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Effects of Rumen-Protected Arginine Supplementation on Ewe Serum-Amino-Acid Concentration, Circulating Progesterone, and Ovarian Blood Flow[1]

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Summary

The objectives of this research were to determine if rumen-protected arginine supplemented to ewes on d 8 to d 13 of the estrous cycle affected serum-amino-acid concentration, ovarian blood flow, and circulating progesterone. Nineteen multiparous Dorset ewes (63.8 kg ± 1.1 kg initial BW) were individually housed and randomly allocated to one of four rumen-protected arginine treatments: 0 (CON; n = 5), 90 mg/kg BW supplemental arginine (90 ARG; n = 4), 180 mg/kg BW supplemental arginine (180 ARG; n = 5), or 360 mg/kg BW supplemental arginine (360 ARG; n = 5). Following estrous synchronization, ewes were individually fed rumen-protected arginine blended into 150 g ground corn, which was immediately followed with 650 g of a pelleted diet (2.40 Mcal ME/kg and 12.9 percent CP; DM basis) on d 8 to d 12 of the estrous cycle. Ewes fed 360 ARG generally had greater serum- arginine concentrations than CON, 90 ARG, and 180 ARG on d 11 ($P \leq 0.07$) and d 12 ($P \leq 0.03$). On d 11, arginine as a percent of total amino acid concentration was greater in 360 ARG compared with CON and 90 ARG ($P \leq 0.05$). Total essential amino-acid concentration was elevated in 360 ARG compared with 90 ARG and 180 ARG ($P \leq 0.03$) on d 12. Arginine supplementation increased peak systolic velocity in the corpus luteum (CL) for 360 ARG and 90 ARG compared to CON ($P \leq 0.04$). Flow time (milliseconds) in the ovarian hilus was increased and CL was generally increased in 360 ARG compared to all other treatments ($P \leq 0.04$ and $P \leq 0.09$, respectively). Supplemental rumen-protected arginine had no effect on serum concentration of progesterone ($P > 0.50$). Results indicate that rumen-protected arginine supplemented to ewes at the rate of 360 mg/kg BW may increase circulating serum arginine concentration, in addition to increasing ovarian blood flow.

Key Words: Arginine, Ovarian Hemodynamics, Sheep

Introduction

As a precursor for nitric oxide, polyamines, creatine, proteins, urea, and glutamate, the amino acid arginine plays a vital role in metabolism and reproduction (Wu and Morris, 1998). Nitric oxide is the endothelium-derived relaxing factor essential for increasing systemic vasodilation (Ignarro et al., 2001; Martin et al., 2001). Supplemental arginine has been reported to increase the number of live pigs born per sow (Mateo et al., 2007). Furthermore, pregnant rats supplemented with arginine throughout gestation exhibited an increase in embryonic survival and litter size (Zeng et al., 2008). Recent research by Luther et al. (2008) indicated that ewes injected with L-arginine during the first 15 d post-breeding had increased ovarian blood flow, serum progesterone, and fetal number, despite similarities in ovulation rates to control ewes. Collectively, these studies provide evidence that reproductive efficiency can be enhanced via supplementation of supranutritional levels of arginine.

In previous studies, arginine supplementation has been investigated only within monogastric species due to the catabolic fate of arginine within the rumen. To protect arginine from ruminal degradation, the amino acid must be encapsulated in a ruminal-protected product to partially escape the rumen, followed by being catabolized in the small intestine for absorption. Due to the lack of available rumen-protected arginine, research in ruminants has been limited. We hypothesize that feeding rumen-protected arginine will increase circulating levels of arginine in addition to increasing systemic blood flow through its role in nitric oxide synthesis. Our specific objectives were to determine the effects of feeding rumen-protected arginine to ewes on serum amino acids, ovarian hemodynamics, and serum progesterone.

Materials and Methods

Animals and Experimental Design

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

Nineteen mature, multiparous Dorset ewes (63.8 kg ± 1.1 kg initial BW) were randomly allocated to one of four rumen-protected L-arginine treatments: 0 (CON; n = 5), 90 mg/kg BW supplemental arginine (90 ARG; n = 4), 180 mg/kg BW supplemental arginine (180 ARG; n = 5), or 360 mg/kg BW supplemental arginine (360 ARG; n = 5). Rumen-protected L-arginine (ARG 60; Eurhema Srl., Carviago, Itlay) was a 60 percent L-Arginine HCL product, calculated to have a minimum intestinal availability of 50 percent. Calculation of the dosage used assumed that 40 percent of arginine reaching the small intestine would be catabolized in this tissue (Wu and Morris, 1998), resulting in 30 percent of the consumed rumen-protected arginine reaching circulation. The 90 ARG treatment was estimated to deliver 27 mg L-arginine/kg BW to circulation, which was the injected dose used in previous studies (Luther et al., 2008).

For estrous synchronization, all ewes received a vaginally inserted, controlled-internal-drug release (CIDR-G®; 300 mg progesterone; Pharmacia & Upjohn Limited Co., Auckland, New Zealand) device for 12 d. Following CIDR removal, a single injection of 400 IU equine chorionic gondotropin (eCG®; Novormon 5000, Syntex S.A., Buenos Aires, Argentina) was given to initiate follicular development and ensure ovulation. After synchronization, ewes were moved into the Animal Nutrition and Physiology Center at NDSU (approximately 46.9° latitude and 96.8° longitude), where they were individually housed in 0.91-m x 1.2-m pens. The facility was temperature controlled (12°C to 21°C) and ventilated with lighting automatically timed to mimic daylight patterns.

Diet

Ewes were allowed a 7-d acclimation period to the facility and diet before beginning rumen-protected arginine supplementation on d 8 of the estrous cycle (d 0 = estrus). For 5 d, ewes were fed rumen-protected arginine blended into 150 g of ground corn, which was immediately followed with 650 g of a pelleted diet (44.9 percent beet pulp, 25.0 percent alfalfa meal, 19.7 percent soyhulls, 6.7 percent corn, 3.7 percent soybean meal; pelleted diet: 2.23 Mcal ME/kg and 13.6 percent CP, DM basis; total diet: 2.40 Mcal ME/kg and 12.9 percent CP, DM basis).

Ovarian Hemodynamics

On d 12 of the estrous cycle, color Doppler ultrasonography (Aloka SSD 3500, Tokyo, Japan) was used to determine ovarian hilus and luteal resistance index [(Peak systolic velocity – End diastolic velocity) / Peak systolic velocity], pulsatility index [(Peak systolic velocity – End diastolic velocity) / Time-averaged maximum velocity], peak systolic velocity, end diastolic velocity, mean velocity, and flow time in both ovaries.

Serum Analyses

Blood samples were collected via jugular venipuncture every 12 h from d 8 to d 13 of the estrous cycle. Serum was analyzed for progesterone concentration using a solid-phase, competitive, chemiluminescent enzyme immunoassay (Immulite 1000, Diagnostics Products Corp. Diagnostic Products Corp., Los Angeles, Calif.). All samples were analyzed as a single assay in duplicate form, with the intraassay CV 9.1 percent. Amino acid concentration (35 AA and metabolites) was determined using the HPLC MassTrak Amino Acid Analysis Solution developed by Waters Corporation (ACQUITY Ultra Performance LC, Waters Corporation, Milford, Mass.). Blood samples were refrigerated and allowed to coagulate for 2 h; thereafter, samples were centrifuged at 2,750 x g for 20 min at 4°C. Serum was removed and stored at -20°C for further amino acid and progesterone analyses.

Statistical Analysis

Ewe ovarian hemodynamic data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute, Inc., Cary, N.C.) with arginine treatment as the fixed effect, pen serving as block, and animal serving as the experimental unit. Repeated measures was used to analyze day and treatment x day effects for serum data. The model specifications included treatment, day, and treatment

x day interaction, with ewe serving as the random effect. The covariance structure used was 1st Order Antedependence. Simple covariance structure was used. Means were separated using LSD and were considered significant when $P \leq 0.10$.

Results

Serum Arginine Concentration

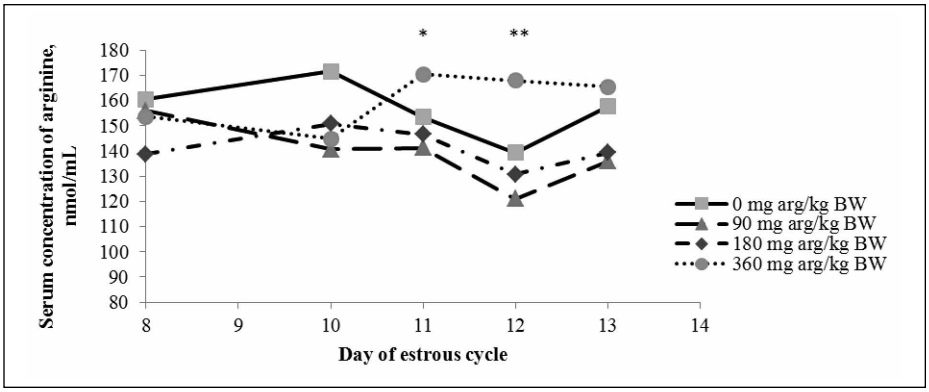
There was no effect of day or treatment x day for serum arginine concentration, total essential AA concentration, total AA concentration, arginine as a percent of total essential AA, or arginine as a % of total AA ($P \geq 0.14$). Ewes fed 360 ARG had greater serum arginine concentration than CON, 90 ARG, and 180 ARG on d 11 (175.5 nmol/mL vs. 153.2 nmol/mL, 132.3 nmol/mL, and 145.4 nmol/mL ± 8.6 nmol/mL, respectively; $P \leq 0.07$; Figure 1) and d 12 (166.4 nmol/mL vs. 142.7 nmol/mL, 121.7 nmol/mL, and 128.2 nmol/mL ± 7.4 nmol/mL, respectively; $P \leq 0.03$). On d 11, arginine as a percent of total amino acid concentration was greater in 360 ARG compared with CON and 90 ARG (7.16 nmol/mL vs. 6.19 nmol/mL, 5.70 nmol/mL ± 0.34 nmol/mL, respectively; $P \leq 0.05$; Table 1). Total essential amino acid concentration was elevated in 360 ARG compared with 90 ARG and 180 ARG ($P \leq 0.03$) on d 12. Supplemental rumen-protected arginine had no effect on citrulline or ornthine levels throughout the treatment period (data not reported; $P > 0.15$).

Ovarian Hemodynamics and Circulating Serum Progesterone

Arginine supplementation increased peak systolic velocity in the CL for 360 ARG and 90 ARG compared to CON (30.53 cm/s and 32.59 cm/s vs. 22.63 cm/s ± 2.48 cm/s, respectively; $P \leq 0.04$; Table 2). Flow time (milliseconds) in the ovarian hilus and corpus luteum was increased in 360 ARG compared to all other treatments ($P \leq 0.04$ and $P \leq 0.09$, respectively). Pulsatility index and resistance index did not differ among treatments for the CL and ovarian hilus ($P \geq 0.18$).

Supplemental, rumen-protected arginine had no effect on serum concentration of progesterone (CON, 6.17 ± 0.24; 90 ARG 6.14 ± 0.31; 180 ARG 5.93 ± 0.39 and 360 ARG 5.41 ± 0.44; $P \geq 0.50$).

Figure 1. Effects of feeding graded amounts of rumen-protected L-arginine on serum arginine concentration (nmol/mL) in Dorset ewes (*P = 0.01; **P = 0.002) from d 8 to d 12 of the estrous cycle (SEM for arginine concentration = 4.54 nmol/ml; SEM for day of estrous cycle = 5.07 nmol/ml).



Discussion

Arginine supplementation has primarily been evaluated in non-ruminant species. Limited research investigating arginine supplementation in ruminants has been conducted because of the high degree of ruminal arginine catabolism and lack of rumen-protected products. Research in pigs (Wu, 1997) and sheep (Luther et al., 2008) has indicated that intravenous injection of arginine at the rate of 27 mg of arginine/kg BW increased serum arginine within one hour of injection. Data published herein provides seminal information on the effects of rumen-protected arginine on serum arginine concentrations and ovarian hemodynamics in sheep. The 90 ARG treatment used in this study was estimated to deliver 27 mg arginine/kg BW to circulation over a 24 h period. This is in contrast to other studies (Wu, 1997; Luther et al., 2008), which used intravenously injected arginine. In the current study, only ewes supplemented with the largest dose (360 ARG) had greater serum-arginine concentrations, which occurred on d 11 and d 12 after 3 d and 4 d of supplementation, respectively.

Nitric oxide is produced when the enzyme nitric oxide synthase catalyzes the oxidation of L-arginine to L-citrulline and is considered the endothelium-derived relaxing factor essential for increasing systemic vasodilation (Ignarro et al., 2001; Martin et al., 2001; Gouge et al., 1998). Increased vascular permeability at the site of blastocyst attachment has been demonstrated to be a requirement for implantation in many species (Gouge et al., 1998). Nitric oxide is an important factor involved in the initiation of implantation due to its ability to increase blood flow (Gouge et al., 1998). Nitric oxide is produced in pre-implantation embryos, and its production is required for normal embryonic development (Gouge et al., 1998). In addition to nitric oxide's ability to regulate embryonic development, the embryo may also produce nitric oxide as a signal to the uterus to stimulate local vasodilation and capillary permeability required for successful implantation (Gouge et al., 1998). In the present study, rumen-protected arginine supplementation increased peak systolic velocity in the CL for 360 ARG and 90 ARG compared to CON on d 12 of the estrous cycle. These findings are similar to previous research (Luther et al., 2008), in which vascular resistance in the ovarian artery was reduced on d 12 following L-arginine injection.

Polyamines and nitric oxide are important for placental growth and angiogenesis. More specifically, they are essential for cellular proliferation and differentiation (Wu and Morris, 1998). The enzyme arginase regulates the availability of arginine for the synthesis

Table 1. Effects of supplemental, rumen-protected L-arginine on serum amino acid (AA) concentration in Dorset ewes from d 8 to d 12 of the estrous cycle.

	Dietary Arginine, mg/kg BW[1]					
Serum AA[2]	**0**	**90**	**180**	**360**	**SEM[3]**	**P-value[4]**
Day 8						
Total essential AA, nmol/mL	1,030	915	938	949	82	0.78
Total AA, nmol/mL	2,447	2,196	2,264	2,360	163	0.72
Arginine, % of total essential AA	15.5	15.1	15.0	16.4	1.3	0.86
Arginine, % of total AA	6.43	6.32	6.08	6.55	0.49	0.92
Day 10						
Total essential AA, nmol/mL	1,085	920	973	1,081	79	0.39
Total AA, nmol/mL	2,600	2,323	2,422	2,599	187	0.66
Arginine, % of total essential AA	15.6	14.4	15.6	15.0	1.1	0.83
Arginine, % of total AA	6.47	5.70	6.13	6.27	0.39	0.54
Day 11						
Total essential AA, nmol/mL	987	932	895	1,055	63	0.34
Total AA, nmol/mL	2,502	2,345	2,260	2,464	125	0.51
Arginine, % of total essential AA	15.7	14.3	16.4	16.8	0.9	0.29
Arginine, % of total AA	6.19[a]	5.70[a]	6.44[ab]	7.16[b]	0.34	0.04
Day 12						
Total essential AA, nmol/mL	936[ab]	828[a]	809[a]	1,014[b]	58	0.08
Total AA, nmol/mL	2,320	2,057	2,037	2,378	118	0.12
Arginine, % of total essential AA	15.9	15.0	16.1	16.6	0.9	0.62
Arginine, % of total AA	6.21	5.99	6.34	7.02	0.32	0.15
Day 13						
Total essential AA, nmol/mL	963	943	885	1,028	95	0.77
Total AA, nmol/mL	2,430	2,384	2,260	2,443	185	0.89
Arginine, % of total essential AA	16.1	15.9	16.5	17.1	0.9	0.80
Arginine, % of total AA	6.34	6.19	6.37	7.14	0.35	0.26

[1] Treatments: 0, 90, 180, and 360 mg/kg BW of rumen-protected L-arginine supplemented from d 8 to d 12 of the estrous cycle (n = 5, 4, 5 and 5 respectively).
[2] Day refers to day of estrous cycle (day 0 = estrus). An initial sample taken on d 8 prior to rumen-protected arginine supplementation.
[3] Standard error of mean.
[4] P-value for F-test for treatment.
[a, b] Means with different superscripts differ *($P \leq 0.10$)* within row.

of ornithine. Polyamines are synthesized from ornithine via ornithine decarboxylase (ODC) and arginase. In the current study, no differences were observed in circulating serum ornithine concentration.

Several studies have reported that low concentrations of progesterone can lead to a greater incidence of embryonic loss in sheep and ultimately result in decreased ewe productivity (Casida and Warwick, 1945; Dixon et. al., 2007). Although rumen-protected arginine in the present study exhibited stimulatory effects on ovarian hemodynamics, it did not affect serum progesterone concentrations, which is in contrast to our previous data on intravenous arginine supplementation (Luther et al., 2008). Additionally, we cannot explain why the treatments did not respond in a linear fashion. In many cases the 180 mg/kg treatment responded similar to the 0 mg/kg treatment, instead of in a linear fashion with the 90 and 360 mg/kg treatments.

Conclusions

Results of this study indicate that rumen-protected arginine supplemented to ewes may increase circulating serum-arginine concentration in addition to increasing ovarian blood flow. These preliminary data indicate that biological responses to rumen-protected arginine may be obtained without changing circulating arginine concentration. Additional research is needed to determine the potential of rumen-protected arginine as a component of strategic supplementation programs. Moreover, the ability of rumen-protected arginine to successfully reach the small intestine and enter circulation needs to be determined *in vivo*.

Table 2. Effects of supplemental rumen-protected L-arginine on ovarian hemodynamics in Dorset ewes from d 8 to d 12 of the estrous cycle

	Dietary Arginine, mg/kg BW[1]					
Hemodynamics	**0**	**90**	**180**	**360**	**SEM[2]**	**P-value[3]**
Corpus luteum						
Peak systolic velocity, cm/s	22.6[a]	32.5[b]	28.4[ab]	30.5[b]	2.4	0.07
Pulsatility index[4]	0.32	0.39	0.30	0.33	0.04	0.48
Resistance index[5]	0.26	0.32	0.25	0.28	0.03	0.42
Mean velocity, cm/s	20.1[a]	26.7[b]	24.4[ab]	25.7[b]	1.9	0.13
Flow time, ms	566[a]	596.00[a]	489[a]	753[b]	61	0.06
Hilus						
Peak systolic velocity, cm/s	31.3	22.3	31.9	29.0	3.3	0.21
Pulsatility index[4]	0.40	0.51	0.40	0.47	0.046	0.30
Resistance index[5]	0.32	0.39	0.31	0.37	0.027	0.18
Mean velocity, cm/s	25.1[b]	17.0[a]	25.8[b]	22.5[ab]	2.5	0.12
Flow time, ms	579[a]	595[a]	514[a]	736[b]	43	0.02

[1] Treatments: 0, 90, 180, and 360 mg/kg BW of rumen-protected L-arginine supplemented from d 8 to 12 of the estrous cycle (n = 5, 4, 5 and 5 respectively).
[2] Standard error of mean.
[3] *P-value* for F-tests for treatment.
[4] Pulsatility index = (Peak systolic velocity – End diastolic velocity) / Time-averaged maximum velocity.
[5] Resistance index = (Peak systolic velocity – End diastolic velocity) / Peak systolic velocity.
[a, b] Means with different superscripts differ ($P < 0.10$) within each row.

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