

Serum micronutrient concentrations in beef cows before and after the summer grazing season

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Van De Weyer, L. M., Hendrick, S. and Waldner, C. L. 2010. **Serum micronutrient concentrations in beef cows before and after the summer grazing season.** *Can. J. Anim. Sci.* **90**: 563–574. Serum concentrations of Cu, Mn, Mo, Se, vitamin A, and vitamin E were measured at the start and at the end of the summer grazing season in an observational field study of 791 commercial beef cows from 40 cow-calf herds from five community pastures. Copper and vitamin A were higher in the fall ($P < 0.001$), and Mo ($P < 0.001$) and selenium ($P < 0.001$) were lower in the fall compared with the spring. Cows aged 10–14 yr had lower concentrations of vitamin A ($P < 0.001$) and vitamin E (spring: $P = 0.010$; fall: $P < 0.001$) than cows aged 4–9 yr. Cows with thin pre-breeding body condition scores were more likely to have lower pre-breeding Cu concentrations than cows that were not thin ($P = 0.044$). Cows that were thin at the end of the grazing season were more likely to have lower vitamin A concentrations at this time than cows that were not thin ($P = 0.033$). Trace mineral supplementation given before calving was associated with higher spring concentrations of Cu ($P = 0.007$). Season of sample collection and the physiological status of the animals should be considered when interpreting the serum micronutrient status of beef cows and developing supplementation programs.

Key words: Cattle, beef, micronutrients, grazing, nutrition, epidemiology

Van De Weyer, L. M., Hendrick, S. et Waldner, C. L. 2010. **Concentration d'oligoéléments dans le sérum des vaches de boucherie avant et après la paissance estivale.** *Can. J. Anim. Sci.* **90**: 563–574. Les auteurs ont dosé la concentration de Cu, de Mn, de Mo, de Se, de vitamine A et de vitamine E dans le sérum de 791 vaches de boucherie commerciales au début et à la fin de la période estivale de mise à l'herbe. Les animaux venaient de 40 troupeaux de naissance broutant dans 5 pâturages communautaires. La concentration de cuivre et de vitamine A est plus élevée à l'automne ($P < 0,001$) qu'au printemps, alors que celle de Mo ($P < 0,001$) et de sélénium ($P < 0,001$) est plus faible. La concentration de vitamine A ($P < 0,001$) et de vitamine E (printemps — $P = 0,010$, automne — $P < 0,001$) est plus basse chez les vaches de 10 à 14 ans que chez celles de 4 à 9 ans. Les vaches au corps mince avant l'accouplement sont plus susceptibles d'avoir une plus faible concentration de Cu avant la saillie que les vaches plus enveloppées ($P = 0,044$). Les vaches maigres à la fin de la période de paissance sont plus enclines que les autres à présenter une concentration inférieure de vitamine A ($P = 0,033$). L'administration d'un supplément d'oligoéléments avant le vêlage présente un lien avec la concentration supérieure de cuivre relevée au printemps ($P = 0,007$). On devrait tenir compte du moment où les échantillons sont prélevés et de l'état physiologique de l'animal avant d'interpréter le bilan des oligoéléments dans le sérum des vaches de boucherie et d'élaborer un plan de supplémentation.

Mots clés: Bovins, bœuf, oligoéléments, paissance, nutrition, épidémiologie

Micronutrients, such as Cu, Mn, Se, vitamin A, and vitamin E, are important for maintaining cow health and maximizing reproductive efficiency. Although below-adequate serum concentrations of these trace minerals and vitamins have been reported in beef cattle throughout North America (Campbell et al. 1995; Dargatz and Ross 1996; Dargatz et al. 1999; Hoff et al. 2001), few scientific studies have examined to what extent serum micronutrient concentrations in beef cows differ according to the season, the animal's physiologic state, herd management, and pasture conditions during the grazing season.

Fluctuations in beef cow serum micronutrient concentrations are expected given the seasonal differences that encompass the cow-calf production cycle in western Canada. Most extensively managed beef cows are bred in late spring and early summer during the first part of the grazing season, and cows remain on pasture with their calves until plant growth stops in the fall. During the winter, through gestation and calving, these animals are typically fed stored forages. The physiologic state of an individual animal, including age, body condition, and pregnancy status, could also potentially affect micronutrient concentrations by altering micronutrient absorption, distribution, or metabolism.

Cow-calf herd management varies by the demands of the production cycle as well as the season. Trace minerals and vitamins are typically easier to effectively supplement during the winter feeding period, due to the

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accessibility of the animals and the opportunity to incorporate oral supplements with feed. Furthermore, because stored feed has substantially lower concentrations of vitamins A and E than fresh forage, animals receive less of these micronutrients in unsupplemented winter rations (Block and Farmer 1987; Hidioglou et al. 1994). These herd management factors may have more influence on the micronutrient concentrations of cows at the start of the grazing season.

The pasture conditions, including pasture water quality and meteorological conditions are more likely to affect fall-measured micronutrient concentrations. The quality of water sources available to cattle on pasture could influence the availability and adsorption of particular micronutrients, notably Cu, through the grazing period. Precipitation and temperature during the growing season influence plant growth, and could affect the micronutrient concentrations in grazed forages or the consumption patterns of animals.

Available studies examining the effect of these factors on beef cow micronutrient concentrations under western Canadian conditions are limited. One report has observed that beef cow serum Cu concentrations are lowest in late winter and highest in fall (Smart et al. 1992). Although several studies have examined associations between season and Se and vitamin status in dairy cows (Ropstad et al. 1988; Miller et al. 1995; Katamoto et al. 2003; Wichtel et al. 2004), the differences in feeding and management of dairy and beef animals make it difficult to generalize these results to cow-calf herds.

A few studies have examined the associations between age, body condition and pregnancy status on Cu concentrations in small numbers of beef cattle (Smart and Christensen 1985; Gooneratne and Christensen 1989; Littledike et al. 1995). However, the effect of an animal's physiologic parameters and Mn, Mo and Se concentrations has not been reported in the literature, and the effect of age on vitamin A and vitamin E has only been reported for dairy animals (Katsoulos et al. 2005).

Researchers have examined the effect of supplementing trace minerals on serum Se and Cu concentrations in beef cows measured after the summer grazing season (Campbell et al. 1995; Dargatz and Ross 1996; Dargatz et al. 1999). However, there were no identified reports of the effects of supplementation on serum micronutrient concentrations measured before the start of the breeding season.

Although geographic differences in the Cu and Se status of beef cattle have been reported (Gooneratne and Christensen 1989; Campbell et al. 1995; Hintze et al. 2002), the effects of differences in water quality and meteorological conditions between grazing pastures have not been examined in western Canada. Sulphate is a frequent contaminant of livestock water sources in the Canadian prairies and north-central regions of the United States. High iron concentrations are also common, particularly in deep well water (Gould et al. 2002; Olkowski 2009). High dietary levels of

sulphate, Mo, and iron adversely affect the absorption, utilization, and excretion of Cu in ruminants, potentially resulting in secondary Cu deficiency (Suttle 1991; Gooneratne et al. 1994).

Understanding what factors are linked to serum micronutrient status would improve our ability to interpret measured serum micronutrient concentrations and target supplementation programs for animals most at risk for below-adequate micronutrient concentrations. Based on this, the three objectives of this study were: first, to examine serum micronutrient concentrations for paired samples collected before and after the summer grazing season; second, to examine cow-, herd-, and pasture-level risk factors associated with micronutrient concentrations before the summer grazing season and; third, to examine the cow-, herd-, and pasture-level risk factors associated with micronutrient concentrations after summer grazing.

MATERIALS AND METHODS

Study Population and Community Pastures

Forty study herds were recruited in February and March 2008 from five southern Saskatchewan community pastures. The community pastures involved in this study were federally owned and managed rangelands that provide grazing and breeding service to beef cattle producers for a fee between May and October of each year (Anonymous 2007a). On-site management of each community pasture is provided by federally employed pasture managers and seasonal labour.

The selected community pastures represented a range of ecosystems (Smith and Marshall 1995) and soil types (Anonymous 2008). Two of the pastures were located in the Moist Mixed Grassland ecoregions, two in the Mixed Grassland, and one in the Aspen Parkland ecoregion. The reported soil types for each pasture were as follows: two Brown Chernozemic, one Dark Brown Chernozemic, one Dark Brown Solonchic, and one Black Chernozemic. The forage on community pastures is composed of tame and native perennial mixed-species grasses. The median distance between these five community pastures was 250 km (range 75 to 550 km).

Researchers presented the objectives of the study and requirements for participation to the herd owners who use each of the local community pastures. Study participation rates of the patrons that use each of the pastures was as follows: Pasture 1, 17.6% (9/51 patrons); Pasture 2, 26.7% (8/30 patrons); Pasture 3, 14.5% (10/69 patrons); Pasture 4, 35.8% (8/23); and Pasture 5, 18.5% (5/27 patrons). Each of the 40 recruited herd owners placed an allotment of cows on the community pastures closest to their home location in the spring of 2008.

Serum Sample Collection

The size of each herd's allotment was unknown before sample collection; therefore, the first 20 cows

through the chute were selected for sampling from each participating herd on arrival at the pasture in May 2008. If the herd owner sent fewer than 20 cows to pasture then their entire allotment of cows was sampled. Ear tag identification was recorded to uniquely identify each animal; if the producer tag was insufficient, researchers supplied a uniquely numbered ear tag for the animal. Individual animal identification was linked to serum samples through the use of a number code duplicated on the serum tubes and the field record sheets. Animals sampled in May were identified and re-sampled as they were moved off pasture in October 2008.

Three full 10-mL red top vacutainer tubes without anticoagulant (Becton-Dickinson, Franklin Lakes, NJ) of blood were collected from the jugular vein of each cow. The blood was allowed to clot at room temperature and then refrigerated at 4°C. The serum was separated and frozen at -20°C within 24 h of collection. Serum samples were frozen to -70°C within 7 d of collection and stored at this temperature until analysis for micronutrient concentrations. Blood and serum samples were protected from light during transport and storage.

Trace Mineral Analysis

Serum Cu, Mn, Mo, and Se concentrations were measured by a commercial laboratory (Prairie Diagnostic Laboratory, Saskatoon, SK). The trace mineral concentrations were determined following wet digestion in concentrated nitric acid in a pressurized microwave, using the Microwave Accelerated Reaction System-Xpress system (CEM Corporation, Matthews, NC). One millilitre of serum was placed in the appropriate digestion vessel, then 2.5 mL concentrated nitric acid and 2.5 mL of de-ionized distilled (DD) water were added. The samples were placed into the microwave for 15 min at 120°C and 830 kPa. Digested samples were transferred to a 25-mL volumetric flask. The flasks were brought to 25 mL with DD water, covered and mixed. Trace mineral quantification was performed immediately following acid digestion by analyzing samples using an inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Jarrel Ash Corporation, Franklin, MA). All samples were analyzed in duplicate, where sufficient serum was available.

Vitamin A and E Analysis

Serum vitamin A (retinol) (Milne and Botnem 1986) and vitamin E (α -tocopherol) (Catignani and Biere 1983) concentrations were determined by high-pressure liquid chromatography at a commercial laboratory (Prairie Diagnostic Laboratory, Saskatoon, SK). Extraction and analysis of individual vitamins was conducted separately, but the procedure was identical. One millilitre of serum was added to a 15-mL glass-stoppered centrifuge tube along with 1 mL of a 1% solution of bovine serum albumin (Calbiochem, La Jolla, CA), 1.6 mL of ethanol, and 0.4 mL of internal standard (retinyl acetate at a concentration of 0.4 $\mu\text{g mL}^{-1}$ or

α -tocopherol acetate at a concentration of 20 $\mu\text{g mL}^{-1}$). The sample was mixed using a vortex for 10 s, and a 4-mL aliquot of petroleum ether was added. The mixture was vortically mixed for another 45 s and centrifuged at 550 $\times g$ for 5 min. Three-quarters of the ether phase was transferred to a clean 12 \times 75 mm glass tube and evaporated to dryness under air. The residue was dissolved in 500 μL filtered high pressure liquid chromatography grade methanol, and vitamin was detected by high pressure liquid chromatography using a 5 μm UltrasphereTM ODS column (International Equipment Co., Needham, MA) and a fluorescent detector at 325 or 285 nm for vitamins A and E, respectively. Samples and standards were protected from light at all times.

Participating Cow and Herd Data

Detailed information was requested for each enrolled cow including: age, calving date, and any history of exposure to bulls in 2008 prior to the start of the community pasture breeding season.

Researchers assessed and recorded body condition score for each cow when the animals arrived at the community pasture in May and during removal of the animals from each pasture in October. Cows were condition scored using a scale from 1 to 5 (Marx 2009), where 1 corresponds with 1 (emaciated animal) on the more commonly published 9-point scale for beef cattle (Rice 1991), and the scale subsequently increases in 0.5 point increments so that 5 corresponds with 9 (obese animal).

Each herd owner completed a written survey in June 2008 documenting their herd size and trace mineral and vitamin supplementation program for the winter of 2007–2008.

In October 2008, the enrolled cows were trans-rectally palpated (L.V. and S.H.) to determine their pregnancy status. In four of the five pastures there was a minimum of 40 d between bull removal and the date of pregnancy determination. In one of the five pastures, the bulls were removed from the breeding field 14 d prior to the pregnancy examination.

Researchers also assessed cow dentition in October to classify the age of animals with missing age records from the producer (Anonymous 2007b). Five herds had all or some cows aged in this manner.

Pasture Data

The pasture managers completed a written survey in November 2008 documenting details of management on all breeding fields with enrolled cows, including: bull breed, bull identification, bull and cow numbers, breeding season dates, breeding field size and location, the numbers and types of water sources, and the availability of trace mineral blocks for each breeding field with enrolled cows.

The primary water sources on each grazing field with enrolled cows were sampled by the pasture managers in

July 2008. Water sample containers and sample collection procedures were provided by the laboratory that performed the water analysis (Saskatchewan Research Council Analytical Laboratory, Saskatoon, SK). Sulphate and iron concentrations in water samples were measured using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Thermo Jarrel Ash Corporation, Franklin, MA). When two or more water sources were sampled from the same breeding field, the mean sulphate and mean iron concentrations were used as a composite measure for the breeding field.

Data from Environment Canada weather stations closest to each community pasture (Bratts Lake, Val Marie, Wynyard, and Weyburn) were accessed to measure cumulative precipitation amounts from 2008 Apr. 01 to Oct. 31 for each pasture (Anonymous 2010). Two of the five pastures were located near the same Environment Canada weather station and the same data were used for both of these pastures.

Statistical Analysis

Study data were described for individual animals and herds using a commercial software program (MLwiN, version 2.11, University of Bristol, Bristol, United Kingdom). The unconditional associations between serum Mo concentrations (high or low) and serum Cu concentrations (above or below adequate) for spring and fall samples were examined using published cut-off values for Mo and Cu (Puls 1994a)

and generalized linear mixed models with a binomial distribution, logit link function, and random intercepts to account for clustering of cows within herds and pastures and repeated measures on individual animals.

The primary study objectives were then addressed by examining a series of factors potentially associated with each micronutrient concentration using generalized linear mixed models with a normal distribution, identity link function, and random intercepts to account for grouping of cows within herds and pastures and repeated measures as necessary (Table 1) (Dohoo et al. 2003).

The potential impact of season on micronutrient concentrations was evaluated by determining if there was a difference between spring and fall values for paired serum samples (Objective 1), after accounting for repeated measures on individual animals and clustering of outcomes by herd and pasture groups using random intercepts (Table 1).

The factors associated with spring micronutrient concentrations were examined after accounting for clustering within herd and pasture (Objective 2). Researchers first estimated the associations between age and spring body condition for each serum micronutrient concentration measured in the spring. Next the associations between trace mineral and vitamin supplements given to herds the winter before the 2008 grazing season and each spring micronutrient concentration were described (Table 1).

Data from the final spring models were used to calculate the proportion of remaining variance

Table 1. Summary of the potential risk factors examined for association with each serum micronutrient concentration for the three primary study objectives

	Outcome of interest for each objective		
	Objective 1: Paired micronutrient concentrations	Objective 2: Spring micronutrient concentrations	Objective 3: Fall micronutrient concentrations
Risk factors considered in each series of models			
<i>Season</i> (spring, fall)	x		
<i>Physiologic parameters</i>			
Age category (1–3 yr, 4–9 yr, 10–14 yr)		x	x
Body condition score (<2.5/5, ≥2.5/5)			
Spring		x	x
Fall			x
Pregnancy status (pregnant, not pregnant)			x
<i>Herd management</i>			
Pre-calving trace mineral supplement (Y, N)		x	
Post-calving trace mineral supplement (Y, N)		x	
Pre-calving vitamin supplement (Y, N)		x	
Post-calving vitamin supplement (Y, N)		x	
Injectable vitamin A (Y, N)		x	
Injectable vitamin E and selenium (Y, N)		x	
<i>Pasture conditions</i>			
Sulphate concentration in pasture water (mg L ⁻¹) ^z			x
Iron concentration in pasture water (mg L ⁻¹) ^z			x
Cumulative precipitation, Apr. 01 to Oct. 31(mm) ^y			x
Trace mineral supplementation on pasture (Y, N)			x

^zSulphate and iron concentrations were each categorized as quartiles of the measured values.

^yCumulative precipitation was categorized using the four measured values from the five pastures.

in serum micronutrient concentrations explained within herd ($\rho_h = \sigma_h^2 / (\sigma_c^2 + \sigma_h^2 + \sigma_p^2 + \pi^2/3)$) and then within pasture ($\rho_p = \sigma_p^2 / (\sigma_c^2 + \sigma_h^2 + \sigma_p^2 + \pi^2/3)$) (Dohoo et al. 2003).

Objective 3 was examined as for Objective 2, but the outcomes of interest were fall-measured serum micronutrient concentrations and the variables of interest were risks measured during the grazing season that could potentially affect fall micronutrient concentrations (Table 1). Pasture water concentrations of sulphate and iron and cumulative precipitation received by each pasture were examined as categorical variables. Researchers examined the linearity assumption of these variables by looking for an increasing (or decreasing) series of estimates as compared with the referent (lowest) category. Estimates that suggested the association increased or decreased and then plateaued would also be consistent with a potentially causal association.

For Objectives 2 and 3, variables that were unconditionally associated ($P < 0.25$) with each outcome were considered for inclusion in the final multivariable linear regression model, given that no two variables entered into the same model were highly correlated (Spearman's $\rho > 0.7$). Manual backward selection was used to develop a main effects model for each micronutrient measure, retaining only variables where $P < 0.05$ or removal of the variable from the model changed the effect estimate for the exposure by $\geq 10\%$; these latter variables were retained in the model as important confounders. Biologically reasonable first-order interactions were considered after establishing a main effect model. Interaction terms were retained in the model if $P < 0.05$.

The adequacy of the models was evaluated using plots of residuals compared with predicted values. Plots were used to assess the normality of the residuals, homogeneity of variance, the importance of outliers, and to examine the impact of any influential data points.

RESULTS

Description of Participating Cows and Herds

Cows from 40 herds from five pastures participated in the study (range, 5–10 herds/pasture). Thirty-five of the 40 participating herds enrolled 20 cows each, three herds enrolled 21 cows, one herd enrolled 17 cows, and one herd enrolled 11 cows. The median size of participating herds was 133 breeding females (range, 11 to 370 females).

Blood samples for micronutrient analysis were collected from 791 mixed-breed beef cows in May 2008 and 781/791 (98.7%) of these cows in October 2008. Ten cows were missing from the fall round-up because they had died on pasture, had been removed early from pasture, or could not be located. Twenty cows from one herd (12/20 were 4–9 yr, 7/20 were 1–3 yr, and 1/20 was 10–14 yr) could not be positively identified in October due to illegible numbers on ear tags, resulting in samples

from 761 cows from 39 herds that were suitable for inclusion in the fall analysis.

Most of the cows (63%, 500/791) were 4–9 yr of age, 22% (171/791) were 1–3 yr of age, and 15% (115/791) were 10–14 yr of age. Five animals were missing an age record. Of the 10 missing cows in October, five were 4–9 yr of age and five were missing an age record. The majority of cows (83.1%, 657/791) had a moderate or good body condition score ($BCS \geq 2.5/5$) in May 2008. During the grazing season the body condition of the cows improved ($P < 0.001$) and 89% (677/761) of cows were in moderate to good body condition in the fall. In October, 781 of the 791 enrolled cows were pregnancy tested; 93% (726/781) of the cows were pregnant.

Owners provided free-choice trace mineral supplements to 83% (33/40) of the herds (651/791 cows) before calving and 58% (23/40) of the herds (394/791 cows) after calving in 2008. Additional free-choice vitamin ADE premix was provided to 10% (4/40) of herds (80/791 cows) both before calving and after calving. No herd was provided vitamin premix in the absence of a trace mineral supplement. Injectable vitamin A/D was given to 38% (15/40) of the herds (293/791 cows) at least once throughout the winter of 2007–2008. One herd of 20 cows received injectable vitamin A/D without other trace mineral supplementation. Ten percent (4/40) of herds (80/791 cows) received an injection of Se/vitamin E at least once throughout the winter of 2007–2008; these herds were also provided with oral trace mineral supplements.

Description of the Pasture Conditions

Trace mineral-fortified block mineral (containing 60 mg kg^{-1} I, 2000 mg kg^{-1} Fe, 12 mg kg^{-1} Co, 750 mg kg^{-1} Cu, 1580 mg kg^{-1} Mn, 2400 mg kg^{-1} Zn, a maximum of 600 mg kg^{-1} F, 8.7 mg kg^{-1} Se, a minimum of 140 000 IU kg^{-1} vitamin A, a minimum of 30 000 IU kg^{-1} vitamin D, and a minimum of 120 IU kg^{-1} vitamin E) was accessible to all animals on one of the five pastures. The other four pastures had iodized salt blocks, without trace mineral, available to all cows.

Thirty-one primary water sources from the five pastures with enrolled cows were sampled in July 2008. The median sulphate concentration of the water samples was 167 mg L^{-1} (range, 1.3 to 1755 mg L^{-1}) and the median iron concentration of the water samples was 0.73 mg L^{-1} (range, 0.06 to 3.89 mg L^{-1}).

The mean of the total precipitation received by each pasture from 2008 Apr. 01 to Oct. 31 was 307 mm (range, 263 to 406 mm).

Serum Micronutrient Analysis

Serum Cu (range, 0.1 to 0.87 mg L^{-1}) was the micronutrient most commonly below adequate concentrations in the cows in spring samples and serum Mn (range, 2 to 47 $\mu\text{g} \text{L}^{-1}$) was the micronutrient most

commonly below adequate concentrations in fall samples (Table 2). Very few animals came on to pasture or left pasture with below-adequate concentrations of Se, vitamin A or vitamin E (Table 2).

High concentrations of Mo (≥ 0.10 mg L⁻¹) were present in 19% (143/769) of cows in the spring (Table 2). No association was found between below-adequate spring serum Cu concentrations and high spring serum Mo concentrations ($P=0.4$), while above-adequate fall Cu concentrations were associated with high fall Mo ($P=0.02$).

Comparison of Micronutrient Concentrations in Paired Serum Samples Collected in the Spring and Fall

Serum concentrations of Cu and vitamins A and E were higher in the fall and Mo and Se concentrations were lower in the fall (Table 3). Paired serum concentrations of Mn were not associated with season.

Risk Factors Associated with Serum Micronutrient Concentrations Measured Before the Start of the Summer Grazing Season (May 2008)

Each potential risk factor was examined individually (unconditionally) with each micronutrient concentration measured at the start of the grazing season. Trace mineral supplementation before calving was unconditionally associated with the serum concentrations of Cu ($P=0.007$), Mn ($P=0.012$), and Se ($P=0.048$) measured at the start of the grazing season. Spring body condition was unconditionally associated with spring serum concentrations of Cu ($P=0.046$). Age was unconditionally associated with spring serum

concentrations of vitamin A ($P<0.001$) and vitamin E ($P=0.033$). There were no risk factors for spring serum Mo concentrations with a P value <0.25 .

After adjusting for the significant unconditional risk factors together in a series of multivariable models, final models were developed for each micronutrient measured before the start of the breeding season. Serum Cu concentrations measured in May were 0.08 mg L⁻¹ higher for cows from herds supplemented with trace minerals before calving than for cows from herds not supplemented before calving, and 0.02 mg L⁻¹ lower in thin cows than in cows with moderate to good condition (Table 4). In the final model, between-herd differences accounted for 24.5% of the remaining variation in spring serum Cu concentrations and between-pasture differences accounted for 9.6%.

Cows from herds that were supplemented with trace minerals before calving had lower serum concentrations of Mn and Se at the start of the grazing season than cows from herds which were not supplemented (Table 4). In these final models, between-herd differences accounted for 8.2% of the remaining variation in spring serum Mn and 59.3% of spring serum Se concentrations and between-pasture differences accounted for 15.1% of Mn and 1.5% of Se.

After considering the other significant risk factors, both vitamin A and vitamin E were lower at the start of the grazing season in 10- to 14-yr-old cows than in 4- to 9-yr-old cows (Table 4). Vitamin A concentrations in 1- to 3-yr-old cows were higher than in 4- to 9-yr-cows, but there was no difference between the spring vitamin E serum concentrations of these same age groups. Spring vitamin A and vitamin E concentrations were higher in

Table 2. Proportion of beef cows with lower-than-adequate copper, manganese, selenium, vitamin A, and vitamin E concentrations and higher-than-adequate molybdenum concentrations and the distribution of the serum micronutrient concentrations.

Micronutrient	Lowest serum concentration considered adequate ^z	Season	No. of cows (%) below adequate concentration	Percentiles of serum micronutrient concentration				
				5th	25th	50th	75th	95th
Copper (mg L ⁻¹)	0.60	Spring	586/790 (74)	0.29	0.43	0.52	0.59	0.72
		Fall	506/761 (66)	0.40	0.49	0.55	0.62	0.73
Manganese (μ g L ⁻¹)	6	Spring	527/789 (67)	2	3	4	6	10
		Fall	561/761 (74)	3	4	5	6	8
Selenium (mg L ⁻¹)	0.08	Spring	4/789 (<1)	0.11	0.13	0.15	0.18	0.23
		Fall	54/761 (7)	0.08	0.10	0.13	0.15	0.17
Vitamin A (mg L ⁻¹)	0.30	Spring	99/789 (13)	0.26	0.34	0.42	0.51	0.69
		Fall	14/760 (2)	0.33	0.41	0.46	0.53	0.61
Vitamin E (mg L ⁻¹)	4.0	Spring	46/790 (6)	4.05	5.75	7.63	9.70	13.1
		Fall	0/759	7.52	9.23	10.60	12.4	15.2
Micronutrient	Highest concentration considered adequate ^z	Season	No. of cows (%) above adequate concentration	Percentiles of serum micronutrient concentration				
Molybdenum (mg L ⁻¹)	0.10	Spring	146/789 (19)	0.004	0.010	0.019	0.065	0.200
		Fall	94/761 (12)	0.006	0.014	0.027	0.053	0.114

^z(Puls 1994a, b).

Table 3. Impact of season on each serum micronutrient concentration for 761 beef cows from 39 herds accounting for repeated measures on individual cows, and herd- and pasture-effects

Micronutrient	Differences between fall and spring serum concentration measurements		
	Estimate	95% CI ^z	<i>P</i> value
Copper (mg L ⁻¹)	0.048	0.038 to 0.058	<0.001
Manganese (µg L ⁻¹)	-0.140	-0.384 to 0.104	0.26
Molybdenum (mg L ⁻¹)	-0.015	-0.020 to -0.010	<0.001
Selenium (mg L ⁻¹)	-0.031	-0.034 to -0.028	<0.001
Vitamin A (mg L ⁻¹)	0.030	0.021 to 0.039	<0.001
Vitamin E (mg L ⁻¹)	3.064	2.838 to 3.290	<0.001

^z95% CI represents the 95% confidence interval.

1- to 3-yr-old cows than in 10- to 14-yr-old cows. Between-herd differences accounted for 49.4% of the remaining variation in spring serum vitamin A and 36.3% of spring serum vitamin E concentrations and between-pasture differences accounted for 15.1% of vitamin A and 22.6% of vitamin E.

Risk Factors Associated with Serum Micronutrient Concentrations Measured at the End of the Grazing Season (October 2008)

Each potential risk factor was examined individually (unconditionally) with each micronutrient concentration measured at the end of the grazing season. Age was unconditionally associated ($P < 0.001$) with fall serum concentrations of vitamin A and vitamin E, spring body condition was unconditionally associated with fall serum concentrations of vitamin E ($P = 0.048$), and pregnancy status was unconditionally associated with

serum concentrations of manganese ($P = 0.0019$) and selenium ($P = 0.040$) measured at the end of the grazing season. The sulphate concentration in pasture water was unconditionally associated with fall serum concentrations of copper ($P = 0.015$), molybdenum ($P = 0.019$), and vitamin A ($P = 0.024$). Iron concentration in pasture water was unconditionally associated with fall serum molybdenum ($P = 0.015$) and vitamin A ($P = 0.0061$) concentrations. The cumulative precipitation received from 2008 Apr. 01 to Oct. 31 by each pasture was unconditionally associated with fall serum concentrations of selenium ($P = 0.0036$), vitamin A ($P = 0.0051$), and vitamin E ($P = 0.0028$). Trace mineral supplementation on pasture was associated with serum vitamin E concentrations ($P = 0.0045$) measured at the end of the grazing season.

After adjusting for the significant unconditional risk factors together in a series of multivariable models, final models were developed for each micronutrient measured at the end of the breeding season. Sulphate and iron concentrations in pasture water were associated with the serum Cu concentrations at the end of the grazing season (Table 5). Although sulphate concentrations were associated with an overall difference in Cu concentrations, there was no linear trend and no significant difference between the highest and lowest sulphate quartiles. Cows on pasture where the iron concentrations in the water were above the 25th percentile had lower fall serum Cu concentrations than cows exposed to lower iron concentrations; however, no apparent linear trend was observed. The proportion of random variability in the final model for fall Cu concentrations explained by between-herd differences

Table 4. Final multivariable model estimates of the impact of exposure to cow-level and herd-level variables on serum micronutrient concentrations measured in the spring for 791 beef cows in 40 herds after accounting for herd and pasture effects

Factors associated with micronutrient concentrations measured in the spring	Estimate	95% CI ^z	<i>P</i> value
<i>Copper (mg L⁻¹)</i>			
Thin spring BCS (<2.5/5.0)	-0.023	-0.045 to -0.001	0.044
Trace mineral supplementation before calving	0.079	0.022 to 0.136	0.007
<i>Manganese (µg L⁻¹)</i>			
Trace mineral supplementation before calving	-1.052	-1.877 to -0.227	0.012
<i>Selenium (mg L⁻¹)</i>			
Trace mineral supplementation before calving	-0.023	-0.046 to 0.000	0.048
<i>Vitamin A (mg L⁻¹)</i>			
Age category (yr)			<0.001 ^y
1-3 vs. 4-9	0.017	0.000 to 0.034	0.051
10-14 vs. 4-9	-0.05	-0.068 to -0.032	<0.001
1-3 vs. 10-14	0.067	0.045 to 0.089	<0.001
<i>Vitamin E (mg L⁻¹)</i>			
Age category (yr)			0.033 ^y
1-3 vs. 4-9	-0.030	-0.422 to 0.362	0.88
10-14 vs. 4-9	-0.546	-0.96 to -0.132	0.010
1-3 vs. 10-14	0.517	0.005 to 1.029	0.048

^z95% CI represents the 95% confidence interval.

^y*P* values testing overall association between age group (2 d.f.) and micronutrient concentrations.

Table 5. Final multivariable model estimates of the impact of exposure to cow-level and herd-level variables and serum micronutrient concentrations measured in the fall for 761 beef cows in 39 herds after accounting for herd and pasture effects

Factors associated with micronutrient concentrations measured in the fall	Estimate	95% CI ^z	P value
<i>Copper (mg L⁻¹)</i>			
Sulphate concentration in pasture water source (mg L ⁻¹)			0.0051 ^y
64–166 vs. 1.3–63	0.059	–0.002 to 0.12	0.058
167–412 vs. 1.3–63	–0.046	–0.099 to 0.007	0.092
413–1755 vs. 1.3–63	–0.037	–0.067 to 0.022	0.21
Iron concentration in pasture water source (mg L ⁻¹)			<0.001 ^y
0.15–0.72 vs. 0.0157–0.14	–0.056	–0.107 to –0.005	0.032
0.73–1.15 vs. 0.0157–0.14	–0.118	–0.172 to –0.064	<0.001
1.16–3.89 vs. 0.0157–0.14	–0.073	–0.135 to –0.011	0.023
<i>Manganese (µg L⁻¹)</i>			
Not pregnant in fall	1.043	0.387 to 1.699	0.0018
Sulphate concentration in pasture water source (mg L ⁻¹)			0.046 ^y
64–166 vs. 1.3–63	–1.107	–1.987 to –0.227	0.014
167–412 vs. 1.3–63	–0.147	–1.018 to 0.724	0.74
413–1755 vs. 1.3–63	–0.998	–2.011 to 0.015	0.054
<i>Molybdenum (mg L⁻¹)</i>			
Sulphate concentration in pasture water source (mg L ⁻¹)			0.0060 ^y
64–166 vs. 1.3–63	–0.043	–0.072 to –0.014	0.0039
167–412 vs. 1.3–63	–0.037	–0.063 to –0.011	0.0051
413–1755 vs. 1.3–63	–0.042	–0.070 to –0.014	0.0030
Iron concentration in pasture water source (mg L ⁻¹)			0.0051 ^y
0.15–0.72 vs. 0.0157–0.14	0.028	0.003 to 0.053	0.031
0.73–1.15 vs. 0.0157–0.14	0.048	0.020 to 0.074	<0.001
1.16–3.89 vs. 0.0157–0.14	0.027	–0.002 to 0.056	0.065
<i>Selenium (mg L⁻¹)</i>			
Not pregnant in fall	0.011	0.000 to 0.022	0.042
Cumulative precipitation on pasture (mm)			0.0064 ^y
275.8 vs. 263	–0.013	–0.042 to 0.016	0.40
306.8 vs. 263	–0.050	–0.078 to –0.022	<0.001
406.4 vs. 263	–0.014	–0.041 to 0.013	0.87
<i>Vitamin A (mg L⁻¹)</i>			
Age category (yr)			<0.001 ^y
1–3 vs. 4–9	0.024	0.010 to 0.038	<0.001
10–14 vs. 4–9	–0.038	–0.053 to –0.023	<0.001
Thin fall BCS (<2.5/5.0)	–0.020	–0.038 to –0.002	0.033
Iron concentration in pasture water source (mg L ⁻¹)			0.0086 ^y
0.15–0.72 vs. 0.0157–0.14	0.047	0.006 to 0.088	0.026
0.73–1.15 vs. 0.0157–0.14	0.056	0.023 to 0.089	<0.001
1.16–3.89 vs. 0.0157–0.14	0.037	–0.013 to 0.087	0.14
Cumulative precipitation on pasture (mm)			<0.001 ^y
275.8 vs. 263	–0.117	–0.166 to –0.068	<0.001
306.8 vs. 263	–0.001	–0.050 to 0.048	0.97
406.4 vs. 263	–0.005	–0.036 to 0.026	0.76
<i>Vitamin E (mg L⁻¹)</i>			
Age category (years)			<0.001 ^y
1–3 vs. 4–9	–0.050	–0.413 to 0.313	0.79
10–14 vs. 4–9	–0.765	–1.147 to –0.383	<0.001
Thin spring BCS (<2.5/5.0)	0.368	0.000 to 0.764	0.059

^z95% CI represents the 95% confidence interval.

^yP values testing overall association between age group (2 d.f.) and micronutrient concentrations.

was 12.8% and the proportion explained by between-pasture differences was 10.9%.

At the end of the grazing season, non-pregnant cows had higher Mn concentrations than pregnant cows when all significant factors were adjusted for (Table 5). Cows on pastures with water sulphate concentrations between the 25th and 50th percentile also had lower serum Mn measured after the grazing season than cows on pastures

with concentrations less than the 25th percentile; no other significant differences were observed. Between-herd differences accounted for 3.7% of the remaining variation in serum Mn at the end of the grazing season and between-pasture differences accounted for 15.5%.

At the end of the grazing season, cows on pastures with sulphate concentrations in sampled water sources above the 25th percentile had the lowest serum

Mo concentrations (Table 5); however, there was no clear linear trend. Cows pastured where water iron concentrations were between the 25th and 75th percentile had higher Mo concentrations than those below the 25th percentile, but there was no difference between the highest and lowest quartile. The proportion of random variability in the final model for fall Mo concentrations explained by between-herd differences was 33.3% and the proportion explained by between-pasture differences was 14.4%.

After considering other factors, Se was higher in the fall in non-pregnant cows compared with the pregnant cows (Table 5). Cows on pastures with cumulative precipitation up to 263 mm had lower fall serum Se concentrations than cows on pastures that received 307 mm, the third highest level of precipitation. There was, however, no difference between cows in the pastures that received the highest and lowest recorded amounts of precipitation. The proportion of random variability in the final model for fall Se concentrations explained by between-herd differences was 18.1% and the proportion explained by between-pasture differences was 4.4%.

Age, fall body condition, iron concentration in pasture water, and cumulative precipitation on pasture were all associated with serum vitamin A concentrations measured at the end of the grazing season (Table 5). Vitamin A concentrations were higher in young cows than in mature cows and the concentration in mature cows was higher than in old cows. Cows that were thin in the fall had lower vitamin A concentrations than cows in moderate to good condition. Iron concentrations in pasture water between the 25th and 75th percentile were associated with higher fall serum vitamin A concentrations compared with cows from pastures with lower iron concentrations. However, there was no difference in vitamin A between cows exposed to the highest and lowest observed concentrations of iron from pasture water. Cows on pasture with a cumulative precipitation of 276 mm had lower vitamin A concentrations in October than cows on pastures that received 263 mm; however, there was no difference between pastures receiving even higher levels of precipitation and those on the driest pastures. Between-herd differences accounted for 21.0% of the remaining variation in serum vitamin A concentrations at the end of the grazing season, but between-pasture differences did not account for any of the remaining variation.

At the end of the grazing season, old cows had serum vitamin E concentrations that were lower than both mature cows and young cows (Table 5). Cows that were thin coming onto pasture in May had more vitamin E in October than cows that came onto pasture in moderate to good body condition. The proportion of random variability in the final model for fall serum vitamin E concentrations explained by between-herd differences was 39.4%, and the proportion explained by between-pasture differences was 8.2%.

DISCUSSION

This study described differences in serum concentrations of several micronutrients measured before and after the summer grazing season in beef cows and the cow-, herd-, and pasture-level risk factors associated with the trace mineral and vitamin concentrations of these animals.

The practical constraints of identifying a single sample suitable for evaluating micronutrient concentrations on a large number of privately owned cows at long distances from a diagnostic laboratory contributed to the decision to measure serum concentrations. There are, however, factors that limit the interpretation of serum measurements. Homeostatic control mechanisms can limit changes in the serum concentrations of some trace minerals, notably Cu and Mn, until liver or other endogenous reserves are depleted (Gooneratne et al. 1989; Kincaid 1999; Hansen et al. 2006). The measurement of Se in whole blood is preferred to serum by some investigators because serum selenium concentrations are more likely to be influenced by recent changes in the animal's intake than whole blood measurements (Maas et al. 1992); however, agreement between serum and whole blood Se status was observed to be very good ($\alpha = 0.79$) at the herd level and good at the individual animal level ($\alpha = 0.68$) for samples collected from beef cows in the fall of the year (Waldner et al. 1998).

Season

Seasonal differences in Cu and Se concentrations in cattle have previously been reported. Consistent with the findings of this study, Smart et al. (1992) found the lowest Cu concentrations occurred in late winter and the highest concentrations occurred in late summer under western Canadian conditions. In contrast, serum Se concentrations decreased during the grazing season in the present study. Season has previously been associated with Se concentrations in dairy cows (Ropstad et al. 1988; Miller et al. 1995; Wichtel et al. 2004), but no consensus exists on the season of lowest Se concentration. The differences in dairy cattle feeding and supplementation practices make it difficult to generalize these studies to extensively managed beef cows.

Serum Mo concentrations also decreased during the grazing season. Seasonal differences in serum Mo concentrations have not previously been documented in cattle. Since Mo concentrations in blood reflect Mo dietary intake (Ward 1978; Wittenburg and Devlin 1987), lower Mo concentrations after the grazing season than before likely indicate lower Mo concentrations in pasture forage than in feed sources fed before arrival at pasture. This study also demonstrated that cows with high serum Mo concentrations in the fall were more likely to have above adequate serum copper concentrations in the fall. Serum Cu concentrations will be especially difficult to interpret in these animals, as serum Cu concentrations have been reported to increase, at least initially, in Mo-supplemented animals, while liver Cu levels declined (Yuan et al. 1988; Wikse et al. 1992).

The increase in vitamin A and E serum concentrations during the grazing season, was consistent with previous observations in both dairy cows (Block and Farmer 1987; Miller et al. 1995) and beef cows (Bass et al. 2001). Vitamin A and E concentrations are lower in stored forage compared with growing plant material (Hidiraglou and Williams 1986) and, therefore, cows on grazing pasture would be expected to have higher vitamin A and E concentrations.

Physiological Factors

Older cows were more likely to have lower concentrations of vitamins A and E in both the spring and fall samples. These results are similar to those from other published reports in dairy animals. Block and Farmer (1987) found higher plasma vitamin A levels in first-calf heifers than in older dairy cows. Katsoulos et al. (2005) also reported that dairy cows 4 yr and younger had higher mean serum concentrations of vitamins A and E than dairy cows older than 4 yr and speculated that the older cows have greater rates of oxidation of vitamins A and E.

Cows that entered pasture in thin body condition in May 2008 had lower spring serum Cu concentrations than cows in moderate or good condition. Likewise, Littledike et al. (1995) reported that an increase in carcass lipid was associated with increased serum Cu concentrations for beef cows aged 6 to 14 years. However, the present study did not observe an association between fall body condition and fall serum Cu concentrations. It is possible that there was a decrease in power to detect an association in the fall because there was less variation in the fall serum Cu concentrations as well as fewer thin cows compared to the spring.

Cows with thin body condition in the fall had lower vitamin A concentrations in the fall than cows in moderate or good condition. A negative relationship between body weight and plasma vitamin A concentrations has previously been reported in dairy animals (Block and Farmer 1987); however, body condition scores for the animals were not reported, and it is possible that body weight was correlated with the age of animals since heifers were included in the study.

The other physiological factor that could potentially affect trace mineral storage in the animals was pregnancy. Cows that were not pregnant had higher serum Mn and Se concentrations in the fall than cows that were pregnant. While there were no published studies on the associations between Se and Mn concentrations and pregnancy status, there is some information on the effect of pregnancy on Cu in the cow. The bovine fetus accumulates Cu exponentially at the expense of maternal Cu and a progressive decline in maternal liver Cu during gestation has previously been documented (Gooneratne et al. 1989). No significant association between pregnancy status and serum Cu was measured in the fall in this study, but it is possible that a similar biological mechanism could account for the difference

between pregnant and non-pregnant cows in fall-measured Mn and Se concentrations.

Herd Management

Trace mineral supplementation before calving was the one identified herd management factor associated with spring trace mineral concentrations. Although Cu concentrations were higher in cows from herds supplemented with trace minerals before calving compared with unsupplemented herds, the Mn and Se concentrations in cows from the supplemented herds were lower. Previous Mn supplementation trials in beef animals have reported consistently low plasma Mn concentrations that do not change in response to supplementation (Legleiter et al. 2005; Hansen et al. 2006). The very small changes this study detected in spring Mn concentrations may not be clinically relevant.

Serum Se concentrations measured at the time of arrival on pasture would not be expected to reflect pre-calving trace mineral supplementation. Previous work has shown that serum Se concentrations reflect recent exposure to dietary Se (Stowe and Herdt 1992). Almost all the cows in this study calved 1 to 4 mo before spring sample collection. Post-calving supplementation reported in more than half of the study herds was not associated with micronutrient concentrations measured at the start of the pasture season.

Management interventions at the farm of origin are most likely to affect micronutrient concentrations at the start of the breeding season. After adjusting for all risk factors identified in this study, approximately half of the remaining variation in Se and vitamin A concentrations measured in the spring and one-quarter of the variation in Cu and vitamin E concentrations were accounted for by unmeasured differences between herds. Feed and water sources available to these animals before spring sample collection were not analyzed as part of this study, and this information should be included in future research or when investigating the potential causes of micronutrient deficiencies in a herd.

Pasture Conditions

Neither water quality nor cumulative precipitation was consistently associated with micronutrient concentrations measured in the fall. High dietary concentrations of sulphur and iron have been associated with secondary Cu deficiency in cattle (Humphries et al. 1983; Bremner et al. 1987; Gould et al. 2002); however, the concentrations of sulphate and iron in the pasture water sources in the present study may have been too low to add substantially to the sulphur or iron intake of these cows. Olkowski (2009) cites water sulphate concentrations above 1000 mg L⁻¹ and water iron concentrations above 10 mg L⁻¹ as potentially contributing significant amounts of dietary sulphate and iron in cattle. Only 2 of the 18 breeding fields in this study had water sulphate concentrations greater than 1000 mg L⁻¹, and no breeding fields had water iron concentrations above

4 mg L⁻¹. The power of the study to examine associations between micronutrient concentrations and water sulphate and iron concentrations was, therefore, limited.

The amount of precipitation received by each pasture was also inconsistently associated with serum concentrations of Se and vitamin A measured in the fall, and there were no observed trends between increasing precipitation levels and micronutrient concentrations that would be expected with a plausible causal relationship. Climatic conditions, such as precipitation and temperature, influence plant growth and can alter the ability of plants to translocate minerals from the soil to the plant, changing their mineral composition (Greene 2000). The small difference in precipitation amounts received by each pasture in this study (143 mm difference between the driest and the wettest pasture over 7 mo) may have been insufficient to substantially affect plant growth and associated mineral uptake between pastures, lowering the power of this study to examine associations between pasture level precipitation and serum micronutrient concentrations.

Differences in pasture conditions and composition throughout the grazing season are most likely to affect fall-measured micronutrient concentrations. After adjusting for the significant risk factors for each of the fall serum micronutrient concentrations, less than 15% of the remaining variation in Cu, Mn, Mo, Se, and vitamin E concentrations was accounted for by differences between pastures. The close geographical proximity of the pastures likely limited this study's ability to examine differences between pastures. Future research in this area should investigate a wider geographical distribution of pastures, including areas known to have high levels of sulphate and iron in livestock water sources.

The micronutrient concentrations of pasture forage were not evaluated in this study. Collecting representative samples of what the cows were actually eating throughout the grazing season was not logistically possible, given the number and large size of the pasture fields (130 to 1500 ha) spread over the five study locations. However, we would expect most pastures to be deficient in copper and zinc. Previous work summarizing the trace mineral content of Saskatchewan forage reported that 60% of the alfalfa hay, silage and brome hay analyzed at the provincial feed testing laboratory was deficient in copper (less than 10 mg kg⁻¹ of dry matter), and 100% were deficient in zinc (less than 40 mg kg⁻¹ of dry matter) (Smart et al. 1992). Three of the five pastures were located on Dark Brown and Black soil types and would also have been at risk for selenium deficiency (Smart et al. 1992).

This study identified factors affecting the micro nutrient concentrations of beef cows during two common management points in the production cycle, pre-breeding entry onto grazing pasture and fall round up from the pasture after the breeding season. Season and age, body condition, and pregnancy status should

be considered when interpreting the micro nutrient status of beef cows and developing supplementation programs. Further investigations into the effects of pasture composition, water quality and meteorological conditions would help determine the extent to which these factors may affect the micronutrient status of beef cows, increasing knowledge about the conditions most likely to be associated with low micronutrient concentrations.

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