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EFFECTS OF MATERNAL NUTRITIONAL PLANE AND SELENIUM SUPPLY ON CELLULARITY ESTIMATES OF NEONATAL LAMB JEJUNAL MUCOSA, HEART, AND SKELETAL MUSCLE

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ABSTRACT: Objectives were to investigate the effects of maternal nutrition and Se supply during gestation on lamb jejunal mucosa, heart, and skeletal muscle RNA, DNA, and protein. Rambouillet ewe lambs (n = 84) were allotted to a 2 x 3 factorial design including dietary factors of Se [adequate Se (**ASe**; 11.5 µg/kg BW) or high Se (**HSe**; 77.0 µg/kg BW)] and nutritional plane [60% (**RES**), 100% (**CON**), or 140% (**HIGH**)]. At breeding Se treatments were initiated followed by nutritional treatments on d 40 of gestation. At birth, lambs (n = 13, 14, 14, 12, 13, and 15 for ASe-RES, ASe-CON, ASe-HIGH, HSe-RES, HSe-CON, and HSe-HIGH, respectively) were removed from ewes before nursing, placed in a common pen, and group fed until necropsy at 20.6 ± 0.9 d of age. Maternal nutritional plane affected ($P \leq 0.07$) offspring jejunal mucosal scrape concentration (mg/g) and total content (mg) of DNA where RES was least, HIGH greatest, and CON intermediate. Plane of nutrition also affected ($P = 0.07$) right ventricle DNA content where RES (189.8 ± 11.8 mg) was least, HIGH (208.2 ± 11.2 mg) intermediate, and CON (227.7 ± 11.3 mg) greatest. Maternal Se supplementation decreased ($P = 0.08$) left ventricle protein:DNA in offspring. For lamb right ventricle, RNA concentration was greatest ($P = 0.05$) for ASe-RES and least for HSe-RES with all other treatments intermediate. However when lamb right ventricle RNA was expressed as total content, HSe-RES was least ($P = 0.02$), ASe-HIGH intermediate, and all other treatments were greater. When RNA:DNA was calculated in right ventricle, ASe-RES and HSe-HIGH were greatest ($P = 0.02$), ASe-CON intermediate, and ASe-HIGH, HSe-RES, and HSe-CON least. Skeletal muscle RNA concentration and RNA:DNA were least ($P < 0.05$) for ASe-HIGH, intermediate for HSe-RES and HSe-CON, and greatest for ASe-RES, ASe-CON, and HSe-HIGH. These data indicate cellularity estimates have tissue specific responses to maternal nutritional plane and Se supply.

KEYWORDS: cellularity, maternal nutrition, neonatal lambs, selenium

Introduction

Selenium, an essential trace mineral, is important for normal growth and development (Sunde, 1997). Selenium is regulated by the FDA to an inclusion limit less than 0.3 ppm (FDA 21CFR573.920), however many plants grown on rangelands in North and South Dakota contain much higher levels of Se due to the geographic formations in these areas (Rosenfeld and Beath, 1964). Global nutrient restriction or overfeeding during adolescence in ewe lambs alters normal growth and development of the fetus and placenta (Wallace et al., 2000; Reed et al., 2007). The lifelong regulation of normal growth, development, and nutrient utilization are likely programmed *in utero* (Wu et al., 2006).

Selenium supplementation and nutrient restriction have had varying results on cellularity estimates in near term fetuses in several studies (Reddy et al., 2006; Reed et al., 2007; Neville et al., 2008). Chemical form of Se supplement (selenate vs. high Se wheat) and level (3 vs. 15 ppm) affected small intestine RNA:DNA and protein:DNA in fetuses at d 134 of gestation (Neville et al., 2008). Fetal skeletal muscle and heart tissue had decreased protein:DNA due to maternal dietary restriction in fetuses at d 135 of gestation (Reed et al., 2007). Maternal nutrient restriction from d 50 to 90 of gestation increased RNA:DNA in the small intestine of fetuses at d 135 of gestation (Reddy et al., 2006). Higher jejunal mucosal DNA was reported in lambs (180 d of age) born to ewes that were either non-Se supplemented control fed or Se supplemented nutrient restricted (unpublished, Caton). The effects of nutrient restriction or overfeeding in conjunction with Se supplementation on neonatal lambs reared independent of their dams have not been studied; therefore, the objective of this study was to investigate the influence of maternal plane of nutrition and Se supplementation on cellularity estimates in jejunal mucosa, heart, and skeletal muscle from 20 d old lambs.

Materials and Methods

Animals and Diets. This experiment was approved by the Institutional Animal Care and Use Committee at North Dakota State University. Eighty-four pregnant Rambouillet ewe lambs (52.1 ± 6.2 kg; d 40 ± 3 d of gestation) were individually housed in 0.91 × 1.2 m pens. Ewes were randomly allotted to 1 of 6 treatments in a 2 x 3 factorial array. Main effects evaluated were dietary levels of Se [initiated at breeding; adequate (**ASe**; 11.5 µg Se•kg BW^{-1}•d^{-1}) vs. high (**HSe**; 77.0 µg Se•kg BW^{-1}•d^{-1})], and nutritional plane [initiated at d 40 of gestation; 60% (**RES**), 100% (**CON**), and 140% (**HIGH**) of requirements for gestating ewe lambs].

All diets were fed once daily in a complete pelleted form (0.48 cm diameter; based on wheat middlings, beet pulp, alfalfa meal, and ground corn). Three pellet formulations (basal, high Se, and concentrated Se pellets) were blended to meet Se and ME intake based upon the Se treatment and nutritional plane of each ewe. The basal pellet contained 15.9% CP and 2.81 Mcal/kg ME DM basis. Selenium sources used were Se-enriched wheat mill run to

replace wheat middlings in basal diet to make a high Se pellet (16.6% CP and 2.82 Mcal/kg ME DM basis) and purified seleno-methionine added to achieve 37.1 ppm Se in the concentrated Se pellet (16.2% CP and 3.01 Mcal/kg ME DM basis). Nutrient requirements were based on NRC (1985) recommendations for 60 kg pregnant ewe lambs during mid to late gestation (weighted ADG of 140 g). Body weight was measured every 14 d and diets were adjusted accordingly.

Parturition to Necropsy. Upon parturition, lambs were immediately removed from dams before nursing occurred. Lambs were fed artificial colostrum (Acquire, APC, Inc., Ankeny, IA) by bottle within 30 min of birth. Colostrum was fed in six feedings over the first 20 h after birth (providing 10.64 g IgG/kg BW). From 24 h until harvest, lambs were fed milk replacer (Super Lamb Instant Milk Replacer, Merrick's Inc., Middleton, WI) and had full access to fresh creep feed and water. At harvest (20.6 ± 0.9 d of age), lambs were stunned by captive bolt (Supercash Mark 2, Acceles and Shelvoke Ltd., England), exsanguinated, and necropsied. Small intestine and heart weights were recorded. A 10-cm sample of small intestine was removed, gently washed in PBS buffer, weighed, placed on a polyethylene cutting board, and opened luminal side up. Mucosal tissue was separated (scraped) from the remaining tissue with a glass histological slide. Remaining tissue was re-weighed to calculate the percentage of mucosa. Five 1-g samples of the mucosa, left and right heart ventricles, and skeletal muscle (loin) were snap frozen in super-cooled isopentane (submerged in liquid nitrogen), and stored at -80°C until analysis.

Cellularity Estimates. Tissue homogenates were analyzed for concentrations of DNA and RNA using the diphenylamine (Johnson et al., 1997) and orcinol procedures (Reynolds et al., 1990). Protein in tissue homogenates was determined with Coomassie brilliant blue G (Bradford, 1976), with bovine serum albumin (Fraction V; Sigma, St. Louis, MO) as the standard (Johnson et al., 1997). Concentration of DNA was used as an index of hyperplasia, and RNA:DNA and protein:DNA ratios were used as an index of hypertrophy (Swanson et al., 2000; Scheaffer et al., 2003; Soto-Navarro et al., 2004). Tissue DNA, RNA, and protein contents were calculated by multiplying DNA, RNA, and protein concentration by fresh tissue weights (Swanson et al., 2000; Scheaffer et al., 2003; Scheaffer et al., 2004). Fresh heart weight was utilized for the left and right ventricles content calculations and weight of mucosal scrape was calculated by multiplying percentage mucosa by small intestinal weight.

Statistics. Data were analyzed as a completely randomized design with a 2 × 3 factorial arrangement of treatments using GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The model contained effects for Se (ASe vs. HSe), nutritional plane (RES, CON, and HIGH), and Se × nutritional plane interaction. Only singleton lambs were utilized in the analysis resulting in 13, 14, 12, 10, 13, and 13 lambs for ASe-RES, ASe-CON, ASe-HIGH, HSe-RES, HSe-CON, and HSe-HIGH, respectively. Sex was included in the model and retained when significant ($P < 0.15$). When main effects or interactions were present ($P < 0.10$), means were separated by least significant difference. Main effects were considered significant when $P < 0.10$.

Results

When interactions were present they will be discussed; however when absent, main effect differences will be presented. Maternal nutritional plane in conjunction with Se supplementation resulted in skeletal muscle having the greatest ($P \leq 0.04$; Table 1) RNA concentration (mg/g) and RNA:DNA in lambs born to ewes from ASe-RES, ASe-CON, or HSe-HIGH treatments and the least RNA concentration and RNA:DNA in lambs from ASe-HIGH ewes. No differences ($P \geq 0.42$) in skeletal muscle DNA, protein, or protein:DNA were found.

Mucosal scrape from lambs born to HIGH fed ewes had the greatest ($P \leq 0.07$) DNA concentration and content with CON offspring intermediate and RES least (5.17, 5.30, and 5.71 ± 0.18 mg/g and 1382, 1556, and 1719 ± 93 mg for DNA concentration and content in RES, CON, and HIGH, respectively). No differences ($P \geq 0.11$) were found in RNA, protein, RNA:DNA, or protein:DNA.

For heart tissue, right and left ventricles were analyzed separately. In the left ventricle, Se supplementation decreased ($P = 0.08$) protein:DNA (35.7 and 29.3 ± 2.6 for ASe and HSe, respectively). In the right ventricle, lambs born to CON ewes had greatest ($P = 0.07$) DNA content with HIGH intermediate and RES the least (189.8, 227.7, and 208.2 ± 11.8 mg for RES, CON, and HIGH, respectively). Right ventricle RNA concentration was least ($P = 0.05$) in HSe-RES offspring and greatest in ASe-RES offspring with all other treatments intermediate. When right ventricle RNA was expressed as total content HSe-RES was least ($P = 0.02$) and ASe-HIGH intermediate compared to all other treatments. The calculation of right ventricle RNA:DNA indicated the greatest ($P = 0.02$) ratio was from ASe-RES and HSe-HIGH offspring with ASe-CON intermediate and all other treatments least.

Discussion

The increase in RNA:DNA ratio in skeletal muscle of ASe-RES, ASe-CON, and HSe-HIGH is an indicator of increased hypertrophy, an increase in the size of cells. This result is dissimilar to previous results where at d 135 of gestation, fetuses had decreased hypertrophy due to maternal nutrient restriction (Reed et al., 2007) and at 180 d of age lambs had no differences in skeletal muscle hypertrophy (unpublished, Maddock-Carlin). Perhaps these differences are due to stage of growth or age relative to the maternal nutritional insults during gestation.

Our current study demonstrated increased hyperplasia in mucosal scrape of offspring from HIGH fed ewes. Jejunal mucosal scrape DNA concentration was altered due to maternal nutritional plane and Se supplementation in lambs harvested at 180 d of age where lambs from ASe-CON and HSe-RES had increased DNA concentrations compared to HSe-CON and HSe-HIGH (unpublished, Caton). Hyperplasia may result in the gross enlargement of an organ; however no differences were reported in small intestinal weight in the current study (Meyer et al., 2009). Because weight of the small intestine at d 20 is similar among treatments, the increased hyperplasia may be

indicative of larger villi that would provide increased surface area for absorption of nutrients. This could alter the health status and performance of the animal as it grows and into adult life.

In the right ventricle, increased RNA:DNA indicates increased hypertrophy in ASe-RES and HSe-HIGH. In previous research, decreased protein:DNA was reported due to ewe dietary restriction during gestation (Reed et al., 2007). Ventricular hypertrophy can be associated with diseased states. The stress resulting from maternal nutrient restriction or overfeeding along with level of Se supplementation on the lamb *in utero* may be the factors driving increased right ventricular hypertrophy. Other researchers (Vonnahme et al., 2003) have demonstrated bilateral ventricular hypertrophy due to maternal nutrient restriction and they hypothesized this to be the result of placental vascular resistance. Additionally, Long et al. (2009) reported increased heart and right ventricle weights per unit of BW in d 125 bovine fetuses due to intrauterine growth restriction from nutrient restriction. These alterations in hypertrophy may have long term consequences on heart health (Godfrey and Barker, 2000; Wu et al., 2006). In the left ventricle, maternal Se supplementation decreased cell size, perhaps moderating cell growth.

In summary, tissue specific differences in hypertrophy and hyperplasia were observed. Impacts of these changes, which resulted from maternal nutritional plane and/or Se supplementation, on life long performance remain to be determined.

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Table 1. Interactive means of maternal selenium supply and nutritional plane on cellularity estimates in skeletal muscle, mucosal scrape, and right and left heart ventricles of lambs at 20 d of age

	Treatments[1]							*P*-values[2]		
Item	ASe-RES	ASe-CON	ASe-HIGH	HSe-RES	HSe-CON	HSe-HIGH	SE	Nut	Se	Nut*Se
Loin										
RNA, mg/g	2.08[a]	2.00[a]	1.63[b]	1.82[ab]	1.91[ab]	1.99[a]	0.14	0.42	0.98	0.04
DNA, mg/g	1.32	1.28	1.33	1.33	1.28	1.26	0.07	0.76	0.68	0.81
Protein, mg/g	82.8	79.5	89.9	76.0	83.1	81.9	6.7	0.55	0.46	0.56
RNA:DNA	1.59[a]	1.58[a]	1.28[b]	1.38[bc]	1.49[ac]	1.59[a]	0.09	0.44	0.97	0.007
Protein:DNA	65.3	63.7	70.7	58.9	66.0	66.8	6.5	0.54	0.58	0.74
Mucosal Scrape										
RNA, mg/g	5.19	5.35	6.10	5.46	5.65	5.25	0.34	0.54	0.71	0.11
RNA, mg	1365	1529	1910	1513	1775	1561	177	0.18	0.91	0.14
DNA, mg/g[3]	4.91	5.36	5.94	5.42	5.25	5.48	0.27	0.07	0.93	0.15
DNA, mg[3]	1281	1494	1815	1484	1617	1621	140	0.04	0.67	0.26
Protein, mg/g	27.1	28.6	29.2	27.1	27.0	27.7	3.1	0.90	0.65	0.95
Protein, mg	7335	8379	9344	7704	8468	8317	1337	0.56	0.85	0.83
RNA:DNA	1.06	1.01	1.03	1.02	1.11	0.97	0.05	0.29	0.90	0.12
Protein:DNA	5.62	5.47	5.00	5.01	5.32	5.12	0.59	0.80	0.62	0.80
Right Ventricle										
RNA, mg/g	2.22[a]	2.08[ab]	1.80[bc]	1.69[b]	1.98[ab]	2.13[ac]	0.19	0.89	0.48	0.05
RNA, mg	146.5[a]	136.0[a]	124.4[ab]	103.5[b]	150.7[a]	154.8[a]	14.7	0.36	0.95	0.02
DNA, mg/g	2.99	3.21	3.10	3.00	3.25	2.77	0.20	0.21	0.53	0.53
DNA, mg[3]	194.6	211.7	213.7	185.0	243.8	202.7	16.2	0.07	0.77	0.30
Protein, mg/g	31.4	29.4	27.7	30.6	34.9	25.1	3.8	0.21	0.80	0.45
Protein, mg	2089	1901	1930	1884	2659	1821	308	0.31	0.51	0.16
RNA:DNA	0.73[a]	0.67[ab]	0.60[b]	0.57[b]	0.64[b]	0.78[a]	0.06	0.78	0.92	0.02
Protein:DNA	10.52	9.40	9.08	10.31	11.62	8.81	1.32	0.34	0.55	0.48
Left Ventricle										
RNA, mg/g	2.22	2.11	2.11	2.38	2.13	2.23	0.16	0.43	0.37	0.87
RNA, mg	144.7	142.4	147.7	145.0	160.0	161.5	14.9	0.77	0.34	0.80
DNA, mg/g	3.40	3.16	3.54	3.91	3.33	3.77	0.36	0.32	0.25	0.86
DNA, mg	216.6	216.4	243.4	235.0	252.2	267.6	26.3	0.44	0.18	0.93
Protein, mg/g	115.3	93.3	111.0	97.4	93.0	103.0	11.1	0.29	0.28	0.68
Protein, mg	7555	6291	7740	6038	7348	7711	912	0.44	0.81	0.30
RNA:DNA	0.70	0.73	0.65	0.65	0.69	0.65	0.07	0.64	0.57	0.92
Protein:DNA[3]	38.4	33.3	35.4	27.6	29.4	30.8	4.9	0.90	0.08	0.71

[1]ASe-RES = offspring from ewes fed 11.5 ug/kg BW Se (ASe: no added Se), restricted to 60% of control;
ASe-CON = offspring from ewes fed 11.5 ug/kg BW Se, consuming requirement level of energy (control);
ASe-HIGH = offspring from ewes fed 11.5 ug/kg BW Se, fed to 140% of control;
HSe-RES = offspring from ewes fed 77 ug/kg BW Se (HSe; high Se) , restricted to 60% of control;
HSe-CON = offspring from ewes fed 77 ug/kg BW Se, consuming requirement level of energy (control);
HSe-HIGH = offspring from ewes fed 77 ug/kg BW Se, fed to 140% of control.
[2]Probablity values for effects of Se supply (Se), nutritional plane (Nut), and the interaction.
[3]Main effect means presented in text.
[abc]Means differ by $P < 0.10$.