01	2.76	1.53	1.51	0.70	0.22
Methionine	3.94	3.42	3.07	3.24	0.60	0.33

[a]Treatments included: ad libitum grass hay (CON), CON hay with 27-mg L-Arg-HCl/kg BW injected twice daily (54 mg/kg BW daily; Arg-INJ), CON hay with 90-mg rumen-protected L-Arg/kg BW supplemented twice daily (180 mg/kg BW daily; Arg-RP180), and CON hay with 180-mg rumen-protected L-Arg/kg BW supplemented twice daily (360 mg/kg BW daily; Arg-RP360).

[b,c,d]Means within a row without a common letter superscript differ ($P \leq 0.05$).

among treatments in the flow of Orn, Pro, Glu, Lys, His, or Met to the duodenum. Ileal flow of Orn was affected ($P = 0.01$) and Glu tended to be affected ($P = 0.09$) by treatment, where steers fed Arg-RP360 had greater Orn ($P < 0.007$) and tended to have greater Glu ($P < 0.08$) compared with all other treatments. There were no differences ($P \geq 0.12$) in ileal flow Pro, Lys, His, or Met due to treatment. Small intestinal disappearance (g/d, Table 1; and %, data not shown) of Orn, Pro, Glu, Lys, His, and Met was unaffected ($P \geq 0.13$) by treatment.

Other Nutrient Intake and Site of Digestion

There was no effect of treatment on total or hay DMI ($P \geq 0.29$; Table 2). By design, intake of rumen-protected Arg was greatest for steers fed Arg-RP360 ($P < 0.001$), followed by Arg-RP180 ($P < 0.001$), and least for CON and Arg-INJ ($P < 0.001$; Table 2). Intake, small intestinal flow, and fecal flow, as well as site and extent of digestion of OM (Table 2), NDF (data not shown), and ADF (data not shown) did not differ ($P \geq 0.12$) among treatments.

Although N intake from hay (Table 3) was not different among treatments ($P = 0.36$), the differences in N intake from rumen-protected Arg caused a treatment effect for total N intake ($P = 0.009$); steers fed Arg-RP360 had greater ($P < 0.01$) total N intake than all other treatments. Duodenal N flow followed ($P = 0.02$) N intake, where steers fed Arg-RP360 had greater ($P < 0.02$) N flow compared with CON, Arg-INJ, and Arg-RP180. This was driven in part by apparent feed N flow, which followed the same pattern ($P = 0.009$), and bacterial N flow was unaffected ($P = 0.39$) by treatment. Apparent ruminal N digestibility tended ($P = 0.08$) to be affected by treatment, where N digestibility was greater ($P \leq 0.05$) for steers fed Arg-RP360 compared with CON and Arg-INJ and tended to be greater ($P = 0.09$) than Arg-RP180, although true ruminal N digestibility and microbial efficiency were not different ($P \geq 0.10$) among treatments.

Ileal N flow (Table 3) was greater ($P < 0.03$) for steers fed Arg-RP360 than all other treatments. Additionally, Arg-RP180 had greater ($P = 0.05$) ileal N flow than CON, but there were no differences ($P = 0.16$) in apparent small intestinal N digestibility due to treatment. Treatment tended to affect ($P = 0.09$) fecal N output, where steers fed Arg-RP360 also had greater ($P = 0.02$) fecal N compared with CON and tended to have greater ($P = 0.06$) fecal N than Arg-RP180. Although apparent large intestinal N digestibility was not different ($P = 0.43$) among treatments, apparent total tract digestibility was greatest for steers fed Arg-RP360 ($P < 0.03$), followed by Arg-RP180 ($P < 0.05$), and least for CON and Arg-INJ ($P < 0.05$).

Ruminal Fill and Fermentation

There was no effect of treatment ($P \geq 0.13$) on total ruminal fill, ruminal DM fill, or fluid dilution rate (Table 4). Ruminal pH was not affected ($P \geq 0.19$) by treatment, hour of sampling, or their interaction. Ruminal ammonia was affected by both hour of sampling ($P < 0.001$) and treatment ($P = 0.004$), but not their interaction ($P = 0.20$).

Table 2. Effects of rumen-protected L-Arg (RP-Arg) supplementation or intravenously injected L-Arg-HCl on DMI and OM intake and site of digestion in forage-fed steers

	Treatments[a]					
Item	CON	Arg-INJ	Arg-RP180	Arg-RP360	SEM	*P*-value
Total DMI, kg/d	7.76	8.09	7.25	8.92	0.95	0.29
Hay DMI, kg	7.76	8.09	7.12	8.67	0.95	0.34
RP-Arg DMI, g/d	0[b]	0[b]	122[b]	242[c]	1.0	<0.001
Total OM intake, kg/d	7.03	7.32	6.45	7.85	0.86	0.34
Duodenal OM flow, kg/d	3.21	3.11	3.22	3.65	0.36	0.48
Bacterial OM flow	0.543	0.477	0.523	0.551	0.047	0.31
Apparent feed OM	2.67	2.64	2.70	3.09	0.32	0.51
Ileal OM flow, kg/d	2.62	2.90	2.66	3.06	0.28	0.53
Fecal OM output, kg/d	2.38	2.68	2.51	2.88	0.30	0.20
OM digestibility, % of intake						
Apparent ruminal	53.1	57.5	48.5	53.8	3.2	0.20
True ruminal[e]	61.1	64.0	56.2	59.4	3.1	0.26
Apparent small intestinal	17.2	8.6	14.8	14.8	3.2	0.14
Apparent large intestinal	−5.20	−2.66	−3.93	−5.71	3.41	0.82
Apparent total tract	65.6	63.7	59.8	62.0	2.6	0.12

[a]Treatments included: ad libitum intake of grass hay (CON), CON with 27 mg L-Arg-HCl/kg BW injected intravenously twice daily (54 mg/kg BW daily; Arg-INJ), CON with 90 mg rumen-protected L-Arg/kg BW supplemented twice daily (180 mg/kg BW daily; Arg-RP180), and CON with 180 mg rumen-protected L-Arg/kg BW supplemented twice daily (360 mg/kg BW daily; Arg-RP360).

[b,c]Means within a row without a common letter superscript differ ($P \leq 0.05$).

[e]Corrected for OM of bacterial origin.

Table 3. Effects of rumen-protected L-Arg (RP-Arg) supplementation or injected L-Arg-HCl on N intake and site of digestion and microbial efficiency in forage-fed steers

	Treatments[a]					
Item	CON	Arg-INJ	Arg-RP180	Arg-RP360	SEM	*P*-value
Total N intake, g/d	94[b]	95[b]	106[b]	144[c]	18	0.009
N intake from hay	94.1	94.6	86.5	105.2	18.2	0.36
N intake from RP-Arg	0.0[b]	0.0[b]	19.6[c]	38.7[d]	0.2	<0.001
Duodenal N flow, g/d	90.9[b]	91.8[b]	95.7[b]	117.8[c]	11.7	0.02
Bacterial N flow	43.5	40.4	41.4	48.0	4.7	0.39
Apparent feed N flow	46.7[b]	51.9[b]	53.6[b]	69.6[c]	7.3	0.009
Ileal N flow, g/d	54.6[b]	59.0[b,c]	65.3[c]	78.3[d]	7.0	0.01
Fecal N output, g/d	36.2[b]	41.0[b,c]	38.9[b,c]	47.3[c]	4.7	0.09
N digestibility, % of intake						
Apparent ruminal	−4.20[b]	1.35[b]	3.98[b,c]	16.44[c]	8.07	0.08
True ruminal[e]	46.5	44.0	45.0	51.1	5.5	0.72
Apparent small intestinal	39.0	35.0	31.1	31.5	6.2	0.16
Apparent large intestinal	22.5	20.3	25.5	18.1	9.3	0.43
Apparent total tract	58.2[b]	56.7[b]	61.4[c]	65.5[d]	3.0	0.003
Microbial efficiency, g microbial N/kg OM truly fermented[f]	10.5	8.6	12.3	10.4	1.0	0.10

[a]Treatments included: ad libitum grass hay (CON), CON hay with 27-mg L-Arg-HCl/kg BW injected twice daily (54 mg/kg BW daily; Arg-INJ), CON hay with 90-mg rumen-protected L-Arg/kg BW supplemented twice daily (180 mg/kg BW daily; Arg-RP180), and CON hay with 180-mg rumen-protected L-Arg/kg BW supplemented twice daily (360 mg/kg BW daily; Arg-RP360).

[b,c,d]Means within a row without a common letter superscript differ ($P < 0.05$).

[e]Corrected for N of bacterial origin.

[f]Corrected for OM of bacterial origin.

Table 4. Effects of rumen-protected L-Arg supplementation or injected L-Arg-HCl on ruminal fill, pH, ammonia, and volatile fatty acids in forage-fed steers

	Treatments[a]					*P*-values		
Item	CON	Arg-INJ	Arg-RP180	Arg-RP360	SEM[b]	Treatment	Hour	Treatment × Hour
Total ruminal fill, kg	73.0	71.7	65.2	69.1	4.3	0.30	–	–
Ruminal DM fill, kg	9.83	8.71	8.55	9.94	0.73	0.40	–	–
Fluid dilution rate, %/h	7.99	6.15	4.87	7.98	1.44	0.13		
Ruminal pH	6.98	6.94	7.05	6.98	0.07	0.19	0.39	0.60
NH_3, mM	2.43[b]	2.73[b]	3.59[b,c]	4.52[c]	0.44	0.004	<0.001	0.20
Total VFA, mM	77.9	78.3	78.1	79.5	1.0	0.28	0.05	0.82
VFA concentration, mol/100 mol								
Acetate	51.0	50.2	50.7	51.0	1.1	0.44	<0.001	0.44
Propionate	24.6	25.5	24.0	24.6	0.8	0.10	<0.001	0.96
Butyrate	18.0	18.1	18.4	18.7	0.6	0.43	0.005	0.38
Isobutyrate	1.92[c,d]	1.75[b,c]	1.97[d]	1.64[b]	0.10	0.01	0.16	0.58
Valerate	1.81	1.78	1.90	1.81	0.09	0.21	<0.001	0.79
Isovalerate	2.63[b,c]	2.47[b]	2.92[c]	2.26[b]	0.23	0.03	0.003	0.28
Acetate:Propionate	2.09[c]	1.96[b]	2.11[c]	2.08[c]	0.09	<0.001	<0.001	0.69

[a]Treatments included: ad libitum grass hay (CON), CON hay with 27-mg L-Arg-HCl/kg BW injected twice daily (54 mg/kg BW daily; Arg-INJ), CON hay with 90-mg rumen-protected L-Arg/kg BW supplemented twice daily (180 mg/kg BW daily; Arg-RP180), and CON hay with 180-mg rumen-protected L-Arg/kg BW supplemented twice daily (360 mg/kg BW daily; Arg-RP360).

[b,c]Means within a row without a common letter superscript differ ($P \leq 0.05$).

Steers fed Arg-RP360 had a greater ($P < 0.004$) ammonia concentration than CON and Arg-INJ, and steers fed Arg-RP180 tended to have greater ($P = 0.06$) ammonia than CON.

There was no effect ($P > 0.27$) of the treatment × hour of sampling interaction on ruminal VFA concentrations (Table 4), although hour affected ($P \leq 0.05$) total VFA, acetate:propionate,

and the concentrations of all VFA except for isobutyrate. Isobutyrate concentrations were greater ($P < 0.05$) for steers fed Arg-RP180 than Arg-INJ and Arg-RP360, and CON had greater ($P = 0.01$) isobutyrate concentrations than Arg-RP360. Similarly, steers fed Arg-RP180 had greater ($P < 0.05$) isovalerate concentrations than Arg-RP360 and Arg-INJ, and CON tended to have greater ($P < 0.10$) isovalerate than Arg-RP360. Total VFA concentration and all other VFA concentrations were not different ($P \geq 0.10$) among treatments, although acetate:propionate was greater ($P < 0.002$) for CON, Arg-RP180, and Arg-RP360 compared with Arg-INJ.

DISCUSSION

In keeping with our hypothesis, rumen-protected Arg used in the current study increased small intestinal Arg delivery and disappearance in a dose-dependent manner. This is congruent with circulating Arg concentration data from this study, where rumen-protected Arg increased serum Arg in steers fed Arg-RP360 (Meyer et al., 2011a). Additionally, Arg-RP180 improved carotid and caudal artery hemodynamics, suggesting greater tissue blood perfusion (Meyer et al., 2011b), despite having similar serum Arg to both Arg-RP360 and CON.

Supplementation with rumen-protected Arg increased Arg flow to the duodenum by 9.8 and 23.7 g and small intestinal disappearance of Arg by 5.3 and 12.1 g for Arg-RP180 and Arg-RP360 treatments compared with CON, respectively. Increased delivery of Arg to the small intestine occurred despite relatively high rates of ruminal Arg disappearance, which indicated greater ruminal degradation than predicted by manufacturer provided data. It should be noted that the rumen-protected Arg product was believed to be 60% Arg on an as fed basis (data supplied by manufacturer). Laboratory analysis of product N suggests that this may have been 50% Arg, which could contribute to small intestinal Arg delivery being lower than expected. Ruminal disappearance of Arg consumed was likely due to deamination by ruminal microbes, which is supported by increased ruminal ammonia concentrations in both rumen-protected Arg treatments, or conversion to other metabolites such as Orn by ruminal microbes. There was a net appearance of Orn between the diet and the duodenum that was not affected by treatment, which suggests a net synthesis of Orn by ruminal microbes, perhaps from conversion by arginase or synthesis from other precursors. This lack of treatment effect on Orn synthesis suggests that it was independent of ruminal Arg supply, however.

Apparent small intestinal absorption of Arg reaching the duodenum was approximately 50% for all treatments. Arginine absorption reported here was less than previously reported values of 58% to 63% (Caton et al., 1991), 63% to 75% (Coomer et al., 1993), and 73% to 81% (Santos et al., 1984), although dietary CP was greater in these studies compared with the current study and Arg present was completely of plant or animal RUP sources. Our data suggest that duodenal flow of Arg from hay and from the rumen-protected product was absorbed to similar extents in the small intestine, suggesting that Arg source did not affect small intestinal disappearance in the current study. The amount of Arg unabsorbed in the small intestine increased with supplementation of the rumen-protected product. This may be due to a lack of release of the rumen-protected Arg, or delayed release of the rumen-protected product, which could have decreased intestinal absorption, but this was not measured in the current study. Alternatively, cationic AA transporters may have been approaching saturation as Arg delivery increased, and thus further increases in Arg transport may not have been possible. The adaptive repressive theory of Ferraris and Diamond (1989) states that transporter down-regulation will occur to decrease energy expenditure for transport of nutrients supplied in excess. Liao et al. (2009) reported that increased luminal supply of AA decreased jejunal mRNA expression of four cationic AA transporters in steers, indicating that Arg absorption may have been limited by AA transporter presence.

Increased ornithine in the small intestinal lumen was likely due to the presence of high arginase activity in the small intestine, which can be released from enterocytes into the intestinal lumen in nonruminants (Wu et al., 2009). This arginase would have had more substrate for the synthesis of ornithine in steers fed Arg-RP360. One pathway for Arg degradation through arginase results in Glu synthesis via ornithine aminotransferase (Flynn et al., 2002), and Glu may be spared from conversion to citrulline in the small intestine of animals fed high-Arg diets (Wu et al., 2009). Thus, the tendency for increased ileal flow of Glu may have been due to greater endogenous losses or decreased small intestinal uptake caused by sparing in the

Arg-RP360 treatment. Additionally, ileal flows of other AA studied here were also numerically increased for Arg-RP360, indicating their potential contribution to increased ileal N flow outside of Arg.

Ruminal and total tract digestibility of OM, NDF, and ADF were not affected by treatment, as has been previously reported for rumen-protected Met or Lys (Oke et al., 1986; Berthiaume et al., 2001). Ruminal ammonia concentrations may have been limiting for microbial growth in CON and Arg-INJ steers based upon data of Satter and Slyter (1974), but concentrations were greater and most likely adequate in steers fed rumen-protected Arg, likely a result of degradation of Arg by ruminal microbes. The addition of rumen-protected Arg did not increase ruminal OM digestion or microbial N flow, suggesting that ruminal N availability in the CON hay diet did not limit microbial growth.

Apparent total tract N digestibility was affected by treatment, which is not surprising given the contribution of Arg to dietary N intake. The Arg-RP360 treatment increased duodenal N flow, which was a result of increased apparent feed N flow. It is unclear why the Arg-RP180 did not increase duodenal N flow, given the increase in duodenal Arg flow for this treatment, but may have been due to numerically lowest hay intakes by steers given Arg-RP180. Although true ruminal and apparent intestinal N digestibilities were not affected by treatment, apparent total tract N digestibility was increased with each dose of rumen-protected Arg. This effect on total tract digestibility is likely due to greater total tract digestion of N from the rumen-protected Arg product than in the hay. Intestinal flow of N and Arg (duodenal vs. ileal) do not match quantitatively, suggesting that increased ileal N flow was from N sources other than Arg. Likewise, increased fecal N flow in the Arg-RP360 treated steers was greater than N from Arg, Orn, and Glu at the terminal ileum; thus, additional N sources must have been present. It is also possible that N recycling stimulated hindgut fermentation or altered endogenous losses, although large intestinal apparent digestion was not affected by treatment. Arginine supplementation at high doses has been known to cause diarrhea or other digestive upsets in humans (Grimble, 2007), but fecal DM was not affected by treatment (18.5 ± 0.05%; $P = 0.74$) and no adverse side effects were noted in the current study.

Although microbial efficiency and fluid dilution rate were not affected by treatment in this study, high variation was present in these parameters despite good fecal marker recovery. Volatile fatty acid concentrations were only minimally affected. Brooks et al. (2011) also observed a decrease in branched-chain VFA concentrations in response to in vitro Arg supplementation, which may have been due to increased uptake of the branched-chain VFA or decreased degradation of branch-chain AA by ruminal microbes when Arg was in excess. The reduction in the acetate:propionate ratio for Arg-INJ is difficult to explain, but was only a 6% change and may not be biologically relevant.

In conclusion, rumen-protected Arg used in this study resulted in increased delivery of Arg to the small intestine and increased intestinal uptake of Arg in forage-fed cattle, as hypothesized. Furthermore, in keeping with our hypothesis, ruminal fermentation and nutrient digestibility were minimally affected by rumen-protected Arg supplementation or intravenous injection of Arg-HCl.

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