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Ruminant Nutrition

RUMINANT NUTRITION

Rumen-protected arginine in ewe lambs: effects on circulating serum amino acids and carotid artery hemodynamics

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Abstract

Sixty nonpregnant, nulliparous Rambouillet ewes (51 ± 1.4 kg initial body weight) were used in a completely randomized design to determine 1) if rumen-protected L-Arg (**RP-ARG**) supplementation would increase serum concentrations of amino acids resulting from Arg supplementation and metabolism, and decrease serum concentrations of amino acids that compete with Arg for transporters, 2) if RP-ARG supplementation would alter carotid artery hemodynamics, and 3) the most effective oral dose of RP-ARG to positively increase both circulating amino acids and improve peripheral tissue blood perfusion as measured by carotid hemodynamics. Ewes were penned individually in a temperature-controlled facility. Ewes were randomly assigned to one of four treatments: a control group that received no supplemental Arg (**CON**; 50 g of finely ground corn, only), or Arg-supplemented groups that received 90 (**90**), 180 (**180**), or 360 (**360**) mg RP-ARG·kg BW^{-1}·d^{-1} mixed in 50 g of finely ground corn. Supplements were administered once daily for 14 d and fully consumed before the delivery of a total pelleted diet at 0630 and 1830 hours daily. Baseline and final blood samples were collected at days 0 (before treatment initiation) and 15, respectively. Doppler ultrasound was used to assess carotid arterial hemodynamics at 0600 hours on days 0 (before treatment initiation), 5, 8, 12, and 15. After 14 d of supplementation, ewes fed 180 had greater Arg ($P = 0.05$) and Orn ($P = 0.05$) and tended ($P = 0.08$) to have greater Asp in serum than ewes fed 90, and for these amino acids, ewes fed 180 were similar ($P \geq 0.16$) compared with ewes fed 360. All supplemented ewes (90, 180, and 360) had a negative change ($P = 0.02$) from baseline when normalized to CON for the pulsatility and resistance indices, which indicate greater distal tissue blood perfusion and lower vascular resistance of blood flow, respectively. Additionally, there were quadratic responses for the pulsatility and resistance indices ($P = 0.03$ and 0.01, respectively) where ewes fed 180 had the greatest change from baseline when normalized to CON. Results indicate that Arg supplementation increased serum amino acid concentrations and improved peripheral tissue blood perfusion. The 180 mg·kg BW^{-1}·d^{-1} RP-ARG dose was determined to be the optimal dose for nonpregnant, nulliparous Rambouillet ewes.

Key words: amino acids, arginine, blood flow, nutrition, sheep

Abbreviations

ADF	acid detergent fiber
BCS	body condition score
BW	body weight
CON	control
CP	crude protein
CSA	cross-sectional area
DM	dry matter
EDV	end-diastolic velocity
MnV	mean velocity
NDF	neutral detergent fiber
PI	pulsatility index
PSV	peak systolic velocity
RI	resistance index
RP-ARG	rumen-protected L-Arg
SV	stroke volume

Introduction

The amino acid Arg has many important functions, including serving as the immediate precursor for nitric oxide and polyamine synthesis (Wu and Morris, 1998; Wu et al., 2009). In ruminants, strategically supplementing Arg during the periconceptual period or later during gestation has yielded mixed pregnancy outcomes (Luther et al., 2009; Lassala et al., 2010, 2011; Saevre et al., 2011; Crane et al., 2016). Saevre et al. (2011) reported improved pregnancy rates using intravenous L-Arg HCl (27 mg L-Arg·kg body weight (**BW**)$^{-1}$·d^{-1}) from day 9 to 14 post-laparoscopic artificial insemination. Lassala et al. (2010, 2011) found intravenous Arg (81 mg Arg·kg BW^{-1}·d^{-1}) infusion to nutrient-restricted, pregnant ewes from day 60 until parturition resulted in enhanced lamb birth weights, and intravenous Arg (180 mg L-Arg·kg BW^{-1}·d^{-1}) to ewes carrying multiple fetuses from day 100 to 121 of gestation improved fetal survival. Conversely, others have reported no differences for pregnancy or lambing rates when supplementing with injectable (Luther et al., 2009; Crane et al., 2016) or oral (Crane et al., 2016) Arg from day 0 to 14 post-breeding. However, Crane et al. (2016) did find increased weaning rate (lambs weaned per ewe lambing) and a tendency for increased litter weaning weight when ewes received intravenous Arg (30 mg L-Arg·kg BW^{-1}·d^{-1}). Recently, Peine et al. (2018) reported lamb birth weight, average daily gain from day 14 to 19 of age, girth measurements at birth and days 19 and 54 of age, and curved crown-rump measurements at day 54 of age were responsive to a 180-mg rumen-protected L-Arg (**RP-ARG**) supplement.

Due to amino acid breakdown in the rumen, dietary amino acid supplementation in ruminants requires direct intravenous injection, post-ruminal infusion, or use of a protective agent to permit the amino acid to bypass the rumen and be absorbed in the small intestine. Meyer et al. (2018) showed increased Arg delivery to the small intestine and intestinal Arg uptake while minimally affecting ruminal fermentation and nutrient digestibility for forage-fed steers that consumed RP-ARG. Because dietary supplementation of Arg is more practical in ruminant livestock production than intravenous injections or post-ruminal infusions, the current research strategy focused on feeding RP-ARG to ewes.

Arginine serves as a substrate for nitric oxide synthase, which in turn synthesizes nitric oxide (Wu et al., 2009, 2013). Nitric oxide is a potent vasodilator that increases blood flow by regulating vascular tone (Martín et al., 2001). Because the carotid artery is one of the first major vessels leaving the aorta, the carotid artery can be used as an indicator of systemic blood flow. Strategic RP-ARG supplementation has been shown to influence carotid artery hemodynamics in steers through increased distal tissue perfusion and decreased resistance in the vessel itself (Meyer et al., 2011b). We hypothesized that RP-ARG supplementation to nulliparous ewes would alter carotid artery hemodynamics and circulating serum amino acids related to Arg and its metabolism, but reduce circulating amino acids that share Arg transporters. We further hypothesized that observed responses to RP-ARG would be RP-ARG dose-dependent.

Materials and Methods

This research was approved by the North Dakota Institutional Animal Care and Use Committee protocol number A12070.

Animals and facility

Nulliparous Rambouillet-cross ewe lambs (n = 60; 51 ± 1.4 kg initial BW; approx. 6 mo of age) from North Dakota State University Hettinger Research Extension Center were housed individually in a temperature-controlled facility that allowed visual and physical contact between ewes (12 to 21 °C; North Dakota State University Animal Nutrition and Physiology Center, Fargo, ND) with free access to water. The lighting in the facility mimicked normal daylight patterns for late June to early July in North Dakota.

Experimental design and treatments

All ewes were fed a complete pelleted diet (Table 1) twice daily (0630 and 1830 hours) to meet or exceed NRC (2007) recommendations. Dietary intake for each ewe was calculated based on BW and metabolizable energy requirements. Diets were analyzed for dry matter, ash, and crude protein following AOAC (1990), and neutral and acid detergent fibers using an Ankom Fiber Analyzer (Ankom Technology, Fairport, NY). In addition to the pelleted diet, ewes were randomly assigned to one of four treatments: a control group that received no supplemental Arg (**CON**; n = 15), or RP-ARG-supplemented

Table 1. Ingredient and nutrient composition of pelleted diet fed to ewe lambs[1]

Item	%	Arginine, % DM basis[2]
Ingredient		
Alfalfa meal, dehydrated	34.0	1.18
Beet pulp, dehydrated	27.0	0.40
Wheat middlings	25.0	1.29
Ground corn	8.4	0.16
Soybean meal	5.0	5.50
Trace mineral premix[3]	0.6	—
Nutrient composition[4]		
DM	89.9	—
CP	15.5	—
NDF	37.2	—
ADF	21.5	—

[1]Diets were fed to ewes twice daily at 0630 and 1830 hours.
[2]Arginine values were obtained from the NASEM, 2016.
[3] Premix: 18% to 21% Ca, 9% P, 10% to 11% NaCl, 49.3 ppm Se, 700,000 IU/kg Vitamin A, 200,000 IU/kg Vitamin D, and 400 IU/kg Vitamin E.
[4]DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber.

groups that received 90 (**90**; *n* = 15), 180 (**180**; *n* = 15), or 360 (**360**; *n* = 15) mg rumen-protected RP-ARG·kg BW^{-1}·d^{-1} (based on initial BW). Rumen-protected Arg (Kemin Industries, Des Moines, IA; Meyer et al., 2018; Peine et al., 2018) was mixed with 50 g of finely ground corn and fed once daily before offering the pelleted diet at 0630 hours. Control ewes (0 mg/kg RP-ARG) were provided 50 g of finely ground corn without the added RP-ARG just before feeding the pelleted diet at 0630 hours. A 7-d adaptation period to the pelleted diet was implemented before initiating the RP-ARG treatments; the supplementation period continued for a total of 14 days. Ewe BW and body condition score (**BCS**) were measured at initiation (day 0) and conclusion (day 15) of the study. A 1 to 5 scale (1 = emaciated and 5 = obese) was used by two independent evaluators to measure BCS.

Blood sample collection and analysis

Blood samples were obtained at 0600 hours on days 0 (baseline, initial) and 15 (final) of the supplementation period. Collections were obtained from the right jugular vein via venipuncture to avoid puncturing the left carotid artery, which was used for Doppler ultrasonography. Blood (10 mL) was collected with Corvac serum separator vacuum tubes with thrombin (Tyco Healthcare, Mansfield, MA), which were placed on ice and held a minimum of 45 min. Whole blood was centrifuged at 1,500 × *g* for 30 min at 4 °C, then the supernatant was pipetted into 2-mL screw-cap vials and stored at -20 °C. Serum amino acid concentrations were evaluated using ultra-performance liquid chromatography methods similar to those of Crouse et al. (2019).

Doppler ultrasonography

Carotid artery hemodynamics were evaluated using duplex B-mode and D-mode programs of the color Doppler ultrasound instrument (model SSD-4000; Aloka America, Wallingford, CT) with an attached 7.5-MHz finger transducer probe (Aloka UST-995). Ultrasound assessments occurred on day 0 (baseline, initial) and days 5, 8, 12, and 15 of the supplementation period. All scans were obtained without the use of anesthesia, approximately 10 cm below the mandible on the left carotid artery. Before scanning, the area of interest was sheared of wool and cleaned, and Aquasonic transmission gel (Parker Laboratories, Fairfield, NJ) was utilized. Using B-mode, a longitudinal section of the carotid artery was visualized by manually turning the transducer of the probe. The probe was aligned to the carotid artery at an average angle of insolation of 68.9 ± 0.5°, and arterial pulsatility was confirmed using a duplex view of B-mode and D-mode. The duplex view allowed for the collection of final hemodynamic measurements using B-mode for visualization while simultaneously using D-mode to record pulsatile waves. As described by Lemley et al. (2012), three comparable cardiac cycle waveforms from three separate ultrasonography assessments were averaged per ewe within a sampling day (i.e., 9 waveform measurements per day). Hemodynamic measurements were calculated using peak systolic velocity (**PSV**; cm/s), end-diastolic velocity (**EDV**; cm/s), mean velocity (**MnV**; cm/s), and cross-sectional area of the vessel (**CSA**; cm^2). These parameters were used to calculate pulsatility index (**PI**; PI = [PSV - EDV]/MnV), an indicator of tissue blood perfusion; resistance index (**RI**; RI = [PSV - EDV]/PSV), an indicator of vascular resistance; and flow volume (flow volume = MnV * CSA * 60 s; mL/min). Additionally, heart rate (beats/min), stroke volume (**SV**; SV = velocity-time interval × CSA; mL), and cardiac output (cardiac output = SV × heart rate; L/min). All calculations given here were performed by the preprogrammed Doppler software.

Statistical analysis

BW, BCS, and amino acid data, including initial (day 0), final (day 15), and change (final - initial), were analyzed as a completely random design using the general linear models procedure of SAS (SAS Inst. Inc., Cary, NY) with ewe serving as an experimental unit with contrasts used to address specific questions. The contrasts evaluated whether the overall RP-ARG supplementation had an effect on performance measurements and circulating serum amino acid concentrations (CON vs. 90, 180, and 360), whether 180 differed from 90 (180 vs. 90), and whether 180 differed from 360 (180 vs. 360).

For hemodynamic data, there were four treatments (*n* = 15 lambs/treatment) and four sampling periods (after baseline hemodynamic measurements). Data were calculated as percent change from baseline and then normalized to the CON treatment by setting it to zero and analyzed with the general linear models procedure of SAS. The model contained the effects of RP-ARG treatment, sampling period (days 5, 8, 12, and 15), and the interaction. No treatment × sampling period interactions were present, so the interaction was dropped from the model, and the main effect least square means are reported. Contrasts were used to address if there was an overall effect of RP-ARG (CON vs. 90 + 180 + 360), if the RP-ARG effects were linear with increasing levels of RP-ARG, and if the RP-ARG effects were quadratic with increasing levels of RP-ARG. For the linear and quadratic contrasts, unequal spacing coefficients were generated using the interactive matrix language procedure of SAS for the contrast statements.

Results

Ewe performance data

There were no differences ($P \geq 0.77$; Table 2) for initial or final BW. When the change in BW was calculated, there was a tendency ($P = 0.06$) for ewes fed 180 to gain less than ewes fed 90. There were no differences ($P \geq 0.31$) for initial or final BCS. When the change in BCS was calculated, there was a tendency ($P = 0.10$) for ewes fed 180 to gain condition while ewes fed 360 lost condition.

Serum amino acids

Specific amino acids of interest, Arg, Orn, Cit, Asp, Pro, Glu, Gln, Met, Lys, and His, are presented in Table 3 and discussed herein. Serum concentrations of Cit, Pro, and Met were not altered ($P \geq 0.12$) due to RP-ARG supplementation or level of RP-ARG supplement.

Serum Arg concentrations tended ($P = 0.07$) to be greater for RP-ARG-supplemented vs. CON ewes at the start of the study. At the conclusion of the study, ewes fed 180 had greater ($P = 0.05$) serum Arg than ewes fed 360. There were no differences ($P \geq 0.17$) in the change of serum Arg across the duration of the study.

There were no differences ($P \geq 0.46$) in serum Orn concentrations due to RP-ARG supplementation compared with CON ewes. Concentrations of serum Orn tended ($P = 0.07$) to be greater for 180 vs. ewes fed 90 at the start of the study. At the end of the study, serum Orn was greater ($P = 0.05$) for ewes fed 180 vs. 90. There were no differences ($P \geq 0.17$) in the change of serum Orn across the duration of the study.

Rumen-protected L-Arg supplementation did not affect ($P \geq 0.23$) serum Asp concentrations compared with CON ewes. Serum Asp concentrations were not different ($P \geq 0.10$) at the

Table 2. Initial, final, and change in BW and BCS measurements of nulliparous ewe lambs supplemented varying doses of rumen-protected L-Arg (RP-ARG) for 14 d

	Treatments[1]				Contrast *P*-values[2]		
Item	CON	90	180	360	SEM	CON vs. 90, 180, 360	180 vs. 90	180 vs. 360
BW, kg								
Initial	50.4	49.6	50.0	49.5	1.9	0.77	0.89	0.86
Final	52.3	52.5	51.7	51.7	2.0	0.90	0.78	0.98
Change[3]	1.9	2.9	1.8	2.2	0.4	0.47	0.06	0.50
BCS[4]								
Initial	2.70	2.73	2.63	2.72	0.07	0.95	0.31	0.39
Final	2.70	2.75	2.67	2.68	0.08	0.99	0.44	0.88
Change[3]	0.00	0.02	0.03	–0.03	0.03	0.87	0.67	0.10

[1]Control received no supplemental Arg (CON; 50 g fine ground corn). Arginine supplemented groups received 90 (90), 180 (180), or 360 (360) mg RP-ARG·kg BW^{-1}·d^{-1} mixed in 50 g of fine ground corn (n = 15 ewe lambs/treatment).

[2]The contrasts evaluated whether overall RP-ARG supplementation affected performance measurements (CON vs. 90, 180, and 360), whether 180 differed from 90 (180 vs. 90), and whether 180 differed from 360 (180 vs. 360). For the standard error of the mean (SEM), n = 15.

[3]Change = final – initial.

[4]BCS was measured on a 1 to 5 scale (1 = emaciated and 5 = obese) by two independent evaluators.

onset of the study. Aspartate concentrations in the serum from ewes fed 180 tended to be greater (P = 0.08) than ewes fed 90 at the conclusion of the study. Furthermore, Asp concentrations tended (P = 0.07) to increase across the duration of the study for ewes fed 180, whereas ewes fed 360 had decreased Asp concentrations.

Serum Glu concentrations were not affected ($P \geq 0.14$) by RP-ARG supplementation compared with CON ewes. Ewes fed 180 mg rumen-protected RP-ARG·kg BW^{-1}·d^{-1} had greater serum Glu than ewes fed 90 at both the onset and conclusion of the study (P = 0.04 and 0.02, respectively); consequently, there was no change (P = 0.22) in Glu concentrations between ewes fed 180 and 90 across the duration of the study. However, change in serum Glu concentrations across the duration of the study was greater (P = 0.05) for ewes fed 180 vs. 360.

Rumen-protected L-Arg supplementation did not alter ($P \geq 0.66$) serum Gln concentrations compared with CON ewes. Serum Gln concentrations tended to be greater (P = 0.06) for ewes fed 360 vs. 180 at the onset of the study. However, that tendency disappeared (P = 0.23) by the conclusion of the study, and no differences ($P \geq 0.22$) were noted between the level of RP-ARG supplementation for change of Gln across the duration of the study.

Serum Lys was not affected ($P \geq 0.26$) by RP-ARG supplementation compared with CON ewes. At the onset of the study, serum Lys concentrations were greater (P = 0.02) for ewes fed 180 vs. 90. This same pattern was observed (P = 0.04) at the conclusion of the study. Consequently, Lys concentrations between ewes fed 180 and 90 across the duration of the study were not different (P = 0.99). For ewes fed 360, serum Lys tended to be lower (P = 0.06) at the conclusion of the study when compared with ewes fed 180. Because ewes fed 360 and 180 were not different (P = 0.92) at the onset of the study, change in serum Lys across the duration of the study tended to increase (P = 0.06) for ewes fed 180 and decrease for ewes fed 360.

Rumen-protected L-Arg supplementation did not affect ($P \geq 0.58$) serum His concentrations compared with CON. For initial or final His concentrations, there were no differences ($P \geq 0.12$) in the assessed contrasts. However, when the change in serum His was calculated across the duration of the study, ewes fed 180 had a positive change (P = 0.02) while ewes fed 360 had a negative change.

Carotid artery hemodynamics

Peak systolic velocity tended to be greater (P = 0.08; Table 4) for RP-ARG-supplemented ewes while EDV and MnV were greater (P = 0.01) for RP-ARG when compared with CON ewes. There was a linear increase for PSV and MnV (P = 0.03 and 0.02, respectively) and a tendency (P = 0.06) for a linear increase of EDV due to increasing RP-ARG supplementation levels. Cross-sectional area of the carotid artery decreased (P < 0.01) due to RP-ARG supplementation compared with CON. Furthermore, the decreased CSA was linear (P < 0.01) based on increasing RP-ARG supplementation level.

Rumen-protected L-Arg-supplemented ewes had lower (P = 0.02) PI and RI compared with CON ewes, which indicates greater distal tissue perfusion and decreased blood flow resistance, respectively. For supplemented ewes, there was a quadratic effect of the Rumen-protected L-Arg supplement level for PI and RI (P = 0.03 and P = 0.01, respectively). RP-ARG supplementation or level of RP-ARG supplement did not alter ($P \geq 61$) the flow volume.

Rumen-protected L-Arg supplementation increased (P = 0.02) ewe heart rate compared with CON ewes. Further, the increase

Table 3. Serum amino acid initial, final, and change in concentrations (μmol/L) of nulliparous ewes supplemented with varying doses of rumen-protected L-Arg (RP-ARG) for 14 d

	Treatment[1]					Contrast *P*-values[2]		
	CON	90	180	360	SEM	CON vs. 90, 180, 360	180 vs. 90	180 vs. 360
Arg								
Initial	164.0	173.1	180.6	175.6	5.9	0.07	0.37	0.55
Final	160.3	158.5	178.3	176.1	7.1	0.20	0.05	0.83
Change[3]	−3.7	−14.6	−2.4	0.4	6.2	0.80	0.17	0.75
Orn								
Initial	45.3	39.4	45.0	46.5	2.1	0.46	0.07	0.70
Final	48.8	45.5	53.2	48.6	2.7	0.93	0.05	0.23
Change[3]	3.5	6.1	8.2	2.4	2.7	0.51	0.59	0.14
Cit								
Initial	149.9	153.1	143.9	148.1	7.8	0.86	0.41	0.71
Final	134.5	137.5	138.6	155.0	8.4	0.35	0.93	0.17
Change[3]	−15.4	−15.5	−5.3	6.9	6.7	0.17	0.29	0.20
Asp								
Initial	10.9	10.4	12.1	12.5	0.7	0.32	0.10	0.65
Final	10.3	10.7	13.0	11.1	0.9	0.23	0.08	0.16
Change[3]	−0.5	0.2	0.9	−1.4	0.9	0.65	0.60	0.07
Pro								
Initial	89.0	82.9	91.2	93.9	3.7	0.94	0.12	0.62
Final	95.1	94.3	99.3	91.8	3.5	0.99	0.31	0.13
Change[3]	6.0	11.4	8.1	−2.0	4.6	0.96	0.61	0.12
Glu								
Initial	54.9	44.1	52.2	54.6	2.7	0.14	0.04	0.53
Final	63.7	59.0	75.1	64.5	4.6	0.64	0.02	0.11
Change[3]	8.7	15.0	22.9	9.9	4.5	0.17	0.22	0.05
Gln								
Initial	472.7	464.2	446.3	486.0	14.3	0.66	0.38	0.06
Final	300.1	295.5	293.8	309.0	9.9	0.95	0.89	0.23
Change[3]	−172.6	−168.7	−152.5	−177.0	13.9	0.68	0.41	0.22
Met								
Initial	17.3	17.3	18.5	19.3	0.9	0.30	0.38	0.52
Final	18.1	17.3	19.2	17.6	1.1	0.94	0.24	0.31
Change[3]	0.8	−0.0	0.7	−1.8	1.1	0.36	0.65	0.13
Lys								
Initial	98.2	91.6	115.7	114.7	7.0	0.26	0.02	0.92
Final	102.9	100.1	124.1	102.6	7.9	0.51	0.04	0.06
Change[3]	4.7	8.5	8.4	−12.2	7.5	0.72	0.99	0.06
His								
Initial	78.9	77.1	77.3	83.3	2.7	0.91	0.97	0.12
Final	80.2	78.7	80.7	76.7	2.7	0.64	0.60	0.30
Change[3]	1.3	1.6	3.5	−6.6	2.9	0.58	0.64	0.02

[1]Control received no supplemental Arg (CON; 50 g fine ground corn). Arginine supplemented groups received 90 (90), 180 (180), or 360 (360) mg RP-ARG·kg BW^{-1}·d^{-1} mixed in 50 g of fine ground corn (n = 15 ewe lambs/treatment).
[2]The contrasts evaluated whether overall RP-ARG supplementation affected circulating serum amino acid concentrations (CON vs. 90, 180, and 360), whether 180 differed from 90 (180 vs. 90), and whether 180 differed from 360 (180 vs. 360). For the standard error of the mean (SEM), n = 15.
[3]Change = final – initial.

Table 4. Carotid artery hemodynamics[1] of nulliparous ewes supplemented with varying doses of rumen-protected L-Arg (RP-ARG) normalized to the control (CON) treatment

	Treatments[2]				Contrast *P*–values[3]		
Item	CON	90	180	360	SEM	CON vs.90, 180, 360	Linear	Quadratic
	% change from baseline							
PSV, cm/s	0.00	5.49	2.88	10.04	2.99	0.08	0.03	0.89
EDV, cm/s	0.00	15.84	13.99	15.71	4.80	0.01	0.06	0.11
MnV[4], cm/s	0.00	11.74	10.20	14.53	3.72	0.01	0.02	0.23
CSA, cm^3	0.00	–7.03	–9.99	–11.51	1.82	0.001	0.001	0.03
Pulsatility index[5]	0.00	–6.99	–8.30	–5.10	2.53	0.02	0.27	0.03
Resistance index[6]	0.00	–3.28	–3.60	–0.92	0.93	0.02	0.85	0.01
Flow volume[7], mL/min	0.00	2.75	–4.12	0.74	4.06	0.97	0.91	0.61
Heart rate, beats/min	0.00	0.80	3.49	8.92	1.70	0.02	0.001	0.52
SV[8], mL	0.00	3.25	–7.12	–8.88	4.22	0.38	0.05	0.99
Cardiac output[9], L/m	0.00	1.81	–4.24	–0.12	4.09	0.86	0.83	0.61

[1]Three comparable cardiac cycle waveforms from three separate Doppler ultrasonography assessments were averaged per ewe within a collection day (9 waveform measurements per day).
[2]Control received no supplemental Arg (CON; 50 g fine ground corn). Arginine supplemented groups received 90 (90), 180 (180), or 360 (360) mg RP-ARG·kg BW^{-1}·d^{-1} mixed in 50 g of fine ground corn (n = 15 ewe lambs/treatment).
[3]There were four treatments (n = 15 lambs/treatment) and four sampling periods (after baseline hemodynamic measurements). Data were calculated as percent change from baseline and then normalized to the control treatment by setting it to zero. Contrasts were used to address specific questions: if there was an overall effect of RP-ARG (CON vs. 90 + 180 + 360), if the RP-ARG effects were linear with increasing levels of RP-ARG, and if the RP-ARG effects were quadratic with increasing levels of RP-ARG. For the linear and quadratic contrasts, unequal spacing coefficients were generated using the IML procedure of SAS for the contrast statements. For the standard error of the mean (SEM), n = 60.
[4]MnV = Velocity time integral (cm) / Flow time (ms).
[5]Pulsatility index = (PSV – EDV) / MnV.
[6]Resistance index = (PSV – EDV) / PSV.
[7]Flow volume = MnV × CSA × 60 s.
[8]SV = Velocity time integral (cm) × CSA (cm^2).
[9]Cardiac output = SV × Heart rate.

in heart rate due to increasing RP-ARG supplement level was linear ($P = 0.001$; Table 4). There was no change in SV for RP-ARG-supplemented vs. CON ewes; however, a linear decrease in SV ($P = 0.05$) was found for increased RP-ARG levels. Cardiac output was not altered ($P \geq 0.61$) by RP-ARG supplementation or level of RP-ARG supplement.

Discussion

Ewe performance data

The current ewe BW data are similar to Peine et al. (2018), where no improvement in ewe BW was noted due to ewes fed 180 mg RP-ARG·kg $BW^{-1}·d^{-1}$ that received 60% nutrient restriction from day 54 of gestation to term when compared with CON. However, that study did not compare different levels of RP-ARG supplementation. Three other short-duration Arg supplementation studies (40 d or less) reported no differences for BW due to Arg supplementation via jugular infusion to nutrient-restricted ewes (McCoard et al., 2013; Satterfield et al., 2013; Guo et al., 2017). In a longer-term study where ewes received 10 g/d of L-Arg as a RP-ARG supplement from day 35 to 105 of gestation, maternal BW was not altered within nutrient-restricted ewes (Zhang et al., 2016).

In the current study, changes to BCS were small, and due to the short, 15-d supplementation period, these changes were probably not due to RP-ARG supplementation. McCoard et al. (2013) fed ewes at 100% NRC requirements and supplemented Arg via tarsal vein catheter infusions three times daily (345 µmol·kg BW^{-1}·infusion^{-1}) from day 100 to 140 of gestation and found in cohort 1, Arg-infused ewes tended to have greater BCS on day 120 and did have greater BCS at days 134 and 140 vs. control ewes, and in cohort 2, Arg-infused ewes tended to have greater BCS only at day 113. Most research with Arg supplementation focuses on relatively short-term supplementation strategies. Applications of short-term supplementation studies to longer-term scenarios should be approached with caution. Additional research with longer-term Arg supplementation during pregnancy in ruminants would provide useful insight and add to the literature.

Serum amino acids

When looking specifically at the concentration of amino acids involved in the urea cycle, the Arg and Orn results from the current study are similar to the findings by Southern and Baker (1982), in which dietary Arg supplementation increased circulating Arg and Orn concentrations in pigs. Additionally, in beef steers, Meyer et al. (2011a) found that 360 mg RP-ARG·kg $BW^{-1}·d^{-1}$ resulted in increased serum Arg and Orn concentrations (expressed as area under the curve over 12 h post-supplementation or 14 d of supplementation) when compared with control-fed steers, but supplementation of Arg via jugular infusion (54 mg L-Arg-HCl·kg $BW^{-1}·d^{-1}$) resulted in the greatest circulating Arg and Orn concentrations. Meyer et al. (2011a) reported increased serum Cit concentrations regardless of route or level of Arg supplementation. In this same study, Meyer et al. (2018) reported increasing ruminal and small intestinal disappearance and duodenal and ileal flow of Arg with the increasing levels of RP-ARG from 0 to 180 to 360 mg RP-ARG·kg $BW^{-1}·d^{-1}$. These authors further reported increased ileal flow of Orn when steers were fed 360 mg RP-ARG·kg $BW^{-1}·d^{-1}$ (Meyer et al., 2018). For nutrient-restricted ewes fed at 50% of NRC recommendations, Arg supplementation equivalent to 81 mg L-Arg·kg $BW^{-1}·d^{-1}$ via jugular infusion resulted in greater plasma Arg and Orn but similar Cit and Asp concentrations compared with saline-infused, underfed ewes (Satterfield et al., 2013). Similarly, McCoard et al. (2013) reported greater plasma Arg and Orn but similar Cit and Asp concentrations for ewes fed at 100% NRC concentrations that were supplemented with infusions of 345 µmol Arg·kg BW^{-1} three times daily. In nutrient-restricted ewes fed at 50% NRC recommendations, RP-ARG supplementation at 20 g/d increased circulating serum Arg, Orn, and Cit but did not alter Asp concentrations in comparison to nutrient-restricted ewes not supplemented with Arg (Zhang et al., 2016). It should be noted that daily RP-ARG supplementation occurred for 14 d and final serum samples were collected at 0600 hours on day 15, or approximately 24 h after the last RP-ARG dose. Consequently, final serum sample concentrations are reflective of baseline changes in circulating amino acids and not necessarily driven by the half-life of specific amino acids associated with a single daily dose. Therefore, differences in final serum amino acid concentrations would likely be small and percentage changes from initial to final sampling points are reflective of baseline changes in circulating amino acids. In the present study, while final serum amino acid concentrations were often responsive to dietary treatments, percentage change from initial to final sampling points were not.

In mammals, Arg synthesis occurs when Cit from the small intestine is taken up by the kidney, and by joining Asp, arginosuccinate is formed and then cleaved to fumarate and Arg that enters the circulation (Mistry et al., 2002; Marini et al., 2017). There were no differences in Cit concentrations, so the greater final Arg concentration for ewes fed 180 vs. 90 would be due to the passage of RP-ARG from the rumen for systemic absorption. Because Orn is a product of Arg catabolism (Wu and Morris, 2004), the greater Orn concentrations in ewes fed 180 vs. 90 may be driven by the elevated Arg concentrations.

Citrulline and Asp are substrates for Arg synthesis, and Arg is needed for nitric oxide production in the reaction with nitric oxide synthase. In the urea cycle, when Asp and Cit join to produce Arg via argininosuccinate, Arg interacts with reduced nicotinamide adenine dinucleotide phosphate, H^+, and O_2 in a reaction catalyzed by nitric oxide synthase to produce Cit, nitric oxide, H_2O and nicotinamide adenine dinucleotide phosphate. Given our differences in PI and RI, which are assumed to be caused by NO, and elevated Arg concentration, one might expect increased serum Cit concentrations in ewes supplemented with RP-ARG. However, no differences were seen in Cit concentrations, which is likely because only a small percentage of arginine present is used for NO synthesis.

Glutamate and Gln, two closely related amino acids, participate in additional reactions to assist in Arg metabolism. Previous literature in steers noted increases in serum Gln for the 360 mg RP-ARG·kg $BW^{-1}·d^{-1}$dose of RP-ARG supplementation compared with control, 180 mg RP-ARG·kg $BW^{-1}·d^{-1}$, and 54 mg·kg $BW^{-1}·d^{-1}$ Arg-HCl jugular infusion, but there was no change in serum Glu concentration (Meyer et al., 2011a). These authors further reported increased ileal flow of Glu when steers were fed 360 mg RP-ARG·kg $BW^{-1}·d^{-1}$ (Meyer et al., 2018). Zhang et al. (2016) reported no change in serum Glu or Gln from nutrient-restricted ewes supplemented with 20 g/d RP-ARG. Similarly, neither Glu nor Gln plasma concentrations were altered in nutrient-restricted ewes that received 81 mg L-Arg·kg $BW^{-1}·d^{-1}$ jugular Arg supplementation (Satterfield et al., 2013).

The amino group of Glu is released by glutamate dehydrogenase to form ammonia, which along with HCO_3^- and adenosine triphosphate, produces carbamoyl phosphate. In the current study, serum Glu concentration was greater for ewes

fed 180 compared with ewes fed 90 at both the initial and final collections and greater for ewes fed 180 compared with 360 for change in Glu across the study. The decrease measured for ewes fed 360 may suggest a threshold for urea cycle activity induced with RP-ARG supplementation above 180 mg RP-ARG·kg BW^{-1}·d^{-1}. Because Glu is a product of Arg catabolism (Wu and Morris, 2004), the greater Glu concentrations in ewes fed 180 vs. 90 may be driven by the elevated Arg concentrations. Glutamine, which in the current study was not altered due to RP-ARG supplementation or level of RP-ARG supplementation, is deaminated to produce Glu via glutaminase in many tissues, including the central nervous system. This deamination produces NH_3 for urea synthesis.

When considering amino acids involved in polyamine synthesis, Zhang et al. (2016) reported Met was elevated due to Arg supplementation in nutrient-restricted ewes supplemented with RP-ARG. However, Satterfield et al. (2013) supplemented nutrient-restricted ewes with a jugular Arg infusion and reported no difference in plasma Met concentration. In our study, no differences were reported in circulating Met concentrations. In polyamine synthesis, Arg is converted to Orn, both of which were elevated in the 180- vs. 90-fed ewes. Because Cit was not altered, the upregulation of the polyamine synthesis pathway may have occurred.

When looking specifically at cationic amino acids, Southern and Baker (1982) reported when pigs received increasing supplemental Arg levels from 0.67% to 2.00% Arg, they had a linear decrease of plasma Lys and His (exp. 1). Nutrient restriction decreased serum Lys and His concentrations in ewes, and further, Arg supplementation did not change those decreases (Zhang et al., 2016). Meyer et al. (2011a) reported steers receiving 180 mg RP-ARG/kg BW had a greater serum Lys concentrations than steers supplemented at 360 mg RP-ARG/kg BW. Similarly, in the current study, there was a tendency for circulating serum Lys concentrations to be decreased in ewes fed 360 compared with 180 across the duration of the study. Furthermore, change in serum His decreased in ewes fed 360 vs. 180. These reductions suggest a possible threshold in doses above 180 mg RP-ARG/kg BW that induced antagonism of intestinal transport because these cationic amino acids use the same transport systems as Arg (Closs and Mann, 2000). Rumen-protected L-Arg supplementation did not alter change in circulating Lys or His concentrations across the supplementation period for non-supplemented vs. RP-ARG-supplemented ewes (0 vs. 90, 180, and 360). For the 180 mg RP-ARG-treated ewes, because of the lack of differences in Lys and His concentrations from the supplemental RP-ARG, no evidence for antagonism of amino acid transport was found.

Carotid artery hemodynamics

In the current study, PI had a quadratic response where ewes fed 180 were lower than CON ewes. This finding is similar to Meyer et al. (2011b), in which steers receiving 180 mg RP-ARG/kg BW had decreased caudal PI than steers receiving no supplement or the 360 mg RP-ARG/kg dose and lower carotid artery PI in steers fed 180 mg RP-ARG/kg BW compared with control-fed or Arg jugular-infused steers. However, Yunta et al. (2015) reported no difference in uterine artery PI between intraperitoneal Arg-infused (40 mg Arg/kg BW) vs. saline-infused heifers, although PI decreased throughout pregnancy. A decrease in PI indicates greater distal tissue blood perfusion, suggesting a potential for increased vascular perfusion to systemic tissues including digestive or reproductive tissues, including the gravid uterus.

Similar to PI, RI had a quadratic response to RP-ARG supplementation where ewes fed 90 and 180 had decreased RI when compared with CON ewes, and ewes fed 360 had a greater RI than ewes fed 180. Likewise, Meyer et al. (2011b) reported steers receiving 180 mg RP-ARG/kg BW had decreased carotid RI compared with steers receiving control or 360 mg RP-ARG/kg BW and caudal RI was decreased in 180 mg RP-ARG/kg BW fed steers compared with all other treatments (Meyer et al., 2011b). Yet, Yunta et al. (2015) did not report differences in uterine artery RI due to the intraperitoneal Arg infusion. Nonetheless, the current study indicates decreased vascular resistance in ewes fed 180 mg RP-ARG/kg BW.

Arginine is oxidized to nitric oxide and Cit by nitric oxide synthases with reduced nicotinamide adenine dinucleotide phosphate and O_2 as cosubstrates (Marletta, 1993). In smooth muscle, such as blood vessels, nitric oxide causes vasodilation (Chowdhary and Townend, 2001). Therefore, the linear decrease in CSA was unexpected for RP-ARG-supplemented ewes. In previous research, both MnV and CSA at the carotid and caudal arteries were unaffected by Arg supplementation in steers fed increasing levels of RP-ARG (Meyer et al., 2011b). Similarly, the uterine artery diameter was not altered due to intraperitoneal Arg infusion vs. control heifers (Yunta et al., 2015).

Because cardiac output is determined based on both heart rate and SV, their opposing effects reported in the current study may explain why there was no effect on cardiac output due to RP-ARG supplementation or level in the current study. Previous research noted no change in heart rate, SV, or cardiac output due to RP-ARG supplementation or level (Meyer et al., 2011b). In contrast, Yunta et al. (2015) reported a decreased heart rate of heifers that received an intraperitoneal Arg infusion from day 41 to 146 of gestation.

Implications

Serum amino acid and carotid blood flow data indicate that 180 mg rumen-protected L-Arg·kg BW^{-1}·d^{-1} was optimal regarding Arg metabolism. Data demonstrated increased final serum Arg and Orn concentrations, along with elevated Lys for the 180 mg rumen-protected L-Arg·kg BW^{-1}·d^{-1} dose compared with the 360 mg rumen-protected L-Arg·kg BW^{-1}·d^{-1} dose. Further, the 180 mg rumen-protected L-Arg·kg BW^{-1}·d^{-1} dose demonstrated low RI and PI to permit blood flow with less resistance and improve distal tissue perfusion. Therefore, for short-term RP-ARG supplementation in ewe lambs, the 180 mg rumen-protected L-Arg/kg BW dose was optimal.

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Conflict of interest statement

No authors have any perceived conflict of interest that would affect their ability to objectively present or review this research.

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