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Effects of level and source of dietary selenium on maternal and fetal body weight, visceral organ mass, cellularity estimates, and jejunal vascularity in pregnant ewe lambs¹

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ABSTRACT: Pregnant Targhee ewe lambs (n = 32; BW = 45.6 ± 2.2 kg) were allotted randomly to 1 of 4 treatments in a completely randomized design to examine the effects of level and source of dietary Se on maternal and fetal visceral organ mass, cellularity estimates, and maternal jejunal crypt cell proliferation and vascularity. Diets contained (DM basis) either no added Se (control) or supranutritional Se from high-Se wheat at 3.0 ppm Se (SW) or from sodium selenate at 3 (S3) or 15 (S15) ppm Se. Diets were similar in CP (15.5%) and ME (2.68 Mcal/kg of DM) and were fed to meet or exceed requirements. Treatments were initiated at 50 ± 5 d of gestation. The control, SW, S3, and S15 treatment diets provided 2.5, 75, 75, and 375 µg of Se/kg of BW, respectively. On d 134 ± 10 of gestation, ewes were necropsied, and tissues were harvested. Contrasts, including control vs. Se treatments (SW, S3, and S15), SW vs. S3, and S3 vs. S15, were used to evaluate differences among Se levels and sources. There were no differences in ewe initial and final BW. Full viscera and liver mass (g/kg

of empty BW and g/kg of maternal BW) and maternal liver protein concentration (mg/g) and content (g) were greater ($P < 0.04$) in Se-treated compared with control ewes. Maternal liver protein concentration was greater ($P = 0.01$) in SW vs. S3 ewes, and content was greater ($P = 0.01$) in S15 compared with S3 ewes. Maternal jejunal mucosal DNA concentration (mg/g) was greater ($P = 0.08$) in SW compared with S3 ewes. Total number of proliferating cells in maternal jejunal mucosa was greater ($P = 0.02$) in Se-fed compared with control ewes. Capillary number density within maternal jejunal tissue was greater ($P = 0.08$) in S3 compared with SW ewes. Selenium treatment resulted in reduced fetal heart girth ($P = 0.08$). Fetal kidney RNA ($P = 0.04$) and protein concentrations (mg/g; $P = 0.03$) were greater in Se-treated compared with control ewes. These results indicate that supranutritional dietary Se increases cell numbers in maternal jejunal mucosa through increased crypt cell proliferation. No indications of toxicity were observed in any of the Se treatments.

Key words: cellularity, fetal, maternal, pregnancy, selenium, sheep

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INTRODUCTION

Selenium is an essential trace mineral for normal growth and development in livestock and humans (Sunde, 1997; McDowell, 2003). Selenomethionine is metabolized differently than inorganic salt forms (Thompson et al., 1982). Selenomethionine, an organically bound form of Se, can be either reduced to form selenide or directly incorporated into proteins in place of Met (McConnell and Hoffman, 1972; Waschulewski and Sunde, 1988; Butler et al., 1989). Potential health benefits of supranutritional dietary Se in animals and humans include improved immune response and thyroid function, as well as reduction of certain types of cancer (Clark et al., 1996; Awadeh et al., 1997; Beck et al., 2001). Increased apoptosis and reduced angiogen-

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esis contributed to inhibited tumor growth in mammary tissue of rats provided supranutritional dietary Se (Combs and Lü, 2001). Research using rodent cancer models has demonstrated that positive responses to supranutritional Se (2 to 3 ppm) may be dependent upon molecular form provided (Finley et al., 2000; Whanger et al., 2000; Finley and Davis, 2001). Soto-Navarro et al. (2004) evaluated effects of supranutritional Se from high-Se wheat on intestinal mass, crypt cell proliferation, and vascular density in finishing steers. They reported that the percentage of jejunal cellular proliferation was unaffected by high Se; however, jejunal mass was increased in steers fed high-Se wheat compared with controls. Consequently, when cellular proliferation estimates were coupled with jejunal mass, total proliferating cells were increased by 84% in steers fed high-Se wheat. Further investigation is needed to evaluate effects of level and source of dietary Se on rapidly growing healthy tissues. Therefore, objectives of this study were to determine effects of level and source of dietary Se on maternal and fetal visceral mass, growth, cellularity, and proliferation and vascularity in maternal jejunal tissues in pregnant ewe lambs.

MATERIALS AND METHODS

Animals and Diets

The North Dakota State University Institutional Animal Care and Use Committee approved care and use of the animals.

Thirty-two pregnant Targhee ewe lambs (45.6 ± 2.2 kg) were allotted randomly to 1 of 4 treatments in a completely randomized design. Ewes were individually housed in 0.91×1.2 -m pens in a temperature-controlled (12°C) and ventilated facility for the duration of the study. Lighting within the facility was automatically timed to mimic daylight patterns.

Treatments (initiated on $d 50 \pm 5$ d gestation) were as follows: control (0.1 ppm Se), Se wheat (SW; 3 ppm Se), selenate fed at 3 ppm (S3), and selenate fed at 15 ppm (S15). The SW and S3 diets provided $75 \mu\text{g}/\text{kg}$ of BW of Se, whereas the S15 treatment provided $375 \mu\text{g}/\text{kg}$ of BW of Se. Diets contained 5% soybean hulls, 33.5% beet pulp, 2.5% soybean meal, 27% alfalfa, and 32% wheat (DM basis). The SW diet was formulated by replacing wheat in the control diet with a high (9 ppm)-Se wheat from a seleniferous region near Pierre, South Dakota. Ewes on selenate treatments received control pellets top-dressed with a tap water-based selenate solution. Diets (DM basis) were similar in CP (15.5%) and energy (2.68 Mcal of ME/kg) and were fed to meet or exceed the NRC requirements (NRC, 1985). All diets were delivered in a complete pelleted form (0.48-cm diam.) and were fed twice daily. Ewes were allowed free access to water and Se-free trace mineralized salt (American Stockman, Overland Park, KS). Ewes were fed at a rate of 2.5% BW (as fed) of their respective treatment diets daily, with BW measured every 14 d.

Necropsy Procedures and Tissue Harvesting

Ewes were assigned a necropsy date randomly, resulting in an average gestation length of 134 ± 10 d. Four animals were assigned for each necropsy date. On each respective necropsy day, the ewes were weighed, blood-sampled, and injected via jugular venipuncture with 5-bromo-2-deoxy-uridine (5 mg/kg of BW; Sigma-Aldrich, St. Louis, MO) 1 h before necropsy. Bromodeoxyuridine is a thymidine analog that incorporates into cellular DNA during the S phase of the cell cycle (Jablonka-Shariff et al., 1993; Jin et al., 1994), thereby allowing for the rate of cellular proliferation to be measured histologically. The first necropsy time was scheduled for 0800 h, with the remaining necropsy times scheduled approximately 2 h apart. Ewes were stunned by captive bolt (Supercash Mark 2, Aceles and Shelvoke Ltd., Birmingham, UK) and exsanguinated. Blood was collected and weighed. After exsanguination, maternal and fetal tissues were immediately harvested, and dissection proceeded concurrently. Maternal tissue dissection procedures were similar to those previously described (Scheaffer et al., 2004; Soto-Navarro et al., 2004).

Pre-necropsy plasma samples were collected with sterile EDTA (K_3) Vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ). Samples were centrifuged at $1,500 \times g$, for 28 min. The supernatant was pipetted into 2-mL screw-cap vials and stored at -20°C . Plasma Se was analyzed by the Utah State Veterinary Diagnostic Laboratory, Logan, utilizing inductively coupled plasma mass spectrometry techniques (Taylor, 2005).

Five samples (approximately 1 g) of maternal kidney, liver, jejunum, jejunal mucosa, and mammary tissues were harvested and preserved for RNA, DNA, and protein analysis. Tissue samples were snap-frozen as quickly as possible in supercooled isopentane (submerged in liquid N) and stored at -80°C until analysis (Reynolds et al., 1990; Reynolds and Redmer, 1992). Maternal jejunal tissue samples were collected after measuring 15 cm distal along the mesenteric vein from the mesenteric vein-ileocecal vein junction and then following the mesenteric arcade to the point of the junction with the intestinal tissue. Approximately 10 cm of maternal jejunum was removed from the same location, to subsample mucosal tissue. To obtain mucosal tissue, the subsample of intact jejunal tissue was submersed in PBS buffer solution, and the digesta was gently removed. The tissue was weighed and placed on a polyethylene cutting board and opened with lumen side up. A glass histological slide was used to scrape the mucosal tissue from remaining jejunal tissue. The mucosal sample was prepared for preservation as previously described, and the remaining jejunum tissue was weighed again to determine the quantity of jejunal mucosa sampled.

Individual fetal and total fetal mass, curved crown-rump length (the length from the crown of the head to

Table 1. Effect of level and source of dietary Se on maternal BW measures and on digesta and gravid uterine weights of pregnant ewe lambs

Item	Treatment ¹				SEM	Contrast		
	Control	SW	S3	S15		Control vs. Se ²	SW vs. S3	S3 vs. S15
Initial BW, kg	45.4	46.0	45.7	46.1	2.2	0.84	0.98	0.89
Final BW, kg	69.9	69.2	70.0	68.7	2.9	0.85	0.85	0.75
Carcass, ³ kg	44.7	45.3	44.3	43.0	1.9	0.80	0.73	0.61
EBW, ⁴ kg	63.3	62.3	63.0	61.5	2.7	0.75	0.85	0.68
MBW, ⁵ kg	56.9	57.0	56.8	55.3	2.9	0.87	0.97	0.70
Digesta, kg	6.7	6.9	7.0	7.2	0.68	0.63	0.94	0.77
g/kg of EBW	107	112	110	118	11	0.64	0.90	0.62
g/kg of MBW	122	123	122	132	14	0.85	0.98	0.64
Gravid uterus, kg	6.0	5.3	6.2	6.2	0.63	0.91	0.29	0.93
g/kg of EBW	96	86	99	101	10	0.95	0.37	0.87
g/kg of MBW	108	95	110	114	13	0.92	0.39	0.83

¹SW = Se wheat, 3 ppm Se; S3 = selenate, 3 ppm Se; S15 = selenate, 15 ppm Se.

²Control vs. the average of the Se treatments (SW, S3, and S15).

³Carcass = empty carcass, including head, hide, and hooves.

⁴EBW = empty BW = final BW – digesta.

⁵MBW = maternal BW = final BW – (digesta + gravid uterus).

the end of the rump, as measured along the backbone), and heart girth length was measured. Fetal organs were harvested and weighed as described above. Samples were collected from fetal liver, kidney, and the middle portion of small intestine and preserved as previously described.

Cellularity Estimates

Freshly thawed tissue samples (0.5 g) were homogenized using a Polytron fitted with a PT-10s probe (Brinkmann, Westbury, NY) in a Tris aminomethane, Na, and EDTA buffer (0.05 M Tris, 2.0 M NaCl, 2 mM EDTA, pH 7.4). Samples were then analyzed for concentrations of DNA and RNA by using the diphenylamine (Johnson et al., 1997) and orcinol (Reynolds et al., 1990) procedures. Protein in tissue homogenates was determined with Coomassie Brilliant Blue G (Bradford, 1976) with BSA (Fraction V, Sigma Chemical) as the standard (Johnson et al., 1997). Prepared samples were analyzed with a spectrophotometer (Beckman DU 640, Beckman Coulter Inc., Fullerton, CA) and were assessed against standard curves of known concentrations.

Jejunal Cell Proliferation

To measure cellular proliferation in maternal jejunal tissue, cross sections of fresh intestinal tissue were made from a section of jejunum. Tissue sections were immersed in Carnoy's solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid, vol/vol/vol) for 3 h. The tissues were subsequently transferred to a 70% (vol/vol) ethanol solution until embedded in paraffin (Reynolds and Redmer, 1992). Tissue sections (4- μ m thick) were made from the paraffin blocks, mounted on glass slides, and prepared for counterstaining proce-

dures, as described by Fricke et al. (1997) and Soto-Navarro et al. (2004). Prepared slides were incubated with anti-5-bromo-2-deoxy-uridine formalin grade mouse IgG monoclonal antibody (Clone BMC, Roche Diagnostics, Indianapolis, IN) at 9 μ L/1.8 mL of dilution buffer. Primary antibody was detected by using 3,3'-diaminobenzidine (Vector Laboratories, Burlingame, CA) to stain cells undergoing proliferation, specifically in the S stage of the cell cycle. Hematoxylin (EMD Chemicals Inc., Gibbstown, NJ) was used to counterstain the nondividing nuclei, and periodic acid-Schiff's staining procedures (Luna, 1968) were utilized to highlight other structures present within the jejunal tissue cross section. Cellular proliferation was quantified using image analysis software (Image Pro Plus 5.0, Media-Cybernetics Inc., Silver Spring, MD).

Small Intestine Vascularity

To measure the vascularity of the small intestine, a portion of the freshly excised gastrointestinal tract was perfusion-fixed. Procedures used for tissue harvesting and preparation were similar to those previously described (Scheaffer et al., 2004; Soto-Navarro et al., 2004), except that epoxy casting resin [Mercox, consisting of 0.8 mL of catalyst, 5 mL of diluent (methyl methacrylate), and 5 mL of resin, which were all from Ladd Industries, Williston, VT] was allowed to set for 30 min rather than for 75 min. Cross sections of perfused intestinal tissue were processed in a similar fashion as the jejunal tissues (described above). The process of measuring vascularity of the jejunal tissue required 4- μ m-thick tissue sections that were stained using periodic acid-Schiff's staining procedures (Luna, 1968) to provide contrast to the vascular tissue. Capillary area, number, and mean capillary circumference measurements were made using image analysis software (Image

Table 2. Effect of level and source of dietary Se on visceral organ weights of pregnant ewe lambs

Item	Treatment ¹				SEM	Contrast		
	Control	SW	S3	S15		Control vs. Se ²	SW vs. S3	S3 vs. S15
Full viscera, ³ kg	13.8	15.5	14.9	16.0	1.0	0.13	0.62	0.41
g/kg of EBW ⁴	216	251	235	261	20	0.02	0.36	0.14
g/kg of MBW ⁵	237	275	261	290	15	0.03	0.50	0.17
Stomach, ⁶ kg	1.44	1.43	1.52	1.32	0.082	0.88	0.46	0.10
g/kg of EBW	23.0	23.2	23.9	21.7	1.3	0.94	0.68	0.22
g/kg of MBW	25.8	25.4	26.5	24.2	1.6	0.84	0.61	0.28
Liver, g	958	1,076	1,038	1,027	48	0.12	0.58	0.87
g/kg of EBW	15.1	17.4	16.4	16.9	0.59	0.02	0.26	0.57
g/kg of MBW	16.7	19.1	18.2	18.9	0.79	0.03	0.45	0.57
Pancreas, g	75.0	65.9	79.3	72.1	4.3	0.59	0.03	0.23
g/kg of EBW	1.21	1.07	1.24	1.19	0.091	0.68	0.18	0.68
g/kg of MBW	1.32	1.18	1.38	1.33	0.11	0.85	0.18	0.77
Spleen, g	115.9	110.9	123.2	119.1	6.8	0.82	0.21	0.67
g/kg of EBW	1.88	1.81	1.97	1.97	0.16	0.82	0.46	0.99
g/kg of MBW	2.17	1.98	2.19	2.21	0.20	0.87	0.44	0.93
Omental fat, kg	2.30	2.85	3.59	2.54	0.49	0.23	0.29	0.14
g/kg of EBW	36	45	60	41	8.5	0.22	0.23	0.13
g/kg of MBW	38	48	66	45	10	0.21	0.21	0.14
Adrenals, g	4.16	3.56	3.56	3.25	0.31	0.06	0.99	0.46
mg/kg of EBW	68.1	56.9	56.8	53.5	5.1	0.04	0.99	0.63
mg/kg of MBW	76.6	62.2	63.1	59.8	6.4	0.05	0.91	0.68
Blood, kg	3.06	2.40	2.45	2.61	0.19	0.02	0.85	0.56
g/kg of EBW	48.9	38.7	39.1	43.9	4.1	0.09	0.93	0.41
g/kg of MBW	55.8	42.3	43.5	49.2	5.4	0.09	0.85	0.41
Heart and lung, g	851	873	854	900	49	0.66	0.80	0.51
g/kg of EBW	13.7	14.1	13.6	14.7	0.94	0.69	0.71	0.41
g/kg of MBW	15.5	15.5	15.1	16.4	1.2	0.91	0.83	0.44
Kidney, g	137.9	144.5	139.1	138.5	6.2	0.70	0.55	0.94
g/kg of EBW	2.19	2.32	2.21	2.26	0.063	0.34	0.25	0.63
g/kg of MBW	2.42	2.54	2.46	2.51	0.082	0.37	0.44	0.93
Perirenal fat, kg	1.06	1.37	1.11	1.04	0.22	0.64	0.42	0.82
g/kg of EBW	16.1	21.2	17.5	16.3	2.7	0.48	0.34	0.75
g/kg of MBW	17.7	23.2	19.4	18.1	3.1	0.49	0.38	0.75
Mammary, g	548	456	502	565	65	0.58	0.62	0.49
g/kg of EBW	8.6	7.5	7.8	9.2	0.98	0.70	0.80	0.31
g/kg of MBW	9.8	8.3	8.7	10.3	1.2	0.58	0.77	0.32

¹SW = Se wheat, 3 ppm Se; S3 = selenate, 3 ppm Se; S15 = selenate, 15 ppm Se.

²Control vs. the average of the Se treatments (SW, S3, and S15).

³Full viscera = full visceral mass = (large intestine + small intestine + stomach complex + spleen + pancreas + liver + gallbladder), including digesta.

⁴EBW = empty BW = final BW – digesta.

⁵MBW = maternal BW = final BW – (digesta + gravid uterus).

⁶Stomach complex = (reticulum + rumen + omasum + abomasum), without digesta.

Pro Plus 5.0, MediaCybernetics), as previously described (Borowicz et al., 2007).

Calculations

All tissue masses are reported on a fresh tissue basis, because previous data has suggested little variation among fresh and dry weights for visceral organs (Jin et al., 1994; Swanson, 1996; Swanson et al., 2000). Organ mass was expressed as grams of fresh tissue, grams per kilogram of empty BW, and grams per kilogram of maternal BW. Empty BW was equal to BW minus total digesta weight, whereas maternal BW was defined as empty BW minus gravid uterine weight (Ratray et al., 1974; Robinson et al., 1978). To express organ mass on

an empty BW basis, fresh organ mass (g) was divided by empty BW (kg). Likewise, expression of organ mass on a maternal BW basis was accomplished by dividing fresh organ mass (g) by maternal BW (kg). Fetal organ mass data are expressed as grams of fresh tissue and grams per kilogram of empty carcass weight (BW minus visceral and thoracic organ mass). Total digesta was not measured in the fetus; therefore, fresh organ mass was divided by empty carcass weight (including head, hide, and hoof mass).

Percentage of jejunal mucosa was calculated by dividing the mucosa scrape mass by the jejunal sample mass before the scrape. Total jejunal mucosa (g) was calculated by multiplying percentage of mucosa by total jejunal mass (g).

Concentration of DNA was used as an index of hyperplasia, with protein:DNA and RNA:DNA ratios used as indices of hypertrophy (Swanson et al., 2000; Scheaffer et al., 2003; Soto-Navarro et al., 2004). Total DNA, RNA, and protein contents were calculated by multiplying DNA, RNA, and protein concentrations by fresh tissue weights (Swanson et al., 2000; Scheaffer et al., 2003, 2004).

Percentage of proliferating cells was estimated by dividing the number of 3,3'-diaminobenzidine-stained nuclei by the total number of nuclei present within the area of tissue analyzed. Total proliferating cells was calculated by dividing total tissue DNA (mg) by 6.6×10^{-12} g then multiplying that value by the percentage of proliferating cells (Baserga, 1985; Zheng et al., 1994).

Capillary area density was determined by dividing the total capillary area by the area of tissue analyzed (Scheaffer et al., 2004; Soto-Navarro et al., 2004; Borowicz et al., 2007; Reed et al., 2007). Capillary number density was calculated by dividing the total number of vessels counted by tissue area (Borowicz et al., 2007). To estimate the capillary surface density, mean capillary circumference (μm) was divided by tissue area (Reynolds et al., 2005a; Borowicz et al., 2007). Although capillary surface density actually represents the circumference of the capillary cross sections, it is nevertheless proportional to their surface area (Borowicz et al., 2007). Mean area per capillary was determined by dividing the capillary area density by the capillary number density. Total vascularity (mL) of the respective tissue was calculated by multiplying the percentage capillary area density by tissue mass, assuming that 1 g of tissue is equivalent to 1 mL of volume (Reynolds and Redmer, 2001).

Statistical Analysis

Maternal and fetal data were analyzed using ANOVA (PROC GLM, SAS Inst. Inc., Cary, NC). Fetal number was included in the model and was retained when significant ($P \leq 0.10$) and dropped when not significant ($P > 0.10$). Contrasts were used to evaluate differences between level and source of dietary Se. Specifically, contrasts were made between control vs. Se treatments (SW, S3, and S15), SW vs. S3, and S3 vs. S15 and were considered different at $P \leq 0.10$.

RESULTS AND DISCUSSION

Maternal BW and Organ Mass

Initial BW (d 50 of gestation), final BW (d 134 of gestation), carcass weight, empty BW, and maternal BW were not different ($P \geq 0.61$) among treatments (Table 1). Likewise, total digesta mass and gravid uterine mass were not affected by treatment ($P \geq 0.29$). Other investigators have reported no differences in final BW, gains, or efficiencies in Se-supplemented finishing cattle and sheep (Lawler et al., 2004; Schauer

et al., 2005; Taylor, 2005). In the current study, Se treatments were effective in elevating plasma Se concentrations at necropsy (0.27, 0.55, 0.47, 1.28 ± 0.06 ppm for control, SW, S3, and S15, respectively), indicating that our treatments changed the supply of Se to tissues.

Expressing organ mass on an empty BW or maternal BW basis reduces variation and provides a more accurate assessment of tissue mass as it pertains to body size and metabolically active tissue (Koong et al., 1985; Sainz and Bently, 1997; NRC, 2000). Full viscera mass, encompassing the stomach complex, liver, gallbladder, pancreas, spleen, small intestine, large intestine, and digesta, was greater ($P = 0.02$) in Se-supplemented ewes compared with control ewes when expressed per unit of empty BW (g/kg; Table 2). Given that total digesta mass was similar ($P > 0.62$; Table 1) among treatments, the change in full visceral mass is attributed to changes in visceral organ masses, indicating that supranutritional Se supplementation may increase mass of metabolically active visceral tissues. Maternal stomach complex mass was not affected by treatment, although supranutritional levels of dietary Se resulted in greater liver mass ($P = 0.02$; g/kg of empty BW; Table 2) compared with control. Lawler et al. (2004) reported no differences in liver mass in steers fed 2.80 to 2.86 ppm from both inorganic and organically bound Se sources compared with controls (0.38 ppm). Likewise, Reed et al. (2007) reported no differences in liver mass of pregnant first-parity ewes fed 6 vs. 80 μg of Se/kg of BW from breeding until d 135 of gestation. Discrepancies in these and the current study are likely explained by differing dietary intake and form, growth, physiological state, and possibly form of Se provided.

Spleen and omental fat were all unaffected by treatment ($P \geq 0.13$). Gross pancreatic mass (g) was decreased ($P = 0.03$) in SW vs. S3 ewes. Adrenal gland mass was greater ($P \leq 0.06$) for control compared with Se-treated ewes when expressed on a gross, empty BW, or maternal BW basis (Table 2). Blood mass (kg) was greater ($P = 0.02$) in control vs. Se-treated ewes. Reduced blood mass could indicate reduced blood flow. Supranutritional levels of Se have been implicated in reduced capillary size in placental tissue (Borowicz et al., 2005), altered angiogenesis in mammary tumors (Jiang et al., 1999), and altered expression of angiogenic factors in normal tissues (Carlin et al., 2007; Carlson et al., 2007; Neville et al., 2007). Heart and lung mass was not affected ($P = 0.41$) by level or source of dietary Se. Likewise, kidney, perirenal fat, and mammary gland masses were unaffected ($P \geq 0.25$) by dietary Se.

Maternal Liver, Kidney, and Mammary Gland Cellularity

In maternal liver, DNA and RNA concentrations, content, and ratios were unaffected ($P \geq 0.13$) by dietary Se (Table 3). Protein concentration (mg/g) was greater ($P = 0.02$) in Se-treated vs. control ewes and greater

Table 3. Effect of level and source of dietary Se on cellularity estimates in maternal liver, kidney, and mammary tissues of pregnant ewe lambs

Item	Treatment ¹				SEM	Contrasts		
	Control	SW	S3	S15		Control vs. Se ²	SW vs. S3	S3 vs. S15
Liver								
DNA, mg/g	2.15	2.15	2.57	2.53	0.22	0.31	0.20	0.90
DNA, g	2.07	2.24	2.66	2.71	0.26	0.13	0.27	0.89
RNA, mg/g	3.66	3.69	4.13	3.76	0.23	0.47	0.18	0.25
RNA, g	3.53	3.87	4.23	4.01	0.28	0.13	0.36	0.57
RNA:DNA	1.90	1.78	1.63	1.64	0.20	0.33	0.58	0.95
Protein, mg/g	95.7	111.9	101.2	121.7	5.7	0.02	0.01	0.18
Protein, g	90.6	114.5	103.5	130.0	6.3	0.01	0.21	0.01
Protein:DNA	50.0	51.1	39.8	52.2	5.5	0.70	0.10	0.15
Kidney								
DNA, mg/g	2.13	2.05	2.49	2.29	0.13	0.32	0.02	0.28
DNA, g	0.29	0.30	0.35	0.31	0.021	0.32	0.09	0.25
RNA, mg/g	2.73	2.37	2.92	2.60	0.15	0.54	0.01	0.13
RNA, g	0.37	0.34	0.40	0.36	0.024	0.82	0.08	0.22
RNA:DNA	1.29	1.18	1.19	1.16	0.080	0.24	0.95	0.80
Protein, mg/g	59.3	53.6	68.9	66.9	5.1	0.52	0.04	0.79
Protein, g	8.12	7.79	9.58	9.11	0.75	0.43	0.10	0.66
Protein:DNA	27.5	26.8	28.4	29.9	2.6	0.77	0.66	0.69
Mammary								
DNA, mg/g	2.24	2.65	2.45	3.05	0.29	0.16	0.62	0.15
DNA, g	1.22	1.15	1.03	1.62	0.14	0.78	0.55	0.01
RNA, mg/g	2.82	2.79	2.89	2.99	0.26	0.82	0.78	0.78
RNA, g	1.53	1.22	1.40	1.62	0.18	0.54	0.47	0.38
RNA:DNA	1.28	1.21	1.27	0.99	0.13	0.43	0.78	0.15
Protein, mg/g	53.8	62.6	49.4	52.3	6.0	0.89	0.12	0.73
Protein, g	30.2	27.1	28.6	31.5	5.3	0.83	0.84	0.69
Protein:DNA	25.5	28.3	24.3	18.9	4.7	0.75	0.54	0.42

¹SW = Se wheat, 3 ppm Se; S3 = selenate, 3 ppm Se; S15 = selenate, 15 ppm Se.

²Control vs. average of Se treatments (SW, S3, and S15).

($P = 0.01$) in SW vs. S3 ewes. Total protein content (g) was greater ($P = 0.01$) in Se-treated vs. control ewes and greater ($P = 0.01$) in S15 compared with S3 ewes.

Maternal kidney DNA and RNA concentrations (mg/g) were greater ($P \leq 0.02$; Table 3) in S3 compared with SW ewes. Greater DNA ($P = 0.09$) and RNA ($P = 0.08$) content (g) and greater ($P = 0.04$) protein concentration (mg/g) was observed in S3 vs. SW ewes. Changes in kidney DNA, RNA, and protein concentrations and contents are reflective of increased cell number, protein synthetic capacity, and likely metabolic activity in kidneys of ewes fed S3 compared with those receiving SW. No differences were observed in kidney cellularity estimates between S3- and S15-treated ewes. Ratio of RNA to DNA, protein content (g), and protein:DNA were not different ($P > 0.10$) among treatments in maternal kidney.

Ewes provided 15 ppm Se from selenate (S15) had greater ($P < 0.01$) DNA content (g) in their mammary tissue compared with S3 (Table 3), indicating greater hyperplasia in mammary tissue from S15-fed ewes. This response appears to be driven by small, nonsignificant changes in DNA concentrations and tissue mass. In lactating animals, Se is readily transferred to milk, especially in colostrum (Underwood and Suttle, 2001; McDowell, 2003). Selenium concentrations in

milk have been directly correlated with dietary Se intake of the dam (Pehrson et al., 1999). Ewes in this study were necropsied at approximately 135 d of gestation, and milk production was already visually evident within the mammary tissue. Increases in hyperplasia noted in the S15-treated ewes may have been the result of added metabolic activity in the mammary gland due to the additional Se load. Other cellularity measurements in the mammary gland were not different ($P \geq 0.12$) among contrasts evaluated.

Maternal Intestinal Cellularity

Small intestine, duodenum, jejunum, and ileum masses were not different among treatments (Table 4). In contrast, Soto-Navarro et al. (2004) reported greater jejunal mass in finishing steers fed 2.81 ppm from a high-Se wheat source compared with controls (0.39 ppm Se). In the current study, jejunal mucosal percentage was greater in S3 vs. SW ($P = 0.06$) and in S3 vs. S15 ($P = 0.05$)-treated ewes. These data indicate that jejunal mucosal mass, as a proportion of total mass, responds to differing source and level of dietary Se; however, contrasts comparing controls with the combined effect of Se treatments were not significant ($P = 0.84$), indicating that control-fed ewes had mucosal proportions that

Table 4. Effect of level and source of dietary Se on intestinal organ weights of pregnant ewe lambs

Item	Treatment ¹				SEM	Contrasts		
	Control	SW	S3	S15		Control vs. Se ²	SW vs. S3	S3 vs. S15
Small intestine, g	639	696	622	597	41	0.99	0.21	0.68
g/kg of EBW ³	10.04	11.30	9.84	9.94	0.76	0.71	0.16	0.92
g/kg of MBW ⁴	11.25	12.40	10.95	11.10	0.99	0.84	0.24	0.90
Duodenum, g	94	119	111	112	11	0.13	0.62	0.96
g/kg of EBW	1.49	1.92	1.78	1.87	0.20	0.12	0.63	0.75
g/kg of MBW	1.69	2.11	1.98	2.08	0.22	0.18	0.69	0.75
Jejunum, g	251	285	262	256	18	0.41	0.36	0.82
g/kg of EBW	4.01	4.68	4.19	4.25	0.38	0.41	0.36	0.92
g/kg of MBW	4.62	5.13	4.66	4.75	0.50	0.69	0.45	0.88
Mucosa, %	72.6	68.4	78.9	68.0	3.7	0.84	0.06	0.05
Mucosa, g	176	195	206	175	16	0.39	0.58	0.15
g/kg of EBW	2.80	3.21	3.31	2.90	0.32	0.36	0.82	0.34
g/kg of MBW	3.20	3.53	3.68	3.25	0.42	0.55	0.77	0.42
Ileum, g	300	292	249	229	29	0.21	0.30	0.65
g/kg of EBW	4.69	4.71	3.86	3.82	0.43	0.27	0.17	0.94
g/kg of MBW	5.13	5.16	4.30	4.26	0.53	0.37	0.23	0.96
Large intestine, g	377	379	422	373	20	0.56	0.14	0.10
g/kg of EBW	5.97	6.14	6.65	6.12	0.26	0.28	0.18	0.16
g/kg of MBW	6.52	6.74	7.39	6.82	0.33	0.23	0.15	0.20

¹SW = Se wheat, 3 ppm Se; S3 = selenate, 3 ppm Se; S15 = selenate, 15 ppm Se.

²Control vs. average of Se treatments (SW, S3, and S15).

³EBW = empty BW.

⁴MBW = maternal BW.

were intermediate to Se treatments. Conversely, other recent work (Reed et al., 2007) indicates that jejunal mucosal mass is not altered by greater levels of dietary Se. Differences in these studies may be related to diet type, level of intake, or both. To what extent Se affects the primary sites of nutrient absorption in the small intestine or visceral energy consumption needs further investigation. Large intestinal mass was greater ($P = 0.10$) in S15 compared with S3-treated ewes. Combined effects in the small and large intestine indicate that rapidly proliferating tissues are responsive to elevated dietary Se.

Maternal jejunal DNA concentration (mg/g) was decreased ($P = 0.01$) in Se-treated compared with control ewes, (Table 5; 4.74 vs. 5.49 ± 0.25 mg/g, respectively). Within Se treatments, ewes fed S15 had greater ($P = 0.06$) jejunal DNA concentration (mg/g) compared with S3-fed ewes, whereas SW-treated ewes had greater ($P = 0.10$) total DNA (g) in jejunal tissues compared with S3-treated ewes. The RNA:DNA was greater ($P = 0.10$) in Se-treated compared with control ewes (0.93 vs. 0.73 ± 0.08 , respectively). In addition, S15-treated ewes had a lower ($P = 0.06$) RNA:DNA compared with S3. Maternal jejunal RNA and protein concentrations (mg/g) and content (g) and protein:DNA were unaffected ($P = 0.14$ to 0.18) by dietary Se.

Responses in jejunal mucosa did not follow patterns observed in whole jejunal tissues for estimates of hyperplasia (DNA) and hypertrophy (RNA:DNA and protein:DNA). Reasons for this are unclear but likely are related to changes in mucosal tissue in relation to total

jejunal tissue. As discussed below, proliferating nuclei in jejunal crypts were increased by Se supplementation, likely resulting in increased total mucosal cells as reflected in mucosal DNA measurements. Others (Soto-Navarro et al., 2004) have also reported increases in total jejunal proliferating cells. In contrast, Reed et al. (2007) indicated that high dietary Se did not alter jejunal mucosal DNA or crypt proliferating cells; however, in their study, total small intestinal mass was increased by dietary Se. Reasons for these differing results are unclear and additional research in this direction is needed, especially considering the effect of intestinal mucosal tissue on nutrient uptake, utilization, and whole body function. In the current study, ewes fed SW and S15 had decreased ($P \leq 0.06$) proportions of jejunal mucosa tissue compared with ewes fed the S3 treatment (Table 4). Ewes provided SW had a greater ($P = 0.08$) concentration (mg/g) of DNA in the jejunal mucosa compared with S3 (Table 5). Mucosal DNA data are supported by the work of Soto-Navarro et al. (2004), who reported greater jejunal DNA concentrations (mg/g) in steers fed a high-Se wheat source. It appears that ruminants provided supranutritional levels of Se in the form of high-Se wheat grain demonstrate greater hyperplasia in the jejunal tissues. Ratio of RNA to DNA in maternal jejunal mucosa was decreased ($P = 0.02$) in SW compared with S3, indicating that SW-fed ewes had reduced cell synthetic capacity or possibly reduced size. Additionally, protein:DNA in maternal jejunal mucosa was decreased ($P = 0.04$) in S3-compared with S15-fed ewes.

Table 5. Effect of level and source of dietary Se on cellularity estimates in maternal jejunal tissues of pregnant ewe lambs

Item	Treatment ¹				SEM	Contrasts		
	Control	SW	S3	S15		Control vs. Se ²	SW vs. S3	S3 vs. S15
Jejunum								
DNA, mg/g	5.49	4.85	4.35	5.03	0.25	0.01	0.16	0.06
DNA, g	1.40	1.38	1.14	1.27	0.10	0.28	0.10	0.35
RNA, mg/g	4.12	4.40	4.64	4.04	0.46	0.66	0.37	0.72
RNA, g	1.00	1.26	1.22	1.05	0.16	0.36	0.85	0.43
RNA:DNA	0.75	0.90	1.07	0.82	0.089	0.10	0.18	0.06
Protein, mg/g	43.1	35.0	38.4	42.7	3.4	0.26	0.45	0.35
Protein, g	10.9	10.0	9.8	11.0	1.3	0.67	0.88	0.45
Protein:DNA	7.9	7.4	9.2	8.6	0.93	0.67	0.14	0.61
Jejunal mucosa								
DNA, mg/g	5.19	6.39	5.53	5.16	0.33	0.21	0.08	0.45
DNA, g	0.91	1.25	1.14	0.90	0.12	0.17	0.52	0.12
RNA, mg/g	4.43	4.41	4.64	3.84	0.27	0.67	0.56	0.05
RNA, g	0.76	0.88	0.96	0.68	0.10	0.51	0.59	0.05
RNA:DNA	0.86	0.69	0.86	0.76	0.048	0.13	0.02	0.15
Protein, mg/g	40	38	34	42	2.8	0.56	0.39	0.06
Protein, g	7.0	7.4	7.0	7.5	0.95	0.83	0.76	0.68
Protein:DNA	7.8	5.9	6.4	8.2	0.55	0.15	0.46	0.04

¹SW = Se wheat, 3 ppm Se; S3 = selenate, 3 ppm Se; S15 = selenate, 15 ppm Se.

²Control vs. average of Se treatments (SW, S3, and S15).

Maternal Jejunal Proliferation and Vascularity

Pregnant ewe lambs fed supranutritional levels of Se for the last two-thirds of gestation had a greater ($P = 0.05$; 15.3 vs. $10.4 \pm 2.1\%$) percentage of proliferating crypt cells in the jejunum at time of necropsy compared with controls (Table 6). Total cells ($P = 0.49$) and total proliferating cells ($P = 0.48$) in the jejunum were unaffected by treatment. However, in the jejunal mucosa, total cells present tended ($P = 0.11$) to be greater in Se-fed compared with control ewes. Total proliferating crypt cells within the jejunal mucosa were greater ($P = 0.02$) in Se-treated compared with control ewes (24.0 vs. 13.6 ± 3.8 cells $\times 10^9$). This large increase (76% greater in Se-treated compared with controls) in jejunal crypt cell proliferation resulting from supranutritional Se supplementation is supported by recent data in

steers (Soto-Navarro et al., 2004). These investigators reported that finishing steers fed a high-Se diet (2.81 ppm from high Se wheat) had an 84% increase in crypt cell proliferation in the jejunum compared with tissues from steers fed a control diet (0.39 ppm). These large increases in total proliferating jejunal mucosal cells are reflective of increased proliferating jejunal crypt cell nuclei at necropsy. These changes may have ramifications on nutrient absorption, tissue energy consumption, and gut health. In the current study, increases in total jejunal mucosal proliferating cells were observed independent of Se source (Se containing wheat or selenate) and level (3 and 15 ppm). It is unknown if lower levels of Se will affect crypt cell proliferation. Recently, Reed et al. (2007) reported that Se supplementation from Se-containing yeast failed to alter proliferating cells in the jejunum; however, there were many other

Table 6. Effect of level and source of dietary Se on cellular proliferation of maternal jejunal tissue of pregnant ewe lambs

Item	Treatment ¹				SEM	Contrasts		
	Control	SW	S3	S15		Control vs. Se ²	SW vs. S3	S3 vs. S15
Proliferating nuclei, %	10.4	12.6	17.4	15.8	2.1	0.05	0.11	0.58
Jejunum								
Total cells, $\times 10^9$	197	209	173	192	16	0.77	0.13	0.41
Total cell proliferating, $\times 10^9$	21.3	25.8	29.6	31.1	4.8	0.18	0.58	0.83
Jejunal mucosa								
Total cells, $\times 10^9$	136	189	154	140	17	0.11	0.15	0.57
Total cell proliferating, $\times 10^9$	13.6	23.0	26.9	22.1	3.8	0.02	0.46	0.36

¹SW = Se wheat, 3 ppm Se; S3 = selenate, 3 ppm Se; S15 = selenate, 15 ppm Se.

²Control vs. average of Se treatments (SW, S3, and S15).

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Table 7. Effect of level and source of dietary Se on vascularity of maternal jejunal tissue of pregnant ewe lambs

Item	Treatment ¹				SEM	Contrasts		
	Control	SW	S3	S15		Control vs. Se ²	SW vs. S3	S3 vs. S15
Capillary area density, ³ %	5.81	5.89	5.25	4.95	0.67	0.54	0.49	0.74
Capillary number density, ⁴ per mm ²	0.61	0.56	0.70	0.81	0.05	0.17	0.08	0.11
Capillary surface density, ⁵ μm	2,152	1,437	2,483	2,123	360	0.73	0.04	0.46
Area/capillary, ⁶ μm ²	134	123	81	67	24	0.11	0.22	0.67
Total jejunal vascularity, ⁷ mL	14.8	17.3	14.0	12.7	2.0	0.93	0.24	0.65
Jejunal mucosa vascularity, ⁸ mL	11.5	11.8	11.2	9.2	1.8	0.70	0.82	0.37

¹SW = Se wheat, 3 ppm Se; S3 = selenate, 3 ppm Se; S15 = selenate, 15 ppm Se.

²Control vs. average of Se treatments (SW, S3, and S15).

³Capillary area density = (capillary area/tissue area evaluated) × 100.

⁴Capillary number density = (capillary number/tissue area evaluated) × 1,000.

⁵The mean surface area of vessels present within the tissue area.

⁶Area/capillary = capillary area density/capillary number density.

⁷Total jejunal vascularity = capillary area density (%) × jejunal mass (g).

⁸Total mucosal vascularity = capillary area density (%) × mucosal mass (g).

differences, besides source of Se, among these studies, and it is currently impossible to define why the jejunal responses were observed in some but not all instances.

Capillary area density determines, to a large extent, capacity for localized tissue blood flow because flow is governed by cross-sectional area and velocity (Reynolds et al., 2005b). Capillary area density (%) was not different ($P \geq 0.54$) among treatments (Table 7), indicating that total capillary area and likely blood flow to the jejunum were not affected by dietary level or source of Se. Capillary number density was decreased ($P = 0.08$) in SW vs. S3. Capillary surface density (μm) and area per capillary (μm²) are parameters indicating nutrient exchange capacity and capillary size (Reynolds et al., 2005b). These respective measurements were largely unaffected by treatment, although ewes fed elevated

levels of Se tended ($P = 0.11$) to have less area per capillary compared with controls (Table 7; 90 vs. 134 ± 24 μm²). When total jejunal vascularity (mL) was calculated, there were no treatment differences in the jejunum or jejunal mucosa tissues. These data differ slightly from Soto-Navarro et al. (2004), who reported no differences in total jejunal vascularity (mL), although they did report a decreased percentage of vascularity in the jejunum of steers fed high dietary Se compared with controls. Jiang et al. (1999) reported a reduction in expression of angiogenic factors in mammary tumors in rats fed 3 ppm Se compared with controls. In their model, reductions in vascularity corresponded with decreases in mammary cellular proliferation. Conversely in the current study, Se from inorganic sources appears to increase capillary number, indicating a po-

Table 8. Effect of level and source of dietary Se on fetal weight and organ mass of pregnant ewe lambs

Item	Treatment ¹				SEM	Contrasts		
	Control	SW	S3	S15		Control vs. Se ²	SW vs. S3	S3 vs. S15
Fetal BW, kg	3.82	3.38	3.54	3.24	0.30	0.17	0.69	0.43
Carcass, ³ kg	2.85	2.59	2.57	2.39	0.26	0.23	0.95	0.60
GRT, ⁴ cm	34.7	32.7	32.8	32.4	1.1	0.08	0.89	0.74
CRL, ⁵ cm ²	53.9	51.4	51.2	50.4	1.8	0.13	0.91	0.74
Full viscera, ⁶ g	968	788	972	846	121	0.44	0.28	0.43
g/kg of ECW ⁷	367	308	438	372	79	0.94	0.24	0.52
Liver, g	118	112	118	113	12	0.74	0.70	0.76
g/kg of ECW	43.1	46.4	49.0	48.5	6.6	0.49	0.77	0.95
Kidney, g	23.2	22.1	21.2	19.4	1.5	0.16	0.67	0.36
g/kg of ECW	8.5	8.9	8.7	8.3	0.75	0.88	0.84	0.69

¹SW = Se wheat, 3 ppm Se; S3 = selenate, 3 ppm Se; S15 = selenate, 15 ppm Se.

²Control vs. average of Se treatments (SW, S3, and S15).

³Carcass = BW - visceral and thoracic tissues.

⁴GRT = heart girth = the total distance around the animal measured directly behind the front legs.

⁵CRL = crown-rump length = the distance from the crown of the head to the tail head, as measured along the backbone.

⁶Full viscera = (large intestine + small intestine + stomach complex + spleen + pancreas + liver + gallbladder) including digesta.

⁷ECW = empty carcass weight.

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Table 9. Effect of level and source of dietary Se on cellularity estimates in fetal small intestine, liver, and kidney tissues of pregnant ewe lambs

Item	Treatment ¹				SEM	Contrasts		
	Control	SW	S3	S15		Control vs. Se ²	SW vs. S3	S3 vs. S15
Small intestine								
DNA, g/g	2.50	2.49	2.10	2.44	0.36	0.65	0.39	0.47
RNA, mg/g	2.75	2.77	3.14	2.54	0.42	0.88	0.52	0.33
RNA:DNA	1.11	1.17	1.61	1.03	0.19	0.39	0.09	0.04
Protein, mg/g	37.2	35.5	37.8	31.0	4.2	0.55	0.67	0.24
Protein:DNA	17.6	15.0	19.8	12.7	2.9	0.52	0.21	0.08
Liver								
DNA, mg/g	4.07	4.71	4.33	5.12	0.49	0.21	0.58	0.24
DNA, g	0.48	0.53	0.52	0.59	0.083	0.49	0.91	0.52
RNA, mg/g	4.89	4.10	3.98	3.81	0.44	0.05	0.84	0.77
RNA, g	0.56	0.46	0.47	0.43	0.062	0.11	0.88	0.59
RNA:DNA	1.19	0.94	0.96	0.90	0.13	0.09	0.91	0.73
Protein, mg/g	51.8	54.9	55.8	64.1	4.3	0.17	0.87	0.15
Protein, g	6.1	6.0	6.3	7.4	0.78	0.60	0.76	0.32
Protein:DNA	12.4	12.6	13.6	13.9	1.3	0.50	0.57	0.87
Kidney								
DNA, mg/g	3.20	3.53	3.41	3.88	0.28	0.18	0.77	0.22
DNA, mg	75.8	77.5	71.7	73.9	7.9	0.87	0.61	0.83
RNA, mg/g	2.36	2.60	2.63	2.85	0.15	0.04	0.87	0.23
RNA, mg	55.6	57.5	55.8	55.0	5.3	0.94	0.82	0.92
RNA:DNA	0.76	0.78	0.81	0.77	0.075	0.75	0.81	0.68
Protein, mg/g	24.4	31.6	31.4	36.5	3.7	0.03	0.97	0.31
Protein, g	0.58	0.72	0.67	0.69	0.10	0.29	0.72	0.88
Protein:DNA	7.9	9.5	9.2	9.4	1.0	0.17	0.85	0.86

¹SW = Se wheat, 3 ppm Se; S3 = selenate, 3 ppm Se; S15 = selenate, 15 ppm Se.

²Control vs. average of Se treatments (SW, S3, and S15).

tential for increased expression of angiogenic factors in the small intestine. Recently, Neville et al. (2007) reported that angiogenic factors and their receptors are responsive to both high dietary Se and nutrient restriction. Additional work directed toward understanding relationships between vascular and mucosal cellular responses to nutritional changes should provide insight into efficiency of nutrient use.

Ewes provided 15 ppm of Se from selenate had increased hyperplasia in jejunal tissue compared with S3. The S15-treated ewes also had greater jejunal capillary number density compared with S3, which may explain the increased concentrations of DNA in the jejunal tissue of these ewes. Mechanistic reasons for these responses remain unclear but may be related to expression of angiogenic factors in respective tissues.

Fetal Organ Mass and Cellularity

Fetal BW, curved crown-rump length, and heart girth were unaffected ($P \geq 0.13$) by Se treatments (Table 8). Lambs from ewes fed Se treatments had decreased ($P = 0.08$) heart girth. Total fetal viscera, liver, and kidney masses were unaffected (g or g/kg of empty carcass weight; $P \geq 0.16$) by dietary Se treatment of the ewe.

In fetal small intestine (Table 9), S3 had greater RNA:DNA compared with both SW ($P = 0.09$) and S15 ($P = 0.04$) ewes. In addition, the S15 treatment resulted in lower ($P = 0.08$) protein:DNA compared with S3 ewes.

Maternal Se supplementation resulted in lower ($P \leq 0.09$) fetal liver RNA (mg/g) and RNA:DNA compared with controls. In fetal kidney, RNA and protein concentrations were increased ($P \leq 0.04$) by Se treatments relative to control (2.69 vs. 2.36 ± 0.15 and 33.2 vs. 24.4 ± 3.6 mg/g for RNA and protein, respectively). No other differences were noted in fetal variables measured. These data indicate that fetal tissue cellularity, particularly in intestinal, liver, and kidney tissue, is responsive to maternal Se supplementation and that level and not source may be an important determinant of this response in the intestine. These data also provide evidence of developmental programming of fetal internal organ cellularity in response to maternal Se supply. Ramifications of these responses on postnatal responses are currently unknown.

Summary

In summary, when cellular response variables such as the content and concentration of DNA, RNA, protein, crypt cell proliferation, and jejunal mucosal vascularity were measured in maternal small intestine, supranutritional-dietary Se influenced cell number and capillary networks within maternal jejunum. Observed increases in total proliferating jejunal mucosal cells resulting from Se supplementation in this study are supported by previously published data (Soto-Navarro et al., 2004). No indications of toxicity were observed

in any of the Se treatments. Evaluating angiogenic factors, rate of cell turnover, and metabolic hormones may better elucidate the involvement of Se in organ mass, morphology, and function. Maternal dietary supranutritional Se supplementation decreased fetal heart girth and resulted in altered cellularity estimates in fetal intestine, liver, and kidney, providing evidence for developmental programming of fetal responses resulting from maternal Se supply.

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