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Effects of different sources of selenium supplementation on antioxidant indices, biochemical parameters, thyroid hormones and Se status in transition cows

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ABSTRACT. The aim of this study was to determine the effects of supplementing close-up and fresh dairy cows' diets with sodium selenite or organic sources of selenium (Se) on the serum biochemical parameters and antioxidant indicators and Se status. Twenty-four multiparous Holstein dairy cows were balanced by body condition score (BCS), previous lactation milk yield and expected calving date and randomly assigned to 1 of 4 experimental treatments. Treatments were: control (basal diet without Se supplementation), sodium selenite supplementation (0.50 mg of Se kg^{-1} DM; Se-S), selenium yeast supplementation (0.50 mg of Se kg^{-1} DM; Se-Y) and selenomethionine supplementation (0.50 mg of Se kg^{-1} DM; Se-M). Serum Se concentrations were higher for cows in Se-Y (72.34 µg dL^{-1}) and Se-Met (72.34 µg dL^{-1}) than control (59.93µg/dl) and Se-S (64.79µg/dl). The Se supplementation and sources did not affect serum metabolites or indices of antioxidant pre and postpartum, except serum total protein and albumin concentrations. Cows in Se-M had greater serum total protein and albumin concentrations than those in control. The results of present study showed that serum Se concentrations increased in Se-Y and Se–M more effectively than Se-S, indicating that selenomethionine could replace Se-S as an effective organic Se source for transition dairy cows.

Keywords: Prepartum; Postpartum; selenomethionine; sodium selenite; selenium yeast.

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Introduction

During early lactation, a large increase in nutrient requirement occurs to support lactation resulting in negative nutrient balance. Dairy cows often experience a declined antioxidant capacity and therefore an increased oxidative stress associating with more production of reactive oxygen species (Gong & Xiao, 2016; Sordillo & Aitken, 2009). Over generation and repletion of reactive oxygen species (ROS) lead transition cows to metabolic disorders and infectious diseases (Gong & Xiao, 2016). Thus, promoting antioxidant status could reduce consequences of oxidative stress and improve health and productive performance during the transition period. The pivotal role of selenium (Se) as a trace element to maintain antioxidant status in humans and animals has been well known and it has been often supplemented in the diet of animals (Schwarz & Foltz, 1957). The diets of dairy cows could be supplemented with selenium in inorganic (sodium selenite and sodium selenite) or organic forms (Se yeast and Se methionine). Inorganic forms of Se are widely supplemented in diet due to their low prices. Whereas, compared to inorganic Se, organic Se is absorbed with a higher rate, and has a greater biological activity, a higher accumulation rate in tissue, and lower toxicity (Boldižárová, Grešáková, Faix, Mellen, & Leng, 2005; Briens, Mercier, Rouffineau, Mercerand, & Geraert, 2013; Ortman & Pehrson, 1999).

The metabolisms of organic and inorganic forms of Se are different in the body (Calamari, Petrera, & Bertin, 2010). Some studies reported supplementing of selenium yeast (SY) increased whole blood and milk Se concentrations (Slavik et al., 2008), improved antioxidant status (Gong, Ni, Wang, Shi, & Yan, 2014), and promoted the Se status than sodium selenite (Doucha, Lívanský, Kotrbáček, & Zachleder, 2009). Likewise, Calamari et al. (2010) reported a greater milk Se concentration in cows fed SY than sodium selenite. Sun et al. (2017) reported supplementing hydroxyselenomethionine in mid lactation diets for 10 weeks did not affect dry matter intake (DMI), milk yield and composition, or blood biochemical parameters compared to those supplemented with selenite selenium. However, they observed that cows fed hydroxyselenomethionine had

higher serum activity of glutathione peroxidase, total antioxidant capacity, superoxide dismutase and greater total Se in milk and plasma than the selenite selenium group.

We hypothesized that cows fed organic sources of selenium during pre and postpartum would have higher serum Se concentrations and consequently have improved antioxidant indices, health and production performance than cows fed inorganic source (selenite). Therefore, aim of this study was to investigate the effects of selenomethionine, selenium yeast and sodium selenium supplementation on DMI, milk production and composition, the selenium concentrations in serum and milk, serum biochemical parameters and antioxidant indicators over the transition period.

Material and methods

Feeding, experimental design and management of cows

The experiment was carried out on a commercial dairy herd in Iran (FKA Animal Husbandry and Agriculture Co., Isfahan, Iran) from May to July 2016. The animal care and used procedures were approved by the Standard Committee of Animal Protection Committee of the Iranian Institute for Animal Research (15-11-90; 10938-5-16-17 protocol). Twenty-four multiparous dairy Holstein cows were used in a completely randomized design with 4 dietary treatments from 21 d before expected calving date to 21 days in milk (DIM). At enrollment, cows averaged 820 ± 65.25 kg of body weight (BW), 3.79 ± 0.38 of BCS, and 14585.70 ± 1196.90 kg of 305-d mature equivalent milk. Animals were housed in individual 4 × 4 m2 stalls bedded with sand with free access to water from 20± 5 d before calving to 21 DIM. During the close-up and after calving period, cows received basal isoenergetic and isonitrogenic diets with 0.1 and 0.15 mg of Se kg^{-1} DM, respectively (Table 1) for ad libitum intake thrice daily at 0800, 1600, and 2400h. The diets offered to close-up and fresh cows (Table 1) were formulated according to the National Academies Press (NRC, 2001) model. Sodium selenite as inorganic source and Se-enriched yeast (Biorigin., Brazil – Selemax 2000 ppm) and Se-methionine (Arkop., Poland – Amino Selstar 2000 ppm) as inorganic source were supplemented into close-up and fresh diets to provide 0.50 mg of supplemental Se kg^{-1}, respectively. After calving, cows received the same sources and concentration of supplemental Se as those fed during the close-up period. Treatments were: control (basal diet without Se supplementation with 0.1 and 0.15 mg of Se kg^{-1} DM in close-up and fresh diets, respectively; CO), sodium selenite supplementation (0.5 mg of Se kg^{-1} of DM; Se-S), Se yeast supplementation (0.5 mg of Se kg^{-1} of DM; Se-Y) and selenomethionine supplementation (0.5 mg of Se kg^{-1} of DM; Se-M).

Sampling and data collection

Samples of total mixed ration (TMR) and orts were weekly taken for dry matter (DM) measurement, and were dried at 60°C for 48h, and then composited by treatment. Dried pooled samples of TMR diets and refusal were ground through a 1-mm screen in a Wiley Mill and analyzed for analytical DM (Association Official Analytical Chemist [AOAC], 2005), method 930.15, crud protein (CP) by the Kjeldahl method (AOAC, 2005), method 984.13), ether extract by the Soxhlet extraction method with diethyl ether (AOAC, 2005), method 920.39), Ash (ignition at 600°C for 2h (AOAC, 2005), method 942.05), and ADF by the cetyl-trimethyl-ammonium bromide H2SO4 (CTAB) and 1N method (AOAC, 2005), method 973.18). The NDF content was determined by heat-stable α-amylase and sodium sulfite (Van Soest, Robertson, & Lewis, 1991). The amounts of Se concentrations in diets and milk were measured by an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) device (using a Varian Model SpectrAA 220 atomic absorption spectrometer, Mulgrave, Victoria, Australia).

Blood samples were taken 4h after morning feeding from the coccygeal vein using an evacuated tube without anticoagulant (Vacumed ® no additive, FL medical, Italy) at -21, -10, 0, +3, +7, +14 and +21 d relative to calving. Serum samples were collected following centrifugation at 2,500 × g for 10 min, and were stored at −20°C for later analysis. Serum samples were analyzed for concentrations of glucose (glucose oxidase-phenol 4-aminoantipyrine peroxidase method), albumin (bromocresol green method at acidic pH), total protein (biuret method), blood urea nitrogen (BUN; berthelot method), cholesterol (cholesterol oxidase-phenol 4-aminoantipyrine peroxidase method), and triacylglycerol (TAG; glycerol-3-phosphate oxidase-phenol 4 aminoantipyrine peroxidase method), using commercial kits (Pars Azmoon Laboratory, Tehran, Iran). Globulin concentration was obtained as the difference between total protein and albumin. Serum BHB concentrations (Enzymatic method; based on 3-hydroxybutyrate dehydrogenase) were measured by Randox Kits (Randox Laboratories Ltd., Crumlin, County Antrim, UK), using a

serum spectrophotometer (UNICCO, 2100, Zistchemi Co., Tehran, Iran). Triiodothyronine (T3) and thyroxine (T4) were measured by ELISA commercial IMMULITE® 2000 kit (Immulite 2000 Total T3/L2KT36; Immulite 2000 Total T4/L2KT46; Immulite 2000 Third Generation TSH/L2KTS6; Siemens, Italy).

Glutathione peroxides activity (GPX) was determined by a kinetic method with a commercial kit (RANSEL by Randox laboratories Ltd., Antrim, United Kingdom). Serum total antioxidant capacity was determined by a commercial kit (ZellBio GmbH, Germany), based on ferric reducing antioxidant power assay (FRAP) method. Serum and milk Se concentrations were measured by an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) device (using a Varian Model SpectrAA 220 atomic absorption spectrometer, Mulgrave, Victoria, Australia).

Statistical analysis

The data were analyzed by PROC MIXED of Statistical Analysis System (SAS, 2004). Serum biochemical parameters and antioxidant indices data (pre and postpartum) were analyzed as repeated measures and Time (DIM and week) was included in the model as a repeated variable. Based on the lowest Akaike information criterion, corrected Akaike information criterion, and Bayesian information criterion values for each variable analyzed the most suitable covariance structure were used (Littell, Henry, & Ammerman, 1998). The following model was used: $Y_{ijk} = \mu + Ti + Timej + (T \times Time)ij + Cow(i)k + eijk$, where Yijk is the dependent variable, μ is the overall mean, Ti is the fixed effect of treatment, Timej is the fixed effect of sampling time, (T × Time)ij is fixed interaction between treatment and sampling time, Cow(i)k is random effect of cow nested within treatment, and eijk is the error term. Previous lactation yield and the concentrations of serum metabolites and antioxidant indices obtained at −21 d relative to expected calving date were used as covariates, which were excluded from the model if they were not significant ($p > 0.1$). Data are reported as LSM and statistical significances were indicated at $p \leqslant 0.05$ and $0.05 < p \leqslant 0.10$ as trends toward significance using the Tukey's multiple comparison test.

Results and discussion

Diet composition

Ingredients and nutrient chemical composition of diets are presented in Table 1. The close-up and fresh diets were formulated to meet NRC (2001) requirements for 1.60 and 1.70 Mcal NEL, 13.70 and 16.10% CP, and 34.60 to 32.20% NDF per kg DM, respectively. To provide 0.30 and 0.35 mg of Se $kg^{-}1$ DM in excess NRC (2001) recommendation, close-up and fresh diets were supplemented by different sources of Se.

Prepartum period

Serum biochemical and antioxidant Indicators

The effects of dietary supplementation of different Se sources on serum biochemical and antioxidant indicators prepartum are presented in Table 2. Serum biochemical and antioxidant indicators prepartum were not affected by dietary supplementing different source of Se during late gestation. Likewise, serum Se concentrations were not different across treatments at -21 relative to calving (63.49, 64.24, 64.47 and 63.23 μg L^{-1} for control, Se-S, Se-Y, and Se-M, respectively) that showed a marginally deficient of Se (51-80 μg L^{-1}) for dairy cows at the beginning of the experiment according to Dargatz and Ross (1996). The experimental treatments did not affect the concentrations of serum Se prepartum which were lower than reported by Weiss and Hogan (2005). In the present study, the lack of response in Se status with either Se sources or Se supplementation may be a result of exceed S (from sulfate) as an antagonist of Se with prepartum diets (0.35 S of %DM). However, there was a tendency for interaction of treatment by week ($p = 0.07$) on serum Se concentrations during the prepartum period (Figure 1), so cows allocated to Se-M group had higher serum Se than control cows at -7 d ($p = 0.01$), and Se-Y cows at -14 d ($p = 0.05$) relative to calving.

Postpartum period

Serum Se concentrations postpartum

The effects of dietary Se supplementation with inorganic and organic forms on total Se concentrations in serum are presented in Table 3. The concentrations of total Se in serum postpartum were affected by supplementation of Se and sources of dietary Se ($p = 0.01$). Cows in Se-Y (68.16 μg L^{-1}) and Se-Met (72.34 μg L^{-1}) had higher serum Se concentrations compared to control (59.93 μg L^{-1}), but this response was not observed for selenite (64.79 μg L^{-1}) compared to control.

Table 1. Feed ingredients and chemical composition of the diets fed during close-up, and fresh periods (% of DM).

	Basal diets	
Ingredient	Close-up	Fresh
Legume forage hay, mature	12.4	17.04
Corn silage, normal	47.5	21.31
Sugar beet pulp	0	9.47
Barley grain, ground, dry	10.08	11.89
Corn grain, ground, dry	11.49	14.5
Soybean meal, solvent	1.94	8.14
Canola meal, mechanical extraction	5.42	0
Extruded full-fat soybean	1.16	2.58
Fish meal	1.55	1.72
Cottonseed, whole with lint	2.39	8.81
Corn gluten meal	1.55	1.58
Mineral premix[1]	0.38	0.25
Vitamin premix[2]	0.38	0.23
Magnesium oxide	0.29	0.23
Calcium carbonate	1.36	0.19
Dicalcium phosphate	0	0.33
potassium carbonate	0	0.19
$NaHCO_3$	0	0.57
Calcium chloride	0.58	0
Magnesium oxide	0.69	0
Biotin	0.004	0.004
Choline chloride	0.37	0.24
Niacin	0.04	0.05
Avila 4	0.06	0.03
Monensin	0.01	0.01
Live yeast	0.004	0.003
Bentonite	0.352	0.393
Salt	0	0.24
Chemical composition		
Dry Matter (%)	41	59
NE_L (Mcal kg^{-1} DM) [3]	1.6	1.7
Protein (%)	13.7	16.1
NDF (%)[4]	34.6	32.2
Ca (%)	1.31	0.94
P (%)	0.39	0.43
DCAD (meq kg^{-1}) [5]	-57	288
Se (ppm)[6]	0.1	0.15

[1]Premix contained 50 g of Ca kg^{-1}, 11 g of Mg kg^{-1}, 15 g of Zn kg^{-1}, 3 g of Cu kg^{-1}, 0.15 g of I kg^{-1}, 0.05 g of Co kg^{-1}. [2]Premix contained 1800000 IU of vitamin A kg^{-1}, 200000 IU of vitamin D kg^{-1}, 15000 IU of vitamin E kg^{-1} and 1.25 g of butylated hydroxytoluene kg^{-1}as a synthetic antioxidant. [3]Estimated from NRC (2001). [4]neutral detergent fiber. [5]dietary cation-anion differences. [6]Selenium were measured by an Inductively Coupled Plasma-Mass Spectrometry.

Table 2. The effects of supplementing inorganic and organic selenium sources on serum antioxidant indexes, thyroid hormones and biochemical indices prepartum.

Items	Experimental treatments[1]				SEM	P- values		
	CO	Se-S	Se-Y	Se-M		Treat	Time	Time×Treat
Glucose (mg dL^{-1})	62.32	57.94	62.89	63.09	3.05	0.35	0.01	0.20
BUN (mg dL^{-1})[2]	10.28	10.88	11.2	10.87	1.22	0.89	0.44	0.96
Total protein (g dL^{-1})	7.25	7.4	7.35	7.17	0.16	0.53	0.17	0.86
Albumin (g dL^{-1})	3.15	3.2	3.18	3.23	0.069	0.75	0.94	0.59
Globulin (g dL^{-1})	4.1	4.22	4.18	4.09	0.16	0.82	0.01	0.99
Triglyceride (mg dL^{-1})	28.79	29.97	31.02	27.95	2.22	0.54	0.03	0.18
Cholesterol (mg dL^{-1})	96.19	94.45	96.23	94.86	5.1	0.98	0.42	0.34
T_3 (µg dL^{-1})[3]	113.05	120.14	109.76	106.14	7.8	0.29	0.02	0.38
T_4 (µg dL^{-1})[4]	4.42	4.72	4.92	4.14	0.39	0.22	0.14	0.37
TAC (mmol dL^{-1})[5]	0.118	0.116	0.116	0.119	0.004	0.91	0.24	0.47
GPX (U mL^{-1})[6]	128.83	141.18	172.3	165.1	19.23	0.16	0.38	0.12
Se (µg dL^{-1})	62.40	64.17	66.48	68.41	2.13	0.18	0.53	0.07

[1]Treatments were: control (basal diet without Se supplementation with 0.1 and 0.15 mg of Se kg^{-1} DM in close- up and fresh diets, respectively), sodium selenite supplementation (0.5 mg of Se kg^{-1} of DM; Se-S), selenium yeast supplementation (.0.5 mg of Se kg^{-1} of DM; Se-Y) and selenomethionine supplementation (0.5 mg of Se kg^{-1} of DM; Se-M). [2]Blood urea nitrogen. [3]Triiodothyronine. [4] thyroxine. [5]Total antioxidant capacity. [6]Glutathione peroxides activity.

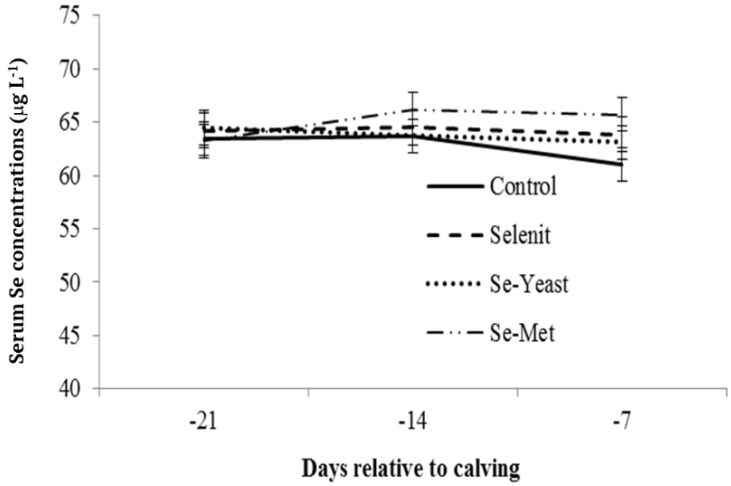

Figure 1. The effect of supplementing inorganic and organic selenium sources on serum Se prepartum. Treatment, p = 0.18; Time, p = 0.53; Treatment × Time, p = 0.07.

The concentrations of Se in whole blood or serum and plasma are an important indicator of animal health (Pavlata, Pechova, & Illek, 2000). Gong and Xiao (2016) suggested that cows with a range from 70 to 79 µg L^{-1} of Se in plasma had an adequate Se status. However, serum Se concentrations across experimental treatments were lower than the normal range (Gong & Xiao, 2016), cows in control had lowest serum Se suggesting control diet could not meet the required minimum level for Se. The results of the current study were supported by the fact that organic forms of Se are more effective than inorganic Se at increasing serum Se concentrations (Givens, Allison, Cottrill, & Blake, 2004; Juniper, Phipps, Jones, & Bertin, 2006; Sun et al., 2017).

Serum antioxidant indices

Total antioxidant capacity (TAC; p = 0.70) and glutathione peroxidase (GPX, p = 0.30) in serum were not affected by supplementation and sources of Se (Table 3). Total antioxidant capacity is known as a biomarker of the body ability for resistance to oxidative stress (Cao, Guo, Zhang, Dong, & Gong, 2014). In opposite our results, Gong et al. (2014) and Sun et al. (2017) reported supplementing Se in organic forms (Se yeast and hydroxy-selenomethionine) increased TAC in mid lactation cows compared to cows fed selenite. The concentrations of Se in serum or plasma are generally a direct index of selenium status, while determining GPX activity, a Se-containing enzyme, and an antioxidant, in blood and tissues is an indirect index of it (Gong & Xiao, 2016). The concentrations of GPX in whole blood and erythrocyte reflect the long-period Se status, whereas plasma or serum GPX concentrations indicate the short-period Se status (Rowntree et al., 2004). Although GPX activity in serum was not affected by Se sources, cows in Se-Met and Se-yeast showed a numerical increase in serum GPX activity compared to selenite and control cows (251.62 and 188.65 vs. 174.64 and 166.83 U mL^{-1}). In parallel to our results, Rutigliano et al. (2008) observed no effects of Se supplementation as selenite and Se-yeast from 25 d before expected calving until 80 d postpartum on plasma GPX activity. Some authors (Juniper et al., 2006; Phipps et al., 2008; Sun et al., 2017; Weiss & Hogan, 2005) reported Se sources (Se-yeast and hydroxyselenomethionine) effectively elevated the whole blood GPX activity than selenite, but others observed no difference among Se sources (Calamari et al., 2010; Knowles, Grace, Wurms, & Lee, 1999; Rutigliano et al., 2008).

Thyroid hormones and Biochemical indices

Thyroid hormones and biochemical indices postpartum were not affected by Se sources and supplementation of Se (Table 3). Only serum total protein and albumin concentrations were affected by treatments, as cows in Se-Met had greater serum albumin than control, but this response was not observed for cows in Se-Y and selenite (Table 3). In the current study, although, Se supplementation using different sources did not affect antioxidant indices, increased concentrations of serum albumin in Se-M cows relative to control cows as a negative acute phase protein implying cows in Se-M experienced lower inflammatory conditions (Bertoni, Trevisi, Han, & Bionaz, 2008). Therefore, it is likely that supplementing organic form of Se might have improved immune status by mitigating inflammatory conditions during the transition period. In agreement with the present study results, some previous studies reported that Se supplementation using organic sources slightly affected blood chemistry (Juniper et al., 2006; Phipps et al., 2008; Sun et al., 2017).

Table 3. The effect of supplementing inorganic and organic selenium sources on serum antioxidant indexes, thyroid hormones, biochemical indices and serum Se postpartum.

	Experimental Treatments[1]					P-Value		
Variables	CO	Se-S	Se-Y	Se-M	SEM	Treat	Time	Time ×Treat
Glucose (mg dL^{-1})	59.7	68	60.16	67.37	4.43	0.16	0.11	0.72
BUN (mg dL^{-1})	10.06	11.86	12.27	12.79	1.56	0.35	0.11	0.9
Total protein (g dL^{-1})	6.33^{b}	7.18^{ab}	6.92^{ab}	7.36^{a}	0.35	0.03	0.48	0.28
Albumin (g dL^{-1})	2.87^{b}	3.07^{ab}	3.25^{ab}	3.35^{a}	0.16	0.05	0.63	0.28
Globulin (g dL^{-1})	3.73	4.01	3.65	4.18	0.32	0.32	0.24	0.26
Triglyceride (mg dL^{-1})	30.36	22.96	24.93	19.98	5.8	0.36	0.9	0.3
Cholesterol (mg dL^{-1})	82.66	88.25	101.58	94.25	12.73	0.5	0.12	0.43
T_3 (µg dL^{-1})	108.32	97.65	125.51	92.09	13.58	0.13	0.12	0.42
T_4 (µg dL^{-1})	3.16	2.51	3.4	3.19	0.68	0.59	0.3	0.39
TAC (mmol dL^{-1})	0.114	0.111	0.115	0.122	0.011	0.76	0.09	0.75
GPX (U mL^{-1})	166.83	174.64	188.65	251.62	32.3	0.30	0.13	0.24
Se (µg dL^{-1})	59.93^{b}	64.79^{ab}	68.16^{a}	72.34^{a}	2.17	0.01	0.31	0.23

a-b Means in the same column with no common superscripts are significantly different ($p < 0.05$). [1]Treatments were: control (basal diet without Se supplementation with 0.1 and 0.15 mg of Se kg^{-1} DM in close- up and fresh diets, respectively), sodium selenite supplementation (0.5 mg of Se kg^{-1} of DM; Se-S), selenium yeast supplementation (.0.5 mg of Se kg^{-1} of DM; Se-Y) and selenomethionine supplementation (0.5 mg of Se kg^{-1} of DM; Se-M). [2]Blood urea nitrogen. [3]Triiodothyronine. [4]thyroxine. [5]Total antioxidant capacity. [6]Glutathione peroxides activity.

Conclusion

In general, Se supplementation and Se sources did not affect antioxidant indices over the transition period. Increased Serum Se concentration and albumin in selenomethionine (Se-M) and selenium yeast (Se-Y) cows could suggest an improvement in immune status. Due to the potential to improve Serum Se concentration by organic sources of Se, the effects of different sources of Se in transition cows on mammary and uterus health after calving, and metabolic disorders warrant further evaluation.

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