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Effects of maternal nutrition and rumen-protected arginine supplementation on ewe and postnatal lamb performance[1]

J. L. Peine,[*] G. Q. Jia,[*] M. L. Van Emon,[†] T. L. Neville,[*] J. D. Kirsch,[*] C. J. Hammer,[*] S. T. O'Rourke,[‡] L. P. Reynolds,[*] and J. S. Caton[*2]

[*]Department of Animal Sciences and [‡]Department of Pharmaceutical Sciences, North Dakota State University, Fargo 58108; [†]Hettinger Research Extension Center, Hettinger ND 58639

ABSTRACT: Our hypothesis was that arginine supplementation would overcome the negative effects of restricted maternal intake during the last two-thirds of gestation on ewe and lamb performance. To test this hypothesis, multiparous, Rambouillet ewes (n = 32) were allocated to 3 treatments in a completely random design at 54 ± 3.9 d of gestation. Dietary treatments were 100% of requirements (control, CON), 60% of CON (restricted, RES), or RES plus a rumen-protected arginine supplement dosed at 180 mg/kg BW once daily (RES-ARG). Ewes were penned individually in a temperature-controlled facility. At parturition, lambs were immediately removed from their dam and reared independently. Ewe BW from d 75 of gestation through parturition was greater ($P \leq 0.05$) in CON compared with RES or RES-ARG. Similarly, ewe BCS from d 82 of gestation through parturition was greater ($P \leq 0.02$) in CON than either RES or RES-ARG. Total ewe colostrum (g) at 3 h after parturition was greater ($P \leq 0.0001$) in CON than RES or RES-ARG. Lamb birth weight was greater ($P = 0.04$) in CON than RES ewes, and tended ($P = 0.10$) to be greater in CON vs. RES-ARG. Lambs born to CON ewes had greater ($P \leq 0.03$) BW than lambs from RES ewes at 7, 14, and 33 d postpartum. On d 19, lambs from CON and RES-ARG ewes had greater ($P \leq 0.04$) BW than lambs from RES ewes (12.0 and 11.5 vs. 10.3 ± 0.41 kg, respectively). Lambs born to CON and RES-ARG ewes had greater ($P \leq 0.04$) ADG than lambs from RES ewes on d 19 (355.0 and 354.0 vs. 306.4 ± 15.77 g, respectively). Lambs from CON and RES-ARG ewes also had greater ($P \leq 0.02$) girth circumference than lambs from RES ewes on d 19 (55.4 and 54.6 vs. 51.3 ± 0.97 cm, respectively). On d 54, lambs from RES-ARG ewes had greater ($P = 0.003$) curved crown rump length than lambs from RES ewes (99.8 vs. 93.9 ± 1.28 cm, respectively). These results confirm our hypothesis that arginine supplementation during the last two-thirds of gestation can mitigate some negative consequences associated with restricted maternal nutrition in the offspring, but not in the underfed dams themselves.

Key words: arginine, developmental programming, gestation, nutrition, offspring

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[2] Corresponding author's e-mail address: joel.caton@ndsu.edu

INTRODUCTION

Fetal intrauterine growth restriction (**IUGR**) has been implicated as the cause of many deleterious postnatal offspring performance defects or traits, including lower birth weights and poor neonatal growth and body composition (Wu et al., 2006; Caton and Hess, 2010; Reynolds and Caton, 2012). A major cause of fetal IUGR is compromised maternal nutrition, which may occur in extensive grazing systems. In the Western U.S., grazing ewes often receive less than 50% of NRC recommendations, resulting in loss of BW during pregnancy and reduced lactation performance (Wu et al., 2006). A potential supplement to offset IUGR is arginine, a semi-essential AA. Arginine contributes to nitric oxide and polyamine production, both of which play key roles in placental growth and function (Martin et al., 2001; Kwon et al., 2003; Wu et al., 2009). Nitric oxide and polyamines play unique roles in regulating fetal development throughout gestation, and contribute to nutrient delivery to and use by the developing fetus despite maternal under nutrition. Improved fetal growth has been demonstrated in ovine models of IUGR in response to intravenous arginine administration (Wu et al., 2009). Use of current rumen protection technologies allows for oral administration of specific AA with subsequent delivery to the small intestine, which is a practical approach for strategic supplement delivery to ruminants. In this study, we tested the hypothesis that arginine supplementation would mitigate the negative effects of compromised maternal nutrition during the last two-thirds of gestation on both ewe and lamb performance. We expected lambs from nutrient-restricted, arginine-supplemented dams to present as normal and therefore to be similar to lambs from control-fed ewes.

MATERIALS AND METHODS

Protocols described herein were approved by the North Dakota State University Institutional Animal Care and Use Committee. Multiparous Rambouillet-cross ewes (n = 32; 67.7 ± 6.2 kg initial BW) were confirmed pregnant via ultrasound on 41 ± 6.0 d after mating. Ewes were housed individually in a climate-controlled facility with free access to water. Ewes were fed a pelleted diet daily at 0800 h. Weekly ewe BW measurements allowed monitoring of ewe BW change to determine if dietary adjustments were needed. Body condition scores were assessed every 2 wk by 2 or 3 independent observers.

Experimental Design and Treatments

The experiment was a completely random design. Ewes were randomly assigned to 1 of 3 treatments at 54 ± 3.9 d of gestation: 100% of dietary requirements (control, **CON**; based on NRC, 1985, 2007), 60% of CON (restricted, **RES**), or RES with the addition of a rumen-protected arginine supplement (**RES-ARG**). Supplement provided to the RES-ARG ewes contained 180 mg arginine/kg BW (based on initial BW). Arginine was mixed with 50 g of fine ground corn and fed once daily at 0800 h before offering the pelleted diet. Both CON and RES ewes were also provided 50 g (as-fed basis) of fine-ground corn daily, without the added rumen-protected arginine. Pelleted diets (Table 1) were fed once daily to ewes on an individual basis, with amounts specific to ewe BW and targeted nutrient supply. Pelleted diets were consumed within 2 h of feeding. Treatments were continued until parturition. Two CON and 1 RES ewe died (2 unknown causes and 1 pneumonia) before parturition; corresponding data were included in the analyses up to when ewes were removed from the study.

Table 1. Ingredient and nutrient composition (DM basis) of the pelleted diet fed to ewes

Item	%
Ingredient	
Alfalfa meal, dehydrated	34.0
Beet pulp, dehydrated	27.0
Wheat middlings	25.0
Ground corn	8.4
Soybean meal	5.0
Trace mineral premix[1]	0.6
Nutrient composition	
DM	91.9
CP	15.5
NDF	35.8
ADF	20.9

[1]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, 400 IU/kg vitamin E.

Parturition and Lamb Management

A closely monitored, 24-h lambing protocol was implemented during the expected dates of parturition. At parturition, lambs were not permitted to suckle from ewes, and were removed from dams immediately and reared independently. There were 4 sets of twins (2 CON, 1 RES, and 1 RES-ARG). At 3 h post parturition, ewes were administered a 1 mL (20 USP units) intramuscular injection of oxytocin (Vet Tek, Blue Springs, MO) and milked out to determine colostrum weight.

Following removal from the ewe, lambs were towel dried and weighed. Lambs received an intramuscular injection of vitamin A, D, and E (0.5 mL/lamb; 100,000 IU of A, 10,000 IU of D_3, 300 IU of E/mL; Stuart Products, Bedford, TX), and 1 mL of *Clostridium perfringens* types C and D and tetanus vaccine (Essential 3+T, Colorado Serum, Denver, CO) subcutaneously. Finally, the umbilical cord was clipped and dipped in 7% iodine tincture.

Lambs received artificial colostrum (Lifeline Rescue Colostrum, APC, Ankeny, IA), administered at 19.1 mL/kg of lamb birth weight at 0 and 2 h post birth, and 25.5 mL/kg of lamb birth weight at 4, 8, 12, 16, and 20 h post birth to achieve 10.64 g IgG/kg lamb birth weight, as previously described (Meyer et al., 2010; Neville et al., 2010).

Lambs were group housed in a climate-controlled facility with free access to water. At 24 h post birth, lambs received milk replacer (Super Lamb Milk Replacer, Merrick's Inc., Middleton, WI; DM basis: 24% CP, 30% fat, 0.10% crude fiber, 0.5 to 1.0% Ca, 0.65% P, 0.3 ppm Se, 66,000 IU/kg vitamin A, 22,000 IU/kg vitamin D, and 330 IU/kg vitamin E) ad libitum via bottle until a strong suckling response was observed. Lambs were then transitioned to a teat-bucket system (Meyer et al., 2010; Neville et al., 2010). In addition to milk replacer, a mixture of long-stem mid-bloom alfalfa hay and creep feed (DM basis: 20% CP, 6% fat, 8% crude fiber, 1.4 to 1.9% Ca, 0.4% P, 0.5% to 1.5% NaCl, 0.3 ppm Se, 11,000 IU/kg vitamin A, 6,000 IU/kg vitamin D, and 100 IU/kg vitamin E) were available ad libitum. At 7 d, all tails were docked and male lambs were castrated by banding. At 40 ± 3 d, lambs received an additional 2-mL injection of vitamin A, D, and E as previously described. Lamb BW was measured at birth, 24 h, and 3, 7, and 14, 19, 33, 40, 47, and 54 ± 3 d. Curved crown rump length, measured as the distance from the crown of the head to the rump along the backbone, and girth, measured as the circumference around the rib cage just behind the forelegs, were determined at birth, and at 19 and 54 d of age. Two lambs died during the experiment of unrelated causes, 1 at 7 d (RES-ARG), and another at 45 d (RES) of age; corresponding data were included in the analyses up to when lambs were removed from the study.

Statistical Analysis

Data were analyzed as a completely random design using the GLM procedure of SAS (v. 9.2; SAS Inst. Inc., Cary, NY), with ewe or lamb serving as the experimental unit. Fetal number was included in the model statement and retained if $P \leq 0.10$. After protection with an overall F-test for treatment ($P \leq 0.10$), means were separated using the LSMEANS procedure of SAS and P-values ≤ 0.05 were considered different.

RESULTS AND DISCUSSION

Ewe Performance

Restricted (RES and RES-ARG) ewes weighed less ($P \leq 0.05$) than CON ewes from d 75 of pregnancy until parturition (Table 2). Similarly, RES and RES-ARG ewes had lower ($P \leq 0.02$) BCS than CON ewes from d 82 throughout parturition, and by d 68 CON ewes had greater ($P \leq 0.03$) BCS than RES-ARG ewes (Table 3). These results are similar to those reported by Meyer et al., (2010). Changes in ewe BW and BCS in CON and RES ewes indicated that our experimental model was performing as predicted and appropriate for testing our hypothesis regarding supplementation of rumen-protected arginine. In this study, the arginine treatment had no rescue effect ($P \geq 0.82$) on ewe BW or BCS.

Colostrum produced at 3 h postpartum by CON ewes was greater ($P \leq 0.0001$) compared with RES and RES-ARG ewes (753.7 vs. 298.6 and 105.4 ± 88.31 g, respectively). Differences observed between CON and RES were expected and reported previously (Wu et al., 2006; Swanson et al., 2008; Meyer et al., 2011). Results also indicated that rumen-protected arginine supplementation did not rescue colostrum yield in restricted ewes. Effects of the arginine supplement used in this study on longer-term lactation responses were not determined.

Lamb Birth Weight and Performance

Lambs from CON ewes had greater ($P = 0.04$) BW at birth than lambs from RES ewes, with lambs from RES-ARG fed ewes being intermediate and similar to both CON and RES (Table 4). This same response was observed for lamb BW on d 7, 14, and 33. On d 3, lambs from CON ewes weighed more ($P \leq 0.02$) than lambs from RES and RES-ARG ewes. On d 19, lambs from CON and RES-ARG ewes weighed more ($P \leq 0.04$) than lambs from RES ewes. Keeping with our hypothesis, data indicated that arginine may play a role in recovering postnatal BW in lambs from nutritionally compromised dams.

Lamb ADG followed a similar pattern as BW. Lambs from CON and RES-ARG ewes had greater ($P \leq 0.04$) ADG than lambs from RES ewes on d 19 (Table 5).

Girth measurements at birth and d 54 were greater ($P = 0.03$) in lambs from CON compared with RES ewes, with RES-ARG being intermediate and similar to both CON and RES (Table 6). However, on d 19 lambs from CON and RES-ARG ewes had greater ($P \leq 0.02$) girth measurements than lambs from RES ewes. Lambs from RES-ARG ewes had greater ($P = 0.003$) curved crown rump measurements than lambs from RES ewes on d 54, with lambs from CON ewes being intermediate and similar to both RES and RES-ARG. These data support the potential role arginine may play in enhancing offspring growth from underfed dams.

IMPLICATIONS

Ewe performance outcomes were inconsistent with our hypothesis and were not responsive to supplemental rumen-protected arginine during gestation. However, in keeping with our hypothesis, lamb BW, ADG, and body size measurements were responsive to maternal rumen-protected arginine supplementation, but not at all times measured. Additional research is needed to further define the effects of supplementation of rumen-protected arginine during gestation on offspring heath and performance outcomes.

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Table 2. Influence of nutrient restriction and arginine supplementation on ewe BW (kg) throughout gestation

	Treatment[1]				
d	CON	RES	RES-ARG	SEM	*P* - value
54	63.8	63.7	64.2	1.95	0.98
61	64.1	60.3	60.9	1.95	0.32
68	62.9	57.8	57.5	1.94	0.09
75	62.3[b]	56.8[a]	56.7[a]	2.00	0.07
82	64.1[b]	57.9[a]	57.3[a]	1.99	0.03
89	65.3[b]	58.1[a]	57.6[a]	2.09	0.02
96	65.5[b]	57.9[a]	57.9[a]	2.20	0.02
103	65.7[b]	57.7[a]	57.4[a]	2.13	0.01
110	66.4[b]	57.7[a]	57.7[a]	2.06	0.005
117	67.0[b]	56.9[a]	56.5[a]	2.16	0.002
124	67.6[b]	56.3[a]	56.7[a]	2.17	0.001
131	67.9[b]	56.0[a]	56.3[a]	2.13	0.001
138	69.6[b]	56.6[a]	56.9[a]	2.12	0.001
145	69.6[b]	56.3[a]	56.1[a]	2.23	0.001
152	67.0[b]	56.5[a]	56.6[a]	3.30	0.06

[1]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 10).

[a,b]Means within a row with different superscripts differ ($P \leq 0.05$).

Table 3. Influence of nutrient restriction and arginine supplementation on ewe BCS throughout gestation[1]

	Treatment[2]				
d	CON	RES	RES-ARG	SEM	*P* - value
54	2.90	2.91	2.88	0.075	0.94
68	2.94[b]	2.78[ab]	2.71[a]	0.073	0.08
82	2.99[b]	2.65[a]	2.66[a]	0.094	0.02
96	2.90[b]	2.47[a]	2.42[a]	0.093	0.001
110	2.93[b]	2.40[a]	2.34[a]	0.137	0.006
124	2.98[b]	2.26[a]	2.26[a]	0.108	<0.0001
138	2.90[b]	2.01[a]	2.05[a]	0.133	<0.0001
152	2.75[b]	1.65[a]	1.79[a]	0.199	0.001

[1]BCS structured by scale of 1 = thin to 5 = over conditioned

[2]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 10).

[a,b]Means within a row with different superscripts differ ($P \leq 0.05$).

Table 4. Influence of maternal nutrient restriction and arginine supplementation on offspring BW (g) over time

	Maternal treatment[1]				
d	CON	RES	RES-ARG	SEM	*P* - value
0	5,228[b]	4,450[a]	4,603[ab]	257	0.09
3	6,045[b]	4,693[a]	4,990[a]	298	0.01
7	7,112[b]	6,101[a]	6,321[ab]	313	0.07
14	9,882[b]	8,811[a]	9,459[ab]	309	0.05
19	11,973[b]	10,272[a]	11,500[b]	405	0.01
33	17,360[b]	15,278[a]	16,202[ab]	574	0.04
40	19,971	18,072	19,488	706	0.14
47	21,765	20,606	22,028	797	0.41
54	23,830	21,870	23,656	890	0.24

[1]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 11).

[a,b]Means within a row with different superscripts differ ($P \leq 0.05$).

Table 5. Influence of maternal nutrient restriction and arginine supplementation on offspring ADG (g) over time

	Maternal treatment[1]				
d	CON	RES	RES-ARG	SEM	*P* - value
3	272.4	81.0	129.1	73.0	0.17
7	269.1	235.9	245.4	19.5	0.47
14	332.4	311.5	334.7	13.1	0.37
19	355.0[b]	306.4[a]	354.0[b]	15.8	0.05
33	367.6	328.1	346.3	13.3	0.11
40	368.6	340.6	367.9	14.5	0.28
47	351.9	342.3	367.1	15.0	0.51
54	344.5	321.4	349.7	15.0	0.37

[1]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 11).

[a,b]Means within a row with different superscripts differ ($P \leq 0.05$).

Table 6. Influence of maternal nutrient restriction and arginine supplementation on offspring girth (cm) and curved crown rump (cm) length over time

	Maternal treatment[1]				
Item	CON	RES	RES-ARG	SEM	*P* - value
Girth					
0d	42.2[b]	38.6[a]	39.4[ab]	1.14	0.08
19d	55.4[b]	51.3[a]	54.6[b]	0.98	0.01
54d	70.8[b]	67.0[a]	69.7[ab]	1.24	0.10
CCR					
0d	54.9	52.6	55.1	1.49	0.43
19d	73.7	69.4	72.9	1.86	0.21
54d	96.3[ab]	93.9[a]	99.8[b]	1.28	0.01

[1]CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with rumen-protected arginine supplement (n = 11).

[a,b]Means within a row with different superscripts differ ($P \leq 0.05$).