Selenium-fertilized forage as a way to supplement lactating dairy cows

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ABSTRACT

Fertilization with Se improves forage organic Se concentration, but comparisons with other forms of Se supplementation in feeding lactating dairy cows are scarce. Our objective was to compare the effect of Se-enriched forages to dietary sources of inorganic and organic Se. Digestibility, retention, and balance were assessed by measuring Se concentrations in feces, urine, milk, and blood. The resulting effect on antioxidant status and lactation performance of dairy cows was also determined. High-Se silages [1.72 mg of Se/kg of dry matter (DM)] were produced following a spring application of 2.5 kg/ha of Selcote Ultra, whereas low-Se silages (0.05 mg of Se/kg of DM) were produced in the Se-unfertilized portion of the same fields. After a 77 ± 17 d period of Se depletion, 33 late-lactation primiparous Holstein cows were blocked and randomly assigned for 43 d to 1 of 4 experimental total mixed rations fed for ad libitum intake in an unbalanced randomized block design. Treatments consisted of 4 diets: control with low-Se silages, without Se supplement (0.12 ± 0.04 mg of Se/kg of DM); ISe with low-Se silages and inorganic Se (0.80 ± 0.14 mg of Se/kg of DM); YSe with low-Se silages and organic Se from yeast (0.70 ± 0.11 mg of Se/kg of DM); and FSe with high-Se silages, without Se supplement (0.79 ± 0.14 mg of Se/kg of DM). Organic Se, either as YSe or FSe, was more available and more effective to increase blood and milk Se concentrations than ISe. Moreover, FSe was more available than YSe, as cows fed FSe excreted 16 and 22% less Se (as percentage of intake) in feces and urine, respectively, had higher Se apparent absorption (17%), retention (37%), and balance (45%), and had greater concentration of Se in serum (16%) and milk (11%) than cows fed YSe. Antioxidant status (whole blood and plasma glutathione peroxidase, and milk thioredoxin reductase and malondialdehyde) was not affected by treatments. Dry matter intake, yield of actual, energy-corrected, and fat-corrected milk, as well as milk fat and lactose concentrations, were not affected by the

dietary treatments. Cows fed ISe had lower milk protein concentration (3.44%) than cows fed YSe (3.58%) or FSe (3.51%). Cows fed Se-supplemented diets had a lower milk somatic cell count than cows fed the control diet. Results from the current study showed that the production of Se-enriched forages is an effective method to supplement dairy cows in Se as it was more available than YSe, and did not alter antioxidant status and performances of lactating dairy cows.

Key words: dairy cow, forage, selenium

INTRODUCTION

Selenium is an essential micronutrient for both livestock and humans (Schwarz and Foltz, 1957). It is an integral component of several functional selenoenzymes, including glutathione peroxidase (**GSH-Px**), which plays a key role in cellular antioxidant systems (Rotruck et al., 1973). Selenium also regulates redox (McKenzie et al., 2002) and immune functions (Salman et al., 2009), and supports thyroid hormone metabolism (Spears, 2000; Ibeagha et al., 2007). In dairy cows, an adequate level of Se intake is known to minimize the incidence of mastitis (Smith et al., 1984), improve immune functions (Gerloff, 1992; Weiss, 2003; Ibeagha et al., 2009), reduce the prevalence of retained fetal membranes (Allison and Laven, 2000; Spears and Weiss, 2008), and prevent calf mortalities (Maas, 1983).

In numerous areas of the world, feeds have low Se concentrations because of low soil Se contents (Filley et al., 2007). Therefore, it is a common practice in the dairy industry to supplement dairy cows to provide adequate levels of Se and avoid deficiencies. Additionally, supplementation of dairy cows enhances milk Se concentration, which could be a way of increasing human intake for this mineral and subsequently benefit human health (Combs et al., 2001; Givens et al., 2004).

Two forms of supplemental Se are commonly used: inorganic Se such as sodium selenite or selenate, and organic forms such as selenized yeast, in which selenomethionine (**SeMet**) is the predominant form. Regardless of the forms, Se can be added in concentrations up to 0.3 mg/kg of DM in dairy cow rations in Canada (Canadian Food Inspection Agency, 2015) and the

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United States (Food and Drug Administration, 2003). In recent years, the utilization of organic Se supplements has gained considerable interest because several studies have demonstrated a greater bioavailability to dairy cows than in inorganic forms, making it interesting considering the functional effect of this trace element on physiological mechanisms (Juniper et al., 2006; Calamari et al., 2010; Cun et al., 2015). In his review, Weiss (2005) showed that cows fed organic Se have greater concentrations of this mineral in whole blood (+20%) and milk (+90%), and a greater whole blood GSH-Px activity (+16%) than cows fed inorganic supplement.

The enrichment of forages is an alternative way to supply Se to dairy cows. Enriched forages can be harvested after a spring application of an inorganic slow-release Se fertilizer (Tremblay et al., 2015). When inorganic Se is applied to field crops, plants have the capacity to incorporate this mineral to organic compounds (Filley et al., 2007). As a result, Se in fertilized forages is more bioavailable to cows than the inorganic supplement commonly used (sodium selenite), as recently demonstrated by Cun et al. (2015). Forage Se-enrichment by fertilization has been studied in several countries for many years (Gupta and MacLeod, 1994; Filley et al., 2007; Tremblay et al., 2015). To our knowledge, however, the bioavailability of Se-enriched forages, and their effect on the performance and the antioxidant status of lactating cows have not been compared with other forms of organic Se. We hypothesized that (1) supplementing dairy cows with Se increases Se in blood and milk, (2) organic sources of Se are more digestible and result in a better Se balance than inorganic sources, and (3) Se-enriched forages are as digestible as other organic sources of Se in dairy cows. The objective of this study was to compare the effect of Se-enriched forages to inorganic and organic dietary supplementation on the Se digestibility, retention, and balance, and the resulting effect on antioxidant status and performances of lactating dairy cows.

MATERIALS AND METHODS

Silage Production

Five silages were produced at the Centre de Recherche en Sciences Animales de Deschambault, QC, Canada (46°40'N, 71°55'W). Two low-Se, and 2 high-Se silages were produced in June of 2011 on a 9.8-ha field of pure timothy (*Phleum pratense* L.) and an 8.9-ha field of mixed alfalfa (*Medicago sativa* L.):timothy (75:25). A low-Se silage was also produced in June of 2012 on the mixed alfalfa/timothy field (Table 1). This forage was used solely in blocks 3 and 4, because

not enough good quality silage of the mixed alfalfa/timothy was produced in 2011. Diets were adjusted to account for this modification (Table 2). Before the start of spring growth in early May, both fields received 75.6 kg of N/ha, 9.4 kg of P/ha, and 68.7 kg of K/ha. The Se-enriched forages were produced by fertilizing 25% of each field with a slow-release Se fertilizer (Selcote Ultra, Terralink, Vancouver, BC, Canada) containing 10 g of Se/kg as Na_2SeO_4 and BaSeO_4 (90% minimum), and Na_2SeO_3 and BaSeO_3 (10% maximum). The slow-release Se fertilizer was mixed with the N fertilizer (ammonium nitrate), and applied at a rate of 25 g of Se/ha in early May. The remaining part of each field was used to produce the low-Se silages. Timothy and alfalfa from the spring growth were respectively at the early-heading and early-bloom stages of development when they were cut using a disc mower equipped with a roller-type conditioner (New Holland Discbine, model 1432, New Holland, PA). The forages were wilted in the field until they reached 35% DM. They were then chopped and stored in plastic bag silos (Bag All, Klerk's Plastic Products Manufacturing Inc., Richburg, SC) with a silage compactor (Roto Press, Sioux Automation, Sioux City, IA).

Cows, Diet, and Experimental Design

The experiment was conducted at the Centre de Recherche en Sciences Animales de Deschambault (Deschambault, QC, Canada) throughout the year of 2012. All procedures involving dairy cows followed the regulations of the Canadian Council on Animal Care (1993), and were approved by the Université Laval Animal Care Committee. Thirty-three primiparous Holstein cows (average $\pm$ SD; 187 ± 54 DIM; 585 ± 45 kg of BW; 27.2 ± 4.2 kg of milk/d) housed in a tie stall barn were used in an unbalanced randomized block design (3 blocks of 8 cows and 1 block of 9 cows). Each block of cows entered the experiment when enough animals with similar DIM were available. Before the start of each experimental block, cows underwent a period of 77 ± 17 d of Se depletion when each animal received the same basal diet not supplemented with Se. Our target for the depletion period was a Se level in serum considered in the zone of marginal deficiencies (40–70 $\mu\text{g/L}$; Gerloff, 1992). Blood samples were taken on d 60 of the depletion period, and cows were kept on the depletion diet until blood serum analyses confirmed that the target value had been reached. This target value was reached for all blocks except one, for which the group of cows started the experiment at an average of 86.3 ± 8.5 $\mu\text{g/L}$ after extending the length of the depletion period to d 102. Cows were then assigned to 1 of the 4 experimental treatments for 43 d. Within each

Table 1. Chemical composition of silages (g/kg of DM, unless otherwise noted) fed to the 4 blocks of cows

Chemical component	Low-Se silages ¹			High-Se silages ¹	
	Timothy ²	Alfalfa/timothy ³	Alfalfa/timothy ⁴	Timothy ²	Alfalfa/timothy ²
DM	345 (45)	295 (7)	380 (28)	338 (29)	303 (17)
OM	902 (2)	899 (4)	901 (1)	904 (9)	892 (2)
ADF	351 (2)	318 (14)	359 (2)	369 (9)	325.3 (15)
NDF	496 (9)	458 (47)	470 (1)	544 (21)	478 (18)
CP	190 (5)	222 (11)	154 (1)	165 (15)	215 (17)
Se, mg/kg of DM	0.06 (0.03)	0.05 (0.01)	0.03 (0.02)	1.49 (0.49)	1.97 (0.27)

¹Mean (SD).²Silages used to feed the cows of blocks 1 to 4.³Silages used to feed the cows of blocks 1 and 2.⁴Silages used to feed the cows of blocks 3 and 4.

block, cows were fed a TMR containing low- or high-Se alfalfa/timothy silage, low- or high-Se timothy silage, corn silage, ground corn, corn gluten meal, canola meal, and a mixture of minerals and vitamins (Table 2). The 4 experimental treatments were (1) a control diet based on low-Se forages; (2) a diet based on low-Se forages supplemented with inorganic Se (**ISe**; sodium selenite, Nutreco, Guelph, ON, Canada); (3) a diet based on low-Se forages supplemented with yeast Se (**YSe**; Sel-Plex, Alltech, Nicholasville, KY); and (4) a diet based on high-Se forages (**FSe**). Different silages were used for blocks 1 and 2, and for blocks 3 and 4 (Table 1). Experimental diets were formulated accordingly (Table 2) to meet the nutrient requirements of dairy cows

based on NRC (2001) recommendations (Table 3). The control diet was formulated to provide 0.1 mg of Se/kg, whereas the Se-supplemented diets (ISe, FSe, and YSe) were formulated to provide 0.6 mg of Se/kg. Small quantities of Se-enriched timothy silage were added to the control, ISe, and YSe diets fed to cows of blocks 1 and 2 to reach targeted Se concentrations. Sodium selenite and Sel-Plex were premixed with the canola meal to ensure homogeneity during diet mixing. Throughout the experiment, TMR were offered to the cows once daily and pushed up in front of the cows several times per day. The amount of feed was adjusted to ensure 10% refusals. Free access to water was provided at all times.

Table 2. Ingredients of experimental diets (g/kg of DM, unless otherwise noted) with low-Se silages without supplement (control), with low-Se silages and inorganic Se (ISe), with low-Se silages and yeast Se (YSe), or with high-Se silages without supplement (FSe) fed to the 4 blocks of cows

Ingredient	Blocks 1 and 2				Blocks 3 and 4			
	Se-supplemented				Se-supplemented			
	Organic Se				Organic Se			
	Control (n = 4)	ISe (n = 5)	YSe (n = 4)	FSe (n = 4)	Control (n = 4)	ISe (n = 4)	YSe (n = 4)	FSe (n = 4)
Low-Se alfalfa/timothy silage	209	209	209	0	218	218	218	0
High-Se alfalfa/timothy silage	0	0	0	250	0	0	0	252
Low-Se timothy silage	124	124	124	0	126	126	126	0
High-Se timothy silage	18	18	18	104	0	0	0	123
Corn silage	340	340	340	340	338	338	338	338
Ground corn	211	211	211	211	211	211	211	210
Corn gluten meal	30	30	30	30	43	43	43	30
Canola meal	45	0	0	45	45	0	0	45
Canola meal + inorganic Se ¹	0	45	0	0	0	45	0	0
Canola meal + yeast Se ²	0	0	45	0	0	0	45	0
Mineral and vitamin mix ^{3,4}	23	23	23	23	24	24	24	24

¹Inorganic Se: sodium selenite, Nutreco, Guelph, ON, Canada.²Sel-Plex, Alltech, Nicholasville, KY.³No added Se.⁴Contained: Ca = 167 g/kg; P = 274 g/kg; Mg = 393 g/kg; Zn = 2.2 g/kg; Cu = 0.5 g/kg; Mn = 1.7 g/kg; vitamin A = 195.57 kIU/kg; vitamin D = 49.20 kIU/kg; vitamin E = 1,378.76 IU/kg.

Measurements and Sampling

Silage, TMR, Orts, and BW. Feed intake was recorded on the last 3 d of the Se-depletion period (d -3 to -1), and on d 41 to 43 of the experimental period by weighing feeds offered to and refused by the cows. Silages, offered TMR, and Orts were sampled on d -3, -2, -1, 41, 42, and 43, whereas Orts were sampled the following days. Dry matter concentration of these samples was determined by oven drying at 55°C for 72 h. Dried samples were kept at -20°C until chemical analyses. Cow BW was recorded after the morning milking on d -3, -2, -1, 40, and 43.

Feces. Total excreted feces were collected and weighed twice daily (10 h postfeeding and at feeding the next morning) on d 41, 42, and 43 of the experimental period. Samples representing 1% of the total amount excreted per collection time were immediately frozen at -20°C for further analysis.

Urine. Catheters (24 mm, 75-mL balloon, Bardex Lubricath, C. R. Bard Inc., Covington, GA) were installed into the bladder of each cow on d 40, and were connected to a container using polyvinyl chloride tubing at the time of feeding on the next day. Containers were kept on ice throughout the sampling period. Urine was collected and weighed twice daily (10 h after feeding and at feeding the next morning) on d 41, 42, and 43 of the experimental period. Volume mass was determined by weighing 1 L of urine. Two 50-mL samples per cow per sampling were immediately frozen at -20°C until further analysis.

Milk. Throughout the experiment, cows were milked twice daily at 0700 and 1700 h. Milk yield was recorded, and milk was sampled on d -3, -2, -1, 41, 42, and 43 using calibrated milk meters (Flomaster Pro, DeLaval, Tumba, Sweden). Samples from the p.m. milking were stored at 4°C until they were mixed with samples from the next a.m. milking, proportionally to milk yield. A first aliquot was stored at 4°C, with bronopol as a preservative, for further analysis of milk components. Three other aliquots were stored at -20°C without preservative for further analysis. Energy-corrected milk was calculated according to the equation reported by Madsen et al. (2008): $ECM = [(38.9 \times \text{kg of fat/d}) + (23.8 \times \text{kg of protein/d}) + (16.3 \times \text{kg of lactose/d})]/3.14$. Fat-corrected milk was calculated by the following equation: $FCM = (0.4 \times \text{kg of milk/d}) + (15 \times \text{kg of milk fat/d})$ (Gaines, 1928).

Blood Samples. Blood samples were taken from the tail vein of all cows 3 h after feeding on d -1, 21, and 43. Blood samples were collected into nonheparinized and heparinized vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). A first series of nonheparinized tube was used to collect whole blood, of which different aliquots were stored at -20 and -90°C. The heparinized tubes were centrifuged at $2,000 \times g$ for 30 min at 4°C to collect plasma, of which different aliquots were also stored at -20 and -90°C. A second series of nonheparinized tubes were allowed to clot at room temperature for 30 min and then centrifuged as described above to harvest serum, which was stored at -20°C until chemical analyses.

Table 3. Chemical composition of experimental diets (g/kg of DM unless otherwise noted) with low-Se silages without supplement (control), with low-Se silages and inorganic Se (ISe), with low-Se silages and yeast Se (YSe), or with high-Se silages without supplement (FSe) fed to the 4 blocks of cows^{1,2}

Chemical component	Blocks 1 and 2					Blocks 3 and 4				
	Se-supplemented					Se-supplemented				
	Control	ISe	Organic Se		SE	Control	ISe	Organic Se		SE
			YSe	FSe				YSe	FSe	
DM	390	388	391	400	4.0	363	357	347	328	14.1
NE _L , Mcal/kg of DM ³	1.61	1.61	1.61	1.64	—	1.60	1.60	1.60	1.63	—
OM	920	923	921	922	2.6	919	922	914	920	3.6
CP	155	151	154	150	4.8	145	145	145	150	4.0
ADF	237	229	237	242	17.0	26.26	270	262	253	5.7
NDF	355	346	358	359	9.5	377	368	373	365	1.9
Ca	8.4	8.0	8.1	8.1	2.3	8.5	8.4	8.4	7.8	1.9
Mg	3.0	2.7	2.9	2.9	1.3	2.6	2.6	2.7	2.4	1.7
K	21.4	19.7	21.3	21.3	8.7	18.7	19.2	19.4	22.7	4.4
Cu, mg/kg of DM	20.50	21.31	22.34	19.47	1.35	18.85	19.89	20.60	19.11	0.56
Zn, mg/kg of DM	94.09	107.36	100.17	98.47	5.65	77.53	87.37	90.72	79.86	0.33
Mn, mg/kg of DM	89.39	84.28	81.04	80.86	11.8	58.73	64.57	70.10	60.74	6.80
Se, mg/kg of DM	0.15	0.84	0.71	0.89	0.05	0.10	0.69	0.77	0.70	0.11

¹Inorganic Se: sodium selenite, Nutreco, Guelph, ON, Canada.

²Yeast Se: Sel-Plex, Alltech, Nicholasville, KY.

³Calculated based on tabular values from NRC (2001).

Chemical Analyses

Silages, TMR, and Orts. Dried silages, TMR, and Orts were first ground to pass through a 2-mm screen using a Wiley mill (model 4, Arthur M. Thomas Co., Philadelphia, PA), and ground again to pass a 1-mm screen using a Cyclotec 1093 Sample Mill (Tecator Inc., Höganäs, Sweden). Samples from d 41, 42, and 43 of TMR were analyzed per treatment per day and Orts were analyzed per cow per day. Concentrations of analytical DM and ash in dried and ground subsamples of silages, TMR, and Orts were determined using a thermogravimetric analyzer (Leco model TGA 701, Leco Corp., St. Joseph, MI). Fiber extractions were done using the Ankom filter bag technique (Ankom200 Fiber Analyzer, Fairport, NY). The ADF was determined according to Association of Official Analytical Chemists (1990; method 973.18). The NDF was analyzed following Mertens (2002) with addition of sodium sulfite and heat-stable α -amylase. Concentrations of Se, Ca, Cu, K, Mn, Mg, and Zn were determined following a $\text{HNO}_3\text{-H}_2\text{O}_2$ microwave (Multiwave 3000, Anton Paar GmbH, Graz, Austria) assisted digestion adapted from Hernandez et al. (2007). Before analysis, reduction of Se^{VI} to Se^{IV} was performed by adding HCl into samples and heating on digestive block (DigiPREP MS, SCP Science). Selenium concentration was measured by hydride-generation inductively coupled plasma optical emission spectrometry (Optima4300DV, Perkin Elmer, Norwalk, CT) according to Bosnak and Davidowski (2004). Daily intake of Se was calculated by subtracting the amount in Orts from the amount in offered TMR. Concentrations of Ca, Mg, K, Cu, Zn, and Mn were measured by inductively coupled plasma optical emission spectrometry (ICP-OES). Subsamples were mineralized using a mixture of sulfuric and selenious acids as described by Isaac and Johnson (1976). This extract was used to determine total N (method 13–107–6–2–E; Lachat Instruments, 2013) using a Lachat QuikChem8000 flow injection analysis system (Zellweger Analytics Inc., Lachat Instruments Division, Milwaukee, WI). Crude protein concentration was calculated as total N $\times$ 6.25.

Feces. Fecal samples were freeze-dried and ground to pass a 1-mm screen using a Cyclotec 1093 Sample Mill (Tecator Inc.). Subsamples were pooled per cow and per day in proportion to the amount excreted. Feces were then analyzed for DM, ash, N, ADF, NDF, Ca, Mg, K, Cu, Zn, Mn, and Se concentrations using procedures previously described. Apparent digestibility of OM and Se was calculated by subtracting daily excretion in feces from daily intake.

Urine. Subsamples of urine were thawed and pooled by cow and day, proportionally to the amount of urine excreted. Analysis of N, Ca, Mg, K, Cu, Zn, Mn, and

Se concentrations were then completed without mineralization and using procedures previously described. Apparent retention of Se was calculated by subtracting daily excretions in feces and urine from daily intake.

Milk. Milk samples with preservative were analyzed at Valacta (Dairy Production Center of Expertise, Québec and Atlantic, Sainte-Anne-de-Bellevue, QC, Canada) for fat, protein, lactose, MUN, and SCC. Analyses were performed using a Foss MilkoScan FT 6000 for infrared evaluation of milk component, combined with a Fossomatic FC for SCC analysis (Foss Electric A/S, Hillerød, Denmark).

Subsamples of milk, without preservative, were thawed and pooled by cow and day proportionally to the milk yield. Analyses of Ca, Mg, K, Cu, Zn, Mn, and Se were then completed without mineralization, and using procedures previously described. Selenium balance was calculated by subtracting daily excretion in feces, urine, and milk from daily intake.

Milk malondialdehyde (MDA) was measured following the method of Ermis et al. (2005) as an index of lipid peroxidation and oxidative status (Michel et al. 2008). Phosphate-buffered saline (800 μL , pH 7.4) and butylated hydroxytoluene solution (25 μL , 0.88%) were added to 500 μL of milk and mixed thoroughly. Trichloroacetic acid (500 μL , 30%) was added and the samples were placed on ice for 2 h. After 15 min of centrifugation at $2,000 \times g$, 1 mL of supernatant was mixed with 75 μL of 0.1 M EDTA and 250 μL of 1% thiobarbituric acid in 0.05 N NaOH. The samples were then placed in boiling water (100°C) for 15 min, followed by cooling to room temperature. Absorbance was measured at 532 nm. Intra- and inter-assay CV values were 6.0 and 5.5%, respectively.

Blood. Whole blood, plasma, and serum samples were thawed and analyzed for Ca, Mg, K, Cu, Zn, Mn, and Se concentrations without mineralization and using procedures previously described. Assay of erythrocyte GSH-Px activity was based on the method of Paglia and Valentine (1967) using a commercial Ransel glutathione peroxidase kit (Randox Laboratories, Crumlin, UK). Thioredoxin reductase (TRX-R1) activity was measured in plasma using a bovine cytoplasmic ELISA kit (MyBioSource Inc., San Diego, CA). The plasma MDA concentration was measured following the procedures described for milk samples, using 200 μL of plasma instead of 500 μL as for milk.

Statistical Analysis

Data were analyzed as a randomized unbalanced block design using PROC GLIMMIX of SAS (SAS Institute Inc., 2003), where $n = 8$ for treatments control, YSe, and FSe, and $n = 9$ for treatment ISe. Measure-

ments obtained at the end of the depletion period (i.e., before the start of experimental treatments) were used as covariates in the statistical analysis. Raw data were transformed [$\log_{10}(x)$, $1/x$] when it was deemed appropriate to meet the homogeneity of variance criteria. A model considering heterogeneity of variance was used for TRX-R1 evaluation, Se intake (mg/d), Se in feces (mg/d), Se absorbed (mg/d), Se in urine (mg/d), Se retained (mg/d), and Se in milk (percentage of intake and percentage of absorbed). Treatment was included as a fixed effect and block as a random effect. Akaike's information criterion was used to select the best covariance structure. Orthogonal contrasts were defined a priori and used to compare (1) control versus Se-supplemented diets (ISe, YSe, and FSe), (2) ISe versus organic Se (YSe and FSe), and (3) YSe versus FSe. Differences between treatments were declared significant when P values were ≤ 0.05 , and tendencies were noted when $0.05 < P < 0.10$. One cow suffered from mastitis at the end of the experimental period. Data from this cow were considered as missing for DMI, milk production and composition, blood parameters, and Se balance in the statistical analysis.

Cost Comparison of Organic Sources of Se

To evaluate the economic interest of using Se-fertilized forages, the relative costs of organic sources were estimated. The cost of adding 0.3 mg/kg of DM of Se from the yeast supplement was calculated. The forage Se needed in the diet to reach the same level of absorbed Se as for the yeast supplement was evaluated using the Se digestibility observed in this study. The level of Se fertilization required to obtain the targeted forage Se concentration was estimated using the equation reported by Tremblay et al. (2015) as follows: forage Se concentration (mg/kg of DM) = $0.0124 + 0.0292 \times \text{Se applied in spring (g/ha)}$. We assumed an average forage yield of 6 t of DM/ha, and actual intake and proportion of forage in the diet similar to those in the current experiment. The prices of the commercial products in 2013 were obtained from the main suppliers in Québec (Canada).

RESULTS AND DISCUSSION

Se Diet Concentration and Intake

All diets provided similar levels of CP, ADF, NDF, Ca, Mg, K, Cu, Zn, and Mn (Table 3). Diets had greater Se concentrations than the targeted 0.1 mg of Se/kg for the control treatment, and 0.6 mg of Se/kg for the Se-supplemented (ISe, YSe, and FSe) treatments (Table 3). All Se-supplemented diets had similar concentra-

tions of Se, and greater Se concentrations than the control treatment. As expected, cows fed the control diet ingested less Se than cows fed the supplemented diets ($P < 0.001$), whereas the Se intake did not differ among cows fed the Se inorganic and organic supplemented diets (Table 4). However, cows fed the FSe tended ($P = 0.06$) to ingest more Se than cows fed the YSe diet, which is attributed to the higher Se concentration in the FSe compared with the YSe diet in blocks 1 and 2 (Table 3).

Se Output, Apparent Absorption, Retention, and Balance

Treatments did not significantly differ in OM digestibility ($68.6 \pm 0.04\%$; $P = 0.44$; data not shown). Determination of Se intake and Se outputs in feces, urine, and milk allowed calculations of Se apparent absorption, retention, and daily balance (Table 4). Cows fed the Se-supplemented diets excreted more Se in feces than cows fed the control treatment ($P < 0.001$), reflecting their greater Se intake. As previously mentioned by Cun et al. (2015), due to differences in dietary intake, Se output should be expressed as a percentage of intake or as a percentage of absorbed Se to compare its availability as affected by different dietary treatments. As compared with cows fed the control diet, the increase in Se intake for cows fed the supplemented diets was associated with a tendency (control vs. Se-supplemented diets, $P = 0.07$) to decrease the proportion of ingested Se, Se excreted in feces, or an increase in proportion absorbed by the animal (control vs. Se-supplemented diets, $P = 0.02$). This result is probably related to the lower dilution of endogenous Se in the control diet than in the Se-supplemented diets because endogenous excretions are included in the calculation when measurements are made using total feces collection as was done in this experiment. A significant effect of dietary form of Se was also observed as cows fed organic Se excreted less, or absorbed more Se, expressed as percentage of intake, than cows fed ISe ($P = 0.02$). Moreover, cows fed FSe diet excreted less, or absorbed more Se, when expressed as percentage of Se intake, than cows fed YSe diet ($P = 0.04$).

Organic and inorganic dietary forms of Se are absorbed and metabolized differently. In the rumen, some of the selenite can be used to synthesize seleno-amino acids (predominantly SeCys) that are incorporated into microbial protein. However, an important proportion of selenite can be converted into low-molecular-weight forms of Se that are not well absorbed and used by the cow, and are mostly retrieved in feces (Weiss, 2005). Finally, the remaining selenite will leave the rumen and can be absorbed in the small intestine via a passive

mechanism. Similarly, SeMet can also be used in the rumen to synthesize seleno-amino acids but a higher proportion of SeMet will leave the rumen and reach the small intestine and be effectively absorbed via the same mechanism as methionine (Weiss, 2005). As a consequence, cows fed organic Se excreted less Se in feces and had a higher Se apparent absorption than cows fed ISe.

Moreover, our results suggest that Se from the FSe diet was more digestible than Se from YSe. Although the Se intake of cows fed FSe diet was greater ($P = 0.06$) than that of cows fed the YSe diet, this difference in Se intake is unlikely to solely explain the greater Se digestibility of the FSe diet. Two hypotheses were therefore formulated to explain this result. First, forages have a longer retention time in the rumen than feeds of smaller particle size. Therefore, Se provided by forages is likely to remain longer in the rumen. In addition, Se brought by forages allows a better Se dispersion in the ration and in the rumen. Both phenomena could have led to a better incorporation of Se into microbial proteins, and to a better distribution of Se in the intestinal digesta, which would have increased the efficiency of Se absorption in the intestine. The second hypothesis is that there could be a difference in digestibility between

yeast walls and forages, giving an advantage to forages and leading to a higher Se digestibility for the FSe treatment.

Urinary Se excretion followed a different general pattern than Se excretion in feces when expressed as percentage of intake. Cows fed the Se-supplemented diets excreted more Se than cows fed the control diet ($P < 0.001$). Urine excretion being the primary mechanism to decrease Se retention and maintain Se homeostasis (NRC, 1983), it was therefore expected that Se supplementation would result in larger amounts of Se excreted in urine. Excretion of Se in urine was similar for cows fed ISe and organic-Se. However, cows fed FSe excreted less Se than cows fed YSe ($P < 0.01$). A same pattern was observed when urine output was expressed as percentage of absorbed Se. The fate of Se in urine and feces is of interest because concern is growing about pollution arising from trace elements produced by agricultural systems (Juniper et al., 2006; Cun et al., 2015). Our results suggest that cows fed FSe excreted less Se in the environment, via feces and urine, than cows fed the other Se-supplemented diets. The greater apparent absorption and the lower urine excretion of Se in cows fed FSe as compared with YSe resulted in higher apparent retention as a percentage of

Table 4. Intake; outputs in feces, urine, and milk; apparent absorption, and apparent retention of Se in cows fed diets with low-Se silages without supplement (control), with low-Se silages and inorganic Se (ISe), with low-Se silages and yeast Se (YSe), or with high-Se silages without supplement (FSe)^{1,2}

Item	Treatment				SE	P-value			
	Se-supplemented					Contrast			
	Control	ISe	Organic Se			Treatment	Control vs. Se-supplemented	ISe vs. Organic Se	YSe vs. FSe
			YSe	FSe					
Intake, mg/d	2.9	17.1	14.8	17.3	1.39	<0.001	<0.001	0.30	0.06
Feces, mg/d	1.4	9.2	7.5	7.5	0.54	<0.001	<0.001	<0.01	0.92
Feces, % of intake	56.0	55.3	52.1	43.8	4.68	0.01	0.07	0.02	0.04
Absorbed, ³ mg/d	1.2	7.9	7.3	9.7	1.09	<0.001	<0.001	0.47	0.02
Absorbed, % of intake	42.0	44.6	47.9	56.0	4.48	<0.01	0.02	0.02	0.04
Urine, mg/d	0.2	2.7	2.4	2.2	0.25	<0.001	<0.001	0.03	0.43
Urine, % of intake	6.1	15.7	16.7	13.1	1.52	<0.001	<0.001	0.37	<0.01
Urine, % of absorbed	20.5	39.5	37.4	23.5	6.35	<0.001	<0.001	<0.01	<0.01
Retained, ⁴ mg/d	1.1	5.3	4.9	7.5	1.04	<0.001	<0.001	0.21	<0.01
Retained, % of intake	34.9	28.9	31.2	42.8	5.50	0.02	0.86	0.02	<0.01
Retained, % of absorbed	79.5	60.5	62.6	75.7	6.10	<0.001	<0.001	<0.01	<0.01
Milk, mg/d	0.4	0.8	1.4	1.6	0.07	<0.001	<0.001	<0.001	<0.01
Milk, % of intake	18.3	4.6	9.6	9.3	0.98	<0.001	<0.001	<0.001	0.74
Milk, % of absorbed	60.5	12.0	21.9	17.1	4.98	<0.001	<0.001	0.07	0.32
Balance, ⁵ mg/d	0.6	4.5	3.7	5.9	1.03	<0.001	<0.001	0.69	0.02
Balance, % of intake	14.8	24.2	22.9	33.2	5.58	<0.001	<0.001	0.20	<0.01
Balance, % of absorbed	17.6	48.4	43.5	59.2	10.2	<0.001	<0.001	0.63	0.04

¹Inorganic Se: sodium selenite, Nutreco, Guelph, ON, Canada.

²Yeast Se: Sel-Plex, Alltech, Nicholasville, KY.

³Se absorbed = intake – feces.

⁴Se retained = absorbed – urine.

⁵Se balance = retained – milk.

intake or absorbed Se. The difference in retention level, expressed as percentage of absorbed Se, between cows fed inorganic and organic Se sources, being higher for the latter, mainly came from the high value (75.7%) obtained for those fed the FSe in comparison to ISe (60.4%) and YSe (62.7%).

Daily amount of Se transferred to milk was higher for cows fed Se-supplemented diets than for cows fed the control diet ($P < 0.001$), and greater for cows fed organic- than inorganic-Se diets ($P < 0.001$). Consistently, increased Se excretion in milk of cows fed an organic as compared with an inorganic source was previously reported (Givens et al., 2004; Cun et al., 2015). Results also showed that Se transferred to milk, expressed as percentages of intake or absorbed Se, was higher for cows of the control treatment compared with cows of the Se-supplemented treatments. This result could be related to the low concentration of Se in the control diet for which a small variation in the amount of Se absorbed could translate in large differences when expressed as a function of intake. Moreover, organic Se supplements increased the transfer of Se to milk compared with inorganic supplement. Similar results were reported by Cun et al. (2015) when the transfer of Se to milk in cows fed enriched forage was compared with transfer in cows fed sodium selenite. Selenium transfer to milk expressed as percentage of intake and as a percentage of absorbed Se were similar among the organic treatments.

More Se was retained in animal tissues when cows were fed Se-supplemented than control diets as seen with the greater Se balance expressed as milligram per day, as percentage of intake, or as percentage of absorbed Se. No difference was found between inorganic and organic sources of Se for this parameter, but cows fed FSe obtained the greater Se balance, expressed as milligram per day, as a percentage of intake, or as a percentage of absorbed Se, as compared with YSe. This suggests that it could be advantageous to use FSe diets, not only to increase Se absorption, but also to achieve a greater final Se balance. This could be beneficial especially in the situations of high Se demand, and for transfer of this trace element to newborn calves (Weiss, 2005).

Se Concentration in Milk

Milk Se concentration was significantly affected by the treatments (Table 5). Cows fed Se-supplemented diets had a higher milk Se concentration than cows fed the control diet ($P < 0.001$), which is in line with our results on daily Se transfer to milk (Table 4) and previous reports (Givens et al., 2004; Weiss, 2005). Cows fed organic-Se diets produced milk with greater

Se concentration than cows fed ISe ($P < 0.001$). This result supports the concept that organic forms of Se are more effective to increase milk Se concentration than inorganic forms, as previously reported by Givens et al. (2004), Weiss (2005), and Gong et al. (2014). The source of organic Se also affected milk Se concentration. Cows fed FSe produced milk with a higher Se concentration than cows fed YSe ($P = 0.02$). This result is probably explained by the greater Se absorption, expressed as a proportion of intake, observed in cows fed FSe as compared with those fed YSe (Table 4). It finally appears that Se in the FSe diet was more available and effective in increasing Se concentration in milk than Se in the YSe diet and the use of Se-enriched forages can ultimately lead to a greater Se intake in humans (Givens et al., 2004). This is of interest because an elevated Se intake and more specifically a high selenized milk casein may be associated with a diminution of cancer risks (Cobo-Angel et al., 2014).

Se Concentration in Blood

Variation in blood parameters were in accordance with the results obtained for Se absorption, retention, and balance (Table 5). Cows fed Se-supplemented diets had higher Se concentration in whole blood, serum, and plasma than cows fed the control diet ($P < 0.001$). Cows fed organic Se (YSe and FSe) had higher ($P < 0.01$) Se concentrations in whole blood and serum (averaging 218.7 and 96.1 $\mu\text{g/L}$, respectively) than cows fed the ISe diet (195.0 and 80.2 $\mu\text{g/L}$, respectively). These results indicate that organic Se supplements are more effective to increase blood Se concentration than inorganic Se supplements, which is consistent with other studies that also reported higher Se concentrations in whole blood (Calamari et al., 2010; Gong et al., 2014) and serum (Weiss and Hogan, 2005) when organic rather than inorganic Se supplements were given to cows. The type of supplementation, however, did not affect plasma Se concentration. Selenium is mainly found in plasma in 2 selenoproteins, selenoprotein P, and GSH-Px as selenocysteine (Behne and Kyriakopoulos, 2001). Burk et al. (2001) mentioned that selenoprotein P and GSH-Px do not increase in response to either source of Se because they are genetically regulated. In plasma, the remaining Se is found in various proteins where SeMet nonspecifically replace sulfur Met (Behne and Kyriakopoulos, 2001). Therefore, a change in SeMet/methionine ratio should have led to an increase in the plasma Se concentration compared with inorganic treatments, as previously described by Calamari et al. (2010) when yeast was supplemented to cows for 140 d. It is therefore reasonable to hypothesize that the duration of treatment could have limited the capacity to observe a variation

Table 5. Selenium concentration in milk, whole blood, serum, and plasma, and antioxidant status of dairy cows fed diets with low-Se silages without supplement (control), with low-Se silages and inorganic Se (ISe), with low-Se silages and yeast Se (YSe), or with high-Se silages without supplement (FSe)^{1,2}

Item	Treatment					P-value			
	Se-supplemented					Contrast			
	Control	ISe	Organic Se		SE	Treatment	Control vs. Se-supplemented	ISe vs. Organic Se	YSe vs. FSe
			YSe	FSe					
Milk									
Milk Se, µg/L	17.4	28.4	54.1	60.0	2.2	<0.001	<0.001	<0.001	0.02
Milk MDA, ³ µmol/L	5.87	4.03	5.53	4.06	1.14	0.53	0.34	0.57	0.37
Blood									
Whole blood Se, µg/L	148.6	195.0	213.1	224.3	13.1	<0.001	<0.001	<0.01	0.25
Serum Se, µg/L	53.8	80.2	89.0	103.1	5.2	<0.001	<0.001	<0.01	0.04
Plasma Se, µg/L	71.0	106.5	117.9	116.7	6.4	<0.001	<0.001	0.13	0.89
Whole blood GSH-Px, ⁴ U/mL	38.75	41.77	40.42	41.41	2.16	0.48	0.17	0.60	0.60
Plasma TRX-R1, ⁵ U/L	0.65	2.53	0.62	1.06	1.03	0.11	0.10	0.11	0.27
Plasma MDA, µmol/L	0.83	0.82	0.70	0.56	0.22	0.51	0.42	0.27	0.44

¹Inorganic Se: sodium selenite, Nutreco, Guelph, ON, Canada.²Yeast Se: Sel-Plex, Alltech, Nicholasville, KY.³MDA = malondialdehyde.⁴GSH-Px = glutathione peroxidase.⁵TRX-R1 = thioredoxin reductase.

in plasma Se concentration. Cows fed FSe had higher serum Se concentration than cows fed YSe ($P = 0.04$). Although this source effect could partly come from the difference in Se intake between the 2 treatment diets, it also suggests that feeding a FSe diet is potentially a more effective way than feeding YSe diet to increase Se availability for animal metabolism. However, cows fed FSe and YSe had similar whole blood Se concentration ($P = 0.25$). In this regard, Scholz and Hutchinson (1979) reported that Se in whole blood responds more slowly to Se supplementation than does the Se in serum because most whole blood Se is incorporated into red blood cells during erythropoiesis. It is therefore reasonable to hypothesize that an increased Se concentration in whole blood could have been observed if cows were fed FSe for a longer experimental period.

Antioxidant Status

Dietary treatments did not affect whole blood GSH-Px concentration (Table 5). This enzyme functions as an antioxidant by reducing hydrogen peroxides to water, thus protecting cells against free radical induced oxidative stress (Gong et al., 2014). Whole blood GSH-Px is considered to reflect the long-term Se status (Rowntree et al., 2004). An increased whole blood GSH-Px activity was expected in Se-supplemented cows compared with control cows as previously reported in several studies (Ortman and Pehrson, 1999; Gunter et al., 2003; Ran et al., 2010). Between d 0 and 21 of the experimental period of the current study, a decrease in the whole

blood GSH-Px concentration was observed in cows of all treatments, and an increase was observed between d 22 and 43 in cows fed ISe, YSe, and FSe treatments (data not shown). The early reduction in blood GSH-Px was probably a collateral effect of the Se-depletion period during which all cows received the same basal diet that was not supplemented in Se. This hypothesis is supported by the decrease observed during the experiment in the whole blood GSH-Px concentration of cows fed the control diet as previously observed by Calamari et al. (2010). Moreover, the same authors reported that whole blood GSH-Px activity started to significantly increase only after 42 d of Se supplementation. Our experimental period lasted 43 d, and this can account for the lack of effect of Se supplementation on whole blood GSH-Px activity. Additionally, no significant difference was found in the whole blood GSH-Px concentration between cows fed organic Se diets (YSe and FSe) and ISe diet as previously reported by Weiss (2005), Calamari et al. (2010), and Ran et al. (2010). In addition to GSH-Px, TRX-R1 also plays a key role in the antioxidant system in dairy cows. Many in vitro studies showed that increased Se intake increases TRX-R1 activity (Miller et al., 2001; Bruzelius et al., 2008). In addition, Gong et al. (2014) showed that a Se yeast supplementation for 60 d markedly improved the TRX-R1 activity in dairy cows. However, the present study indicated that dietary treatments had no significant effect on plasma TRX-R1 activity. Little information is available on the effect of different Se sources on plasma TRX-R1 concentration. The lack of effect in our study

Table 6. Body weight, DMI, milk yield, milk composition, milk component yield, ECM, and FCM of lactating dairy cows fed diets with low-Se silages without supplement (control), with low-Se silages and inorganic Se (ISe), with low-Se silages and yeast Se (YSe), or with high-Se silages without supplement (FSe)^{1,2}

Item	Treatment				P-value				
	Se-supplemented				Contrast				
	Control	ISe	Organic Se		SE	Treatment	Control vs. Se-supplemented	ISe vs. Organic Se	YSe vs. FSe
			YSe	FSe					
BW, kg	603	597	611	606	7.8	0.08	0.71	0.02	0.35
DMI, kg/d	21.0	20.5	21.5	20.3	1.07	0.55	0.71	0.62	0.19
Milk yield, kg/d									
Actual	26.7	28.2	26.8	27.2	1.01	0.32	0.34	0.14	0.72
Energy corrected ³	26.7	27.7	28.7	28.2	1.03	0.12	0.03	0.32	0.56
Fat corrected ⁴	27.2	28.8	27.5	28.3	1.03	0.31	0.20	0.27	0.42
Milk composition, %									
Fat	4.25	4.19	4.22	4.36	0.10	0.15	0.89	0.11	0.08
Protein	3.52	3.44	3.58	3.51	0.07	0.04	0.08	0.01	0.15
Lactose	4.57	4.63	4.74	4.62	0.05	0.11	0.12	0.38	0.09
Milk component yield, g/d									
Fat	1,111	1,172	1,133	1,169	44.9	0.37	0.16	0.54	0.40
Protein	921	948	941	940	43.0	0.85	0.41	0.79	0.99
Lactose	1,171	1,317	1,242	1,263	60.2	0.12	0.05	0.21	0.74
Milk SCC, $\times 10^3$ /mL	65	40	29	39	12.4	0.03	0.01	0.40	0.27
MUN, mg/dL	8.72	9.10	9.03	10.44	0.61	0.07	0.13	0.25	0.05

¹Inorganic Se: sodium selenite Nutreco, Guelph, ON, Canada.

²Yeast Se: Sel-Plex, Alltech, Nicholasville, KY.

³ECM = [(38.9 $\times$ kg of fat/d) + (23.8 $\times$ kg of protein/d) + (16.3 $\times$ kg of lactose/d)]/3.14 (Madsen et al., 2008).

⁴FCM = (0.4 $\times$ kg of milk/d) + (15 $\times$ kg of milk fat/d) (Gaines, 1928).

could also be a consequence of the Se depletion period and could have been caused by the short length of our feeding trial. Dietary Se treatments had no significant effect on plasma and milk MDA concentrations (Table 5), most likely for the same reasons as mentioned for whole blood GSH-Px and plasma TRX-R1 concentrations.

Animal Performance

Dietary treatments tended to affect BW at the end of the experimental period ($P = 0.08$) with cows fed the ISe diet having a significantly lower BW than cows fed organic Se diets (YSe and FSe; Table 6). The relationship between the Se-supplementation form and BW is not clear mostly because many factors can influence BW in late-lactation cows. Dry matter intake, milk yield, energy-corrected milk yield, fat-corrected milk yield, milk fat and lactose concentrations, and milk component yields were not altered by dietary treatments. This is in accordance with the general view that Se supplementation, irrespective of the source and dose, is unlikely to markedly affect milk yield or milk composition when cows are not deficient in Se (Juniper et al., 2006; Ran et al., 2010; Gong et al., 2014).

Cows fed Se-supplemented diets tended ($P = 0.08$) to produce milk with a higher protein concentration

than cows with the control diet. This agrees with the findings of Calamari et al. (2010) who also observed a higher milk protein concentration in Se-supplemented cows. Additionally, cows fed the ISe diet had a lower milk protein concentration ($P = 0.01$) than cows fed organic Se diets (YSe and FSe). Given that milk protein yield was comparable between cows fed these treatments, the difference in milk concentration is most likely attributed to the numerically lower actual milk production in cows fed the YSe and FSe treatments.

Treatments affected milk SCC with cows fed Se-supplemented diets having lower milk SCC than control cows. This result suggests an improvement in the global mammary gland environment in cows fed Se-supplemented diets. The relationship between Se supplementation and SCC is probably related to the positive effects of Se on several measures of immune functions (Gerloff, 1992). Silvestre et al. (2007) observed that milk SCC was significantly lower when cows were fed organic Se compared with inorganic Se diets. Our results do not support this finding, perhaps because values of milk SCC were already low for cows of Se-supplemented treatments. Milk urea nitrogen was higher for cows fed FSe compared with cows fed the YSe diet ($P = 0.05$). Although forages were grown in the same fields, slight differences in their nutrient compositions are normally expected (Table 1), and these

variations might be the main source of differences in MUN between cows fed YSe and FSe.

Cost Comparison of Organic Sources of Se

With the diet forage proportion used in this study along with the observed DMI and the potentially higher digestibility of Se for FSe in comparison to YSe, the difference in cost between Se-fertilized forage and yeast was very small (\$0.023 vs. \$0.025 per cow and per day for forage and yeast, respectively). We conclude that Se-fertilized forages and yeast are 2 economically comparable sources of Se for cows.

CONCLUSIONS

The results of this study indicate that, at similar dietary concentrations, Se from organic sources (YSe and FSe diets) was significantly more available as compared with an inorganic source (ISe diet), and was more effective in increasing milk and blood Se concentrations. Furthermore, Se from the FSe is potentially more available than Se from the YSe diet, as it was significantly less excreted in feces and urine, resulting in better apparent absorption and retention, and higher concentrations in serum and milk. The use of Se-fertilized forages ultimately resulted in a better Se balance compared with other Se-supplemented treatments. Selenium dietary treatments did not alter the antioxidant status and overall performance of the cows. Therefore, the production of enriched forages is an effective method to supplement dairy cows in Se.

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REFERENCES

- Allison, R. D., and R. A. Laven. 2000. Effect of vitamin E supplementation on the health and fertility of dairy cows: A review. *Vet. Rec.* 147:703–708. <http://dx.doi.org/10.1136/vr.147.25.703>.
- Association of Official Analytical Chemists. 1990. *Official Methods of Analysis*. Vol. I. 15th ed. Association of Official Analytical Chemists, Arlington, VA.
- Behne, D., and A. Kyriakopoulos. 2001. Mammalian selenium-containing proteins. *Annu. Rev. Nutr.* 21:453–473. <http://dx.doi.org/10.1146/annurev.nutr.21.1.453>.
- Bosnak, C. P., and L. Davidowski. 2004. Continuous flow hydride generation using the Optima 4300DV ICP. Field application report. Perkin-Elmer Instruments, Shelton, CT.
- Bruzeliuss, K., S. Purup, P. James, G. Onning, and B. Åkesson. 2008. Biosynthesis of selenoproteins in cultured bovine mammary cells. *J. Trace Elem. Med. Biol.* 22:224–233. <http://dx.doi.org/10.1016/j.jtemb.2008.03.009>.
- Burk, R. F., K. E. Hill, and A. K. Motley. 2001. Plasma selenium in specific and non-specific forms. *Biofactors* 14:107–114. <http://dx.doi.org/10.1002/biof.5520140115>.
- Calamari, L., F. Petrerá, and G. Bertin. 2010. Effects of either sodium selenite or Se yeast (Sc CNCM I-3060) supplementation on selenium status and milk characteristics in dairy cows. *Livest. Sci.* 128:154–165. <http://dx.doi.org/10.1016/j.livsci.2009.12.005>.
- Canadian Council on Animal Care. 1993. *Guide to the care and use of experimental animals*. Vol. 1. 2nd ed. CCAC, Ottawa, ON, Canada.
- Canadian Food Inspection Agency. 2015. Selenium supplementation of livestock feeds. Regulatory guidance. RG-1 chapter 4. Accessed Feb. 11, 2016. <http://www.inspection.gc.ca/animals/feeds/regulatory-guidance/rg-1/chapter-4/eng/1329341411340/1329341520337?chap=6>.
- Cobo-Angel, C., J. Wichtel, and A. Ceballos-Márquez. 2014. Selenium in milk and human health. *Anim. Front.* 4:38–43. <http://dx.doi.org/10.2527/af.2012-0013>.
- Combs, G. F. Jr., L. C. Clark, and B. W. Turnbull. 2001. An analysis of cancer prevention by selenium. *Biofactors* 14:153–159. <http://dx.doi.org/10.1002/biof.5520140120>.
- Cun, G. S., P. H. Robinson, and S. E. Benes. 2015. Bioavailability of selenium in 'Jose' tall wheatgrass (*Thinopyrum ponticum* var 'Jose') hay as a substitute for sodium selenite in the diets of dairy cattle. *Sci. Total Environ.* 518–519:159–167. <http://dx.doi.org/10.1016/j.scitotenv.2015.02.091>.
- Ermis, B., A. Yildirim, R. Ors, A. Tastekin, B. Ozkan, and F. Akcay. 2005. Influence of smoking on serum and milk malondialdehyde, superoxide dismutase, glutathione peroxidase, and antioxidant potential levels in mothers at the postpartum seventh day. *Biol. Trace Elem. Res.* 105:27–36. <http://dx.doi.org/10.1385/BTER:105:1-3:027>.
- Filley, S. J., A. Peters, C. Bouska, G. Pirelli, and J. Oldfield. 2007. Selenium fertilization of pastures for improved forage selenium content. *Prof. Anim. Sci.* 23:144–147.
- Food and Drug Administration. 2003. Food additives permitted in feed and drinking water of animals: Selenium yeast. Rules and Regulation. Federal Register 68(170):39561–39562. Accessed Sep. 16, 2015. <http://www.fda.gov/OHRMS/DOCKETS/98fr/E7-13954.pdf>.
- Gaines, W. L. 1928. The energy basis of measuring milk yield in dairy cows. *Tech. Bull. No. 308*. Agric. Exp. Sta., Champaign, IL.
- Gerloff, B. J. 1992. Effect of selenium supplementation on dairy cattle. *J. Anim. Sci.* 70: 3934–3940.
- Givens, D. I., R. Allison, B. Cottrill, and J. S. Blake. 2004. Enhancing the selenium content of bovine milk through alteration of the form and concentration of selenium in the diet of the dairy cow. *J. Sci. Food Agric.* 84:811–817. <http://dx.doi.org/10.1002/jsfa.1737>.
- Gong, J., L. Ni, D. Wang, B. Shi, and S. Yan. 2014. Effect of dietary organic selenium on milk selenium concentration and antioxidant and immune status in midlactation dairy cows. *Livest. J.* 170:84–90. <http://dx.doi.org/10.1016/j.livsci.2014.10.003>.

- Gunter, S. A., P. A. Beck, and J. M. Phillips. 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. *J. Anim. Sci.* 81:856–864.
- Gupta, U. C., and J. A. MacLeod. 1994. Effect of various sources of selenium fertilization on the selenium concentration of feed crops. *Can. J. Soil Sci.* 74:285–290. <http://dx.doi.org/10.4141/cjss94-040>.
- Hernandez, P. C., J. F. Tyson, P. C. Uden, and D. Yates. 2007. Determination of selenium by flow injection hydride generation inductively coupled plasma optical emission spectrometry. *J. Anal. At. Spectrom.* 22:298–304. <http://dx.doi.org/10.1039/B612658H>.
- Ibeagha, A. E., E. M. Ibeagha-Awemu, J. Mehrzad, B. Baurhoo, P. Kgwatalala, and X. Zhao. 2009. The effect of selenium sources and supplementation on neutrophil functions in dairy cows. *Animal* 3:1037–1043. <http://dx.doi.org/10.1017/S1751731109004303>.
- Ibeagha, A. E., E. M. Ibeagha-Awemu, J. Mehrzad, and X. Zhao. 2007. Selenium, immune functions and health of dairy cattle. Pages 289–303 in *Nutritional Biotechnology in Feed and Food Industries*. T. P. Lyons, K. A. Jacques, and J. M. Hower, ed. Nottingham University Press, Nottingham, UK.
- Isaac, R. A., and W. C. Johnson. 1976. Determination of total nitrogen in plant tissue, using a block digester. *J. Assoc. Off. Anal. Chem.* 59:98–100.
- Juniper, D. T., R. H. Phipps, A. K. Jones, and G. Bertin. 2006. Selenium supplementation of lactating dairy cows: Effect on selenium concentration in blood, milk, urine, and feces. *J. Dairy Sci.* 89:3544–3551. [http://dx.doi.org/10.3168/jds.S0022-0302\(06\)72394-3](http://dx.doi.org/10.3168/jds.S0022-0302(06)72394-3).
- Lachat Instruments. 2013. Methods list for automated ion analyzers (flow injection analyses, ion chromatography). Lachat Instruments, Loveland, CO. Accessed Jun. 6, 2015. www.lachatinstruments.com/download/LL022-Rev-7.pdf.
- Maas, J. P. 1983. Diagnosis and management of selenium responsive diseases in cattle. *Compend. Contin. Educ. Pract. Vet.* 5:S383.
- Madsen, T. G., M. O. Nielsen, J. B. Andersen, and K. L. Ingvarsen. 2008. Continuous lactation in dairy cows: Effect on milk production and mammary nutrient supply and extraction. *J. Dairy Sci.* 91:1791–1801. <http://dx.doi.org/10.3168/jds.2007-0905>.
- McKenzie, R. C., J. R. Arthur, and G. J. Beckett. 2002. Selenium and the regulation of cell signaling, growth, and survival: Molecular and mechanistic aspects. *Antioxid. Redox Signal.* 4:339–351. <http://dx.doi.org/10.1089/152308602753666398>.
- Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: Collaborative study. *J. AOAC Int.* 85:1217–1240.
- Michel, F., D. Bonnefont-Rousselot, E. Mas, J. Draï, and P. Thérond. 2008. Biomarqueurs de la peroxydation lipidique: aspects analytiques. *Ann. Biol. Clin. (Paris)* 66:605–620. <http://dx.doi.org/10.1684/abc.2008.0283>.
- Miller, S., S. W. Walker, J. R. Arthur, F. Nicol, K. Pickard, M. H. Lewin, A. F. Howie, and G. J. Beckett. 2001. Selenite protects human endothelial cells from oxidative damage and induces thioredoxin reductase. *Clin. Sci.* 100:543–550. <http://dx.doi.org/10.1042/cs1000543>.
- NRC. 1983. *Selenium in Nutrition*. Rev. ed. Natl. Acad. Press, Washington, DC.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Ortman, K., and B. Pehrson. 1999. Effect of selenate as a feed supplement to dairy cows in comparison to selenite and selenium yeast. *J. Anim. Sci.* 77:3365–3370.
- Paglia, D. E., and W. N. Valentine. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 70:158–169.
- Ran, L., X. Wu, X. Shen, K. Zhang, F. Ren, and K. Huang. 2010. Effects of selenium form on blood and milk selenium concentrations, milk component and milk fatty acid composition in dairy cows. *J. Sci. Food Agric.* 90:2214–2219. <http://dx.doi.org/10.1002/jsfa.4073>.
- Rotruck, J. T., A. L. Pope, H. E. Ganther, A. B. Swanson, D. G. Hafeman, and W. G. Hoekstra. 1973. Selenium biochemical role as a component of glutathione peroxidase. *Science* 179:588–590. <http://dx.doi.org/10.1126/science.179.4073.588>.
- Rowntree, J. E., G. M. Hill, D. R. Hawkins, J. E. Link, M. J. Rincker, G. W. Bednar, and R. A. Kreft Jr. 2004. Effect of Se on selenoprotein activity and thyroid hormone metabolism in beef and dairy cows and calves. *J. Anim. Sci.* 82:2995–3005.
- Salman, S., A. Khol-Parisini, H. Schafft, M. Lahrssen-Wiederholt, H. W. Hulan, D. Dinse, and J. Zentek. 2009. The role of dietary selenium in bovine mammary gland health and immune function. *Anim. Health Res. Rev.* 10:21–34. <http://dx.doi.org/10.1017/S1466252308001588>.
- SAS Institute Inc. 2003. *SAS User's Guide*, version 9.3, 1st ed. SAS Institute Inc., Cary, NC.
- Scholz, R. W., and L. J. Hutchinson. 1979. Distribution of glutathione peroxidase activity and selenium in the blood of dairy cows. *Am. J. Vet. Res.* 40:245–249.
- Schwarz, K., and C. M. Foltz. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.* 79:3292–3293. <http://dx.doi.org/10.1021/ja01569a087>.
- Silvestre, F. T., H. M. Rutigliano, W. W. Thatcher, J. E.-P. Santos, and C. R. Staples. 2007. Effect of selenium source on production, reproduction, and immunity of lactating dairy cows. Pages 48–61 in *Proc. 18th Annu. Florida Rumin. Nutr. Symp.* Gainesville, FL. Univ. of Florida, Gainesville.
- Smith, K. L., J. H. Harrison, D. D. Hancock, D. A. Todhunter, and H. R. Conrad. 1984. Effect of vitamin E and selenium supplementation on incidence of clinical mastitis and duration of clinical symptoms. *J. Dairy Sci.* 67:1293–1300. [http://dx.doi.org/10.3168/jds.S0022-0302\(84\)81436-8](http://dx.doi.org/10.3168/jds.S0022-0302(84)81436-8).
- Spears, J. W. 2000. Micronutrients and immune function in cattle. *Proc. Nutr. Soc.* 59:587–594. <http://dx.doi.org/10.1017/S0029665100000835>.
- Spears, J. W., and W. P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. *Vet. J.* 176:70–76. <http://dx.doi.org/10.1016/j.tvjl.2007.12.015>.
- Tremblay, G. F., G. Bélanger, J. Lajeunesse, P. Y. Chouinard, and É. Charbonneau. 2015. Timothy response to increasing rates of selenium fertilizer in Eastern Canada. *Agron. J.* 107:211–220. <http://dx.doi.org/10.2134/agronj14.0397>.
- Weiss, W. P. 2003. Selenium nutrition of dairy cows: Comparing responses to organic and inorganic selenium forms. Pages 333–343 in *Proc. 19th Alltech Annual Symp. Nutr., Biotechnol. Feed Food Ind.* P. T. Lyons and K. A. Jacques, ed. Nottingham University Press, Nottingham, UK.
- Weiss, W. P. 2005. Selenium sources for dairy cattle. Pages 61–72 in *Proc. Tri-State Dairy Nutr. Conf.*, Ft. Wayne, IN. The Ohio State Univ., Columbus.
- Weiss, W. P., and J. S. Hogan. 2005. Effect of selenium source on selenium status, neutrophil function, and response to intramammary endotoxin challenge of dairy cows. *J. Dairy Sci.* 88:4366–4374. [http://dx.doi.org/10.3168/jds.S0022-0302\(05\)73123-4](http://dx.doi.org/10.3168/jds.S0022-0302(05)73123-4).