SERUM MICRONUTRIENT CONCENTRATIONS IN WESTERN CANADIAN BEEF CATTLE AT PRE-BREEDING AND PREGNANCY TESTING

A Thesis Submitted to the College of

Graduate Studies and Research

In Partial Fulfillment of the Requirements

For the Degree of Master of Science

In the Department of Large Animal Clinical Sciences

University of Saskatchewan

Saskatoon

By

Leanne Van De Weyer, BSA, BEd, DVM

Permission to Use

In presenting this thesis in partial fulfillment of the requirement for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor of professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Large Animal Clinical Sciences

University of Saskatchewan

Saskatoon, Saskatchewan, S7N 5B4

ABSTRACT

This thesis described the results of two studies that measured the concentrations of serum micronutrients in western Canadian beef cows at key production points, and examined associations between reproductive outcomes and micronutrient concentrations. The first study reported the serum copper and molybdenum concentrations at pregnancy testing time in cows from 66 cow-calf herds that were enrolled in a study of factors affecting productivity in 205 herds from western Canada. A relatively small proportion of cows had serum copper and molybdenum concentrations outside of adequate levels (16.2% of cows had below adequate serum copper, < 0.60 ppm; 12% of cows had high serum molybdenum, ≥ 0.10 ppm). There were no associations between copper and molybdenum concentrations measured at the end of the grazing season and reproductive outcomes measured in these cows, with the exception that cows with the lowest serum copper concentrations at pregnancy testing were more likely to be pregnant than cows with higher copper concentrations. The practice of comparing serum copper and molybdenum concentrations between pregnant and non-pregnant animals in the fall as a tool in investigating poor pregnancy rates was not supported by this study.

In the second study, serum micronutrient concentrations of beef cows (n = 791) in southern Saskatchewan were measured before placement onto summer grazing and breeding pastures and again at the end of the grazing season. Pre-breeding serum copper concentrations were less than adequate (< 0.60 ppm) in 75% of cows. High concentrations of serum molybdenum (≥ 0.10 ppm) were present in 19% of cows at pre-

breeding. Cows < 10 years of age with lower pre-breeding serum copper concentrations were at increased odds of nonpregnancy. The greatest effect on pregnancy rates was observed for pre-breeding serum copper concentrations < 0.4 ppm. Season of measurement influenced the concentrations of serum micronutrients in these cows. Copper and vitamins A and E were higher in the fall, and molybdenum and selenium concentrations were lower in the fall.

These studies described serum micronutrient concentrations from healthy cows in western Canada at two production points, pre-breeding placement onto grazing pastures and pregnancy testing when cows are removed from grazing pasture. Identifying increased odds of nonpregnancy in cows with below adequate serum copper at pre-breeding emphasizes the importance of ensuring adequate copper concentrations in breeding females during this critical production phase.

ACKNOWLEDGMENTS

There are two separate research studies comprising this thesis. The trace mineral data from the 2001 – 2002 Western Canadian Beef Productivity study was given to me by Dr. Cheryl Waldner. This study was funded by the Western Interprovincial Scientific Studies Association (WISSA). The 2008 study evaluating micronutrient concentrations in beef cows in southern Saskatchewan was funded by the WCVM Vitamin Class Action Settlement Fund, Agriculture and Agri-Food Canada, and the Alberta Beef Producers. I would sincerely like to thank the PFRA patrons, pasture managers and staff that cooperated with this project, as well as the veterinarians and WCVM students that helped with sample collection and processing, especially Drs. Wendy Wilkins, Richard Kennedy, Colleen Pollack, Fritz Schumann, Leigh Rosengren, Sheryl Gow, Lori Zemlak and Roberta Templeton.

Throughout my graduate program, I received unwavering support from my supervisor, Dr. Cheryl Waldner. Thank you for taking me on as a non-resident student and believing that there is always more than one way to get a job done. Your guidance, sense of humour, and focus on research priorities despite setbacks or complications were the best (not to mention those spontaneous statistics lessons over the phone). I would also like to thank my fantastic committee members: Drs. Steve Hendrick, Leigh Rosengren, and Janet Hill for their energy, ideas and support throughout all phases of my field work and thesis preparation.

I am grateful to the Department of Large Animal Clinical Studies for allowing me to pursue my M.Sc. as a non-resident student. Although graduate-level veterinary epidemiology courses are increasingly available on-line, they cannot offer “hands-on” field experience. Ultimately, the research process is best learned by doing and I truly appreciated the opportunity to be involved in a large observational field study that brought together academia, government agencies, and private producers.

Finally, I would like to thank my family for their ongoing love and encouragement. Learning truly is a life-long endeavour!

ORIGINAL CONTRIBUTION

Two separate studies comprise this thesis. Data for the first study, an examination of the serum copper and molybdenum concentrations of beef cows in western Canada, were collected as part of the Western Canadian Beef Productivity Study (WCBPS). The concept, design, management, and field data collection of this 2001-2003 study were undertaken by Dr. Cheryl Waldner. Data from the WCBPS presented in this thesis were given to me by Dr. Waldner. I was responsible for the trace mineral data analysis and literature review presented in this thesis.

The second study, evaluating micronutrient concentrations in beef cows in southern Saskatchewan, was an independent research project. I contributed to the project design and in preparing and submitting grant proposals for part of the funding for the project. I was involved in herd enrolment, sample collection, survey design and information collection, and communication of results to PFRA patrons and staff involved in the study. I coordinated sample submission to the laboratories, entered and cleaned much of the data, and conducted all of the data analysis for this study.

I did not perform any of the laboratory analysis for either of these studies. The Western College of Veterinary Medicine (WCVM) Toxicology Laboratory and Prairie Diagnostic Services (PDS), Saskatoon, Saskatchewan analyzed the serum micronutrient concentrations and provided the *Campylobacter fetus* cultures, *Tritrichomonas foetus* polymerase chain reactions (PCR), and bovine viral diarrhea virus serum neutralization

titers for both of these studies. Drs. Janet Hill and Bonnie Chaban, and Ms. Kristyna Musil of the WCVM Molecular Biology Laboratory developed and conducted the polymerase chain reaction (PCR) analysis for *Campylobacter fetus* for the second study. The Animal Health Laboratory, University of Guelph, Guelph, Ontario performed the *Leptospira* serovar serum antibody titrations for the second study. Water sample analysis for the second study was provided by the Saskatchewan Research Council (SRC) Analytical Laboratory, Saskatoon, Saskatchewan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BCS	Body Condition Score
ºC	Degrees Celsius
CBI	Calving to breeding Interval
Co	Cobalt
CI	Confidence Interval
Cu	Copper
F	Fluoride
ha	Hectare
I	Iodine
IU	International Units
Fe	Iron
km	Kilometre
L	Litre
µg	Microgram
µL	Microlitre
ml	Millilitre
mm	Millimetre
Mn	Manganese
Mo	Molybdenum
n	Number
nm	Nanometre
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PDS	Prairie Diagnostic Services
ppm	Parts per million
S, SD	Standard Deviation
Se	Selenium
Y, N	Yes, No
Zn	Zinc

CHAPTER 1

INTRODUCTION

Reproductive efficiency is critical to the financial success of cow-calf operations. Each breeding female in the herd should give birth to one calf each year in a defined calving period to ensure a uniform calf crop with high weaning weights to sell and from which to choose replacement heifers, and to ensure that the cow herd is actively cycling before the start of the next breeding season. In western Canada, breeding activity typically coincides with the grazing season for extensively managed cow-calf herds. The success of the breeding season is determined at the end of summer pasture turnout through examination for pregnancy or observation of cows for estrus activity. When the pregnancy rate of a herd is lower than expected, common causes related to bull performance, breeding pasture management, infectious disease, and nutrition are considered.

There are many aspects of nutrition that can affect reproductive outcomes. Clear evidence exists for an association between low energy and protein levels in beef cattle and poor pregnancy rates (Yavas and Walton 2000; Larson 2007; Crowe 2008). However, the role of micronutrients in fertility is less conclusive despite reports of micronutrient deficiency in beef cows throughout North America (Campbell et al. 1995; Dargatz and Ross 1996; Dargatz et al. 1999; Hoff et al. 2001). There is a lack of clinically relevant studies examining the relationships between micronutrient concentrations and reproductive outcomes in beef cows, leaving veterinarians without the

necessary scientific evidence to examine the impact of vitamin and trace mineral status in herds with low pregnancy rates.

The following sections briefly describe the prevalence of micronutrient deficiencies in North American soils, forages and feedstuff, and beef cattle. Then the available evidence for associations between micronutrient concentrations and reproductive outcomes is reviewed for beef cows and heifers. The objectives of this introduction are first to identify the potential for below adequate micronutrient concentrations in western Canadian beef herds, and second to summarize previous research examining the associations between the micronutrient status of beef cows and their reproductive outcomes.

1.1 Scope of micronutrient deficiencies in North America

1.1.1 Soils

Trace elements in soil are generally derived from the mineral material from which the soil originated. These trace elements become plant available by ongoing weathering of the mineral material (Steinnes 2009). The soil texture, organic matter, pH, and other nutrients of the soil affect the trace mineral uptake by the plant (Malhi and Karamanos 2006). Plants react to inadequate supplies of trace minerals in the soil either by reducing the concentration of the deficient element in their tissue, or by reducing growth, or both (Berger 1996). Maps illustrating the geographical distribution of mineral material and soil classification within Canada are readily available (Anonymous 2010).

Kruger et al. (1985) reported that copper deficiencies in Saskatchewan soils tended to be clustered in Grey soil types with lighter textures. This was suggested to be due to lower amounts of organic matter and a low copper content of the quartz, the primary parent material of these Grey soils (Kruger et al. 1985). Geological formations containing molybdenum-rich shales result in high soil molybdenum, as documented in northwestern Manitoba (Boila et al. 1984). Molybdenum uptake also increases as soil pH increases; therefore, alkaline soils are more likely to have plants with high molybdenum levels (Berger 1996).

The trace mineral in plants that is most affected by soil concentrations is probably selenium (Berger 1996). In North America, selenium deficiency occurs widely: northeastern and northwestern regions, regions east of the Mississippi river, and in areas where sulfur-based fertilizers depress plant selenium uptake. Selenium deficiency may be particularly pronounced in acidic soils and heavily leeched soils. In acid soils, a ferric-iron-selenite complex is formed, which is only slightly available to plants (Gupta and Gupta 2000). Soils containing less than 0.6 mg/kg of selenium are likely to produce crops with insufficient selenium to protect livestock from selenium deficiency (Gupta and Gupta 2000).

Manganese deficiency occurs in calcareous soils or alkaline soils that favour manganese oxidation and immobilization of the element (Cailliatte et al. 2010). In addition, when iron occurs in excess in soil, it can compete with manganese and trigger a manganese deficiency.

1.1.2 Plants

The trace mineral concentrations of common feedstuffs is highly variable. Even within a particular geographical region, wide variations in the trace mineral profiles of plant material can occur (Berger 1996). The particular plant species grown and the stage of maturity affect trace mineral concentrations. Generally, there is a rapid uptake of minerals during early growth and a gradual dilution as the plant matures.

The use of National Research Council (NRC) feed composition values to calculate beef cattle diet formulations could result in errors for specific trace minerals if the available feedstuff is substantially different. Variations in plant trace mineral concentrations are difficult to predict: plant species and variety, stage of plant maturity, and seasonal climatic conditions affect feed values, in addition to the trace mineral concentration of the soil (Berger 1996). Western Canadian forages, cereal hay, and cereal grains have been reported to have copper and selenium levels below the suggested NRC requirement for beef cattle (Anonymous 1996; Boila et al. 1984; Kruger et al. 1985; Gooneratne and Christensen 1989; Smart et al. 1992; Suleiman et al. 1997). Secondary copper deficiency due to high molybdenum levels in forage has occurred in localized regions of western Canada (Smart et al. 1992; Gardner et al. 2003). Red clover and grass silages in western Canada have been associated with manganese deficiency in cattle (Hidiroglou et al. 1990), and manganese concentrations in alfalfa hay and forage grown in Alberta have been reported to be below the level required by cattle (Suleiman et al. 1997).

Fresh green forage is the primary source of vitamins A and E. Hay cut during the summer months may lose up to 95% of its vitamin A content by late winter (Blakley 2010). Hidiroglou et al. (1994) reported that stored feedstuffs typically contain only 17-20% of the vitamin E found in fresh forages, and that stored hay had less vitamin E than silage. Grain contains very little vitamin A or E (Weiss 1998).

1.1.3 Beef cattle

Copper deficiency has been reported to be the most commonly recognized micronutrient deficiency in domestic livestock (Wikse et al. 1992). Of the 2000 cows and heifers sampled across the United States for the National Animal Health Monitoring System (NAHMS) Cow-Calf Health and Productivity Audit, 38.9% were identified as having marginally deficient serum copper concentrations [0.250 to 0.649 μg/g] and 1.7% as having severely deficient concentrations [< 0.250 μg/g]. Canadian studies support these findings. Korsrud et al. (1985) reported that 35% of slaughter cattle had marginal liver copper concentrations [10 - 30 ppm wet weight] and 28.7% had deficient concentrations [< 10 ppm wet weight]. Two studies of slaughter cattle from Saskatchewan abattoirs also reported a high percentage of cattle deficient in copper. Brockman (1977) identified 67% of all livers examined were deficient in copper [< 10 ppm wet weight], and Gooneratne and Christensen (1989) reported that 54% of pregnant cows, 52% of heifers, and 77% of nonpregnant cows had deficient copper liver concentrations at slaughter [< 25 mg/kg dry matter].

Selenium deficiency has also been frequently recognized in North American beef cattle. A NAHMS survey identified selenium as being marginally deficient [0.051-0.080 ppm whole blood] and deficient [< 0.051 ppm whole blood] for 10.4% of beef cows and 7.8% of heifers across the United States (Dargatz and Ross 1996). Similarly, Campbell et al. (1995) reported 9% of 335 cows sampled in Alberta to be marginal or deficient in selenium [< 1.27 μmol/L whole blood].

A small survey of 55 cull beef cows in Ontario reported 96% of the animals deficient in selenium, 73% deficient in copper, and 51% deficient in manganese (Hoff et al. 2001). This study also identified below adequate vitamin E levels, although the proportion of animals below adequate was not reported [mean serum vitamin E 3.13 μg/mL; range 0.39-8.20 μg/mL] (Hoff et al. 2001). No other surveys of North American beef cattle were identified that reported manganese or vitamin E concentrations, and no surveys reported vitamin A concentrations. However, vitamin A and E deficiencies in livestock have been reported to be a common problem identified by a Saskatchewan veterinary diagnostic laboratory each spring (Blakley 2010). Liver stores of vitamin A built up in the summer grazing season are reported to be depleted in two to three months, without ongoing supplementation (Puls 1994).

1.2 Associations between micronutrients and reproductive performance

Micronutrients are required for the synthesis of many proteins and are essential components of an array of enzyme systems. As such, micronutrients are necessary for normal tissue formation and metabolism (Hostetler et al. 2003). Trace elements,

including copper, selenium, manganese, and molybdenum, are required in low concentrations in mammalian diets. Cattle also have specific dietary requirements for fat soluble vitamins, including A and E, in contrast with water soluble vitamins which are provided by the rumen microbiota (Allison and Laven 2000). Below adequate (or marginally deficient) micronutrient concentrations are more commonly encountered in livestock operations than severe deficiencies, and theoretically may compromise pregnancy outcomes by affecting the development of the preovulatory oocyte, embryo, placenta, or offspring (Ashworth and Antipatis 2001). However, field data linking below adequate micronutrient concentrations and poor reproductive outcomes in beef cows are lacking for many of the micronutrients.

1.2.1 Copper and molybdenum

The copper-containing enzyme ceruloplasmin is associated with iron metabolism and is required for normal hematopoiesis (Hostetler 2003). Copper is also a co-factor in cytochrome *c* oxidase, lysyl oxidase, and tyrosinase enzymes involved in neutrophil function, bone formation and melanin production (Gooneratne et al. 1989; Galyean et al. 1999). Copper deficiency in cattle has been associated with immunosuppression, anemia, alterations in hair colour, skeleton abnormalities, and growth retardation (Smart et al. 1992; Wikse 1992). The extent of clinical signs resulting from copper deficiency depends on the degree of deficiency, and whether the deficiency is primary or secondary. Molybdenum-induced copper deficiency appears more likely to result in clinical signs of lameness and coat colour alterations than primary copper deficiency (Humphries et al. 1983; Phillippo et al. 1987a).

Copper entering the bloodstream from the gastrointestinal tract after ingestion becomes bound to albumin and another protein, transcuprein. This protein-bound copper is rapidly cleared by the liver. The liver is the primary storage organ of copper, and is important in copper metabolism and homeostasis (Gooneratne et al. 1989; Wikse et al. 1992). Blood copper concentrations may not accurately reflect below adequate copper status until liver copper concentrations fall below 40 ppm on a dry weight basis (Claypool et al. 1975). Serum copper concentrations of 0.45 ppm have been previously correlated with low liver copper concentrations (Claypool et al. 1975; Tessman et al. 2001).

The effect of below adequate copper concentrations on reproductive outcomes in cattle is controversial. Although some researchers have suggested a link between low copper and early embryonic loss in cattle (Hidiroglou 1979b; Muhlenbein et al. 2001), this has not been substantiated by other studies. A case-control study comparing herd-level concentrations of copper and other micronutrients in dairy and beef herds with and without reproductive disorders reported that copper deficient herds [plasma copper < 8 μmol/L] did not have increased odds of low fertility or abortion compared to non-deficient herds (Enjalbert et al. 2006).

Copper deficiency can be primary, the result of inadequate dietary intake, or secondary, due to interference of dietary copper absorption or utilization by the presence of other minerals (Wikse et al. 1992). Molybdenum, sulfur, and iron can bind copper in

the rumen or blood, making it unavailable to the animal (Suttle 1991). Molybdenum is readily absorbed from dietary sources and as molybdenum concentrations in the diet increase, molybdate and sulphide interact in the rumen to form thiomolybdates, which may react with copper to form physiologically unavailable complexes (Suttle 1991). The ratio of copper to molybdenum in ruminant diets is important for copper availability; and copper deficiency may occur at ratios of 2:1 or less (Ward 1978).

Lowered conception rates, failure to ovulate, and anestrous have been associated with excess dietary molybdenum regardless of the animal's copper status, raising debate about the relationship between low copper and fertility (Phillippo et al. 1987b). However, other reports have indicated that cattle can graze pastures containing high molybdenum without adverse health or reproductive effects providing they receive copper supplementation or have adequate copper status going onto high molybdenum pastures (Gardner et al. 2003; Raisbeck et al. 2006).

1.2.2 Selenium and vitamin E

Selenium and vitamin E have complementary actions protecting cells from destructive oxidation reactions, and large amounts of vitamin E in the diet have been reported to reduce the requirement for selenium (Maas 1983). However, evidence suggests that requirements for vitamin E and selenium are mostly independent (Hurley and Doane 1987; Gerloff 1992). Selenium is an integral component of several selenoproteins, including glutathione peroxidase. These enzymes catabolize the peroxides generated during lipid oxidation (Rotruck et al. 1973; Gerloff 1992; Hostetler 2003).

Vitamin E oxidation prevents the degradation of other lipid materials to free radicals and peroxides, and because it is lipid soluble its activity is primarily associated with the cell membranes (Frye 1991; Gerloff 1992).

The highest concentration of selenium in the body is found in kidneys, followed by the liver (Olson 1996). Both whole blood and serum can be used to evaluate the selenium status of animals. Serum selenium concentrations reflect current selenium status, while whole blood selenium and glutathione-peroxidase activity better reflect longer term status (Puls 1994; Olson 1996; Waldner et al. 1998). Relatively little storage of vitamin E occurs in the body and the liver is not a storage organ for this nutrient (Frye 1991).

Selenium deficiency has long been associated with congenital white muscle disease in calves from 1 to 4 weeks of age (Muth 1955; Maas 1983). Skeletal and cardiac muscle necrosis are present, and clinical signs can range from stiffness and lameness to acute death (Radostits 2007). Selenium deficiency has also been associated with myocardial necrosis and heart failure in aborted bovine fetuses (Orr and Blakley 1997). Some studies have reported an improvement in conception rates following selenium supplementation (Tasker et al. 1987; Allan et al. 1993), but this finding has not been consistent (Spears et al. 1986; Gunter et al. 2003).

There is limited evidence for an effect of vitamin E supplementation on the reproductive efficiency of dairy cows (Allison and Laven 2000; Hemingway 2003).

Studies in beef cows have focused on the effects of vitamin E supplementation on calves rather than the reproductive parameters of cows (Bass et al. 2001; Maas et al. 2008).

1.2.3 Manganese

Manganese may act as a cofactor for enzymes involved in the production of squalene, a precursor of cholesterol and an intermediate in the production of progesterone (Hansen et al. 2006). Manganese is also required to activate glycosyltransferase, an enzyme necessary for the synthesis of cartilage. Manganese is essential for formation of chondroitin sulfate as a component of mucopolysaccharides in the matrix of bone (Hostetler et al. 2003); as such, it also plays an essential role in long bone and epiphyseal growth plate development (Hidiroglou 1979a; Hansen et al. 2006).

The highest concentration of manganese occurs in the liver, pancreas and kidneys (Hidiroglou 1979a). Manganese concentrations in the blood are tightly regulated; manganese is removed from blood very efficiently by the liver and increased biliary excretion corresponds to increased dietary manganese (Hall and Symonds 1981; Legleiter et al. 2005).

In the adult animal, reproductive functions have been reported to be the most sensitive to manganese deficiency, occurring ahead of other physiological and biochemical changes (Radostits 2007). Much of the research on dietary manganese and reproductive function in beef cattle was done in the 1970's or earlier. This work suggests that manganese deficiency can result in delayed puberty, anestrus or weak estrus, failure

to conceive, increased abortion rates, and the birth of deformed calves (Rojas et al. 1965; Hidiroglou 1979a). Wilson (1966) reported an increase in the conception rates of dairy cows supplemented with manganese. However, a recent study by Hansen et al. (2006) found no significant improvement in pregnancy rates, age at conception, and services to conception of beef heifers fed a low-manganese diet that was supplemented with manganese compared to non-supplemented. Hansen et al. (2006) noted, however, that several of the calves born to control heifers exhibited dwarfism and superior brachygnathism, signs previously linked to manganese deficiency.

1.2.4 Vitamin A

Vitamin A is synthesized from carotenoid precursors, most notably β-carotene. Since animals are unable to synthesize carotenoids, cattle rely on fresh green forage or dietary supplements to supply these compounds (Gomez et al. 2006). Cattle store 70% to 90% of their total vitamin A in the liver, but important reserves also exist in fat tissue (Gomez et al. 2006). Dietary intake of vitamin A has been positively correlated with serum vitamin A (Block and Farmer 1987), although other studies have reported serum concentrations to be a poor indicator of vitamin A intake (Oldham et al. 1991; Michal et al. 1994). There is an established relationship between increasing severity of clinical signs of vitamin A deficiency and decreasing serum vitamin A (Frye et al. 1991).

Vitamin A is necessary to support growth and tissue maintenance in animals. Vitamin A and its metabolites affect ovarian follicular growth and steroidogenesis, oviduct and uterine environments, immune functions, oocyte maturation, and conceptus

development (Ikeda et al. 2005). Deficiency of vitamin A and its natural precursor, β-carotene, has been reported to result in reduced conception rates, increased abortion rates, and increased rates stillborn, weak or blind calves (Hurley and Doane 1987; Frye 1991). Block and Farmer (1987) identified associations between higher vitamin A plasma concentrations and shorter calving intervals, and fewer breedings per conception in dairy cows.

1.3 Impetus for further research

There are very few epidemiologic studies examining associations between micronutrient status and reproductive outcomes especially in North American beef herds, although the impact of micronutrient supplementation on reproductive outcomes has been examined in published feeding trials (Olson et al. 1999; Bass et al. 2001; Muehlenbein et al. 2001; Gunter et al. 2003; Ahola et al. 2004; Black and French 2004; Siciliano-Jones et al. 2008). The wide variations among these trials in supplement type, supplement concentration, and the length and timing of supplementation within the production cycle make it difficult to draw conclusions about a specific micronutrient and its effect on the reproductive performance in client-owned herds. Many of the small trials also lack adequate power to demonstrate a significant difference in pregnancy outcomes between treatment groups (Olson et al. 1999; Muehlenbein et al. 2001; Gunter et al. 2003). Conclusions from these controlled feeding trials, especially those conducted in dairy cattle (Black and French 2004; Siciliano-Jones et al. 2008), are therefore difficult to generalize to extensively managed beef herds.

The timing of micronutrient concentration measurement within the cow herd's production cycle is also an important consideration for veterinarians working with beef herds with low pregnancy rates. Given the wide variations in season and corresponding management throughout the cow-calf production cycle in western Canada, it is likely that serum micronutrient levels sampled at the end of the grazing season might not be representative of micronutrient levels during the pre-breeding phase. 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 (Gooneratne and Christensen 1989; Smart et al. 1992; Campbell et al. 1995; Littledike et al. 1995). Understanding the factors that 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.

This thesis was written to address some of the deficiencies in our knowledge of micronutrient status and reproductive efficiency in beef cows. Copper, selenium, manganese, vitamin A, and vitamin E were examined because previous studies have indicated the potential for these micronutrients to be deficient in North American beef animals (Campbell et al. 1995; Dargatz and Ross 1996; Dargatz et al. 1999; Hoff et al. 2001). Molybdenum status was also examined because of the potential for it to interact with dietary copper in ruminants, potentially resulting in secondary copper deficiency in cattle (Gooneratne et al. 1989; Suttle 1992).

The specific objectives of this thesis were addressed in a series of three papers. Chapter 2 describes the serum concentrations of copper and molybdenum at fall pregnancy testing of a cohort of beef cows located in Saskatchewan and Alberta. This study examined animal and environmental factors associated with these concentrations, and determined whether copper and molybdenum concentrations measured at the end of the grazing season were associated with various reproductive outcomes. Chapter 3 examines the associations between serum concentrations of copper, molybdenum, selenium, vitamin A, and vitamin E in commercial beef cows in southern Saskatchewan measured before the start of the breeding season and pregnancy outcome measured at the end of the summer pasture season. Chapter 4 is a companion paper to Chapter 3; it examines the effect of season on serum micronutrient concentrations in the same group of southern Saskatchewan cows before and after the summer grazing season. It also examines cow-, herd-, and pasture-level risk factors associated with the micronutrient concentrations before and after the summer grazing season. The concluding chapter (Chapter 5) reviews and discusses the important findings of this thesis, closing with identified research needs for future micronutrient studies.

Each chapter of this thesis was written as an independent paper and formatted for publication in different scientific journals. Although this has made the study results more usable, it has resulted in some repetition of study description between Chapters 3 and 4 and in format style differences between chapters.

1.4 References

Ahola, J. K., Baker, D. S., Burns, P. D., Mortimer, R. G., Enns, R. M., Whittier, J. C., Geary, T. W., and Engle, T. E. 2004. Effect of copper, zinc, and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two-year period. J. Anim. Sci. **82:** 2375-2383.

Allan, C. L., Hemingway, R. G., and Parkins, J. J. 1993. Improved reproductive performance in cattle dosed with trace element/vitamin boluses. The Vet. Rec. **132:** 463-464.

Allison, R. D. and Laven, R. A. 2000. Effect of vitamin E supplementation on the health and fertility of dairy cows: a review. The Vet. Rec. **147:** 703-708.

Anonymous. 1996. National Research Council. Nutrient requirements of beef cattle, 7th ed. National Academy Press, Washington, DC.

Anonymous. 2010. Interactive Maps. Soils of Canada. [Online] Available at: http://sis.agr.gc.ca/cansis/publications/webmaps.html. [23 May 2011].

Ashworth, C. J. and Antipatis, C. 2001. Micronutrient programming of development throughout gestation. Repro. **122:** 527-535.

Bass, R. T., Swecker, W. S., and Eversole, D. E. 2001. Effects of oral vitamin E supplementation during late gestation in beef cattle that calved in late winter and late summer. Am. J. Vet. Res. **62:** 921-927.

Berger, L. L. 1996. Variation in the trace mineral content of feedstuffs. The Prof. Anim. Scientist. **12:** 1-5.

Black, D. H. and French, N. P. 2004. Effects of three types of trace element supplementation on the fertility of three commercial dairy herds. The Vet. Rec. **154:** 652-658.

Blakley, B. 2010. Vitamin A (retinol) testing. Anim. Health Perspectives. **6:** 1-4.

Block, E. and Farmer, B. 1987. The status of beta-carotene and vitamin A in Quebec dairy herds: factors affecting their status in cows and their effects on reproductive performance. Can. J. Anim. Sci. **67:** 775-788.

Boila, R. J., Devlin, T. J., Drysdale, J. M., and Lillie, L. E. 1984. Geographical variation in the copper and molybdenum content of forages grown in northwestern Manitoba. Can. J. Anim. Sci. **64:** 899-918.

Brockman, R. P. 1977. Concentration of copper in livers of Saskatchewan cattle at slaughter. Can. Vet. J. **18:** 168-170.

Cailliatte, R., Schikora, A., Briat, J., Mari S., and Curie, C. 2010. High-affinity manganese uptake by the metal transporter NRAMP1 is essential for *Arabidopsis* growth in low manganese conditions. The Plant Cell. **22:** 904-917.

Campbell, J. R., Jim, G. K., Booker, C. W., and Guichon, P. T. 1995. A survey of the selenium status of beef cows in Alberta. Can. Vet. J. **36:** 698-702.

Claypool, D. W., Adams, F. W., Pendell, H. W., Harmann Jr., N. A., Bone, J. F. 1975. Relationship between the level of copper in the blood plasma and the liver of cattle. J. Anim. Sci. **41:** 911-914.

Crowe, M. A. 2008. Resumption of ovarian cyclicity in post-partum beef and dairy cows. Repro. Dom. Anim. **43:** 20-28.

Dargatz, D. A. and Ross, P. F. 1996. Blood selenium concentrations in cows and heifers on 253 cow-calf operations in 18 states. J. Anim. Sci. **74:** 2891-2895.

Dargatz, D. A., Garry, F. B., Clark, G. B., and Ross, P. F. 1999. Serum copper concentrations in beef cows and heifers. J. Am. Vet. Med. Assoc. **215:** 1828-1832.

Enjalbert, F., Lebreton, P., and Salat, O. 2006. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study. J. Anim. Physiol. and Anim. Nutr. **90:** 459-466.

Frye, T. M., Williams, S. N., and Graham, T. W. 1991. Vitamin deficiencies in cattle. Vet. Clin. Fd. Anim. **7:** 217-275.

Galyean, M. L., Perino, L. J., and Duff, G. C. 1999. Interaction of cattle health/immunity and nutrition. J. Anim. Sci. **77:** 1120-1134.

Gardner, W. C., Broersma, K., Popp, J. D., Mir, Z., Mir, P. S., and Buckley, W. T. 2003. Copper and health status of cattle grazing on high-molybdenum forage from a reclaimed mine tailing site. Can. J. Anim. Sci. **83:** 479-485.

Gerloff, B. J. 1992. Effect of selenium supplementation on dairy cattle. J. Anim. Sci. **70:** 3934-3940.

Gomez, E., Caamano, J. N., Rodriguez, A., DeFurtos, C., Facal, N., and Diez, C. 2006. Bovine early embryonic development and vitamin A. Reprod. Dom. Anim. **41 (Suppl. 2):** 63-71.

Gooneratne, S. R., Buckley, W. T., and Christensen, D. A. 1989. Review of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci. **69:** 819-845.

Gooneratne, S. R. and Christensen, D. A. 1989. A survey of maternal copper status and fetal tissue copper concentrations in Saskatchewan bovine. Can. J. Anim. Sci. **69:** 141-150.

Gunter, S. A., Beck, P. A., and Phillips, J. M. 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. J. Anim. Sci. **81:** 856-864.

Gupta, U. C. and Gupta, S. C. 2000. Selenium in soils and crops, its deficiencies in livestock and humans: implications for management. Commun. Soil Sci. Plant Anal. **31:** 1791-1807.

Hall, E. D. and Symonds, H. W. 1981. The maximum capacity of the bovine liver to excrete manganese in bile, and the effects of a manganese load on the rate of excretion of copper, iron and zinc in bile. Br. J. Nutr. **45:** 605-611.

Hansen, S. L., Spears, J. W., Lloyd, K. E., and Whisnant, C. S. 2006. Growth, reproductive performance, and manganese status of heifers fed varying concentrations of manganese. J. Anim. Sci. **84:** 3375-3380.

Hemingway, R. G. 2003. The influences of dietary intakes and supplementation with selenium and vitamin E on reproduction diseases and reproductive efficiency in cattle and sheep. Vet. Res. Commun. **27:** 159-174.

Hidiroglou, M. 1979a. Manganese in ruminant nutrition. Can. J. Anim. Sci. **59:** 217-236.

Hidiroglou, M. 1979b. Trace element deficiencies and fertility in ruminants: a review. J. Dairy Sci. **62:** 1195-1206.

Hidiroglou, M. Ivan, M., Bryan, M. K., Ribble, C. S., Janzen, E. D., Proulx, J. G., and Elliot, J. I. 1990. Assessment of the role of manganese in congenital joint laxity and dwarfism in calves. Ann. Rech. Vet. **21:** 281-284.

Hidiroglou, M., Batra, T. R., and Roy, G. L. 1994. Changes in plasma α–tocopherol and selenium of gestating cows fed hay or silage. J. Dairy Sci. **77:** 190-195.

Hoff, B., Schrier, N., Boermans, H., Faulkner, H., and Hussein, A. 2001. Assessment of trace mineral and vitamin E status beef cows in Ontario. Can. Vet. J. **42:** 384-385.

Hostetler, C. E., Kincaid, R. L., and Mirando, M. A. 2003. The role of essential trace elements in embryonic and fetal development of livestock. The Vet. J. **166:** 125-139.

Humphries, W. R., Phillippo, M., Young, B. W., and Bremner, I. 1983. The influence of dietary iron and molybdenum on copper metabolism in calves. Br. J. Nutr. **49:** 77-86.

Hurley, W. L. and Doane, R. M. 1987. Recent developments in the roles of vitamins and minerals in reproduction. J. Dairy Sci. **72:** 784-804.

Ideda, S., Kitagawa, M., Imai, H., and Yamada, M. 2005. The roles of vitamin A for cytoplasmic maturation of bovine oocytes. J. Repro. And Develop. **51:** 23-35.

Korsrud, G. O., Meldrum, J. B., Salisbury, C. D., Houlahan, B. J., Saschenbrecker, P. W., and Tittiger, F. 1985. Trace element levels in liver and kidney from cattle, swine and poultry slaughtered in Canada. Can. J. Comp. Med. **49:** 159-163.

Kruger, G. A., Karamanos, R. E., and Singh, J. P. 1985. The copper fertility of Saskatchewan soils. Can. J. Soil Sc. **65:** 89-99.

Larson, R. L. 2007. Heifer development: reproduction and nutrition. Vet. Clin. Fd. Anim. **23:** 53-68.

Legleiter, L. R., Spears, J. W., and Lloyd, K. E. 2005. Influence of dietary manganese on performance, lipid metabolism, and carcass composition of growing and finishing steers. J. Anim. Sci. **83:** 2434-2439.

Littledike, E. T., Wittum, T. E., and Jenkins, T. G. 1995. Effect of breed, intake, and carcass composition on the status of several macro and trace minerals of adult beef cattle. J. Anim. Sci. **73:** 2113-2119.

Maas, J. 1983. Diagnosis and management of selenium-responsive diseases in cattle. Comp. Cont. Ed. Pract. Vet. **5 (S393): 393**-399.

Maas, J. Hoar, B. R., Myers, D. M., Tindall, J. P, and Puschner, B. 2008. Vitamin E and selenium concentration in month-old beef calves. J. Vet. Diagn. Invest. **20:** 86-89.

Malhi, S. S. and Karamanos, R. E. 2006. A review of copper fertilizer management for optimum yield and quality of crops in the Canadian prairie provinces. Can. J. Plant Sci. **86:** 605-619.

Michal, J. J., Heirman, L. R., Wong, T. S., Chew, B. P., Frigg, M. and Volker, L. 1994. Modulatory effects of dietary b-carotene on blood and mammary leukocyte function in periparturient dairy cows. J. Dairy Sci. **77:** 1408-14421.

Muehlenbein, E. L., Brink, D. R., Deutscher, G. H., Carlson M. P., and Johnson A. B. 2001. Effects of inorganic and organic copper supplemented to first-calf cows on cow reproduction and calf health and performance. J. Anim. Sci. **79:** 1650-1659.

Muth, O. H. 1955. White muscle disease (myopathy) in lambs and calves. Occurrence and nature of the disease under Oregon conditions. J. Amer. Vet. Med. Assoc. **126:** 355-361.

Oldham, E. R., Eberhart, R. J., and Muller, L. D. 1991. Effects of supplemental vitamin A or B-carotene during the dry period and early lactation on udder health. J. Dairy Sci. **74:** 3775-3781.

Olson, P. A., Brink, D. R., Hickok, D. T., Carlson, M. P., Schneider, N. R., Deutscher, G. H., Adams, D. C., Colburn, D. J., and Johnson, A. B. 1999. Effects of supplementation of organic and inorganic combinations of copper, cobalt, manganese, and zinc above nutrient requirement levels on postpartum two-year-old cows. J. Anim. Sci. **77:** 522-532.

Orr, J. P. and Blakley, B. R. 1997. Investigation of the selenium status of aborted calves with cardiac failure and myocardial necrosis. J. Vet. Diagn. Invest. **9:** 172-179.

Phillippo, M., Humphries, W. R., and Garthwaite, P. H. 1987a. The effect of dietary molybdenum and iron on copper status and growth in cattle. J. Agric. Sci. **109:** 315-320.

Phillippo, M., Humphries, W. R., Atkinson, T., Henderson, G. D., and Garthwaite, P. H. 1987b. The effect of dietary molybdenum and iron on copper status, puberty, fertility and oestrous cycles in cattle. J. Agric. Sci. **109:** 321-336.

Puls R. 1994. Vitamin levels in animal health: diagnostic data and bibliographies. Sherpa International, Clearbrook, British Colombia. 15-18.

Raisbeck, M. F., Siemion, R. S., and Smith, M. A. 2006. Modest copper supplementation blocks molybdenosis in cattle. J. Vet. Diagn. Invest. **18:** 566-572.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W., and Constable, P. D. 2007. Veterinary Medicine, 10th edit. pp. 1735-1755.

Rojas, M. A., Dyer, I. A., and Cassatt, W. A. 1965. Manganese efficiency in the bovine. J. Anim. Sci. **24:** 664-667.

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., and Haekstra, W. G. 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science. **179:** 588-590.

Siciliano-Jones, J. L., Socha, M. T., Tomlinson, D.J., and DeFrain, J. M. 2008. Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle. J. Dairy Sci. **91:** 1985-1995.

Smart, M. E., Cymbaluk, N. F., and Christensen, D. A. 1992. A review of copper status of cattle in Canada and recommendations for supplementation. Can. Vet. J. **33:** 163-170.

Spears, J. W., Harvey, R. W., and Segerson, E. C. 1986. Effects of marginal selenium deficiency and winter protein supplementation on growth, reproduction and selenium status of beef cattle. J. Anim. Sci. **63:** 586-594.

Steinnes, E. 2009. Soils and geomedicine. Environ. Geochem. Health. **31:** 523-535.

Suleiman, A., Okine, E., and Goonewardene, L. A. 1997. Relevance of National Research Council feed composition tables in Alberta. Can. J. Anim. Sci. **77:** 197-203.

Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annu. Rev. Nutri. **11:** 121-140.

Tasker, J. B., Bewick, T. D., Clark, R. G., and Fraser, A. J. 1987. Selenium response in dairy cattle. N. Z. Vet. J. **35:** 139-140.

Tessman, R. K., Lakritz, J., Tyler, J. W., Casteel, S. W., Williams, J. E., Dew, R. K. 2001. Sensitivity and specificity of serum copper determination for detection of copper deficiency in feeder calves. J. Amer. Vet. Med. Assoc. **218:** 756-760.

Waldner, C., Campbell, J., Jim, K. G., Guichon, P. T., and Booker, C. W. 1998. Comparison of three methods of selenium assessment in cattle. Can. Vet. J. **39:** 225-231.

Ward, G. M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: a review. J. Anim. Sci. **46:** 1078-1085.

Weiss, W. P. 1998. Requirements of fat-soluble vitamins for dairy cows: a review. J. Dairy Sci. **81:** 2493-2501.

Wikse, S. E., Herd, D., Field, R., and Holland, P. 1992. Diagnosis of copper deficiency in cattle. J. Amer. Vet. Med. Assoc. **200:** 1625-1629.

Wilson, J. G. 1966. Bovine functional infertility in Devon and Cornwall: response to manganese therapy. The Vet. Rec. **79:** 562-566.

Yavas, Y. and Walton, J. S. 2000. Postpartum acyclicity in suckled beef cows: a review. Therio. **54:** 25-55.

CHAPTER 2

GEOGRAPHIC DETERMINANTS OF COPPER AND MOLYBDENUM CONCENTRATIONS IN SERUM AT THE END OF THE GRAZING SEASON AND ASSOCIATIONS WITH REPRODUCTIVE PERFORMANCE IN BEEF COWS FROM WESTERN CANADA

The paper presented in Chapter 2 has been accepted for publication in the Canadian Journal of Animal Science, 2011, article in press. Data for this paper were collected as part of the Western Canadian Beef Productivity Study (WCBPS). The concept, design, management, and field data collection of this 2001-2003 study were undertaken by Dr. Cheryl Waldner. Data from the WCBPS presented in Chapter 2 were given to me by Dr. Waldner. I was responsible for the trace mineral data analysis and literature review presented in this chapter.

2.1 Introduction

The breeding season often occurs when cattle are grazing for many extensively managed cow-calf herds in western Canada. The success of the breeding season is assessed at the end of summer pasture turnout through examination for pregnancy or observation of the cow herd for signs of estrus activity. When the pregnancy rate is lower than expected, common causes related to bull performance, breeding pasture management, body condition, and infectious disease should be considered. If there is no apparent diagnosis, micronutrient deficiency is often suggested as a potential contributor to poor pregnancy rates (Sanders 2005; Maas 2007). This study investigates the relationship between reproductive health and copper and molybdenum serum concentrations.

The effect of inadequate copper liver and serum concentrations on fertility and early pregnancy is controversial (Phillippo et al. 1987; Muehlenbein et al. 2001; Enjalbert et al. 2006; Van De Weyer et al. 2011). While copper is an essential component of proper fetal development (Hostetler 2003), the fetus accumulates copper exponentially at the expense of maternal copper (Gooneratne and Christensen 1989; Gooneratne et al. 1989; Graham et al. 1994). A relationship exists between liver copper concentrations in dams and liver copper concentrations in their calves at birth (Gooneratne et al. 1989; Gengelbach et al. 1994). However, the question of whether inadequate maternal copper status increases the risk of abortion or stillbirth has not been examined.

Excess dietary molybdenum has also been reported to cause decreased conception rates either directly or through secondary copper deficiency (Phillippo et al. 1987). High dietary molybdenum and sulphate concentrations lead to the formation of thiomolybdates, compounds that adversely affect the absorption, utilization, and excretion of copper in ruminants (Suttle 1991; Gooneratne et al. 1994).

Copper deficiency, measured in liver and serum samples, has been identified in beef cows throughout North America (Gooneratne and Christensen 1989; Dargatz et al. 1999; Hoff et al. 2001). Differences between animals and factors in the environment can each affect the likelihood of deficiency and subsequent clinical effects. For example, Continental cattle breeds require more copper than British breeds (Smart and Christensen 1985; Gooneratne et al. 1994; Ward et al. 1995; Mullis et al. 2003). Primary copper deficiency has also been related to low copper concentrations in forages, cereal hay, and cereal grains (Gooneratne et al. 1989; Suleiman et al. 1997) which are reported to be more likely with some soil types (Kruger et al. 1985).

Molybdenum excess is sporadic but not uncommon. Forages that are high in molybdenum are often associated with distinctive natural geologic features, such as the molybdenum-rich shales in northwest Manitoba (Boila et al. 1984). Mining can also result in excess molybdenum in the soil, and forages grown on reclaimed land may be very high in molybdenum (Gardner et al. 2003; Raisbeck et al. 2006). Most cases of molybdenosis have been reported in grazing cattle because high molybdenum plant

material is more likely to cause toxicity in cattle when eaten fresh as compared to stored (Ward 1991; Majak et al. 2004).

The objectives of this paper were to describe the concentrations of copper and molybdenum in serum collected from western Canadian beef cows at fall pregnancy testing, to examine animal and environmental factors that were associated with copper and molybdenum concentrations, and to determine whether copper and molybdenum concentrations measured at the end of the grazing season were associated with important reproductive outcomes.

2.2 Materials and Methods

2.2.1 Herd and animal selection

Samples were collected from 66 cow-calf herds that were enrolled in a study of factors affecting productivity in 205 herds from western Canada (Waldner 2008). From this larger study, all herds with pregnancy rates < 90% in the fall of 2001 (n = 31) were recruited for an in-depth investigation of the role of infectious disease in low conception (Waldner 2005). Another 35 herds were randomly selected from the remaining herds with pregnancy rates ≥ 90% using a computer generated random numbers list.

Blood samples were collected from all accessible open cows and a systematic random sample of pregnant cows from each of the 66 herds in the study (Dohoo et al. 2003). Following analysis for infectious causes of reproductive failure (Waldner 2005), half of the remaining samples were then randomly allocated to this analysis. Only

aliquots containing a sufficient volume of non-hemolyzed serum were submitted to the laboratory. The selection was stratified by herd and pregnancy status. The number of samples analyzed was determined by funding availability.

2.2.2 Laboratory methods

The blood samples were allowed to clot. The serum was separated and frozen at -70 C within 48 hours of collection. Individual animal samples were coded so that laboratory staff was unaware of the animal's identity and the location of the herd.

A commercial laboratory (Prairie Diagnostic Laboratory, Saskatoon, SK) measured the copper and molybdenum concentrations using an inductively coupled plasma mass spectrometer (Thermo Jarrel Ash Corporation, Franklin, MA) (Van De Weyer et al. 2010).

2.2.3 Reproductive outcome data

The pregnancy status of individual cows was determined by the herd veterinarian using trans-rectal palpation in the fall of 2001. Cow age and body condition score based on a 9-point scale were also recorded (Rice 1991).

All animals were accounted for using detailed individual animal calving records maintained by the herd owner. An abortion was defined as an observed premature calving, judged to be at least one month prior to full term, or as an assumed calf loss from a cow that was diagnosed pregnant but which failed to calve. A stillborn calf was defined

as a calf born dead at, or within one hour of, birth that appeared to be within one month of full-term gestation.

2.2.4 Description of herd grazing management

Herd owners provided information about the availability of trace mineral supplementation for cows during the summer grazing season.

All land locations used for pasture during the 2001 growing season were categorized into ecoregions (Wiken 2008) using a geographic information system (ArcView GIS 3.2, ESRI Inc., Redlands, CA). Soil data were provided by Agriculture and Agri-Food Canada: the Canadian Soil Information System (Anonymous 2010a) and the National Soil Database (Anonymous 2008). Each herd was classified based on the dominant soil type for the pastures to which the cattle had access during the 2001 grazing season. Soil type categories were then further summarized by soil colour. Information on total accumulated precipitation from the 2001 growing season was obtained for the period April 1 to August 31, 2001 from the website Drought Watch (Anonymous 2010b).

2.2.5 Statistical analysis

All analyses were completed with a commercial software package (Stata for Windows, version 10, StataCorp, College Station, TX). The serum trace mineral concentrations were categorized on the basis of published reference cutoffs (Puls 1994) used by the testing laboratory. Serum concentrations of copper were classified as

adequate (≥ 0.6 ppm) or less than adequate. Serum concentrations of molybdenum were described as excessive (≥ 0.10 ppm) or acceptable.

Potential risk factors for serum copper and molybdenum concentrations were assessed using linear mixed models with a random intercept to account for clustering within herd (Dohoo et al. 2003). Cow age, breed type, body condition score prior to breeding and at pregnancy testing, history of trace mineral supplementation during the summer grazing season, and environmental characteristics, including ecoregion, soil type, soil colour, and precipitation, were individually evaluated for their association with serum micronutrient concentrations in a series of unconditional, or single variable, models. Cow age, cow breed and body condition score were categorized as follows: age (bred heifers – 1.5 y; bred 1st calf heifers – 2.5 y; mature cows 3.5 to 9.5 y; old cows > 10 y); cow breed (Continental breeds; Continental crossed with British breeds; and British breeds); and body condition score (BCS) at pre-breeding and at pregnancy testing (< 5/9 or ≥ 5/9).

Multivariable models were explored only if more than one risk factor was potentially important in the model ($P < 0.20$). Manual backwards elimination was used to achieve a final model containing statistically significant risk factors ($P < 0.05$) and important confounders. A factor was judged to be a confounder if removing or adding the factor from the model changed other effect estimates by more than 10%. After establishing the final summary main-effect model, biologically reasonable first-order interaction terms were added and assessed for their association with the outcome.

Residuals were examined graphically to assess model fit with assumptions of normality and homogeneous variance. Because soil type was considered in the determination of ecoregion, these two variables are highly correlated; therefore, these two factors were not considered together in the same model. Where both of these variables were significant, the competing models were compared for fit and complexity using Akaike's Information Criteria (AIC) (Dohoo et al. 2003). After adjusting for all important measured risk factors, investigators estimated the proportion of the remaining variance in copper and molybdenum concentrations that was explained by differences among herds ($\rho_h = \sigma^2_h / (\sigma^2_c + \sigma^2_h)$).

The unconditional associations between animal and herd level variables and reproductive outcomes were examined using a generalized linear mixed model (GLMM) with a binomial distribution, logit link function, and random intercepts to account for clustering by herd (Dohoo et al. 2003). Fall serum concentrations of copper and molybdenum were individually assessed for association with each of the reproductive outcomes, including non-pregnancy, abortion, and stillbirth. The linearity of the relationships between micronutrient concentrations and each reproductive outcome was examined by categorizing the continuous micronutrient concentrations into hierarchical indicator variables (quartiles). The log odds of the reproductive outcome were plotted against the quartiles for serum concentration and visually assessed for a plausible monotonic dose response relationship (Dohoo et al. 2003). Any unconditional models suggesting a potential association ($P < 0.25$) were examined further to adjust for other known risk factors for reproductive failure including age group and body condition at

pregnancy testing and to determine the adjusted log odds of the outcome. The reported odds ratios for copper represent the odds of each reproductive outcome in the cows with trace mineral concentrations in the lower quartiles relative to the odds of that outcome in cows with the highest quartile of trace mineral concentration. For molybdenum, the odds ratios compare the odds of each event in cows with the higher quartiles of serum concentration to those in the lowest quartile.

2.3 Results

2.3.1 Study Population

In the fall of 2001, 2516 cows and heifers were selected for sample collection from the 12,073 animals pregnancy tested in the 66 study herds. The average number of animals pregnancy tested per herd was 183 (range, 63 to 391). Serum micronutrient concentrations were analyzed for 783 breeding females from 66 herds (average, 12 per herd). This included 380 cows from 31 herds with pregnancy rates < 90% and 403 cows from 35 herds with pregnancy rates $\geq$ 90%; 479 of the 783 sampled cows were pregnant.

2.3.2 Description of Participating Cows

Of the 783 cows included in this analysis, 146 (18.6%) bred heifers [~1.5 y], 128 (16.3%) bred first-calf heifers [~2.5 y], 438 (55.9%) mature cows [~3.5 to 9.5 y], 46 (5.9%) old cows [> 10 y], and 25 (3.2%) unknown age. The breeds represented included 311 (40.3%) Continental, 365 (47.3%) Continental x British, 95 (12.3%) British, and 12 (1.5%) unknown. Body condition scores were available for 746 (95.3%) of the 783 cows

in the spring and 762 (97.3%) in the fall at pregnancy testing; 107 (14.3%) cows were thin (< 5 of 9) in the spring and 99 (13.0%) were thin in the fall.

Of the 443 pregnant cows that were retained after pregnancy testing, 8 (1.8%) subsequently aborted. Of the 435 remaining cows, 10 (1.3%) delivered a stillborn calf.

2.3.3 Description of Herd Management and Environment

Mineral supplementation practices on summer pasture varied by herd; 27% (18/66) provided loose mineral and 33% (22/66) provided only blocks or tubs, and 3 herd owners used both methods. Twenty-nine herd owners (44%) did not report providing supplementation on pasture.

Herds were located in 8 different ecoregions and the grazing pastures for these herds were located in 9 different soil zones **(Table 1)**. The amount of rainfall received by the pastures used during the 2001 growing season (April 1 to August 31) varied from less than 100 mm received to more than 300 mm **(Table 1)**.

2.3.4 Serum Copper and Molybdenum Concentrations Measured After the Summer Grazing Season

The mean serum copper concentration for individual cows was 0.82 ppm (*S,* 0.30 ppm). There was a substantial range in copper concentrations (5^{th} percentile, 0.45 ppm; 95^{th} percentile, 1.29 ppm) and 127/783 (16.2%) of cows had below adequate serum copper (< 0.60 ppm). The mean serum molybdenum concentration was 0.056 ppm (*S,*

0.055 ppm) and there was also substantial variation in molybdenum concentrations (5^{th} percentile, 0.005 ppm; 95^{th} percentile, 0.14 ppm). Serum molybdenum concentrations were high ($\geq$ 0.10 ppm) in 94/783 (12%) of cows.

The mean copper for cows with high molybdenum was 0.91 ppm (*S*, 0.29 ppm) and the mean copper for cows with < 0.10 ppm molybdenum was 0.81 ppm (*S*, 0.30 ppm). There was an association between increasing serum molybdenum and increasing serum copper (OR, 2.15; 95% CI, 1.46 to 3.16; $P < 0.001$).

Of the 66 herds in the study, 29 had at least 2 sampled cows with serum copper concentrations categorized as less than adequate (Figure 2.1). The mean herd average copper concentration was 0.82 ppm (*S*, 0.30 ppm), and the herd copper means ranged between 0.52 ppm (*S*, 0.14 ppm) and 1.27 ppm (*S*, 0.24 ppm). Of the 66 herds in the study, 23 had at least 2 sampled cows with serum molybdenum concentrations categorized as high (Figure 2.2). The mean herd average molybdenum concentration was 0.056 ppm (*S*, 0.055 ppm), and the herd molybdenum means ranged between 0.013 ppm (*S*, 0.013 ppm) and 0.21 ppm (*S*, 0.31 ppm).

2.3.5 Factors Associated with Serum Copper Concentrations

No significant associations were found between age, breed, BCS before breeding, BCS at pregnancy testing, trace mineral supplementation on summer pasture, pregnancy status, soil type, soil colour, or total precipitation during the growing season and fall serum copper concentrations ($P > 0.20$).

Ecoregion was unconditionally associated ($P = 0.01$) with fall serum copper concentration. Herds from the Northern Continental Divide ecoregion had lower copper concentrations than herds on Aspen Parkland ($P = 0.04$), Boreal Transition ($P = 0.001$), Fescue Grassland ($P = 0.009$), Mixed Grassland ($P = 0.003$) and Peace Lowland ($P = 0.02$) ecoregions. Herds from the Moist Mixed Grassland ecoregion had lower copper concentrations than herds in the Boreal Transition ($P = 0.006$) and Mixed Grassland ($P = 0.02$) ecoregions. After adjusting for ecoregion, the proportion of remaining variation in serum copper concentrations accounted for by between-herd differences was 22.6%.

2.3.6 Factors Associated with Serum Molybdenum Concentrations

There were no significant associations between age, breed, BCS before breeding, BCS at pregnancy testing, trace mineral supplementation on summer pasture, or pregnancy status and fall serum molybdenum concentrations ($P \geq 0.19$). Ecoregion ($P = 0.002$), soil type ($P = 0.01$), soil colour ($P = 0.02$), and total precipitation during the growing season ($P = 0.004$) were each unconditionally associated with fall serum molybdenum concentrations.

Ecoregion, soil type, soil colour, and total precipitation during the growing season were examined in separate models. Herds from the Western Alberta Upland ecoregion had higher molybdenum concentrations than herds in the Aspen Parkland ($P = 0.003$), Boreal Transition ($P = 0.005$), Fescue Grassland ($P = 0.006$), Mixed Grassland ($P = 0.046$) and Moist Mixed Grassland ($P < 0.0001$) ecoregions. Herds from Peace Lowland

had higher Mo concentrations than herds in the Aspen Parkland ($P = 0.01$), Boreal Transition ($P = 0.02$), Fescue Grassland ($P = 0.03$), and Moist Mixed Grassland ($P = 0.001$) ecoregions. Herds on the Mixed Grassland ecoregion had higher Mo concentrations than herds in the Moist Mixed Grassland ecoregion ($P = 0.03$) **(Table 1)**. After adjusting for ecoregion, 26.6%.of the total variation in molybdenum concentrations was explained by differences among herds.

Herds from Dark Gray soil types (Chernozemic or Luvisolic) had higher molybdenum concentrations than herds on Black Chernozemic ($P = 0.005$), Dark Brown Chernozemic ($P = 0.003$), Dark Brown Solonetzic ($P = 0.03$), and Regosolic ($P = 0.03$) soil types. Herds from Gray Luvisolic soil type had higher serum molybdenum concentrations than herds from Black Chernozemic ($P = 0.004$) and Dark Brown Chernozemic soils ($P = 0.003$) **(Table 1)**. After adjusting for soil type, 27.8% of the total variation in molybdenum concentrations was explained by differences among herds.

Herds from Gray soils had higher serum molybdenum concentrations than herds from Black soils ($P = 0.003$) and herds from Brown soils ($P = 0.004$). After adjusting for soil colour, 30.4% of the total variation in molybdenum concentrations was explained by differences among herds.

Herds from areas with total precipitation of 301-350 mm had higher serum molybdenum concentrations than herds from areas with 75-100 mm ($P = 0.04$), 100-150 mm ($P = 0.001$), 151-200 mm ($P < 0.001$), 201-250 mm ($P < 0.001$), and 251-300 mm (P

= 0.008) total precipitation between April 1 and August 31, 2001 **(Table 1)**. After adjusting for total precipitation, 27.9% of the total variation in molybdenum concentrations was explained by differences among herds.

The models examining the association between ecoregions, soil colour, and total precipitation and serum molybdenum concentrations were slightly superior based on calculated AIC values to the model exploring the differences in serum molybdenum concentrations across soil types.

2.3.7 Associations Between Serum Copper and Molybdenum Concentrations Measured After the Summer Grazing Season and Reproductive Outcomes

Serum copper concentrations, categorized by quartiles, were not associated with the odds of non-pregnancy ($P = 0.09$). However, cows with serum copper concentrations in the lowest quartile (0.17 to 0.63 ppm) were more likely to be pregnant than cows with copper concentrations in the highest quartile (0.94 to 2.81 ppm) (**Table 2**). Adjusting for age and BCS had relatively little effect on the difference in the odds of non-pregnancy between the cows with the lowest and highest concentration of serum copper (OR, 0.56; 95%CI, 0.35 to 0.92; $P = 0.02$).

There were no detectable associations between the concentration of serum copper in the fall and the odds of abortion or stillbirth **(Table 2)**. There were also no detectable associations between serum molybdenum concentrations at pregnancy testing and the odds of non-pregnancy, abortion, or stillbirth in these cows **(Table 2)**.

2.4 Discussion

The percentage of cows (16%) with below adequate (≤ 0.60 ppm) copper concentrations was half that reported in previous studies of beef cattle in North America (Gooneratne and Christensen 1989; Dargatz et al. 1999). This difference may be due to variations in the season of sample collection, changes in meteorological and pasture conditions between years, or systematic differences in nutritional management. Serum copper concentrations in beef cows have been reported to be higher in the fall than in the spring (Smart et al. 1992; Van De Weyer 2010), possibly accounting for higher copper concentrations in this population. It is also possible that the herd owners who enrolled in this study were more likely to supplement than other beef producers.

The present study also identified 12% of cows with elevated (≥ 0.10 ppm) serum molybdenum; however, no reports of serum molybdenum concentrations were identified from previous field studies for comparison. While concentrations in feed and water were not available for these herds, blood molybdenum concentrations are reported to reflect dietary intake (Ward 1978; Wittenberg and Devlin 1987). In this study, cows with high serum molybdenum also had higher serum copper concentrations than cows with serum molybdenum concentrations less than 0.1 ppm. A diet high in molybdenum will ultimately decrease liver copper, through the formation of molybdenum and sulphur compounds called thiomolybdates (Gooneratne et al. 1994). However, some studies have reported a transient increase in circulating copper concentrations in animals fed high molybdenum diets, although this increase was less evident as liver copper reserves

declined (Mason 1986; Wang et al. 1987; Gooneratne et al. 1994). Research has indicated that the increase in circulating copper is likely to be of systemic origin and may reflect some of the mechanisms by which thiomolybdates deplete copper reserves (Mason 1986). Thiomolybdates can cause copper deficiency via several mechanisms, which include limiting copper absorption in the gut; increasing copper-binding to albumin and inhibiting the copper-containing enzymes in plasma; changing the availability of copper in tissues; and increasing excretion of copper via bile, urine and feces (Mason 1986; Wang 1987; Gooneratne et al. 1989; Gooneratne et al. 1994).

While none of the measured individual cows' characteristics explained differences in copper or molybdenum serum concentration observed at pregnancy testing in these herds, previous studies have identified relationships between breed and body condition score and serum copper concentration. Simmental and Charolais cattle have been reported to have lower serum copper than British breeds when fed diets with marginal amounts of copper (Smart and Christensen 1985, Gooneratne et al. 1989; Ward et al. 1995; Mullis et al. 2003). Most of the animals included in this study were from mixed commercial herds and only breed type was reported. Littledike et al. (1995) reported that higher concentrations of serum copper were associated with higher carcass lipid concentrations in beef cows aged 6 to 14 years, and Van De Weyer (2011) reported thin cows as being more likely to have lower serum copper concentrations. Since the majority of cows in this study were in good body condition, there may have been limited power to assess differences in copper concentrations. There were no similar studies examining molybdenum concentrations.

Trace mineral supplementation on summer pasture was not associated with serum concentrations of copper in individual animals measured at pregnancy testing. The intake of free-choice supplements was not measured and could vary substantially in a pasture setting. In a large survey of beef cow-calf producers and herds in the United States, Dargatz et al. (1999) reported that the percentage of cow serum samples classified as above adequate for copper was, in general, higher when trace mineral supplements were used. However, a substantial proportion (39%) of cows from herds supplemented with trace minerals had below adequate copper concentrations (Dargatz et al. 1999), indicating that herd-level supplementation practices are not reliable predictors of individual animal copper status. It is also difficult to correlate the timing of copper supplementation to serum copper concentrations in cattle with adequate copper reserves. The liver is the primary storage organ for copper and maintains copper homeostasis for the animal (Gooneratne et al. 1989) and inadequate copper intake is not consistently reflected as below adequate serum copper concentrations until liver copper concentrations fall below 40 ppm on a dry weight basis (Claypool et al. 1975).

Soil type and ecoregion should be considered when evaluating the need for feed testing and supplementation. Cows from herds located on the Northern Continental Divide ecoregion had significantly lower copper concentrations than herds from all other ecoregions examined, except the Moist Mixed Grassland. The Northern Continental Divide is a mountainous region spanning the southern Alberta-British Columbia border. Gray soils within the Northern Continental Divide ecoregion have previously been

associated with low soil copper (Kruger et al. 1985) and with low serum copper in cattle located within these areas (Gooneratne and Christensen 1989). Ecoregions encompass plant and climatic factors that might also affect the bioavailability of copper (Soon and Abboud 1990; Berger 1996), potentially explaining why this variable was associated with copper concentrations in cows when soil type was not.

Cows located in areas with gray soils also had higher molybdenum concentrations than cows located in areas with black or brown soils. Cows in the Western Alberta Upland ecoregion and in areas receiving the greatest amounts of precipitation also had higher serum molybdenum concentrations. Secondary copper deficiency can result from high concentrations (> 100 ppm) of molybdenum in the diet of ruminants (Ward 1978; Phillippo et al. 1987; Suttle 1991), as well as from a low dietary ratio of copper to molybdenum (< 3 parts copper to 1 part molybdenum) (Ward 1978).

There were no associations between copper and molybdenum concentrations measured at the end of the grazing season and reproductive outcomes measured in these cows, with the exception that cows with the lowest serum copper concentrations at pregnancy testing were more likely to be pregnant than cows with higher copper concentrations. This finding was not unexpected as pregnancy status could also be considered as a risk factor for copper status. The bovine fetus accumulates copper exponentially at the expense of maternal copper and maternal liver copper declines during gestation (Gooneratne and Christensen 1989; Gooneratne et al. 1989). Low maternal serum copper can result if maternal liver concentrations fall below a critical

threshold (Claypool et al. 1975). The number of abortions and stillbirths in the animals that were sampled was very low, limiting the power of this study to detect an association between micronutrient concentrations and these reproductive events. The relatively small proportion of cows with serum copper and molybdenum concentrations outside of adequate levels also limited study power. The only association suggesting further research might be warranted was that between high molybdenum and the occurrence of stillbirth where the odds ratios were above 1 and the confidence intervals were very wide.

The practice of comparing serum copper and molybdenum concentrations between pregnant and non-pregnant animals in the fall as a tool in investigating poor pregnancy rates is not supported by the present study. In a recent study of beef cows in southern Saskatchewan, cows less than 10 years of age with low pre-breeding serum copper concentrations (< 0.40 ppm) were observed to have increased odds of non-pregnancy compared to cows with higher copper concentrations (Van De Weyer et al. 2011). Consequently, veterinarians working with herds experiencing poor reproductive performance in areas of potential copper deficiency should consider testing serum copper concentrations well before the start of the subsequent breeding season rather than at pregnancy-testing time so that appropriate supplements can be provided. Geographic location can help determine the risk of primary and secondary copper deficiency, as well as inform the need for trace mineral testing of feed samples and careful consideration of trace mineral supplementation practices.

2.5 References

Anonymous. 2008. National Soil DataBase. [Online] Available at: http://sis.agr.gc.ca/cansis/nsdb/index.html [15 March 2010].

Anonymous. 2010a. Canadian Soil Information System. [Online] Available at: http://sis.agr.gc.ca/cansis/index.html. [15 March 2010].

Anonymous. 2010b. Drought Watch. [Online] Available at: http://www.agr.gc.ca/pfra/drought/index_e.htm. [6 September 2010].

Berger, L. L. 1996. Variation in the trace mineral content of feedstuffs. The Prof. Anim. Scientist. **12:** 1-5.

Boila, R. J., Devlin, T. J., Drysdale, J. M., and Lillie, L. E. 1984. Geographical variation in the copper and molybdenum content of forages grown in northwestern Manitoba. Can. J. Anim. Sci. **64:** 899-918.

Claypool, D. W., Adams, F. W., Pendell, H. W., Hartmann, N. A., and Bone, J. F. 1975. Relationship between the level of copper in the blood plasma and the liver of cattle. J. Anim. Sci. **41:** 911-914.

Dargatz, D. A., Garry, F. B., Clark, G. B., and Ross, P. F. 1999. Serum copper concentrations in beef cows and heifers. J. Am. Vet. Med. Assoc. **215:** 1828-1832.

Dohoo, I., Martin, W., and Stryhn, H. 2003. Veterinary epidemiologic research. Charlottetown, PEI, Canada: AVC Inc. Pages 32, 502-504.

Enjalbert, F., Lebreton, P., and Salat, O. 2006. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study. J. Anim. Physiol. and Anim. Nutr. **90:** 459-466.

Gardner, W. C., Broersma, K., Popp, J. D., Mir, Z., Mir, P. S., and Buckley, W. T. 2003. Copper and health status of cattle grazing on high-molybdenum forage from a reclaimed mine tailing site. Can. J. Anim. Sci. **83:** 479-485.

Gengelbach, G.P., Ward, J.D., and Spears, J.W. 1994. Effect of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. J. Anim. Sci. **72:** 2722-2727.

Gooneratne, S. R. and Christensen, D. A. 1989. A survey of maternal copper status and fetal tissue copper concentrations in Saskatchewan bovine. Can. J. Anim. Sci. **69:** 141-150.

Gooneratne, S. R., Buckley, W. T., and Christensen, D. A. 1989. Review of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci. **69:** 819-845.

Gooneratne, S. R., Symonds, H. W., Bailey, J. V., and Christensen, D. A. 1994. Effects of dietary copper, molybdenum and sulfur on biliary copper and zinc excretion in Simmental and Angus cattle. Can. J. Anim. Sci. **74:** 315-325.

Graham, T. W., Thurmond, M. C., Mohr, C. F., Holmberg C. A., Anderson, M. L., and Keen, C. L. 1994. Relationships between maternal and fetal liver copper, iron, manganese, and zinc concentrations and fetal development in California Holstein dairy cows. J. Vet. Diagn. Invest. **6:** 77-87.

Hoff, B., Schrier, N., Boermans, H., Faulkner, H., and Hussein, A. 2001. Assessment of trace mineral and vitamin E status beef cows in Ontario. Can. Vet. J. **42:** 384-385.

Hostetler, C. E., Kincaid, R. L., and Mirando, M. A. 2003. The role of essential trace elements in embryonic and fetal development of livestock. The Vet. Journal. **166:** 125-139.

Kruger, G. A., Karamanos, R. E., and Singh, J. P. 1985. The copper fertility of Saskatchewan soils. Can. J. Soil. Sci. **65:** 89-99.

Littledike, E. T., Wittum, T. E., and Jenkins, T. G. 1995. Effect of breed, intake, and carcass composition on the status of several macro and trace minerals of adult beef cattle. J. Anim. Sci. **73:** 2113-2119.

Maas, J. 2007. Diagnostic considerations for evaluating nutritional problems in cattle. Vet. Clin. North Am. Food Anim. Pract. **23:** 527-539.

Majak, W., Steinke, D., McGillivray, J., and Lysyk, T. 2004. Clinical signs in cattle grazing high molybdenum forage. J. Range Manage. **57:** 269-274.

Mason, J. 1986. Thiomolybdates: mediators of molybdenum toxicity and enzyme inhibitors. Toxicology. **42:** 99-109.

Muehlenbein, E. L., Brink, D. R., Deutscher, G. H., Carlson M. P., and Johnson A. B. 2001. Effects of inorganic and organic copper supplemented to first-calf cows on cow reproduction and calf health and performance. J. Anim. Sci. **79:** 1650-1659.

Mullis, L. A., Spears, J. W., and McCraw, R. L. 2003. Estimated copper requirements of Angus and Simmental heifers. J. Anim. Sci. **81:** 865-873.

Phillippo, M., Humphries, W. R., Atkinson, T., Henderson, G. D., and Garthwaite, P. H. 1987. The effect of dietary molybdenum and iron on copper status, puberty, fertility and oestrous cycles in cattle. J. Agric. Sci. **109:** 321-336.

Puls, R. 1994. Mineral levels in animal health: diagnostic data, 2nd ed. Sherpa International, Clearbrook, British Columbia. Pages 83, 230.

Raisbeck, M. F., Siemion, R. S., and Smith, M. A. 2006. Modest copper supplementation blocks molybdenosis in cattle. J. Vet. Diagn. Invest. **18:** 566-572.

Rice, L. E. 1991. The effects of nutrition on reproductive performance of beef cattle. In: Maas, J. (editor). Beef cattle nutrition. Vet. Clin. North Am. Food Anim. Pract. **7:** 1-26.

Sanders, D. E. 2005. Troubleshooting poor reproductive performance in large herds. Vet. Clin. North Am. Food Anim. Pract. **21:** 289-304.

Smart, M. E. and Christensen, D. A. 1985. The effect of cow's dietary copper intake, sire breed, age on her copper status and that of her fetus in the first ninety days of gestation. Can. J. Comp. Med. **49:** 156-158.

Smart, M. E., Cymbaluk, N. F., and Christensen, D. A. 1992. A review of copper status of cattle in Canada and recommendations for supplementation. Can. Vet. J. **33:** 163-170.

Soon, Y. K. and Abboud, S. 1990. Trace elements in agricultural soils of northwestern Alberta. Can. J. Soil Sci. **70:** 277-288.

Suleiman, A., Okine, E., and Goonewardene, L. A. 1997. Relevance of National Research Council feed composition tables in Alberta. Can. J. Anim. Sci. **77:** 197-203.

Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annu. Rev. Nutri. **11:** 121-140.

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.

Van De Weyer, L. M., Hendrick, S. , and Waldner, C. L. 2011. Associations between pre-breeding serum micronutrient concentrations and pregnancy outcome in beef cows. J. Am. Vet. Med. Assoc. (in press).

Waldner, C. L. 2005. Serological status for N. caninum, Bovine Viral Diarrhea Virus, and Infectious Bovine Rhinotracheitis Virus at pregnancy testing and reproductive performance in beef herds. Anim. Repro. Sci. **90:** 219-242.

Waldner, C. L. 2008. Western Canada study of animal health effects associated with exposure to emissions from oil and natural gas field facilities. Study design and data collection I. Herd performance records and management. Arch. of Environ. and Occup. Health. **63:** 167-186.

Wang, Z. Y., Poole, D., and Mason, J. 1987. The uptake and intracellular distribution of [^{35}S] trithiomolybdate in bovine liver in-vivo. J. Inorg. Biochem. **31:** 85-93.

Ward, G. M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: a review. J. Anim. Sci. **46:** 1078-1085.

Ward, G. M. 1991. Acceptable limits of molybdenum for ruminants exist. Feedstuffs. **63:** 15-22.

Ward, J. D., Spears, J. W., and Gengelbach, G. P. 1995. Differences in copper status and copper metabolism among Angus, Simmental, and Charolais cattle. J. Anim. Sci. **73:** 571-577.

Wiken, E., Director. 2008. A National Ecological Framework for Canada. [Online] Available at: http://sis.agr.gc.ca/cansis/publications/ecostrat/intro.html. [17 April 2010].

Wittenberg, K. M. and Devlin, T. J. 1987. Effects of dietary molybdenum on productivity and metabolic parameters of lactating beef cows and their offspring. Can. J. Anim. Sci. **67**: 1055-1066.

Table 2.1 The distribution of mean serum copper and mean serum molybdenum concentrations for 783 cows from 66 herds sampled in the fall of 2001, by ecoregion, soil type, and level of precipitation for the herds of origin.

Geographic location	Number of herds represented in each region	No. of cows represented in each region	Mean serum copper concentration and standard deviation (*S*), in ppm	Mean serum molybdenum concentration and standard deviation (*S*), in ppm
Ecoregion				
Aspen Parkland	10/66	116	0.80 (0.31)*b,c*	0.044 (0.037)*b,d*
Boreal Transition	7/66	83	0.95 (0.36)*b*	0.046 (0.034)*b,d*
Fescue Grassland	12/66	134	0.87 (0.31)*b,c*	0.052 (0.049)*b,d*
Mixed Grassland	10/66	112	0.86 (0.32)*b*	0.062 (0.050)*b,c*
Moist Mixed Grassland	11/66	129	0.73 (0.21)*a,c*	0.035 (0.032)*b,d*
Northern Continental Divide	3/66	39	0.63 (0.20)*a,c*	0.048 (0.035)*a,b,c,d*
Peace Lowland	8/66	127	0.82 (0.27)*b,c*	0.073 (0.080)*a,c*
Western Alberta Upland	4/66	43	0.76 (0.18)*a,c*	0.12 (0.081)*a,c*
Predominate Soil type[z]				
Black Chernozemic	24/66	277	0.85 (0.34)	0.047 (0.043)*b*
Black Solonetzic	3/66	64	0.80 (0.27)	0.067 (0.046)*a,c*
Brown Chernozemic	8/66	80	0.90 (0.35)	0.062 (0.040)*a,c*
Brunisolic Gray Luvisolic	3/66	40	0.82 (0.13)	0.058 (0.028)*a,c*
Dark Brown Chernozemic	10/66	117	0.73 (0.23)	0.042 (0.049)*b*
Dark Brown Solonetzic	2/66	28	0.79 (0.17)	0.036 (0.023)*b,c*
Dark Gray Chernozemic or Dark Gray Luvisolic	8/66	53	0.71 (0.21)	0.095 (0.083)*a,c*
Gray Luvisolic	10/66	92	0.86 (0.32)	0.077 (0.089)*a,c*
Regosolic	4/66	32	0.79 (0.16)	0.036 (0.029)*b,c*
Total precipitation received between April 1 and August 31, 2001				
75-100 mm	2/66	21	0.78 (0.19)	0.055 (0.037)*b*
101-150 mm	8/66	77	0.81 (0.30)	0.045 (0.042)*b*
151-200 mm	20/66	244	0.80 (0.35)	0.045 (0.043)*b*
201-250 mm	18/66	196	0.84 (0.25)	0.051 (0.042)*b*
251-300 mm	15/66	208	0.84 (0.28)	0.064 (0.068)*b*
301-350 mm	3/66	37	0.74 (0.19)	0.131 (0.079)*a*

[z] Several herd owners used land from more than 1 soil type.
a-c Means in a given column not sharing the same letter differ ($P < 0.05$); column sections without letters have no differences between any location.

Table 2.2 The unconditional associations between serum copper and molybdenum concentrations (divided into quartiles) measured after the 2001 grazing season and 3 adverse reproductive outcomes, including non-pregnancy (n=783 cows), abortion (443 cows), and stillbirth (435 cows) from 66 herds.

Reproductive Outcome: Trace Mineral Concentration	Odds Ratio (OR)	95% Confidence Interval for OR		*P* value
		Lower	Upper	
Non-pregnancy: Cu quartiles (ppm)				0.09[y]
0.17-0.63 vs. 0.94-2.81	0.55	0.34	0.89	0.02[z]
0.64-0.76 vs. 0.94-2.81	0.88	0.57	1.37	0.57[z]
0.77-0.93 vs. 0.94-2.81	0.88	0.57	1.37	0.31[z]
Abortion: Cu quartiles (ppm)				0.89[y]
0.17-0.63 vs. 0.94-2.81	2.28	0.19	27.9	0.52[z]
0.64-0.76 vs. 0.94-2.81	1.55	0.12	20.7	0.74[z]
0.77-0.93 vs. 0.94-2.81	0.96	0.05	17.0	0.98[z]
Stillbirth: Cu quartiles (ppm)				0.94[y]
0.17-0.63 vs. 0.94-2.81	0.86	0.17	4.35	0.86[z]
0.64-0.76 vs. 0.94-2.81	0.65	0.11	4.0	0.64[z]
0.77-0.93 vs. 0.94-2.81	0.60	0.01	3.7	0.58[z]
Non-pregnancy: Mo quartiles (ppm)				0.42[y]
0.022-0.044 vs. 0.001-0.021	0.95	0.61	1.49	0.82[z]
0.045-0.073 vs. 0.001-0.021	0.77	0.49	1.20	0.25[z]
0.074-0.84 vs. 0.001-0.021	1.12	0.71	1.76	0.64[z]
Abortion: Mo quartiles (ppm)				0.83[y]
0.022-0.044 vs. 0.001-0.021	1.13	0.12	10.6	0.92[z]
0.045-0.073 vs. 0.001-0.021	1.86	0.25	13.7	0.54[z]
0.074-0.84 vs. 0.001-0.021	0.60	0.04	8.60	0.71[z]
Stillbirth: Mo quartiles (ppm)				0.43[y]
0.022-0.044 vs. 0.001-0.021	1.96	0.18	22.0	0.58[z]
0.045-0.073 vs. 0.001-0.021	4.68	0.55	40.8	0.16[z]
0.074-0.84 vs. 0.001-0.021	2.00	0.18	22.4	0.57[z]

[y] *P* values testing the overall association between the concentration of each micronutrient when categorized into quartiles and the odds of each reproductive outcome.
[z] *P* values for the comparison of the relative difference in the odds of the reproductive outcome between the two listed quartiles of micronutrient concentration.

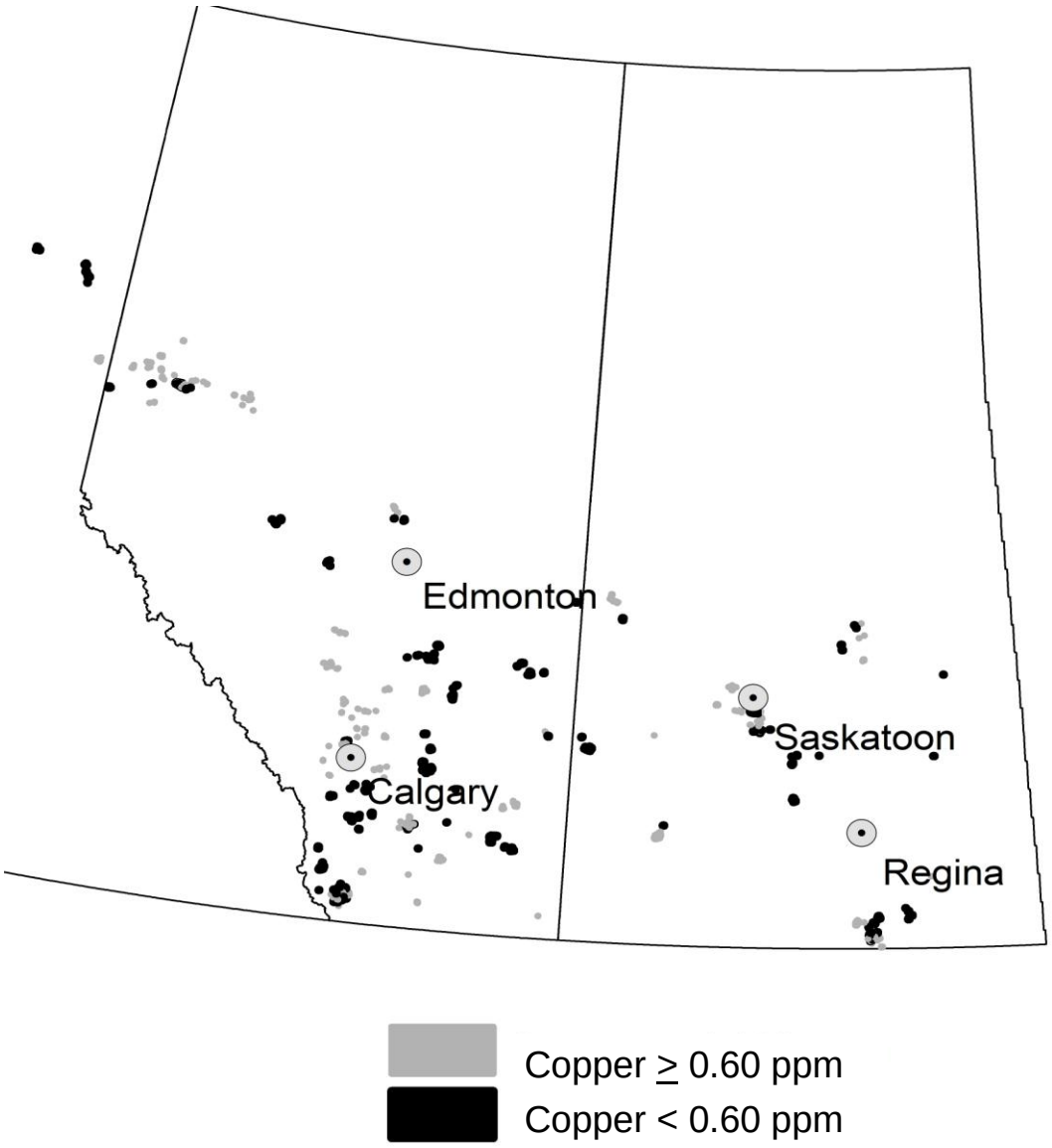

Figure 2.1 Map of pasture locations throughout western Canada (British Columbia, Alberta, Saskatchewan) used by 29 herds that had ≥ 2 sampled cows with less than adequate serum copper concentrations (< 0.60 ppm) and 37 herds that had fewer than 2 sampled cows with less than adequate copper concentrations, measured at the end of the grazing season.

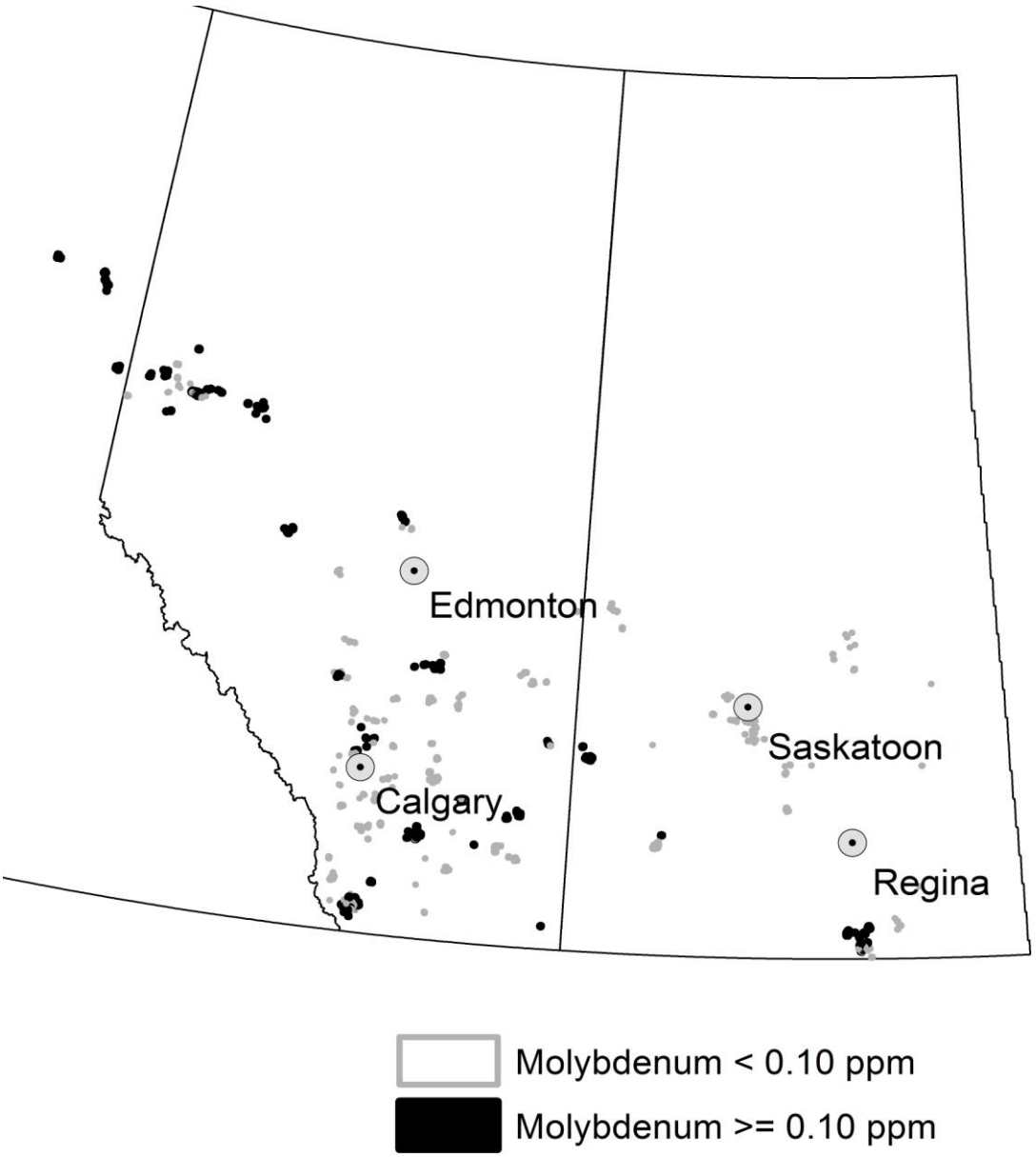

Figure 2.2 Map of pasture locations throughout western Canada (British Columbia, Alberta, Saskatchewan) used by 23 herds that had ≥ 2 sampled cows with high serum molybdenum concentration (≥ 0.10 ppm) and 43 herds that had fewer than 2 sampled cows with high serum molybdenum concentrations, measured at the end of the grazing season.

CHAPTER 3

ASSOCIATIONS BETWEEN PREBREEDING SERUM MICRONUTRIENT CONCENTRATIONS AND PREGNANCY OUTCOME IN BEEF COWS

The paper presented in Chapter 3 has been published and can be found under: **L. M. Van De Weyer, S. H. Hendrick, C. L. Waldner. 2011.** Associations between prebreeding serum micronutrient concentrations and pregnancy outcome in beef cows. J. of the Amer. Vet. Med. Assoc. **238:** 1323-1332.

Together with Chapter 4, Chapter 3 describes the results of an independent research study evaluating micronutrient concentrations in beef cows in southern Saskatchewan. I contributed to the project design and in preparing and submitting grant proposals for part of the funding for the project. I was involved in herd enrolment, sample collection, survey design and information collection, and communication of results to PFRA patrons and staff involved in the study. I coordinated sample submission to the laboratories, entered and cleaned much of the data, and conducted all of the data analysis. I did not perform any of the laboratory analysis for this paper. The WCVM Toxicology Laboratory analyzed the serum micronutrient concentrations The WCVM Toxicology Laboratory and Prairie Diagnostic Services (PDS), Saskatoon, SK analyzed the serum micronutrient concentrations and provided the *C. fetus* cultures, *Tritrichomonas. foetus* polymerase chain reactions (PCR), and BVDV serum neutralization titers. The WCVM Molecular Biology Laboratory developed and conducted the PCR analysis for *C. fetus*; although these results are not described in the following paper due to word number restrictions by the publishing journal, the methodology is outlined in Appendix A.3.

3.1 Introduction

Micronutrient deficiencies are frequently suggested as risk factors for reproductive failure in beef herds (Sanders 2005; Maas 2007). However, the impact of inadequate circulating concentrations of micronutrients on the reproductive performance of cattle remains unclear, despite evidence of less than adequate blood and serum concentrations of copper, selenium, manganese, and vitamin E in beef cows in many regions of North America (Campbell et al. 1995; Dargatz and Ross 1996; Dargatz et al. 1999; Hoff et al. 2001). To the authors' knowledge, the effect of prebreeding serum micronutrient concentrations on the subsequent pregnancy outcome of beef cows has not been examined under field conditions.

Poor pregnancy rates are among the most common complaints presented to the Outbreak Investigation Unit at the Western College of Veterinary Medicine. Reproductive problems are typically recognized during fall months when more cows than expected are found to be open (nonpregnant), or when a high number of cows are determined to have signs of estrus when they are moved off summer pasture. After common risk factors for nonpregnancy such as compromised health of the bull, breeding management, cow age and body condition, and common infectious diseases are ruled out, the micronutrient status of breeding cows is often questioned as a potential contributor to the problem. However, the lack of applicable studies examining associations between micronutrient concentrations and reproductive outcomes in beef cattle leaves veterinarians without the necessary scientific evidence to address this question.

The effects of dietary micronutrient supplementation on reproductive outcomes in cattle have been examined in feeding trials (Olson et al. 1999; Bass et al. 2001; Muehlenbein et al. 2001; Gunter et al. 2003; Ahola et al. 2004; Black and French 2004; Siciliano-Jones et al. 2008). The variations in supplement type and concentration, as well as duration and timing of supplementation within the production cycle make it difficult to draw conclusions about a specific micronutrient and its effect on the reproductive performance in client-owned herds. Many small trials also lacked adequate power to demonstrate a significant difference in pregnancy outcomes between treatment groups (Olson et al. 1999; Muehlenbein et al. 2001; Gunter et al. 2003). Conclusions from these controlled feeding trials, especially those conducted in dairy cattle (Black and French 2004; Siciliano-Jones et al. 2008), are therefore difficult to generalize to extensively managed herds of beef cattle.

Very few epidemiologic studies have been published in which investigators examined associations between micronutrient concentrations and reproductive outcomes. Results of 1 large case-control study (Enjalbert et al. 2006) revealed associations between low herd-level plasma zinc and selenium concentrations and herd-level reproductive disorders (low fertility, abortions, and retained placentas) in dairy and beef cattle. However, because other known risk factors for reproductive failure were not considered in that analysis, the reported associations between micronutrient concentrations and reproductive outcomes were potentially biased. Also, the timing of serum collection relative to bull exposure, insemination and pregnancy assessment was not described, so it

is not possible to compare prebreeding plasma concentrations of the trace minerals to reproductive outcomes by use of the published results.

The objective of the study reported here was to determine associations between serum concentrations of copper, molybdenum, selenium, vitamin A, and vitamin E measured in beef cows prior to breeding and pregnancy status at the end of the summer pasture season.

3.2 Materials and Methods

3.2.1 Selection of herds

Owners of beef herds were contacted to recruit cattle for the study between February 1, 2008 and March 28, 2008. The cattle were being sent to 5 southern Saskatchewan community pastures with histories of poor herd-level pregnancy rates. Researchers presented objectives of the study and requirements for participation to herd owners who used each of the community pastures. Forty herd owners agreed to participation at each of the 5 pastures as follows: pasture 1, 9 of 51 (17.6%); pasture 2, 8 of 30 (26.7%); pasture 3, 10 of 69 (14.5%); pasture 4, 8 of 23 (34.8%); and pasture 5, 5 of 27 (18.5%). Each of the herd owners that agreed to study participation sent a group of cows to the community pasture closest to their home location between May 14 and 22, 2008. Of 40 herds offered for enrolment, 771 beef cows from 39 cow-calf herds were included in the study; 1 herd from pasture 5 was omitted because it only comprised yearling heifers. At the end of the study, blood samples were collected from 205 calves (not vaccinated against BVDV) that shared pastures with the study cows in order to

evaluate exposure to BVDV. These calves belonged to the owners of the breeding cattle, and consent was obtained for their use in the study. The study was approved by the University of Saskatchewan Committee on Animal Care and Supply.

3.2.2 Community pastures

Five community pastures located in rangelands owned and managed by the Canadian federal government were selected for the present study. Fee-based services, including grazing and breeding services for cattle, were provided to livestock producers between May and October as part of an ongoing program (Anonymous 2007a). Onsite management of each community pasture was provided by federally employed pasture managers and seasonal labourers. Each pasture manager determined the cattle stocking rate after consideration of soil moisture levels, water supplies, and carryover and vigour of forage (comprised of tame [introduced] and native perennial mixed-species grasses).

The selected community pastures comprised a range of ecosystems and soil types, and were located in 3 ecoregions (Anonymous 2008): 2 were in moist mixed grasslands (pastures 1 and 2), 2 were in mixed grasslands (pastures 3 and 4), and 1 was in aspen parkland (pasture 5). The reported soil types for the 5 pastures (reflecting various amounts of moisture and organic matter content) were as follows: 2 brown chernozemic (pastures 3 and 4), 1 dark brown chernozemic (pasture 2), 1 dark brown solonetzic (pasture 1), and 1 black chernozemic (pasture 5) (Anonymous 2008). The median distance between these 5 community pastures was 250 km (range, 75 km to 550 km).

3.2.3 Precipitation and temperature

Data from Environment Canada weather stations (Anonymous 2010) closest to each community pasture were accessed to determine daily precipitation measurements (in millimeters) and daily maximum temperatures (in ºC) for the period April 1, 2008 to October 31, 2008. Two pastures were located near the same weather station, and data from this station were used for both of these pastures.

3.2.4 Herd management survey data

Herd owners provided answers to open-ended questions regarding herd size, overall calving outcomes for 2008, trace mineral and vitamin supplementation programs for the spring of 2008, and vaccination programs for control of infectious diseases that could affect reproductive health (BVDV, IBR, *Leptospira* spp, and *Campylobacter fetus*).

3.2.5 Individual cow survey data

Herd owners were requested to provide detailed information for each cow enrolled in the study. This data included age, calving date, and any history of exposure to bulls in 2008 prior to the start of the community pasture breeding season.

3.2.6 Breeding field management survey data

Each community pasture was divided into breeding fields to accommodate client needs and pasture limitations. Professional pasture managers provided answers to open-ended questions regarding management of the breeding fields in which enrolled cows

were kept, including bull breed, bull identification, bull and cow numbers, breeding season dates, breeding field size, and location.

Allocation of herds to particular breeding fields was dictated by the herd owners, who specified the breed of bull and breeding season dates within the range of choices offered at each pasture. Each owner's cows typically remained together and were placed in a breeding field either as a solitary group or together with cattle owned by others. The number of bulls in each breeding field was determined by the pasture manager, according to the number of cows. Once cattle were placed on a breeding field together, they remained together until the bulls were removed at the end of the breeding season between August 14 and September 22, and then when cows and calves from different herds were separated at the end of the grazing season between October 6 and 23, 2008. Exceptions would have occurred if injury or sickness necessitated an animal's removal from pasture or if pasture conditions did not support a constant numbers of animals throughout the grazing season. No changes to the structure and management of the community pastures' breeding and grazing program were made for purposes of the study.

3.2.7 Bull evaluation

All bulls used for breeding at the community pastures were owned by the Canadian federal government and managed by its employees. Each bull, regardless of whether it was in a breeding field with enrolled cows, was required to have a satisfactory breeding evaluation completed by a veterinarian in private practice in April 2008. The minimum standard for a successful semen evaluation included 70% or greater morphologically

normal sperm, sufficient scrotal circumference for age and breed, and absence of obvious anatomic abnormalities (Barth 2000).

Each bull ≥ 2 years old (250 bulls) was also tested for *Tritrichomonas foetus* and *C. fetus* at the same time the semen evaluations were performed. The prepuce of each bull was scraped with an artificial insemination pipette attached to a 20 mL-syringe with suction applied. Separate scrapings were obtained from each bull and a new pipette was used for each test. The preputial aspirate was inoculated into a proprietary trichomonad selection media (InPouch TF, Biomed Diagnostics, White City, OR, United States) and transported at 18º to 25 ºC to a commercial veterinary laboratory (Prairie Diagnostic Laboratory, Saskatoon, SK, Canada) within 24 hours. After arrival at the laboratory, contents of the pouch were incubated for 24 hours and examined for the presence of *T. foetus* by means of a polymerase chain reaction assay (Parker et al. 2001).

Cultures performed by the same laboratory (Prairie Diagnostic Laboratory, Saskatoon, SK, Canada) were used to screen bulls for *C. fetus*. Preputial material collected as described for *T. foetus* assays was inoculated into 2.5 mL of modified Weybridge transport enrichment media (deLisle et al. 1982) and transported to the laboratory at 18º to 25 ºC within 24 hours of collection. After arrival at the laboratory, the inoculated transport media was incubated at 37 ºC for 3 days and then transferred onto *Campylobacter* selective medium for further incubation under microaerophilic conditions at 37 ºC for 3 to 5 days (deLisle et al. 1982).

3.2.8 Body condition scoring of cows

Researchers assessed and recorded body condition score for each cow at the time of arrival at the community pasture. Cows were assigned a body condition score on a scale of 1 to 5 (Anonymous 2009), in which 1 corresponded with 1 on the more commonly published 9-point scale for beef cattle (Rice 1991), and the scale subsequently increased in 0.5-point increments so that 5 corresponded with 9 on the traditional scale. Body condition scores were assessed by 3 researchers (LMV, SHH, and CLW), who jointly reviewed the scoring system prior to use and who each worked with a field reference that outlined the scoring parameters. It was not possible to have 1 researcher assess the scores for all cows because of the distance between community pastures and overlapping arrival dates for different groups of cows at different pastures.

3.2.9 Serum sample collection

The number of cattle allocated to each pasture was not known prior to sample collection; therefore, blood samples were collected from the first 20 cows through the chute for each participating herd on arrival at each pasture (ie, start of the breeding season). If a herd owner sent < 20 cows to the pasture, samples were obtained from all cows in the group. Three 10 mL blood samples were collected from a jugular vein of each cow into vacuum tubes without anticoagulant. Blood was allowed to clot at ambient temperature (18 ºC [64 ºF]) and then refrigerated at 4 ºC. Within 24 hours of collection, samples were centrifuged at 1,500 × *g* for 15 minutes, and serum was removed and frozen at –20 ºC. Serum samples were transferred to storage at –70 ºC within 7 days of collection and maintained at this temperature until analysis for micronutrient

concentrations. Blood and serum samples were protected from light during transportation and storage.

3.2.10 Trace mineral analysis

Serum copper, selenium and molybdenum concentrations were determined at a commercial laboratory (Prairie Diagnostic Laboratory, Saskatoon, SK, Canada). Trace mineral concentrations were determined following wet digestion in concentrated nitric acid in a pressurized microwave, using a microwave-accelerated reaction system (CEM Corp, Mathews, NC, United States) system. Briefly, an aliquot (1 mL) of serum was placed in the supplied digestion vessel and concentrated nitric acid (2.5 mL) and deionized distilled water (2.5 mL) were added. Samples were placed in the microwave for 15 minutes (120 ºC, 830 kPa). Digested samples were transferred to a 25 mL volumetric flask. The digestion vessels were rinsed with deionized distilled water and the rinse was added to the flask. The flasks were brought to 25 mL with deionized distilled water, covered, and mixed by hand. Copper, molybdenum, and selenium were quantified immediately after acid digestion by use of an inductively coupled plasma-mass spectrometer (Thermo Jarrel Ash Corp. Franklin, MA, United States).

3.2.11 Vitamin A and Vitamin E analysis

Serum vitamin A (retinol) (Milne et al. 1986) and vitamin E (α-tocopherol) (Catignani and Bieri 1983) concentrations were determined via HPLC at a commercial laboratory (Prairie Diagnostic Laboratory, Saskatoon, SK, Canada). Extraction and analysis of individual vitamins was conducted separately by means of identical

procedures. A 1 mL aliquot of serum was placed in a 15 mL, glass-stoppered centrifuge tube and 1 mL of 1% bovine serum albumin solution (Calbiochem, La Jolla, CA, United States), 1.6 mL of ethanol, and 0.4 mL of internal standard (retinol acetate or α-tocopherol) were added. The sample was vortexed for 10 seconds, and a 4-mL aliquot of petroleum ether was added. The mixture was vortexed for another 45 seconds and centrifuged at 550 × *g* for 5 minutes. Three-quarters of the ether phase was transferred to a clean 12 × 75 mm glass tube and evaporated to dryness with air. The residue was dissolved in 500 μL of filtered, HPLC-grade methanol. The vitamin was detected via HPLC with a 5 μm HPLC column (International Equipment Co, Needham, MA, United States) (4.6 mm × 15 cm) and a fluorescence detector set at 325- or 285-nm wavelength for vitamins A and E, respectively. Samples and standards were protected from light at all times.

3.2.12 Dental examination for age estimation

Researchers (LMV and SHH) assessed dentition (Anonymous 2007b) in between October 6 and 23, 2008 to estimate the ages of cattle for which the owners could not provide this information; ages of some or all cows in 5 of the 39 herds were determined in this manner. Cows with only intermediate or central permanent incisors present were classified as 2 to 3 years old. Cows with only lateral incisors or with no incisors remaining were classified as ≥ 10 years old. The remaining cows were classified as 4 to 9 years old.

3.2.13 Pregnancy determination

Between October 6 and 23, 2008, enrolled cows were transrectally palpated by 2 researchers (LMV and SHH) to determine pregnancy status. In 4 of the 5 pastures, there was an interval of ≥ 40 days between bull removal from the breeding field and the date of pregnancy determination. In 1 of the 5 pastures, the bulls were removed 14 days before pregnancy examinations were performed.

3.2.14 Assessment of exposure to BVDV

From October 6 to 23, 2008, researchers (LMV and SHH) collected blood samples from ≥ 10 calves from each breeding field shared with cows of the present study. None of these calves had been vaccinated against BVDV. Blood samples (5 mL) were collected via jugular or coccygeal venipuncture into 10 mL vacuum tubes without anticoagulant. The blood was allowed to clot, the serum was separated via centrifugation at 1500 × *g* for 15 minutes at ambient temperature, and the separated serum was frozen at –20^{o}C within 24 hours of collection. Serum samples were stored at this temperature for approximately 1 month until analyzed by a commercial veterinary laboratory (Prairie Diagnostic Laboratory, Saskatoon, SK, Canada) for analysis of serum anti-BVDV antibody concentrations. IgG antibody concentrations were determined via virus neutralization assays with BVDV types 1 and 2 as described in another study (Waldner and Kennedy 2008) and were expressed as the highest dilution of serum to exert a neutralizing effect.

3.2.15 Statistical analysis

Data for individual animals and herds were evaluated by use of a commercially available software program (Stata for Windows, Version 10, StatCorp, College Station, TX, United States). Micronutrient concentrations were categorized as adequate or below adequate (copper, selenium, vitamin A, vitamin E) and excessive or not excessive (molybdenum) on the basis of published (Puls 1994a; Puls 1994b) reference cutoffs used by the testing laboratory. Acceptable limits of the micronutrient ranges were as follows: copper, ≥ 0.60 ppm; selenium, ≥ 0.08 ppm; vitamin A, ≥ 0.30 ppm; vitamin E, ≥ 4.0 ppm; and molybdenum, < 0.10 ppm. Null mixed models were used to calculate the proportion of variance in micronutrient concentrations measured on arrival at pasture that was attributable to herd of origin according to the following formula: $\rho = \sigma^2_h /(\sigma^2_h + \sigma^2)$, where ρ represents the intraclass correlation coefficient, σ^2_h represents the variance between herds, and σ^2 represents the remaining variance not attributable to herd (Dohoo et al. 2003).

Associations between animal-, herd-, and pasture-level variables and nonpregnancy were examined by use of a generalized linear mixed model with a binomial distribution, logit link function, and random intercepts to account for clustering by herd and community pasture (Dohoo et al. 2003). Springtime serum concentrations of copper, selenium, molybdenum, vitamin A, and vitamin E were individually assessed as continuous variables for association with pregnancy outcome. Linearity of relationships between micronutrient concentrations and pregnancy outcome was examined by categorizing continuous micronutrient concentrations into hierarchical indicator variables

(quartiles). The log odds of pregnancy were plotted against the quartiles for serum concentration. Researchers then visually assessed the form of the relationship between micronutrient exposure and pregnancy outcome for a plausible monotonic dose-response relationship (Dohoo et al. 2003).

Eight other potential risk factors for nonpregnancy were assessed. Four of these factors were categorized into binary or categorical variables according to biologically plausible values: body condition score (< 2.5/5 or ≥ 2.5/5), age (2 to 3 years, 4 to 9 years, and 10 to 14 years), calving-to-breeding interval (≤ 50 days or > 50 days), and length of breeding season (≤ 84 days or > 84 days) were individually examined for unconditional association with pregnancy outcome. The 6 continuous variables assessed were as follows: proportions of calves in each breeding field with anti-BVDV type 1 and then type 2 antibody titers greater than the overall median antibody titer; proportions of calves with anti-BVDV type 1 and then type 2 antibody titers > 1,000; cumulative amount of precipitation determined for each breeding field during the first 21 days of the breeding season; and the mean daily maximum temperature determined for each breeding field during the first 21 days of the breeding season.

Variables representing other potential risk factors were screened for examination in the final model on the basis of their association with pregnancy status ($P < 0.20$) in unconditional analysis. A final model was developed for the association between each micronutrient and pregnancy status via manual backwards elimination. Variables were retained in the final model if they were statistically significant ($P < 0.05$) or acted as

important confounders (ie, removal of the variable from the model changed the effect estimate for the exposure of interest by ≥ 10%). Biologically reasonable, first-order interaction terms were tested after a main effect model was established.

3.3 Results

3.3.1 Rainfall and temperature

Mean total precipitation determined for each of the community pastures during the first 21 days of the breeding season was 29.6 mm (range, 12.1 mm to 42.5 mm). Mean maximum daily temperature of the community pastures during the first 21 days of the breeding season was 23.6 ºC (74 ºF) with a range of 19.5º to 27.2 ºC (67º to 81ºF).

3.3.2 Management and history of study herds and cows

Cows from 39 herds kept in 5 pastures were included in the final analysis: 9 herds from Pasture 1, 8 herds from Pasture 2, 10 herds from Pasture 3, 8 herds from Pasture 4, and 4 herds from Pasture 5. The median number of breeding females in participating herds on January 1, 2008 was 133 (range, 11 to 370).

Vaccinations (either modified-live or inactivated) against BVDV and IBR were administered to 611 of 771 cows in 32 of 39 (82%) herds before start of the May 2008 grazing season; *Leptospira* vaccine, given as a multivalent vaccine in combination with the BVDV and IBR vaccines, was administered to 313 of 771 cows in 17 of 39 (44%) herds; and *Campylobacter* vaccine was administered as a single-antigen or multivalent vaccine to 470 of 771 cows in 25 of 39 (64%) herds.

Owners reported that a free-choice trace-mineral dietary supplement was provided to 631 of 771 cows in 32 of 39 (82%) herds before calving in the winter of 2007–2008 and to 374 of 771 cows in 22 of 39 (56%) herds after calving in 2008. An additional premix of vitamins A, D, and E was provided free-choice to 80 of 771 cows in 4 of 39 (10%) herds before calving and to 60 or 771 cows in 3 of 39 (8%) herds after calving; no herds were provided this vitamin premix in the absence of a trace-mineral dietary supplement. A vitamin A and D solution (500,000 and 75,000 IU/mL, respectively) was administered to 273 of 771 cows in 14 of 39 (36%) herds via injection (5 mL/cow, IM) at least once during the winter of 2007–2008; 20 cows in 1 of these herds did not receive a trace-mineral supplement. Fifty-one of 771 cows in 3 of 39 (8%) herds were administered a selenium and vitamin E solution (3 mg/mL and 136 IU/mL, respectively) via injection (10 mL/cow, IM) at least once during the winter of 2007–2008; these herds were also provided trace-mineral dietary supplements.

The start of the community pasture breeding season was > 50 days after the 2008 calving date for 679 of 762 (89%) cows. Nine cows had no calving-to-breeding interval recorded (8/9 were heifers that had not previously calved).

3.3.3 Breeding field management and bull evaluation

Thirty-four (87%) of the 39 herds were placed in the pasture breeding fields with cattle from at least 1 other herd. Cows from herds enrolled in the present study were kept in 4 breeding fields in pasture 1, 3 breeding fields in pasture 2, 4 breeding fields in

pasture 3, 6 breeding fields in pasture 4, and 1 breeding field in pasture 5. Trace mineral-fortified blocks were accessible to all cows in 1 of the 5 pastures. Iodized salt blocks without other trace mineral supplements were available to all cows in the other 4 pastures.

All bulls kept in community pasture breeding fields with cows of the present study had satisfactory semen evaluations, and bulls ≥ 2 years old were tested negative for *T. foetus* and *C. fetus*. All bulls were vaccinated against BVDV, IBR, and *C. fetus* before the start of the 2008 breeding season. The median cow-to-bull ratio for community pasture breeding fields was 27:1.

The median duration of the breeding seasons was 72 days (range, 64 to 126 days). Two hundred and sixty-three of 771 (34%) cows were exposed to bulls for ≤ 84 days; the remaining cows were exposed to bulls for > 84 days.

3.3.4 Body condition score and age evaluation of cows

The majority of cows (637/771 [83%]) had moderate to good body condition scores (≥ 2.5/5) upon arrival at pasture. Most cows in the study (500/771 [65%]) were 4 to 9 years old, 151 (20%) were 2 to 3 years old, and 115 (15%) were 10 to 14 years old. Five cows were missing an age record. The cows appeared healthy and all mature (≥ 2 year-old) cows were accompanied by offspring from the previous breeding season.

3.3.5 Pregnancy assessment and pregnancy-associated variables

In October, 761 of the 771 enrolled cows were evaluated for pregnancy via palpation per rectum; 708 (93%) were pregnant. Ten cows were not examined because they had died on pasture, had been removed early from pasture, or could not be located at roundup time. Body condition score (assessed on arrival at the pasture in May; P = 0.001), the number of days from calving to the start of the breeding season (ie, calving-to-breeding interval; P = 0.049), and the amount of precipitation received during the first 21 days of the breeding season (P = 0.017) were associated with pregnancy status in the unconditional analysis **(Table 3.1)**. Precipitation was not significantly associated with pregnancy status after other potential risk factors for nonpregnancy were accounted for in the model.

3.3.6 Exposure to BVDV

Blood samples from non-BVDV vaccinated calves were collected in October from 10 calves kept in each of 15 breeding fields, 15 calves kept in 1 breeding field, and 20 calves kept in 2 breeding fields. The median antibody titer was 1:36 for both type 1 and type 2 BVDV. Of 205 calves tested, 87 (42%) of the calves had anti-BVDV type 1 antibody titers > 1:36 and 57 (28%) of the calves had anti-BVDV type 2 antibody titers > 1:36. Ten (5%) of the tested calves had anti-BVDV type 1 antibody titers > 1:1,000 and 21 (10%) had anti-BVDV type 2 antibody titers > 1:1,000 (values suggestive of BVDV exposure).

3.3.7 Serum micronutrient analysis

Serum copper concentrations were less than adequate (< 0.60 ppm) in 580 of 771 (75%) cows **(Table 3.2)**. Three hundred eighty-eight of 500 (78%) 4 to 9 year-old cows, 108 of 151 (72%) 2 to 3 year-old cows, and 80 of 115 (70%) 10 to 14 year-old cows had serum copper concentrations < 0.60 ppm. Copper concentration was not reported for 1 serum sample due to laboratory error and 4 cows had less than adequate copper concentrations but did not have ages recorded.

High concentrations of serum molybdenum (≥ 0.10 ppm) were present in 143 of 769 (19%) cows (Table 2). Molybdenum concentration was not reported for 2 serum samples due to laboratory error. The mean ± SD serum copper concentration for cows with high concentrations of serum molybdenum was 0.54 ± 0.11 ppm, compared with 0.51 ± 0.14 ppm for cows with serum molybdenum concentrations < 0.10 ppm. There was no association between high serum molybdenum concentrations (≥ 0.10 ppm) and less than adequate serum copper concentrations (< 0.6 ppm) ($P = 0.96$). Only 4 of 769 (0.5%) cows had concentrations of serum selenium considered less than adequate (< 0.08 ppm).

Ninety-nine of 769 (13%) cows had less than adequate vitamin A concentrations (< 0.30 ppm), and 38 of 770 (5%) of cows had less than adequate vitamin E concentrations (< 4.0 ppm). Laboratory error resulted in the loss of results for 2 samples for vitamin A and selenium analyses, and 1 sample for vitamin E analysis.

Serum micronutrient concentrations for individual cows were evaluated to determine the amount of variance attributable to the herd of origin. Approximately 40% or more of the variation in serum micronutrient concentrations was attributable to differences among the herds **(Table 3)**.

3.3.8 Associations between serum micronutrient concentration and pregnancy status

Serum copper and vitamin E concentrations were associated with pregnancy status in the unconditional analysis **(Table 4)**; after accounting for the other identified risk factors for nonpregnancy, copper was the only micronutrient significantly ($P < 0.001$) associated with pregnancy status and retained in the final model **(Table 5)**. The odds of nonpregnancy in cows within the lowest quartile of vitamin E concentrations (range, 2.62 to 5.74 ppm) were 2.8 (95% CI, 1.0 to 7.8) times that of cows in the highest quartile (range, 9.62 to 19.7 ppm); however, this association was not significant ($P = 0.05$).

In the final model, which accounted for body condition score and length of the breeding season, a significant association was detected between serum copper concentrations and ages of cows (**Figure 1; Table 5**). Decreased serum copper concentration was associated with increased odds of nonpregnancy in 2 to 3 year-old and 4 to 9 year-old cows, but was not associated with pregnancy outcome in 10 to 14 year-old cows.

3.4 Discussion

Serum copper concentrations below established reference ranges are common in cattle (Dargatz et al. 1999; Hoff et al. 2001), but the effect of these copper concentrations on reproductive parameters remains controversial (Muehlenbein et al. 2001; Enjalbert et al. 2006; Phillippo et al. 1987). In the present study, cows < 10 years of age with lower serum copper concentrations were at increased odds of nonpregnancy. The greatest effect was observed for prebreeding serum copper concentrations < 0.4 ppm.

In a large case-control study (Enjalbert et al. 2006) in which herd-level plasma concentrations of copper and other micronutrients were compared between herds with and without reproductive disorders, investigators reported that copper-deficient herds did not have increased odds of low fertility. This is in contrast with results of the study reported here. Compared with serum copper concentrations of cows in the present study, plasma copper concentrations (evaluated after transformation into serum values as described elsewhere (Claypool et al. 1975) reported for herds with low fertility were considerably higher in the earlier study, which may possibly explain the lack of any observed association between copper and reproductive performance in that study. Furthermore, the timing of sample collections relative to the production cycle of the animals was not described in that report; thus, it is difficult to assess how appropriate the measured copper concentrations were for predicting the reported reproductive outcomes.

Serum copper concentrations measured before the start of the breeding season may be a more useful predictor of pregnancy outcome than copper concentrations measured at

other phases of the production cycle. Muehlenbein et al. (2001) speculated that dietary copper supplements may increase early conception rates in cows that are extremely copper deficient before and during the breeding season. Seasonal fluctuations in serum copper concentrations have been previously described (Smart et al. 1992) and were reported to be at their lowest values in February and March in western Canada. Survey results from owners of cattle in the present study indicated that cows in only 22 of 39 (56%) herds received dietary trace mineral supplements after calving, compared with 32 of 39 (82%) herds that received these supplements before calving. While the reasons for this change in dietary management have not been studied, the authors hypothesize that it may be because beef producers typically move cows and calves onto larger nursery fields after winter calving, dispersing the animals to reduce the opportunity for calf diseases to spread; however, this important management practice can increase time and labor required to provide trace mineral supplements to breeding females after calving.

Primary copper deficiency results from an inadequate intake of dietary copper. Forage copper concentrations vary depending on plant species, soil type, and growing conditions (Smart et al. 1992). Western Canadian forages, cereal hay, and cereal grains have frequently been reported to have copper levels below the suggested NRC requirement for beef cattle (National Research Council 1984; Gooneratne et al. 1989; Suleiman et al. 1997). Feed tests were not done as part of the present study, but it is likely that primary copper deficiency contributed to the less than adequate copper status detected in the majority of cows.

Secondary copper deficiency can result from high concentrations of molybdenum or sulfur in the diet of ruminants (Phillippo et al. 1987; Suttle 1991; Olkowski 2009). Low conception rates, failure to ovulate, and anestrous have been associated with excess dietary molybdenum regardless of the animal's copper status, causing debate about the relationship between low circulating copper concentrations and fertility (Phillippo et al. 1987). However, other reports (Gardner et al. 2003; Raisbeck et al. 2006) have indicated that cattle can graze pastures that contain high molybdenum concentrations without adverse health or reproductive effects, providing they receive copper supplementation or have adequate circulating concentrations of copper when placed in such pastures. One hundred forty-three of 769 (19%) cows in the present study had high prebreeding serum molybdenum concentrations (≥ 0.1 ppm), but high molybdenum concentrations did not increase the odds of nonpregnancy and were not associated with less than adequate copper status.

High dietary concentrations of sulfates can reduce copper bioavailability independent of dietary molybdenum through the formation of insoluble copper sulfide in the rumen (Raisbeck et al. 2006). High sulfate concentrations are common in water throughout the Great Plains region of North America and are additive to the sulfur content in feed (Olkowski 2009; Olkowski et al. 1991; Gould et al. 2002); however, water and feed sources available to cows in the present study immediately prior to blood sample collection were not analyzed. The possibility of secondary copper deficiency in these cows as a result of high concentrations of dietary sulfate cannot be ruled out.

Although not significant in the final model, serum vitamin E concentrations measured in cows on arrival at community pastures were associated with pregnancy in the unconditional analysis. Cow-calf producers in northern climates rely on hay and silage for winter feed, and these stored feedstuffs typically contain < 20% of the vitamin E concentrations found in fresh forages (Hidiroglou et al. 1993). Cattle do not store vitamin E for any extended period and without supplementation, circulating vitamin E concentrations decrease during the winter feeding period (Maas 2007). When growing conditions are good, a very rapid increase in circulating vitamin E concentrations can be expected after cows are placed on pasture with green forage (Puls 1994). Prolonged periods of low serum vitamin E concentrations during the initial phase of the breeding season could, however, occur in cattle placed on dormant or drought-stricken pasture after winter feeding (Frye et al. 1991). No published papers were identified that examined the association between serum vitamin E concentrations and pregnancy outcome in beef cows, and the results of unconditional analysis in the study reported here suggest that further research in this area may be warranted.

Most of the cows in this study had serum concentrations of selenium, vitamin A, and vitamin E within accepted reference ranges, potentially decreasing this study's power to examine the associations between various concentrations of these micronutrients and pregnancy outcome in beef cattle. Additional field studies should examine animals from a wider range of geographical areas, including known selenium-deficient areas in which beef cows are more likely to have less than adequate concentrations of these micronutrients. Future research should also evaluate the micronutrient concentrations of

winter feed and water sources for breeding females in order to assess their contribution to low prebreeding serum micronutrient concentrations. The potential usefulness of serum vitamin concentrations measured on arrival at pasture may be limited because in years with good pasture conditions, vitamin A and E status of cows would be expected to improve rapidly following ingestion of fresh forage (Puls 1994).

The practical constraints of conducting a study on 771 privately-owned cows led to the decision to evaluate serum micronutrient concentrations instead of performing liver biopsies. The liver is the primary storage organ for copper and maintains copper homeostasis (National Research Council 1984). Inadequate copper intake is not consistently reflected by low serum copper concentrations until liver copper concentrations are < 40 ppm on a dry weight basis (Claypool et al. 1975). Serum copper concentrations of 0.45 ppm have been correlated with low copper concentrations in the liver in cattle (Claypool et al. 1975; Tessman et al. 2001). It is interesting that the strongest association with the odds of nonpregnancy in the present study was detected for serum copper concentrations < 0.40 ppm.

Finally, it would be beneficial to examine more cows in the youngest (2 to 3 year-old) and oldest (≥ 10 year-old) age categories to further examine the described interaction between serum copper and age and its association with pregnancy outcome. The number of cows ≥ 10 years old in the present study may have been insufficient to detect an association between their serum copper concentrations and pregnancy outcomes because of a likely increase in other factors that diminish fertility and conception rates, such as

age-related reproductive pathology and concurrent disease. Results of a large study (Waldner 2008) performed in the same geographical region as the present study revealed a significant ($P < 0.001$) increase in the risk of nonpregnancy in cows > 10 years old as compared to mature cows between 4 and 10 years old.

The association between decreased prebreeding serum copper concentrations and increased odds of nonpregnancy represents new information for beef cattle producers. Results of the present study suggest that less than adequate prebreeding serum copper concentrations can impede the reproductive performance of younger beef cows. Identification and correction of low serum copper concentrations in breeding females may enhance the reproductive efficiency of cow-calf herds.

3.5 References

Anonymous. 2007a. Community Pasture Program. [Online] Available at: http://www4.agr.gc.ca/AAFC-AAC/display-afficher.do?id=1183493052855&lang=eng. [16 February 2010].

Anonymous. 2007b. Using dentition to age cattle. [Online] Available at: http://www.fsis.usda.gov/Fact_Sheets/Bovine_Spongiform_Encephalopathy_BSE. [9 November 2009].

Anonymous. 2008. Soil landscape illustrations of the prairie provinces. [Online] Available at: http://sis.agr.gc.ca/cansis/taxa/landscape/slc_prairie.html. [11 March 2010].

Anonymous. 2009. Body condition scoring your cow herd. [Online] Available at: www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/beef8822. [15 February 2010].

Anonymous. 2010. Canadian climate data and information archive. [Online] Available at: http://climate.weatheroffice.gc.ca/advanceSearch/searchHistoricData_e.html. [1 February 2011].

Ahola, J. K., Baker, D. S., Burns, P. D., Mortimer, R. G., Enns, R. M., Whittier, J. C., Geary, T. W., and Engle, T. E. 2004. Effect of copper, zinc, and manganese supplementation and source on reproduction, mineral status, and performance in grazing beef cattle over a two-year period. J. Anim. Sci. **82:** 2375-2383.

Barth, A. D. 2000. Bull breeding soundness evaluation, 2nd ed. Saskatoon, SK, Canada: The Western Canadian Association of Bovine Practitioners Pages 1–285.

Bass, R. T., Swecker, W. S., and Eversole, D. E. 2001. Effects of oral vitamin E supplementation during late gestation in beef cattle that calved in late winter and late summer. Am. J. Vet. Res. **62:** 921-927.

Black, D. H. and French, N. P. 2004. Effects of three types of trace element supplementation on the fertility of three commercial dairy herds. The Vet. Rec. **154:** 652-658.

Campbell, J. R., Jim, G. K., Booker, C. W., and Guichon, P. T. 1995. A survey of the selenium status of beef cows in Alberta. Can. Vet. J. **36:** 698-702.

Catignani, G. L. and Bieri, J. G. 1983. Simultaneous determination of retinol and α -tocopherol in serum or plasma by liquid chromatography. Clin. Chem. **29:** 708-712.

Claypool, D. W., Adams, F. W., Pendell, H. W., Harmann Jr., N. A., and Bone, J. F. 1975. Relationship between the level of copper in the blood plasma and the liver of cattle. J. Anim. Sci. **41:** 911-914.

Dargatz, D. A. and Ross, P. F. 1996. Blood selenium concentrations in cows and heifers on 253 cow-calf operations in 18 states. J. Anim. Sci. **74:** 2891-2895.

Dargatz, D. A., Garry, F. B., Clark, G. B., and Ross, P. F. 1999. Serum copper concentrations in beef cows and heifers. J. Am. Vet. Med. Assoc. **215:** 1828-1832.

deLisle, G. W., Stephens, D. J., and Bird, M. M. 1982. Transport media for *Campylobacter fetus venerealis*. N. Z. Vet. J. **30:** 31–32.

Dohoo, I., Martin, W., and Stryhn, H. 2003. Veterinary epidemiologic research. Charlottetown, PEI, Canada: AVC Inc. Pages 502-504.

Enjalbert, F., Lebreton, P., and Salat, O. 2006. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study. J. Anim. Physiol. and Anim. Nutr. **90:** 459-466.

Frye, T. M., Williams, S. N., and Graham, T. W. 1991. Vitamin deficiencies in cattle. Vet. Clin. Fd. Anim. **7:** 217-275.

Gardner, W. C., Broersma, K., Popp, J. D., Mir, Z., Mir, P. S., and Buckley, W. T. 2003. Copper and health status of cattle grazing on high-molybdenum forage from a reclaimed mine tailing site. Can. J. Anim. Sci. **83:** 479-485.

Gooneratne, S. R., Buckley, W. T., and Christensen, D. A. 1989. Review of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci. **69:** 819-845.

Gould, D. H., Dargatz, D. A., Garry, F. B., and Hamar, D. W. 2002. Potentially hazardous sulfur conditions on beef cattle ranches in the United States. J. Am. Vet. Med. Assoc. **221:** 673-677.

Gunter, S. A., Beck, P. A., and Phillips, J. M. 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. J. Anim. Sci. **81:** 856-864.

Hidiroglou, M., Batra, T. R., and Roy, G. L. 1994. Changes in plasma α–tocopherol and selenium of gestating cows fed hay or silage. J. Dairy Sci. **77:** 190-195.

Hoff, B., Schrier, N., Boermans, H., Faulkner, H., and Hussein, A. 2001. Assessment of trace mineral and vitamin E status beef cows in Ontario. Can. Vet. J. **42:** 384-385.

Maas, J. 2007. Diagnostic considerations for evaluating nutritional problems in cattle. Vet. Clin. North Am. Food Anim. Pract. **23:** 527-539.

Milne, D. B. and Botnem, J. 1986. Retinol, α-tocopherol, lycopene, and α- and β-carotene simultaneously determined in plasma by isocratic liquid chromatography. Clin. Chem. **32:** 874-876.

Muehlenbein, E. L., Brink, D. R., Deutscher, G. H., Carlson M. P., and Johnson A. B. 2001. Effects of inorganic and organic copper supplemented to first-calf cows on cow reproduction and calf health and performance. J. Anim. Sci. **79:** 1650-1659.

National Research Council. 1984. Nutrient requirements of beef cattle. 6th ed. Washington, DC: National Academy Press.

Olkowski, A. A. 2009. Livestock water quality-a field guide for cattle, horses, poultry, and swine. [Online] Available at: http://www.agriculture.gov.sk.ca/Livestock-Feeds-Nutrition. [28 August 2009].

Olkowski, A. A., Rousseaux, C. G., and Christensen, D. A. 1991. Association of sulfate-water and blood thiamine concentration in beef cattle: field studies. Can. J. Anim. Sci. **71:** 825–832.

Olson, P. A., Brink, D. R., Hickok, D. T., Carlson, M. P., Schneider, N. R., Deutscher, G. H., Adams, D. C., Colburn, D. J., and Johnson, A. B. 1999. Effects of supplementation of organic and inorganic combinations of copper, cobalt, manganese, and zinc above nutrient requirement levels on postpartum two-year-old cows. J. Anim. Sci. **77:** 522–532.

Parker, S., Lun, Z-R, and Gajadhar, A. 2001. Application of a PCR assay to enhance the detection and identification of *Tritrichomonas foetus* in cultured preputial samples. J. Vet. Diagn. Invest. **13:** 508–513.

Phillippo, M., Humphries, W. R., Atkinson, T., Henderson, G. D., and Garthwaite, P. H. 1987. The effect of dietary molybdenum and iron on copper status, puberty, fertility and oestrous cycles in cattle. J. Agric. Sci. **109:** 321-336.

Puls, R. 1994a. Mineral levels in animal health: diagnostic data, 2nd ed. Sherpa International, Clearbrook, British Columbia. Pages 83, 135, 192, 230.

Puls, R. 1994b. Vitamin levels in animal health: diagnostic data and bibliographies. Sherpa International, Clearbrook, British Colombia. Pages 15, 98.

Raisbeck, M. F., Siemion, R. S., and Smith, M. A. 2006. Modest copper supplementation blocks molybdenosis in cattle. J. Vet. Diagn. Invest. **18:** 566-572.

Rice, L. E. 1991. The effects of nutrition on reproductive performance of beef cattle. In: Maas, J. (editor). *Beef cattle nutrition*. Vet. Clin. North Am. Food Anim. Pract. **7:** 1-26.

Sanders, D. E. 2005. Troubleshooting poor reproductive performance in large herds. Vet. Clin. North Am. Food Anim. Pract. **21:** 289-304.

Siciliano-Jones, J. L., Socha, M. T., Tomlinson, D.J., and DeFrain, J. M. 2008. Effect of trace mineral source on lactation performance, claw integrity, and fertility of dairy cattle. J. Dairy Sci. **91:** 1985-1995.

Smart, M. E., Cymbaluk, N. F., and Christensen, D. A. 1992. A review of copper status of cattle in Canada and recommendations for supplementation. Can. Vet. J. **33:** 163-170.

Suleiman, A., Okine, E., and Goonewardene, L. A. 1997. Relevance of National Research Council feed composition tables in Alberta. Can. J. Anim. Sci. **77:** 197-203.

Suttle, N. F. 1974. Effects of organic and inorganic sulphur on the availability of dietary copper to sheep. Br. J. Nutr. **32:** 559–568.

Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annu. Rev. Nutri. **11:** 121-140.

Tessman, R. K., Lakritz, J., Tyler, J. W., Casteel, S. W., Williams, J. E., and Dew, R. K. 2001. Sensitivity and specificity of serum copper determination for detection of copper deficiency in feeder calves. J. Amer. Vet. Med. Assoc. **218:** 756-760.

Waldner, C. L. and Kennedy, R. I. 2008. Associations between health and productivity in cow-calf beef herds and persistent infection with bovine viral diarrhea virus, antibodies against bovine viral diarrhea virus, or antibodies against infectious bovine rhinotracheitis virus in calves. Amer. J. Vet. Res. **69:** 916–927.

Waldner, C. L. 2008. Western Canada study of animal health effects associated with exposure to emissions from oil and natural gas field facilities. Study design and data collection I. Herd performance records and management. Arch. of Environ. and Occup. Health. **63:** 167-186.

Table 3.1 Herd-adjusted and pasture-adjusted associations between cow- and pasture-related variables and the odds of nonpregnancy determined via unconditional analysis of data for 761 cows from 39 herds.

Variable	**OR**	**95% CI**	***P* value**
Body condition score			
< 2.5/5.0	3.03	1.59 - 5.77	0.001
≥ 2.5/5.0	Reference category		
Age*			0.067**
2 - 3 years	1.99	0.98 - 4.08	0.058
4 – 9 years	Reference category		
10 – 14 years	2.03	0.96 - 4.27	0.063
Calving-to-breeding interval†			
≤ 50 days	2.20	1.00 - 4.84	0.049
> 50 days	Reference category		
Length of breeding season			
≤ 4 cycles (≤ 84 days)	2.02	0.89 - 4.59	0.093
> 4 cycles (> 84 days)	Reference category		
Proportion of calves‡ with BVDV type 1 antibody titre > 1:36	1.02	0.37 - 2.79	0.98
Proportion of calves‡ with BVDV type 1 antibody titre > 1:1000	0.27	0.01 - 6.81	0.43
Proportion of calves‡ with BVDV type 2 antibody titre > 1:36	0.77	0.24 - 2.47	0.66
Proportion of calves‡ with BVDV type 2 antibody titre > 1:1000	0.65	0.10 - 4.01	0.64
Precipitation (mm) received in first 21 days of breeding season	0.97	0.95 - 0.99	0.017
Mean daily maximum temperature (°C) in first 21 days of breeding season	1.26	0.99 - 1.24	0.074

Cows were kept in 5 Saskatchewan community pastures during the 2008 breeding season. Results for 10 cows that died at pasture, were removed from pasture early, or could not be located at the end of breeding season roundup were excluded.
*Excludes 5 cows for which age was not determined.
** *P* value testing the overall association between age, categorized into age groups, and the odds of non-pregnancy. †Excludes 8 heifers that did not calve in 2008 and 1 cow that had a missing calving record. ‡Antibody titers were determined in samples from 205 calves (not vaccinated against BVDV) that shared breeding fields with study cows.
BCS = Body condition score. CI = Confidence interval. OR = Odds ratio. — = Reference category.

Table 3.2 Summary of serum micronutrient concentrations assessed in blood samples collected from 771 cows of 39 herds at the start of the community pasture breeding season (May 2008).

Micronutrient	**Mean ± SD serum concentration**	**Median**	**Percentile**			
			5th	**25th**	**75th**	**95th**
Copper (ppm)*	0.51 ± 0.13	0.52	0.29	0.43	0.59	0.72
Molybdenum (ppm)†	0.051 ± 0.067	0.019	0.004	0.010	0.065	0.200
Selenium (ppm)†	0.16 ± 0.04	0.15	0.11	0.13	0.18	0.23
Vitamin A (ppm)†	0.44 ± 0.13	0.42	0.26	0.34	0.51	0.69
Vitamin E (ppm)*	7.98 ± 2.78	7.63	4.05	5.86	9.70	13.10

Blood samples were collected from the first 20 cows of each herd through the chute on arrival at 1 of 5 Saskatchewan community pastures. Results for all analyses were not available for all cows. *n = 770 cows. †n = 769 cows.

Table 3.3 Herd-level serum micronutrient concentrations for 39 beef herds evaluated at the start of the community pasture breeding season and the percentage of variability in these values attributable to between-herd differences.

Micronutrient	**Mean ± SD (mean ± SD range) of herd mean serum micronutrient concentrations (ppm)**	**Variability attributable to herd of origin (%)**
Copper	0.51 ± 0.13 (0.24 ± 0.13 – 0.67 ± 0.10)	39.8
Molybdenum	0.051 ± 0.067 (0.003 ± 0.001 – 0.173 ± 0.088)	72.1
Selenium	0.16 ± 0.04 (0.11 ± 0.02 – 0.26 ± 0.02)	62.7
Vitamin A	0.44 ± 0.13 (0.26 ± 0.06 – 0.61 ± 0.10)	61.1
Vitamin E	7.98 ± 2.78 (4.13 ± 1.15 – 12.09 ± 2.21)	52.8

Table 3.4 Herd-adjusted and pasture-adjusted associations between serum copper, molybdenum, selenium, vitamin A, and vitamin E concentrations and the odds of nonpregnancy determined via unconditional analysis of data for 761 cows from 39 herds.

Variable	**OR**	**95% CI**	***P* value**
Copper concentration (ppm)	0.007	0.001 - 0.071	< 0.001
Interquartile comparison	NA	NA	0.002*
(0.52 – 0.59) compared to (0.60 – 0.87)	1.95	0.65 - 5.85	0.23
(0.43 – 0.51) compared to (0.60 – 0.87)	2.52	0.88 - 7.27	0.086
(0.10 - 0.42) compared to (0.60 - 0.87)	5.53	2.02 - 15.12	0.001
Molybdenum concentration (ppm)	0.23	0.001 - 64.28	0.61
Interquartile comparison	NA	NA	0.19*
(0.020 - 0.069) compared to (0.070 – 0.550)	1.08	0.43 - 2.71	0.87
(0.010 - 0.019) compared to (0.070 – 0.550)	2.08	0.87 - 4.96	0.10
(0.000 - 0.009) compared to (0.070 – 0.550)	2.34	0.68 - 8.02	0.18
Selenium concentration (ppm)	4.68	0.001 - 22701	0.72
Interquartile comparison	NA	NA	0.87*
(0.15 - 0.17) compared to (0.18 – 3.00)	1.03	0.46 - 2.30	0.92
(0.13 - 0.14) compared to (0.18 – 3.00)	1.38	0.58 - 3.24	0.47
(0.011 - 0.12) compared to (0.18 – 3.00)	1.08	0.41 - 2.83	0.88
Vitamin A concentration (ppm)	0.54	0.04 - 6.53	0.63
Interquartile comparison	NA	NA	0.15*
(0.42 - 0.51) compared to (0.52 - 0.90)	0.75	0.31 - 1.82	0.53
(0.34 - 0.41) compared to (0.52 - 0.90)	1.77	0.78 - 3.97	0.17
(0.16 - 0.33) compared to (0.52 - 0.90)	0.92	0.34 - 2.47	0.87
Vitamin E concentration (ppm)	0.86	0.76 - 0.97	0.013
Interquartile comparison	NA	NA	0.11*
(7.55 – 9.61) compared to (9.62 - 19.69)	2.61	0.96 - 7.09	0.060
(5.75 – 7.54) compared to (9.62 - 19.69)	2.86	1.05 - 7.80	0.040
(2.62 – 5.74) compared to (9.62 - 19.69)	3.47	1.26 - 9.59	0.016

Serum samples were collected at the start of the community pasture breeding season (May 2008) and pregnancy status was evaluated via palpation per rectum at the end of the season (October 2008); results were not available for 10 of 771 cows. Serum micronutrient concentrations were evaluated as continuous variables and as categorical variables categorized according to quartiles. Interquartile comparisons were made between the 3 lower quartiles and the highest quartile.

**P* values testing the overall association between micronutrient concentrations categorized into quartiles and the odds of nonpregnancy.

NA = Not applicable.

See Table 1 for remainder of key.

Table 3.5 Herd-adjusted and pasture-adjusted associations between serum copper concentrations at the start of the community pasture breeding season and pregnancy status at the end of the season determined via conditional analysis of data for 761 cows from 39 herds.

Variable	**Regression Coefficient (β)**	**95% CI**	***P* value**
Thin cow (BCS < 2.5/5.0)	1.10	0.45 to 1.76	0.001
Age category (y)*	NA	NA	0.007**
2 to 3	4.00	0.72 to 7.28	0.017
4 to 9	—	—	—
10 to 14	–2.18	–5.00 to 0.64	0.130
Serum copper (ppm)	–5.57	–8.66 to –2.47	< 0.001
Age-Copper interaction	NA	NA	0.003**
2 and 3	–8.69	–16.88 to –0.49	0.038
4 to 9	—	—	—
10 to 14	6.24	0.69 to 11.78	0.028
Short calving- to- breeding interval (< 50 days)†	1.18	0.39 to 1.98	0.004

Conditional analysis accounted for BCS at the start of the breeding season, age of cows, first-order interaction between serum copper concentration and age, and calving-to-breeding interval.

** *P* values testing the overall association between age and age-copper interaction, categorized by age groups, and the odds of nonpregnancy.

See Table 1 for remainder of key.

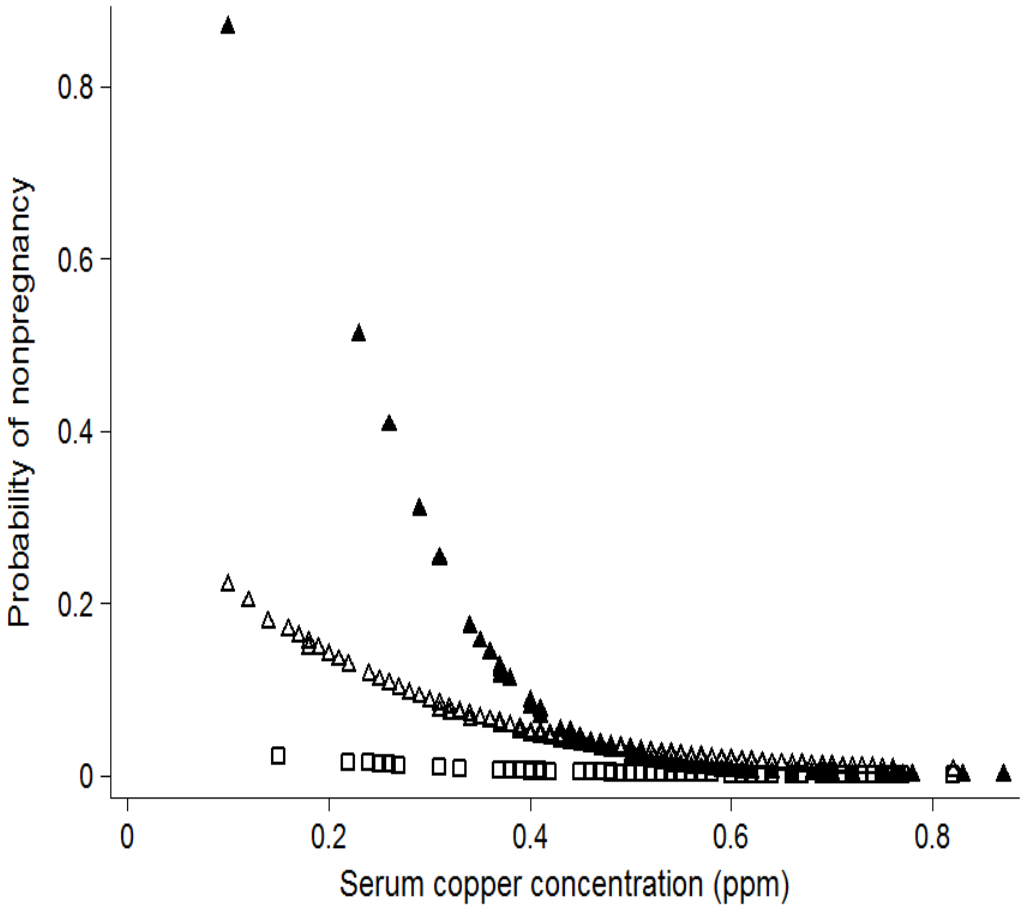

Figure 3.1 Predicted probabilities of nonpregnancy for 2 to 3 year-old cows (black triangles), 4 to 9 year-old cows (white triangles), and 10 to 14 year-old cows (white squares) across the range of prebreeding serum copper concentrations measured in 557cows that had body condition scores ≥ 2.5 (on a scale of 1.0 to 5.0) at the start of the community pasture breeding season and calving-to-breeding intervals of > 50 days.

CHAPTER 4

SERUM MICRONUTRIENT CONCENTRATIONS IN BEEF COWS BEFORE AND AFTER THE SUMMER GRAZING SEASON

The paper presented in Chapter 4 has been published and can be found under: **L. M. Van De Weyer, S. Hendrick, C. L. Waldner. 2010.** Serum micronutrient concentrations in beef cows before and after the summer grazing season. Can. J. of Anim. Sci. **90:** 563–574.

Together with Chapter 3, Chapter 4 describes the results of an independent research study evaluating micronutrient concentrations in beef cows in southern Saskatchewan. I contributed to the project design and in preparing and submitting grant proposals for part of the funding for the project. I was involved in herd enrolment, sample collection, survey design and information collection, and communication of results to PFRA patrons and staff involved in the study. I coordinated sample submission to the laboratories, entered and cleaned much of the data, and conducted all of the data analysis. I did not perform any of the laboratory analysis for this paper. The Western College of Veterinary Medicine (WCVM) Toxicology Laboratory analyzed the serum micronutrient concentrations. Water sample analysis was provided by the Saskatchewan Research Council (SRC) Analytical Laboratory, Saskatoon, Saskatchewan.

4.1 Introduction

Micronutrients, such as copper, manganese, selenium, 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 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; Hidiroglou 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 copper, 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 copper concentrations are lowest in late winter and highest in fall (Smart et al. 1992). Although several studies have examined associations between season and selenium 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 copper 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 selenium and copper concentrations in beef cows measured after the summer grazing season (Campbell et al. 1995; Dargatz et al. 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 copper and selenium 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. Sulfate 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 sulfate, molybdenum, and iron adversely affect the absorption, utilization, and excretion of copper in ruminants, potentially resulting in secondary copper 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 to animals most at risk for below adequate micronutrient concentrations. Based on this, the three objectives of this study were to: first, 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.

4.2 Materials and Methods

4.2.1 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 Solonetzic, and one Black Chernozemic. The forage on community pastures is comprised 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.

4.2.2 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, United States) 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 ºC. The serum was separated and frozen at -20 ºC within 24 hours of collection. Serum samples were frozen to -70 ºC within 7 days of collection and stored at this temperature until analysis for micronutrient concentrations. Blood and serum samples were protected from light during transport and storage.

4.2.3 Trace Mineral Analysis

Serum copper, manganese, molybdenum, and selenium concentrations were measured by a commercial laboratory (Prairie Diagnostic Laboratory, Saskatoon, SK, Canada). 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, United States). One mL 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 minutes at 120 ºC 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 determined 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.

4.2.4 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, Canada). Extraction and analysis of individual vitamins was conducted separately but the procedure was identical. One mL 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, United States),1.6 mL of ethanol, and 0.4 mL of internal standard (retinyl acetate at a concentration of 0.4 μg/mL or α-tocopherol acetate at a concentration of 20 μg/mL). The sample was mixed using a vortex for 10 seconds, and a 4 mL aliquot of petroleum ether was added. The mixture was vortically mixed for another 45 seconds and centrifuged at 550 × *g* for 5 minutes. Three-quarters of the ether phase was transferred to a clean 12 × 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, United States) 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.

4.2.5 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 one to five (Marx 2009), where one corresponds with one (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 five corresponds with nine (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 days between bull removal and the date of pregnancy determination. In one of the five pastures, the bulls were removed from the breeding field 14 days 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 2009b). Five herds had all or some cows aged in this manner.

4.2.6 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, Canada). Sulfate and iron concentrations in water samples were measured using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (Thermo Jarrel Ash Corporation, Franklin, MA, United States). When two or more water sources were sampled from the same breeding field, the mean sulfate 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 April 01 to October 31, 2008 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

4.2.7 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 molybdenum concentrations (high or low) and serum copper concentrations (above or below adequate) for spring and fall samples were examined using published cut-off values for molybdenum and copper (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 4.1) (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 4.1).

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 4.1).

Data from the final spring models were used to calculate the proportion of remaining variance in serum micronutrient concentrations explained within herd ($\rho_h = \sigma^2_h / (\sigma^2_c + \sigma^2_h + \sigma^2_p)$ and then within pasture ($\rho_p = \sigma^2_p / (\sigma^2_c + \sigma^2_h + \sigma^2_p)$ (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 4.1). Pasture water concentrations of sulfate 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 to 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.

4.3 Results

4.3.1 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 years, 7/20 were 1-3 years, and 1/20 was 10-14 years) 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 years of age, 22% (171/791) were 1-3 years of age, and 15% (115/791) were 10-14 years of age. Five animals were missing an age record. Of the 10 missing cows in October, 5 were 4-9 years of age and 5 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.

4.3.2 Description of the pasture conditions

Trace mineral-fortified block mineral (containing 60 mg/kg I, 2000 mg/kg Fe, 12 mg/kg Co, 750 mg/kg Cu, 1580 mg/kg Mn, 2400 mg/kg Zn, a maximum of 600 mg/kg F, 8.7 mg/kg Se, a minimum of 140 000 IU/kg vitamin A, a minimum of 30 000 IU/kg vitamin D, and a minimum of 120 IU/kg 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 sulfate 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 April 01 to October 31, 2008 was 307 mm (range, 263 to 406 mm).

4.3.3 Serum micronutrient analysis

Serum copper (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 manganese (range, 2 to 47 μg 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 selenium, vitamin A or vitamin E (Table 4.2).

High concentrations of molybdenum ($\geq$ 0.10 mg L^{-1}) were present in 19% (143/769) of cows in the spring (Table 4.2). No association was found between below adequate spring serum copper concentrations and high spring serum molybdenum concentrations (P = 0.4), while above adequate fall copper concentrations were associated with high fall molybdenum (P = 0.02).

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

Serum concentrations of copper and vitamins A and E were higher in the fall and molybdenum and selenium concentrations were lower in the fall (Table 4.3). Paired serum concentrations of manganese were not associated with season.

4.3.5 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 copper ($P = 0.007$), manganese ($P = 0.012$), and selenium ($P = 0.048$) measured at the start of the grazing season. Spring body condition was unconditionally associated with spring serum concentrations of copper ($P = 0.046$). 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 molybdenum 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 copper 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 4.4). In the final model, between-herd differences accounted for 24.5% of the remaining variation in spring serum copper 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 manganese and selenium at the start of the grazing season

than cows from herds which were not supplemented (Table 4.4). In these final models, between-herd differences accounted for 8.2% of the remaining variation in spring serum manganese and 59.3% of spring serum selenium concentrations and between-pasture differences accounted for 15.1% of manganese and 1.5% of selenium.

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 year old cows than in 4 to 9 year old cows (Table 4.4). Vitamin A concentrations in 1 to 3 year old cows were higher than in 4 to 9 year 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 1 to 3 year old cows than in 10 to 14 year 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.

4.3.6 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 sulfate 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 April 1 to October 31, 2008 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. Sulfate and iron concentrations in pasture water were associated with the serum Copper concentrations at the end of the grazing season (Table 4.5). Although sulfate concentrations were associated with an overall difference in copper concentrations, there was no linear trend and no significant difference between the highest and lowest sulfate quartiles. Cows on pasture where the iron concentrations in the water were above the 25^{th} percentile had lower fall serum copper 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 copper concentrations explained by between-herd differences 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 manganese concentrations than pregnant cows when all significant factors were adjusted for (Table 4.5). Cows on pastures with water sulfate concentrations between the 25th and 50th percentile also had lower serum manganese 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 manganese 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 sulfate concentrations in sampled water sources above the 25th percentile had the lowest serum molybdenum concentrations (Table 4.5); however, there was no clear linear trend. Cows pastured where water iron concentrations were between the 25th and 75th percentile had higher molybdenum 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 molybdenum concentrations explained by between-herd differences was 33.3% and the proportion explained by between-pasture differences was 14.4%.

After considering other factors, selenium was higher in the fall in non-pregnant cows compared to pregnant cows (Table 4.5). Cows on pastures with cumulative precipitation up to 263 mm had lower fall serum selenium 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 selenium 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 4.5). 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 25^{th} and 75^{th} percentile were associated with higher fall serum vitamin A concentrations compared to 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 4.5). 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%.

4.4 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 copper and manganese, until liver or other endogenous reserves are depleted (Gooneratne et al. 1989; Kincaid 1999; Hansen 2006). The measurement of selenium 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 selenium status was observed to be very good ($\kappa = 0.79$) at the herd level and good at the individual animal level ($\kappa = 0.68$) for samples collected from beef cows in the fall of the year (Waldner et al. 1998).

4.4.1 Season

Seasonal differences in copper and selenium concentrations in cattle have previously been reported. Consistent with the findings of this study, Smart et al. (1992) found the lowest copper concentrations occurred in late winter and the highest concentrations occurred in late summer under western Canadian conditions. In contrast, serum selenium concentrations decreased during the grazing season in the present study. Season has been previously associated with selenium 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 selenium concentration. The differences in dairy cattle feeding and supplementation practices make it difficult to generalize these studies to extensively managed beef cows.

Serum molybdenum concentrations also decreased during the grazing season. Seasonal differences in serum molybdenum concentrations have not been previously documented in cattle. Since molybdenum concentrations in blood reflect molybdenum dietary intake (Ward 1978; Wittenburg and Devlin 1987), lower molybdenum concentrations after the grazing season than before likely indicate lower molybdenum concentrations in pasture forage than in feed sources fed before arrival at pasture. This

study also observed that cows with high serum molybdenum concentrations in the fall were more likely to have above adequate serum copper concentrations in the fall. Serum copper concentrations will be especially difficult to interpret in these animals, as serum copper concentrations have been reported to increase, at least initially, in molybdenum supplemented animals while liver copper 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 to growing plant material (Hidiraglou and Williams 1986) and, therefore, cows on grazing pasture would be expected to have higher vitamin A and E concentrations.

4.4.2 Physiological factors

Older cows were more likely to have lower concentrations of vitamin 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 years and younger had higher mean serum concentrations of vitamins A and E than dairy cows older than 4 years and speculated that the older cows have greater oxidation rates of vitamins A and E.

Cows that entered pasture in thin body condition in May 2008 had lower spring serum copper 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 copper 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 copper 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 copper 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 been previously 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 manganese and selenium concentrations in the fall than cows that were pregnant. While there were no published studies on the associations between selenium and manganese concentrations and pregnancy status, there is some information on the effect of pregnancy on copper in the cow. The bovine fetus accumulates copper exponentially at the expense of maternal copper and a progressive decline in maternal liver copper during gestation has been

previously documented (Gooneratne et al. 1989). There was no significant association between pregnancy status and serum copper 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 manganese and selenium concentrations.

4.4.3 Herd management

Trace mineral supplementation before calving was the one identified herd management factor associated with spring trace mineral concentrations. Although copper concentrations were higher in cows from herds supplemented with trace minerals before calving compared to unsupplemented herds, the manganese and selenium concentrations in cows from the supplemented herds were lower. Previous manganese supplementation trials in beef animals have reported consistently low plasma manganese 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 manganese concentrations may not be clinically relevant.

Serum selenium 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 selenium concentrations reflect recent exposure to dietary seleniuim (Stowe and Herdt, 1992). Almost all the cows in this study calved one to four months 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 selenium and vitamin A concentrations measured in the spring and one-quarter of the variation in copper 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.

4.4.4 Pasture conditions

Neither water quality nor cumulative precipitation was consistently associated with micronutrient concentrations measured in the fall. High dietary concentrations of sulfur and iron have been associated with secondary copper deficiency in cattle (Humphries et al. 1983; Bremner et al. 1987; Gould 2002); however, the concentrations of sulfate and iron in the pasture water sources in the present study may have been too low to add substantially to the sulfur or iron intake of these cows. Olkaowski (2009) cites water sulfate concentrations above 1000 mg L^{-1} and water iron concentrations above 10 mg L^{-1} as potentially contributing significant amounts of dietary sulfate and iron in cattle. Only two of the eighteen breeding fields in this study had water sulfate 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 sulfate and iron concentrations was, therefore, limited.

The amount of precipitation received by each pasture was also inconsistently associated with serum concentrations of selenium 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 months) 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 copper, manganese, molybdenum, selenium, 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 sulfate 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 ha 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 were deficient in copper (less than 10 mg/ kg of dry matter), and 100% were deficient in zinc (less than 40 mg/ kg of dry matter) (Smart et al. 1992). Three of the 5 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 micronutrient 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 micronutrient 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.

4.5 References

Anonymous. 2007a. Community Pasture Program. [Online] Available at: http://www4.agr.gc.ca/AAFC-AAC/display-afficher.do?id=1183493052855&lang=eng. [25 March 2010].

Anonymous. 2007b. Using dentition to age cattle. [Online] Available at: http://www.fsis.usda.gov/Fact_Sheets/Bovine_Spongiform_Encephalopathy_BSE. [25 March 2010].

Anonymous. 2008. Soil landscape illustrations of the prairie provinces. [Online] Available at: http://sis.agr.gc.ca/cansis/taxa/landscape/slc_prairie.html. [5 August 2010].

Anonymous. 2010. Canadian climate data and information archive. [Online] Available at: http://climate.weatheroffice.gc.ca/advanceSearch/searchHistoricData_e.html. [23 August 2010].

Bass, R. T., Swecker, W. S., Eversole, D. E. 2001. Effects of oral vitamin E supplementation during late gestation in beef cattle that calved in late winter and late summer. Am. J. Vet. Res. **62:** 921-927.

Block, E. and Farmer, B. 1987. The status of beta-carotene and vitamin A in Quebec dairy herds: factors affecting their status in cows and their effects on reproductive performance. Can. J. Anim. Sci. **67:** 775-788.

Bremner, I., Humphries, W. R., Phillippo, M., Walker, M. J., Morrice, P. C. 1987. Iron-induced copper deficiency in calves: dose-response relationships and interactions with molybdenum and sulphur. Anim. Prod. **45:** 403-414.

Campbell, J. R., Jim, G. K., Booker, C. W., Guichon, P. T. 1995. A survey of the selenium status of beef cows in Alberta. Can. Vet. J. **36:** 698-702.

Catignani, G. L. and Bieri, J. G. 1983. Simultaneous determination of retinol and α -tocopherol in serum or plasma by liquid chromatography. Clin. Chem. **29:** 708-712.

Dargatz, D. A. and Ross, P. F. 1996. Blood selenium concentrations in cows and heifers on 253 cow-calf operations in 18 states. J. Anim. Sci. **74:** 2891-2895.

Dargatz, D. A., Garry, F. B., Clark, G. B., Ross, P. F. 1999. Serum copper concentrations in beef cows and heifers. J. Am. Vet. Med. Assoc. **215:** 1828-1832.

Dohoo, I., Martin, W., Stryhn, H. 2003. Veterinary epidemiologic research. Charlottetown, PEI, Canada: AVC Inc. Pages 502-504.

Gooneratne, S. R., Buckley, W. T., Christensen, D. A. 1989. Review of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci. **69:** 819-845.

Gooneratne, S. R. and Christensen, D. A. 1989. A survey of maternal copper status and fetal tissue copper concentrations in Saskatchewan bovine. Can. J. Anim. Sci. **69:** 141-150.

Gooneratne, S. R., Symonds, H. W., Bailey, J. V., Christensen, D. A. 1994. Effects of dietary copper, molybdenum and sulfur on biliary copper and zinc excretion in Simmental and Angus cattle. Can. J. Anim. Sci. **74:** 315-325.

Gould, D. H., Dargatz, D. A., Garry, F. B., Hamar, D. W. 2002. Potentially hazardous sulfur conditions on beef cattle ranches in the United States. J. Am. Vet. Med. Assoc. **221:** 673-677.

Greene, L. W. 2000. Designing mineral supplementation of forage programs for beef cattle. J. Anim. Sci. **77:** 1-9.

Hansen, S. L., Spears, J. W., Lloyd, K. E., Whisnant, C. S. 2006. Growth, reproductive performance, and manganese status of heifers fed varying concentrations of manganese. J. Anim. Sci. **84:** 3375-3380.

Hidiroglou, M. and Williams, C. J. 1986. Interrelationships among liposoluble vitamins in ruminants. Am. J. Vet. Res. **47:** 1767-1771.

Hidiroglou, M., Batra, T. R., Roy, G. L. 1994. Changes in plasma α–tocopherol and selenium of gestating cows fed hay or silage. J. Dairy Sci. **77:** 190-195.

Hintze, K. J., Lardy, G. P., Marchello, M. J., Finley, J. W. 2002. Selenium accumulation in beef: effect of dietary selenium and geographical area of animal origin. J. of Agric. and Food Chem. **50:** 3938-3942.

Hoff, B., Schrier, N., Boermans, H., Faulkner, H., Hussein, A. 2001. Assessment of trace mineral and vitamin E status beef cows in Ontario. Can. Vet. J. **42:** 384-385.

Humphries, W. R., Phillippo, M., Young, B. W., Bremner, I. 1983. The influence of dietary iron and molybdenum on copper metabolism in calves. Br. J. Nutr. **49:** 77-86.

Katamoto, H., Yamada, Y., Nishizaki, S., Hashimoto, T. 2003. Seasonal changes in serum vitamin A, vitamin E and β-carotene concentrations in Japanese black breeding cattle in Hyogo prefecture. J. Vet. Med. Sci. **65:** 1001-1002.

Katsoulos, P. D., Roubies, N., Panousis, N., Karatzanos, P., Karatzias, H. 2005. Long-term fluctuations and effect of age on serum concentrations of certain fat-soluble vitamins in dairy cows. Vet. Clin. Path. **34:** 362-367.

Kincaid, R. L. 1999. Assessment of trace mineral status of ruminants: A review. Proc. of Amer. Soc. Anim. Sci. **E22:** 1-10.

Legleiter, L. R., Spears, J. W., Lloyd, K. E. 2005. Influence of dietary manganese on performance, lipid metabolism, and carcass composition of growing and finishing steers. J. Anim. Sci. **83:** 2434-2439.

Littledike, E. T., Wittum, T. E., Jenkins, T. G. 1995. Effect of breed, intake, and carcass composition on the status of several macro and trace minerals of adult beef cattle. J. Anim. Sci. **73:** 2113-2119.

Maas, J. Galey, F. D., Peauroi, J. R., Case, J. T., Littlefield, E. S., Gay, C. C., Koller, L. D., Crisman, R. O., Weber, D. W., Warner D. W., Tracy M. L. 1992. The correlation between serum selenium and blood selenium in cattle. J. Vet. Diagn. Invest. **4:** 48-52.

Marx, T. (Ed.) 2009. Body condition scoring your cow herd. [Online] Available at: http://www1.agric.gov.ab.ca/Information/Livestock/Beef/Feeding. [25 March 2010].

Miller, G. Y., Bartlett, P. C., Eskine, R. J., Smith, K. L. 1995. Factors affecting serum selenium and vitamin E concentrations in dairy cows. J. Am. Vet. Med. Assoc. **206:** 1369-1373.

Milne, D. B. and Botnem, J. 1986. Retinol, α-tocopherol, lycopene, and α- and β-carotene simultaneously determined in plasma by isocratic liquid chromatography. Clin. Chem. **32:** 874-876.

Olkowski, A. A. 2009. Livestock water quality-a field guide for cattle, horses, poultry, and swine. [Online] Available at: http://www.agriculture.gov.sk.ca/Livestock-Feeds-Nutrition. [28 August 2009].

Puls, R. 1994a. Mineral levels in animal health: diagnostic data, 2nd ed. Sherpa International, Clearbrook, British Columbia. Pages 83, 135, 192, 230.

Puls, R. 1994b. Vitamin levels in animal health: diagnostic data and bibliographies. Sherpa International, Clearbrook, British Colombia. Pages 15, 98.

Rice, L. E. 1991. The effects of nutrition on reproductive performance of beef cattle. In: Maas, J. (editor). *Beef cattle nutrition*. Vet. Clin. North Am. Food Anim. Pract. **7:** 1-26.

Ropstad, E., Osteras, O., Overnes, G., Froslie, A. 1988. Seasonal variation of selenium status of Norwegian dairy cows and effects of selenium supplementation. Acta Vet. Scand. **29:** 159-164.

Smart, M. E. and Christensen, D. A. 1985. The effect of cow's dietary copper intake, sire breed, age on her copper status and that of her fetus in the first ninety days of gestation. Can. J. Comp. Med. **49:** 156-158.

Smart, M. E., Cymbaluk, N. F., Christensen, D. A. 1992. A review of copper status of cattle in Canada and recommendations for supplementation. Can. Vet. J. **33:** 163-170.

Smith, S. and Marshall, I. (Eds.) 1995. A National Ecological Framework for Canada. [Online] Available at: http://sis.agr.gc.ca/cansis/publications/ecostrat/intro.html. [5 August 2010].

Stowe, H. D. and Herdt, T. H. 1992. Clinical assessment of selenium status of livestock. J. Anim. Sci. **70:** 3928-3933.

Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annu. Rev. Nutri. **11:** 121-140.

Waldner, C., Campbell, J., Jim, K. G., Guichon, P. T. and Booker, C. W. 1998. Comparison of 3 methods of selenium assessment in cattle. Can. Vet. J. **39:** 225–231.

Ward, G. M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: a review. J. Anim. Sci. **46:** 1078-1085.

Wichtel, J. J., Keefe, G. P., Van Leeuwen, J. A., Spangler, E., McNiven, M. A., Ogilvie, T. H. 2004. The selenium status of dairy herds in Prince Edward Island. Can. Vet. J. **45:** 124-132.

Wikse, S. E., Herd, D., Field, R., Holland, P. 1992. Diagnosis of copper deficiency in cattle. J. Amer. Vet. Med. Assoc. **200:** 1625-1629.

Wittenberg, K. M. and Devlin, T. J. 1987. Effects of dietary molybdenum on productivity and metabolic parameters of lactating beef cows and their offspring. Can. J. Anim. Sci. **67:** 1055-1066.

Yuan, W. Z., Poole, D. B. R., Mason, J. 1988. The effects of supplementation of the diet of young steers with Mo and S on the intracellular distribution of copper in liver and on copper fractions in blood. Br. Vet. J. **144:** 543-551.

Table 4.1 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			
Season (spring, fall)	x		
Physiologic parameters			
Age category (1-3 years, 4-9 years, 10-14 years)		x	x
Body condition score (< 2.5/5, ≥ 2.5/5)			
spring		x	x
fall			x
Pregnancy status (pregnant, not pregnant)			x
Herd management			
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	
Pasture conditions			
Sulfate concentration in pasture water (mg L^{-1})[z]			x
Iron concentration in pasture water (mg L^{-1})[z]			x
Cumulative precipitation, April 01-October 31(mm)[y]			x
Trace mineral supplementation on pasture (Y, N)			x

[z] sulfate and iron concentrations were each categorized as quartiles of the measured values.
[y] cumulative precipitation was categorized using the four measured values from the five pastures.

Table 4.2 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 conc. considered adequate[z]	Season	No. of cows (%) below adequate conc.	Percentiles of serum micronutrient concentration				
				5th	25th	50th	75th	95th
Copper	0.60	Spring	586/790 (74)	0.29	0.43	0.52	0.59	0.72
(mg L^{-1})		Fall	506/761 (66)	0.40	0.49	0.55	0.62	0.73
Manganese	6	Spring	527/789 (67)	2	3	4	6	10
(μg L^{-1})		Fall	561/761 (74)	3	4	5	6	8
Selenium	0.08	Spring	4/789 (< 1)	0.11	0.13	0.15	0.18	0.23
(mg L^{-1})		Fall	54/761 (7)	0.08	0.10	0.13	0.15	0.17
Vitamin A	0.30	Spring	99/789 (13)	0.26	0.34	0.42	0.51	0.69
(mg L^{-1})		Fall	14/760 (2)	0.33	0.41	0.46	0.53	0.61
Vitamin E	4.0	Spring	46/790 (6)	4.05	5.75	7.63	9.70	13.1
(mg L^{-1})		Fall	0/759	7.52	9.23	10.60	12.4	15.2

Micronutrient	Highest conc. considered adequate[z]	Season	No. of Cows (%) Above Adequate Concentration	Percentiles of Serum Micronutrient Concentration				
Molybdenum	0.10	Spring	146/789 (19)	0.004	0.010	0.019	0.065	0.200
(mg L^{-1})		Fall	94/761 (12)	0.006	0.014	0.027	0.053	0.114

[z] (Puls 1994a,b)

Table 4.3 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.

	Differences between fall and spring serum concentration measurements		
Micronutrient	Estimate	95% CI[z]	*P* 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

[z] 95% CI – represents the 95% confidence interval

Table 4.4 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]	*P* value
Copper (mg L^{-1})			
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
Manganese (μg L^{-1})			
Trace mineral supplementation before calving	-1.052	-1.877 to -0.227	0.012
Selenium (mg L^{-1})			
Trace mineral supplementation before calving	-0.023	-0.046 to 0.000	0.048
Vitamin A (mg L^{-1})			
Age category (years)			< 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
Vitamin E (mg L^{-1})			
Age category (years)			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

[z] 95% CI – represents the 95% confidence interval

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

Table 4.5 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
Copper (mg L^{-1})			
Sulfate concentration in pasture water (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 (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
Manganese (μg L^{-1})			
Not pregnant in fall	1.043	0.387 to 1.699	0.0018
Sulfate concentration in pasture water (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
Molybdenum (mg L^{-1})			
Sulfate concentration in pasture water (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 (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
Selenium (mg L^{-1})			
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
Vitamin A (mg L^{-1})			
Age category (years)			< 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			0.0086[y]
0.15-0.72 vs. 0.0157-0.14 (mg L^{-1})	0.047	0.006 to 0.088	0.026
0.73-1.15 vs. 0.0157-0.14 (mg L^{-1})	0.056	0.023 to 0.089	< 0.001
1.16-3.89 vs. 0.0157-0.14 (mg L^{-1})	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
Vitamin E (mg L^{-1})			
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

[z] 95% CI – represents the 95% confidence interval

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

CHAPTER 5

CONCLUSIONS

This thesis describes the serum micronutrient concentrations measured at the start and the end of the grazing season of commercial beef cows in western Canada. Individual animal, herd, and environmental factors were examined for associations with serum concentrations of copper, molybdenum, manganese, selenium, vitamin A, and vitamin E, and the relationship between micronutrient concentrations and reproductive outcomes was assessed. Approximately 70% of Canada's breeding beef cows and heifers are located in Saskatchewan and Alberta (Anonymous 2011), and the cow-calf production and management cycle in this region is intimately connected to the season and available feedstuffs. The seasonal changes in feed and often water sources, combined with animal, herd and environmental factors, may result in a variable micronutrient supply at different production phases. To date, no large surveys have measured the micronutrient status of western Canadian beef cows at common handling times in their production cycle; therefore, the associations between micronutrient concentrations and reproductive outcomes have not been examined. This concluding chapter presents the key findings of this thesis and considers the strengths and limitations of the two projects from which the conclusions were drawn. Unanswered questions and future research directions are also discussed.

To address the objectives of this thesis, the results of two studies were presented. The first study (Chapter 2) examined data from a subset of cow-calf herds enrolled in a

cohort study of factors affecting productivity in 205 herds from western Canada (Waldner 2008). Blood samples were collected at pregnancy testing time from all accessible open cows and a systematic random sample of pregnant cows from each of 66 herds. Following analysis for infectious causes of reproductive failure (Waldner 2005), half of the remaining samples were then randomly allocated to an analysis of the copper and molybdenum serum concentrations. Information on the individual animals and trace mineral supplementation practices was collected from the producers. Environmental information about each herd's primary grazing pasture was extracted using a geographic information system and publicly accessible data provided by Agriculture and Agri-Food Canada.

The second study (Chapters 3 and 4) was a cohort study conducted in a convenience sample of beef cows from 40 privately-owned cow-calf herds placed on five Saskatchewan community pastures in 2008. The copper, molybdenum, selenium, manganese, vitamin A, and vitamin E serum concentrations were measured at two points in the production cycle; as the cows entered (pre-breeding) and exited the community pastures for the summer grazing season. Enrolled cows were pregnancy tested at the end of the grazing season. Surveys were used to collect individual animal, herd management information, and breeding and grazing pasture information from producers and pasture managers.

Chapter 2 described copper and molybdenum concentrations for serum samples collected at pregnancy testing. This was a larger survey of copper concentrations in beef

cattle than studies previously done in Canada (Smart and Christensen 1985; Gooneratne and Christenson 1989), and was the only survey of molybdenum concentrations in beef cattle in a non-disease situation (Smart et al. 1992; Majak et al. 2004).

Only 16% of cows in these herds had less than adequate (< 0.60 ppm) copper concentrations, half the proportion of cows with below adequate serum copper in previous surveys of beef cattle in North America, including a small study conducted in Saskatchewan (Gooneratne and Christensen 1989; Dargatz et al. 1999). The reasons for the higher copper concentrations in this population may be due to season of sampling, feed and water quality, or herd supplementation practices. High molybdenum concentrations ($\geq$ 0.10 ppm) were found in 12% of cows, potentially predisposing these animals to secondary copper deficiency. There were no similar studies to which to compare the molybdenum data.

Cattle diets containing a copper to molybdenum ratio of less than 2:1 can also produce secondary copper deficiency (Ward 1978; Olson 2007). Secondary copper deficiency due to the presence of minerals, including molybdenum, sulphates, and iron, in feed or water that antagonize copper absorption and metabolism in ruminants is probably the most common form of copper deficiency in cattle (Wikse 1992; Smart et al. 1992; Olson 2007). The lack of water and forage analysis from the pasture used immediately prior to sampling limited this study from analyzing the potential for secondary copper deficiency due to low copper:molybdenum ratios or the presence of other antagonistic minerals.

Chapter 2 described the relationships between geographic location of the primary grazing pasture of each herd and the copper and molybdenum concentrations of cows located throughout Saskatchewan and Alberta. Previously, one small study had described serum copper concentrations of beef cows originating in central soil zones of Saskatchewan (Gooneratne and Christensen 1989); however, this study examined slaughter animals and the exact location of herds could not be determined. Sporadic localized reports of clinical molybdenosis and secondary copper deficiency related to western Canadian geographic locations have also been reported (Smart et al. 1992; Majak et al. 2004).

By measuring copper and molybdenum concentrations in a large number of herds located across Saskatchewan and Alberta, this study provided more evidence that geographic location can help determine the risk of primary and secondary copper deficiency in beef herds. Furthermore, by examining various components of geographic information, including soil type, precipitation amount, and ecoregion, for associations with copper and molybdenum serum concentrations, several potentially useful ways of distinguishing high risk areas for copper deficiency were described. Cows from herds located on the Northern Continental Divide ecoregion had significantly lower copper concentrations than herds from all other ecoregions examined, except the Moist Mixed Grassland. Cows located in areas with gray soils had higher molybdenum concentrations than cows located in areas with black or brown soils. Cows in the Western Alberta Upland ecoregion and in areas receiving the greatest amounts of precipitation also had

higher serum molybdenum concentrations. Producers with herds located in these higher risk areas for primary and secondary copper deficiency should ensure that adequate trace mineral supplementation is provided.

One limitation of the study described in Chapter 2 is that the micronutrient concentrations were only measured at pregnancy testing time. There were no associations between copper and molybdenum concentrations measured at the end of the grazing season and reproductive outcome in these cows, with the exception that cows with the lowest serum copper concentrations at pregnancy testing were more likely to be pregnant than cows with higher copper concentrations. This was not surprising since the bovine fetus accumulates copper exponentially at the expense of maternal copper and maternal liver copper declines during gestation (Gooneratne and Christensen 1989; Gooneratne et al. 1989). Seasonal fluctuations in serum copper concentrations have been previously documented and were found to be at their lowest in February and March under western Canadian conditions (Smart et al. 1992). Therefore, pregnancy testing may not be the most appropriate time to measure serum copper concentrations. Serum copper concentrations measured before the start of the breeding season may more accurately reflect the time period when the cow's micronutrient status has the greatest potential effects on pregnancy rates.

Chapter 3 addressed this limitation from Chapter 2, and described the association between serum concentrations of micronutrients measured at the start of the breeding season and pregnancy outcome after the summer grazing season in cows placed on five

different community pastures in southern Saskatchewan. Very few epidemiological studies have examined the relationship between trace mineral concentrations and reproductive outcomes in beef cattle. One case-control study conducted in France reported associations between low herd-level zinc and selenium concentrations and herd-level reproductive disorders (low fertility, abortions, and retained placentas) in dairy and beef animals; however, this study did not account for other potential risk factors for poor reproductive outcomes (Enjalbert et al. 2006). A strength of the studies described in Chapters 2 and 3 is that they control for common potential risk factors for non-pregnancy at the animal and herd levels.

Serum copper concentrations were less than adequate (< 0.60 ppm) in 75% (580/771) of cows examined in Chapter 3. Seventy-eight percent of the 4 to 9 year old cows (388/500), 72% of the 2 and 3 year old cows (108/151), and 70% of the 10 to 14 year old cows (80/115) had serum copper concentrations < 0.60 ppm. Decreasing serum copper concentrations increased the risk of non-pregnancy in cows less than 10 years of age. In contrast, copper deficient herds described in Enjalbert's study (2006) did not have increased odds of low fertility. Compared to the serum copper concentrations of cows in Chapter 3, the copper concentrations reported for herds with low fertility were considerably higher in the Enjalbert study, possibly explaining the lack of any observed association in that study between copper and reproductive performance.

This study was unable to determine whether the low copper concentrations of these cows were due to primary or secondary copper deficiency because feed and water tests

were not done. Primary copper deficiency results from an inadequate intake of dietary copper. This is a possible contributor to the low copper status because western Canadian forages, cereal hay, and cereal grains have frequently been reported to have copper levels below the suggested NRC requirement for beef cattle (Anonymous 1996; Gooneratne et al. 1989; Suleiman et al. 1997). Secondary copper deficiency due to the presence of minerals, including molybdenum, sulphates, and iron, in feed or water that antagonize copper absorption and metabolism in ruminants is probably the most common form of copper deficiency in cattle (Wikse et al. 1992; Smart et al. 1992; Olson 2007) and cannot be ruled out as a contributing factor to the low copper concentrations. High sulfate in water is common throughout the Great Plains region of North America and is additive to the sulfur content in feed (Olkowski et al. 1991; Suttle 1991; Gould et al. 2002). Future research should also consider the micronutrient concentrations of winter feed and water sources for the breeding females in order to assess their contribution to low pre-breeding serum micronutrients.

Another potential limiting factor for both studies described in this thesis is that the micronutrient concentrations were determined solely from serum measurements. The practical constraints of conducting measurements on privately-owned cows led to the decision in both studies described in this thesis to sample serum micronutrient concentrations instead of performing liver biopsies. The liver is the primary storage organ and homeostasis regulator for copper and vitamin A, and one of the main storage and regulating organs for selenium and manganese (Goonteratne et al. 1989; Olson 1996; Legleiter et al. 2005; Gomez 2006). Inadequate copper intake in particular is not

consistently reflected as below adequate serum copper concentrations until liver copper concentrations fall below a critical threshold (40 ppm on a dry weight basis) (Claypool et al. 1975). However, previous researchers have noted that serum copper concentrations of 0.45 ppm are correlated with low liver copper concentrations (Claypool et al. 1975; Tessman et al. 2001). It is interesting that the strongest association with the odds of non-pregnancy described in Chapter 3 were observed for serum copper concentrations less than 0.40 ppm.

Chapter 3 did not identify associations between serum selenium, molybdenum, vitamin A, and vitamin E concentrations and pregnancy status after accounting for other potential risk factors for non-pregnancy. Most of the cows in this study had serum concentrations of selenium, vitamin A, and vitamin E within recommended ranges, potentially decreasing this study's power to examine the associations between various concentrations of these micronutrients and pregnancy outcome in beef cattle. Additional field studies should examine animals from a wider range of geographical areas, including known selenium-deficient areas, where beef cows are at greater risk for below adequate concentrations of these micronutrients.

Chapter 4 is the companion paper to Chapter 3. Chapter 4 explored the factors that were associated with serum copper, manganese, selenium, molybdenum, vitamin A, and vitamin E status before and after the summer grazing season. The seasonal difference between micronutrient concentrations of paired serum samples was described, and cow-,

herd-, and pasture-level risk factors associated with the micronutrient concentrations at each of these time periods were examined.

Available studies examining the effect of season on beef cow micronutrient concentrations under western Canadian conditions are limited. One report has observed that beef cow serum copper concentrations are lowest in late winter and highest in fall (Smart et al. 1992). Several studies have examined associations between season and selenium and vitamin status in dairy cows (Ropstad et al. 1988; Miller et al.1995; Katamoto et al. 2003; Wichtel et al. 2004), but it is difficult to generalize these results to beef herds. A few previous studies had examined the associations between age, body condition and pregnancy status on copper 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 manganese, molybdenum and selenium concentrations has not been previously 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). Chapter 4 concluded that season and individual animal factors including age, body condition, and pregnancy status should be considered when interpreting the micronutrient status of beef cows in western Canada and developing supplementation programs.

The effectiveness of trace mineral supplementation programs is of interest to producers and veterinarians. Chapter 4 described trace mineral supplementation before calving as one herd management factor associated with spring trace mineral

concentrations. Although copper concentrations were higher in cows from herds supplemented with trace minerals before calving compared to unsupplemented herds, the manganese and selenium concentrations in cows from the supplemented herds were lower. Almost all the cows in this study calved one to four months before spring sample collection. This delay between pre-calving supplementation and measurement of pre-breeding serum micronutrient concentrations in this study increases the likelihood that feed differences or other unmeasured management differences between herds could explain the differences in copper, manganese, and selenium concentrations in cows from supplemented and non-supplemented herds. Serum selenium 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).

This study did not show any consistent association between pasture water quality or cumulative precipitation over the growing season and micronutrient concentrations measured in the fall. The concentrations of sulfate and iron in the pasture water sources may have been too low to add substantially to the sulfur or iron intake of these cows. The pastures enrolled in this study used primarily surface water sources, and these are less likely than deep well water sources to contain high concentrations of sulfur and iron (Olkowski 2009). 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 months) 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. This study's ability to examine environmental differences was likely limited by the close geographical proximity of the pastures. Future research should investigate a wider geographical distribution of pastures, including areas known to have high levels of sulfate and iron in livestock water sources.

Demonstrating a relationship between micronutrients and reproductive outcomes is a critical first step in determining whether beef cow veterinarians and producers in western Canada should spend time and money investigating and correcting below adequate micronutrient concentrations. A clearer understanding of the factors that are linked to serum micronutrient status would improve the ability of veterinarians to interpret measured serum micronutrient concentrations and target supplementation programs to animals most at risk for below adequate micronutrient concentrations.

References

Anonymous. 1996. National Research Council. Nutrient requirements of beef cattle, 7th ed. National Academy Press, Washington, DC.

Anonymous. 2011. Statistics Canada. [Online] Available at: http://www.statcan.gc.ca/pub/23-012-x/2010002/aftertoc-aprestdm1-eng.htm. [10 April 2011].

Claypool, D. W., Adams, F. W., Pendell, H. W., Harmann Jr., N. A., Bone, J. F. 1975. Relationship between the level of copper in the blood plasma and the liver of cattle. J. Anim. Sci. **41:** 911-914.

Dargatz, D. A., Garry, F. B., Clark, G. B., Ross, P. F. 1999. Serum copper concentrations in beef cows and heifers. J. Am. Vet. Med. Assoc. **215:** 1828-1832.

Enjalbert, F., Lebreton, P., and Salat, O. 2006. Effects of copper, zinc and selenium status on performance and health in commercial dairy and beef herds: retrospective study. J. Anim. Physiol. and Anim. Nutr. **90:** 459-466.

Gomez, E., Caamano, J. N., Rodriguez, A., DeFurtos, C., Facal, N., and Diez, C. 2006. Bovine early embryonic development and vitamin A. Reprod. Dom. Anim. **41 (Suppl. 2):** 63-71.

Gooneratne, S. R. and Christensen, D. A. 1989. A survey of maternal copper status and fetal tissue copper concentrations in Saskatchewan bovine. Can. J. Anim. Sci. **69:** 141-150.

Gooneratne, S. R., Buckley, W. T., and Christensen, D. A. 1989. Review of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci. **69:** 819-845.

Gould, D. H., Dargatz, D. A., Garry, F. B., Hamar, D. W. 2002. Potentially hazardous sulfur conditions on beef cattle ranches in the United States. J. Am. Vet. Med. Assoc. **221:** 673-677.

Katamoto, H., Yamada, Y., Nishizaki, S., Hashimoto, T. 2003. Seasonal changes in serum vitamin A, vitamin E and β-carotene concentrations in Japanese black breeding cattle in Hyogo prefecture. J. Vet. Med. Sci. **65:** 1001-1002.

Katsoulos, P. D., Roubies, N., Panousis, N., Karatzanos, P., Karatzias, H. 2005. Long-term fluctuations and effect of age on serum concentrations of certain fat-soluble vitamins in dairy cows. Vet. Clin. Path. **34:** 362-367.

Legleiter, L. R., Spears, J. W., Lloyd, K. E. 2005. Influence of dietary manganese on performance, lipid metabolism, and carcass composition of growing and finishing steers. J. Anim. Sci. **83:** 2434-2439.

Littledike, E. T., Wittum, T. E., Jenkins, T. G. 1995. Effect of breed, intake, and carcass composition on the status of several macro and trace minerals of adult beef cattle. J. Anim. Sci. **73:** 2113-2119.

Majak, W., Steinke, D., McGillivray, J., and Lysyk, T. 2004. Clinical signs in cattle grazing high molybdenum forage. J. Range Manage. **57:** 269-274.

Miller, G. Y., Bartlett, P. C., Eskine, R. J., Smith, K. L. 1995. Factors affecting serum selenium and vitamin E concentrations in dairy cows. J. Am. Vet. Med. Assoc. **206:** 1369-1373.

National Research Council. *Nutrient requirements of beef cattle*. 6th ed. Washington, DC: National Academy Press, 1984.

Olkowski, A. A., Rousseaux C. G., Christensen D. A. 1991. Association of sulfate-water and blood thiamine concentration in beef cattle: Field studies. Can. J. Anim. Sci. **71:** 825-832.

Olkowski, A. A. 2009. Livestock water quality-a field guide for cattle, horses, poultry, and swine. [Online] Available at: http://www.agriculture.gov.sk.ca/Livestock-Feeds-Nutrition. [28 August 2009].

Olson, J. D. 1996. The role of selenium and vitamin E in mastitis and reproduction of dairy cattle. Irish Vet J. **49:** 362-364.

Olson, K. C. 2007. Management of mineral supplementation programs for cow-calf operations. Vet. Clin. Food Anim. **23:** 69-90.

Ropstad, E., Osteras, O., Overnes, G., Froslie, A. 1988. Seasonal variation of selenium status of Norwegian dairy cows and effects of selenium supplementation. Acta Vet. Scand. **29:** 159-164.

Smart, M. E. and Christensen, D. A. 1985. The effect of cow's dietary copper intake, sire breed, age on her copper status and that of her fetus in the first ninety days of gestation. Can. J. Comp. Med. **49:** 156-158.

Smart, M. E., Cymbaluk, N. F., Christensen, D. A. 1992. A review of copper status of cattle in Canada and recommendations for supplementation. Can. Vet. J. **33:** 163-170.

Stowe, H. D. and Herdt, T. H. 1992. Clinical assessment of selenium status of livestock. J. Anim. Sci. **70:** 3928-3933.

Suleiman, A., Okine, E., and Goonewardene, L. A. 1997. Relevance of National Research Council feed composition tables in Alberta. Can. J. Anim. Sci. **77:** 197-203.

Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annu. Rev. Nutri. **11:** 121-140.

Tessman, R. K., Lakritz, J., Tyler, J. W., Casteel, S. W., Williams, J. E., Dew, R. K. 2001. Sensitivity and specificity of serum copper determination for detection of copper deficiency in feeder calves. J. Amer. Vet. Med. Assoc. **218:** 756-760.

Waldner, C. L. 2005. Serological status for N. caninum, Bovine Viral Diarrhea Virus, and Infectious Bovine Rhinotracheitis Virus at pregnancy testing and reproductive performance in beef herds. Anim. Repro. Sci. **90:** 219-242.

Waldner, C. L. 2008. Western Canada study of animal health effects associated with exposure to emissions from oil and natural gas field facilities. Study design and data collection I. Herd performance records and management. Arch. of Environ. and Occup. Health. **63:** 167-186.

Ward, G. M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: a review. J. Anim. Sci. **46:** 1078-1085.

Wichtel, J. J., Keefe, G. P., Van Leeuwen, J. A., Spangler, E., McNiven, M. A., Ogilvie, T. H. 2004. The selenium status of dairy herds in Prince Edward Island. Can. Vet. J. **45:** 124-132.

Wikse, S. E., Herd, D., Field, R., Holland, P. 1992. Diagnosis of copper deficiency in cattle. J. Amer. Vet. Med. Assoc. **200:** 1625-1629.

APPENDIX A
DATA COLLECTION TOOLS

A.1 Herd Data Provided By Herd Owners

A. Herd Management:

1. How many cows (including heifers that calved spring 2008) did you have in your herd as of January 1st, 2008? _____
2. How many of those cows/heifers calved by May 15th, 2008? _____
3. How many calves died within 24hrs of birth? _____
4. How many calves died between birth and May 15th? _____

B. Nutrition:

5. Did you feed a TRACE MINERAL SUPPLEMENT to your replacement heifers or cows?
   (please check all boxes that apply)

   Heifers - ☐ no ☐ pre-calving ☐ post-calving/pre-breeding
   Cows - ☐ no ☐ pre-calving ☐ post-calving/pre-breeding

   If you answered 'no' to both of the above, skip to Question 8.

6. What is the name of the TRACE MINERAL SUPPLEMENT you fed?

   Heifers - _____

   Cows - _____

7. How was the TRACE MINERAL SUPPLEMENT fed?

   Heifers - ☐ free choice ☐ mixed with other feed ☐ top dress
   Cows - ☐ free choice ☐ mixed with other feed ☐ top dress

8. Did you feed a VITAMIN SUPPLEMENT to your replacement heifers or cows?
   (please check all that apply)

   Heifers - ☐ no ☐ pre-calving ☐ post-calving/pre-breeding
   Cows - ☐ no ☐ pre-calving ☐ post-calving/pre-breeding

   If you answered 'no' to both of the above, skip to Question C.

9. What is the name of the VITAMIN SUPPLEMENT you fed?

Heifers - _____

Cows - _____

How was the **VITAMIN** SUPPLEMENT fed?

Heifers - ☐ free choice ☐ mixed with other feed ☐ top dress
Cows - ☐ free choice ☐ mixed with other feed ☐ top dress

10. Did you inject your cows or replacement heifers with any of the following?
(please check all that apply)

Heifers - ☐ Selenium ☐ Vitamin A + D ☐ Vitamin E
Cows - ☐ Selenium ☐ Vitamin A + D ☐ Vitamin E

C. Vaccination: Please provide as much vaccination information as possible. "Heifers" refers to females born in 2006 that calved spring 2008, and "calves" refers to calves born spring 2008.

Vaccinated For:		Not Sure?	Not Vaccinated For	Month and Year Last Vaccinated?	Brand Name of Vaccine?
Blackleg	Cows	□	□		
	Heifers	□	□		
	Calves	□	□		
BVD	Cows	□	□		
	Heifers	□	□		
	Calves	□	□		
Leptospirosis	Cows	□	□		
	Heifers	□	□		
Scours	Cows	□	□		
	Heifers	□	□		
Vibrio	Cows	□	□		
	Heifers	□	□		

A.2 Pasture Data Provided by PFRA Pasture Managers

A. Length of Breeding Season:

1. What date were bulls placed onto breeding fields with the cows?

   _____

2. What date will bull be pulled off of the breeding field?

   _____

B. Supplements:

3. Is any TRACE MINERAL SUPPLEMENT included in the salt on the breeding fields?

   ☐ no ☐ yes

   If you answered 'no' to the above, skip to Question 5.

4. What is the name of the TRACE MINERAL SUPPLEMENT you feed?

   _____

C. Water:

5. Has a water analysis been done on the primary water sources in the pasture within the last year?

   ☐ no ☐ yes

   If yes, can you provide the date and summary of the results (either list or send copy)?

   _____

   _____

D. Cow Treatment:

6. Have any of the cows enrolled in the study been treated for infections (footrot, pneumonia, other)? If yes, please fill in the information listed on the table below for each cow.

	Patron	Cow Description	Cow ID Tag	Infection treated	Date of Treatment
1.					

E. Patron Grazing Information – Please fill out for each patron enrolled in the study.

Patron	Field Number	Total # of Cows on Field	ID of Bulls on Field (Please provide brands)	Date Moved Onto Field	Date Moved Off of Field	Approximate Field Size (Acres)	Water Source on Pasture		
							Dugout	Slough	Well

A.3 *Campylobacter fetus* Screening of Breeding Bulls

All mature bulls on fields with enrolled study cows were screened with a culture test offered by a commercial veterinary laboratory and a polymerase chain reaction (PCR) test performed by a research laboratory. An enzyme-linked immunosorbent assay (ELISA) test was used in addition to the culture and PCR tests for a subset of 40 bulls from two community pastures, including 28 bulls from a pasture with previous fertility problems suggestive of *C. fetus* subspecies *venerealis*.

Preputial material for the *C. fetus* culture test was inoculated into 2.5 mL of modified Weybridge transport enrichment media and transported to the laboratory at 18 to 25 C within 24 hours of collection. Following transport, the inoculated transport media was incubated at 37 C for 3 days and then transferred onto Campylobacter selective medium for further incubation under microaerophilic conditions at 37 C for 3 to 5 days.

Preputial material for the *C. fetus* PCR test was inoculated into 2.5 mL of phosphate-buffered saline (PBS) and stored on ice until frozen at -80 C within 24 hours of collection. Following thawing and vortical mixing, 0.5 ml of preputial material was transferred for DNA extraction. A real-time quantitative PCR assay was used for *C. fetus* detection in these samples.

Forty mature bulls were screened for *C. fetus* using Clark's transport enrichment media and subsequent monoclonal antibody-based capture enzyme-linked immunosorbent assay (ELISA). Preputial samples from these bulls were inoculated into

duplicate vials of Clark's transport enrichment media and submitted to the Canadian Food Inspection Agency Ottawa laboratory. The samples were incubated at the laboratory at 35 to 37 C for 4 to 5 days. A separate sterile swab was then used to inoculate fluid from each vial onto a *C. fetus* selective medium, without polymyxin B, for culture. One mL of the incubated Clark's media was collected for testing by ELISA for detection of *C. fetus*. These 40 bulls were tested for *T. foetus* in November 2008 so that they were not subjected to more than three consecutive preputial scrapings during April 2008.