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Update on copper and selenium in Canadian cow–calf herds: regional differences and estimation of serum reference values

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Abstract

Trace mineral supplementation of beef cattle is essential for efficient reproduction and herd health. Understanding regional differences in cow trace mineral status could inform decisions about risks of deficiencies and supplementation management. Cow–calf surveillance projects provided three opportunities to evaluate the trace mineral status of Canadian beef cow herds. Blood samples were collected at pregnancy testing in 2014 from 102 cow–calf herds and in 2016 from 86 cow–calf herds in Western Canada. In 2019, blood samples were collected at pregnancy testing from cows in 163 cow–calf herds from Eastern and Western Canada. Serum samples were analyzed for copper, selenium, and molybdenum concentrations using a plasma mass spectrometer. The prevalence of copper deficient cows sampled from the Western provinces ranged from 24% to 43% across the three periods, and was 20% from Eastern Canada in 2019. The prevalence of selenium deficient cows ranged from 0.2% to 0.4% across the three projects in Western Canada, but was higher in Eastern Canada at 4.6% in 2019. High serum molybdenum was identified in 9.4% to 14% of cows across the three periods in Western Canada and in 15% of cows sampled in Eastern Canada in 2019. Serum copper, selenium, and molybdenum concentrations varied by cow age and month of sample collection. Serum selenium and molybdenum concentrations, but not copper, varied by soil type associated with the location of the farm. A subsample of samples from cows from Western Canadian herds provided body condition score (**BCS**) data, pregnancy status, and calf survival data and were used to estimate updated serum reference values for adequate concentrations. Age-specific values were required for selenium and molybdenum. Reference intervals (80%) were estimated from 2,406 pregnant beef cows from 99 herds with each cow having a BCS ≥ 2.5/5 and a live calf at 3 wk with no retained placenta: copper for all cows (0.379 to 0.717 ppm), selenium for cows <4 yr (0.052 to 0.152 ppm), and selenium for cows ≥4 yr (0.064 to 0.184 ppm). Upper 90% reference limits were also estimated for serum molybdenum for cows <4 yr (>0.104 ppm) and cows ≥4 yr (>0.110 ppm). The lower limits for the reference intervals for adequate copper and selenium are below those previously reported; nevertheless, they represent a large sample that was specifically applicable to extensively managed beef animals in western Canada.

Key words: copper, cow–calf, molybdenum, reference intervals, selenium

Introduction

Nutrition is a key determinant of productivity in beef herds. Protein and energy nutrition are well-documented determinants of reproductive performance (Waldner and Garcia Guerra, 2013; Waldner, 2014a, 2014b); however, micronutrient status is also very important. Because the most recent large Canadian survey of micronutrients in beef cattle was carried out in 2001 (Van De Weyer and Waldner, 2011; Waldner and Van De Weyer, 2011; Waldner and Blakley, 2014; Waldner and Uehlinger, 2017), with some additional regional work in 2008 (Van De Weyer et al., 2010, 2011), the findings of previous studies will not fully reflect current herd status and management. Herd size and management practices have changed (Sheppard et al., 2015; Jelinski and Waldner, 2018), creating a need for more recent data. With increasing herd sizes, there has, for example, been increasing use of more extensive feeding systems such as swath, bale, and corn grazing as alternatives to more confined feeding systems and targeted distribution of hay or silage, particularly in western Canada. These less intensive feeding systems offer many potential benefits to producers, but typically increase the challenges of effectively managing micronutrient intake. With more extensive management, herd owners often have fewer options to provide controlled amounts of minerals mixed with other supplements and rely on free-choice feeding for critical times during the production cycle.

Relying on intake of free choice supplement alone is often not sufficient in areas where trace minerals in forage are less than adequate to achieve health and performance targets. While most producers utilize free choice supplementation in their cow–calf herds, copper and selenium deficiencies continue to be recognized (Arthington and Ranches, 2021). Previous studies have documented significant variation in free-choice mineral and vitamin intake in beef cows (Manzano et al., 2012; Patterson et al., 2013; Arthington and Ranches, 2021; Ranches et al., 2021), with average intakes sometimes less than 40% of recommended intake leaving many cows at risk of deficiency (Patterson et al., 2013).

While other research has shown that deficiencies in forage are more common in certain soil types (Smart et al., 1992), soil type does not provide sufficient information to predict the risk of deficiency (Van De Weyer and Waldner, 2011; Waldner and Van De Weyer, 2011) and to inform

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more intensive and often costly supplementation programs. Knowing concentrations in feed alone similarly does not provide sufficient information due to the difficulty in measuring free choice intake of supplements, the biological availability of ingested supplements, and predicting interactions, such as those among dietary molybdenum, sulfates, and iron that can result in secondary copper deficiency (Herdt and Hoff, 2011).

Measuring the cow's trace mineral status provides the most direct assessment of whether additional intervention is required. Serum concentrations provide a less ideal metric of a cow's trace mineral status when compared to liver biopsies; however, serum samples can provide a practical surveillance option for screening large numbers of animals for important micronutrient deficiencies and have been shown to predict reproductive and health outcomes in observational studies of commercial beef herds (Van De Weyer et al., 2011; Waldner and Van De Weyer, 2011). However, most laboratory references used to interpret these results date back to the 1980s or earlier when laboratory technology was less able to measure very low concentrations (Puls, 1994). The evolution of beef herd genetics and management since that time could also impact expected values.

More recent micronutrient data collected from surveillance of commercial cows together with individual animal observations of overall health status and body condition score (**BCS**), pregnancy status, and subsequent calving outcomes, provided a very unique opportunity to re-evaluate the values currently being used to define adequate serum concentrations for beef herds. In 2014 and 2016 cows from 102 and 86 herds from across western Canada were tested for micronutrient status at pregnancy testing. In 2019, cows from 163 herds were sampled at pregnancy testing, but this time from herds across Canada. Using the 2014 and 2016 data including complete follow-up data on calving outcomes, the objective of this study was to estimate reference values and then use those values, in addition to existing standards, to classify serum micronutrient data collected in 2019. The 2019 data also provided the opportunity to compare trace mineral nutrition challenges in eastern and western Canadian herds and target producer education. The second objective of this study was to use the combined data from 2014 to 2019 to map the observed micronutrient concentrations to better identify geographical areas of focus for nutritional investigations and interventions.

Materials and Methods

This study was approved and then reviewed annually by the University Animal Care Committee and Research Ethics Board at the University of Saskatchewan (Protocol # 2014003).

Herd recruitment and sample collection 2014 and 2016

Cow–calf producers with more than 100 breeding cows were recruited from the western Canadian provinces of Alberta, Saskatchewan, and Manitoba to participate in a herd health and productivity study known as the Western Canadian Cow-Calf Surveillance Network (**WCCCSN**). To determine a geographically representative sample, national census data (2011) were used to describe the distribution of cow–calf herds in each province. Census data informed goals for recruitment from each province and the total sample size for the WCCCSN was determined based on available funding.

Producers were recruited to the WCCCSN through their veterinarians in 2014 (Johnson et al., 2022a). Enrollment criteria included herds that routinely pregnancy checked their cows and kept basic calving and production records. Veterinarians were asked to identify and contact eligible producers, and then producers were contacted by phone to confirm their eligibility and interest. A mailed-out survey and consent form were sent to interested producers to collect baseline production and management information from the 2013 calving season and to gain consent for participation. Producers that returned the initial survey and consent form were enrolled in the WCCCSN. Herd owners were asked to provide access to their herd for sampling at pregnancy testing in the fall of 2014 and again in 2016. In 2016, producers and veterinarians were specifically asked to target sampling to young cows, aged 2 and 3 yr, as having previously demonstrated the highest risk of low copper-related pregnancy risks (Van De Weyer et al., 2011).

Local veterinarians of the herd owners' choice collected blood and submitted blood samples from a systematic random sample of 20 pregnant cows regardless of herd size, as the objective was to collect a cross-section of samples from participating beef herds rather than providing diagnostic data to individual herd owners. The samples were shipped on ice by overnight courier to the diagnostic laboratory. The serum was separated by the diagnostic laboratory and frozen at −80 °C until testing.

The date of pregnancy testing, the animal's identification, age, BCS (5-point scale), and pregnancy status were recorded with each vial number. Age at the time of sampling was categorized as <4 yr of age or ≥4 yr. BCS was coded as <2.5, ≥2.5, or missing (Beef Cattle Research Council).

Herd recruitment and sample collection 2019

Participants were enrolled from the Canadian Cow-Calf Surveillance Network (**C3SN**) (Johnson et al., 2022b). The purpose of the C3SN was to estimate the prevalence of production-limiting diseases in beef herds across Canada to improve herd health and productivity. Producers were initially recruited to the network in mid to late 2018 through veterinarians, social media, provincial beef associations, and fellow producers. Criteria for recruitment included operations that conducted pregnancy checking, had greater than 40 breeding animals and access to email. Baseline management information was collected for each herd at the time of enrollment in the C3SN.

Blood samples were collected in the fall of 2019 during pregnancy testing by private veterinarians selected by the herd owners. Veterinarians were instructed to collect a systematic random sample of 20 cows in each herd. Information was also collected on age and BCS of each cow as well as the date of sample collection. Following collection, whole blood was sent to the diagnostic laboratory in insulated coolers via courier (shipping time 1 to 4 d depending on point of origin) for processing and analysis. Ten samples per herd were randomly selected for analysis and testing results were provided by the diagnostic lab directly to the submitting veterinarians.

Laboratory analysis

All samples were prepared and analyzed by Prairie Diagnostic Services Ltd, Saskatoon, SK for trace minerals. The laboratory methods used by the PDS toxicology lab have been previously reported (Van De Weyer et al., 2010, 2011).

Briefly, 0.5 mL of serum was added to a microwave vessel containing 2.5 mL of double-distilled water, 1.5 mL trace mineral grade nitric acid, and 0.15 mL internal standard [1 ppm yttrium (Y) and indium (In)]. Samples were digested in Microwave Accelerated Reaction System (MARS 5) Digestion Microwave System (CEM Corporation, Matthews, NC) and diluted to 15.0 mL with double-distilled water in a trace mineral-free tube. Analysis was performed on Thermo Scientific™ iCAP™ Q inductively-coupled plasma mass spectrometer (Thermo Fisher Scientific, Waltham, MA) using standard curves for magnesium, manganese, iron, cobalt, copper, zinc, selenium, and molybdenum. Samples were analyzed in duplicate where sufficient serum was available. Copper (Cu), selenium (Se), and molybdenum (Mo) were targeted for investigation in this study due to the established clinical relevance of serum concentrations in Canadian beef herds (Van De Weyer et al., 2010, 2011; Van De Weyer and Waldner, 2011; Waldner and Van De Weyer, 2011; Waldner and Blakley, 2014; Waldner and Uehlinger, 2017).

Quality control procedures for the lab utilize a previously diagnostically analyzed liver chosen at random. Ten subsamples (~0.5 g each), were then each digested in 5 mL 50% nitric acid, in a Multiwave 5000 microwave digestion platform (Anton Paar Canada Inc., Montreal, QC). After digestion, these subsamples were pooled into a 1 L acid-washed volumetric flask and filled to capacity with ultrapure, deionized water. An aliquot of this pool was analyzed with all mineral panel analyses. Averages over time were assessed and monitored within a 3 standard deviation acceptance level. For external verification, three subsamples of this liver were sent to Animal Health Laboratory, Guelph, ON.

Assessment of winter nutrition and calving outcomes

In a multiple choice and open-ended, paper-based survey distributed in the late spring of 2015, herd owners were asked to describe what, if any, supplements (trace mineral, vitamin, salt) were provided in the period from breeding season leading up to sample collection and the form and method of supplementation (block/loose/combined with salt or other supplement, free choice or force-fed) in 2014. The herd owner was also asked to describe what, if any, supplements (trace mineral, vitamin, salt) were provided from pregnancy testing through calving 2015, including the form and method of supplementation.

Soil zone

A map of the soil zones in western Canada (Government of Canada) was imported into GIS software (ArcMap, Esri, Redlands, CA). The location of participating herds in 2014 and 2016 was then plotted and the most appropriate soil zone was extracted for each herd. The soil zone for each herd was categorized as grey, dark grey, black, dark brown, or brown (Natural Resources Conservation Service—USDA).

Statistical analysis

In the first step, participating herds and sampled animals were characterized for 2014, 2016, and 2019. The overall and herd average concentrations for copper, selenium, and molybdenum were also summarized. Serum concentrations were categorized as deficient (<0.5 ppm) and less than adequate for copper (<0.6 ppm) and deficient (< 0.025 ppm) and less than adequate (<0.08 ppm) for selenium and high for molybdenum (>0.10 ppm) based on commonly used reference values in North American diagnostic laboratories (Puls, 1994).

The prevalence of micronutrient deficiencies for each herd for young and mature cows, combined across all years, were mapped by geographic region using natural neighbor interpolation (ArcGIS Pro, version 2.9.2, Esri, Redlands, CA) with herd location estimated by postal code centroids. Interpolated surfaces were limited to the data extent for the eastern and western regions and a buffer of 100 km around herd locations within the data extent.

Reported trace mineral supplementation practices were graphed to illustrate variability over the 2015 production cycle. Soil type during the preceding grazing season were described as well as history of micronutrient supplementation in the period before sample collection for herds from western Canada in 2014 and 2016.

Differences in the prevalence of copper deficiency, selenium deficiency, less than adequate selenium concentrations, and high molybdenum concentrations were compared for samples collected in eastern and western herds using generalized linear mixed models with a binomial distribution and logit link function accounting for clustering by herd with a random intercept (StataSE version 17, StataCorp LLC, College Station, TX).

Other factors of interest associated with serum trace mineral concentrations for 2014 and 2016 were examined using generalized linear mixed models to account for clustering of outcomes within herd. For copper, a normal distribution was used and then the residuals were checked for normality and homogeneity of variance. Selenium and molybdenum concentrations were log transformed prior to analysis as there was a substantial right skew apparent when the data were viewed using histograms. Null or intercept only models were used to estimate the intracluster correlation coefficient (**ICC**) as a measure of the variation in concentrations explained by differences between herds. All models were checked for extreme outliers by graphing the residuals. Factors considered in the exploratory analysis included: cow age, BCS, herd size, whether the herd sold any seed stock or was only commercial, month of testing, soil type, and history of supplementation. The association between molybdenum and copper concentrations was also examined using this model. The results for copper were reported as absolute differences (β) between risk factor categories with 95% confidence intervals (**95% CI**) and the results for selenium and molybdenum were reported as relative differences after back transforming the regression coefficient from the log scale.

Reference intervals and limits

A subset of the data for 2014 and 2016 using the best available data to identify healthy animals was selected for use in estimating normal reference intervals for copper and selenium and an upper reference limit for molybdenum. Reference intervals suggesting an adequate range of concentrations expected in healthy animals for copper and selenium were reported because values that are both potentially too low and too high are of interest. For molybdenum, only the upper reference limit was estimated as only concentrations that are too high are a concern.

Cows considered for inclusion were those with complete pregnancy testing, BCS, and follow-up data on calving outcomes and retained placentas. Only pregnant animals with adequate body condition (BCS ≥ 2.5) that produced a calf that survived to at least 3 wk of age and from cows that did not have a retained placenta were included in estimation of reference intervals and limits. Only the 2014 and 2016 data were used for herds from western Canada, as information on subsequent individual animal calving outcomes was not collected for the cows sampled in 2019.

Nonparametric 95% (2.5%, 97.5% limits), 90% (5%, 95% limits), and 80% (10%, 90% limits) reference intervals for copper and selenium and corresponding upper 97.5%, 95%, and 90% reference limits for molybdenum were estimated using commercial software (Analyze-It Version 3.0; Analyze-It Software, Leeds, UK) as previously recommended by the American Society for Veterinary Clinical Pathology (Friedrichs et al., 2012). The criteria proposed by Lahti were used to determine whether age-specific intervals and limits were appropriate when age was associated with serum concentrations (Gowans et al., 1988; Lahti et al., 2002a, 2002b, 2004).

The 80% reference intervals were reported for copper and selenium in the core results text to allow for comparison in the discussion to previous reference intervals proposed for cattle based on laboratory data (Herdt and Hoff, 2011). The corresponding upper 90% reference limit was reported for molybdenum.

The 95% and 90% reference intervals for copper and selenium and corresponding 97.5% and 95% upper reference limits for molybdenum were reported for completeness in supplemental files and documentation of the values used for comparison to the criteria proposed by Lahti et al. (Gowans et al., 1988; Lahti et al., 2002a, 2002b, 2004),

The raw data used for the reference intervals for copper and selenium and reference limits of molybdenum were summarized in histograms and box and whisker plots in the supplemental files.

The resulting lower limits of the 80% reference intervals for copper and selenium and the 90% upper reference limit for molybdenum were then applied to the 2019 concentration data for copper, selenium, and molybdenum and results compared to those from the previously published reference values (Puls, 1994). The prevalence of 2019 results below the lower limit for the 80% reference intervals for copper and selenium and above the 90% upper reference limit for molybdenum was summarized with 95% confidence intervals accounting for clustering by herd with a random intercept and a fixed effect for region using mixed effects logistic regression and ICC was reported to estimate the amount of variation explained by herd (StataSE version 17, StataCorp LLC).

Results

Summary of participating herds and sampled cows

Of the producers recruited to the WCCCSN, 99 were eligible to participate in the trace mineral study as they had submitted a consent form and the initial recruitment survey before September 30, 2014. Two of these producers opted out of testing their cows. Samples were collected from another five producers later in the fall by local veterinarians and the producers submitted their completed recruitment surveys and consent to use the data at the time of sample submission. Most herds (70/102) that were sampled in 2014 were resampled in 2016; the remaining 16 of the 86 herds sampled in 2016 were herds more recently recruited to the network.

The WCCCSN was expanded into the C3SN in 2018. Of the 181 producers that were initially enrolled in the C3SN, 178 provided complete baseline information of which 176 identified they were also willing to participate in blood and fecal sample collection as part of a sample banking project for infectious disease and trace mineral studies. Samples from 163 herds were tested for micronutrients after sample collection in the fall of 2019.

The attributes of herds and cows sampled within these herds during 2014, 2016, and 2019 are summarized in Table 1.

Most of the 102 herds from western Canada sampled in 2014 were in the black soil zone (45, 44%); 17 (17%) were in the brown soil zone, 13 (13%) in the dark brown zone, 18 (18%) in the gray zone, and 9 (9%) were in the dark gray zone. Similarly, most of the 86 herds from 2016 were in the black soil zone (44%, 38/86); 14 (16%) were in the brown soil zone, 10 (12%) in the dark brown zone, 18 (21%) in the gray zone, and 6 (7%) were in the dark gray zone. The 2019 herds were not mapped to soil zone.

Supplementation practices, feed and water testing in participating herds for 2014–2015

Of the 102 producers providing samples in 2014 from western Canada, all 87 who completed the follow-up survey and answered the questions on supplementation practices reported using trace mineral supplements. Almost all (97%, 84/87) reported providing free-choice trace mineral supplements at some time during the year. Three producers only provided minerals mixed with grain or silage and did not provide free choice. Less than half (41%, 36/87) reported ever providing trace minerals mixed with either grain or silage. Only 15% (13/87) of producers reported supplementing vitamin A and vitamin E.

The proportion of the 87 producers who reported feeding any trace mineral and adding trace mineral to supplement varied based on the time of year (Figure 1). The proportion of producers providing vitamin supplement was lower than for trace minerals, but also increased slightly in the winter-feeding period ($n = 87$) (Figure 1). For example, while 99% of producers provided any trace mineral at calving, 36% mixed trace mineral with supplement to ensure more consistent intake, but only 9% provided vitamins A and E.

Only 5% (4/87) of herd owners reported using selenium injections to supplement their cows. The proportion of herd owners that reported using vitamin A injections in their adult cows was slightly higher (14 %, 12/87).

In the spring of 2015, 80 producers provided information on the use of injectable supplements in their neonatal calves; 44 (55%) reported using selenium injections and 40 (50%) reported using vitamin A/D injections.

In the previous 5 years, samples from the primary water source for the cattle had been submitted for laboratory testing by only 15% (13/87) of participating producers who completed this survey question. However, 69% (60/87) of producers reported lab testing at least some of their feed for quality in 2014.

Summary of serum trace mineral concentrations

The number of herds tested, the number of cows sampled, and serum concentrations of copper, selenium, and molybdenum are summarized in Table 2.

The percentage of cows that were copper deficient or less than adequate fluctuated between 2014 and 2019 in western

Table 1. Attributes of 226 Canadian cow–calf herds submitting serum samples for trace mineral status[1]

Year samples collected	2014	2016	2019
Sample collection dates	Oct 2014 to Feb 2015	Oct 2016 to Mar 2017	Sep 2019 to Feb 2020
Number of herds	102	86	163
Herd location			
Alberta/Northern BC	52% (52/102)	51% (44/86)	36% (58/163)
Saskatchewan	31% (32/102)	31% (27/86)	21% (34/163)
Manitoba	17% (18/102)	17% (15/86)	13% (21/163)
Eastern Canada	NA	NA	31% (50/163)
Primarily commercial herds	75% (76/102)	77% (66/86)	67% (109/163)
Sales of some seed stock	25% (26/102)	23% (20/86)	33% (54/163)
Herd size (median, 5th and 95th percentile)	240 (107,942)	228 (107,796)	142 (39,511) West: 212 (63,551) East: 76 (30,381)
Herd size > 300 cows	39% (40/102)	37% (32/86)	20% (33/163) West: 27% (30/113) East: 6% (3/50)
Number of cows sampled	2,030	1,604	1,629
Cow age at sampling			
• Mature (≥4 yr)	69% (1,397/2,030)	0%	77% (1,260/1,629)
• Young (< 4 yr)	31% (633/2,030)	100% (1,604/1,604)	23% (369/1,629)
Body condition score < 2.5/5.0	3.7% (76/2,030)	1.3% (21/1,604)	8.3% (136/1,629)
Percentage with live calf[2]	95% (1,472/1,549)	98% (1,108/1,130)	NA

[1]Some herds were sampled in more than 1 yr.
[2]Not all herds provided follow up individual animal records at calving for all animals sampled.

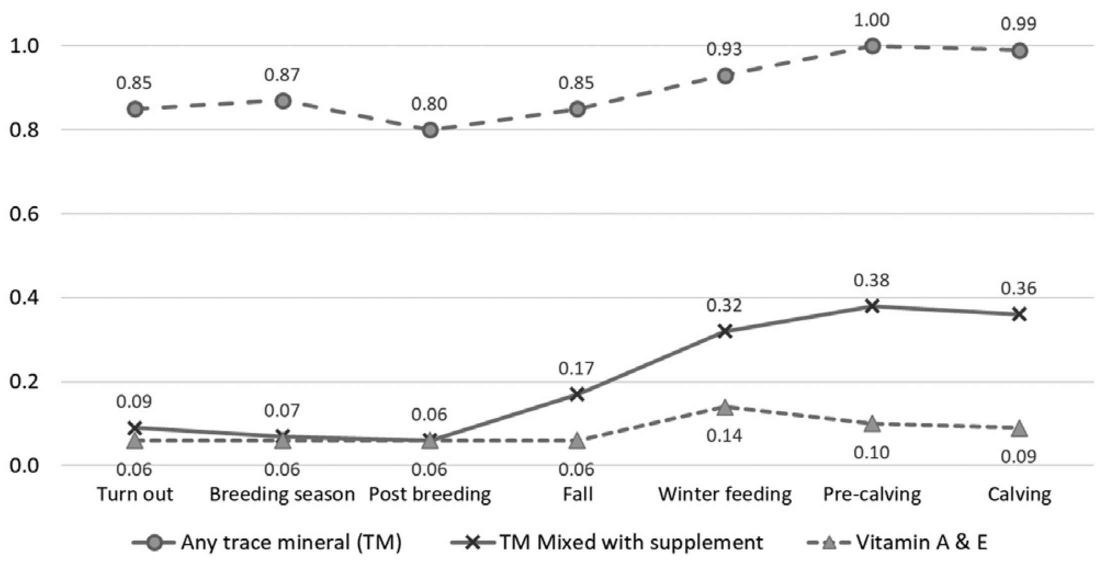

Figure 1. Variation in the proportion of producers who provided any trace mineral (TM), measured quantities of trace minerals mixed with grain or silage, and vitamin A and E during different parts of the production cycle in 2014–2015 ($n = 87$).

Canada (Table 3). The prevalence of cows tested in eastern Canada that were copper deficient (21%, 95% CI 15, 27%) was slightly lower than for western Canada (29%, 95% CI 24, 33%) in 2019 after accounting for clustering by herd ($P = 0.04$).

A similar fluctuation was seen for selenium concentrations in western Canada from 2014 to 2019 (Table 3). However, samples collected from 2016 were from cows 2 and 3 yr of age at the time of testing (Table 2). The prevalence of cows that were selenium deficient in 2019 was higher ($P = 0.03$) after accounting for clustering by herd in eastern Canada (6.3%, 95% CI 0.0, 14%) than in western Canada (0.1%, 95% CI 0.0, 0.2%). Similarly, the prevalence of cows that had less than adequate selenium in 2019 was higher ($P < 0.0001$) in eastern Canada (72%, 95% CI 67, 77%) after accounting for clustering by herd than in western Canada (30%, 95% CI 27, 33%).

Table 2. Summary trace mineral concentration data for 5,263 serum samples collected from 226 Canadian cow–calf herds from 2014 to 2019[1]

Year of collection	Number of herds	Number of samples	Mean serum concentration (SD)		
			Copper, ppm	Selenium, ppm	Molybdenum, ppm
Western provinces					
2014	102	2,030	0.528 (0.134)	0.119 (0.048)	0.052 (0.058)
2016	86	1,604	0.592 (0.174)	0.080 (0.037)	0.043 (0.060)
2019	113	1,130	0.568 (0.196)	0.098 (0.038)	0.056 (0.088)
Eastern provinces					
2019	50	499	0.595 (0.145)	0.071 (0.063)	0.053 (0.061)

[1]Some herds were sampled in more than 1 yr.

Table 3. Summary of trace mineral concentrations as compared to existing thresholds for adequate, deficient and high for 5,263 serum samples collected from 226 cow–calf herds from 2014 to 2019[1]

Copper less than adequate (<0.6 ppm) (Puls, 1994)	% cows with less than adequate copper	% of herds with ≥1 cows with less than adequate copper	Mean herd prevalence less than adequate (SD)
Western provinces: 2014	75.4% (1530/2,030)	100% (102/102)	0.75 (0.22)
2016	54.3% (871/1,604)	100% (86/86)	0.55 (0.26)
2019	63.6% (719/1,130)	100% (113/113)	0.64 (0.22)
Eastern provinces: 2019	59.7% (298/499)	100% (50/50)	0.60 (0.24)
Copper deficient (<0.5 ppm) (Puls, 1994):	**% copper deficient cows**	**% of herds with ≥1 deficient cow(s)**	**Mean herd prevalence deficient (SD)**
Western provinces: 2014	42.9% (871/2,030)	92% (94/102)	0.43 (0.29)
2016	24.4% (391/1,604)	85% (73/86)	0.25 (0.27)
2019	29.0% (328/1,130)	85% (96/113)	0.29 (0.26)
Eastern provinces: 2019	20.4% (102/499)	76% (38/50)	0.20 (0.18)
Selenium less than adequate (<0.08 ppm) (Puls, 1994):	**% cows with less than adequate selenium**	**% of herds with ≥1 cows with less than adequate selenium**	**Mean herd prevalence less than adequate (SD)**
Western provinces: 2014	21.8% (442/2,030)	51% (52/102)	0.22 (0.33)
2016	60.0% (962/1,604)	81% (70/86)	0.59 (0.40)
2019	33.8% (382/1,130)	55% (62/113)	0.34 (0.40)
Eastern provinces: 2019	73.1% (365/499)	94% (47/50)	0.73 (0.32)
Selenium deficient (<0.025 ppm) (Puls, 1994):	**% selenium deficient cows**	**% of herds with ≥1 deficient cow(s)**	**Mean herd prevalence deficient (SD)**
Western provinces: 2014	0.4% (9/2,030)	2.9% (3/102)	0.004 (0.028)
2016	0.3% (5/1,604)	2.3% (2/86)	0.003 (0.022)
2019	0.2% (2/1,130)	0.9% (1/113)	0.002 (0.019)
Eastern provinces: 2019	4.6% (23/499)	8% (4/50)	0.046 (0.181)
Molybdenum high (>0.10 ppm) (Puls, 1994):	**% high molybdenum cows**	**% of herds with ≥1 high molybdenum cow(s)**	**Mean herd prevalence high molybdenum (SD)**
Western provinces: 2014	13.2% (268/2,030)	44% (45/102)	0.13 (0.23)
2016	9.4% (151/1,604)	27% (23/86)	0.11 (0.25)
2019	13.9% (157/1,126)	33% (37/113)	0.14 (0.27)
Eastern provinces: 2019	15.0% (75/499)	22% (11/50)	0.15 (0.32)

[1]Western provinces: 2014—102 herds, 2,030 cows, 2016—86 herds, 1,604 cows, 2019—113 herds, 1,130 cows; Eastern provinces: 2019 —50 herds, 499 cows; some herds were sampled more than once.

There was no difference in the prevalence of sampled cows with high molybdenum between eastern and western Canada in 2019 after accounting for clustering by herd ($P = 0.38$).

Maps of the proportion of sampled cows from each herd tested in 2014, 2016, and 2019 with deficient copper, less than adequate and deficient selenium, and high molybdenum (Table 3) suggest more targeted regions at higher risk for trace mineral imbalances (Supplementary Figures S1 to S4). Southeastern Saskatchewan appeared to be at highest risk for copper deficiency. Low selenium was most prevalent in much of eastern Canada and western Alberta. Smaller regional higher-risk areas of molybdenum excess were focused in Alberta, southern Saskatchewan, Manitoba, and Quebec.

Differences among herds accounted for a substantial percentage of the variation in the serum concentrations for copper (ICC 0.286, 95% CI 0.225 to 0.355), selenium (ICC 0.540, 95% CI 0.466 to 0.613), and molybdenum (ICC 0.556, 95% CI 0.482 to 0.629).

Cow and herd attributes potentially associated with trace mineral status fall/winter 2014–2015 and 2016–2017

Serum copper concentrations were 0.052 ppm (95% CI 0.041, 0.062, $P < 0.0001$) or 11% higher for cows less than 4 yr of age compared to older cows. Conversely, selenium concentrations for young cows were on average 1.34 times (95% CI 1.31, 1.37, $P < 0.0001$) or 34% lower than for cows 4 yr of age or greater at the time of sampling, and molybdenum concentrations for young cows were 1.27 times (95% CI 1.21 to 1.34, $P < 0.0001$) or 27% lower than for older cows.

BCS < 2.5/5 was not associated with serum copper ($P = 0.45$) or molybdenum ($P = 0.62$) concentrations. BCS was similarly not associated with serum selenium concentrations after accounting for cow age ($P = 0.13$).

Whether the herd was commercial or sold at least some seed stock was not associated with measured serum copper concentrations ($P = 0.85$) or serum selenium concentrations ($P = 0.82$). The size of the herd was not associated with serum copper concentrations ($P = 0.75$). Similarly, herd size was not associated with serum selenium ($P = 0.08$) after accounting for cow age.

Serum copper concentrations varied based on the month samples were collected ($P < 0.001$). The cows sampled in November had lower serum copper concentrations than cows sampled in October (difference 0.067 ppm, 95% CI 0.048 to 0.087, $P < 0.001$), December (difference 0.072 ppm, 95% CI 0.052 to 0.091, $P < 0.001$), and January (difference 0.066 ppm, 95% CI 0.037 to 0.095, $P < 0.001$).

Serum selenium concentrations ($P < 0.001$) also varied by month of sample collection. The cows sampled in November had lower serum selenium concentrations than cows sampled in October (relative difference 1.13, 95% CI 1.07 to 1.18, $P < 0.001$), December (relative difference 1.12, 95% CI 1.07 to 1.18, $P < 0.001$), February (relative difference 1.45, 95% CI 1.30 to 1.62, $P < 0.001$), and March (relative difference 1.62, 95% CI 1.01 to 2.59, $P = 0.04$).

Serum molybdenum concentrations ($P < 0.001$) also varied by month of sample collection. The cows sampled in October had higher serum molybdenum concentrations than cows sampled in later months.

Serum selenium concentrations were lower on black (relative difference 1.28 times, 95% CI 1.12 to 1.45, $P < 0.001$) and grey soils (relative difference 1.15 times, 95% CI 1.01 to 1.30, $P = 0.03$) than on brown soils. Serum molybdenum concentrations were higher on black (relative difference 1.61 times, 95% CI 1.18 to 2.21, $P = 0.003$) and grey soils (relative difference 1.56 times, 95% CI 1.18 to 2.07, $P = 0.002$) than on brown soils. There was no association in this sample between soil color and serum copper ($P = 0.38$).

Reported provision of any trace mineral supplement in either the post-breeding or fall period immediately before sampling was not associated with serum copper ($P = 0.11$), but was associated with increased serum selenium concentrations (relative difference 1.27 times, 95% CI 1.21 to 1.33, $P < 0.001$) in this sample.

Serum molybdenum concentrations were not associated with serum copper concentrations with the difference in copper per ppm difference in molybdenum estimated at −0.053 ppm (95% CI −0.175, 0.069; $P = 0.39$). The interaction between cow age (younger vs. older cows) and serum molybdenum was also examined, and there was no difference in the association based on age ($P = 0.62$).

There was, however, an association between serum selenium and serum copper concentrations after accounting for cow age and the differences between collection years. Copper increased an average of 0.167 ppm (95% CI 0.004, 0.330; $P = 0.045$) for every ppm increase in serum selenium concentration.

Development of reference intervals

Reference intervals (80%) with lower 10% and upper 90% limits were developed for serum copper and selenium concentrations for a subset of 2,406 cows (Table 4). Upper reference limits (90%) were also developed for serum molybdenum (Table 4). The 1,326 pregnant cows from 78 herds in 2014 and 1,080 pregnant cows from 67 herds in 2016 included in the analysis had a BCS of at least 2.5/5.0 reported by the veterinarian at the time of pregnancy testing and complete individual animal records provided by the herd owners in the late spring and early summer of 2015 and 2017 that confirmed the cow subsequently delivered a live calf that survived to 3 wk of age and that the cow did not have a retained placenta. For completeness, 95% and 90% reference intervals were also reported for copper and selenium and corresponding 97.5% and 95% upper reference limits for molybdenum (Supplementary Tables S1 and S2).

One reference interval (Table 4, Supplementary Figure S5) was reported for copper without creating separate values based on cow age. While there was a difference in copper concentrations based on age, 1.9% of serum copper concentrations in young cows and 2.0% in older cows were below the lower 95% reference interval (2.5th percentile) estimated using data from all cows (Table S1). As such the criteria proposed by Lahti et al. (Gowans et al., 1988; Lahti et al., 2002a, 2002b, 2004) intended to target 2.5% of values outside the cutoff in any subgroup with no greater than 4.1% and no less than 0.9% outside the lower interval were met, and a single reference interval for all cow ages was considered sufficient.

There were also differences in selenium and molybdenum concentrations for young and older cows and the criteria proposed by Lahti et al. (Gowans et al., 1988; Lahti et al., 2002a, 2002b, 2004) for a single reference interval were not met. For selenium, 0.88% of the serum concentrations in older cows were below the lower 95% reference interval (2.5th percentile) estimated using data for all cows (Supplementary Table

Table 4. Eighty percent reference intervals estimated from 2,406 pregnant beef cows from 99 herds[1] with a BCS of at least 2.5/5, with a reported live calf at birth that lived to 3 wk of age and no retained placenta

	Median (10th and 90th percentile)	80% reference interval	
		Lower 10% reference limit (90% CI)[2]	Upper 90% reference limit (90% CI) [2]
Copper, ppm	0.541 (0.379, 0.717)	0.379 (0.374 to 0.385)	0.717 (0.709 to 0.729)
Selenium, ppm			
Young cows <4 yr	0.081 (0.052, 0.151)	0.052 (0.050 to 0.053)	0.152 (0.146 to 0.159)
Older cows ≥ 4 yr	0.115 (0.064, 0.184)	0.064 (0.061 to 0.066)	0.184 (0.179 to 0.190)
		90% reference limit (90% CI) – upper	
Molybdenum, ppm			
Young cows <4 yr	0.027 (0.006, 0.103)	0.104 (0.095 to 0.116)	
Older cows ≥4 yr	0.037 (0.008, 0.110)	0.110 (0.105 to 0.117)	

[1]Seventy-eight herds sampled in 2014 and 67 herds sampled in 2016 with a total of 99 unique herds sampled at least once across the 2 yrs and 46 of them sampled twice.
[2]Nonparametric limits based on (N + 1)p quantile with 90% confidence intervals (90% CI).

S1). Therefore, separate reference intervals were proposed for selenium for cows < 4 yr (Table 4, Supplementary Figure S6) and cows ≥4 yr (Table 4, Supplementary Figure S7).

Similarly, age-specific upper reference limits were estimated for molybdenum (Table 4, Supplementary Figures S8 and S9). For molybdenum, 4.8% of the serum concentrations for young cows and 5.0% from older cows were higher than the estimated 97.5% reference limit for all cows (Supplementary Table S1) (Gowans et al., 1988; Lahti et al., 2002a, 2002b, 2004).

Application of reference intervals to 2019 trace mineral data

Using the 80% reference interval for serum copper (Table 4) established with data from 2014 and 2016, there were 7.4% (95% CI 4.9, 10.0%) cows that were below the lower 10% limit for copper in the cross-Canada 2019 data after accounting for clustering by herd; 3.3% (95% CI 0.3, 6.2%) from eastern Canada and 9.1% (95% CI 5.8%, 12.5%) from western Canada ($P = 0.02$). These values were substantially lower than those based on existing criteria for less than adequate for the same 2019 data (Table 3). There was substantial variation between herds in the percentage of cows classified as below the lower limit of the 80% reference interval for serum copper both before (ICC 0.708, 95% CI 0.574 to 0.814) and after accounting for regional differences (ICC 0.688, 95% CI 0.550 to 0.799).

Using the 80% reference interval for serum selenium (Table 4) established with data from 2014 and 2016, there were 24% (95% CI 20, 27%) cows that were below their age-specific appropriate lower 10% limit for selenium in the cross-Canada 2019 data after accounting for clustering by herd; 43% (95% CI 33, 53%) from eastern Canada and 15% (95% CI 11%, 19%) from western Canada ($P < 0.001$). These values were substantially greater than those based on existing criteria for less than adequate for the same 2019 data (Table 3). There was substantial variation between herds in the percentage of cows classified as below the 80% reference interval for serum selenium both before (ICC 0.827, 95% CI 0.745 to 0.886) and after accounting for regional differences (ICC 0.782, 95% CI 0.693 to 0.851).

Using the age-specific upper 90% reference limit for serum molybdenum (Table 4) established with data from 2014 and 2016, there were 14% (95% CI 9.5, 19%) cows that were above the reference limit for molybdenum in the cross-Canada 2019 data after accounting for clustering by herd; 12% (95% CI 5.4, 20%) from eastern Canada and 15% (95% CI 10, 21%) from western Canada ($P = 0.52$). These values were not substantially different than those based on existing criteria for the same 2019 data (Table 3). There was substantial variation between herds in the percentage of cows classified as above the 90% reference limit for serum molybdenum (ICC 0.888, 95% CI 0.810 to 0.936).

Discussion

This report summarizes the first field studies of micronutrients in beef cattle in western Canada since 2008 (Van De Weyer et al., 2010, 2011) and the first to include herds from across Canada in a single study. The surveillance network provides snapshots of the trace mineral status of beef cows in British Columbia, Alberta, Saskatchewan, and Manitoba in three different years and contrasts to data from Ontario, Quebec, and the Maritimes in a single year. The most common deficiency identified was copper. While low selenium affected far fewer cows than low copper, selenium deficiency was more likely in cows sampled from eastern Canada versus western Canada.

Copper deficiency was slightly more common in western Canada than in the east, but impacted at least one tested animal in more than 82% of herds regardless of location. Copper deficiency as based on the current laboratory reference levels (Puls, 1994) was identified in 26% of more than 1,629 cows sampled from 163 herds in 2019. This is likely an underestimate of the true extent of copper deficiency in these herds as serum samples are not considered a sensitive indicator of low copper status; plasma status has been reported to not drop significantly until liver concentrations fall below 40 mg/kg dry matter (Spears et al., 2022). Greater than recommended concentrations of molybdenum were present in more than 14% of 2019 serum samples. While serum copper varied slightly across the country, there appeared to be specific geographic locations with high molybdenum concentrations and further elevated risks of secondary copper deficiency. This study did not have information on water quality and the role of sulfates in water or feed which could have played a role in secondary copper deficiency. Water quality is becoming an increasing challenge in many areas of western Canada associated with recurring droughts and reliance on surface water in many summer pastures. Evaporative losses with repeated cycles of drought have increased total dissolved solids concentrations in these water sources, often leading to reduced mineral consumption and risk of primary copper deficiency as well as increased sulfate concentrations and associated secondary copper deficiency.

The very high prevalence of cows with less than adequate serum copper in this study was consistent with a previous Saskatchewan study that reported 66% of cows were less than adequate (<0.6 ppm) in the fall of 2008 (Van De Weyer et al., 2010). Both the current findings and those from 2008 were considerably higher than the 16% of 781 cows from 66 herds that were less than adequate for copper status in the fall of 2001 (Van De Weyer and Waldner, 2011). The 13% of cows reported in the present study as having high molybdenum concentrations, however, was very similar to the 12% reported in each of the previous studies (Van De Weyer et al., 2010; Van De Weyer and Waldner, 2011).

Selenium deficiency was less common with less than 2% of all cows classified as deficient across the country in 2019. The risk of selenium deficiency decreased substantially in western Canada for the period between 2014 and 2019 and as compared to 11% of deficient cows and 87% that were less adequate reported for 781 cows from 66 herds in 2001 (Waldner and Van De Weyer, 2011). However, a third of cows in western Canada and three quarters of cows in eastern Canada were classified as below the level considered to be adequate suggesting continuing opportunities for improvement in many regions. While much of the change in western Canada could be due to improved supplementation practices, the 2001 survey did include relatively more herds in the higher-risk region from western Alberta (Waldner and Van De Weyer, 2011) visible on the maps generated in the current study.

The differences in serum selenium concentrations and copper concentrations between eastern and western Canada suggest the importance of differences in feed and water quality, as well as potential differences in supplementation practices. Soil type alone did not explain the geographic variability in the results within western Canada, particularly for copper. In western Canada, selenium concentrations from 2014 and 2016 were slightly lower on black and grey soils than on brown soils, and molybdenum concentrations were higher. The results support previous findings that low selenium concentrations were most common on black or grey soil types (Waldner and Van De Weyer, 2011), and high molybdenum had previously been associated with grey and dark grey soils (Van De Weyer and Waldner, 2011). The lack of association between serum copper concentrations and soil type is also consistent with previous reports from western Canada (Van De Weyer et al., 2010; Van De Weyer and Waldner, 2011). Soil type does not account for purchased hay and trucking cattle to more remote pastures particularly in drought years that have been common in western Canada, further emphasizing the importance of evaluating animal copper status.

In the present study, serum selenium concentrations were higher at pregnancy testing in herds that were supplemented in the period prior to sample collection. There was, however, no association between reported trace mineral supplementation in the fall and serum copper concentrations at pregnancy testing for 2014 and 2016 in western Canadian herds. Because most herd owners reported providing supplement during the grazing season, the power to address this question was limited. Previous studies had reported supplementation during summer pasture was not associated with fall serum copper and selenium concentrations (Van De Weyer and Waldner, 2011; Waldner and Van De Weyer, 2011). These earlier studies were limited by lack of data on supplementation in the period immediately before sample collection. In the present study, that information was available.

While participants did provide information on the presence or absence of supplementation, there was no practical option to collect information on the amount of supplement provided or consumed by the cattle. Regardless, the lack of associations between reported free choice supplementation and serum copper concentration is not necessarily surprising. Consumption is highly variable and does not consistently increase serum micronutrient concentrations (Manzano et al., 2012; Hendrick, 2013; Patterson et al., 2013; Ranches et al., 2021). This is not to say that provision of supplement is not effective, just that it is difficult to consistently measure its effectiveness under field conditions. We do have evidence that the provision of supplement before calving was associated with higher serum copper concentrations at the start of the breeding season in one study (Van De Weyer et al., 2010). The differences in serum concentrations of copper and selenium based on month of sample collection observed in the present study are consistent with an improvement in trace mineral status prior to calving that would be expected based on reported supplementation practices.

All herds provided trace mineral supplements before calving; however, not all herds provided trace mineral supplements during the breeding season. Measured amounts of supplement were provided mixed with grain or silage by less than half of herd owners. Very few herds provided supplements for vitamin A or E. The advantages of direct feeding measured amounts of trace minerals as compared to free-choice feeding are probably best determined using controlled clinical trials. In an experimental trial of 169 cows and bred heifers in a Saskatchewan research herd randomized to compare trace mineral force-fed in silage vs. free choice before and after calving, cows that were fed free-choice pre-calving were 6 times more likely to be below adequate levels of copper at calving than cows in the force-fed group (Hendrick, 2013). Cows force-fed mineral before calving subsequently conceived 5 d earlier than free-choice fed cattle.

Finally, other animal and herd-level factors including supplementation management were examined to find ways to more effectively target producer education and recommendations to optimize intake. Neither herd size nor commercial as compared to purebred herd type were predictors of trace mineral status. BCS was not associated with either serum copper or selenium concentrations in this sample, although the study in 2001 found lower serum selenium in cows that were thin at the time of sample collection (Waldner and Van De Weyer, 2011). While serum copper concentrations were higher in young cows, both selenium and molybdenum concentrations were lower in young cows. One explanation might be that cows preferentially sequester copper in the fetus during pregnancy (Smart et al., 1992). Multiparous cows might, therefore, be at higher risk of low copper status particularly in the fall at the time of pregnancy testing. The other possibility is that the older cows with lower copper are somehow more resilient to the impacts of low copper on reproductive performance and are the survivors of the resulting selective culling pressure. A previous study examining the association between serum copper and pregnancy outcomes found associations between copper concentrations and lower pregnancy rates, but only in younger cows (Van De Weyer et al., 2011).

One limitation of this study was that not all of the herds participating in sample collection and analysis completed the management questionnaire detailing supplementation practices. There was also a potential for errors in the classification of soil color as participating herds were mapped to closest town rather than the actual herd locations to maintain participant confidentiality. However, the results are consistent with early studies that tracked individual cow locations and used actual pasture locations (Van De Weyer and Waldner, 2011; Waldner and Van De Weyer, 2011).

Another limitation is that serum concentrations are not an optimal measure of a cow's trace mineral status. Liver tissue levels provide a more sensitive indication of trace mineral status when compared to serum, particularly for copper status (Herdt and Hoff, 2011; Spears et al., 2022). However, submitted liver biopsies can sometimes be very small in weight, limiting the capacity of the lab to run duplicate analyses and impacting their reliability. For selenium, whole blood can also provide a better long-term indication of animal status than serum (Waldner et al., 1998). However, serum is a more practical option for collecting and shipping large numbers of samples under field conditions for large-scale surveillance projects in beef herds spread across large geographic regions. While low serum concentrations can be masked by recent dietary intake (Spears et al., 2022), low serum concentrations are a specific indicator of low animal trace mineral status and have been associated with health outcomes in large field studies (Van De Weyer and Waldner, 2011; Waldner and Van De Weyer, 2011). If anything, using serum to measure copper status will underestimate the true extent of the problem. Further, as the serum samples in the present study were acid digested prior to analysis, copper functionally bound in thiomolybdates would be reflected in the analysis potentially over representing the copper available to the animal (Herdt and Hoff, 2011). The serum samples from this study suggest that the problem is widespread and there are some areas where herds are more likely to be more seriously affected. The interpretation of serum data is, nevertheless, complicated by a lack of recent data informing thresholds for identifying deficiencies.

The present paper reported 80% reference intervals with lower 10% and upper 90% limits for copper and selenium and the upper 90% reference limits for molybdenum to be consistent with the metrics previously reported by Herdt and Hoff (2011) that included the 10th and 90th percentiles. However, given differences from previously reported cut points for adequate concentrations reported in Puls (1994), the reference used by many diagnostic laboratories in Canada and the United States, the 95% and 90% reference intervals and 97.5% and 95% reference limits were also estimated and provided in the Supplementary data. Reference ranges based on healthy randomly selected animals should provide a baseline for defining a range of adequate concentrations (Herdt and Hoff, 2011). The previously reported cutoffs for less than adequate copper (0.60 ppm) and selenium (0.08 ppm) (Puls, 1994) most closely approached but were higher than the lower 80% reference intervals (or 10th percentiles) for serum copper and selenium estimated from the present data. The cutoff for deficient serum copper (0.50 ppm) also exceeded the 10th percentile; however, the cutoff for deficient serum selenium (0.025 ppm) was less than 10th percentile. The 90% reference limits (or 90th percentile) identified in the present data very closely matched the cutoff for high molybdenum concentrations reported by Puls (1994).

Herdt and Hoff (2011) also reported reference ranges bounded by the lower 10th percentiles for serum copper (0.6 ppm) and selenium (0.065 ppm) and an upper limit reference for molybdenum of 0.035 ppm from a mixed breed data set of 1,386 adult animals. While the serum selenium cut point was very similar to that reported for mature cows in the present study, the value for serum copper in the present study was much lower and the value for molybdenum was higher. There was no information on the proportion of dairy animals included or range of age of the animals or current diet (total mixed ration as compared to grazing or other forage ration with or without supplement). The present study has the advantage that only visibly healthy pregnant cows in good body condition and that subsequently delivered a live calf with no retained placenta contributed to the dataset. Ideally, the data from the present study might have been compared to liver concentrations and included follow-up on calf health and performance, but that was not practical given the cost and logistical constraints of the surveillance network.

Optimum reference values for assessing trace mineral concentrations, whether from serum or liver, would be determined based on epidemiologic data demonstrating increased risks of adverse health and productivity outcomes. While the previous study from the primary author in a different group of beef cows from western Canada identified serum copper values of < 0.4 ppm prior to breeding as being most strongly associated with the risk of nonpregnancy (Van De Weyer et al., 2011), the increase in risk was not uniform across all age groups. The value of 0.4 ppm reported in this 2008 study varies little from the lower 80% reference interval of 0.379 ppm reported in the present study. There are very few other studies that document risk-based cutoffs for measures of trace mineral status in beef cattle and cutoffs would be necessary for each specific health or productivity outcome of interest.

In summary, the present study provides an update on the status of important serum micronutrients in Canadian beef cows. The samples were collected at pregnancy testing and low values detected at this time do provide an opportunity to enhance supplementation prior to calving. Age differences were identified for both serum selenium and molybdenum concentrations with young cows having lower concentrations than older cows. Serum copper concentrations were, conversely, slightly higher in younger cows. Serum copper concentrations were slightly higher in eastern Canada and serum selenium was substantially higher in western Canada. Care should be taken to ensure appropriate trace mineral supplementation particularly in younger cows due to the tendency to have lower selenium and increased susceptibility to the impacts of low copper on reproductive performance. Higher-risk areas for low selenium, low copper, and high molybdenum were identified based on both regional comparisons and mapping.

Individual animal data on age, BCS, pregnancy status, and calving outcomes allowed identification of 2,406 beef cows from western Canada that were used to estimate reference intervals for serum copper and selenium. The resulting reference interval for copper was lower than those previously reported; however, the estimated values were the first that were specifically applicable to extensively managed beef animals from this geographic region and were almost identical to the value previously associated with an increased risk of nonpregnancy in beef cows. The reference interval for selenium matched closely to that reported in another study using similar methods and the reference limits for molybdenum were equivalent to those used in most diagnostic laboratories.

Supplementary Data

Supplementary data are available at *Translational Animal Science* online.

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Conflict of Interest Statement

The authors declare no known conflicts of interest.

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