Effect of source and concentration of supplemental trace minerals on apparent absorption and retention, performance and physiological indicators of trace mineral status in lactating Holstein dairy cows

by

Nicole Teri Briggs

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Animal Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Inorganic sources of trace minerals are commonly supplemented in dairy cow diets; however, there has been an increase in the supplementation of minerals complexed with organic compounds. Organic sources of trace minerals are thought to be protected from antagonistic interactions within the gastrointestinal tract and therefore have increased bioavailability for absorption and rumen fermentation, enhancing their utility for production. The objective of this study was to investigate the effect of source and concentration of supplemental trace minerals on productivity, apparent absorption, apparent retention, rumen fermentation parameters and physiological indicators of trace mineral status (vitamin B_{12} and glutathione peroxidase) in lactating Holstein dairy cows. Six lactating, cannulated, Holstein cows (129 ± 12 DIM; mid to late lactation) were used in a 6 x 6 Latin square design with a 28-d of experimental period (23-d of adaptation and 5-d of sample and data collection). The same basal diet was fed daily, but with different sources (organic [ORG] versus inorganic [INO]) and concentrations (50%, 100%, and 200% based on NRC recommendations) of supplemented trace minerals (Co, Cu, Mn, Se, and Zn). During the 5-d data and sample collection period, feed intake, water intake, blood, total milk, urine, and feces were collected daily, while rumen fluid and pH were collected during the final two days of the sample collection period. Results from chapter 2 revealed organic trace mineral supplementation decreased milk yield and milk fat yield compared to inorganic supplementation; however, low levels of organic trace mineral supplementation resulted in the same milk yield as all levels of inorganic supplementation. Fecal excretion of all trace minerals increased with increasing concentration of trace minerals in the diet. Organic cobalt supplementation exhibited higher apparent absorption and retention which could be an indication of organic cobalt having increased bioavailability. Selenium apparent absorption and retention were impacted by dietary mineral concentration, where high levels of organic and inorganic

supplementation showed higher absorption and retention than low levels. The results in chapter 3 showed that source and concentration of supplemental trace minerals did not impact serum trace mineral status with the exception of cobalt at high levels (200%) of supplementation and did not impact serum GSH-Px activity or the concentration of vitamin B_{12} concentration in plasma, ruminal fluid or milk. Organic trace mineral supplementation decreased the minimum rumen pH compared to inorganic supplementation. In addition, treatments affected the rumen environment in a dose-dependent manner where high organic supplementation (200%) was not beneficial in the production of total VFA compared to low (50%) organic supplementation and source and concentration of trace minerals caused a shift in the proportions of certain major and minor VFA excluding butyrate and valerate. Low organic could replace inorganic trace mineral supplementation without observing changes in productivity. As production results in low organic and inorganic supplementation are similar, more efficient supplementation of trace minerals to lactating dairy cows would allow producers to be more sustainable (reduce excretion and environmental impact) and profitable (reduce feed costs), while still maintaining performance of the herd.

Preface

The research project conducted in this thesis received ethics approval from the Animal Care and Use Committee of the University of Alberta with all procedures performed in accordance with the guidelines of the Canadian Council of Animal Care (CCAC, Ottawa, ON, Canada, 2009).

This thesis encompasses the original work of Nicole Teri Briggs with collaborations led by Dr. Michael Steele at the University of Alberta. The experiment presented in this thesis was conducted at the Dairy Research and Technology Centre at the University of Alberta. Co-authors for chapters 2 and 3 include Dr. Michael Steele and Dr. Bayissa Hatew of the University of Guelph and University of Alberta, respectively, who contributed to experimental design, experimental sampling and manuscript preparation. Chapter 1 and chapter 4 are the author's original work.

Acknowledgements

I would like to start out by thanking my supervisor, Dr. Michael Steele, for accepting me as his graduate student and providing his expertise, patience and guidance which allowed me to complete this thesis. The countless experiences and extensive knowledge I have gained throughout this degree would not have been possible without you. I would also like to thank Dr. Le Luo Guan for her role as a committee member and allowing us to join your lab. Thank you for providing the opportunity to expand my knowledge in a new field and improve my scientific thinking.

Thank-you to Dr. Doug Korver for his role as a committee member and for the advice and suggestions. Thanks-you to Dr. Heather Bruce for serving as my external examiner and committee chair.

I sincerely appreciate all the help and patience from the staff at the Dairy Research and Technology Centre, especially Courtney Felton, for allowing our six-month trial to run smoothly. Also, a huge thank you to the volunteers and members of Dr. Steele's lab who contributed to sample collection at all hours of the day, this project would not have been possible without all of your help.

A special thanks to Yanhong Chen for her patience and guidance in teaching qRT-PCR, Amanda Tlustos for her countless hours of editing and advice for this thesis, Michelle Edwards for her knowledge into statistics and for answering all my questions at all times of the week, and Bayissa Hatew for your immense help in the experimental setup and sampling.

Also, a special thanks to Jolet Kohler for being there whenever needed, answering every possible question and the countless amount of advice and support you have provided throughout my degree. It would not have been possible without you.

Thank you to Brett Kampjes, for being my rock during this journey. Your love and encouragement kept me grounded. Thanks for always being there when I needed it most.

Lastly, thank you to my parents, Alan and Pauline Briggs, and brother, Stephen Briggs, for all your love, support and encouragement throughout my life and for always giving me the best opportunity to chase my dreams.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Co	Cobalt
CP	Crude protein
CRIP	Cystine-rich intestinal protein
Cu	Copper
DM	Dry matter
DMI	Dry matter intake
DMT	Divalent metal transporter
GIT	Gastrointestinal tract
GSH-Px	Glutathione peroxidase
ICP-MS	Inductively coupled plasma mass-spectrometry
INO	Inorganic
Mn	Manganese
MUN	Milk urea nitrogen
NRC	National Research Council
ORG	Organic
PCR	Polymerase chain reaction
ppm	parts per million
Se	Selenium
TM	Trace mineral
TMR	Total mixed ration
VFA	Volatile fatty acids
Zn	Zinc

1.0 Chapter 1: Literature Review

1.1 Introduction

Dairy is an important component of the food industry. Meeting the nutrient requirements for lactating dairy cattle is crucial to ensure they reach their genetic potential to produce milk without compromising health and welfare (Drackley et al., 2006). Although the dairy industry continually works to optimize their feeding programs to ensure herds produce to the highest potential, there is still a considerable amount of work that needs to be done in regards to trace mineral supplementation. With a substantial variation in mineral concentrations of total mixed ration (TMR) feedstuffs, trace minerals are commonly supplemented at levels above the National Research Council (NRC) recommendations in an attempt to increase total absorption; however, these increased levels do not eliminate antagonistic interactions that occur within the gastrointestinal tract (GIT). Inorganic sources of trace minerals are commonly supplemented, but feed companies have recently begun to complex minerals with organic molecules as organic complexes are believed to be protected from antagonistic interactions with the GIT and increase bioavailability for absorption in the small intestine (Brown and Zeringue, 1994). Due to the limited information regarding the absorption of trace minerals, concentrations in dairy cow rations are commonly formulated to exceed the mineral requirements of the cow, with organic sources supplemented on top or in substitution of their inorganic counterparts.

Over-supplementation of minerals can lead to increased feed costs, increased antagonism with other minerals leading to decreased absorption, and in extreme cases can prove to be toxic to both rumen microbes and the cow itself (Genther and Hansen, 2015). In most cases, over-supplementation of minerals will lead to greater mineral excretion, which can have negative effects on soil and water reservoirs (Spears, 1996). Therefore, optimization of mineral

supplementation is not only of economic benefit due to decreasing feed costs associated with over-supplementation but is also environmentally sustainable through decreasing the excretion of excess minerals.

1.2 Trace Minerals

Trace minerals are minerals required in minute quantities (micrograms or milligrams per day) within the diet from either inorganic or organic sources. There are eight essential trace minerals required by dairy cattle; cobalt (Co), zinc (Zn), copper (Cu), manganese (Mn), selenium (Se), iodine (I), iron (Fe), and molybdenum (Mo) (NRC, 2001). These minerals play critical roles in immune function, oxidative metabolism and energy metabolism of dairy cattle (Arthur, 2000; Andrieu, 2008; Goff, 2018). Trace minerals are present in the body tissues in low concentrations and are part of metalloenzymes, enzyme cofactors, and components of hormones in the endocrine system (Yatoo et al., 2013). There are four specific functions performed by minerals including: structural, physiological, catalytic and regulatory as described in Table 1-1. Of these functions, the two most well-known areas of involvement include oxidative balance and immune cell function (Spears and Weiss, 2008).

Forages and concentrates can have a great variation in the concentration of trace minerals. This may be due to multiple factors including fertilization, soil types and plant species (Lopez-Alonso, 2012). Along with the type of feedstuff, processing can also influence the amount and concentration of certain minerals provided in the diet (Lopez-Alonso, 2012). Although trace minerals are often categorized together in dairy rations, it is important to understand the biological function, absorption and requirements for each individual trace mineral.

1.2.1 Cobalt

1.2.1.1 Biological Function

Cobalt is a component of cobalamin (vitamin B_{12}), which is a necessary component for gluconeogenesis in ruminant animals (NRC, 2001). Ruminants depend on the synthesis of glucose from gluconeogenesis as energy for tissues through the conversion of methylmalonyl CoA to succinyl CoA. Vitamin B_{12} production is dependent on the cobalt status of a ruminant as rumen microbes are able to synthesize all the required vitamin B_{12} with adequate amounts of dietary cobalt. Three percent of cobalt provided in the diet is incorporated into vitamin B_{12} when an animal is in adequate cobalt status, however this number can reach as high as 13% in diets deficient in cobalt (Smith and Marston, 1970). Over-supplementation of dietary cobalt can cause rumen microbes to produce vitamin B_{12} analogs that are not physiologically active and cannot be utilized by the animal for gluconeogenesis (van den Top, 2005). These analogs in the blood and liver result in difficultly in assessing the cobalt and vitamin B_{12} status of cattle (van den Top, 2005).

Vitamin B_{12} plays a role in the breakdown of propionate and the conversion of methlymalonyl-CoA to succinyl CoA (Goff, 2018). A deficiency in cobalt raises the methylmalonic acid concentrations in plasma and can be used as an indicator of cobalt deficiency. Microbial synthesis of vitamin B_{12} in the rumen increases within hours of cobalt supplementation and has been shown to increase ruminal digestion of some feedstuffs, including low quality forages and has increased the total anaerobic bacteria and lactic acid production in the rumen (Saxena and Ranjhan, 1978; Young, 1979; Lopez-Guisa and Satter, 1992).

1.2.1.2 Absorption

Cobalt is only required in the ruminant for vitamin B_{12} synthesis (van den Top, 2005). In mammals, cobalamin is absorbed, transported and utilized by three structurally-related

cobalamin transporting proteins: haptocorrin, intrinsic factor and transcobalamin II (Wuerges et al., 2006). Haptocorrin is found in saliva and binds cobalamin until proteolysis in the duodenum. Intrinsic factor, produced by parietal cells in the abomasum, is released into the gastric juices and binds cobalamin in the proximal jejunum (McKay and McLery, 1981; Wuerges et al., 2006). Cobalamin bound to intrinsic factor enters the mucosal cells in the distal ileum via receptor-mediated endocytosis, where cobalamin is transferred to transcobalamin II transporting protein (Seetheram et al., 1999). Cobalamin bound to transcobalamin II is released into the plasma and enters the cell via endocytosis. In the cell, transcobalamin II-bound cobalamin is dissociated in the lysosome and is transformed into two co-enzymes: methyl-cobalamin and ado-cobalamin within the cytoplasm and mitochondria (Wuerges et al., 2006). Cobalamin bound to transcobalamin accounts for one-quarter of the total cobalamin present in plasma. The remaining cobalamin is bound to haptocorrin, which is only able to enter hepatocytes (Wuerges et al., 2006). Cobalt cations can be absorbed, although their function is unknown. These cations cannot re-enter the rumen, and therefore cannot be utilized by rumen microbes for vitamin B_{12} production (Smith, 1987). These cations are mainly excreted in the urine (Underwood, 1981).

1.2.1.3 Requirements

Cobalt is required at 0.11 mg/kg of dietary DM for a mid-lactation Holstein cow (NRC, 2001). Compared to non-ruminant species, ruminants have higher sensitivity to vitamin B_{12} deficiency as it is required for gluconeogenesis (Goff, 2018). Within the ruminant, rumen microbes are sensitive to cobalt and a lack of cobalt in the diet results in a rapid decrease in the production of vitamin B_{12} (Goff, 2018). Vitamin B_{12} concentrations in ruminal fluid are the first to decline in states of cobalt deficiency due to the decline in rumen microbial production (Suttle, 2010). Within days of low dietary cobalt, there is a shift to low ruminal propionate and increased

succinate (Kennedy et al., 1996). This change may be a result of a blockage in the pathway that converts succinate to propionate or favours a change in the microbial population to produce succinate (Kennedy et al., 1996). Total serum vitamin B_{12} concentrations are the next to deplete as a result of the reduced synthesis of vitamin B_{12} by rumen microbes for absorption and lastly, a decline in liver vitamin B_{12} concentration is observed indicating that vitamin B_{12} stores are mobilized for metabolic purposes (Goff, 2018).

Early cobalt deficiency signs include an inability to grow, weight loss and decreased feed digestibility. Severe cases of cobalt deficiency are characterized by degeneration of liver fat, anemia, pale mucous membranes, impaired reproductive performance, and reduced resistance to infection through impaired neutrophil function (Underwood, 1981; Smith, 1997; Paterson and MacPherson, 1990). Cobalt is considered to be of low toxicity to ruminants, however, in rare cases, cobalt toxicity can lead to reduced feed intake, loss of body weight, and anemia (Ely et al., 1948; Keener et al., 1949).

1.2.2 Copper

1.2.2.1 Biological Function

Copper is a component of many enzymes including: cytochrome oxidase, lysyl oxidase, ceruloplasmin, tyrosinase, and superoxide dismutase (Ammerman et al., 1995; Peña et al. 1999). These enzymes are required for processes including electron transfer in the respiratory chain for energy generation, protection against oxidative stress, immune function, reproduction, bone development, pigmentation and connective tissue development (Peña et al., 1999; Bertinato and Labbé, 2004).

1.2.2.2 Absorption

In mammals, absorption of copper occurs in the small intestine and is facilitated by specific (Crt1) and non-specific divalent metal transport proteins in the intestinal mucosa (Hansen et al., 2010). The transmembrane copper-transporting protein, Ctr1, transports copper across the basolateral membrane and into the portal blood (Lee et al., 2012). Once in the blood, copper is transported to tissues within the body with a portion of copper bound to albumin and the remaining proceeding without binding (Prohaska, 2012). Copper that reaches the liver is absorbed in a two-step process, first by glutathione and then by metallothionein. The liver then partitions copper into storage, ceruloplasmin synthesis and biliary secretion (Wapnir, 1998). The biliary secretion of copper from the liver leads to feces being the main route for excess copper excretion (Prohaska, 2012).

Sulfur, molybdenum and high zinc levels are well-known antagonists of copper. For instance, copper is unavailable for absorption when sulfur binds copper in the rumen and is precipitated as the non-absorbable compound, copper sulphide, and when copper binds with sulfur and molybdenum to create a non-absorbable complex, tetrathiomolybdate (Bird, 1970; Allen and Gawthornet, 1987). Similarly, high zinc diets can also decrease intestinal absorption of copper as it creates increased metallothionein levels on the luminal side of the intestinal cells. Copper is sequestered and bound to the luminal surface by metallothionein rendering it unavailable for absorption. As these cells are sloughed, copper is lost to the feces (Fischer et al., 1981; Fischer et al., 1983).

1.2.2.3 Requirements

The requirement for copper is 15.7 mg/kg of dietary DM for a mid-lactation Holstein cow (NRC, 2001). Copper deficiency is characterized by loss of hair pigmentation (commonly around the eyes), anemia, fragile bones, osteoporosis, cardiac failure, poor growth, reproductive

inefficiency through delayed or depressed estrus, and reduced immune function (Gooneratne et al., 1989; Ammerman et al., 1995; Minatel and Carfagnini, 2000). Deficiencies may be difficult to detect as high molybdenum and sulfur concentrations can promote thiomolybdates in the blood, however, these forms are unavailable for biochemical functions. Copper may accumulate in excessive amounts within the liver before toxicity is known. This is hazardous as stress or illness may allow for release of excess copper from the liver into the bloodstream causing a hemolytic crisis. This is characterized by hemolysis, jaundice, methemoglobinemia, necrosis, and death (Johnston et al., 2014).

1.2.3 Manganese

1.2.3.1 Biological Function

Manganese functions within multiple metalloenzymes including pyruvate carboxylase, manganese superoxide dismutase, and glycosyltransferase (Andrieu, 2008). These manganese metalloenzymes are required for lipid and carbohydrate metabolism, protect cells from oxidative stress associated with inflammation and synthesis of mucopolysaccharides in cartilage, respectively (Andrieu, 2008). Manganese is one of the least abundant minerals in livestock tissues and the majority of manganese in the body is found within the soft tissues, skeleton (where it accumulates in the inorganic matrix of the bone), hair and liver (Hidiroglou, 1979a; Goff, 2018).

1.2.3.2 Absorption

The mechanism by which manganese is absorbed is not well described. In broiler chicks, manganese-specific transporter proteins have been found in the small intestine (Goff, 2018). In rats, divalent metal transporters are responsible for the movement of manganese across the apical membrane (Jenkitkasemwong et al., 2012). Once manganese is absorbed and removed from

circulation by liver, a portion of the available manganese is bound to transferrin and released into the blood circulation to be transported and utilized by body tissues (Goff, 2018). Manganese homeostasis is maintained by the liver; however, the liver has a limited capacity to mobilize manganese during deficiency (Hidiroglou, 1979a). Excess manganese is primarily released into bile and then into the feces, with little manganese being excreted in the urine (Bertinchamps et al., 1966; Papavasiliou et al., 1966).

1.2.3.3 Requirements

Manganese is required at 40 mg/kg of DM (NRC, 2001) for a mid-lactation Holstein cow. Manganese-deficient animals can be difficult to diagnose, since blood and plasma levels can be extremely variable and liver manganese concentrations can remain unchanged even when manganese intakes are low (Hidiroglou, 1979a). Long-term deficiencies in manganese can cause impaired growth, skeletal deformities, disturbed/deformed reproduction and abnormalities of newborns (Hidiroglou, 1979b; Goff, 2018). Toxicity is unlikely as negative effects, namely reduced feed intake and growth, appeared at 1000 mg/kg which has only been used under experimental conditions (Jenkins and Hidiroglou, 1991).

1.2.4 Selenium

1.2.4.1 Biological Function

Selenium is incorporated into selenoproteins which are used for a variety of functions within the mammalian body including: cell proliferation, calcium binding, metal detoxification, selenium transport, conversion of thyroxine to triiodothyronine, and as an antioxidant (Beckett and Arthur, 2005). The most well-known function of selenium is its role as a component of the enzyme glutathione peroxidase (GSHpx). Glutathione peroxidase is used as a cellular antioxidant by metabolizing hydrogen peroxide and a variety of organic peroxides to prevent oxidative

cellular damage (Arthur, 2000). This enzyme is also involved with the metabolism of arachidonic acid, which aids in increasing the killing ability of neutrophils and has been shown to reduce the prevalence and severity of mastitis in lactating dairy cattle (Sordillo et al., 1997).

1.2.4.2 Absorption

In adult ruminants, selenium leaves the rumen in the microbial fraction (Koenig et al., 1991). Inorganic selenium, selenite, can be passively absorbed; however, selenate requires active absorption through a sodium-dependent transport system, which is the same pathway utilized by molybdate and sulfate (Vendeland et al., 1992). Therefore, molybdate and sulfate can be antagonistic to selenite absorption. Organic sources of selenium, such as selenomethionine and selenocysteine, utilize the same transcellular transporters as their amino acids, methionine and cysteine (Nickel et al., 2009). The liver processes all forms of selenium from the portal blood, however, accumulation of selenium in other tissues within the body depends on the form of selenium. These changes in tissue or blood selenium vary and take up to 50 days to adjust in dairy cows, and therefore, do not always accurately represent changes in selenium intake (Ortman and Pherson, 1997). Selenium can be excreted from the body through exhalation, urine, feces, bile and milk (Koenig et al.,1991; Ivancic and Weiss, 2001; Rowntree et al., 2004).

1.2.4.3 Requirements

Selenium is required at 0.3 mg/kg of DM for a mid-lactation Holstein cow (NRC, 2001). Sodium selenite and sodium selenate are the only two inorganic forms approved to be supplemented in diets. The levels supplemented are not to exceed 0.3mg/kg of DM in commercial diets (CFIA, 2018; USFDA, 2018). Other sources of dietary selenium include selenium enriched yeast. As cows consume increased levels of selenium, the concentration of

selenium increases in the milk. Urinary excretion is also dependent on the level of selenium intake (Ivancic and Weiss, 2001).

Concentration of selenium in plants is highly correlated with the selenium concentrations of the soil in which it is grown (Goff, 2018). As such, the levels of selenium vary greatly between regions in which feedstuffs are grown, and thus regions with soils low in selenium must supplement animals at a higher level. Selenium deficiencies can cause diseases such as White Muscle Disease, also known as nutritional muscular dystrophy. The characteristics of this disease include leg weakness and stiffness, flexion of hock joints, muscle tremors, chalky striations of cardiac muscles, and necrosis. Animals with this condition usually die as a result of heart failure (Mehdi and Dufrasne, 2016). Deficiencies in selenium can also cause loss of milk fat yield in lactating dairy cattle and can compromise components of the immune system (Fraser et al., 1987; Wu et al., 2016). Alkali disease and blind staggers are two conditions representative of selenium toxicity. Signs of these conditions include sloughing of hooves, loss of hair and emaciation (Mehdi and Dufrasne, 2016).

1.2.5 Zinc

1.2.5.1 Biological Function

The functions of zinc are numerous, as it is required for the structure and function of thousands of transcription factors involved in almost every metabolic pathway (Salgueiro et al., 2000). Zinc is an important component of metalloenzymes, including copper-zinc superoxide dismutase, carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase and RNA and DNA polymerases which affects metabolism of carbohydrates, proteins, lipids and nucleic acids (Ruckenbusch and Thivend, 1980). However, these are just a small number of the

metalloenzymes zinc is involved in. Many of zinc's functions include gene expression, appetite control, fat absorption and antioxidant defense (Salgueiro et al., 2000).

1.2.5.2 Absorption

Absorption of zinc occurs mainly in the small intestine. The amount of zinc absorbed by the intestinal cells is determined through the up- or down-regulation of metallothionein in the mucosal enterocytes with intestinal metallothionein synthesis being induced in the presence of zinc (Odell, 2009). In rats, zinc enters the enterocytes of the small intestine and is transported across the cell using a cysteine-rich intestinal protein (CRIP) (Odell, 2009). Once transported across the cell using CRIP, zinc is released into the portal circulation where it is carried by transferrin. For animals adequate in zinc status, metallothionein is high and competes with the CRIP for the zinc transported into the cell. Zinc binds to metallothionein and remains inside the enterocyte until the cell is sloughed and the zinc is excreted in the feces (Odell, 2009). When animals are low in zinc, metallothionein is down-regulated in mucosal enterocytes. This decrease in metallothionein can take days to weeks to adjust in low zinc diets (Ordell, 2009). There is little retention of zinc within the body and retention is related directly to the amount of zinc absorbed. Zinc is excreted via pancreatic secretions and through the feces and only a small amount of zinc is excreted through the urine (Miller, 1970).

1.2.5.3 Requirements

Zinc is required at 63 mg/kg of DM in the diet for a mid-lactation Holstein cow (NRC, 2001). Adult ruminants absorb approximately 20% of the zinc that is provided in the diet and zinc status can be well described using plasma or urine concentrations of metallothionein. Zinc deficiency is observed acutely in cattle through reduced feed intake and growth rate with long term deficiencies characterized by parakeratosis of the skin on the legs, head and neck, skeletal

disorders, emaciation, and reproductive disorders (Ammerman et al., 1995; NRC, 2001). High concentrations of zinc are well-tolerated by cattle and there are currently no signs of toxicity documented (NRC, 2001).

1.2.6 Iodine

1.2.6.1 Biological Function

Iodine's only known vital function is a component of thyroid hormones. Iodine is required for the synthesis of the two thyroid hormones, thyroxine and triiodothyronine, which regulate energy metabolism within the body (Panneels et al., 2009). These hormones play a thermoregulatory role in cellular respiration, energy production, metabolism, growth, muscle function, immune defense and circulation (Panneels et al., 2009).

1.2.6.2 Absorption

A large percentage (80-90%) of dietary iodine is absorbed (Miller et al., 1988). When an animal is adequate in iodine, less than 20% of the iodine is taken up by the thyroid gland (Sorenson, 1962), while in times of iodine deficiency this value can increase to 65% (Lengemann and Swanson, 1957). Iodine that is not absorbed by the thyroid gland is excreted in the urine and milk (Miller et al., 1988). This mineral is absorbed efficiently from the GIT and allows for any iodine excreted via the abomasum to be recycled (Miller et al., 1988).

1.2.6.3 Requirements

Iodine is required at 0.45 mg/kg of DM in the diet for a mid-lactation Holstein cow (NRC, 2001). Deficiency in iodine reduces the production of thyroid hormones and is characterized by an enlargement of the thyroid glands, decreased fertility in both males and females, increased morbidity and low milk yield (Hill, 1991). Toxicity can occur in cattle as

well, causing hyperthyroidism, excessive nasal discharge and salivation, a decrease in milk production, coughing, and dry, scaly coats (Olson et al., 1984).

1.2.7 Iron

1.2.7.1 Biological Function

Iron is used as a cofactor in a multitude of enzymes including: those found in the electron transport chain, ferredoxin, myeloperoxidase, catalase, cytochrome oxidase, and cytochrome P-450 (Goff, 2018). The primary function of iron is a component of heme, which is found in both hemoglobin and myoglobin and is used in the transportation and release of oxygen to tissues via arterial blood and the return of carbon dioxide via venous circulation (Morris, 1987).

1.2.7.2 Absorption

In rats, the majority of iron within the diet exists in the ferric form (Fe^{+3}), however, this form is poorly absorbed by the intestinal tract (Wollenberg and Rummel, 1987). Only some of the ferric forms of iron will be converted to the ferrous form (Fe^{+2}) via a reaction with the acid within the abomasum (Wollenberg and Rummel, 1987). In the intestine in mammals, iron is absorbed and enters the cell by a divalent metal transporter (DMT1) in the brush border of the enterocyte (Garrick et al., 2003). If iron is required by the body, it is transported in the cell to the basolateral membrane and is bound to transferrin in the blood to be transported (Goff, 2018). If the iron status of the animal is adequate, iron does not enter the basolateral membrane but instead binds to ferritin, a protein that is produced by enterocytes in time of adequate iron status. This iron is sloughed with the turnover of enterocytes and is lost via the feces (Beard and Dawson, 1987).

1.2.7.3 Requirements

Iron is required at 24 mg/kg of DM in the diet for a mid-lactation Holstein cow and most of this requirement is met through the intake of feedstuffs (NRC, 2001). Iron deficiency can lead to morbidity and mortality due to a depressed immune response and can also lead to anemia as a result of the inability to produce hemoglobin (Mollerberg and Moreno-Lopez, 1975). Iron deficiency is rare in adult cattle as iron is found throughout the environment and a high number of forages contain adequate amounts (Spears, 2003). Excess iron becomes an issue as it can interfere with the absorption of other minerals, mainly copper and zinc (Bremner et al., 1987; Phillippo et al., 1987). Free iron may increase in tissues if the level of iron in the diet exceeds the binding capacity of transferrin and lactoferrin and this highly reactive form causes oxidative stress leading to an increased requirement for antioxidants (Halliwell, 1987). Toxicity of iron is characterized by diarrhea, reduced feed intake and weight gain (Miller, 1981).

1.2.8 Molybdenum

1.2.8.1 Biological Function

Molybdenum is used as a component in sulfide oxidase, aldehyde oxidase and xanthine oxidase as well as enzymes in milk and tissues (Mills and Davis, 1987). The evidence of molybdenum's involvement in these enzymes suggests its importance as an essential trace mineral in the diet (Mills and Bremner, 1980). Overall, the main functions of molybdenum are not well-defined and its main focus is the antagonistic interactions it can have with copper.

1.2.8.2 Absorption

Molybdenum absorption occurs by an active carrier mediated process in the intestinal mucosa which is shared and inhibited by sulfate (Mason and Cardin, 1977). The absorbed molybdenum is transported via the plasma in the free state but is later stored in tissues as molybdopterin in the cytosol (bound to xanthine dehydrogenase and aldehyde oxidase) or in the

mitochondria (bound to sulfide oxidase) in mammals (Johnson, 1997). Excess molybdenum is excreted into the urine via the kidneys (Bishara and Bray, 1978).

1.2.8.3 Requirements

A requirement for molybdenum does not exist, however is suggested to not exceed 10 mg/kg of DM in the diet for mid-lactation Holstein cows. (NRC, 2001). Supplementation is not recommended as the requirement is commonly met with most feedstuffs (NRC, 2001). Molybdenum is antagonistic to copper absorption and toxicity resembles that of copper deficiency (Goff, 2018). Molybdenum interacts with sulfate to create thiomolybdate complexes that have a high affinity for binding copper, therefore rendering copper unavailable for absorption. Deficiencies in molybdenum have been difficult to observe.

1.3 Sources of Trace Mineral Supplementation

1.3.1 Inorganic Trace Minerals

Inorganic trace minerals have been and continue to be the most common source of mineral supplemented in dairy cow rations. The conventional method of supplementation is incorporating inorganic trace minerals as salts (oxides, sulfates and carbonates) (NRC, 2001). The utilization of these inorganic sources by ruminants is not well understood and there are many dietary factors that influence the bioavailability for absorption in the GIT (Spears, 2003). With the dissociation of salts, trace minerals may interact with other feed components to create non-absorbable compounds or interactions among minerals may create chemical reduction into insoluble elements unavailable for absorption. There are also antagonistic interactions between minerals, through competition for cellular transporters and utilization in metabolic pathways which can reduce absorption and retention, leading to increased excretion. To counteract these effects, there is often an increase in supplementation well above the recommended levels,

although this does not eliminate antagonistic interactions. This increase in supplementation can lead to increased excretion of minerals into the environment and could lead to toxicity of the rumen microorganisms or in extreme cases, the animal itself (Genther and Hansen, 2015).

The solubility and bioavailability of inorganic sources of individual trace minerals have been investigated. Inorganic cobalt sources need to be readily soluble in the rumen to be of value to rumen microbes through their availability for absorption. Cobaltous and cobaltic oxides are less soluble compared to cobalt (II) carbonate and cobalt (II) sulfate within the rumen (Kawashima et al., 1997a; Kawashima et al., 1997b). Inorganic sources with higher solubility increase liver cobalt and vitamin B_{12} synthesis by rumen microbes (Kawashima et al., 1997a; Kawashima et al., 1997b). As for selenium, differences between selenate and selenite are not extensively studied in the literature. There is some information suggesting that selenate had a slight advantage in increasing selenium serum concentrations in dairy cattle (Podoll et al., 1992); however, Galibrath et al. (2016) found no difference in the incorporation of selenite and selenate to rumen microorganisms. As for inorganic manganese, manganese sulfate is the most common and most soluble inorganic form of manganese supplemented into the diet of dairy cattle (NRC, 2001). Manganese carbonate, manganese dioxide and manganese monoxide can also be supplemented, however, all three are less efficiently absorbed compared to manganese sulfate in chicks (Ammerman et al., 1995). As shown above, there is a large variation in the results comparing the individual minerals and their inorganic sources and more research is needed to investigate the best form of inorganic trace minerals to be supplemented in the diet of dairy cattle.

1.3.2 Organic Trace Minerals

Recently, there has been an increasing interest in substituting inorganic trace minerals with organic sources in dairy rations in the forms of amino acid chelates, metal complexes, metal methionine hydroxy-analog chelates, metal proteinates and metal propionates (Bach et al., 2015). A chelate is defined as a metal compound (mineral) that is bound with an organic molecule (ligand) with either one or two points (Figure 1-1). The ligand of the chelated molecule is what changes the inorganic mineral into an organic mineral. These organic sources have been shown to increase bioavailability and absorption by the ruminant and could allow for lower inclusion levels in dairy ration to achieve similar absorption and bioavailability compared to inorganic sources (Brown and Zeringue, 1994; Nemec et al., 2012).

Despite the limited information regarding feeding organic trace minerals to dairy cows, recent studies have shown positive results with organic supplementation. This has generated industry interest in the supplementation of organic trace mineral sources and their viability. Partial replacement of inorganic trace minerals by organic sources, and topping up diets with organic sources, is already common practice on most Canadian dairy operations. Fundamental bioavailability research to determine physiological responses and performance of dairy cattle is needed to confirm the benefits of organic trace mineral supplementation. This information would increase nutritionist and producer confidence in the products available and is necessary for a complete substitution of inorganic trace minerals by organic sources.

Recently, a partial replacement of inorganic trace minerals by organic sources supplemented via oral bolus has shown some benefits during the periparturient period on dry matter intake, milk production, and innate immunity in dairy cattle (Osorio et al., 2016). In another study, trace mineral absorption was also observed and found that mineral intake was reduced in an organic trace mineral treatment compared to an inorganic trace mineral treatment,

but there was no difference between treatments regarding excretion or blood plasma concentration (Pino and Heinrichs, 2016). These findings suggest a higher trace mineral absorption of organic sources compared to inorganic sources. In agreement with this, previous studies found that supplementation of organic sources of selenium to ruminants has been found to increase concentrations of selenium in both the blood and tissues as well as increase the activity of glutathione peroxidase compared to inorganic sources (Conrad and Moxon, 1979; Johansson et al., 1990; Nicholson et al., 1991). Similarly, supplementation with organic selenium yeast was found to be more effective at increasing whole blood and serum concentrations in sheep than its inorganic counterparts (sodium selenite and sodium selenate) (Hall et al., 2012). Similar results were found by Awadeh et al. (1998) where 12 crossbred beef cattle were fed diets consisting of inorganic selenite and selenate and selenized yeast. In this study, selenized yeast increased whole blood selenium concentrations, however, there was no difference in blood serum concentrations of selenium.

Organic copper proteinate has been found to be more available for absorption than sulfate sources, however information in the literature has been variable. A study conducted in lambs showed increased ceruloplasmin, red blood cell superoxide dismutase and mediated immune response in copper proteinate supplementation compared to copper sulfate, whereas plasma ceruloplasmin concentrations between organic and inorganic copper in beef cattle exhibited no difference (Arthington et al., 2003; Senthilkumar et al., 2008). Lower milk yield but a higher milk fat content with no difference in milk fat yield or milk protein was observed in dairy cattle fed organic versus inorganic copper. There was a trend for higher body weight in organic copper-supplemented cows than inorganic-supplemented. Overall, there was no difference between the two sources on plasma or hepatic copper concentrations (Sinclair et al., 2013).

Although the findings of organic trace mineral replacement have been relatively positive, there has been certain cases where no difference was observed between the two trace mineral types. For instance, Cortinhas et al. (2012) looked at organic versus inorganic trace minerals and did not detect any differences in milk yield and composition, plasma concentration, dry matter intake or body condition scores of dairy cattle. A recent study conducted by Faulkner et al. (2017) found no difference in apparent absorption between zinc sources in dairy heifers. However, this study had varying results regarding copper and manganese absorption depending on the diet fed. Cobalt specifically had no significant differences between sources in stimulating microbial vitamin B_{12} synthesis when observed in *vitro* and in *vivo* in sheep (Kawashima et al., 1997a; Kawashima et al., 1997b).

1.4 Techniques in Analyzing Trace Minerals

1.4.1 Wet Chemistry

Wet chemistry is a classical analytical method using where at least one substance is in the liquid phase (Otles and Ozyurt, 2014). This analysis involves basic experimentation techniques such as measuring, mixing, and weighing chemicals, conductivity, density, pH, specific gravity, and temperature in order to determine the presence of a specific chemical. It may also involve quantitative techniques such as gravimetry and titrimetry to determine weight or volume of a substance, respectively (Otles and Ozyurt, 2014). Minerals are commonly analyzed by commercial feed companies using wet chemistry using tirtrimetic procedures (Otles and Ozyurt, 2014).

1.4.2 Inductively Coupled Plasma-Mass Spectrometry

Inductively coupled plasma-mass spectrometry (ICP-MS) is an analytical technique that allows for the detection of minerals at extremely low detection limits while also having the

ability to detect minerals at high parts per million concentrations (Thomas, 2011). In brief from Thomas (2011), the ICP-MS analysis is performed by aerosolizing a liquid sample using argon gas. The fine droplets of the aerosol are separated from the larger droplets and transported into the plasma torch via the sample injector. When the droplets enter this area, positively charge ions are formed which then pass into the mass spectrometer via the interface region. Here, the ions are allowed to pass through to the main vacuum chamber which focuses the ion beam toward the mass separation device which prevents unwanted photons, particulates and neutral species from reaching the detector. Following this step, ions are converted into an electrical signal using the dynode detector where the signal is processed by the data handling system and converted into analyte concentration using ICP-MS calibrations standards.

1.5 Techniques in Measuring Mineral Absorption

1.5.1 Apparent Absorption

Apparent absorption is used in evaluating mineral elements and is defined as the total intake minus total fecal excretion (Ammerman et al., 1995). These values are most commonly expressed as a percentage of intake (Ammerman et al., 1995). The difference between intake and excretion represents the disappearance of the mineral from the GIT and does not take in to account any losses from minerals trapped in the mucosal cells or movement back into the GIT (Ammerman et al., 1995). This value is an approximate measure of the absorbability when the animal is in an adequate mineral balance and animals must not be absorbing minerals in times of deficiency or excreting the mineral when intakes are exceeding the requirement to obtain an accurate apparent absorption calculation (Suttle, 2010). Some disadvantages of apparent absorption arise when the GIT is one of the major pathways of excretion. The values of the apparent absorption will be lower than the true values as the feces will indicate a larger amount

of mineral excreted when in fact some has been absorbed and possibly utilized before returning to the GIT.

Apparent absorption is calculated by:

$$\frac{(\text{intake} - \text{feces})}{\text{intake}} \text{ x } 100\%$$

1.5.2 Net Retention

Net retention (apparent retention) is defined as the total intake minus the total excretion of feces, urine, and in lactating animals, milk (Ammerman et al., 1995; Suttle, 2010). This value can be useful in interpreting the retention of minerals; however, caution must be taken in using it to determine bioavailability as minerals excreted in urine may have been involved in biological processes before being excreted (Ammerman et al., 2015).

Retained trace element is calculated by:

$$\frac{(\text{intake} - \text{feces} - \text{urine} - \text{milk})}{\text{intake}} \text{ x } 100\%$$

1.5.3 True Absorption

True absorption is used to correct for the portion of the mineral which has been excreted back into the gastrointestinal tract after being previously absorbed by the body (Ammerman et al., 1995). This parameter accounts for the endogenous losses from the animal's body. These endogenous losses by definition include two fractions: minimal endogenous fraction and variable endogenous fraction. The minimal endogenous fraction is the minimal loss from the animal's body, whereas the variable endogenous fraction is the portion that may not have been involved in functions within the body (ARC, 1980; Ammerman et al., 1995). The variable fraction can be influenced by intake and the bioavailability of the mineral. Although these two fractions exist, it

is not possible to chemically separate them. The endogenous loses in the fecal excretion can be estimated by the use of appropriate radioisotopes or stable isotopes for a specific mineral (Suttle, 2010). Total endogenous fecal excretion is determined by dividing the specific activity of the radioisotope in the feces by the specific activity of the radioisotope of the plasma and multiplying the result by the total fecal excretion (Ammerman et al., 1995). In order for isotopic markers to give an accurate estimation of the true absorption, it must closely resemble the physiological and biochemical properties of the mineral forms present in the feed and must not disturb the normal metabolism of the animal. Providing a highly accessible and soluble mineral in an isotopic form compared to its lower accessible counterpart present in the feed can lead to the over estimation of true absorption (Buckley, 1988). Careful consideration and adequate research must be completed in order to determine the appropriate isotopic markers to accurately determine true absorption. The values of true absorption are greater than those of apparent absorption and true absorption gives a more accurate estimate of the amount of mineral absorbed and available to body tissues for metabolic purposes (Ammerman et al., 1995).

True absorption is calculated by:

$$\frac{(\text{intake} - [\text{total fecal excretion} - \text{total endogenous excretion}])}{\text{intake}} \text{ x } 100\%$$

1.6 Trace Minerals and Rumen Fermentation

Rumen microbes require an optimum supply of minerals for normal growth and function. Minerals are derived from both feeds and forages provided by plant-based ingredients and can also be consumed through the soil. Minerals are released/solubilized in the rumen and are either absorbed or move out of the rumen with digesta. They can also be transported into microbial cells, depending on intracellular or metabolic requirements of the microbes. Cells cannot

passively transport anions or cations across the cell membrane and specific transport systems will be required for each mineral anion or cation needed for growth or metabolic processes. Microbes may also have transport systems for excreting excess ions which are not needed for growth at that time (Mackie and Gilchrist, 1984).

However, there is a lack of data reporting the transport of anions and cations in ruminal bacteria and most information is derived from other prokaryotes (Mackie and Gilchrist, 1984). Transport systems are controlled by the intracellular requirements for certain mineral anions and cations to be utilized for enzymes and metabolic processes within the microorganism, as well as the abundance of that ion from the environment and the availability of the mineral ions. There are highly specific transport systems that have high affinities and high substrate specificity during conditions where growth is limited by the availability of trace minerals (Mackie and Gilchrist, 1984). These will include regulatory feedback control mechanisms that will protect the cell from toxicity in the event that the external environment is high in these trace minerals (Mackie and Gilchrist, 1984).

There are few studies looking at the differences in the ruminal effects of organic versus inorganic trace mineral sources. One study showed organic sources of trace minerals have increased the production of volatile fatty acids (VFA) and decreased rumen pH as compared to inorganic sources (Pino and Heinrichs, 2016). Butyrate levels were significantly higher in treatments containing organic sources and were numerically higher for acetate within the first 8 hours after feeding (Pino and Heinrichs, 2016). There is speculation that organic trace minerals are used most efficiently by amylolytic bacteria and cellulolytic bacteria within the first 8 hours after feeding, however, specific microbial populations responsible for these changes have not yet been identified (Kljak et al., 2017). Other studies in *vitro* have found that increased levels of

inorganic zinc decrease cellulose digestion within the first 24h without a change in total bacteria numbers (Eryavuz and Dehority, 2009). Again, no specific microbial communities were investigated.

Microbial populations have begun to be studied more in depth. Organic trace mineral supplementation led to decreased rumen pH and increased total VFA production, which is believed to be a result of higher bioavailability of the organic trace minerals and increased utilization of rumen microbes (Pino and Heinrichs, 2016). This increased utilization of organic trace minerals is suggested to be from the accelerated replication of rumen microorganisms that stimulate ruminal fermentation and VFA production (Pino and Heinrichs, 2016). Recent studies have begun to look into the differences between organic and inorganic sources of trace minerals and their effects on the changes in microbial populations of the rumen. Effects of a high dose of manganese in both organic and inorganic form was studied in lambs (Kišidayová et al., 2018). At these high doses, there was a significant decrease in the diversity of the rumen microbial population with the organic manganese treatment compared to inorganic. Although there was a change in the diversity of rumen microbes, there was no significant difference in microbial counts between the two treatments (Kišidayová et al., 2018).

Rumen microorganisms were investigated ex *vivo* by Galbraith et al. (2015), which determined their microbial incorporation of two forms of inorganic selenium (sodium selenite and sodium selenate) and organic selenium. The results concluded that there was no difference in the incorporation of two inorganic forms of selenium, but they did find a significant difference in the incorporation of the organic selenium. The formation of elemental selenium was also measured, and results were consistent with those above. Elemental selenium was found to be higher in both inorganic forms, with no difference between sodium selenite and sodium selenate.

Organic selenium was significantly lower in elemental selenium as compared to the two inorganic sources. These results support the hypothesis that inorganic selenium is less bioavailable to rumen microorganisms than its organic counterpart (Galbraith et al., 2015).

An in *vitro* study was conducted by Martinez and Church (1970) to look at the effects of varying concentrations of inorganic trace minerals on cellulose digestion in the rumen. Each mineral was tested individually, and the results indicated a stimulatory effect on cellulose digestion levels of copper at 3 ppm, manganese at 5-30 ppm and zinc at 5-7 ppm. However, inhibitory effects on digestion occurred at different levels in each trace mineral. Copper became inhibitory at 1 ppm, selenium and cobalt at 7 ppm, zinc at 20 ppm and manganese at 100 ppm (Martinez and Church, 1970).

The substitution of inorganic trace minerals by organic sources has the potential to influence specific communities in the rumen microbiome. Overall, the impact of organic trace minerals in the rumen microbiome and fermentations is not well explored. Studies have shown that trace mineral supplements with distinct solubility and bioavailability in the rumen can affect the utilization of trace minerals by rumen microbes, the digestibility of the diet, production of VFA used as energy by the cow and pH of the rumen, and the overall feed intake (Genther and Hansen, 2015; Galbraith et al., 2016; Pino and Heinrichs, 2016; Osorio et al., 2016). The effects on rumen microbes could be the explanation for differences in animal performance that cannot be explained by analytic measures of the mineral status of cows.

1.7 Knowledge Gap

Fundamental knowledge of trace mineral nutrition and absorption is limited with the literature lacking basic information regarding trace minerals for lactating dairy cattle. Literature focuses on beef cattle breeds or dairy heifers, however with the metabolic demands of lactation,

dairy cows need to be looked at specifically. Many of the calculated values and utilization assumptions are based on literature regarding other species and this does not allow for differences that may be observed in a ruminant animal compared to a monogastric species, as well as the source and nature of the trace mineral supplementation (Suttle, 2010). Due to the vast difference in GIT, these assumptions should be cautiously extrapolated to ruminants.

It is unknown how the source of trace minerals (organic versus inorganic) and the level supplemented into the diet relates to the NRC requirements as the literature suggests an increased bioavailability of organic trace minerals. This could allow for decreased supplementation of organic sources to achieve the same absorption as its inorganic counterpart. It is also unknown how the source and level of trace minerals affects rumen fermentation. The absorption and bioavailability of both inorganic and organic trace mineral sources on lactating dairy cows needs to be investigated further.

There is no fundamental information regarding the absorption of organic trace minerals on their own. Previous studies have supplemented organic sources at a level above the recommendation, above supplementation of inorganic trace minerals or with differing diets. There are many assumptions of how these organic sources of trace minerals are absorbed, however there is no literature stating whether these assumptions are true.

1.8 Hypothesis and Objectives

The overall hypothesis for this thesis is that organic sources of trace minerals will have a higher bioavailability, as they will be protected from most antagonistic interactions in the GIT, allowing for increased absorption and utilization for metabolic purposes and by rumen microbes at a lower concentration compared to inorganic sources. Therefore, the objectives of this thesis are to investigate the effects of source and concentration of supplemental trace minerals (organic

versus inorganic) in lactating dairy cows on: 1) apparent absorption and apparent retention, 2) performance, 3) rumen fermentation parameters, and 4) physiological indicators of trace mineral status such as vitamin B_{12} and GSH-Px. This thesis is one of few that investigates the overall effects of organic vs. inorganic trace minerals and the varying concentrations of supplementation on apparent absorption, rumen fermentation and physiological indicators of trace mineral status. This knowledge may be used in allowing for the proper substitution of inorganic trace minerals by organic sources. Additionally, it could allow more consistent and efficient supplementation of trace minerals, which has the potential to increase animal performance, as well as increase the profitability and sustainability of the dairy industry.

1.9 Literature Cited

Allen, J.D., and J.M. Gawthornet. 1987. Involvement of the solid phase of rumen digesta in the interaction between copper, molybdenum and sulphur in sheep. British Journal of Nutrition. 58:265–276. doi:10.1079/bjn19870094.

Ammerman, C.B., D.H. Baker, and A.J. Lewis. 1995. Bioavailability of nutrients for animals amino acids, minerals, and vitamins. San Diego: Academic Press.

Andrieu, S. 2008. Is there a role for organic trace element supplements in transition cow health? The Veterinary Journal,176:77-83. doi:10.1016/j.tvjl.2007.12.022

ARC. 1980. Agricultural Research Council. The Nutrient Requirements of Ruminant Livestock. Technical review by an agricultural research council working party, Common wealth Agricultural Bureau, Farnham Royal, UK.

Arthington, J.D., F.M. Pate, and J.W. Spears. 2003. Effect of copper source and level on performance and copper status of cattle consuming molasses-based supplements. Journal of Animal Science. 81:1357–1362. doi:10.2527/2003.8161357x.

Arthur, J.R. 2000. The glutathione peroxidases. Cellular and Molecular Life Sciences. 57:1825-1835.

Awadeh, F., M. Abdelrahman, R. Kincaid, and J. Finley. 1998. Effect of selenium supplements on the distribution of selenium among serum proteins in cattle. Journal of Dairy Science. 81:1089–1094. doi:10.3168/jds.s0022-0302(98)75670-x.

Bach, A., A. Pinto, and M. Blanch. 2015. Association between chelated trace mineral supplementation and milk yield, reproductive performance, and lameness in dairy cattle. Livestock Science, 182:69-75. doi:10.1016/j.livsci.2015.10.023

Beard, J.L., and H.D. Dawson. 1997. Iron. Pp. 278–284 in Handbook of Nutritionally Essential Mineral Elements, B. L. O'Dell and R. A. Sunde, eds. New York: Marcel Dekker, Inc.

Beckett, G.J., and J.R. Arthur. 2005. Selenium and endocrine systems. Journal of Endocrinology. 184:455–465. doi:10.1677/joe.1.05971.

Bertinato, J., and M.R. Labbé. 2004. Maintaining copper homeostasis: regulation of copper-trafficking proteins in response to copper deficiency or overload. The Journal of Nutritional Biochemistry. 15:316–322. doi:10.1016/j.jnutbio.2004.02.004.

Bertinchamps, A., S. Miller, and G. Cotzias. 1966. Interdependence of routes excreting manganese. American Journal of Physiology-Legacy Content. 211:217–224. doi:10.1152/ajplegacy.1966.211.1.217.

Bird, P.R. 1970. Sulphur metabolism and excretion studies in ruminants. III. The effect of sulphur intake on the availability of copper in sheep. Proceedings of the Australian Society of Animal Production 8:212–218

Bishara, H.N. and A.C. Bray. 1978. Competition between molybdate and sulfate for renal tubular reabsorption in sheep. Proceedings of the Australian Society of Animal Production 12, 123.

Bremner, I., W.R. Humphries, M. Phillippo, M.J. Walker, and P.C. Morrice. 1987. Iron-induced copper deficiency in calves: Dose- response relationships and interactions with molybdenum and sulfur. Animal Production Science. 45:403–414.

Brown, T.F., and L.K. Zeringue. 1994. Laboratory evaluations of solubility and structural integrity of complexed and chelated trace mineral supplements. Journal of Dairy Science. 77:181-189.

Buckley, W.T. 1988. The use of stable isotopes in studies of mineral metabolism. Proceedings of the Nutrition Society. 47:407–416. doi:10.1079/pns19880059.

Conrad, H.R., and A.L. Moxon. 1979. Transfer of dietary selenium to milk. Journal of Dairy Science. 62:404–411.

Cortinhas, C.S., J.E.D.F. Júnior, J.D.R. Naves, M.A.D.F. Porcionato, L.F.P.E. Silva, F.P. Rennó, and M.V.D. Santos. 2012. Organic and inorganic sources of zinc, copper and selenium in diets for dairy cows: intake, blood metabolic profile, milk yield and composition. Revista Brasileira de Zootecnia. 41:1477–1483. doi:10.1590/s1516-35982012000600023.

Drackley, J.K., S.S. Donkin, and C.K. Reynolds. 2006. Major advances in fundamental dairy cattle nutrition. Journal of Dairy Science. 89:1324-1336.

Ely, R.E., K.M. Dunn, and C.F. Huffman. 1948. Cobalt toxicity in calves resulting from high oral administration. Journal of Animal Science. 7:239–243.

Eryavuz, A., and B.A. Dehority. 2009. Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers in vitro. Animal Feed Science and Technology. 151:175–183. doi:10.1016/j.anifeedsci.2009.01.008.

Faulkner, M., N. St-Pierre, and W. Weiss. 2017. Effect of source of trace minerals in either forage- or by-product–based diets fed to dairy cows: 2. Apparent absorption and retention of minerals. Journal of Dairy Science. 100:5368–5377. doi:10.3168/jds.2016-12096.

Fischer, P.W., A. Giroux, and M.R. Labbé. 1981. The effect of dietary zinc on intestinal copper absorption. The American Journal of Clinical Nutrition. 34:1670–1675. doi:10.1093/ajcn/34.9.1670.

Fischer, P.W.F., A. Giroux, and M.R. Labbe. 1983. Effects of zinc on mucosal copper binding and on the kinetics of copper absorption. The Journal of Nutrition. 113:462–469. doi:10.1093/jn/113.2.462.

Fraser, A.J., T.J. Ryan, R. Sproule, R.G. Clark, D. Anderson, and E.O. Pederson. 1987. The effect of selenium on milk production in dairy cattle. Proceedings of the New Zealand Society of Animal Production. 47: 61–64.

Galbraith, M.L., W.R. Vorachek, C.T. Estill, P.D. Whanger, G. Bobe, T.Z. Davis, and J.A. Hall. 2015. Rumen microorganisms decrease bioavailability of inorganic selenium supplements. Biological Trace Element Research. 171:338–343. doi:10.1007/s12011-015-0560-8

Galbraith, M.L., W.R. Vorachek, C.T. Estill, P.D. Whanger, G. Bobe, T.Z. Davis, and J.A. Hall. 2016. Rumen microorganisms decrease bioavailability of inorganic selenium supplements. Biological Trace Element Research. 171:338-343.

Garrick, M.D., K.G. Dolan, C. Horbinski, A.J. Ghoio, D. Higgins, M. Porcubin, E.G. Moore, L.N. Hainsworth, J.N. Umbreit, M.E. Conrad, L. Feng, A. Lis, J.A. Roth, S. Singleton,

and L.M. Garrick. 2003. DMT 1: a mammalian transporter for multiple metals. Biometals 16:41–54.

Genther, O.N., and S.L. Hansen. 2015. The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. Journal of Dairy Science. 98:566-573.

Gibbons, R., S. Dixon, K. Hallis, A. Russell, B. Sansom, and H. Symonds. 1976. Manganese metabolism in cows and goats. Biochimica et Biophysica Acta (BBA) - General Subjects. 444:1–10. doi:10.1016/0304-4165(76)90218-x.

Goff, J.P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of Dairy Science. 101:2763-2813. doi:10.3168/jds.2017-13112

Gooneratne, S.R., W.T. Buckley, and D.A. Christensen. 1989. Review of copper deficiency and metabolism in ruminants. Canadian Journal of Animal Science. 69:819–845. doi:10.4141/cjas89-096.

Government of Canada, (CFIA) Canadian Food Inspection Agency, and Animal Health Directorate. (2017, October 24). Chapter 4 – Labelling and Guarantees. Retrieved from http://www.inspection.gc.ca/animals/feeds/regulatory-guidance/rg-1/chapter-4/eng/1329341411340/1329341520337?chap=6

Hall, J.A., R.J. Van Saun, G. Bobe, W.C. Stewart, W.R. Vorachek, W.D. Mosher, T. Nichols, N.E. Forsberg, and G.J. Pirelli. 2012. Organic and inorganic selenium: I. Oral bioavailability in ewes. Journal of Animal Science. 90: 568–576. doi:10.2527/jas.2011-4075.

Halliwell, B. 1987. Oxidants and human disease: some new concepts. The FASEB Journal. 1:358–364. doi:10.1096/fasebj.1.5.2824268.

Hansen, S.L., N. Trakooljul, H.-C.S. Liu, J.A. Hicks, M.S. Ashwell, and J.W. Spears. 2010. Proteins involved in iron metabolism in beef cattle are affected by copper deficiency in combination with high dietary manganese, but not by copper deficiency alone1. Journal of Animal Science. 88:275–283. doi:10.2527/jas.2009-1846.

Hidiroglou, M. 1979a. Manganese in ruminant nutrition. Canadian Journal of Animal Science. 59:217–236. doi:10.4141/cjas79-028.

Hidiroglou, M. 1979b. Trace element deficiencies and fertility in ruminants: A review. Journal of Dairy Science. 62:1195–1206. doi:10.3168/jds.s0022-0302(79)83400-1.

Hill, R. 1991 Rapeseed meal in the diet of ruminants. Nutrition Abstracts and Reviews, Series B 61:139–155.

Ivancic, J., and W. Weiss. 2001. Effect of dietary sulfur and selenium concentrations on selenium balance of lactating Holstein cows. Journal of Dairy Science. 84:225–232. doi:10.3168/jds.s0022-0302(01)74472-4.

Jenkins, K.J., and M. Hidiroglou. 1991. Tolerance of the preruminant calf for excess manganese or zinc in milk replacer. Journal of Dairy Science. 74:1047–1053.

Jenkitkasemwong, S., C.Y. Wang, B. Mackenzie, and M.D. Knutson. 2012. Physiologic implications of metal-ion transport by ZIP14 and ZIP8. Biometals 25:643–655.

Johansson, E., S.O. Jacobsson, J. Luthman, and U. Lindh. 1990. The biological response of selenium in individual erythrocytes and GSH- px in lambs fed sodium selenite or selenium yeast. Journal of the American Veterinary Medical Association. 37:463–470.

Johnson, J.L. 1997. Molybdenum. In: O'Dell, B.L. and Sunde, R.A. (eds) Handbook of Nutritionally Essential Mineral Elements. Marcel Dekker, New York, pp. 413–438.

Johnston, H., L. Beasley, and N. MacPherson. 2014. Copper toxicity in a New Zealand dairy herd. Irish Veterinary Journal. 67:20–24.

Kawashima, T., P. Henry, D. Bates, C. Ammerman, R. Littell, and J. Price. 1997a. Bioavailability of cobalt sources for ruminants. 2. Estimation of the relative value of reagent grade and feed grade cobalt sources from tissue cobalt accumulation and vitamin B_{12} concentrations. Nutrition Research. 17:957-974. doi:10.1016/s0271-5317(97)00061-4

Kawashima, T., P. Henry, D. Bates, C. Ammerman, R. Littell, and J. Price. 1997b. Bioavailability of cobalt sources for ruminants. 3. In vitro ruminal production of vitamin B_{12} and total corrinoids in response to different cobalt sources and concentrations. Nutrition Research. 17:975-987. doi:10.1016/s0271-5317(97)00062-6

Keener, H.A., G.P. Percival, and K.S. Marrow. 1949. Cobalt tolerance in young dairy cattle. Journal of Dairy Science. 32:527.

Kennedy, D.G., S. Kennedy, and P.B. Young. 1996. Effects of low concentrations of dietary cobalt on rumen succinate concentration in sheep. International Journal of Vitamin Research. 66:86–92

Kennedy, D.G., S. Kennedy, and P.B. Young. 1996. Effects of low concentrations of dietary cobalt on rumen succinate concentration in sheep. International Journal of Vitamin Nutrition Research. 66:86–92.

Kišidayová, S., P. Pristaš, M. Zimovčáková, M.B. Wencelová, L. Homolová, K. Mihaliková, K. Čobanová, Ľ. Grešáková, and Z. Váradyová. 2018. The effects of high dose of two manganese supplements (organic and inorganic) on the rumen microbial ecosystem. Plos One. 13. doi:10.1371/journal.pone.0191158.

Kljak, K., F. Pino, K.J. Harvatine, and A.J. Heinrichs. 2017. Analysis of selected rumen microbial populations in dairy heifers limit fed diets varying in trace mineral form and starch content. Livestock Science. 198:93–96. doi:10.1016/j.livsci.2017.02.012.

Koenig, K.M., W.T. Buckley, and J.A. Shelford. 1991. True absorption of selenium in dairy, cows: Stable isotope tracer methodology and effect of dietary copper. Canadian Journal of Animal Science. 71:175-183.

Lee, J., J.R. Prohaska, and D.J. Thiele. 2001. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. Proceedings of the National Academy of Sciences. 98:6842-6847. doi:10.1073/pnas.111058698

Lengemann, F.W., and E.W. Swanson. 1957. A study of the secretion of iodine in milk of dairy cows, using daily oral doses of ^{131}I. J. Dairy Sci. 40:215–222.

Lopez-Guisa, J.M., and L.D. Satter. 1992. Effect of copper and cobalt addition on digestion and growth in heifers fed diets containing alfalfa silage or corn crop residues. Journal of Dairy Science. 75:247–256.

Mackie, R.I., and F.M.C. Gilchrist. “Herbivore nutrition in the subtropics and tropics.” The Science Press, Herbivore Nutrition in the Subtropics and Tropics, 1984. Influence of mineral interactions on growth efficiency of rumen bacteria. R.I. Mackie and J.J. Therion. Pages 455-477.

Martinez, A., and D.C. Church. 1970. Effect of various mineral elements on in vitro rumen cellulose digestion. Journal of Animal Science. 31:982–990. doi:10.2527/jas1970.315982x

Mason, J. and C.J. Cardin. 1977. The competition of molybdate and sulfate ions for a transport system in the ovine small intestine. Research in Veterinary Science. 22:313–315.

McKay, E.J. and L.M. McLery. 1981. Location and secretion of gastric intrinsic factor in the sheep. Research in Veterinary Science. 30:261–265

Mehdi, Y., and I. Dufrasne. 2016. Selenium in cattle: A review. Molecules. 21:545. doi:10.3390/molecules21040545.

Miller, J.K., N. Ramsey, and F.C. Madsen. 1988. The trace elements. Pp. 342–400 in The Ruminant Animal: Digestive Physiology and Nutrition, D.C. Church, ed. Englewood Cliffs, NJ: Prentice-Hall, Inc.

Miller, W. 1970. Zinc nutrition of cattle: A review. Journal of Dairy Science. 53:1123–1135. doi:10.3168/jds.s0022-0302(70)86355-x.

Miller, W. 1981. Mineral and vitamin nutrition of dairy cattle. Journal of Dairy Science. 64:1196–1206. doi:10.3168/jds.s0022-0302(81)82696-3.

Mills, C.F., and I. Bremner. 1980. Nutritional Aspects of Molybdenum in Animals. M. P. Coughlan, ed. Molybdenum and molybdenum containing enzymes. Pergamon Press, Oxford England.

Mills, C.F., and G.K. Davis. 1987. Molybdenum. Pp. 429–463 in Trace Elements in Human and Animal Nutrition, Vol. 1, W. Mertz, ed. New York: Academic.

Minatel, L., and J.C. Carfagnini. 2000. Copper deficiency and immune response in ruminants. Nutrition Research. 20:1473–1484. doi:10.1016/s0271-5317(00)00236-0.

Mollerberg, L., and J. Moreno-Lopez. 1975. The response of normal and iron anemic calves to nasal infection with an attenuated strain of parainfluenza-3 virus. Acta Veterinaria Scandinavica. 16:186–196.

Morris, E. 1987. Iron. Pp. 79–142 in Trace Elements in Human and Animal Nutrition, Vol. 1, W. Mertz, ed. New York: Academic.

Nemec, L., J. Richards, C. Atwell, D. Diaz, G. Zanton, and T. Gressley. 2012. Immune responses in lactating Holstein cows supplemented with Cu, Mn, and Zn as sulfates or methionine hydroxy analogue chelates. Journal of Dairy Science. 95:4568-4577. doi:10.3168/jds.2012-5404

Nicholson, J.W., R.E. McQueen, and R.S. Bush. 1991b. Response of growing cattle to supplementation with organically bound or inorganic sources of selenium or yeast cultures. Canadian Journal of Animal Science. 71:803–811.

Nickel, A., G. Kottra, G. Schmidt, J. Danier, T. Hofmann, and H. Daniel. 2009. Characteristics of transport of selenoamino acids by epithelial amino acid transporters. Chemico-Biological Interactions. 177:234-241. doi:10.1016/j.cbi.2008.09.008

NRC. 2001. Nutrient Requirements of Dairy Cows, 7th edn. National Academy of Sciences.

Odell, B.L. 2009. Cysteine-rich intestinal protein (CRIP): A new intestinal zinc transport protein. Nutrition Reviews. 50:232-233. doi:10.1111/j.1753-4887.1992.tb01334.x

Olson, W., J. Stevens, J. Anderson, and D.W. Haggard. 1984. Iodine toxicosis in six herds of dairy cattle. Journal of American Veterinary Medical Association. 184:179–179.

Ortman, K., and B. Pehrson. 1997. Selenite and selenium yeast as feed supplements for dairy cows. Journal of Veterinary Medicine Series A. 44:373–380. doi:10.1111/j.1439-0442.1997.tb01121.x.

Osorio, J.S., E. Trevisi, C. Li, J.K. Drackley, M.T. Socha, and J.J. Loor. 2016. Supplementing Zn, Mn, and Cu from amino acid complexes and Co from cobalt glucoheptonate during the peripartal period benefits postpartal cow performance and blood neutrophil function. Journal of Dairy Science. 99:1868- 1883.

Otles, S., and V.G. Ozyurt. 2014. Classical wet chemistry methods. Cheung, P.C.K, and B.M. Metha, ed. Handbook of Food Chemistry. Springer International Publishing, Cham.

Panneels V., G. Juvenal, J.M. Boeynaems, J.E. Dumont, and J. Van Sande. 2009. Iodide effects on the thyroid: biochemical, physiological, pharmacological, and clinical effects of iodide in the thyroid. Pp 303-314 in Comprehensive handbook of iodine: nutritional, biochemical, pathological and therapeutic aspects. Academic, Amsterdam.

Papavasiliou, P., S. Miller, and G. Cotzias. 1966. Role of liver in regulating distribution and excretion of manganese. American Journal of Physiology-Legacy Content. 211:211–216. doi:10.1152/ajplegacy.1966.211.1.211.

Paterson, J.E., and A. MacPherson. 1990. The influence of a low cobalt intake on the neutrophil function and severity of Ostertagia infection in cattle. British Veterinary Journal. 146:519–530.

Peña Maria M.O., J. Lee, and D.J. Thiele. 1999. A delicate balance: homeostatic control of copper uptake and distribution. The Journal of Nutrition. 129:1251–1260. doi:10.1093/jn/129.7.1251.

Phillippo, M., W.R. Humphries, and P.H. Garthwaite. 1987. The effect of dietary molybdenum and iron on copper status and growth in cattle. The Journal of Agricultural Science. 109:315. doi:10.1017/s0021859600080746.

Pino, F., and A. Heinrichs. 2016. Effect of trace minerals and starch on digestibility and rumen fermentation in diets for dairy heifers 1. Journal of Dairy Science. 99:2797–2810. doi:10.3168/jds.2015-10034

Podoll, K.L., J.B. Bernard, D.E. Ullrey, S.R. Debar, P.K. Ku, and W.T. Magee. 1992. Dietary selenate versus selenite for cattle, sheep, and horses. Journal of Animal Science. 70:1965-1970. doi:10.2527/1992.7061965x

Prohaska, J.R. 2012. Copper. In: Filer, L.J. and Ziegler, E.E. (eds) Present Knowledge in Nutrition, 10th edn. International Life Science Institute–Nutrition Foundation, Washington, DC.

Rowntree, J.E., G.M. Hill, D.R. Hawkins, J.E. Link, M.J. Rincker, G.W. Bednar, and R.A. Kreft. 2004. Effect of Se on selenoprotein activity and thyroid hormone metabolism in beef and dairy cows and calves. Journal of Animal Science. 82:2995–3005. doi:10.2527/2004.82102995x.

Ruckenbusch, Y., and P. Thivend. 1980. Digestive physiology and metabolism of ruminants. International Symposium on Ruminant Physiology. 3-7. Lancaster, Lancs. MTP Press.

Salgueiro, M.J., M. Zubillaga, A. Lysionek, M. I. Sarabia, R. Caro, T. De Paoli, A. Hager, and J. Boccio. 2000. Zinc as an essential micronutrient: A review. Nutrition Research. 20:737–755.

Saxena, K.K. and S.K. Ranjhan. 1978. Effect of cobalt and copper supplementation, separately and in combination, on the digestibility of organic nutrients and mineral balances in Hariana calves. Indian Journal of Animal Science. 48:566-571.

Seetharam, B., S. Bose, and N. Li. 1999. Cellular import of cobalamin (Vitamin B_{12}). The Journal of Nutrition. 129:1761–1764. doi:10.1093/jn/129.10.1761.

Senthilkumar, P., D. Nagalakshmi, Y.R. Reddy, and K. Sudhakar. 2008. Effect of different level and source of copper supplementation on immune response and copper dependent enzyme activity in lambs. Tropical Animal Health and Production. 41:645–653. doi:10.1007/s11250-008-9236-0.

Sinclair, L., K. Hart, D. Johnson, and A. Mackenzie. 2013. Effect of inorganic or organic copper fed without or with added sulfur and molybdenum on the performance, indicators of

copper status, and hepatic mRNA in dairy cows. Journal of Dairy Science. 96:4355–4367. doi:10.3168/jds.2012-6322.

Smith, R.M. 1987. Cobalt. in Trace Elements in Human Health and Disease, Vol. 1, W. Mertz, ed. San Diego: Academic Press.

Smith, R.M. 1997. Cobalt. Pp. 357–387 in Handbook of Nutritionally Essential Mineral Elements, B. O'Dell and R. Sunde, eds. New York: Marcel Dekker, Inc.

Smith, R.M., and L.H.R. Marston. 1970. Production, absorption, distribution and excretion of vitamin B_{12} in sheep. British Journal of Nutrition. 24:857–877. doi:10.1079/bjn19700092.

Sordillo, L.M., K. Shefer-Weaver, D. DeRosa. 1997. Immunobiology of the mammary gland. Journal of Dairy Science 80:1851–1865

Sorensen, P. 1962. Studies of thyroid function in cattle and pigs. Pg. 455 in Use of Radioisotopes in Animal Biology and Medical Sciences, Vol. 1. New York: Academic Press.

Spears, J.W. 1996. Optimizing mineral levels and sources for farm animals. E. T. Kornegay, ed. Nutrient Management of Food Animals to Enhance and Protect the Environment. CRC Press, Boca Raton, FL.

Spears, J.W. 2003. Trace mineral bioavailability in ruminants. The Journal of Nutrition. 133. doi:10.1093/jn/133.5.1506s.

Spears, J.W., and W.P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Veterinary Journal. 176:70-76.

Suttle, N.F. 2010. The Mineral Nutrition of Livestock. 4th edn. CAB International, Wallingford, UK.

U.S Food and Drug Administration (USDA). (n.d.) CFR – Code of Federal Regulations Title 21. Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.920

Underwood, E. J. 1981. The Mineral Nutrition of Livestock. 2nd Ed. Slough, England: Commonwealth Agricultural Bureaux.

Thomas, R. 2011. Practical guide to ICP-MS: a tutorial for beginners. CRC Press, Boca Raton.

van den Top, A.M. 2005. Reviews on the mineral provision in ruminants (X): cobalt metabolism and requirements in ruminants. Centraal Veevoederbureau.

Vendeland, S., J. Deagen, and P. Whanger. 1992. Uptake of selenotrisulfides of gluthathione and cysteine by brush border membranes from rat intestines. Journal of Inorganic Biochemistry. 47:131–140. doi:10.1016/0162-0134(92)84049-s.

Wapnir, R.A. 1998. Copper absorption and bioavailability. The American Journal of Clinical Nutrition. 67:1054-1060. doi:10.1093/ajcn/67.5.1054s

Wollenberg, P., and W. Rummel. 1987. Dependence of intestinal iron absorption on the valency state of iron. Naunyn-Schmiedebergs Archives of Pharmacology. 336:578-582. Doi:10.1007/bf00169317.

Wu, L., H. Zhang, C. Xu, and C. Xia. 2016. Critical thresholds of antioxidant and immune function parameters for Se deficiency prediction in dairy cows. Biological Trace Element Research. 172:320–325. Doi:10.1007/s12011-015-0606-y.

Wuerges, J., G. Garau, S. Geremia, S.N. Fedosov, T.E. Petersen, and L. Randaccio. 2006. Structural basis for mammalian vitamin B_{12} transport by transcobalamin. Proceedings of the National Academy of Sciences USA. 103:4386–4391.

Yatoo, M.I., A. Saxena, P.M. Deepa, B.P. Habeab, S. Devi, R.S. Jatav and U. Dimri. 2013. Role of trace elements in animals: a review, Veterinary World. 6: 963-967.

Young, R. 1979. Cobalt in biology and biochemistry. London: Academic Press.

1.10 Tables and Figures

Table 1-1. Specific trace mineral functions and involvement within the body (Suttle, 2010).

Function	Involvement
Structural	Organs and tissues
	Stability of molecules and membranes
Physiological	Electrolytes
	Osmotic pressure
	Acid-base balance
	Nerve impulse transmission
Catalytic	Enzymes
	Metalloenzymes
	Hormones
	Co-enzymes
	Antioxidants
Regulatory	Cell replication
	Cell differentiation

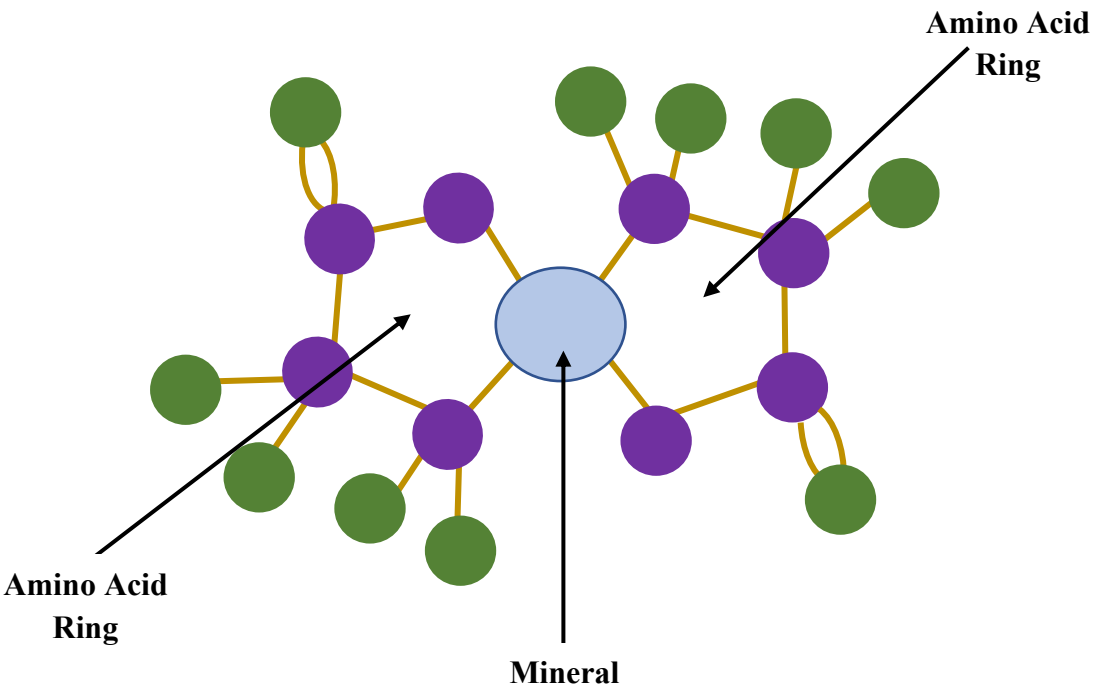

Figure 1-1. Diagram of a chelated mineral.

2.0 Chapter 2: Effect of source and concentration of supplemental trace minerals on performance, apparent absorption and apparent retention of trace minerals in lactating Holstein dairy cows

2.1 Abstract

Recently, there has been a shift from supplementing inorganic trace minerals to feeding their organic counterparts, which may have increased bioavailability for absorption. The objective of this study was to investigate the effect of source and concentration of supplemental trace minerals on productivity, apparent absorption and apparent retention of trace minerals in lactating Holstein dairy cows. Six lactating Holstein cows (129 ± 12 DIM; mid to late lactation) were used in a 6 x 6 Latin square design with a 28-d experimental period (23-d of adaptation and 5-d of sample collection). The same basal diet was fed daily, but using different sources (organic [ORG] versus inorganic [INO]) and concentrations (50%, 100%, and 200% based on NRC recommendations [defined as 50, 100, 200]) of supplemented trace minerals (Co, Cu, Mn, Se, and Zn). Over the 5-d data and sample collection period, feed intake, water intake, total milk, urine, and feces were collected daily. Results demonstrated that DMI, DM digestibility, milk fat, lactose yield and MUN did not differ between treatment or source of supplementation. Milk yield, milk fat and zinc apparent absorption were lower, and cobalt apparent absorption, cobalt apparent retention and milk CP percentage were higher in ORG supplementation than INO supplementation. Milk yield was lower in 200 ORG compared to 50 INO and 100 INO. Milk CP percentage was higher in 200 ORG than 50 INO. Total fecal excretion and total urine excretion did not differ between treatment or source of supplementation. Due to high fecal trace mineral excretion values, apparent absorption and retention values of Co, Cu, Mn, and Zn were highly negative which is physiologically abnormal. Higher apparent absorption was observed in 200

ORG than 50 INO and 100 INO. Selenium had higher apparent absorption in 200 INO and 200 ORG than 50 IN0 and 50 ORG. Cobalt had higher apparent retention in 200 ORG than 50 INO and 100 INO. Selenium had higher apparent retention in 200 INO and 200 ORG than 50 INO and 50 ORG. There was no difference in apparent absorption for Cu, Mn, and Zn and no difference in apparent retention in Cu and Mn. These findings suggest that source and source in combination with concentration have minimal impact on the apparent absorption and retention of trace minerals; however, more work on the validity of the negative absorption and retention results is warranted.

2.2 Introduction

Trace minerals are an important component of the lactating cow diet. Although they are required in minute quantities, they play a crucial role in metabolic functions throughout the body, including immune function, energy metabolism, cell signaling and oxidative balance (Machado et al., 2013; Maret, 2017 and Sun et al., 2019). Recommended levels of Co, Cu, Mn, Se, and Zn have been outlined by the National Research Council (NRC) for lactating dairy cows (NRC, 2001) and are based on inorganic trace mineral supplementation. However, there has been a recent shift from feeding inorganic trace minerals to feeding their organic counterparts through total replacement of inorganic by organic sources, or adding organic trace minerals on top of the inorganic minerals already supplemented in the diet.

There is currently a lack of information regarding the differences in how organic and inorganic sources of trace minerals are absorbed and the amount of mineral retained within the body. In mammals, inorganic trace minerals enter the small intestine and can dissociate, with their ions binding antagonistic metals and producing non-absorbable compounds that are excreted in the feces (Vinus, 2018). The ions themselves may also be excreted in the feces, yet a

portion of the minerals that remain as ions are absorbed through paracellular transport or through inorganic metal transporters into the enterocyte for use in biological processes (Goff, 2018). Minerals that are bound to metals that still allow for absorption are absorbed via transcellular transportation (Goff, 2018). It is speculated that the chelated (metal bound with an organic compound) organic trace minerals are less likely to dissociate and are therefore protected from most antagonistic interactions in the gastrointestinal tract. Organic trace minerals utilize an amino acid transporter in the small intestine for absorption, thus increasing their bioavailability (Vinus, 2017). Similar to inorganic minerals, chelated minerals may also be excreted in the feces. Supplementation concentrations of these minerals vary throughout the industry, with most producers over-supplementing in an attempt to avoid mineral deficiencies or avoid antagonistic reactions within the body. However, over-supplementation of trace minerals can lead to increased excretion via feces and urine, resulting in environmental contamination (Nicholson and Chambers, 2008; Castillo et al., 2013) and economic inefficiency. In extreme cases, over-supplementation of these minerals can lead to toxicity, which can be detrimental to a dairy herd (NRC, 2001).

In order to increase the sustainability and productivity of the dairy industry, knowledge regarding the accurate and efficient supplementation of inorganic and organic sources of trace minerals is required. It is hypothesized that low levels of organic trace mineral supplementation will increase absorption and retention due to their protection from antagonistic interactions within the gastrointestinal tract (GIT). This will ultimately increase bioavailability and production compared to high levels of inorganic trace mineral supplementation. Therefore, the objective of this study was to investigate the effect of source and concentration of supplemental

trace minerals on performance, apparent absorption and retention in lactating Holstein dairy cows.

2.3 Materials and Methods

The experiment was conducted at the Dairy Research and Technology Centre of the University of Alberta. The animal use protocol was approved by University of Alberta Livestock Care Committee (AUP 00002389) following the guidelines of the Canadian Council of Animal Care (CCAC, 2009).

2.3.1 Animal Experiment and Feeding

Six multiparous lactating Holstein cows beginning at 129 ± 12 DIM (mean ± SD), fitted with rumen cannulas (Bar Diamond Inc., Parma, ID; Rumen Cannula, Victoria, AUS) were utilized in a 6 x 6 Latin square design with 28 day periods. Specifically, each period consisted of a 23-d diet adaptation followed by 5-d of data and sample collection. Six biological replicates per treatment were determined from previous studies by Koenig et al. (1991) and Faulkner et al. (2017) to detect a 30% difference with 80% statistical power (Berndston, 1991). A total mixed ration (TMR; Table 2-1) was formulated to meet the nutrient requirements of a 675 kg cow producing 30 kg of milk per day (NRC, 2001) and was fed to cows as a basal diet for the duration of the study. Individual feed ingredients were tested for trace mineral levels and the TMR was designed to utilize ingredients low in trace mineral levels to ensure the basal diet contributed less than 20% of the NRC recommendations (Table 2-1). Cows were allowed to consume the basal diet *ad libitum* during the first 23 days of adaptation. Based on the calculated DMI over the 23 days of adaptation, 95% was fed for the remaining 5-d data and sample collection period. Treatments included the supplementation of trace minerals in either organic (ORG) or inorganic (INO) form at 50, 100 and 200% based on the NRC recommendations

(Table 2-2) (Organic trace minerals, Bio-Plex® and Sel-Plex®, Alltech Canada, Guelph, Ontario, Canada). Trace mineral treatments were mixed with 500 g of ground corn, hand mixed with the top 10 cm of the basal diet and fed individually to each cow in the feed bin at 08:00 h daily. Trace mineral treatments were adjusted to the average DMI of each individual animal every three days during the 23-d adaptation period and were held consistent with the basal diet during the 5-d data and sample collection period. Cows were housed individually in tie stalls designed to allow for total collection of urine, milk and feces. The cows were provided outside access every second day for 3 h without feed during the 23-d adaptation period and remained inside their respective tie stalls for the duration of the 5-d total collection. Water was provided *ad libitum* throughout the duration of the study and water intake was recorded during the 5-d data and sample collection period.

2.3.2 Data and Sample Collection

2.3.2.1 Intake

Feed offered (TMR) and orts were recorded daily throughout the sample and data collection period. During the final 5 days, TMR and orts for each individual cow were weighed and samples collected daily. The TMR and orts were stored at -20°C until further analysis.

2.3.2.2 Storage Container Preparation

All polypropylene collection containers for milk, urine and rumen fluid were acid washed with 20% HCl for 4 h and then rinsed for 2 h with double distilled water to prevent the cross contamination of trace minerals which may have been present in the containers (Moody and Lindstrom, 1977; Faulkner et al., 2017).

2.3.2.3 Fecal Collection

In order to facilitate collection of feces, large stainless-steel trays were placed directly behind each tie stall in the gutter. Four times daily (08:00, 14:00, 20:00 and 02:00 h) fecal output for each individual cow was transferred into a pre-weighed plastic container. Fecal contents were weighed and recorded, and fecal contents were thoroughly mixed. A subsample of 1% of the wet weight was taken and stored at -20°C until thawed and composited per cow, per day.

2.3.2.4 Urine Collection

Urine collection was facilitated using indwelling bladder catheters (Bardex Lubricath Foley bladder catheters, 75 cc ribbed balloon; C. R. Bard Inc., Covington, GA) according to Crutchfield (1968). Bladder catheters were inserted 24-h prior to total collection of urine and urine collection tubing was connected to the catheters following catheter insertion. Urine was collected into pre-weighed 20 L plastic containers beginning on day 24 at 08:00 h. Urine output was weighed and recorded twice daily (08:00 and 20:00). A 5% subsample based on the weight of the urine was taken after urine was thoroughly mixed, composited per cow per day and stored at -20°C until trace mineral analysis.

2.3.2.5 Milk Collection

Cows were milked twice daily during the data and sample collection period in their respective tie stalls at 0400 and 1600 h. Cows were milked individually into their respective pre-weighed stainless-steel collection bucket during the 5-d data and sample collection period. Milk production was recorded daily by weighing collection buckets and a subsample of 0.05% of the total weight was taken at each milking. Milk samples were composited per cow per day and stored at -20°C for further analysis.

2.3.3 Sample Analysis

2.3.3.1 Analysis of Trace Mineral Concentration in Feed, Fecal, Milk, and Urine Samples

Total mixed ration, feed orts and fecal samples were thawed overnight and then dried in a forced air oven at 60°C until a constant weight was reached (~48 h). Feed and fecal samples were ground to pass through a 1mm stainless steel screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Urine and milk samples were freeze dried (Freeze Dryer, HarvestRight, Salt Lake City, Utah) for 2 h prior to hot block digestion. Hot block digestion was performed on all samples by adding 0.5 g of sample and 5 mL of 70% nitric acid to a digestion tube and allowed to digest overnight. The digestion tube was transferred to the block digester and digested at 100°C for 120 min. Following block digestion, samples were left to cool at room temperature and brought to volume using Nanopure water. Trace mineral analysis in all samples were performed by Laboratory Services at the University of Guelph (Guelph, Ontario, Canada) using inductively coupled plasma mass spectrometry (Agilent 7900, inductively coupled plasma- mass spectrometer system, Santa Clara, CA) and ESI prepFAST, M5 auto-sampler (Elemental Scientific, Omaha, Nebraska).

2.3.4 Calculations

Total trace mineral intake was calculated on a DM basis and included the amount of trace mineral provided by both the basal diet and the trace mineral treatments. The trace mineral remaining in the orts were then subtracted from the total amount of trace mineral provided to obtain total trace mineral intake. This calculation was performed per cow, per day during the data and sample collection period to determine the total trace mineral intake. Total trace mineral intake was determined using the following equation:

$$\text{intake} = \left(\text{TM in basal diet } \left(\frac{\text{mg}}{\text{d}}\right) + \text{TM in treatments}\left(\frac{\text{mg}}{\text{d}}\right) + \text{TM in water}\left(\frac{\text{mg}}{\text{d}}\right)\right) - \text{TM in feed orts } \left(\frac{\text{mg}}{\text{d}}\right)$$

Apparent absorption is the total intake minus the total fecal excretion expressed as a percentage of intake (Ammerman et al., 1995). Apparent absorption for each individual trace mineral was calculated using the equation:

$$\frac{(\text{intake} - \text{feces})}{\text{intake}} \text{ x } 100\%$$

Apparent retention is the total intake minus the total fecal, urine and milk excretion, expressed as a percentage of intake (Ammerman et al., 1995; Suttle, 2010). Apparent retention for each individual trace mineral was calculated using the following equation:

$$\frac{(\text{intake} - \text{feces} - \text{urine} - \text{milk})}{\text{intake}} \text{ x } 100\%$$

2.3.5 Statistical Analysis

Data were analyzed using the GLIMMIX procedure of the Statistical Analysis System (version 9.3; SAS institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + C_j + P_i + M_k + D_l + MD_{kl} + E_{ijkl}$$

Where: Y_{ijkl} = dependent variable, μ = overall mean, C_j = random effect of cow j, P_i = fixed effect of period i, M_k = fixed effect of source of trace mineral k, D_l = fixed effect of concentration of trace mineral l, MD_{kl} = interaction between source of trace mineral, k, and concentration of trace mineral, l, and E_{ijkl} = residual error. All reported values are least squares mean (LSM) with significance being declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. The

adjusted p-value reported shows the significance of the main effect of the treatments as a whole, whereas the results reported as least squares means are looking specifically at the differences in individual treatments.

2.4 Results

2.4.1 Effect of source and concentration of supplemental trace minerals on dry matter, trace mineral and water intake, and dry matter apparent digestibility

Dry matter intake and apparent DM digestibility were not affected by trace mineral supplementation treatments (Table 2-3). Water intake decreased ($P < 0.001$) in the 50 ORG treatment compared to the 50 INO, 100 ORG and 200 ORG treatments. All other treatments did not differ in water intake. Source of trace minerals did not have a large effect on dry matter intake and apparent dry matter digestibility, however, there was a tendency ($P = 0.086$) for inorganic trace mineral supplementation to increase water intake by 4.3% compared to organic trace mineral supplementation.

Cobalt, copper, selenium, and zinc intakes were all higher ($P < 0.05$) in the 200 INO and 200 ORG treatments compared to 50 INO, 50 ORG, 100 INO and 100 ORG treatments. They were also higher in the 100 INO and 100 ORG treatments than the 50 INO and 50 ORG treatments. Manganese intake was higher ($P < 0.05$) in 200 INO than all other treatments, and higher in the 100 INO and 100 ORG treatments than the 50 INO and 50 ORG treatments. Trace mineral intake was higher ($P < 0.05$) in inorganic copper, manganese, and zinc supplementation compared to organic trace mineral supplementation. No differences between the sources of trace minerals were observed in cobalt and selenium.

2.4.2 Effect of source and concentration of supplemental trace minerals on milk yield and composition

Milk yield was decreased ($P < 0.05$) in the 200 ORG treatment compared to the 50 INO and 100 INO treatments. Milk yield was higher ($P = 0.001$) in INO than ORG supplementation of trace minerals. Milk fat percentage and milk fat yield over the experimental period were not affected by treatment (Table 2-5). There was no difference among all other treatments. Overall, there were few differences in protein yield and percentages except for where the 200 INO treatment was 7% higher ($P < 0.05$) than the 100 INO treatment for protein yield. No differences were observed between treatments in lactose yield, however, when comparing the milk lactose percentage, 50 INO and 100 ORG treatments were higher ($P = 0.003$) than the 200 ORG treatment. All other treatments did not differ in milk lactose. There was also no difference among treatments observed for milk urea nitrogen (MUN).

There were very few differences in milk composition when comparing the source of trace minerals. Milk fat percentage, milk protein yield, lactose percentage, lactose yield, and MUN displayed no differences. Milk fat yield increased ($P = 0.022$) by 5.6% in the inorganic sources compared to the organic. The opposite was observed for milk protein percentage, where organic was 4.7% higher ($P = 0.02$) than the inorganic treatments.

2.4.3 Effect of source and concentration of supplemental trace minerals on trace mineral excretion in feces, urine and milk

There was no difference in the amount of feces excreted between all treatment groups (Table 2-4). Cobalt excreted in feces was the highest ($P < 0.05$) in the 200 INO treatment compared to all other treatments. Excretion of cobalt in the feces did not differ between the 100 INO and 100 and 200 ORG treatments. The 50 INO and 50 ORG treatments had lower fecal cobalt excretion than all other treatment groups. Fecal excretion of copper, manganese, selenium, and zinc were higher ($P < 0.05$) in the 200 INO and 200 ORG treatments than the 100 INO, 100

ORG, 50 INO, and 50 ORG, and were higher in the 100 INO and 100 ORG than the 50 INO and 50 ORG treatments. There was a 1.1x increase ($P = 0.002$) in fecal cobalt excretion in INO compared to ORG. No differences were observed between the sources of trace minerals on the amount feces excreted, or the amount of copper, manganese, selenium, and zinc excreted in the feces.

Total amount of urine excreted over the experimental period did not differ between treatment groups. Similarly, no difference was observed between treatment groups for the urinary excretion of copper, manganese and zinc. As for urinary excretion of cobalt, excretion was higher ($P < 0.05$) in 200 INO compared to 50 INO, 50 ORG, 100 INO, and 100 ORG. 50 INO and 50 ORG treatments were lower ($P <0.05$) than both the 200 INO and 200 ORG treatments. Urinary excretion of selenium was the highest ($P < 0.001$) in the 200 INO group compared to all other treatments. The 50 INO treatment had lower ($P < 0.001$) selenium urinary excretion compared to the 100 INO, 100 ORG, 200 INO and 200 ORG groups. Supplementation of ORG resulted in lower ($P < 0.001$) selenium urinary excretion than INO supplementation. No differences were observed between the sources of trace minerals and the amount of urine excreted or the urinary excretion of cobalt, copper, manganese, and zinc.

There was no treatment difference in the milk excretion of cobalt, copper, and zinc. Manganese excreted in the milk was higher ($P < 0.05$) in 200 ORG and 200 INO compared to the 50 ORG treatment. No difference was observed between all other treatments in the milk excretion of manganese. Milk excretion of selenium was the highest ($P < 0.05$) in the 200 ORG treatment than 50 INO, 50 ORG, 100 INO, and 200 INO. The 100 ORG treatment also had higher ($P < 0.05$) selenium excretion in milk compared to the 50 INO and 50 ORG treatments. Selenium excretion in milk was 1.1x higher ($P < 0.001$) with ORG supplementation than INO

supplementation. Cobalt, copper, manganese, and zinc excretion in milk showed no differences between ORG and INO.

2.4.4 Effect of source and concentration of supplemental trace minerals on apparent absorption and apparent retention of trace minerals

Based on the high fecal excretion values, apparent absorption and retention values for cobalt, copper, manganese, and zinc were negative. Apparent absorption of cobalt was higher ($P < 0.05$) in 200 ORG treatment compared to the 50 INO and 100 INO treatments, with no other differences between treatments (Figure 2-1). Selenium had higher ($P < 0.05$) apparent absorption in the 200 INO and 200 ORG treatments compared to the 50 INO and 50 ORG treatments. Apparent absorption of copper, manganese and zinc did not differ between treatments over the experimental period. Copper, manganese, and selenium did not differ in apparent absorption between INO and ORG supplementation. Cobalt differed in apparent absorption, where ORG was 1.3 x higher ($P = 0.019$) than INO. In contrast, zinc apparent absorption was lower ($P = 0.030$) in ORG compared to INO.

Apparent retention had similar results to apparent absorption, with only cobalt and selenium showing differences between treatments and no differences in apparent retention between treatments in copper, manganese, and zinc (Figure 2-2). Selenium apparent absorption was higher ($P < 0.05$) in the 200 INO and 200 ORG compared to the 50 INO and 50 ORG. Cobalt apparent retention was lower ($P < 0.05$) in the 50 INO and 100 INO treatments compared to the 200 ORG treatment. When comparing ORG versus INO supplementation, the only mineral that exhibited a difference between sources was cobalt, where ORG had a 1.3x higher ($P = 0.018$) apparent retention than INO supplementation.

2.5 Discussion

This study is the first to investigate performance and apparent absorption and retention of the source, in combination with concentration, of five trace minerals. It was hypothesized that low levels of organic trace mineral supplementation would have increased absorption and increased retention, allowing for increased bioavailability and increased production compared to high levels of inorganic trace mineral supplementation. In contrast to the hypothesis, apparent absorption and retention of trace minerals was minimally affected by source and concentration of supplemental trace minerals, with the exception of selenium where apparent retention and absorption were higher in the 200% levels of supplementation compared to 50%. In addition, organic trace mineral supplementation decreased milk yield and milk fat yield compared to inorganic supplementation; however, low levels of organic trace mineral supplementation provided the same milk yield as all levels of inorganic supplementation.

Prior to the commencement of the trial, care was taken to formulate the basal diet to provide less than 20% of the NRC requirement for each trace mineral and was confirmed using wet chemistry from a commercial feed analysis company. Following the trial, ICP-MS analysis was conducted on the same individual feed ingredients and the TMR fed to each cow during the experimental period, and revealed that values of each trace mineral within the basal diet reached closer to 50-80% above the NRC requirement prior to trace mineral supplementation (minerals provided by diet: Co, 0.53 ppm; Cu, 54.80 ppm; Mn, 253 ppm; Se, 0.84 ppm; Zn, 231 ppm). However, it is important to note that this trial was one of the first to include the basal diet contributions using ICP-MS and treatments of trace minerals for overall trace mineral intake. From this discovery, it may be possible that previous studies have formulated diets and trace mineral supplementation levels that may have exceeded their target trace mineral supplementation levels. Trace mineral contribution from water were also tested via wet

chemistry prior to the commencement of the trial with values providing less than 4% for all trace minerals (Co, < MDL; Cu, 4%; Mn, <MDL; Se, <MDL; Zn, 1%), therefore water trace mineral contribution to the diet was neglected from the calculations. Upon analysis from ICP-MS, water contributions for cobalt, manganese and selenium remained the same, however, copper and zinc were higher (Co, < MDL; Cu, 31%; Mn, <MDL; Se, <MDL; Zn, 4%), therefore, only copper and zinc contributions from water were included in the trace mineral intake calculations.

Studies comparing sources of trace mineral supplementation are consistent in observing no change in DMI between inorganic and organic trace mineral supplementation (Kincaid and Socha, 2004; Yasui et al., 2014; Faulkner and Weiss, 2017). In the present study, cows were restricted to 95% of their expected intake over the 5-d data and sample collection period, and therefore the absence of differences between treatments was by design; however, previous studies show consistent DMI results and it is unlikely that these values would have been different if animals were allowed to consume the feed *ad libitum* over the 5-d of restriction (Kincaid and Socha, 2004; Yasui et al., 2014; Faulkner and Weiss, 2017). Although DM digestibility values were lower than those observed in previous studies (Pino and Heinrichs, 2016; Faulkner and Weiss, 2017), overall results were the same, with no change observed between organic and inorganic supplementation. Diets provided by both Pino and Heinrichs (2016) and Faulkner and Weiss (2017) had lower inclusions of forage, which would lead to the higher DM digestibility than those observed in the present study. Changes in water intake between inorganic and organic supplementation have been observed prior to this study, with Faulkner and Weiss (2017) reporting that free water intake was higher in inorganic supplementation than organic supplementation groups, similar to the results of the present study. Furthermore, Pino and Heinrichs (2016) observed a similar result, where the authors suggested water consumption

would be higher in the inorganic group due to the increased wet weight of feces and urine, although water intake was not directly measured. Since feces and urine output did not change in the present study, the observed increase in milk production in the inorganic supplementation group could be due to the tendency for increased water intake in this study. Another possible explanation for the tendency towards increased water consumption for the inorganic supplementation group could be the sulfate salts provided by the inorganic minerals, as sulfate salts have been shown to stimulate water intake (Weeth and Hunter, 1971; James and Butcher, 1972). Dietary concentration of trace minerals on water intake has not been studied thoroughly and further research is required to understand the differences observed between lower and higher trace mineral supplementation levels.

Increased inorganic intakes of Cu, Mn, and Zn may be due to the numerical difference in DMI and although not significant, the organic trace mineral supplementation was close to showing a tendency for reduced DMI. As trace minerals are measured in milligrams within a diet, minute changes in DMI could display large differences in mineral intake. However, caution must be taken when interpreting these results, as there was no significance within the results of DMI. Even though organic supplementation has lower intake overall in copper, manganese and zinc, only manganese showed a significant difference in intake between the dietary concentration treatments at the 200% level. Thus, the majority of the trace mineral treatments were supplied at consistent amounts in both organic and inorganic supplementation.

The responses to organic trace mineral supplementation on milk composition and performance vary across studies, with results identifying increased milk, fat and protein yield (Griffiths et al., 2007), increased milk protein (Kincaid and Socha, 2004), and no difference in milk yield, fat, milk protein and lactose (Uchida et al., 2001; Yasui et al., 2014; Faulkner and

Weiss, 2017), respectively. This study is one of the first to report decreased milk yield with organic supplementation, however, prior studies did not supplement lactating cows to the same degree as in the present study. The main difference observed was that low levels (50 and 100%) of inorganic supplementation resulted in higher milk production than high (200%) organic supplementation. Therefore, low levels of organic supplementation produce similar milk yield to all levels of inorganic supplementation. However, since the basal diet contributed higher than expected levels of trace minerals (50-80% above NRC recommendations), these low levels (50%) of supplementation might be meeting optimal trace mineral supplementation levels for the performance of dairy cows. It also suggests that cows are equally productive at low or optimal levels of organic supplementation compared to all levels of inorganic supplementation.

The lack of change in milk composition amongst the differing concentrations of trace minerals supplied suggests that milk fat responses are attributed to the source of trace minerals provided in the diet. Milk urea nitrogen (MUN) values of 16 mg/dl were slightly higher than the 12-14 mg/dl range recommended for lactating dairy cows. These values indicate excess protein, rumen degradable protein or poor utilization of protein, yet did not reach extreme levels of 18 mg/dl or higher, which would suggest a protein imbalance or severe lack of carbohydrate in the diet. A previous study observed similar results, with no change in MUN between source of supplementation, but increase in MUN with the forage diet (15 mg/dl) compared to a by-product diet (11 mg/dl) (Faulkner and Weiss, 2017). In order for rumen microbes to convert urea back to protein, carbohydrates are required and thus the results may be attributed to the large inclusion of forage and decreased concentrate within the diet. Overall, the results of the present study indicate no change in the utilization of protein supplied to the cows by the body or through rumen microbial synthesis due to trace mineral treatments or source of supplementation.

Lactose is the driving force behind milk production by attracting water to the udder. Propionate is required for glucose production, which is used in the production of lactose in the mammary gland. Therefore, more glucose (from propionate) increases lactose production, which in turn increases milk production. As lactose percentage decreased at high levels of organic supplementation, milk yield also decreased. Conversely, low levels of inorganic supplementation increased lactose percentage and milk yield. Due to the relationship between lactose and milk production, there would be no change in the lactose yield, as the amount of lactose per kg of milk should remain consistent. However, as our findings confirm, the amount of milk produced effectively changes the lactose percentage.

No differences between total feces and urine excreted due to the source of trace mineral supplementation have been previously reported (Faulkner et al., 2017). Of all the minerals supplemented, selenium fecal excretion was the only mineral that was in the range of a previously reported level (Ivancic and Weiss, 2001), while all other minerals (cobalt, copper, manganese, and zinc) were higher than previously observed (Veirboom et al., 2002; Spears et al., 2004; Weiss and Socha, 2005). When these high fecal excretion values were used to determine the amount of trace minerals excreted as a percentage of intake, values reach levels above 100%. Therefore, cows were excreting more minerals in their feces than consumed. This is a peculiar result and may be due to contamination. Since airborne selenium contamination is rare, and fecal excretion levels for all minerals were high, we suggest that environmental contamination is likely the reason behind these findings (ATSDR, 2003). These findings could have also occurred through contamination of the TMR – and thus we underestimated trace mineral intake – or it could have occurred before fecal sample collection, resulting in overestimation of the trace minerals levels excreted.

Although difficult to quantify, copper, zinc and, to some extent cobalt, as cobalamin, are stored in the liver and other tissues within the body and under stressful conditions stores of these minerals can be mobilized (Czarnek, 2015; Shkurashivska and Ersteniuk, 2019). Although care was taken to reduce stress during the trial, consistent movement of people, sample collection, and confinement to tie stalls for 5 consecutive days may have led to stressful conditions, therefore leading to the mobilization of trace minerals stored during the dietary adaptation period. Biliary excretion is one of the main routes for mobilized mineral excretion in humans (Ishihara and Matsushiro, 1986), however biliary excretion in ruminants is limited (Lopez-Alonso, 2012). Therefore, only limited amounts of mobilized trace minerals would have entered the feces. Increased excretion from stress can only be speculated, and moreover, it seems unlikely that this minimal amount of mobilization would have a large contribution to the excess minerals in the feces. It is more likely that, as noted above, environmental contamination of feed intake and/or feces, once excreted, caused the high fecal excretion values.

Sample collection and excretion values of Co, Cu, Mn and Zn were consistent throughout the treatments, therefore the effects of treatment can still be compared. Increased supplementation of trace minerals, regardless of the source, increased fecal excretion, with the exception of cobalt, where inorganic supplementation exhibited higher fecal excretion than organic. Selenium is the only mineral in which urine, milk and fecal excretion were all impacted by treatment within the study. This suggests that of all the minerals, selenium excretion is the most dependent on the level of intake.

Urinary excretion of trace minerals is similar to studies previously reported in cattle (Veirboom et al., 2002; Spears et al., 2004; Weiss and Socha, 2005; Faulkner et al., 2017). An increase in urine selenium and cobalt excretion with increased level of dietary supplementation

has been observed in prior studies (Walker and Elliot, 1972; Ivancic and Weiss, 2001). When in excess of what the body requires, vitamin B_{12} is excreted in the urine. Therefore, higher supplementation may have led to higher excretion of cobalt via vitamin B_{12} in the urine, which is in agreement with the results.

Similar to previous studies, our results indicate that excretion through milk is dependent on the amount of manganese supplemented in the diet, regardless of the source of the trace mineral (Weiss and Socha, 2005). Similar results have also been reported for selenium where higher selenium milk concentrations are observed with increasing concentration, and in organic supplementation compared to inorganic supplementation (Givens et al., 2004; Juniper et al., 2006). These results indicate that increasing organic supplementation in dairy diets can increase selenium enrichment of milk, which is beneficial for human consumption in areas where dietary selenium deficiencies are prevalent.

The high negative apparent absorption and retention values can be attributed to the high trace mineral excretion via feces. Although values are unexpected, it indicates that there are few differences between the source of trace mineral supplementation in regards to apparent absorption and retention. Organic cobalt supplemented at 200% had higher apparent absorption and apparent retention than the low concentrations (50 and 100%) of inorganic supplementation. This may be an indication of the bioavailability of these trace minerals within the body, where inorganic sources dissociate and bind with antagonists within the gastrointestinal tract rendering them unavailable for absorption in the small intestine. Conversely, organic sources may avoid these interactions, making them more bioavailable. As for selenium, similar results have been observed previously, where the high concentrations of supplementation, regardless of source, exhibited increased apparent absorption and apparent retention (Ivancic and Weiss, 2001).

Therefore, increasing supplementation may induce levels that surpass antagonism within the GIT, allowing more selenium to be absorbed by the body, regardless of the source of supplementation. Since the organic form of selenium supplementation is selenized yeast, it may exhibit properties different from other chelated organic trace minerals, therefore, organic selenium may dissociate within the rumen and bind with antagonists at the same rate of its inorganic counterparts. These results suggest increased concentrations of selenium within the diet are more beneficial in increasing absorption and retention as opposed to the source of supplementation. As for Cu, Mn, and Zn, Faulkner et al. (2017) showed similar results with no difference in apparent absorption and retention between organic and inorganic supplementation. Overall, source and the combination of source and dietary concentration of trace minerals had minimal impact on apparent absorption and retention of trace minerals.

2.6 Conclusion

This study is the first to investigate performance and apparent absorption and retention of the source, in combination concentration, of five trace minerals. Organic trace mineral supplementation decreased milk yield and milk fat yield compared to inorganic supplementation; however, low levels of organic trace mineral supplementation provided the same milk yield as all levels of inorganic supplementation. The difference in source of cobalt supplementation could be an indication of organic cobalt having increased bioavailability to the body. Selenium apparent absorption and retention was impacted by dietary concentration, where high levels of organic and inorganic supplementation showed higher absorption and retention than low levels. In this instance, the increased supplementation may be inducing levels that surpass antagonism within the gastrointestinal tract and allow for more selenium to be absorbed by the body. Overall, source in combination with dietary concentration had minimal impact on the apparent absorption

and retention of trace minerals. In conclusion, supplementation of organic trace minerals at high levels (200% of NRC) in lactating dairy cows may negatively affect performance measures compared to low levels of inorganic supplementation (50% and 100%) and therefore, more efficient supplementation of trace minerals to lactating dairy cows would allow producers to increase sustainability while reducing feed costs and maintaining performance of the herd.

2.7 Literature Cited

Agency for Toxic Substances and Disease Registry. 2003. Public health statement: Selenium. U.S. Department of Health and Human Services. Washington, D.C.

Ammerman, C.B., D.H. Baker, and A.J. Lewis. 1995. Bioavailability of nutrients for animals amino acids, minerals, and vitamins. San Diego: Academic Press.

Berndtson, W.E. 1991. A simple, rapid and reliable method for selecting or assessing the number of replicates for animal experiments. Journal of Animal Science. 69:67. doi:10.2527/1991.69167x.

Castillo, A., N. St-Pierre, N.S.D. Rio, and W. Weiss. 2013. Mineral concentrations in diets, water, and milk and their value in estimating on-farm excretion of manure minerals in lactating dairy cows. Journal of Dairy Science. 96:3388–3398. doi:10.3168/jds.2012-6121.

Crutchfield, W. O. 1968. A technique for placement of an in-dwelling catheter in the cow. Veterinary Medicine Small Animal Clinician. 63:1141-1144.

Czarnek, K., S. Terpiłowska, and A.K. Siwicki. 2015. Review paper Selected aspects of the action of cobalt ions in the human body. Central European Journal of Immunology. 2:236–242. doi:10.5114/ceji.2015.52837.

Faulkner, M., and W. Weiss. 2017. Effect of source of trace minerals in either forage- or by-product–based diets fed to dairy cows: 1. Production and macronutrient digestibility. Journal of Dairy Science. 100:5358–5367. doi:10.3168/jds.2016-12095.

Faulkner, M., N. St-Pierre, and W. Weiss. 2017. Effect of source of trace minerals in either forage- or by-product–based diets fed to dairy cows: 2. Apparent absorption and retention of minerals. Journal of Dairy Science. 100:5368–5377. doi:10.3168/jds.2016-12096.

Givens, D.I., R. Allison, B. Cottrill, and J.S. Blake. 2004. Enhancing the selenium content of bovine milk through alteration of the form and concentration of selenium in the diet of the dairy cow. Journal of the Science of Food and Agriculture. 84:811–817.

Goff, J.P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of Dairy Science. 101:2763–2813. doi:10.3168/jds.2017-13112.

Griffiths, L., S. Loeffler, M. Socha, D. Tomlinson, and A. Johnson. 2007. Effects of supplementing complexed zinc, manganese, copper and cobalt on lactation and reproductive performance of intensively grazed lactating dairy cattle on the South Island of New Zealand. Animal Feed Science and Technology. 137:69–83. doi:10.1016/j.anifeedsci.2006.10.006.

Ishihara, N., and T. Matsushiro. 1986. Biliary and urinary excretion of metals in humans. Archives of Environmental Health: An International Journal. 41:324–330. doi:10.1080/00039896.1986.9936705

Ivancic, J., and W. Weiss. 2001. Effect of Dietary Sulfur and Selenium Concentrations on Selenium Balance of Lactating Holstein Cows. Journal of Dairy Science. 84:225–232. doi:10.3168/jds.s0022-0302(01)74472-4.

James, L.F., and J.E. Butcher. 1972. Halogeton poisoning of sheep: Effect of high level oxalate intake. Journal of Animal Science. 35:1233–1238.

Juniper, D., R. Phipps, A. Jones, and G. Bertin. 2006. Selenium supplementation of lactating dairy cows: effect on selenium concentration in blood, milk, urine, and feces. Journal of Dairy Science. 89:3544–3551. doi:10.3168/jds.s0022-0302(06)72394-3.

Kincaid, R., and M. Socha. 2004. Inorganic versus complexed trace mineral supplements on performance of dairy cows. The Professional Animal Scientist. 20:66–73. doi:10.15232/s1080-7446(15)31274-2.

Koenig, K.M., W.T. Buckley, and J.A. Shelford. 1991. True absorption of selenium in dairy, cows: Stable isotope tracer methodology and effect of dietary copper. Canadian Journal of Animal Science. 71:175-183.

López-Alonso, M. 2012. Trace minerals and livestock: Not too much not too little. ISRN Veterinary Science. 2012:1–18. doi:10.5402/2012/704825.

Machado, V., M. Bicalho, R. Pereira, L. Caixeta, W. Knauer, G. Oikonomou, R. Gilbert, and R. Bicalho. 2013. Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on the health and production of lactating Holstein cows. The Veterinary Journal. 197:451–456. doi:10.1016/j.tvjl.2013.02.022.

Maret, W. 2017. Zinc in cellular regulation: The nature and significance of “Zinc Signals”. International Journal of Molecular Science. 18:2285. doi: 10.3390/ijms18112285

Moody, J.R., and R.M. Lindstrom. 1977. Selection and cleaning of plastic containers for storage of trace element samples. Analytical Chemistry. 49:2264–2267. doi:10.1021/ac50022a039.

Nicholson, F.A., and B.J. Chambers. 2008. Livestock manure management and treatment: implications for heavy metal inputs to agricultural soils. Schlegel, P., S. Durosoy, and A.W. Jongbloed. Trace elements in animal production systems. Wageningen Academic, Wageningen.

NRC. 2001. Nutrient Requirements of Dairy Cows, 7th edn. National Academy of Sciences.

Pino, F., and A. Heinrichs. 2016. Effect of trace minerals and starch on digestibility and rumen fermentation in diets for dairy heifers. Journal of Dairy Science. 99:2797–2810. doi:10.3168/jds.2015-10034.

Shkurashivska, S., and H. Ersteniuk. 2019. The effect of adrenaline on the mineral and trace element status in rats. Open Life Sciences. 14:158–164. doi:10.1515/biol-2019-0018.

Spears, J., P. Schlegel, M. Seal, and K. Lloyd. 2004. Bioavailability of zinc from zinc sulfate and different organic zinc sources and their effects on ruminal volatile fatty acid proportions. Livestock Production Science. 90:211–217. doi:10.1016/j.livprodsci.2004.05.001.

Stangl, G.I., F.J. Schwarz, H. Müller, and M. Kirchgessner. 2000. Evaluation of the cobalt requirement of beef cattle based on vitamin B_{12}, folate, homocysteine and methylmalonic acid. British Journal of Nutrition. 84:645–653. doi:10.1017/s0007114500001987.

Sun, L., S. Gao, K. Wang, J. Xu, M. Sanz-Fernandez, L. Baumgard, and D. Bu. 2019. Effects of source on bioavailability of selenium, antioxidant status, and performance in lactating dairy cows during oxidative stress-inducing conditions. Journal of Dairy Science. 102:311–319. doi:10.3168/jds.2018-14974.

Suttle, N.F. 2010. The Mineral Nutrition of Livestock. 4th edn. CAB International, Wallingford, UK.

Uchida, K., P. Mandebvu, C. Ballard, C. Sniffen, and M. Carter. 2001. Effect of feeding a combination of zinc, manganese and copper amino acid complexes, and cobalt glucoheptonate on performance of early lactation high producing dairy cows. Animal Feed Science and Technology. 93:193–203. doi:10.1016/s0377-8401(01)00279-6.

Vierboom, M.M., T.E. Engle, and C.V. Kimberling. 2003. Effects of Gestational Status on Apparent Absorption and Retention of Copper and Zinc in Mature Angus Cows and Suffolk Ewes. Asian-Australasian Journal of Animal Sciences. 16:515–518. doi:10.5713/ajas.2003.515.

Vinus, S. 2017. Organic minerals in poultry. Advances in Research. 12:1–10. doi:10.9734/air/2017/37878.

Walker, C.K. and Elliot, J.M. 1972. Lactational trends in vitamin B_{12} status on conventional and restricted- roughage rations. Journal of Dairy Science 55, 474–478.

Weeth, H.J., and J.E. Hunter. 1971. Drinking of sulfate-water by cattle. Journal of Animal Science. 32:277–281.

Weiss, W., and M. Socha. 2005. Dietary manganese for dry and lactating Holstein cows. Journal of Dairy Science. 88:2517–2523. doi:10.3168/jds.s0022-0302(05)72929- 5.

Yasui, T., C. Ryan, R. Gilbert, K. Perryman, and T. Overton. 2014. Effects of hydroxy trace minerals on oxidative metabolism, cytological endometritis, and performance of transition dairy cows. Journal of Dairy Science. 97:3728–3738. doi:10.3168/jds.2013-7331.

2.8 Tables and Figures

Table 2-1: Ingredients and nutrient composition of experimental diet (basal diet) fed to lactating dairy cows.

		Trace Mineral Amounts, mg/kg				
Item	Diet	Cobalt	Copper	Manganese	Selenium	Zinc
Ingredient composition, kg/day DM						
Barley silage	12.20	0.027	5.0	12	0.049	25
Straw	4.00	0.065	24	93	0.017	22
Ground corn	0.44	0.045	5.8	18	0.21	34
Concentrate pellet	8.6	0.39	20	130	0.56	150
Corn grain	2.57					
Soybean meal	2.99					
Wheat grain	2.51					
Limestone	0.39					
Canola oil	0.08					
Magnesium oxide	0.03					
Salt	0.03					
Vitamin A, IU/day	75,000					
Vitamin D, IU/day	21,000					
Vitamin E, IU/day	545					
Water	-	<MDL	0.87	<MDL	<MDL	0.63
Nutrient composition of diet, % of DM						
DMI, kg	25.3					
$NE_{L,}$ Mcal/kg	1.49					
CP	15.1					
NDF	42.0					
ADF	27.8					
NFC	34.8					
Ca	0.8					
P	0.3					
Mg	0.25					
Cl	0.35					
Na	0.11					

Table 2-2. Inorganic and organic sources of trace minerals and NRC recommended supplementation concentrations fed as experimental treatments to lactating dairy cows.

	Source of supplemental trace mineral		
Trace Mineral	Inorganic	Organic	100 % NRC (ppm)
Cobalt	Co sulfate	Co proteinate[1]	0.25[3]
Copper	Cu sulfate	Cu proteinate[1]	15.7
Manganese	Mn sulfate	Mn proteniate[1]	40[3]
Selenium	Na selenite	Selenized yeast[2]	0.30
Zinc	Zn sulfate	Zn proteinate[1]	63

[1]Bioplex, Alltech Canada, Guelph, ON.
[2]Sel-Plex, Alltech Canada, Guelph ON.
[3]Levels based off of NRC, increased from NRC 2001 to levels as described by Lopez-Guisa and Satter (1992) and Stangl et al., (2000).

Table 2-3. Dry matter intake, trace mineral intake, water intake and apparent dry matter digestibility of lactating dairy cattle fed a diet supplemented with different source and concentration of trace minerals[1]

	Treatments[2]								
	Inorganic (INO), %			Organic (ORG), %				*P*-value	
Item	50	100	200	50	100	200	SEM	Source x Conc	INO vs. ORG
DMI, kg	18.65	18.22	18.08	18.00	18.37	17.42	0.760	0.367	0.144
Apparent digestibility									
DM, %	59.44	58.73	60.19	59.23	59.23	59.54	1.334	0.837	0.948
Water intake, Litre[3]	80.80^{a}	76.63^{ab}	76.77^{ab}	67.07^{b}	79.56^{a}	76.65^{a}	8.090	< 0.001	0.086
Trace mineral intake, mg/d[4]									
Co	7.94^{c}	9.79^{b}	14.47^{a}	7.79^{c}	9.87^{b}	13.02^{a}	0.344	0.241	0.172
Cu	446.10^{c}	580.38^{b}	833.60^{a}	423.34^{c}	573.32^{b}	748.50^{a}	24.102	0.292	0.022
Mn	1521.32^{d}	1857.89^{c}	2526.54^{a}	1490.70^{d}	1822.86^{c}	2170.70^{b}	76.569	0.088	0.024
Se	8.95^{c}	11.80^{b}	17.20^{a}	8.75^{c}	11.90^{b}	15.64^{a}	0.365	0.221	0.149
Zn	2381.10^{c}	2905.45^{b}	4058.57^{a}	2262.80^{c}	2855.75^{b}	3520.76^{a}	114.359	0.215	0.020

[1] Values are least squares mean obtained by a 6 x 6 Latin square design

[2] Lactating dairy cows were fed a total mixed ration supplemented with organic and inorganic sources of cobalt (Co), copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) at 50, 100, and 200 % of NRC requirements (100% treatment; Co; 0.25, Cu; 15.70, Mn; 40.00, Se; 0.30, and Zn; 63.00 ppm)

[3] Treatments with different superscripts within a row indicate significant differences among the means between treatments at $P < 0.05$.

[4] Trace mineral intake includes basal diet trace mineral contributions in addition to the trace mineral supplementation treatment.

[5] The adjusted p-value reported shows the significance of the main effect of the treatments as a whole, whereas the data reported as least squares means is effect of the differences in individual treatments.

Table 2-4. Milk yield and composition of lactating dairy cattle fed a diet supplemented with different source and concentration of trace minerals[1]

	Treatments[2]								
	Inorganic (INO), %			Organic (ORG), %				*P*-value	
Item	50	100	200	50	100	200	SEM	Source x Conc[4]	INO vs. ORG
Milk yield, kg/d	24.50[a]	24.21[a]	23.18[ab]	22.74[ab]	22.94[ab]	20.90[b]	1.53	0.746	0.001
Milk composition									
Fat, %	3.76	3.76	3.96	4.00	3.78	3.67	0.108	0.007	0.815
Fat yield, kg/d	0.91	0.89	0.86	0.83	0.88	0.81	0.044	0.405	0.022
Crude protein, %	25.32[b]	25.47[ab]	27.94[ab]	27.56[ab]	26.73[ab]	28.28[a]	2.735	0.386	0.02
Crude protein yield, kg/d	0.121[ab]	0.120[b]	0.129[a]	0.124[ab]	0.124[ab]	0.124[ab]	0.006	0.093	0.521
Lactose, %	83.76[a]	75.67[ab]	81.56[ab]	78.52[ab]	82.35[a]	72.07[b]	0.082	0.003	0.168
Lactose yield, kg/d	0.14	0.14	0.15	0.14	0.14	0.14	0.004	0.110	0.485
MUN, mg/100 ml	16.50	16.34	16.71	16.78	16.13	16.72	0.814	0.672	0.907

[1] Values are least squares mean obtained by a 6 x 6 Latin square design

[2] Lactating dairy cows were fed a total mixed ration supplemented with organic and inorganic sources of cobalt (Co), copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) at 50, 100, and 200 % of NRC requirements (100% treatment; Co; 0.25, Cu; 15.70, Mn; 40.00, Se; 0.30, and Zn; 63.00 ppm)

[3] Treatments with different superscripts within a row indicate significant differences among the means between treatments at $P < 0.05$.

[4] The adjusted p-value reported shows the significance of the main effect of the treatments as a whole, whereas the data reported as least squares means is effect of the differences in individual treatments.

Table 2-5. Effects source and concentration of trace mineral supplementation in lactating dairy cows on trace mineral excretion[1]

	Treatments[2]								
	Inorganic (INO), %			Organic (ORG), %				*P*-value	
Item	50	100	200	50	100	200	SEM	Source x Conc[4]	INO vs. ORG
Feces									
Feces excreted, kg/d	49.32	48.83	46.58	46.90	48.34	43.25	2.669	0.626	0.103
Trace minerals excreted in feces, mg/d									
Co[3]	11.46^{d}	14.86^{bc}	19.80^{a}	11.14^{d}	13.76^{c}	16.35^{b}	0.526	0.099	0.002
Cu	463.20^{c}	636.88^{b}	873.78^{a}	465.36^{c}	618.36^{b}	792.54^{a}	25.589	0.442	0.219
Mn	2055.18^{c}	2415.89^{b}	3001.55^{a}	2016.44^{c}	2345.00^{b}	2895.00^{a}	70.134	0.890	0.210
Se	7.07^{c}	8.72^{b}	11.00^{a}	7.28^{c}	8.67^{b}	10.70^{a}	0.388	0.785	0.970
Zn	3848.96^{b}	4275.70^{b}	4999.34^{a}	3832.71^{b}	4197.59^{b}	5148.41^{a}	175.498	0.800	0.900
Urine									
Urine excreted, kg/d	13.01	12.93	12.88	13.87	12.92	12.92	0.161	0.963	0.783
Trace minerals excreted in urine, mg/d									
Co	0.12^{c}	0.17^{bc}	0.25^{a}	0.14^{c}	0.15^{bc}	0.20^{ab}	0.015	0.059	0.271
Cu	0.70	0.73	0.76	0.70	0.72	0.80	0.078	0.707	0.639

Mn	0.15	0.12	0.15	0.12	0.12	0.18	0.020	0.209	0.935
Se	3.87[c]	4.99[b]	6.66[a]	4.44[bc]	4.92[b]	4.77[b]	0.109	< 0.001	< 0.001
Zn	1.55	1.28	1.36	1.32	1.31	1.34	0.122	0.234	0.302
Milk									
Trace minerals excreted in milk, mg/d									
Co	0.02	0.02	0.02	0.02	0.02	0.02	0.003	0.919	0.584
Cu	1.19	2.09	2.09	1.74	1.98	2.41	0.257	0.471	0.982
Mn	0.75[ab]	0.87[ab]	0.96[a]	0.73[b]	0.82[ab]	0.95[a]	0.110	0.912	0.577
Se	1.16[c]	1.25[bc]	1.23[bc]	1.21[c]	1.42[ab]	1.49[a]	0.092	0.016	< 0.001
Zn	109.69	115.70	112.90	106.12	118.92	80.09	16.070	0.285	0.257

[1] Values are least squares mean obtained by a 6 x 6 Latin square design

[2] Lactating dairy cows were fed a total mixed ration supplemented with organic and inorganic sources of cobalt (Co), copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) at 50, 100, and 200 % of NRC requirements (100% treatment; Co; 0.25, Cu; 15.70, Mn; 40.00, Se; 0.30, and Zn; 63.00 ppm)

[3] Treatments with different superscripts within a row indicate significant differences among the means between treatments at $P < 0.05$.

[4] The adjusted p-value reported shows the significance of the main effect of the treatments as a whole, whereas the data reported as least squares means is effect of the differences in individual treatments.

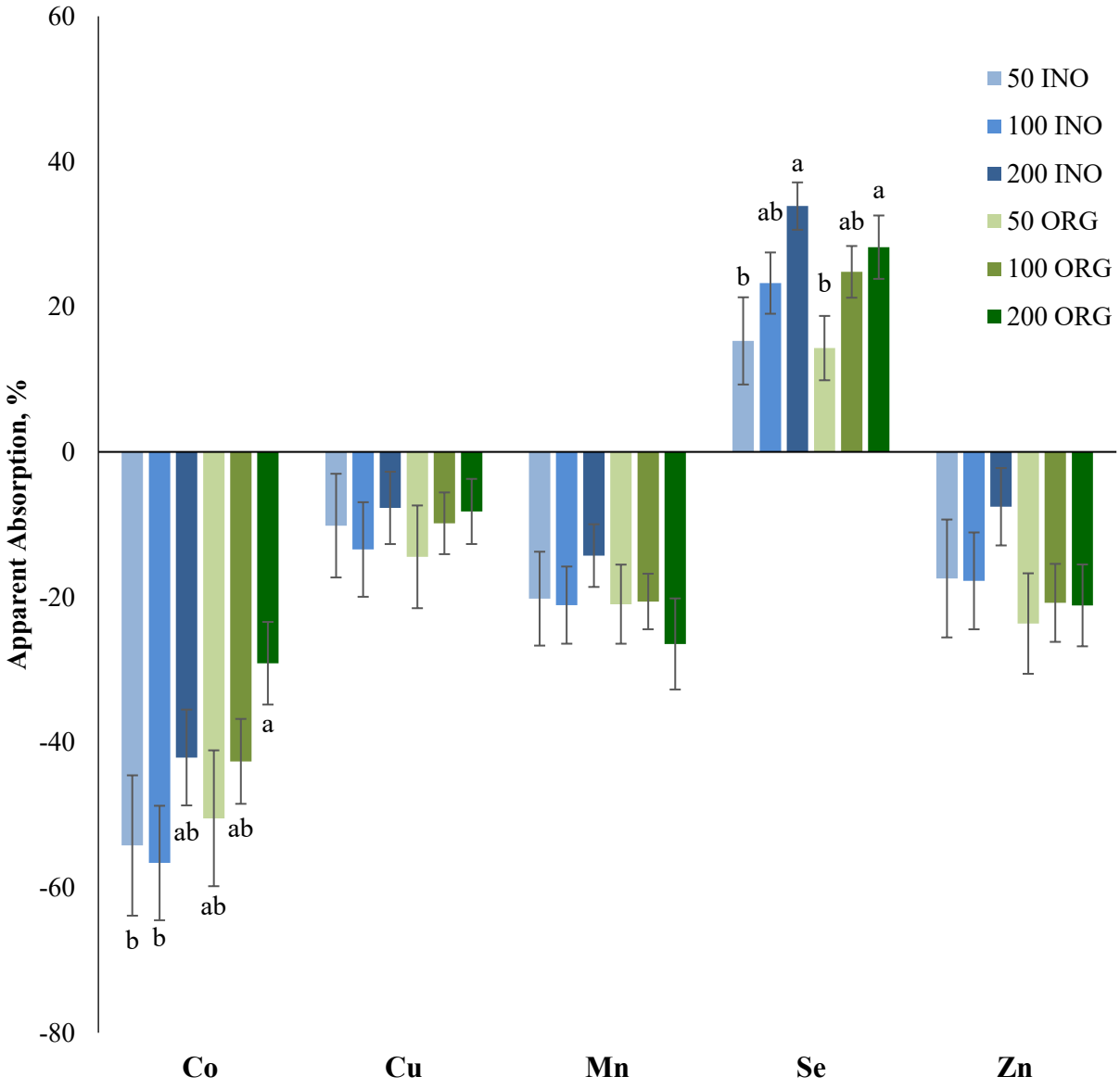

Figure 2-1: Apparent absorption of trace minerals in lactating dairy cows utilized in a 6 x 6 Latin square fed a total mixed ration supplemented with different source and concentration of trace minerals. 50 INO, 100 INO and 200 INO = total mixed ration of dairy cows supplemented with inorganic source of trace minerals (cobalt, copper, manganese, selenium, and zinc) at 50, 100 and 200% of NRC requirement, respectively; 50 ORG, 100 ORG and 200 ORG = total mixed ration of dairy cows supplemented with organic source of trace minerals (cobalt, copper, manganese, selenium, and zinc) at 50, 100 and 200% of NRC requirement, respectively. (100% treatment; Co; 0.25, Cu; 15.70, Mn; 40.00, Se; 0.30, and Zn; 63.00 ppm). Different letters represent differences among treatments at $P < 0.05$. Bars represent the least squares mean ± SEM.

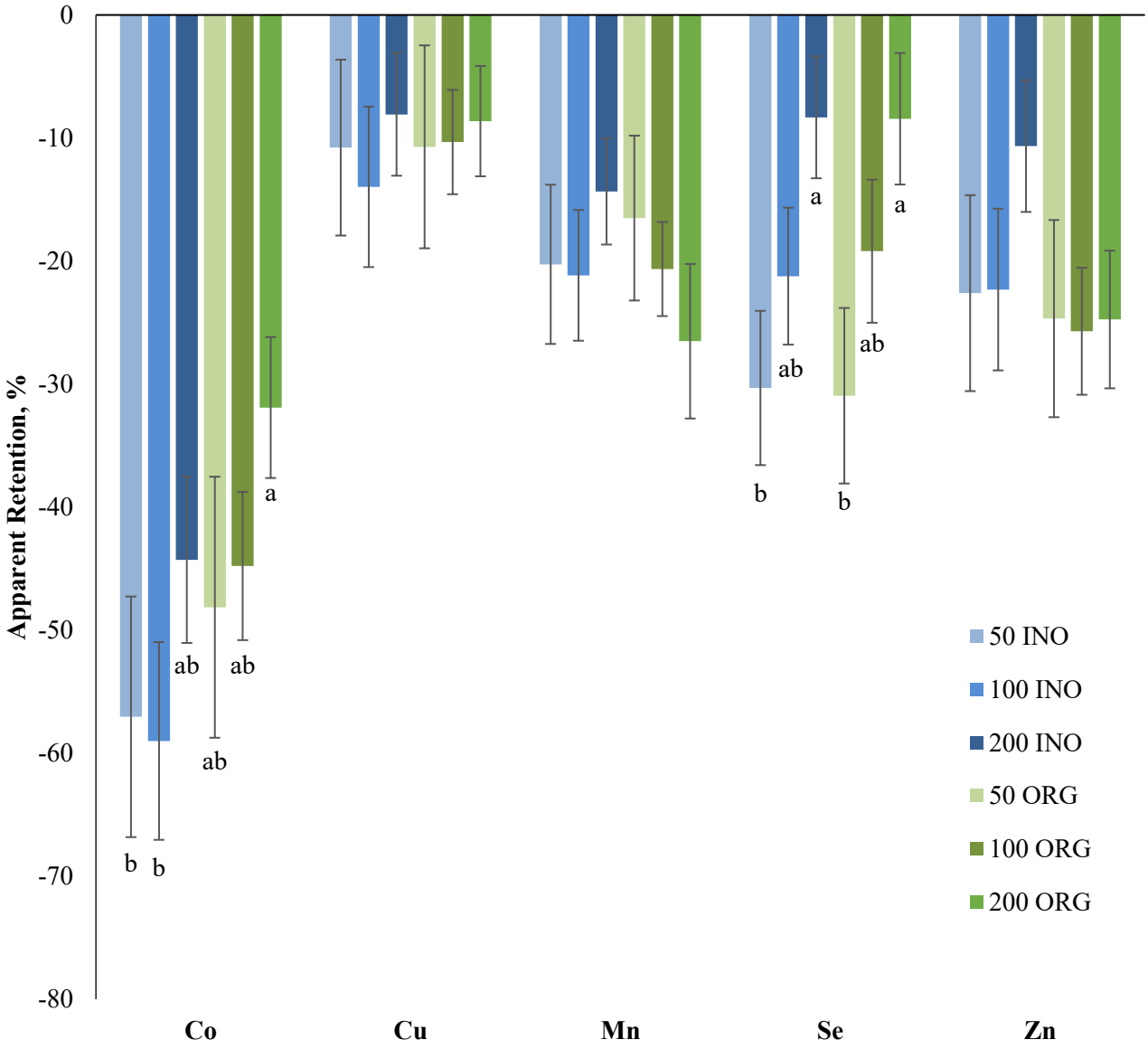

Figure 2-2: Apparent retention of trace minerals in lactating dairy cows utilized in a 6 x 6 Latin square fed a total mixed ration supplemented with different source and concentration of trace minerals. 50 INO, 100 INO and 200 INO = total mixed ration of dairy cows supplemented with inorganic source of trace minerals (cobalt, copper, manganese, selenium, and zinc) at 50, 100 and 200% NRC requirement, respectively; 50 ORG, 100 ORG and 200 ORG = total mixed ration of dairy cows supplemented with organic source of trace minerals (cobalt, copper, manganese, selenium, and zinc) at 50, 100 and 200% NRC requirement, respectively. (100% treatment; Co; 0.25, Cu; 15.70, Mn; 40.00, Se; 0.30, and Zn; 63.00 ppm). Different letters represent differences among treatments at $P < 0.05$. Bars represent the least squares mean ± SEM.

3.0 Chapter 3: Effect of source and concentration of supplemental trace minerals on rumen fermentation parameters, serum trace mineral concentration and physiological indicators of trace mineral status

3.1 Abstract

Inorganic sources of trace minerals are commonly supplemented in dairy cow diets; however, there has been an increase in the supplementation of minerals complexed with organic compounds. These organic trace minerals are thought to have greater bioavailability which may enhance rumen fermentation and absorption. The objective of this study was to investigate the effect of source and concentration of supplemental trace minerals on rumen fermentation parameters and physiological indicators of trace mineral status. Six lactating Holstein cows (129 ± 12 DIM; mid to late lactation) were used in a 6 x 6 Latin square design with a 23-d adaptation and 5-d data and sample collection period. Cows were fed the same basal diet daily except for the difference in source (organic [ORG] versus inorganic [INO]) and concentration (50%, 100%, or 200% based on NRC recommendation) of supplemental trace minerals (Co, Cu, Mn, Se, and Zn). During the data and sample collection period, blood and milk were collected daily. Rumen fluid and rumen pH were collected and recorded on the final two days of the sample collection period. Serum Co concentration was higher in 200 INO and 200 ORG compared all other treatments. Aside from Co, all other mineral concentrations (Cu, Mn, Se, and Zn), vitamin B_{12} concentrations (rumen fluid, plasma, milk), GSH-Px activity, total bacteria, rumen pH, and molar proportions of butyrate and valerate did not differ among the treatments. Total ruminal VFA was increased in the 50 ORG compared to 200 ORG. Molar proportion of propionate was higher in 100 ORG than both 200 ORG and 100 INO and was also higher in 50 ORG than 100 INO. Isovalerate molar proportion was the highest in 200 ORG compared to all other treatments.

Molar proportion of acetate was higher in 100 INO than 50 INO and 100 ORG. Molar isobutyrate proportion was higher in 200 ORG compared to 100 INO, 200 INO, 50 ORG and 100 ORG. Acetate:propionate was higher in 100 INO than 50 ORG and 100 ORG, whereas 100 ORG was lower than 200 INO and 200 ORG. Minimum pH and acetate:propionate was lower, acetate molar proportion tended to be lower and molar proportions of propionate, isobutyrate and isovalerate were higher in ORG compared to INO. These findings demonstrate serum trace mineral status and ruminal pH may not be controlled by the source of trace minerals when supplemented on top of a basal diet at 50, 100, and 200% of the NRC recommendation. However, rumen VFA production may be affected by both the source and concentration of supplemental trace minerals.

3.2 Introduction

The proper function of biological processes within the lactating cow is dependent on multiple nutritional factors, including trace minerals. Recent knowledge of the biological importance of trace minerals has shifted the dairy industry to develop new trace mineral technologies to help increase the bioavailability, absorption and the overall use of these minerals by the body. This has led to a shift from inorganic trace mineral supplementation from sulfates to organic trace minerals. These organic minerals are chelated or complexed with amino acids, proteins or organic acids, which are thought to make them more bioavailable to the animal; however, information regarding the effects of their supplementation in lactating dairy cow rations on ruminal fermentation and physiological indicators of trace mineral status is lacking.

Trace minerals have many bioactive properties including their role in rumen fermentation, energy metabolism, cellular structure, structure and function of transcription factors and their role in antioxidant systems (Arthur 2000; van den Top 2005; Andrieu, 2008)

Studies involving the source of trace mineral supplementation on rumen fermentation has increased in the recent years, however research regarding source in combination with the dietary concentration is lacking. Due to chelation with organic compounds, organic trace minerals are suspected to be more stable and protected from dissociation within the rumen and therefore, protected from antagonistic interactions that would render them unavailable for absorption or use by rumen microbes (Genther and Hansen, 2013). This has resulted in an increasing usage of organic trace minerals in dairy cow diets. However, it is possible that increased availability of organic trace minerals at high levels of supplementation could cause increased uptake of these minerals into the bacterial cell, which may cause reduced efficiency in VFA production through physical and chemical stress as these metals are capable of catalyzing oxidative damage, disrupting signaling pathways and inhibiting the activities of the cell at high intracellular levels (Finney and O'Halloran, 2003). Within the rumen, minerals are crucial for rumen microbial growth and function, and it has been determined that butyrate can be higher and mean pH lower in the organic trace mineral treatments compared to inorganic (Pino and Heinrichs, 2016). Furthermore, changes in specific rumen bacteria, namely *Prevotella bryantii* in dairy heifers have been observed when feeding organic trace minerals compared to inorganic sources (Kljak et al., 2017). Changes that may occur in rumen bacterial populations in response to trace mineral supplementation are important, as these organisms are also the sole producers of vitamin B_{12} in the ruminant animal which is absorbed and utilized for gluconeogenesis for energy production (Goff, 2018). These previous works suggest that rumen fermentation and rumen microbes may be impacted by trace minerals; however, it has not yet been studied in lactating dairy cows.

Trace minerals not utilized by rumen microbes are absorbed by the gastrointestinal tract (GIT) and transported throughout the body for biological processes. The absorption of these

minerals can influence blood trace mineral concentrations prior to the uptake from the bloodstream by tissues within the body. A well-known bioactive property of selenium is its antioxidant properties as a component of the selenium-dependent glutathione peroxidase (GSH-Px), which destroys lipid peroxidases and protects cell membranes from peroxidative damage (Rotruck et al., 1973; Arthur, 2000). Results from the literature regarding source and dietary concentration of selenium on GSH-Px vary showing both increases (Ortman and Pherson, 1997; Gong and Xiao, 2018) and no changes (Ortman and Pherson 1999; Juniper et al., 2006) in the activity of GSH-Px. Although previously studied, there has not been a comparison of multiple concentrations of inorganic and organic supplementation of selenium at one time.

The role of individual trace minerals in specific biological processes is well established; however, how the effect of concentration and source of supplemental trace minerals impacts rumen fermentation and physiological indicators is not well known. The objective of this study was to investigate the effect of source and concentration of supplemental trace minerals on rumen fermentation parameters and physiological indicators of trace mineral status. It was hypothesized that organic trace minerals will have increased bioavailability to rumen microbes thereby increasing VFA production, and also increasing antioxidant activity (GSH-Px) and vitamin B_{12} production in the dairy cow as compared to inorganic sources. Furthermore, it is hypothesized that low organic trace mineral supplementation will provide similar responses to high inorganic supplementation.

3.3 Materials and Methods

3.3.1 Animals, Experimental Design and Treatments

The experiment was conducted at the Dairy Research and Technology Centre of the University of Alberta following the guidelines of the Canadian Council of Animal Care (CCAC,

2009). The animal use protocol was approved by University of Alberta Livestock Care Committee (AUP 00002389). This study has been previously described in chapter 2. In brief, at 129 ± 12 DIM, six multiparous, cannulated, Holstein cows (Bar Diamond Inc., Parma, ID; Rumen Cannula, Victoria, AUS) were enrolled in a 6 x 6 Latin square design. Each period consisted of 28 days; 23-d adaptation and 5-d sample and data collection period. A common basal diet was fed for the duration of the study to meet the National Research Council (NRC) recommendations for lactating dairy cows as described in Table 2-1 (NRC, 2001). Diet ingredients were tested for trace minerals prior to the trial and the diet only incorporated ingredients with low trace mineral levels. Cows consumed the basal diet *ad libitum* throughout the 23-d adaptation period. During the 5-d data and sample collection period, cows were fed 95% of the calculated average intake from the adaptation period. Treatments consisted of organic and inorganic trace minerals supplemented at 50, 100, and 200% based on the NRC recommendations as shown in Table 2-2. Treatments were mixed with ground corn (500 g) and hand mixed with the top 10 cm of the TMR. Basal diet and treatments were fed at 0800 daily and adjusted to average dry matter intake (DMI) of each individual animal every three days during the 23-d adaptation period and DMI was held consistent with the basal diet during the 5-d sample and data collection period. Cows were housed individually in tie stalls designed to allow for total collection of urine, milk and feces and remained in these stalls for the duration of the 5-d data and sample collection period. Water was provided *ad libitum* throughout the duration of the study.

3.3.2 Data and Sample Collection

3.3.2.1 Ruminal pH

Continuous ruminal pH was recorded via the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA). Ruminal pH measurements were continuously recorded every 30 sec for the final 2-d of the sample and data collection period. Loggers were inserted into the rumen 24 h before data collection. Immediately before insertion into the rumen and following removal, pH data loggers were calibrated using buffers at pH 4 and 7. Shifts in millivolt readings from the electrodes between the days are assumed to be linear and millivolt readings were converted to pH units according to Penner et al. (2006).

3.3.2.2 Rumen Digesta and Fluid Collection

Rumen digesta was collected from five locations (cranial dorsal, cranial ventral, central, caudal dorsal and caudal ventral) on the final 2-d of the data and sample collection period before feeding and 1, 2, 3, 4, 5, 6, 12, and 18 hours following feeding. Digesta was placed onto 4 layers of perforated fabric (Cheesecloth Wipers, Uline Canada, Milton, Ontario, Canada) and strained into an aluminum tray to separate the fluid and solid fractions. Fluid and solids were collected into a 15 mL and 50 mL sterile tube, respectively. Remaining fluids and solids were placed back into the rumen via rumen cannula. Tubes were immediately snap frozen in liquid nitrogen and samples to be analyzed for microbial analysis were stored at -80°C, while all other samples were stored at -20°C until further analysis.

3.3.2.3 Blood Sampling

A 6 mL serum sample (BD Vacutainer Trace Element Serum, Franklin Lakes, NJ, USA) and 6 mL plasma sample (BD Vacutainer Trace Element K2 EDTA, Franklin Lakes, NJ, USA) were collected via the coccygeal vein using a needle and vacutainer. Blood sampling occurred daily during the 5-d sample and data collection period at 0800 before feeding and two hours after feeding at 1000 on the final 2-d of the data and sample collection period. Whole blood and

plasma samples were placed on ice until centrifugation and storage. Serum samples were incubated at room temperature for 1 hour to allow for coagulation and whole blood was immediately stored at -20°C. Plasma and serum supernatant were collected following centrifugation at 3,000 x *g* at 4°C for 20 min and transferred in equal volume aliquots into two 1.5 mL microcentrifuge tubes and frozen at -20°C until further analysis.

3.3.2.4 Milk Collection

Cows were milked twice daily in their respective tie stalls at 0400 and 1600 h. Milk production was recorded daily throughout the study. During the 5-d sample and data collection period, each cow was individually milked into a stainless-steel milk collection bucket specific to each cow. Milk was weighed and a subsample of 0.05% of the total weight was collected at each milking. Milk samples were composited per cow per day and stored at -20°C for further analysis.

3.3.3 Sample Analysis

3.3.3.1 Rumen Fluid Volatile Fatty Acid Analysis

Rumen fluid samples were thawed, vortexed for 10 seconds, and immediately preserved with 25% (wt/vol.) meta-phosphoric acid (H_2PO_4) followed by centrifugation at 18,000 x *g* for 15 min at 4°C. Supernatant was collected and centrifuged again at 18,000 x *g* for 15 min at 4°C. A 0.8 mL subsample of the supernatant and 0.2 mL of an internal standard was added to a clean, dry vial. Samples were analyzed for VFA concentrations by gas chromatography as described by Schlau et al. (2011).

3.3.3.2 DNA Extraction from Rumen Fluid and Solid Samples

Total DNA from rumen fluid and solid samples was extracted using the repeated bead beating plus column method (Yu and Morrison, 2004), respectively. Rumen fluid (1.5 mL) and solids (0.5 g) were washed twice with TE buffer followed by the addition of cell lysis buffer

containing 4% SDS. Both fluid and solid samples were physically disrupted using the Biospec Mini Beads Beater 8 (BioSpec, Bartlesville, OK) at 4,800 rpm for 3 min and immediately incubated at 70°C for 15 min. Following incubation, samples were centrifuged at 16,000 x *g* for 5 min and supernatant was collected. Bead beating, incubation and centrifugation was repeated one time with collection of supernatant. Impurities within the supernatant were removed using 7.5 M ammonium acetate, followed by DNA precipitation using isopropanol and incubation at -20°C overnight. Further purification was performed using the QIAmp fast DNA stool mini kit (Qiagen Inc., Germantown, MD). Quantity and purity of DNA was evaluated using NanoDrop 1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE) and stored at -20°C until further use.

3.3.3.3 Quantification of Total Bacteria in Rumen Fluid and Solid Samples using Quantitative Real Time PCR

Rumen fluid and solid samples were analyzed for the densities of total bacteria using quantitative real time PCR (qRT-PCR). Total bacteria from both rumen fluid and solid samples were estimated using the Viia 7 Real-Time PCR System (Applied Biosystems, Thermo-Fischer Scientific, Foster City, CA). Quantitative real time PCR was performed using SYBR green chemistry (Fast SYBR Green Master Mix, Applied Biosystems, Foster City, CA) and the primer pair U2 (forward 5'- ACTCCTACGGGAGGCAG-3'; reverse, 5'-GACTACCAGGGTATCTAATCC-3'; Stevenson and Weimer, 2007) with a product size of 467 bp and an annealing temperature of 62°C to detect the copy number of total bacteria. Amplification was performed with a program including a fast cycle and melting curve section. Specifically, the program used was 95°C for 5 min, followed by 40 cycles at 95°C for 20 s and 62°C for 20 s. Standard curve for total bacteria was generated using 16s rRNA genes of

Butyrivibrio hungatei. Copy number was calculated using the following equation as described by Li et al. (2009):

$$total\ DNA\ (ng) = DNA\ concentration\ \left(\frac{ng}{\mu l}\right) x\ elution\ volume\ (\mu l)$$

$$copy\ number\ per\ g\ (or\ ml) of\ sample = \left[\frac{\frac{total\ DNA(ng)}{qRTPCR\ concentration}\ x\ quanitity\ mean}{weight\ (or\ amount)\ of\ sample\ (g\ or\ ml)}\right]$$

3.3.3.4 Analysis of Milk, Plasma and Ruminal Fluid Vitamin B_{12} Concentrations

All vitamin B_{12} analyses were performed at the Agriculture and Agri-Food Canada Research Centre (Sherbrooke, Quebec, Canada). Milk vitamin B_{12} analysis was performed as described by Duplessis et al. (2015). In short, samples were thawed in a water bath at 37°C for 30 min. In a 15 mL polypropylene tube, 2.5 mL of milk sample, 2.5 mL Na_2HPO_4 (0.1 *M*) and 25 µL of NaCN (1 *M*) were combined and shaken. Once mixed, 100 µL of protease (protease Type XIV: bacteria, from *Streptomyces griseus*; EC number 232-909-5; Sigma Aldrich, Oakville, Ontario, Canada) was added. Samples were incubated at 37°C for 90 min, shaken frequently and autoclaved at 100°C for 5 min following incubation to stop the enzyme activity. Immediately following autoclaving, samples were placed in ice-cold water for 5 min to chill. This was followed by centrifugation at 5,000 x *g* for 10 min at 4°C and 50 µL of supernatant and 150 µL of ultrapure water were placed in a 2 mL centrifuge tube. Samples were frozen at -20°C until analysis.

To prepare the rumen fluid for analysis, 24 mL of solution (13 g anhydrous Na_2HPO_4, 12 g citric acid and 10 g sodium metasulfite, brought to 1 L volume using ultrapure water), 1 mL of ruminal fluid and 150 µL of NaCN (1 M) was combined and mixed into a 50 mL polypropylene tube. Contents were autoclaved for 10 min at 100°C to stop enzyme activity, followed by chilling

in cold water. Immediately after, NaOH 3.3 M (120 µL) was added to the solution to reach a pH between 6.2 and 6.5 and brought to a 30 mL volume with ultrapure water.

All vitamin B_{12} in rumen fluid, plasma and milk were analyzed in duplicate was analyzed in duplicate, according to the method of Allen (1983) with modifications in solutions from Santschi et al. (2005) using a commercial radioimmunoassay kit (SimulTRAC B_{12}, MP Biomedicals). In brief, 450 µL of buffer (containing the albumin), 200 µL of either the standard solutions or the sample, 600 µL of boiled buffer, 50 µL of radiolabeled vitamin B_{12} [(57CO)B12; ICN Biomedicals, Cleveland, OH], 200 µL of binder and 500 µL of charcoal solution (activated charcoal Norit®A; Sigma), to get a final volume of 2000 µL. Cow saliva were used as a binder where salvia contains haptocorrin which non-specifically binds all forms of vitamin B_{12}.

3.3.3.5 Analysis of Serum Trace Minerals

Serum trace mineral analysis was performed by the Animal Health Laboratory at the University of Guelph. Serum samples (1.5 mL) were diluted with a 1% HNO_3/ 1% isopropanol/ 0.01% TritonX-100/ 0.0% EDTA solution (1:20) before analysis with CHEM-162- inductively coupled plasma mass spectrometry (ICP-MS) (Varian/Bruker Inductively Coupled Plasma-Mass Spectrometer, Analytical West, Corona, CA) with Burgener Peek Mira Mist Nebulizer and Cetac ASX-520 auto sampler (CETAC Technologies, Omaha, Nebraska). Quantification was performed against a multi-element standard curve by ICP-MS.

3.3.3.6 Analysis of Serum Glutathione Peroxidase

Glutathione peroxidase activity was measured in serum samples using a commercial kit (Glutathione Peroxidase Assay Kit, Cayman Chemical, Ann Arbor, Michigan) as described by Paglia and Valentine (1967). Serum samples were diluted 1:3 with a sample buffer (50 mM Tris-HCl, pH 7.6, 5 mM EDTA and 1 mg/ml BSA). The diluted sample was combined with an assay

buffer (50 mM Tris-HCl, pH 7.6, 5 mM EDTA), lyophilized glutathione, glutathione peroxidase, NADPH, and cumene hydroperoxide. The oxidation of NADPH to $NADP^+$ was measured by the decrease of absorbance at 340 nm for 7 min at room temperature using an automatic analyzer (Cytation5, BioTek, Winooski, Vermont). Enzyme activity was reported as nmol/min/ml of serum.

3.3.4 Statistical Analysis

Data were analyzed using the GLIMMIX procedure of the Statistical Analysis System (version 9.3; SAS institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + C_j + P_i + M_k + D_l + MD_{kl} + E_{ijkl}$$

Where: Y_{ijkl} = dependent variable, μ = overall mean, C_j = random effect of cow j, P_i = fixed effect of period i, M_k = fixed effect of source of trace mineral k, D_l = fixed effect of concentration of trace mineral l, MD_{kl} = interaction between source of trace mineral, k, and concentration of trace mineral, l, and E_{ijkl} = residual error. All reported values are least squares mean (LSM) with significance being declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. The adjusted p-value reported shows the significance of the main effect of the treatments as a whole, whereas the data reported as least squares means are looking specifically at the differences in individual treatments.

3.4 Results

3.4.1 Effect of source and concentration of supplemental trace minerals on ruminal pH and ruminal total bacteria

Supplementation treatments had no effect on mean, minimum and maximum ruminal pH (Table 3-1). Ruminal pH parameters were largely unaffected by supplementation of trace minerals except for minimum pH where INO was higher ($P = 0.017$) than ORG. Total bacteria in

ruminal fluid and solid fractions were not affected by trace mineral supplementation treatments, nor were they affected by supplemental source of trace minerals.

3.4.2 Effect of source and concentration of supplemental trace minerals on ruminal volatile fatty acids

Feeding supplemental trace minerals at 50 ORG increased ($P = 0.025$) total ruminal VFA concentrations by 9.3% compared to the 200 ORG supplementation, with no differences between all other treatments (Table 3-2). Molar proportion of propionate was higher ($P < 0.001$) in 100 ORG than both 100 INO and 200 ORG treatments and was also higher in the 50 ORG supplementation than 100 INO treatment. Isovalerate molar proportion was higher ($P < 0.001$) in the 200 ORG than all other treatments. Molar concentration of acetate was higher ($P = 0.021$) in 100 INO compared to 50 INO and 100 ORG. There was an increase ($P = 0.001$) in molar isobutyrate proportion in 200 ORG compared to 100 INO, 200 INO, 50 ORG and 100 ORG. Acetate:propionate was higher ($P < 0.001$) in 100 INO than 50 ORG and 100 ORG, whereas 100 ORG was lower than 200 INO and 200 ORG. No difference was observed between treatments in the molar proportions of butyrate and valerate.

In regards to the source of supplemental trace minerals, ORG had higher molar proportions of propionate ($P = 0.036$), isobutyrate ($P = 0.019$) and isovalerate ($P < 0.001$) than the INO. There was a tendency for the molar proportion of acetate in INO to be higher ($P = 0.053$) than ORG and acetate:propionate was higher ($P = 0.024$) in INO compared to ORG. There was no effect of trace mineral source on total ruminal VFA concentration, and molar proportions of butyrate, and valerate.

3.4.3 Effect of source and concentration of supplemental trace minerals on serum trace mineral concentrations and glutathione peroxidase activity

Serum concentrations of cobalt increased ($P < 0.05$) with supplementation of ORG and INO at 200% compared to all other treatments (Table 3-3). Supplementation of ORG and INO trace minerals at 50, 100 and 200% did not change the serum concentration of copper, manganese, selenium, and zinc. No difference was observed between ORG and INO supplementation in serum trace mineral concentrations of cobalt, copper, manganese, selenium, and zinc.

Dietary treatments of source and concentration of trace minerals, particularly selenium, did not influence the serum GSH-Px activity in this study. Correspondingly, there was no effect on serum GSH-Px activity between INO and ORG supplementation.

3.4.4 Effect of source and concentration of supplemental trace minerals on vitamin B_{12} concentrations in serum, rumen fluid and milk

Vitamin B_{12} concentrations in plasma, rumen fluid and milk were unaffected by all treatments (Table 3-4). Source of trace minerals also had no effect on vitamin B_{12} concentrations in plasma, ruminal fluid and milk.

3.5 Discussion

To our knowledge, the present study is the first to investigate the effect of a combination of source and concentration of supplemental trace minerals on ruminal fermentation, serum trace mineral concentrations and physiological indicators of trace mineral status. It was hypothesized that organic trace minerals would be more bioavailable to rumen microbes thereby increasing VFA, antioxidant activity, and vitamin B_{12} production in the dairy cow compared to inorganic sources with low organic trace mineral supplementation providing similar responses as high inorganic supplementation. In contrast to the hypothesis, supplementing organic trace minerals at a higher level (200%) decreased total VFA concentrations compared to a low level (50%) of

organic supplementation with no difference between organic and inorganic supplementation at all levels. Furthermore, there was a shift for increased proportions of propionate, acetate, isobutyrate and isovalerate with organic supplementation. Previous work has found no difference in VFA proportions of propionate, acetate, isobutyrate, and isovalerate (Pino and Heinrichs, 2016) which is in contrast with the results found in the present study. However, in agreement with the present study, the authors found lower minimum pH in the organic trace mineral supplemented group as compared to the inorganic, but conversely observed a lower mean pH in the organic trace mineral supplemented group. It has been shown that the rumen microbial population of dairy cattle shifts with increasing age under identical feeding conditions (Kumar et al., 2015; Liu et al., 2017) and therefore, dairy cows in the present study may utilize trace minerals within the rumen differently, than heifers used by Pino and Heinrichs (2016).

High organic trace mineral supplementation (200%) decreased the total VFA concentration compared to the low level (50%) of organic trace mineral supplementation, which was also observed by Wang et al. (2009) investigating selenium yeast supplementation. The increase in propionate proportion within the rumen is consistent with findings from other studies that have investigated selenium yeast supplementation and organic zinc supplementation (Spears et al., 2004; Wang et al., 2009). Increased proportions of propionate, isobutyrate and isovalerate in the organic trace mineral supplementation experimental treatments support the decrease in the minimum pH of organic supplementation versus inorganic supplementation without a change in the mean pH between the two supplementation types. The reduction in the acetate:propionate ratio resulted from the increased proportion of propionate and the reduction in acetate in the 100% organic treatment with the reverse occurring in the 100% inorganic treatment. It is difficult to conclude why these changes occur without looking into specific microbial populations and the

effect of source and concentration of trace minerals on these populations. Further investigation into the biological implications of long term high organic supplementation of trace minerals on VFA production is required to determine whether these changes impact the productivity of lactating dairy cows.

Changes occurring in rumen fermentation and the production of VFA may be attributed to changes the rumen microbial population or their utilization of differing sources of trace minerals. A study performed by Genther and Hansen (2015) demonstrated that copper and manganese from organic sources were less ruminally soluble compared to inorganic sources. Since microorganisms can use both soluble and insoluble forms of elements in the rumen for bacterial metabolism, it is speculated that increased solubility of inorganic trace minerals provides the opportunity to bind with antagonists within the rumen rendering them unavailable for absorption and use by rumen microbes (Cao et al., 2000). Changes in the individual VFA support the notion that the type of trace mineral provided in the diet alters ruminal fermentation, suggesting rumen microbial populations may use organic trace minerals more effectively. As the total bacteria population is unaffected, it is speculated that the utilization of trace minerals by specific rumen bacteria causes a difference in VFA production, as opposed to a total population change. It is possible that decreased solubility and protection from antagonistic interaction of organic trace minerals within the rumen could be responsible for this result.

Research describing changes in total ruminal bacteria and bacterial populations in dairy cows fed organic versus inorganic trace minerals are scarce, with Kljak et al. (2017) being one of the first to characterize this effect in dairy cattle. Results from the present study are supported by Kljak et al. (2017), in which there was no change in the total ruminal bacteria when feeding inorganic versus organic trace minerals to heifers. Similarly, the same result was observed in a

study using lambs supplemented with inorganic and organic manganese (Kisidayova et al., 2018). In comparison to the current study, Kljak et al. (2017) and Kisidayova et al. (2018) also provided adequate levels of trace minerals in both organic and inorganic form suggesting that both forms of trace mineral supplementation were provided at levels sufficient for the rumen environment without reaching toxic levels that may cause a decrease in the total bacterial population.

Although the utilization of trace minerals from differing sources or concentrations within the rumen is a key component, the post absorptive effect on animal health and productivity is of equal importance. Contradictory to our hypothesis, concentration and source of trace minerals had a minimal impact on serum trace mineral concentrations and no impact on antioxidant activity and vitamin B_{12} levels. When comparing serum trace mineral concentration, previous studies have also noted no change in serum zinc, manganese and copper concentration between inorganic and organic supplementation at various dietary concentrations (Malcolm-Callis et al., 2000; Kinal et al., 2005; Corinthas et al., 2012). Cellular uptake mechanisms are critical in maintaining trace mineral homeostasis through post-translational modification. Ubiquination is a common and highly regulated post-translational regulatory mechanism for the degradation of cobalt, copper, manganese, and zinc trace mineral transporters which allows these transporters to adapt rapidly to cellular and systemic signals to homeostatically regulate ion transport (Hennigar and McClung, 2016). Since trace mineral transporters can quickly respond and regulate uptake at the level of cellular level, homeostasis of blood trace mineral concentration occurs regardless of the concentration of trace minerals provided in the diet.

Similar to the results of the present study, Awadeh et al. (1998) and Corinthas et al. (2012) did not observe a difference in serum or plasma selenium concentrations between various

dietary concentrations of inorganic selenium or organic selenium. In contrast, whole blood concentrations of selenium were higher for organic supplemented selenium compared to inorganic supplemented cows (Ortman and Pherson, 1999; Knowles et al., 1999; Gunter et al., 2003). Differences obtained in the results from previous studies to the current results could be due to serum being examined as opposed to whole blood. Whole blood concentrations of selenium are more indicative of long-term selenium status, due to selenium's involvement in GSH-Px and its incorporation into erythrocytes at the erythropoiesis stage, whereas plasma and serum reflect the short-term status (Gerlof, 1992). Therefore, the lack of change observed in blood selenium could be attributed to the sufficient amounts of selenium provided in the short term by basal diet before supplementation. Higher supplementation levels of organic selenium have shown increased activity of GSH-Px over a longer supplementation period than the present study (Ortman and Pherson, 1997; Gong and Xiao, 2018). However, other studies in lactating dairy cows and heifers have observed similar GSH-Px results as those observed in the present study (Ortman and Pherson, 1999, Juniper et al., 2006; Pino and Heinrichs, 2016). A possible explanation for the differences between studies is that each period in the present study only consisted of 28 days, where as it was noted that a plateau in both selenium blood concentration and GSH-Px was not observed until 119 days and 50 days of supplementation, respectively (Ortman and Pherson, 1997). Therefore, a 28 day period may not have been adequate to allow for selenium balance to occur.

Although selenium serum concentrations and its selenium dependent GSH-Px did not show any difference between treatments, cobalt serum concentrations displayed a different result where the 200% supplementation of inorganic and organic cobalt was higher. In contrast to the present study, there were no differences in serum cobalt concentrations in two separate studies

that compared multiple dietary cobalt supplementation levels (Kincaid et al., 2003; Kincaid and Socha, 2007). The basal diet provided by both studies is similar to the diet in the present study, where levels of trace minerals, including cobalt, exceeded the NRC recommendation prior to supplementation of the treatments (Table 2-1). Vitamin B_{12} synthesis is the only known function of cobalt within the ruminant and it cannot be re-absorbed into the rumen from the body for vitamin B_{12} synthesis (van den Top, 2005). Therefore, there are two possible explanations for the increase in serum concentration when cobalt is supplemented at levels as high as 200% above those provided in the basal diet, with the first being that an overload of the absorption mechanisms occurred, causing an increase in the serum cobalt concentrations directly from the level of absorption. Secondly, cobalt can also be stored to some degree in the heart, liver, kidneys and spleen in humans (Czarnek et al., 2015), but has not been investigated in ruminants. With levels reaching 200%, it is possible that the ruminant also stores cobalt in these areas and reaches a maximum capacity of cobalt storage with excess being excreted, resulting in increased cobalt serum levels. With a large difference between supplemental levels of trace minerals between treatments and no response in serum concentrations, serum trace mineral status may not be a reliable indicator of trace mineral status. In addition to nutrition, other factors such as homeostatic control, physiological state and age can affect serum trace mineral concentrations (Herdt and Hoff, 2011) Therefore, limitations exist in using serum as an indicator of nutritional trace mineral status.

The results of dietary supplementation of organic and inorganic cobalt has shown various results including: increased dietary cobalt supplementation increasing vitamin B_{12} synthesis (Stemme et al., 2008), a tendency for higher plasma vitamin B_{12} concentrations in low organic cobalt supplementation than high organic cobalt supplementation (Akins, 2013), no difference in

serum vitamin B_{12} concentrations (Waterman et al., 2017), and no difference in milk vitamin B_{12} with increasing dietary concentration or differing source of cobalt supplementation (Stemme et al., 2006; Kincaid and Socha, 2007; Akins et al., 2013). In contrast to the present results, Quirk and Norton (1988) found that milk vitamin B_{12} concentration increased in Hereford cows when supplemented with cobalt compared to the control. However, unlike cows in the present study, the control cows were considered to be in inadequate cobalt status indicative of their low serum vitamin B_{12} concentrations before supplementation (Quirk and Norton, 1988). Supplemental cobalt at two levels (0.1 mg/d cobalt to meet the minimum requirement and 4.0 mg/d cobalt to exceed requirements) to deficient sheep stimulated an increase in rumen vitamin B_{12} concentrations 5 days after supplementation (Weise et al., 2007). However, in our study, the basal diet met the minimum requirement of cobalt required for lactating dairy cows, therefore even the 50% supplementation treatment provided enough cobalt for the proper function of rumen microbes. Due to observing no difference with excess supplementation, it is speculated that rumen microbes only use cobalt as required and excess does not have an effect on producing excess vitamin B_{12} in the rumen. In addition studies have also shown a higher proportion of forage in the diet increases vitamin B_{12} synthesis from rumen microbes (Smith and Marston, 1970; Sutton and Elliot, 1972; Schwab et al., 2006). It is possible that the high inclusion of forage within this study was optimal for vitamin B_{12} synthesis by rumen microbes, therefore no change would be observed in vitamin B_{12} within the plasma, milk and rumen fluid, even with varying concentrations and sources of cobalt.

3.6 Conclusion

To our knowledge, this is the first study to compare the effects of concentration and source of trace minerals on rumen fermentation and biological indicators of trace mineral status.

It was determined that concentration and source of trace minerals supplemented to mid- to late-lactation dairy cows does not impact serum trace mineral status with the exception of cobalt at high levels of organic supplementation. From the results of this study, concentration and source of trace minerals does not impact the serum GSH-Px activity or the concentration of vitamin B_{12} concentration in plasma, ruminal fluid or milk. In addition, treatments did affect the rumen environment in a dose dependent manner where high organic supplementation (200%) was not beneficial in the production of total VFA compared to low (50%) organic supplementation. Furthermore, source and concentration of trace minerals cause a shift in the proportions of certain major and minor VFA excluding butyrate and valerate. Organic trace mineral supplementation decreased the minimum rumen pH compared to inorganic supplementation and it is speculated that these changes are due to the increased availability of organic trace minerals by rumen microbes or due to shifts in bacterial populations. In conclusion, trace mineral utilization and impact on the overall function of the rumen and rumen microbiome is largely under-studied and further research is necessary to understand the optimal supplementation of inorganic and organic trace minerals for the ruminant.

3.7 Literature Cited

Akins, M., S. Bertics, M. Socha, and R. Shaver. 2013. Effects of cobalt supplementation and vitamin B_{12} injections on lactation performance and metabolism of Holstein dairy cows. Journal of Dairy Science. 96:1755–1768. doi:10.3168/jds.2012-5979.

Andrieu, S. 2008. Is there a role for organic trace element supplements in transition cow health? The Veterinary Journal,176:77-83. doi:10.1016/j.tvjl.2007.12.022

Arthur, J.R. 2000. The glutathione peroxidases. Cellular and Molecular Life Sciences. 57:1825-1835.

Awadeh, F., M. Abdelrahman, R. Kincaid, and J. Finley. 1998. Effect of selenium supplements on the distribution of selenium among serum proteins in cattle. Journal of Dairy Science. 81:1089–1094. doi:10.3168/jds.s0022-0302(98)75670-x.

Cao, J., P.R. Henry, C.B. Ammerman, R.D. Miles, and R.C. Littell. 2000. Relative bioavailability of basic zinc sulfate and basic zinc chloride for chicks. Journal of Applied Poultry Research. 9:513–517.

Cortinhas, C.S., J.E.D.F. Júnior, J.D.R. Naves, M.A.D.F. Porcionato, L.F.P.E. Silva, F.P. Rennó, and M.V.D. Santos. 2012. Organic and inorganic sources of zinc, copper and selenium in diets for dairy cows: intake, blood metabolic profile, milk yield and composition. Revista Brasileira de Zootecnia. 41:1477–1483. doi:10.1590/s1516-35982012000600023.

Czarnek, K., S. Terpiłowska, and A.K. Siwicki. 2015. Review paper selected aspects of the action of cobalt ions in the human body. Central European Journal of Immunology. 2:236–242. doi:10.5114/ceji.2015.52837.

Duplessis, M., S. Mann, D. Nydam, C. Girard, D. Pellerin, and T. Overton. 2015. Short communication: Folates and vitamin B_{12} in colostrum and milk from dairy cows fed different energy levels during the dry period. Journal of Dairy Science. 98:5454–5459. doi:10.3168/jds.2015-9507.

Finney, L.A., and T.V. O'Halloran. 2003. Transition metal speciation in the cell: Insights from the chemistry of metal ion receptors. Science. 300:931–936. doi:10.1126/science.1085049.

Genther, O.N., and S.L. Hansen. 2015. The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. Journal of Dairy Science. 98:566-573.

Gerloff, B.J. 1992. Effect of selenium supplementation on dairy cattle. Journal of Animal Science. 70:3934–3940. doi:10.2527/1992.70123934x.

Goff, J.P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of Dairy Science. 101:2763–2813. doi:10.3168/jds.2017-13112.

Gong, J., and M. Xiao. 2018. Effect of organic selenium supplementation on selenium status, oxidative stress, and antioxidant status in selenium-adequate dairy cows during the periparturient period. Biological Trace Element Research. 186:430–440. doi:10.1007/s12011-018-1323-0.

Gunter, S.A., P.A. Beck, and J.M. Phillips. 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. Journal of Animal Science. 81:856–864.

Hennigar, S.R., and J.P. Mcclung. 2015. Homeostatic regulation of trace mineral transport by ubiquitination of membrane transporters. Nutrition Reviews. 74:59–67. doi:10.1093/nutrit/nuv060.

Herdt, T.H., and B. Hoff. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Veterinary Clinics of North America: Food Animal Practice. 27:255–283. doi:10.1016/j.cvfa.2011.02.004.

Jaing, B. 2006. The effect of trace elements on the metabolism of methanogenic consortia. Doctoral dissertation. Wageningen University, Wageningen, Gelderland, Netherlands.

Juniper, D., R. Phipps, A. Jones, and G. Bertin. 2006. Selenium supplementation of lactating dairy cows: Effect on selenium concentration in blood, milk, urine, and feces. Journal of Dairy Science. 89:3544–3551. doi:10.3168/jds.s0022-0302(06)72394-3.

Kinal, S., A. Korniewicz, D. Jamroz , R. Zieminski and M. Slupczynska. 2005. Dietary effects of zinc, copper and manganese chelates and sulphates on dairy cows. Journal of Food, Agriculture & Environment. 1:168-172.

Kincaid, R., and M. Socha. 2007. Effect of cobalt supplementation during late gestation and Early Lactation on Milk and Serum Measures. Journal of Dairy Science. 90:1880–1886. doi:10.3168/jds.2006-296.

Kincaid, R., L. Lefebvre, J. Cronrath, M. Socha, and A. Johnson. 2003. Effect of dietary cobalt supplementation on cobalt metabolism and performance of dairy cattle. Journal of Dairy Science. 86:1405–1414. doi:10.3168/jds.s0022-0302(03)73724-2.

Kišidayová, S., P. Pristaš, M. Zimovčáková, M.B. Wencelová, L. Homolová, K. Mihaliková, K. Čobanová, Ľ. Grešáková, and Z. Váradyová. 2018. The effects of high dose of two manganese supplements (organic and inorganic) on the rumen microbial ecosystem. Plos One. 13. doi:10.1371/journal.pone.0191158.

Kljak, K., F. Pino, K.J. Harvatine, and A.J. Heinrichs. 2017. Analysis of selected rumen microbial populations in dairy heifers limit fed diets varying in trace mineral form and starch content. Livestock Science. 198:93–96. doi:10.1016/j.livsci.2017.02.012.

Knowles, S.O., N.D. Grace, K. Wurms, and J. Lee. 1999. Significance of amount and form of dietary selenium on blood, milk, and casein selenium concentrations in grazing cows. Journal of Dairy Science. 82:429– 437.

Kumar, S., N. Indugu, B. Vecchiarelli, and D.W. Pitta. 2015. Associative patterns among anaerobic fungi, methanogenic archaea, and bacterial communities in response to changes in diet and age in the rumen of dairy cows. Frontiers in Microbiology. 6. doi:10.3389/fmicb.2015.00781.

Liu, C., Q. Meng, Y. Chen, M. Xu, M. Shen, R. Gao, and S. Gan. 2017. Role of age-related shifts in rumen bacteria and methanogens in methane production in cattle. Frontiers in Microbiology. 8. doi:10.3389/fmicb.2017.01563.

Malcolm-Callis, K.J., G.C. Duff, S.A. Gunter, E.B. Kegley, and D.A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing beef steers. Journal of Animal Science. 78:2801. doi:10.2527/2000.78112801x.

McDowell, L.R. 2000. Vitamins in animal and human nutrition. 2nd ed. Iowa State University Press, Ames.

NRC. 2001. Nutrient Requirements of Dairy Cows, 7th edn. National Academy of Sciences.

Ortman, K., and B. Pehrson. 1997. Selenite and selenium yeast as feed supplements for dairy cows. Journal of Veterinary Medicine Series A. 44:373–380. doi:10.1111/j.1439-0442.1997.tb01121.x.

Ortman, K., and B. Pehrson. 1999. Effect of selenate as a feed supplement to dairy cows in comparison to selenite and selenium yeast. Journal of Animal Science. 77:3365. doi:10.2527/1999.77123365x.

Paglia, D.E. and Valentine, W.N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory Clinical Medicine. 70:158-169.

Penner, G.B., K.A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. Journal of Dairy Science. 89:2132-2140.

Pino, F., and A. Heinrichs. 2016. Effect of trace minerals and starch on digestibility and rumen fermentation in diets for dairy heifers. Journal of Dairy Science. 99:2797–2810. doi:10.3168/jds.2015-10034

Quirk, M.F., and B.W. Norton. 1988. Detection of cobalt deficiency in lactating heifers and their calves. The Journal of Agricultural Science. 110:465–470. doi:10.1017/s0021859600082010.

Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, and W.G. Hoekstra. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 179:588–590. doi:10.1126/science.179.4073.588.

Schlau, N., L.L. Guan, and M. Oba. 2011. The relationship between rumen acidosis resistance and expression of genes involved in regulation of intracellular pH and butyrate metabolism of ruminal epithelial cells in steers. Journal of Dairy Science. 95:5866- 5875.

Schwab, E., C. Schwab, R. Shaver, C. Girard, D. Putnam, and N. Whitehouse. 2006. Dietary forage and non-fiber carbohydrate contents influence B-vitamin intake, duodenal flow, and apparent ruminal synthesis in lactating dairy cows. Journal of Dairy Science. 89:174–187. doi:10.3168/jds.s0022-0302(06)72082-3.

Schwartzkopf-Genswein, K.S., K.A. Beauchemin, T.A. Mcallister, D.J. Gibb, M. Streeter, and A.D. Kennedy. 2004. Effect of feed delivery fluctuations and feeding time on ruminal acidosis, growth performance, and feeding behavior of feedlot cattle. Journal of Animal Science. 82:3357–3365. doi:10.2527/2004.82113357x

Smith, R.M., and L.H.R. Marston. 1970. Production, absorption, distribution and excretion of vitamin B_{12} in sheep. British Journal of Nutrition. 24:857–877. doi:10.1079/bjn19700092.

Sobhanirad, S., R. Valizadeh, F. Azizi, M. Hedayati, and A. Tahmasebi. 2009. Effect of zinc sources on haematology, enzyme activity and blood serum zinc concentration in Holstein dairy cattle. Proceedings of the British Society of Animal Science. 2009:152–152. doi:10.1017/s1752756200029914.

Spears, J., P. Schlegel, M. Seal, and K. Lloyd. 2004. Bioavailability of zinc from zinc sulfate and different organic zinc sources and their effects on ruminal volatile fatty acid proportions. Livestock Production Science. 90:211–217. doi:10.1016/j.livprodsci.2004.05.001.

Stemme, K., P. Lebzien, G. Flachowsky, and H. Scholz. 2008. The influence of an increased cobalt supply on ruminal parameters and microbial vitamin B_{12} synthesis in the rumen of dairy cows. Archives of Animal Nutrition. 62:207–218. doi:10.1080/17450390802027460.

Stemme, K., U. Meyer, G. Flachowsky, and H. Scholz. 2006. The influence of an increased cobalt supply to dairy cows on the vitamin B_{12} status of their calves. Journal of Animal Physiology and Animal Nutrition. 90:173–176. doi:10.1111/j.1439-0396.2005.00584.x.

Stevenson, D.M., and P.J. Weimer. 2007. Dominance of Prevotella and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Applied Microbiology Biotechnology. 75:165–174.

Sutton, A.L., and J.M. Elliot. 1972. Effect of ratio of roughage to concentrate and level of feed intake on ovine ruminal vitamin B_{12} production. The Journal of Nutrition. 102:1341–1346. doi:10.1093/jn/102.10.1341.

van den Top, A.M. 2005. Reviews on the mineral provision in ruminants (X): cobalt metabolism and requirements in ruminants. Lelystad: Centraal Veevoederbureau.

Vinus, S. 2017. Organic minerals in poultry. Advances in Research. 12:1–10. doi:10.9734/air/2017/37878.

Wang, C., Q. Liu, W. Yang, Q. Dong, X. Yang, D. He, P. Zhang, K. Dong, and Y. Huang. 2009. Effects of selenium yeast on rumen fermentation, lactation performance and feed digestibilities in lactating dairy cows. Livestock Science. 126:239–244. doi:10.1016/j.livsci.2009.07.005.

Waterman, R.C., W.L. Kelly, C.K. Larson, and M.K. Petersen. 2017. Comparison of supplemental cobalt form on fibre digestion and cobalamin concentrations in cattle. The Journal of Agricultural Science. 155:832–838. doi:10.1017/s0021859617000107.

Wiese, S.C., C.L. White, I.H. Williams, and J.G. Allen. 2007. Relationships between plasma methylmalonic acid and ruminal succinate in cobalt-deficient and -repleted sheep. Australian Journal of Agricultural Research. 58:367. doi:10.1071/ar05125.

Yu, Z. and M. Morrison. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques. 36:808-812.

3.8 Tables and Figures

Table 3-1. Ruminal pH parameters and total bacteria in ruminal fluid and solid fraction in lactating dairy cattle fed a diet supplemented with different source and concentration of trace minerals[1]

	Treatments[2]								
	Inorganic (INO), %			Organic (ORG), %				*P*-value	
Item	50	100	200	50	100	200	SEM	Source x Conc	INO vs. ORG
Ruminal pH									
Mean	6.42	6.57	6.56	6.46	6.47	6.52	0.066	0.102	0.243
Min	5.61	5.82	5.86	5.62	5.57	5.66	0.079	0.130	0.017
Max	7.20	7.35	7.40	7.25	7.33	7.35	0.115	0.763	0.940
Total bacteria, copy number/ml (log10)									
Ruminal fluid	11.01	11.00	10.99	10.91	11.04	11.04	0.081	0.634	0.865
Ruminal solids	11.48	11.45	11.48	11.54	11.50	11.40	0.092	0.712	0.877

[1] Values are least squares mean obtained by a 6 x 6 Latin square design

[2] Lactating dairy cows were fed a total mixed ration supplemented with organic and inorganic sources of cobalt (Co), copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) at 50, 100, and 200 % of NRC requirements (100% treatment; Co; 0.25, Cu; 15.70, Mn; 40.00, Se; 0.30, and Zn; 63.00 ppm)

Table 3-2. Ruminal concentrations of VFA of lactating dairy cows fed a diet supplemented with different source and concentration of trace minerals[1]

	Treatments[2]								
	Inorganic (INO), %			Organic (ORG), %				*P*-value	
Item	50	100	200	50	100	200	SEM	Source x Conc	INO vs. ORG
Total VFA, mM[3]	88.82^{ab}	88.41^{ab}	89.51^{ab}	93.16^{a}	92.08^{ab}	84.47^{b}	3.389	0.025	0.525
VFA, mol/100 mol									
Propionate	0.204^{abc}	0.199^{c}	0.203^{abc}	0.206^{ab}	0.209^{a}	0.200^{bc}	0.0042	< 0.001	0.036
Acetate	0.622^{b}	0.633^{a}	0.631^{ab}	0.623^{ab}	0.620^{b}	0.630^{ab}	0.006	0.021	0.053
Butyrate	0.12	0.11	0.11	0.12	0.11	0.11	0.003	0.773	0.979
Isobutyrate	0.011^{ab}	0.010^{b}	0.010^{b}	0.010^{b}	0.010^{b}	0.012^{a}	0.0004	0.001	0.019
Valerate	0.02	0.01	0.01	0.02	0.02	0.01	0.001	0.106	0.267
Isovalarate	0.020^{b}	0.020^{b}	0.020^{b}	0.020^{b}	0.020^{b}	0.022^{a}	0.001	< 0.001	< 0.001
Acetate : Propionate	3.05^{abc}	3.18^{a}	3.10^{ab}	3.02^{bc}	2.96^{c}	3.15^{ab}	0.090	< 0.001	0.024

[1] Values are least squares mean obtained by a 6 x 6 Latin square design

[2] Lactating dairy cows were fed a total mixed ration supplemented with organic and inorganic sources of cobalt (Co), copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) at 50, 100, and 200 % of NRC requirements (100% treatment; Co; 0.25, Cu; 15.70, Mn; 40.00, Se; 0.30, and Zn; 63.00 ppm)

[3] Treatments with different superscripts within a row indicate significant differences among the means between treatments at $P < 0.05$.

Table 3-3. Serum trace mineral concentrations and glutathione peroxidase activity (GSH-Px) of lactating dairy cattle fed a diet supplemented with different source and concentration of trace minerals[1]

	Treatments[2]								
	Inorganic (INO), %			Organic (ORG), %				*P*-value	
Item	50	100	200	50	100	200	SEM	Source x Conc[4]	INO vs. ORG
Serum trace mineral concentration, μg/ml									
Co[3]	1.05[b]	1.14[b]	1.73[a]	1.11[b]	1.23[b]	1.66[a]	0.115	0.373	0.384
Cu	0.99	1.00	0.97	1.03	1.00	0.98	0.055	0.519	0.247
Mn	3.43	3.30	4.04	3.56	3.65	3.63	0.335	0.342	0.839
Se	0.10	0.11	0.11	0.11	0.11	0.11	0.004	0.338	0.444
Zn	0.83	0.77	0.87	0.83	0.83	0.79	0.074	0.028	0.613
GSH-Px activity, mmol/min/ml	158.49	167.57	148.65	166.01	158.44	157.77	23.364	0.661	0.769

[1] Values are least squares mean obtained by a 6 x 6 Latin square design

[2] Lactating dairy cows were fed a total mixed ration supplemented with organic and inorganic sources of cobalt (Co), copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) at 50, 100, and 200 % of NRC requirements (100% treatment; Co; 0.25, Cu; 15.70, Mn; 40.00, Se; 0.30, and Zn; 63.00 ppm)

[3] Treatments with different superscripts within a row indicate significant differences among the means between treatments at $P < 0.05$.

[4] The adjusted p-value reported shows the significance of the main effect of the treatments as a whole, whereas the data reported as least squares means is effect of the differences in individual treatments.

Table 3-4. Vitamin B_{12} concentrations in plasma, ruminal fluid and milk of lactating dairy cattle fed a diet supplemented with different source and concentration of trace minerals[1]

	Treatments[2]								
	Inorganic (INO), %			Organic (ORG), %				*P*-value	
Item	50	100	200	50	100	200	SEM	Source x Conc[3]	INO vs. ORG
Vitamin B_{12}, pg/mL									
Ruminal fluid	35385	38415	35410	30367	34847	41465	3734.8	0.339	0.733
Plasma	261.91	298.81	280.48	291.29	263.87	313.41	23.754	0.069	0.503
Milk	4616.1	4829.9	4978.3	5123.7	4598.0	4584.6	429.70	0.020	0.753

[1] Values are least squares mean obtained by a 6 x 6 Latin square design

[2] Lactating dairy cows were fed a total mixed ration supplemented with organic and inorganic sources of cobalt (Co), copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) at 50, 100, and 200 % of NRC requirements (100% treatment; Co; 0.25, Cu; 15.70, Mn; 40.00, Se; 0.30, and Zn; 63.00 ppm)

[3] The adjusted p-value reported shows the significance of the main effect of the treatments as a whole, whereas the data reported as least squares means is effect of the differences in individual treatments.

4.0 General Discussion

4.1 Major Findings

This experiment was designed to address knowledge gaps pertaining to trace mineral nutrition in regards to absorption, retention, performance, rumen fermentation and physiological indicators of trace mineral status. The present study is the first to investigate the effect of source and concentration of trace mineral supplementation and to our knowledge is the only study to determine the effect of the combined supplementation of five trace minerals in lactating dairy cows. The objectives of this thesis were to investigate the effects of source and concentration of supplemental trace minerals (organic versus inorganic) in lactating dairy cows on: 1) apparent absorption and apparent retention, 2) performance, 3) rumen fermentation parameters, and 4) physiological indicators of trace mineral status such as vitamin B_{12} and GSH-Px. Knowledge regarding the combination of multiple trace minerals, inorganic and organic sources, and concentration is crucial in understanding the effects supplementation may have in the practical application for dairy nutritionists and producers.

The major findings of this thesis include:

1) Apparent absorption and apparent retention of trace minerals is minimally affected by source and concentration of supplemental trace minerals.
2) Higher levels of trace mineral supplementation, regardless of source, increase the amount of trace minerals excreted in the feces.
3) Source and concentration of trace minerals affected the rumen environment in which high levels of organic supplementation were not beneficial in the production of total VFA and organic supplementation caused a shift in the proportions of the major and minor VFA, excluding butyrate and valerate.

4) Organic trace mineral supplementation decreased milk yield and milk fat yield compared to inorganic supplementation; however, low levels of organic trace mineral supplementation provided the same milk yield as all levels of inorganic supplementation.

4.2 Industry Implications

This study is one of the first to document a decrease in milk production with organic trace mineral supplementation and lower milk production at high (200%) organic supplementation compared to lower levels (50% and 100%) of inorganic trace mineral supplementation. However, it must be noted that even 50% supplementation of trace minerals still reached a level above 100% of the NRC recommendations due to the basal diet contributing over 50-80% above the NRC recommendations. As replacement of organic trace mineral supplementation has been gaining popularity within the dairy industry, these results provide nutritionists and producers with the necessary information required to replace inorganic sources of trace minerals with their organic counterparts. When transitioning to total replacement of inorganic trace minerals with organic sources, lower levels can be supplemented without impacting milk production, decreasing both trace mineral inputs and excess excretion.

It is important to note that care must be taken in the supplementation of organic trace minerals by dairy nutritionists and producers. Supplementing high levels of trace minerals is a current strategy used to avoid deficiencies and overcome antagonistic interactions within the gastrointestinal tract, however these high levels of supplementation can increase fecal excretion and have a negative impact on the environment (Lopez-Alonso, 2012). Current industry practices involve supplementing inorganic trace minerals at the high levels provided in this study, which demonstrate that producers may be able to provide low levels of organic supplementation as a

replacement for high levels of inorganic supplementation to obtain similar absorption, retention and productivity of their herds. As over-supplementation occurred under a research setting due to differences in analytical methods, it is highly likely that nutritionists and producers are underestimating the trace mineral intake in the basal diets of their herds. This demonstrates that dairy producers can benefit from more consistent and efficient supplementation of both sources of trace minerals, thereby decreasing feed costs and increasing profitability while maintaining animal performance and health. Additionally, there will be a reduction in the excretion of excess minerals, thereby reducing impact on soils and water reservoirs. Therefore, this research has a positive impact on both the profitability and sustainability of the dairy industry.

4.3 Limitations

Before the experimental trial began, feed ingredients were tested using wet chemistry at a commercial feed analysis company for trace mineral levels. Diets were balanced based on basal diet trace mineral levels, which were originally reported as being less than 20% of the NRC recommendation and less than 4% for water contributions for each trace mineral. Upon further analysis of the individual feed ingredients and water following the trial utilizing ICP-MS, it was determined that trace mineral levels above the NRC recommendations were met before supplementation of the trace mineral treatments ([minerals provided by diet: Co, 0.53 ppm; Cu, 54.80 ppm; Mn, 253 ppm; Se, 0.84 ppm; Zn, 231 ppm] [water contributions: Co, < MDL; Cu, 31%; Mn, <MDL; Se, <MDL; Zn, 4%]).

Although a consistent basal diet was fed throughout the trial, a diet lower in trace minerals might have provided different results where treatments would have been contributing to trace mineral levels at a higher proportion. It would have allowed for the 50% levels of trace mineral supplementation to still be reaching levels below the recommendation in order to give an

accurate representation of a low supplementation level. Throughout all treatments, including the 50% level, cows were over-supplemented and provided trace mineral levels above the NRC recommendations.

The Latin square experimental design is another limitation to this study as the ruminant animal has the ability to store trace minerals, and therefore some storage of trace minerals may have attributed to a carryover effect, even with the 25-d adaptation period to each diet. Furthermore, the location of the total collection stalls within the milking barn could be a contributing factor to the high fecal excretion values reported through the possibility of environmental contamination of samples. Total collection stalls were designed and placed at the far end of the milking barn as to not interfere with regular barn and research activities. However, this placement corresponds with the ventilation system, with all air being drawn to the location of the total collection stalls (Lillie, 1970; Respiratory Health Hazards in Agriculture, 1998). These stalls were also in close proximity to the manure handling system, where gutters draw waste from the rest of the barn. All of these factors may have contributed to the high fecal values and low absorption and retention values that cannot be explained in the results. These values therefore may have contributed to contamination of feed within in the feed bunks, resulting in underestimating feed intake, or contamination of the feces in the collection trays, thus overestimating the output.

A separate 24 h trial was performed in an attempt to determine whether there was any contamination of feed or feces from the feed bunks, stainless steel feces collection trays, or from air within the barn. Feed bunks and stainless steel trays were placed in close proximity to the trial location and samples of feed and feces were obtained in 6 h increments over a 24 h period. Overall, trace mineral levels remained consistent over the 24 h period with no indication of

contamination from the feed bunks, stainless steel collection trays, or in air. However, the original trial occurred over the summer months (May to October) and the contamination trial was performed in late October; therefore, only two ventilation fans were being used at the time of the contamination trial as opposed to all 8 fans used over the summer months. Therefore, increase use of ventilation over the summer months may still be a possibility for increased contamination of the feed and feces that was noted in the original experiment. However, from this contamination trial it is highly unlikely that contamination from materials in the feed bunks and stainless steel trays were a factor that influenced the results (Lillie, 1970; Respiratory Health Hazards in Agriculture, 1998).

Copper, zinc and, to some extent cobalt, as cobalamin, are stored within tissues and under stressful conditions cows may enter a catabolic state, with the mineral stores being mobilized (Czarnek, 2015; Shkurashivska and Ersteniuk, 2019). Cows within the trial were confined to stalls for the 5-d data and sample collection period, and although care was taken to reduce stress during this time period, consistent movement of people, sample collection may have led to stressful conditions, therefore leading to the mobilization of trace minerals stored during the dietary adaptation period. Increased excretion from stress can only be speculated, and is difficult to quantify. However, throughout the trial cows maintained body weight over the sample period with the average weight being 641.5 ± 55 kg (bodyweight ± SD). The standard deviation for the body weight for individual cows ranged from the lowest at 6 kg to the highest at 23 kg. Therefore, it is unlikely that this amount of mobilization would have a large contribution to the excess minerals in the feces, however must be noted as it may have contributed to some excess mineral excretion. It is more likely that, as noted above, environmental contamination of feed intake and/or feces, once excreted, caused the high fecal excretion values.

4.4 Future Research

The proper design of experiments and experimental models is critical in ensuring the success of trace mineral research. The complexity of animal experiments involving total collections to determine trace mineral excretion, absorption and retention often results in difficult experimental design. Future trace mineral research should avoid a Latin square design in efforts to avoid carryover effects which may occur due to storage of minerals in animal tissues. Since minerals can be stored within the body, there is the possibly for cows to utilize these minerals in times of deficiency or stress (Czarnek, 2015; Shkurashivska and Ersteniuk, 2019). Since cows were receiving adequate supplementation in all treatments due to high trace mineral levels in the basal diet, it is more likely that stress would be a greater contributing factor than deficiency. Although the 23-d adaptation period is designed to eliminate a carryover effect, our results suggest that future studies should be completed using a completely randomized design, which eliminates the need for an adaptation period, to validate that results were due to the dietary treatments provided and not due to a carryover effect of minerals stored in the body. In addition, the individual ingredients of the basal diet should be routinely tested using several types of analytical methods for months prior to the study to accurately measure trace mineral levels at the research station, with the basal diet contributing the least amount of trace minerals possible. Trace mineral treatments should therefore be consistent with the suggested supplementation levels in the current trial, allowing for more accurate information regarding supplementation below, at and above NRC recommendations. As selenium balance is one of the few trace minerals to be investigated over time, cows should be fed for at least 50 days as to allow for the body to adapt to dietary treatments in order for differences in selenium to be observed (Ortman

and Pherson, 1997). Other trace mineral balances should be investigated over this same time period in order to determine the time it takes for mineral balances within the body to occur.

In order to avoid the possibility of environmental contamination, cows should be kept in an isolated environment free from contaminants of the milking barn as airborne particles could be a source of contamination in both the feed and feces. Feed should be hand mixed within a separate, clean, plastic bin for each cow to help minimize contamination from other feed ingredients used for non-experimental cows, and of metal which is the structural component of both the feed mixer and feed delivery wagon. There is a possibility that minerals from the other supplements entering the mixer and feed delivery wagon may have contaminated the diet, increasing the amount of mineral provided by the basal diet.

Source and concentration of dietary supplementation of trace minerals had an impact on performance; however minimally impacted apparent absorption and retention in lactating dairy cows. These results suggest differences between the two sources may be attributed to the use within the rumen as opposed to differences in absorption and utilization in biological processes post-absorption. The mechanisms of absorption resulting in changes in performance and physiological responses can only be speculated and how the chemical form of trace minerals is impacted by the rumen environment needs to be investigated further. Currently, it is speculated that organic sources are protected from dissociation and therefore, protected from antagonistic interactions within the GIT compared to their inorganic counterparts (Vinus, 2017). Results from this study lead to the conclusion that there are differences occurring between organic and inorganic sources in specific trace minerals, namely selenium and cobalt, which may occur due to their ability to dissociate and bind with antagonists thus rendering them unavailable for absorption or use by rumen microbes. However, not all minerals displayed the same result and

differences may exist between specific trace minerals. More research is required to understand the differences in dissociation and binding of antagonists within the rumen between inorganic and organic sources.

Since the majority of trace mineral research has been completed in monogastric species, and the NRC recommendations for lactating dairy cows are formulated as such, it is crucial that trace mineral interactions and effects within the rumen are further explored (Suttle, 2010). Results showed differences in total VFA and specific VFA proportions with no change in total bacteria, which has been noted in previous studies (Pino and Heinrichs, 2016; Kljak et al., 2017). To our knowledge, Kljak et al. (2017) is the only study investigating changes in specific bacterial communities within the rumen following inorganic and organic supplementation. However, this experiment only investigated the impact of source on rumen bacterial communities, therefore, changes in specific communities based on both source and dietary concentration trace minerals needs to be investigated further. Organic trace minerals do not dissociate within the rumen as effectively as inorganic sources, making them less ruminally soluble (Genther and Hansen, 2015). It is speculated that the increased dissociation and therefore, increased solubility of inorganic trace minerals, allows them to bind with antagonists within the rumen more readily, rendering them unavailable for absorption or for use by rumen microbes, allowing for increased bioavailability of organic sources (Cao et al., 2000). Further research is required to understand whether these interactions in the rumen are due to the difference between organic and inorganic trace minerals and their bioavailability to both the animal and rumen microorganisms. The differences in rumen microbial interactions, solubility of trace minerals, and the use of trace minerals by microbes before absorption make the rumen a complex system, and therefore more

information is required to accurately supplement ruminants to maximize herd productivity and reduce excretion of excess minerals into the environment.

4.5 Conclusion

It was hypothesized that that organic sources of trace minerals would have a higher bioavailability compared to their inorganic counterparts, as they are protected from most antagonistic interactions in the GIT, allowing for increased absorption, retention, performance and utilization for metabolic purposes and by rumen microbes at a lower concentration compared to inorganic sources. These results suggest, low levels of organic trace mineral supplementation can replace inorganic trace mineral supplementation without observing changes in productivity, apparent absorption or apparent retention. However, high levels of organic trace mineral supplementation may decrease production compared to low levels of inorganic supplementation in lactating dairy cows. By obtaining similar production results in lower levels of supplementation of both inorganic and organic trace mineral sources, it may be possible for dairy producers to supplement more efficiently, thereby decreasing feed costs and increasing profitability while maintaining animal performance and health. Additionally, producers can reduce excretion of excess minerals, thereby reducing the impact on soils and water reservoirs and ultimately increase the sustainability of the dairy industry.

4.6 Literature Cited

Cao, J., P.R. Henry, C.B. Ammerman, R.D. Miles, and R.C. Littell. 2000. Relative bioavailability of basic zinc sulfate and basic zinc chloride for chicks. Journal of Applied Poultry Research. 9:513–517.

Czarnek, K., S. Terpiłowska, and A.K. Siwicki. 2015. Review paper selected aspects of the action of cobalt ions in the human body. Central European Journal of Immunology. 2:236–242. doi:10.5114/ceji.2015.52837.

Genther, O.N., and S.L. Hansen. 2015. The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. Journal of Dairy Science. 98:566-573.

Kljak, K., F. Pino, K.J. Harvatine, and A.J. Heinrichs. 2017. Analysis of selected rumen microbial populations in dairy heifers limit fed diets varying in trace mineral form and starch content. Livestock Science. 198:93–96. doi:10.1016/j.livsci.2017.02.012.

Lillie, R.J. 1970. Air pollutants affecting the performance of domestic animals. Agricultural Handbook No. 380. Agriculture Research Service. U.S.D.A. Washington, D. C.

López-Alonso, M. 2012. Trace minerals and livestock: Not too much not too Little. ISRN Veterinary Science. 2012:1–18. doi:10.5402/2012/704825.

Ortman, K., and B. Pehrson. 1997. Selenite and selenium yeast as feed supplements for dairy cows. Journal of Veterinary Medicine Series A. 44:373–380. doi:10.1111/j.1439-0442.1997.tb01121.x.

Pino, F., and A. Heinrichs. 2016. Effect of trace minerals and starch on digestibility and rumen fermentation in diets for dairy heifers. Journal of Dairy Science. 99:2797–2810. doi:10.3168/jds.2015-10034.

Respiratory Health Hazards in Agriculture. 1998. American Journal of Respiratory and Critical Care Medicine. 158. doi:10.1164/ajrccm.158.supplement_1.rccm1585s1.

Shkurashivska, S., and H. Ersteniuk. 2019. The effect of adrenaline on the mineral and trace element status in rats. Open Life Sciences. 14:158–164. doi:10.1515/biol-2019-0018.

Suttle, N.F. 2010. The Mineral Nutrition of Livestock. 4th edn. CAB International, Wallingford, UK.

Vinus, S. 2017. Organic Minerals in Poultry. Advances in Research. 12:1–10. doi:10.9734/air/2017/37878.

Bibliography

Agency for Toxic Substances and Disease Registry. 2003. Public health statement: Selenium. U.S. Department of Health and Human Services. Washington, D.C.

Akins, M., S. Bertics, M. Socha, and R. Shaver. 2013. Effects of cobalt supplementation and vitamin B_{12} injections on lactation performance and metabolism of Holstein dairy cows. Journal of Dairy Science. 96:1755–1768. doi:10.3168/jds.2012-5979.

Allen, J.D., and J.M. Gawthornet. 1987. Involvement of the solid phase of rumen digesta in the interaction between copper, molybdenum and sulphur in sheep. British Journal of Nutrition. 58:265–276. doi:10.1079/bjn19870094.

Ammerman, C.B., D.H. Baker, and A.J. Lewis. 1995. Bioavailability of nutrients for animals amino acids, minerals, and vitamins. San Diego: Academic Press.

Andrieu, S. 2008. Is there a role for organic trace element supplements in transition cow health? The Veterinary Journal,176:77-83. doi:10.1016/j.tvjl.2007.12.022

ARC. 1980. Agricultural Research Council. The Nutrient Requirements of Ruminant Livestock. Technical review by an agricultural research council working party, Common wealth Agricultural Bureau, Farnham Royal, UK.

Arthington, J.D., F.M. Pate, and J.W. Spears. 2003. Effect of copper source and level on performance and copper status of cattle consuming molasses-based supplements. Journal of Animal Science. 81:1357–1362. doi:10.2527/2003.8161357x.

Arthur, J.R. 2000. The glutathione peroxidases. Cellular and Molecular Life Sciences. 57:1825-1835.

Awadeh, F., M. Abdelrahman, R. Kincaid, and J. Finley. 1998. Effect of selenium supplements on the distribution of selenium among serum proteins in cattle. Journal of Dairy Science. 81:1089–1094. doi:10.3168/jds.s0022-0302(98)75670-x.

Bach, A., A. Pinto, and M. Blanch. 2015. Association between chelated trace mineral supplementation and milk yield, reproductive performance, and lameness in dairy cattle. Livestock Science, 182:69-75. doi:10.1016/j.livsci.2015.10.023

Beard, J.L., and H.D. Dawson. 1997. Iron. Pp. 278–284 in Handbook of Nutritionally Essential Mineral Elements, B. L. O'Dell and R. A. Sunde, eds. New York: Marcel Dekker, Inc.

Beckett, G.J., and J.R. Arthur. 2005. Selenium and endocrine systems. Journal of Endocrinology. 184:455–465. doi:10.1677/joe.1.05971.

Berndtson, W.E. 1991. A simple, rapid and reliable method for selecting or assessing the number of replicates for animal experiments. Journal of Animal Science. 69:67. doi:10.2527/1991.69167x.

Bertinato, J., and M.R. Labbé. 2004. Maintaining copper homeostasis: regulation of copper-trafficking proteins in response to copper deficiency or overload. The Journal of Nutritional Biochemistry. 15:316–322. doi:10.1016/j.jnutbio.2004.02.004.

Bertinchamps, A., S. Miller, and G. Cotzias. 1966. Interdependence of routes excreting manganese. American Journal of Physiology-Legacy Content. 211:217–224. doi:10.1152/ajplegacy.1966.211.1.217.

Bird, P.R. 1970. Sulphur metabolism and excretion studies in ruminants. III. The effect of sulphur intake on the availability of copper in sheep. Proceedings of the Australian Society of Animal Production 8:212–218

Bishara, H.N. and A.C. Bray. 1978. Competition between molybdate and sulfate for renal tubular reabsorption in sheep. Proceedings of the Australian Society of Animal Production 12, 123.

Bremner, I., W.R. Humphries, M. Phillippo, M.J. Walker, and P.C. Morrice. 1987. Iron-induced copper deficiency in calves: Dose- response relationships and interactions with molybdenum and sulfur. Animal Production Science. 45:403–414.

Brown, T.F., and L.K. Zeringue. 1994. Laboratory evaluations of solubility and structural integrity of complexed and chelated trace mineral supplements. Journal of Dairy Science. 77:181-189.

Buckley, W.T. 1988. The use of stable isotopes in studies of mineral metabolism. Proceedings of the Nutrition Society. 47:407–416. doi:10.1079/pns19880059.

Cao, J., P.R. Henry, C.B. Ammerman, R.D. Miles, and R.C. Littell. 2000. Relative bioavailability of basic zinc sulfate and basic zinc chloride for chicks. Journal of Applied Poultry Research. 9:513–517.

Castillo, A., N. St-Pierre, N.S.D. Rio, and W. Weiss. 2013. Mineral concentrations in diets, water, and milk and their value in estimating on-farm excretion of manure minerals in lactating dairy cows. Journal of Dairy Science. 96:3388–3398. doi:10.3168/jds.2012-6121.

Conrad, H.R., and A.L. Moxon. 1979. Transfer of dietary selenium to milk. Journal of Dairy Science. 62:404–411.

Cortinhas, C.S., J.E.D.F. Júnior, J.D.R. Naves, M.A.D.F. Porcionato, L.F.P.E. Silva, F.P. Rennó, and M.V.D. Santos. 2012. Organic and inorganic sources of zinc, copper and selenium in diets for dairy cows: intake, blood metabolic profile, milk yield and composition. Revista Brasileira de Zootecnia. 41:1477–1483. doi:10.1590/s1516-35982012000600023.

Crutchfield, W. O. 1968. A technique for placement of an in-dwelling catheter in the cow. Veterinary Medicine Small Animal Clinician. 63:1141-1144.

Czarnek, K., S. Terpiłowska, and A.K. Siwicki. 2015. Review paper Selected aspects of the action of cobalt ions in the human body. Central European Journal of Immunology. 2:236–242. doi:10.5114/ceji.2015.52837.

Drackley, J.K., S.S. Donkin, and C.K. Reynolds. 2006. Major advances in fundamental dairy cattle nutrition. Journal of Dairy Science. 89:1324-1336.

Duplessis, M., S. Mann, D. Nydam, C. Girard, D. Pellerin, and T. Overton. 2015. Short communication: Folates and vitamin B_{12} in colostrum and milk from dairy cows fed different energy levels during the dry period. Journal of Dairy Science. 98:5454–5459. doi:10.3168/jds.2015-9507.

Ely, R.E., K.M. Dunn, and C.F. Huffman. 1948. Cobalt toxicity in calves resulting from high oral administration. Journal of Animal Science. 7:239–243.

Eryavuz, A., and B.A. Dehority. 2009. Effects of supplemental zinc concentration on cellulose digestion and cellulolytic and total bacterial numbers in vitro. Animal Feed Science and Technology. 151:175–183. doi:10.1016/j.anifeedsci.2009.01.008.

Faulkner, M., and W. Weiss. 2017. Effect of source of trace minerals in either forage- or by-product–based diets fed to dairy cows: 1. Production and macronutrient digestibility. Journal of Dairy Science. 100:5358–5367. doi:10.3168/jds.2016-12095.

Faulkner, M., N. St-Pierre, and W. Weiss. 2017. Effect of source of trace minerals in either forage- or by-product–based diets fed to dairy cows: 2. Apparent absorption and retention of minerals. Journal of Dairy Science. 100:5368–5377. doi:10.3168/jds.2016-12096.

Finney, L.A., and T.V. O'Halloran. 2003. Transition metal speciation in the cell: Insights from the chemistry of metal ion receptors. Science. 300:931–936. doi:10.1126/science.1085049.

Fischer, P.W., A. Giroux, and M.R. Labbé. 1981. The effect of dietary zinc on intestinal copper absorption. The American Journal of Clinical Nutrition. 34:1670–1675. doi:10.1093/ajcn/34.9.1670.

Fischer, P.W.F., A. Giroux, and M.R. Labbe. 1983. Effects of zinc on mucosal copper binding and on the kinetics of copper absorption. The Journal of Nutrition. 113:462–469. doi:10.1093/jn/113.2.462.

Fraser, A.J., T.J. Ryan, R. Sproule, R.G. Clark, D. Anderson, and E.O. Pederson. 1987. The effect of selenium on milk production in dairy cattle. Proceedings of the New Zealand Society of Animal Production. 47: 61–64.

Galbraith, M.L., W.R. Vorachek, C.T. Estill, P.D. Whanger, G. Bobe, T.Z. Davis, and J.A. Hall. 2015. Rumen microorganisms decrease bioavailability of inorganic selenium supplements. Biological Trace Element Research. 171:338–343. doi:10.1007/s12011-015-0560-8

Galbraith, M.L., W.R. Vorachek, C.T. Estill, P.D. Whanger, G. Bobe, T.Z. Davis, and J.A. Hall. 2016. Rumen microorganisms decrease bioavailability of inorganic selenium supplements. Biological Trace Element Research. 171:338-343.

Garrick, M.D., K.G. Dolan, C. Horbinski, A.J. Ghoio, D. Higgins, M. Porcubin, E.G. Moore, L.N. Hainsworth, J.N. Umbreit, M.E. Conrad, L. Feng, A. Lis, J.A. Roth, S. Singleton, and L.M. Garrick. 2003. DMT 1: a mammalian transporter for multiple metals. Biometals 16:41–54.

Genther, O.N., and S.L. Hansen. 2015. The effect of trace mineral source and concentration on ruminal digestion and mineral solubility. Journal of Dairy Science. 98:566-573.

Gerloff, B.J. 1992. Effect of selenium supplementation on dairy cattle. Journal of Animal Science. 70:3934–3940. doi:10.2527/1992.70123934x.

Gibbons, R., S. Dixon, K. Hallis, A. Russell, B. Sansom, and H. Symonds. 1976. Manganese metabolism in cows and goats. Biochimica et Biophysica Acta (BBA) - General Subjects. 444:1–10. doi:10.1016/0304-4165(76)90218-x.

Givens, D.I., R. Allison, B. Cottrill, and J.S. Blake. 2004. Enhancing the selenium content of bovine milk through alteration of the form and concentration of selenium in the diet of the dairy cow. Journal of the Science of Food and Agriculture. 84:811–817.

Goff, J.P. 2018. Invited review: Mineral absorption mechanisms, mineral interactions that affect acid–base and antioxidant status, and diet considerations to improve mineral status. Journal of Dairy Science. 101:2763-2813. doi:10.3168/jds.2017-13112

Gong, J., and M. Xiao. 2018. Effect of organic selenium supplementation on selenium status, oxidative stress, and antioxidant status in selenium-adequate dairy cows during the periparturient period. Biological Trace Element Research. 186:430–440. doi:10.1007/s12011-018-1323-0.

Gooneratne, S.R., W.T. Buckley, and D.A. Christensen. 1989. Review of copper deficiency and metabolism in ruminants. Canadian Journal of Animal Science. 69:819–845. doi:10.4141/cjas89-096.

Government of Canada, (CFIA) Canadian Food Inspection Agency, and Animal Health Directorate. (2017, October 24). Chapter 4 – Labelling and Guarantees. Retrieved from http://www.inspection.gc.ca/animals/feeds/regulatory-guidance/rg-1/chapter-4/eng/1329341411340/1329341520337?chap=6

Griffiths, L., S. Loeffler, M. Socha, D. Tomlinson, and A. Johnson. 2007. Effects of supplementing complexed zinc, manganese, copper and cobalt on lactation and reproductive performance of intensively grazed lactating dairy cattle on the South Island of New Zealand. Animal Feed Science and Technology. 137:69–83. doi:10.1016/j.anifeedsci.2006.10.006.

Gunter, S.A., P.A. Beck, and J.M. Phillips. 2003. Effects of supplementary selenium source on the performance and blood measurements in beef cows and their calves. Journal of Animal Science. 81:856–864.

Hall, J.A., R.J. Van Saun, G. Bobe, W.C. Stewart, W.R. Vorachek, W.D. Mosher, T. Nichols, N.E. Forsberg, and G.J. Pirelli. 2012. Organic and inorganic selenium: I. Oral bioavailability in ewes. Journal of Animal Science. 90: 568–576. doi:10.2527/jas.2011-4075.

Halliwell, B. 1987. Oxidants and human disease: some new concepts. The FASEB Journal. 1:358–364. doi:10.1096/fasebj.1.5.2824268.

Hansen, S.L., N. Trakooljul, H.-C.S. Liu, J.A. Hicks, M.S. Ashwell, and J.W. Spears. 2010. Proteins involved in iron metabolism in beef cattle are affected by copper deficiency in combination with high dietary manganese, but not by copper deficiency alone1. Journal of Animal Science. 88:275–283. doi:10.2527/jas.2009-1846.

Hennigar, S.R., and J.P. Mcclung. 2015. Homeostatic regulation of trace mineral transport by ubiquitination of membrane transporters. Nutrition Reviews. 74:59–67. doi:10.1093/nutrit/nuv060.

Herdt, T.H., and B. Hoff. 2011. The use of blood analysis to evaluate trace mineral status in ruminant livestock. Veterinary Clinics of North America: Food Animal Practice. 27:255–283. doi:10.1016/j.cvfa.2011.02.004.

Hidiroglou, M. 1979a. Manganese in ruminant nutrition. Canadian Journal of Animal Science. 59:217–236. doi:10.4141/cjas79-028.

Hidiroglou, M. 1979b. Trace element deficiencies and fertility in ruminants: A review. Journal of Dairy Science. 62:1195–1206. doi:10.3168/jds.s0022-0302(79)83400-1.

Hill, R. 1991 Rapeseed meal in the diet of ruminants. Nutrition Abstracts and Reviews, Series B 61:139–155.

Ishihara, N., and T. Matsushiro. 1986. Biliary and urinary excretion of metals in humans. Archives of Environmental Health: An International Journal. 41:324–330. doi:10.1080/00039896.1986.9936705

Ivancic, J., and W. Weiss. 2001. Effect of Dietary Sulfur and Selenium Concentrations on Selenium Balance of Lactating Holstein Cows. Journal of Dairy Science. 84:225–232. doi:10.3168/jds.s0022-0302(01)74472-4.

Jaing, B. 2006. The effect of trace elements on the metabolism of methanogenic consortia. Doctoral dissertation. Wageningen University, Wageningen, Gelderland, Netherlands.

James, L.F., and J.E. Butcher. 1972. Halogeton poisoning of sheep: Effect of high level oxalate intake. Journal of Animal Science. 35:1233–1238.

Jenkins, K.J., and M. Hidiroglou. 1991. Tolerance of the preruminant calf for excess manganese or zinc in milk replacer. Journal of Dairy Science. 74:1047–1053.

Jenkitkasemwong, S., C.Y. Wang, B. Mackenzie, and M.D. Knutson. 2012. Physiologic implications of metal-ion transport by ZIP14 and ZIP8. Biometals 25:643–655.

Johansson, E., S.O. Jacobsson, J. Luthman, and U. Lindh. 1990. The biological response of selenium in individual erythrocytes and GSH- px in lambs fed sodium selenite or selenium yeast. Journal of the American Veterinary Medical Association. 37:463–470.

Johnson, J.L. 1997. Molybdenum. In: O'Dell, B.L. and Sunde, R.A. (eds) Handbook of Nutritionally Essential Mineral Elements. Marcel Dekker, New York, pp. 413–438.

Johnston, H., L. Beasley, and N. MacPherson. 2014. Copper toxicity in a New Zealand dairy herd. Irish Veterinary Journal. 67:20–24.

Juniper, D., R. Phipps, A. Jones, and G. Bertin. 2006. Selenium supplementation of lactating dairy cows: effect on selenium concentration in blood, milk, urine, and feces. Journal of Dairy Science. 89:3544–3551. doi:10.3168/jds.s0022-0302(06)72394-3.

Kawashima, T., P. Henry, D. Bates, C. Ammerman, R. Littell, and J. Price. 1997a. Bioavailability of cobalt sources for ruminants. 2. Estimation of the relative value of reagent grade and feed grade cobalt sources from tissue cobalt accumulation and vitamin B_{12} concentrations. Nutrition Research. 17:957-974. doi:10.1016/s0271-5317(97)00061-4

Kawashima, T., P. Henry, D. Bates, C. Ammerman, R. Littell, and J. Price. 1997b. Bioavailability of cobalt sources for ruminants. 3. In vitro ruminal production of vitamin B_{12} and total corrinoids in response to different cobalt sources and concentrations. Nutrition Research. 17:975-987. doi:10.1016/s0271-5317(97)00062-6

Keener, H.A., G.P. Percival, and K.S. Marrow. 1949. Cobalt tolerance in young dairy cattle. Journal of Dairy Science. 32:527.

Kennedy, D.G., S. Kennedy, and P.B. Young. 1996. Effects of low concentrations of dietary cobalt on rumen succinate concentration in sheep. International Journal of Vitamin Research. 66:86–92

Kinal, S., A. Korniewicz, D. Jamroz , R. Zieminski and M. Slupczynska. 2005. Dietary effects of zinc, copper and manganese chelates and sulphates on dairy cows. Journal of Food, Agriculture & Environment. 1:168-172.

Kincaid, R., and M. Socha. 2004. Inorganic versus complexed trace mineral supplements on performance of dairy cows. The Professional Animal Scientist. 20:66–73. doi:10.15232/s1080-7446(15)31274-2.

Kincaid, R., and M. Socha. 2007. Effect of cobalt supplementation during late gestation and Early Lactation on Milk and Serum Measures. Journal of Dairy Science. 90:1880–1886. doi:10.3168/jds.2006-296.

Kincaid, R., L. Lefebvre, J. Cronrath, M. Socha, and A. Johnson. 2003. Effect of dietary cobalt supplementation on cobalt metabolism and performance of dairy cattle. Journal of Dairy Science. 86:1405–1414. doi:10.3168/jds.s0022-0302(03)73724-2.

Kišidayová, S., P. Pristaš, M. Zimovčáková, M.B. Wencelová, L. Homolová, K. Mihaliková, K. Čobanová, Ľ. Grešáková, and Z. Váradyová. 2018. The effects of high dose of two manganese supplements (organic and inorganic) on the rumen microbial ecosystem. Plos One. 13. doi:10.1371/journal.pone.0191158.

Kišidayová, S., P. Pristaš, M. Zimovčáková, M.B. Wencelová, L. Homolová, K. Mihaliková, K. Čobanová, Ľ. Grešáková, and Z. Váradyová. 2018. The effects of high dose of two manganese supplements (organic and inorganic) on the rumen microbial ecosystem. Plos One. 13. doi:10.1371/journal.pone.0191158.

Kljak, K., F. Pino, K.J. Harvatine, and A.J. Heinrichs. 2017. Analysis of selected rumen microbial populations in dairy heifers limit fed diets varying in trace mineral form and starch content. Livestock Science. 198:93–96. doi:10.1016/j.livsci.2017.02.012.

Knowles, S.O., N.D. Grace, K. Wurms, and J. Lee. 1999. Significance of amount and form of dietary selenium on blood, milk, and casein selenium concentrations in grazing cows. Journal of Dairy Science. 82:429– 437.

Koenig, K.M., W.T. Buckley, and J.A. Shelford. 1991. True absorption of selenium in dairy, cows: Stable isotope tracer methodology and effect of dietary copper. Canadian Journal of Animal Science. 71:175-183.

Kumar, S., N. Indugu, B. Vecchiarelli, and D.W. Pitta. 2015. Associative patterns among anaerobic fungi, methanogenic archaea, and bacterial communities in response to changes in diet and age in the rumen of dairy cows. Frontiers in Microbiology. 6. doi:10.3389/fmicb.2015.00781.

Lee, J., J.R. Prohaska, and D.J. Thiele. 2001. Essential role for mammalian copper transporter Ctr1 in copper homeostasis and embryonic development. Proceedings of the National Academy of Sciences. 98:6842-6847. doi:10.1073/pnas.111058698

Lengemann, F.W., and E.W. Swanson. 1957. A study of the secretion of iodine in milk of dairy cows, using daily oral doses of ^{131}I. J. Dairy Sci. 40:215–222.

Lillie, R.J. 1970. Air pollutants affecting the performance of domestic animals. Agriculture Handbook No. 380. Agriculture Research Service. U.S.D.A. Washington, D. C.

Liu, C., Q. Meng, Y. Chen, M. Xu, M. Shen, R. Gao, and S. Gan. 2017. Role of age-related shifts in rumen bacteria and methanogens in methane production in cattle. Frontiers in Microbiology. 8. doi:10.3389/fmicb.2017.01563.

López-Alonso, M. 2012. Trace minerals and livestock: Not too much not too little. ISRN Veterinary Science. 2012:1–18. doi:10.5402/2012/704825.

Lopez-Guisa, J.M., and L.D. Satter. 1992. Effect of copper and cobalt addition on digestion and growth in heifers fed diets containing alfalfa silage or corn crop residues. Journal of Dairy Science. 75:247–256.

Machado, V., M. Bicalho, R. Pereira, L. Caixeta, W. Knauer, G. Oikonomou, R. Gilbert, and R. Bicalho. 2013. Effect of an injectable trace mineral supplement containing selenium, copper, zinc, and manganese on the health and production of lactating Holstein cows. The Veterinary Journal. 197:451–456. doi:10.1016/j.tvjl.2013.02.022.

Mackie, R.I., and F.M.C. Gilchrist. "Herbivore nutrition in the subtropics and tropics." The Science Press, Herbivore Nutrition in the Subtropics and Tropics, 1984. Influence of mineral interactions on growth efficiency of rumen bacteria. R.I. Mackie and J.J. Therion. Pages 455-477.

Malcolm-Callis, K.J., G.C. Duff, S.A. Gunter, E.B. Kegley, and D.A. Vermeire. 2000. Effects of supplemental zinc concentration and source on performance, carcass characteristics, and serum values in finishing beef steers. Journal of Animal Science. 78:2801. doi:10.2527/2000.78112801x.

Maret, W. 2017. Zinc in cellular regulation: The nature and significance of “Zinc Signals”. International Journal of Molecular Science. 18:2285. doi: 10.3390/ijms18112285

Martinez, A., and D.C. Church. 1970. Effect of various mineral elements on in vitro rumen cellulose digestion. Journal of Animal Science. 31:982–990. doi:10.2527/jas1970.315982x

Mason, J. and C.J. Cardin. 1977. The competition of molybdate and sulfate ions for a transport system in the ovine small intestine. Research in Veterinary Science. 22:313–315.

McDowell, L.R. 2000. Vitamins in animal and human nutrition. 2nd ed. Iowa State University Press, Ames.

McKay, E.J. and L.M. McLery. 1981. Location and secretion of gastric intrinsic factor in the sheep. Research in Veterinary Science. 30:261–265

Mehdi, Y., and I. Dufrasne. 2016. Selenium in cattle: A review. Molecules. 21:545. doi:10.3390/molecules21040545.

Miller, J.K., N. Ramsey, and F.C. Madsen. 1988. The trace elements. Pp. 342–400 in The Ruminant Animal: Digestive Physiology and Nutrition, D.C. Church, ed. Englewood Cliffs, NJ: Prentice-Hall, Inc.

Miller, W. 1970. Zinc nutrition of cattle: A review. Journal of Dairy Science. 53:1123–1135. doi:10.3168/jds.s0022-0302(70)86355-x.

Miller, W. 1981. Mineral and vitamin nutrition of dairy cattle. Journal of Dairy Science. 64:1196–1206. doi:10.3168/jds.s0022-0302(81)82696-3.

Mills, C.F., and G.K. Davis. 1987. Molybdenum. Pp. 429–463 in Trace Elements in Human and Animal Nutrition, Vol. 1, W. Mertz, ed. New York: Academic.

Mills, C.F., and I. Bremner. 1980. Nutritional Aspects of Molybdenum in Animals. M. P. Coughlan, ed. Molybdenum and molybdenum containing enzymes. Pergamon Press, Oxford England.

Minatel, L., and J.C. Carfagnini. 2000. Copper deficiency and immune response in ruminants. Nutrition Research. 20:1473–1484. doi:10.1016/s0271-5317(00)00236-0.

Mollerberg, L., and J. Moreno-Lopez. 1975. The response of normal and iron anemic calves to nasal infection with an attenuated strain of parainfluenza-3 virus. Acta Veterinaria Scandinavica. 16:186–196.

Moody, J.R., and R.M. Lindstrom. 1977. Selection and cleaning of plastic containers for storage of trace element samples. Analytical Chemistry. 49:2264–2267. doi:10.1021/ac50022a039.

Morris, E. 1987. Iron. Pp. 79–142 in Trace Elements in Human and Animal Nutrition, Vol. 1, W. Mertz, ed. New York: Academic.

Nemec, L., J. Richards, C. Atwell, D. Diaz, G. Zanton, and T. Gressley. 2012. Immune responses in lactating Holstein cows supplemented with Cu, Mn, and Zn as sulfates or methionine hydroxy analogue chelates. Journal of Dairy Science. 95:4568-4577. doi:10.3168/jds.2012-5404

Nicholson, F.A., and B.J. Chambers. 2008. Livestock manure management and treatment: implications for heavy metal inputs to agricultural soils. Schlegel, P., S. Durosoy, and

A.W. Jongbloed. Trace elements in animal production systems. Wageningen Academic, Wageningen.

Nicholson, J.W., R.E. McQueen, and R.S. Bush. 1991b. Response of growing cattle to supplementation with organically bound or inorganic sources of selenium or yeast cultures. Canadian Journal of Animal Science. 71:803–811.

Nickel, A., G. Kottra, G. Schmidt, J. Danier, T. Hofmann, and H. Daniel. 2009. Characteristics of transport of selenoamino acids by epithelial amino acid transporters. Chemico-Biological Interactions. 177:234-241. doi:10.1016/j.cbi.2008.09.008

NRC. 2001. Nutrient Requirements of Dairy Cows, 7th edn. National Academy of Sciences.

Odell, B.L. 2009. Cysteine-rich intestinal protein (CRIP): A new intestinal zinc transport protein. Nutrition Reviews. 50:232-233. doi:10.1111/j.1753-4887.1992.tb01334.x

Olson, W., J. Stevens, J. Anderson, and D.W. Haggard. 1984. Iodine toxicosis in six herds of dairy cattle. Journal of American Veterinary Medical Association. 184:179–179.

Ortman, K., and B. Pehrson. 1997. Selenite and selenium yeast as feed supplements for dairy cows. Journal of Veterinary Medicine Series A. 44:373–380. doi:10.1111/j.1439-0442.1997.tb01121.x.

Ortman, K., and B. Pehrson. 1999. Effect of selenate as a feed supplement to dairy cows in comparison to selenite and selenium yeast. Journal of Animal Science. 77:3365. doi:10.2527/1999.77123365x.

Osorio, J.S., E. Trevisi, C. Li, J.K. Drackley, M.T. Socha, and J.J. Loor. 2016. Supplementing Zn, Mn, and Cu from amino acid complexes and Co from cobalt glucoheptonate during

the peripartal period benefits postpartal cow performance and blood neutrophil function. Journal of Dairy Science. 99:1868- 1883.

Otles, S., and V.G. Ozyurt. 2014. Classical wet chemistry methods. Cheung, P.C.K, and B.M. Metha, ed. Handbook of Food Chemistry. Springer International Publishing, Cham.

Paglia, D.E. and Valentine, W.N. 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory Clinical Medicine. 70:158-169.

Panneels V., G. Juvenal, J.M. Boeynaems, J.E. Dumont, and J. Van Sande. 2009. Iodide effects on the thyroid: biochemical, physiological, pharmacological, and clinical effects of iodide in the thyroid. Pp 303-314 in Comprehensive handbook of iodine: nutritional, biochemical, pathological and therapeutic aspects. Academic, Amsterdam.

Papavasiliou, P., S. Miller, and G. Cotzias. 1966. Role of liver in regulating distribution and excretion of manganese. American Journal of Physiology-Legacy Content. 211:211–216. doi:10.1152/ajplegacy.1966.211.1.211.

Paterson, J.E., and A. MacPherson. 1990. The influence of a low cobalt intake on the neutrophil function and severity of Ostertagia infection in cattle. British Veterinary Journal. 146:519–530.

Peña Maria M.O., J. Lee, and D.J. Thiele. 1999. A delicate balance: homeostatic control of copper uptake and distribution. The Journal of Nutrition. 129:1251–1260. doi:10.1093/jn/129.7.1251.

Penner, G.B., K.A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. Journal of Dairy Science. 89:2132-2140.

Phillippo, M., W.R. Humphries, and P.H. Garthwaite. 1987. The effect of dietary molybdenum and iron on copper status and growth in cattle. The Journal of Agricultural Science. 109:315. doi:10.1017/s0021859600080746.

Pino, F., and A. Heinrichs. 2016. Effect of trace minerals and starch on digestibility and rumen fermentation in diets for dairy heifers 1. Journal of Dairy Science. 99:2797–2810. doi:10.3168/jds.2015-10034

Podoll, K.L., J.B. Bernard, D.E. Ullrey, S.R. Debar, P.K. Ku, and W.T. Magee. 1992. Dietary selenate versus selenite for cattle, sheep, and horses. Journal of Animal Science. 70:1965-1970. doi:10.2527/1992.7061965x

Prohaska, J.R. 2012. Copper. In: Filer, L.J. and Ziegler, E.E. (eds) Present Knowledge in Nutrition, 10th edn. International Life Science Institute–Nutrition Foundation, Washington, DC.

Quirk, M.F., and B.W. Norton. 1988. Detection of cobalt deficiency in lactating heifers and their calves. The Journal of Agricultural Science. 110:465–470. doi:10.1017/s0021859600082010.

Respiratory Health Hazards in Agriculture. 1998. American Journal of Respiratory and Critical Care Medicine. 158. doi:10.1164/ajrccm.158.supplement_1.rccm1585s1.

Rotruck, J.T., A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, and W.G. Hoekstra. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 179:588–590. doi:10.1126/science.179.4073.588.

Rowntree, J.E., G.M. Hill, D.R. Hawkins, J.E. Link, M.J. Rincker, G.W. Bednar, and R.A. Kreft. 2004. Effect of Se on selenoprotein activity and thyroid hormone metabolism in beef and dairy cows and calves. Journal of Animal Science. 82:2995–3005. doi:10.2527/2004.82102995x.

Ruckenbusch, Y., and P. Thivend. 1980. Digestive physiology and metabolism of ruminants. International Symposium on Ruminant Physiology. 3-7. Lancaster, Lancs. MTP Press.

Salgueiro, M.J., M. Zubillaga, A. Lysionek, M. I. Sarabia, R. Caro, T. De Paoli, A. Hager, and J. Boccio. 2000. Zinc as an essential micronutrient: A review. Nutrition Research. 20:737–755.

Saxena, K.K. and S.K. Ranjhan. 1978. Effect of cobalt and copper supplementation, separately and in combination, on the digestibility of organic nutrients and mineral balances in Hariana calves. Indian Journal of Animal Science. 48:566-571.

Schlau, N., L.L. Guan, and M. Oba. 2011. The relationship between rumen acidosis resistance and expression of genes involved in regulation of intracellular pH and butyrate metabolism of ruminal epithelial cells in steers. Journal of Dairy Science. 95:5866- 5875.

Schwab, E., C. Schwab, R. Shaver, C. Girard, D. Putnam, and N. Whitehouse. 2006. Dietary forage and non-fiber carbohydrate contents influence B-vitamin intake, duodenal flow,

and apparent ruminal synthesis in lactating dairy cows. Journal of Dairy Science. 89:174–187. doi:10.3168/jds.s0022-0302(06)72082-3.

Schwartzkopf-Genswein, K.S., K.A. Beauchemin, T.A. Mcallister, D.J. Gibb, M. Streeter, and A.D. Kennedy. 2004. Effect of feed delivery fluctuations and feeding time on ruminal acidosis, growth performance, and feeding behavior of feedlot cattle. Journal of Animal Science. 82:3357–3365. doi:10.2527/2004.82113357x

Seetharam, B., S. Bose, and N. Li. 1999. Cellular import of cobalamin (Vitamin B_{12}). The Journal of Nutrition. 129:1761–1764. doi:10.1093/jn/129.10.1761.

Senthilkumar, P., D. Nagalakshmi, Y.R. Reddy, and K. Sudhakar. 2008. Effect of different level and source of copper supplementation on immune response and copper dependent enzyme activity in lambs. Tropical Animal Health and Production. 41:645–653. doi:10.1007/s11250-008-9236-0.

Shkurashivska, S., and H. Ersteniuk. 2019. The effect of adrenaline on the mineral and trace element status in rats. Open Life Sciences. 14:158–164. doi:10.1515/biol-2019-0018.

Sinclair, L., K. Hart, D. Johnson, and A. Mackenzie. 2013. Effect of inorganic or organic copper fed without or with added sulfur and molybdenum on the performance, indicators of copper status, and hepatic mRNA in dairy cows. Journal of Dairy Science. 96:4355–4367. doi:10.3168/jds.2012-6322.

Smith, R.M. 1987. Cobalt. in Trace Elements in Human Health and Disease, Vol. 1, W. Mertz, ed. San Diego: Academic Press.

Smith, R.M. 1997. Cobalt. Pp. 357–387 in Handbook of Nutritionally Essential Mineral Elements, B. O'Dell and R. Sunde, eds. New York: Marcel Dekker, Inc.

Smith, R.M., and L.H.R. Marston. 1970. Production, absorption, distribution and excretion of vitamin B_{12} in sheep. British Journal of Nutrition. 24:857–877. doi:10.1079/bjn19700092.

Sobhanirad, S., R. Valizadeh, F. Azizi, M. Hedayati, and A. Tahmasebi. 2009. Effect of zinc sources on haematology, enzyme activity and blood serum zinc concentration in Holstein dairy cattle. Proceedings of the British Society of Animal Science. 2009:152–152. doi:10.1017/s1752756200029914.

Sordillo, L.M., K. Shefer-Weaver, D. DeRosa. 1997. Immunobiology of the mammary gland. Journal of Dairy Science 80:1851–1865

Sorensen, P. 1962. Studies of thyroid function in cattle and pigs. Pg. 455 in Use of Radioisotopes in Animal Biology and Medical Sciences, Vol. 1. New York: Academic Press.

Spears, J., P. Schlegel, M. Seal, and K. Lloyd. 2004. Bioavailability of zinc from zinc sulfate and different organic zinc sources and their effects on ruminal volatile fatty acid proportions. Livestock Production Science. 90:211–217. doi:10.1016/j.livprodsci.2004.05.001.

Spears, J.W. 1996. Optimizing mineral levels and sources for farm animals. E. T. Kornegay, ed. Nutrient Management of Food Animals to Enhance and Protect the Environment. CRC Press, Boca Raton, FL.

Spears, J.W. 2003. Trace mineral bioavailability in ruminants. The Journal of Nutrition. 133. doi:10.1093/jn/133.5.1506s.

Spears, J.W., and W.P. Weiss. 2008. Role of antioxidants and trace elements in health and immunity of transition dairy cows. Veterinary Journal. 176:70-76.

Stangl, G.I., F.J. Schwarz, H. Müller, and M. Kirchgessner. 2000. Evaluation of the cobalt requirement of beef cattle based on vitamin B_{12}, folate, homocysteine and methylmalonic acid. British Journal of Nutrition. 84:645–653. doi:10.1017/s0007114500001987.

Stemme, K., P. Lebzien, G. Flachowsky, and H. Scholz. 2008. The influence of an increased cobalt supply on ruminal parameters and microbial vitamin B_{12} synthesis in the rumen of dairy cows. Archives of Animal Nutrition. 62:207–218. doi:10.1080/17450390802027460.

Stemme, K., U. Meyer, G. Flachowsky, and H. Scholz. 2006. The influence of an increased cobalt supply to dairy cows on the vitamin B_{12} status of their calves. Journal of Animal Physiology and Animal Nutrition. 90:173–176. doi:10.1111/j.1439-0396.2005.00584.x.

Stevenson, D.M., and P.J. Weimer. 2007. Dominance of Prevotella and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Applied Microbiology Biotechnology. 75:165–174.

Sun, L., S. Gao, K. Wang, J. Xu, M. Sanz-Fernandez, L. Baumgard, and D. Bu. 2019. Effects of source on bioavailability of selenium, antioxidant status, and performance in lactating dairy cows during oxidative stress-inducing conditions. Journal of Dairy Science. 102:311–319. doi:10.3168/jds.2018-14974.

Suttle, N.F. 2010. The Mineral Nutrition of Livestock. 4th edn. CAB International, Wallingford, UK.

Sutton, A.L., and J.M. Elliot. 1972. Effect of ratio of roughage to concentrate and level of feed intake on ovine ruminal vitamin B_{12} production. The Journal of Nutrition. 102:1341–1346. doi:10.1093/jn/102.10.1341.

Thomas, R. 2011. Practical guide to ICP-MS: a tutorial for beginners. CRC Press, Boca Raton.

U.S Food and Drug Administration (USDA). (n.d.) CFR – Code of Federal Regulations Title 21. Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.920

Uchida, K., P. Mandebvu, C. Ballard, C. Sniffen, and M. Carter. 2001. Effect of feeding a combination of zinc, manganese and copper amino acid complexes, and cobalt glucoheptonate on performance of early lactation high producing dairy cows. Animal Feed Science and Technology. 93:193–203. doi:10.1016/s0377-8401(01)00279-6.

Underwood, E. J. 1981. The Mineral Nutrition of Livestock. 2nd Ed. Slough, England: Commonwealth Agricultural Bureaux.

van den Top, A.M. 2005. Reviews on the mineral provision in ruminants (X): cobalt metabolism and requirements in ruminants. Centraal Veevoederbureau.

Vendeland, S., J. Deagen, and P. Whanger. 1992. Uptake of selenotrisulfides of gluthathione and cysteine by brush border membranes from rat intestines. Journal of Inorganic Biochemistry. 47:131–140. doi:10.1016/0162-0134(92)84049-s.

Vierboom, M.M., T.E. Engle, and C.V. Kimberling. 2003. Effects of Gestational Status on Apparent Absorption and Retention of Copper and Zinc in Mature Angus Cows and Suffolk Ewes. Asian-Australasian Journal of Animal Sciences. 16:515–518. doi:10.5713/ajas.2003.515.

Vinus, S. 2017. Organic minerals in poultry. Advances in Research. 12:1–10. doi:10.9734/air/2017/37878.

Walker, C.K. and Elliot, J.M. 1972. Lactational trends in vitamin B_{12} status on conventional and restricted- roughage rations. Journal of Dairy Science 55, 474–478.

Wang, C., Q. Liu, W. Yang, Q. Dong, X. Yang, D. He, P. Zhang, K. Dong, and Y. Huang. 2009. Effects of selenium yeast on rumen fermentation, lactation performance and feed digestibilities in lactating dairy cows. Livestock Science. 126:239–244. doi:10.1016/j.livsci.2009.07.005.

Wapnir, R.A. 1998. Copper absorption and bioavailability. The American Journal of Clinical Nutrition. 67:1054-1060. doi:10.1093/ajcn/67.5.1054s

Waterman, R.C., W.L. Kelly, C.K. Larson, and M.K. Petersen. 2017. Comparison of supplemental cobalt form on fibre digestion and cobalamin concentrations in cattle. The Journal of Agricultural Science. 155:832–838. doi:10.1017/s0021859617000107.

Weeth, H.J., and J.E. Hunter. 1971. Drinking of sulfate-water by cattle. Journal of Animal Science. 32:277–281.

Weiss, W., and M. Socha. 2005. Dietary manganese for dry and lactating Holstein cows. Journal of Dairy Science. 88:2517–2523. doi:10.3168/jds.s0022-0302(05)72929- 5.

Wiese, S.C., C.L. White, I.H. Williams, and J.G. Allen. 2007. Relationships between plasma methylmalonic acid and ruminal succinate in cobalt-deficient and -repleted sheep. Australian Journal of Agricultural Research. 58:367. doi:10.1071/ar05125.

Wollenberg, P., and W. Rummel. 1987. Dependence of intestinal iron absorption on the valency state of iron. Naunyn-Schmiedebergs Archives of Pharmacology. 336:578-582. Doi:10.1007/bf00169317.

Wu, L., H. Zhang, C. Xu, and C. Xia. 2016. Critical thresholds of antioxidant and immune function parameters for Se deficiency prediction in dairy cows. Biological Trace Element Research. 172:320–325. Doi:10.1007/s12011-015-0606-y.

Wuerges, J., G. Garau, S. Geremia, S.N. Fedosov, T.E. Petersen, and L. Randaccio. 2006. Structural basis for mammalian vitamin B_{12} transport by transcobalamin. Proceedings of the National Academy of Sciences USA. 103:4386–4391.

Yasui, T., C. Ryan, R. Gilbert, K. Perryman, and T. Overton. 2014. Effects of hydroxy trace minerals on oxidative metabolism, cytological endometritis, and performance of transition dairy cows. Journal of Dairy Science. 97:3728–3738. doi:10.3168/jds.2013-7331.

Yatoo, M.I., A. Saxena, P.M. Deepa, B.P. Habeab, S. Devi, R.S. Jatav and U. Dimri. 2013. Role of trace elements in animals: a review, Veterinary World. 6: 963-967.

Young, R. 1979. Cobalt in biology and biochemistry. London: Academic Press.

Yu, Z. and M. Morrison. 2004. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques. 36:808-812.