Effects of maternal nutrition and rumen-protected arginine supplementation on ewe performance and postnatal lamb growth and internal organ mass¹

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ABSTRACT: The hypothesis of this study was that arginine supplementation would overcome negative effects of restricted maternal feed intake during the last two-thirds of gestation on ewe performance and positively affect postnatal lamb growth and development. 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 nutrient requirements (control, CON), 60% of control (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 dams and reared independently. At day 54 ± 3 of age, lambs were stunned using captive bolt, exsanguinated, and organs were collected and weighed. Ewe BW from day 68 of gestation through parturition was greater ($P \leq 0.03$) in CON compared with RES or RES-ARG. Similarly, ewe BCS from day 68 of gestation through parturition was greater ($P \leq 0.03$) in CON than either RES or RES-ARG. Total ewe colostrum mass (g) at 3 h after parturition was greater ($P \leq 0.001$) 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 day 19, lambs from CON and RES-ARG ewes both 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 day 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 day 19 (55.4 and 54.6 vs. 51.3 ± 0.97 cm, respectively). On day 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). Adrenal glands in lambs from CON dams had greater ($P = 0.01$) mass than adrenal glands in lambs from RES dams. Livers from lambs born to RES-ARG ewes weighed more ($P = 0.05$) than livers from lambs born to RES ewes. These results confirm our hypothesis that arginine supplementation during the last two-thirds of gestation can mitigate offspring, but not maternal negative consequences associated with restricted maternal nutrition.

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

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J. Anim. Sci. 2018.96:3471–3481
doi: 10.1093/jas/sky221

¹Appreciation is expressed to Dr. Chris Schauer, NDSU Hettinger Research and Extension Center, Drs. Reid Redden and Pawel Borowicz, NDSU Department of Animal Sciences, and Fernando Valdez, Kemin Industries, for their assistance with this project. Support for this study was provided, in part,

by a grant from the National Institute of Child Health and Human Development (HD61532).

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Received April 10, 2018.

Accepted May 31, 2018.

INTRODUCTION

Fetal growth restriction (FGR) has been implicated as the cause of 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). One of the major causes of FGR is compromised maternal nutrition, which frequently occurs in extensive grazing systems. In the Western United States, grazing ewes often receive less than 50% of NRC recommendations, resulting in loss of body weight during pregnancy and reduced lactation performance (Wu et al., 2006; Long et al., 2009; Meyer et al., 2011).

A potential supplement to offset FGR is arginine, a semiessential amino acid. 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). Improved fetal growth has been demonstrated in ovine models of FGR in response to intravenous arginine administration (Wu et al., 2009). Arginine may also enhance offspring growth via insulin stimulation or other avenues of glucose metabolism (Schmidt et al., 1992; Floyd et al., 1966; Gannon et al., 2002). Lassala et al. (2010) found arginine administration to underfed ewes enhanced offspring birth weights by 21% when compared with saline-infusion; birth weights of offspring from arginine-infused underfed ewes were equal to those from control-fed ewes.

Use of rumen protection technologies allows for oral administration of specific amino acids, which is a practical approach for strategic supplement delivery to ruminants. Although previous research has used intravenous arginine administration, this would be one of the first studies to use oral rumen-protected arginine administration. 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 performances.

MATERIALS AND METHODS

Animals

Protocols described herein were approved by the North Dakota State University Institutional Animal Care and Use Committee. Multiparous Rambouillet-cross ewes ($n = 32$; 4.4 ± 1.2 years of age; 67.7 ± 6.2 kg initial BW) were confirmed

pregnant via ultrasound on 41 ± 6.0 d after mating. Rams were of similar Rambouillet breed and came from the same related flock as one another. Ewes were housed individually in a climate-controlled facility with free access to water. Ewes were fed a pelleted diet daily at 0800 h (Table 1). Weekly ewe BW measurements allowed monitoring of ewe BW change to determine whether dietary adjustments were needed. Body condition scores were assessed every 2 wk by 2 or 3 independent observers. There were no differences ($P \geq 0.94$) in BW or BCS prior to initiation of treatments.

Experimental Design and Treatments

This experiment was a completely randomized 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 control (restricted, RES), or RES with the addition of a rumen-protected arginine (RP-ARG, Kemin Industries, Des Moines, IA) supplement (RES-ARG); the amount of feed given to each ewe was based on NRC ME requirements (1985, 2007). Supplement provided to 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 of fine ground corn daily, without added RP-ARG. Pelleted diets (Table 1) were fed once daily to ewes on an individual basis, with rations specific to ewe BW and targeted nutrient supply. Pelleted diet rations were consumed within 2 h of feeding. Treatments continued until

Table 1. Ingredient and nutrient composition of 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	89.9
CP	15.5
NDF	37.2
ADF	21.5

Diets administered to ewes daily at 0800 h.

¹Premix: 18 to 21% Ca, 9% P, 10 to 11% NaCl, 49.3 mg/kg Se, 700,000 IU/kg Vitamin A, 200,000 IU/kg Vitamin D, 400 IU/kg Vitamin E.

parturition. Two CON and 1 RES ewe died (2 unknown causes and 1 pneumonia) before parturition. Their data were included in analyses up to removal from the study.

Parturition and Lamb Management

A closely monitored, 24-h lambing protocol was implemented during expected dates of parturition. At parturition, lambs were not permitted to suckle from ewes; they were removed from dams immediately and reared independently of dams. 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 manually milked out to determine colostrum weight.

Following removal from ewes, 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 D3, 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 described previously (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) for ad libitum intake via bottle until a strong suckling response was observed. Lambs 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 ($n = 15$ males; $n = 18$ females). At 40 ± 3 d, lambs received an additional 2-mL injection of vitamin A, D, and E as described previously. 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 19 and 54 d. Two lambs died during the experiment of unrelated causes: one at 7 d (RES-ARG) and another at 45 d (RES). Their data were included in analyses up to removal from the study.

Necropsy

At day 54 ± 3 of age (CON = 53.5, RES = 53.0, RES-ARG = 53.1; $P = 0.81$), lambs were stunned using captive bolt and exsanguinated. Viscera were removed and dissected, and the liver and pancreas were separated from the viscera to obtain individual organ weights. The digestive tract was stripped of digesta and fat, and the ileum, duodenum, and jejunum were separated from the large intestine as described by Soto-Navarro et al. (2004), and each component weighed. The heart was also removed and measured with calipers for ventricle dimensions and thickness. In addition, the brain, adrenal glands, kidneys, gonads, spleen, stomach complex, and thyroid were removed and each component weighed.

Jejunal Histological Analyses

Jejunal tissue was further dissected to a 15-cm segment for mucosal analysis; the segment was cut open flat along the mesenteric side and the luminal surface washed with PBS. After rinsing, mucosa was scraped from the section using a glass microscope slide. Differential weights were taken on the segment before and after mucosal removal to determine percent mucosa in the jejunum.

Further dissection of jejunal tissue yielded sections (<1-cm wide) for immersion-fixation in neutral buffered formalin (NBF), and tissues were ultimately stored in 70% ethanol solution. Following fixation, these tissue sections were embedded in paraffin (Reynolds and Redmer, 1992) and cut to 5- μ m cross sections. These 5- μ m cross sections were mounted on glass slides, deparaffinized in HistoClear (Electron Microscopy Services, Hatfield, PA), and rehydrated through a series of ethanol/water solutions. Antigen retrieval was performed for 15 min with a 10-mM sodium citrate 0.05% tween (pH = 6) buffer in a 2100 retriever (Electron Microscopy Sciences, Hatfield, PA), following antigen retrieval slides were cooled to room

temperature for 20 min. Slides were rinsed twice with tris buffered saline (TBS) containing 0.1% Triton X-100 (TBST) prior to treatment with a blocking buffer consisting of TBS and 10% normal goat serum (Vector Laboratories, Burlingame, CA) for 20 min. Primary antibody against Ki67 (1:100; Clone MM1; Vector Laboratories, CA) was used to treat slides overnight at 4 °C to mark cells that are proliferating (Freetly et al., 2014). The following day, slides were washed in TBST prior to treatment in total darkness using a goat antimouse CF633 secondary antibody (1:200; 89138-632; VWR, Radnor, PA) for 30 min. Finally, slides were washed in distilled water prior to coverslip application using Vectashield Hardset mounting medium (Vector Laboratories, Inc., Burlingame, CA) containing 4',6-diamidino-2-phenylindole (DAPI).

Slides were visualized via photomicrographs taken on Zeiss Imager.M2 epifluorescence microscope using 10× objective and AxioCam HRm camera with a Zeiss piezo automated stage. Photomicrographs were taken at 6 random locations per animal visualizing the crypt region of the intestine, the primary location of intestinal cell proliferation. Images were analyzed using Image-ProPlus 5.0 software (MediaCybernetics Inc., Silver Spring, MD) for percentage of proliferating Ki-67-positive cells of the total number of cells within the analyzed crypt region. Of the 6 photomicrographs, the 3 proliferation percentage calculations within the smallest range of each other were used to calculate an average percent proliferation for each lamb.

Statistical Analysis

Data were analyzed as a completely random design using the GLM procedure of SAS (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 significant ($P \leq 0.10$). Sex was included in the model statement, but only significant for offspring heart weight ($P = 0.03$); growth measurements ($P \geq 0.07$) and organ weights ($P \geq 0.09$) were largely not significant in sex effect; and therefore, it was removed from the model statement. After protection with an overall *F*-test for treatment ($P \leq 0.10$), means were separated using the PDIF procedure of SAS; *P*-values ≤ 0.05 were considered different, and *P*-values ≤ 0.10 were considered tending to be different. Contrasts were also used to address specific questions: was there an effect of maternal nutritional plane (CON vs. RES and RES-ARG), or was there an effect of arginine supplementation (RES vs. RES-ARG)?

Animal numbers by sex and treatment have been included in Table 2.

RESULTS AND DISCUSSION

Ewe Performance

Restricted (RES and RES-ARG) ewes weighed less ($P \leq 0.03$) than CON ewes from day 68 of pregnancy until parturition (Table 3). Similarly, by day 68 CON ewes had greater ($P \leq 0.03$) BCS than RES and RES-ARG ewes (Table 4), and this growth difference continued throughout parturition. These results are similar to those reported by Zhang et al. (2016) and Meyer et al. (2010). These differences in ewe BW and BCS in CON and RES ewes indicate our experimental maternal plane of nutrition model responded as predicted and was appropriate for testing our hypothesis regarding supplementation of rumen-protected arginine. In this study, the arginine treatment had no rescue effect ($P \geq 0.48$) on ewe BW or BCS. Similar maternal performance based on arginine supplementation was observed by Satterfield et al. (2013).

Colostrum produced at 3 h postpartum by CON ewes was greater ($P \leq 0.001$) 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 ($P \geq 0.10$) to both CON and RES (Table 5).

Table 2. Offspring numbers by sex and treatment

	CON	RES ¹	RES-ARG ¹
Male (M)	5	5	5
Female (F)	6	6	6
Total	11	11	11

There are 4 sets of twins: 2 CON, 1 RES, and 1 RES-ARG.

¹1 RES-ARG and 1 RES lamb died of unrelated causes. Their data are included up to their removal from the study (RES-ARG lamb (M) until day 7, RES lamb (M) until day 47).

Table 3. Influence of nutrient restriction and arginine supplementation on ewe BW (kg) throughout gestation

d	Treatment ¹			SEM	P-value ²	P-values ³	
	CON	RES	RES-ARG			CON vs. RES and RES-ARG	RES vs. RES-ARG
54	63.8	63.7	64.2	1.95	0.98	0.93	0.85
61	64.1	60.3	60.9	1.95	0.32	0.14	0.82
68	62.9 ^a	57.8 ^{ab}	57.5 ^b	1.94	0.09	0.03	0.91
75	62.3 ^a	56.8 ^b	56.7 ^b	2.00	0.07	0.02	0.96
82	64.1 ^a	57.9 ^b	57.3 ^b	1.99	0.03	0.01	0.83
89	65.3 ^a	58.1 ^b	57.6 ^b	2.09	0.02	0.01	0.85
96	65.5 ^a	57.9 ^b	57.9 ^b	2.20	0.02	0.01	0.99
103	65.7 ^a	57.7 ^b	57.4 ^b	2.13	0.01	0.003	0.93
110	66.4 ^a	57.7 ^b	57.7 ^b	2.06	0.005	0.001	0.99
117	67.0 ^a	56.9 ^b	56.5 ^b	2.16	0.002	0.001	0.89
124	67.6 ^a	56.3 ^b	56.7 ^b	2.17	0.001	<0.001	0.88
131	67.9 ^a	56.0 ^b	56.3 ^b	2.13	<0.001	<0.001	0.91
138	69.6 ^a	56.6 ^b	56.9 ^b	2.12	<0.001	<0.001	0.92
145	69.6 ^a	56.3 ^b	56.1 ^b	2.23	<0.001	<0.001	0.96
152	67.0 ^a	56.5 ^b	56.6 ^b	3.30	0.06	0.02	0.99
Total BW change ⁴	6.59	-7.23	-7.76	0.755	<0.001	<0.001	0.61

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$).

¹CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with 180 mg rumen-protected arginine supplement per kg BW (n = 10).

²P-value associated with overall F-test for treatment.

³P-values associated with specific contrasts.

⁴Change is calculated between days 54 and 152 of gestation.

Table 4. Influence of nutrient restriction and arginine supplementation on ewe BCS¹ throughout gestation

d	Treatment ²			SEM	P-value ³	P-values ⁴	
	CON	RES	RES-ARG			CON vs. RES and RES-ARG	RES vs. RES-ARG
54	2.90	2.91	2.88	0.075	0.94	0.92	0.75
68	2.94 ^a	2.78 ^{ab}	2.71 ^b	0.073	0.08	0.03	0.48
82	2.99 ^a	2.65 ^b	2.66 ^b	0.094	0.02	0.005	0.96
96	2.90 ^a	2.47 ^b	2.42 ^b	0.093	0.001	<0.001	0.69
110	2.93 ^a	2.40 ^b	2.34 ^b	0.137	0.006	0.002	0.75
124	2.98 ^a	2.26 ^b	2.26 ^b	0.108	<0.001	<0.001	1.00
138	2.90 ^a	2.01 ^b	2.05 ^b	0.133	<0.001	<0.001	0.84
152	2.75 ^a	1.65 ^b	1.79 ^b	0.199	0.001	<0.001	0.61
Total BCS change ⁵	-0.06	-1.03	-1.00	0.145	<0.001	<0.001	0.88

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$).

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

²CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with 180 mg rumen-protected arginine supplement per kg BW (n = 10).

³P-value associated with overall F-test for treatment.

⁴P-values associated with specific contrasts.

⁵Change is calculated between days 54 and 152 of gestation.

This same response was observed for lamb BW on days 7, 14, and 33. On day 3, lambs from CON ewes weighed more ($P \leq 0.003$) than lambs from RES and RES-ARG ewes. On day 19, lambs from CON and RES-ARG ewes weighed more ($P \leq 0.04$) than lambs from RES ewes. Keeping with our hypothesis, these data indicate that arginine may play a role in recovering postnatal BW in lambs from

nutritionally compromised dams. This hypothesis is further supported in data by [Lassala et al. \(2010\)](#), where lambs from ewes on a restricted nutrient diet receiving IV L-arginine were similar in birth weights to lambs from adequately fed ewes, both with heavier birth weights than lambs from ewes on nutrient restriction (50% NRC requirements). This ability for offspring from RES-ARG dams to “catch up”

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

d	Maternal treatment ¹			SEM	<i>P</i> -value ²	<i>P</i> -values ³	
	CON	RES	RES-ARG			CON vs. RES and RES-ARG	RES vs. RES-ARG
0	5,228 ^a	4,449 ^b	4,603 ^{ab}	257.1	0.09	0.03	0.68
3	6,045 ^a	4,692 ^b	4,990 ^b	298.2	0.008	0.003	0.49
7	7,112 ^a	6,100 ^b	6,321 ^{ab}	312.7	0.07	0.03	0.62
14	9,882 ^a	8,811 ^b	9,459 ^{ab}	308.8	0.05	0.05	0.14
19	11,973 ^a	10,272 ^b	11,500 ^a	405.3	0.01	0.03	0.04
33	17,360 ^a	15,278 ^b	16,202 ^{ab}	574.5	0.04	0.02	0.25
40	19,971	18,072	19,488	705.7	0.14	0.16	0.16
47	21,765	20,606	22,028	796.9	0.41	0.64	0.22
54	23,830	21,870	23,656	889.9	0.24	0.32	0.17
Total BW change ⁴	18,602	17,354	18,882	808.9	0.37	0.62	0.19

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$).

¹CON = control, 100% NRC requirements ($n = 11$); RES = restricted, 60% CON nutrients ($n = 11$); RES-ARG = restricted + arginine, 60% CON nutrients with 180 mg rumen-protected arginine supplement per kg BW ($n = 11$).

²*P*-value associated with overall *F*-test for treatment.

³*P*-values associated with specific contrasts.

⁴Change is calculated between birth and 54 d of age.

in growth to offspring from CON dams is especially significant at this age. If the RES-ARG offspring are able to catch up at d 19, which is approximately half way to weaning age, the offspring may be more vigorous at weaning and perform better as mature animals. By day 40, offspring from CON dams were similar to offspring from RES and RES-ARG dams ($P \geq 0.16$), so this catch-up growth is eventually seen in all treatment groups; however, the ability for the RES-ARG offspring to catch up sooner may make them more viable earlier in life. The present study did not examine weaning vigor, but this may be a direction for future studies in this area. Because lambs were euthanized at day 54 of age, we were not able to follow offspring growth long term. Sales et al. (2016) found that female lambs from dams given intravenous boluses of arginine (345- μ mol Arg HCl/kg BW 3 times daily) from days 100 to 140 of pregnancy were heavier at day 153 of age and tended to have heavier psoas major muscles than female lambs from dams not administered arginine boluses. Although we saw offspring from all treatments equal by day 40, future studies could follow growth more long term to evaluate the effects of oral administration of arginine at market weight.

Lamb ADG followed a similar pattern as BW, with lambs from CON and RES-ARG ewes having greater ($P \leq 0.04$) ADG than lambs from RES ewes on day 19 (Table 6). Girth measurements at birth and day 54 were greater ($P = 0.03$) in lambs from CON compared with RES ewes, with RES-ARG being intermediate and similar ($P \geq 0.09$) to both CON and RES (Table 7). However, on day 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 CON and RES ewes on day 54 ($P \leq 0.05$). Pillai et al. (2017) found that offspring from restricted dams (60% NRC requirements) had shorter curve crown rump measurements ($P \leq 0.05$, maternal diet effect) and lower girth measurements ($P \leq 0.004$, maternal diet*day of gestation interaction) than control (100% NRC requirements), which supports that there may be a rescue effect of maternal arginine supplementation on offspring length. These data support the potential role arginine may play in enhancing offspring growth from underfed dams.

Lamb Organ Mass

After organ collection and weighing, no differences ($P \geq 0.44$) were observed based on nutritional plane or arginine supplementation in total GI tract weight (Table 8). These results contradict Meyer et al. (2013), where lambs from adequately fed ewes had greater empty GI tract weights than lambs from nutrient-restricted ewes. These measurements were taken in lambs 19 to 22 d of age, which are significantly younger than the present study including lambs at 54 ± 3 d of age. It is possible that these visceral differences may have been present during fetal development and at birth, but corrected during postnatal growth. To support this, Reed et al. (2007) observed several differences in organ mass of offspring from restricted ewes compared with offspring from control-fed ewes when offspring were

Table 6. Influence of maternal nutrient restriction and arginine supplementation on offspring ADG (g/d) over time

d ¹	Maternal treatment ²			SEM	P-value ³	P-values ⁴	
	CON	RES	RES-ARG			CON vs. RES and RES-ARG	RES vs. RES-ARG
3	272.4	81.0	129.1	72.96	0.17	0.07	0.64
7	269.1	235.9	245.4	19.49	0.47	0.24	0.73
14	332.4	311.5	334.7	13.14	0.37	0.55	0.21
19	355.0 ^a	306.4 ^b	354.0 ^a	15.77	0.05	0.19	0.04
33	367.6	328.1	346.3	13.29	0.11	0.06	0.33
40	368.6	340.6	367.9	14.45	0.28	0.41	0.18
47	351.9	342.3	367.1	15.02	0.51	0.87	0.25
54	344.5	321.4	349.7	14.98	0.37	0.62	0.19

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$).

¹Interval of d for ADG measured between 2 subsequent dates (i.e., d 3 through 7 = d 7 ADG).

²CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with 180 mg rumen-protected arginine supplement per kg BW (n = 11).

³P-value associated with overall F-test for treatment.

⁴P-values associated with specific contrasts.

Table 7. Influence of maternal nutrient restriction and arginine supplementation on offspring girth (cm) and curved crown rump (cm) length over time

Item	Maternal treatment ¹			SEM	P-value ²	P-values ³	
	CON	RES	RES-ARG			CON vs. RES and RES-ARG	RES vs. RES-ARG
Girth							
0d	42.2 ^a	38.6 ^b	39.4 ^{ab}	1.14	0.08	0.03	0.64
19d	55.4 ^a	51.3 ^b	54.6 ^a	0.97	0.01	0.04	0.02
54d	70.8 ^a	67.0 ^b	69.7 ^{ab}	1.23	0.10	0.11	0.14
Change in girth ⁴	28.6	28.5	29.6	1.25	0.79	0.78	0.53
CCR							
0d	54.9	52.6	55.1	1.49	0.43	0.56	0.24
19d	73.7	69.4	72.9	1.86	0.21	0.25	0.18
54d	96.3 ^b	93.9 ^b	99.8 ^a	1.28	0.01	0.70	0.003
Change in CCR ⁴	41.4	40.9	43.8	1.70	0.42	0.63	0.23

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$).

¹CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with 180 mg rumen-protected arginine supplement per kg BW (n = 11).

²P-value associated with overall F-test for treatment.

³P-values associated with specific contrasts.

⁴Change is calculated between birth and 54 d of age.

harvested just before birth at day 135 ± 5 d of gestation. Furthermore, [McMillen et al. \(2001\)](#) found that mass of more “vital” organs, including the brain, may have an inverse relationship with fetal BW, suggesting a specific compensatory effect to preserve vital organs. In contrast, mass of less “vital” organs, such as the kidney and liver, has a more direct relationship with fetal BW until fetal BW drops below 2 to 3 kg, suggesting that these less vital organs are not preserved until the fetus reaches a high level of stress. These differences may have disappeared if offspring were followed to a later age, or if they were allowed to go through the birthing process ([Fowden](#)

[et al., 2006](#)). To support this, [Zhang et al. \(2016\)](#) found that offspring taken at day 110 of gestation from ewes restricted in nutrients (50% NRC requirements) receiving a rumen-protected arginine supplement (10 g/d) had greater organ weights of heart, liver, pancreas, small intestine, and large intestine ($P \leq 0.04$) than offspring from ewes restricted in nutrients without arginine supplementation. If we had measured some offspring organs prior to the birthing process, it is possible that we may have seen more differences among treatments in organ weights.

Adrenal glands in lambs from CON ewes had greater ($P = 0.01$) mass than adrenal glands in

Table 8. Influence of maternal nutrient restriction and arginine supplementation on offspring organ mass at day 54 of age

Organ	Maternal treatments ¹				<i>P</i> -value ²	<i>P</i> -values ³	
	CON	RES	RES-ARG	SEM		CON vs. RES and RES-ARG	RES vs. RES-ARG
Brain, g	93.6	93.4	92.7	2.69	0.97	0.88	0.85
g/kg BW	4.05	4.31	3.96	0.224	0.52	0.75	0.27
Adrenals, g	1.72 ^a	1.46 ^b	1.54 ^{ab}	0.072	0.03	0.01	0.44
g/kg BW	0.074	0.067	0.065	0.0035	0.18	0.07	0.77
Thyroid, g	1.80	1.81	1.93	0.139	0.77	0.69	0.55
g/kg BW	0.076	0.083	0.082	0.0056	0.61	0.33	0.83
Heart, g	147.0	132.3	138.4	5.38	0.16	0.08	0.43
g/kg BW	6.19	6.08	5.87	0.169	0.40	0.29	0.40
Liver, g	481.2	429.5	490.0	21.08	0.11	0.40	0.05
g/kg BW	20.34	19.63	20.70	0.616	0.47	0.81	0.23
Pancreas, g	28.7	24.3	27.2	1.53	0.12	0.10	0.17
g/kg BW	1.20	1.10	1.16	0.058	0.47	0.31	0.48
Kidneys, g	106.1	102.6	108.6	4.67	0.66	0.93	0.37
g/kg BW	4.46	4.68	4.58	0.084	0.17	0.09	0.41
Total visceral adiposity, g	1,021.7	851.2	888.1	102.40	0.45	0.22	0.80
g/kg BW	41.5	38.9	37.5	3.83	0.75	0.48	0.80
Omental fat, g	511.6	424.4	487.8	53.87	0.49	0.39	0.41
g/kg BW	20.69	19.38	20.55	2.013	0.88	0.76	0.68
Perirenal fat, g	510.0	426.8	400.3	55.98	0.34	0.16	0.74
g/kg BW	20.79	19.52	16.97	2.182	0.45	0.33	0.41
Full GI tract, g	3,732.2	3,330.8	3,471.6	186.73	0.30	0.15	0.60
g/kg BW	156.4	152.5	147.2	5.82	0.52	0.35	0.52
Empty GI tract, g	955.9	891.2	920.2	35.35	0.44	0.26	0.57
g/kg BW	40.59	40.91	39.00	1.172	0.48	0.66	0.26
Stomach, g	304.5	267.8	259.0	15.67	0.10	0.04	0.70
g/kg BW	12.78	12.31	11.10	0.630	0.16	0.16	0.18
Small intestine, g	454.7	442.8	470.5	20.55	0.64	0.94	0.35
g/kg BW	19.37	20.31	19.85	0.707	0.65	0.42	0.65
Large intestine, g	190.4	180.6	190.7	9.74	0.71	0.69	0.47
g/kg BW	8.09	8.28	8.05	0.394	0.91	0.88	0.69

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$).

¹CON = control, 100% NRC requirements ($n = 11$); RES = restricted, 60% CON nutrients ($n = 11$); RES-ARG = restricted + arginine, 60% CON nutrients with 180 mg rumen-protected arginine supplement per kg BW ($n = 11$).

²*P*-value associated with overall F-test for treatment.

³*P*-values associated with specific contrasts.

lambs from RES and RES-ARG ewes. Similarly, pancreases in lambs from CON ewes tended to be greater ($P = 0.10$) in mass than lambs from RES and RES-ARG ewes. These organs are major users of glucose and suggest a possible difference in our offspring in terms of glucose metabolism based on maternal plane of nutrition. FGR induced via dietary manipulation may cause low birth weights, which creates ideal conditions for metabolic and endocrine abnormalities in offspring (Fowden et al., 2006). In addition, glucose intolerance, insulin resistance, and type 2 diabetes have been associated with low birth weight offspring (Fowden et al., 2006); Long et al., (2009) reported that circulating glucose was lower in bovine FGR offspring. These

physiological effects may be induced by structural and functional changes during organogenesis in an FGR environment, which primarily occurs in late gestation when our ewes were on different nutritional planes (Fowden et al., 2006). In offspring of smaller body size like those from our restricted ewes, there should be lower maintenance costs and smaller adrenal glands and pancreas are likely adequate for their health and maintenance.

Livers from lambs born to RES-ARG ewes weighed more ($P = 0.05$) than livers from lambs born to RES ewes. This is intriguing, as the liver is the primary organ for ureagenesis, a process needing arginine. Ureagenesis is especially important in ruminants to support urea recycling to the

Table 9. Influence of maternal nutrient restriction and arginine supplementation on offspring small intestine characteristics at day 54 of age

Item	Maternal treatments ¹			SEM	P-value ²	P-values ³	
	CON	RES	RES-ARG			CON vs. RES and RES-ARG	RES vs. RES-ARG
Small intestine, g	454.7	442.8	470.5	20.55	0.64	0.94	0.35
g/kg BW	19.37	20.31	19.85	0.707	0.65	0.42	0.65
Duodenum, g	43.9	41.2	43.5	7.88	0.97	0.87	0.84
g/kg BW	1.84	1.88	1.85	0.316	1.00	0.95	0.95
Ileum, g	263.5	207.6	256.9	21.98	0.16	0.24	0.12
g/kg BW	11.07	9.53	10.70	0.782	0.35	0.31	0.30
Jejunum, g	63.9	95.8	78.0	14.09	0.29	0.19	0.38
g/kg BW	2.72	4.46	3.39	0.629	0.16	0.13	0.24
Jejunal characteristics							
Jejunum, g	63.9	95.8	78.0	14.09	0.29	0.13	0.24
g/kg BW	2.72	4.46	3.39	0.629	0.16		
% mucosa	81.5%	82.0%	84.3%	1.43	0.34	0.35	0.26
Mucosal weight	52.0	79.6	65.9	11.95	0.28	0.17	0.42
Percent cell proliferation	29.9	36.8	30.2	4.10	0.41	0.47	0.27

^{a,b}Means within a row with different superscripts differ ($P \leq 0.05$).

¹CON = control, 100% NRC requirements (n = 11); RES = restricted, 60% CON nutrients (n = 11); RES-ARG = restricted + arginine, 60% CON nutrients with 180 mg rumen-protected arginine supplement per kg BW (n = 11).

²P-value associated with overall F-test for treatment.

³P-values associated with specific contrasts.

digestive tract where it contributes to bacterial and, hence, whole animal protein demands. Urea recycling within nutrient-restricted gestating animals is likely of greater importance than adequately fed animals. The crucial role of maternal protein supply in fetal development has been documented (Wu et al., 2006; Larson et al., 2009). A smaller liver for RES offspring may be sufficient for the amount of arginine they have to contribute to the urea cycle, whereas a RES-ARG lamb may have more arginine to contribute, and therefore would perform the urea cycle more readily. This in turn could allow them to recycle more urea to meet protein requirements more efficiently. This is supported by the aforementioned Zhang et al. (2016), where offspring from restricted dams receiving rumen-protected arginine supplements had greater liver weights at day 110 of gestation than offspring from restricted dams receiving no arginine supplementation. Offspring liver tissue was used to measure oxygen consumption and demonstrated no rescue effect on hepatic energy use due to arginine supplementation (RES and RES-ARG had lower O_2 consumption than CON, $P \leq 0.02$; Prezotto et al., 2015).

Jejunal Characteristics

There were no differences in total jejunum weight, percent mucosa, mucosal tissue weight, or percent cell

proliferation in the jejunum ($P \geq 0.28$; Table 9). This is inconsistent with Yunusova et al. (2013), in which offspring (180 d of age) from nutrient restricted and overfed ewes were compared with those from control-fed ewes in terms of small intestinal biology. Offspring from both nutrient restricted and overfed ewes had decreased crypt cell proliferation compared with offspring from control-fed ewes. It is possible that if we followed our offspring to a later age we may have seen this effect develop. This may also contribute to offspring with fewer differences in organ weights among treatments as age progresses. The intestine of offspring from restricted ewes may adapt to expend less energy on crypt cell regeneration in order to allow organ development to progress.

CONCLUSIONS

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 while organ masses were not responsive to maternal rumen-protected arginine supplementation. Additional research is needed to further define effects of supplementation of rumen-protected arginine during gestation on offspring health and performance outcomes.

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