The Effect of Dietary Trace Minerals During Late Gestation on Health and Performance of the Dam and Progeny

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INTRODUCTION

Trace minerals are essential nutrients required at levels generally less than 100 ppm (Miller et al., 1988) by all species of animals. The most commonly discussed trace minerals are zinc (**Zn**), copper (**Cu**), manganese (**Mn**), selenium (**Se**), cobalt (**Co**), iron (**Fe**) and iodine (**I**). The role of trace minerals in animal production is of great interest to producers and industry alike as they have a wide range of activities and functions within the body. These include but are not limited to, vitamin synthesis, hormone production, enzyme activity, collagen formation, tissue synthesis, oxygen transport, and other physiological processes related to growth, reproduction, and health (Paterson et al., 1999). Trace mineral requirements of animals vary greatly and are affected by many factors including genetics, age, maintenance, growth, reproduction, lactation, and level of production. Balance among the trace minerals is also an important consideration and often poses a large challenge to the trace mineral status of the animal due to antagonist interactions that can occur between minerals (Larson, 2005).

The trace mineral status of an animal is important during times of stress, especially for transition dairy cows because of stress due to fetal growth, colostrum production, as well as other hormonal, physiological, dietary and environmental changes they face (Larson, 2005). A transition cow is generally defined as a cow that is transitioning from the dry period into the next lactation. The transition period for a dairy cow begins two to three weeks prepartum and continues until two to three weeks postpartum. Nockels et al. (1993) studied the effect of stress on copper retention and suggested stress can potentially reduce an animal's ability to retain specific trace minerals. These studies imply that it is important to have cows in adequate trace mineral

status prior to, during and following parturition to avoid sub-clinical problems that may lead to decreased production or reproductive complications. Adequate mineral status is also needed during gestation to provide the developing fetus with sufficient trace minerals for development or tissue accretion. The storage of minerals in fetal tissue reflects fetal demands for growth and the ability of the dam to transfer minerals (Abdelrahman and Kincaid, 1993).

Trace minerals play an important role in maintaining fetal development and immune function during the transition period. Copper, Mn, Zn, and Se are often the most limiting trace elements for the fetus and neonate for normal development (Abdelrahman and Kincaid, 1993). Therefore, supplementation of the pregnant cow with adequate trace minerals is essential as Hidiroglou and Knipfel (1981) stated the fetus relies entirely on the dam for a sufficient supply of trace minerals and other nutrients needed for growth and development. If trace mineral levels are sub-optimal in a transition cow, the dam may not exhibit signs of clinical deficiency, but the transfer of these minerals to the calf may be affected. As the trace mineral status in the calf declines immunity and enzyme functions are compromised first, followed by a reduction in maximum growth and finally normal growth decreases prior to clinical deficiency (Wikse, 1992).

Newborn calves depend not only on mineral reserves acquired from the dam but also on the mineral intake from colostrum. Calves are born with a naïve immune system and they need supplemental help via colostrum before they can mount an effective immune response on their own. Colostrum is the main source of minerals for the calf and mineral content of colostrum is largely affected by mineral supplementation to the cow during the transition period. Therefore, the use of supplemental trace minerals to the

dam prior to parturition to increase the trace mineral content in fetal tissues and colostrum may help to enhance immunity in the calf. For the purpose of this review, the focus will be on Co, Cu, Mn and Zn and the importance of these trace minerals on the health, performance and production of pre-ruminant dairy calves and mature dairy cows.

Chelated minerals

Sources used to supply Co, Cu, Mn, and Zn to dairy animals include reagent grade chemicals, natural ores and by-products from industrial processing. These sources may vary greatly in purity and biological availability. Biological availability is the ability of the element or ion under consideration to support some physiological process (Peeler, 1972). It is well documented that when trace elements are complexed to organic molecules, such as amino acids (AA) their absorption into the animal's body and biological availability is increased compared to inorganic sources (Ashmead, 1970; Wedekind et al., 1992; Nockels et al., 1993).

Commercial organic trace mineral products use peptides or AA from hydrolyzed protein, or individual AA as the organic molecules to complex with the metal ions. These products are classified as metal AA complexes, metal proteinates, or metal AA chelates depending on what compounds are being complexed together. The Association of American Feed Control Officials (2000) defined the following: (1) metal AA complexes are the product of complexing a soluble metal salt with an AA; (2) metal proteinates are the chelation of a soluble metal salt with AA or hydrolyzed proteins; (3) a chelate is the product resulting from the reaction of a metal ion with an amino acid, and a molar ratio of metal to AA of 1:1 to 1:3 to form coordinate covalent bonds. The word chelation refers to a complex in which a metal atom is bound to two or more ligands (Rubin and Princiotto,

1963). The metal complex or chelate is stable in the digestive tract and protected from forming complexes with other dietary compounds that would otherwise inhibit its absorption (Spears, 1996). Metal AA chelates can be absorbed intact from the intestine which reduces the competition between the metal ions for absorption sites. The absorption of AA chelates from the intestine may stimulate certain physiological responses or enter target tissues at higher levels (Ashmead, 1970). In the animal, trace minerals occur and function as organic complexes or chelates and not as free inorganic ions (Spears, 1996). Therefore, inorganic mineral sources must be converted to organic forms for use by the animal (Ashmead, 1993). The trace minerals that occur naturally in feeds exist primarily as organic chelates or complexes (Spears, 1996). The differences in bioavailability between complexed and inorganic sources of trace minerals are confounded by level of antagonists (Wedekind et al., 1992), and stress (Nockels et al., 1993), with antagonists and stress having a greater negative impact on the bioavailability of inorganic trace mineral sources than amino acid complex sources. As a result, chelated trace minerals have traditionally been fed to cows experiencing stress from disease, health, reproduction or the environment with the goal of increasing the bioavailability of minerals to the animal to support performance and production.

COBALT

Function and Metabolism

Cobalt was first discovered to be an essential trace mineral in cattle by researchers studying "wasting disease" (Underwood and Filmer, 1935). Cobalt is known mainly for its function as a component of the vitamin B_{12} molecule. Ruminants very efficiently utilize dietary cobalt and synthesis of vitamin B_{12} responds rapidly to changes in dietary

supply, but efficiency declines and production of non-physiological active vitamin B_{12} analogs increase as dietary cobalt levels increase (Underwood and Suttle, 1999). Vitamin B_{12} is very important to ruminants due to its indirect role in propionate metabolism, through methylmalonyl CoA, and ultimately glucose production through gluconeogenesis. In mature ruminants, gluconeogenesis is extremely important because it may provide up to 90% of the total glucose needs of the animal (Young, 1977). Rumen microbes enable ruminants to use dietary cobalt to produce vitamin B_{12} and meet daily requirements. Neonatal or pre-ruminant calves are unable to synthesize vitamin B_{12} from dietary cobalt and as a result, their vitamin B_{12} requirement must be met by the diet. Smith and Marston (1970) observed when dietary cobalt was fed in sufficient amounts to sheep; up to three percent of the dietary cobalt was incorporated into vitamin B_{12}, but may increase to 13% when dietary cobalt amounts were deficient. Absorption of cobalt is low, 1 to 2 %, in ruminants compared to other trace minerals which may be due to the binding and utilization by rumen microbes (Looney et al., 1976). Ruminants require 0.11 mg per kg (or parts per million; ppm) cobalt of dietary DM to allow for sufficient vitamin B_{12} synthesis by rumen bacteria (NRC, 2001). However, this requirement is based largely on observations from grazing herds. Most feedstuffs are low in cobalt and therefore, additional supplementation is often needed. Research by Schwarz et al., (2000) with Simmental bulls estimated that the cobalt requirement may be 0.26 and 0.24 ppm based on plasma and liver B_{12} concentrations, respectively. Kincaid et al., (2003) observed that supplementing lactating dairy cows with 25 mg per head per day of Co, well in excess of requirements increased fat corrected milk yield. Serum cobalt declines with increased days in milk, although this may have little or no effect on the cow except as an indicator

of vitamin B_{12}. Repletion of cobalt appears to take place during the dry period, but the extent of repletion is dependent on the length of the dry period. Research by Kincaid and Socha (2007) showed serum vitamin B_{12} levels are reduced in the early dry period and that addition of cobalt to the diet may increase ruminal synthesis of vitamin B_{12} increasing vitamin B_{12} levels in colostrum and milk. The authors also hypothesized added cobalt would reduce losses of vitamin B_{12} from maternal liver stores to fetal growth and milk synthesis. In general, older cows appear to benefit more than younger cows from higher dietary cobalt levels. Taking this into account, more research is needed to better define the cobalt requirements of cattle.

Cobalt is widely distributed throughout the body, but the highest concentrations are found in liver, bone and kidney. Cobalt which is not incorporated into vitamin B_{12} or vitamin B_{12} analogues is not readily absorbed and is mostly excreted in the feces. The only known function of cobalt is as a component of vitamin B_{12}. Absorption, metabolism functions, and tissue status of cobalt directly are not well known. Miller et al. (1988) suggest that since cobalt functions mostly as a component of vitamin B_{12}, produced by rumen microbes, vitamin B_{12} levels should be considered as a method of determining cobalt status.

Deficiency and Toxicity

Because of its function in vitamin B_{12}, a cobalt deficiency is therefore a vitamin B_{12} deficiency. Ruminants are very sensitive to a vitamin B_{12} deficiency as propionate metabolism is impaired affecting glucose production from gluconeogenesis (NRC, 2001). Sheep are more sensitive to inadequate cobalt than are cattle, and young animals are more sensitive than mature ones. Symptoms of cobalt deficiency are well defined and include

depressed appetite, severe emaciation, listlessness, depressed weight gain, decreased milk production, rough hair coat, wool with a low breaking point in sheep, and anemia resulting in pale skin and mucous membranes (Miller et al., 1988). Moderate cobalt deficient areas exist primarily in the Central and Northeast portions of the U.S., while the Southeast has a more extreme deficiency. Upon depletion of cobalt in the rumen, vitamin B_{12} in serum begins to fall. Toxic levels of cobalt inhibit iron absorption resulting in anorexia, salivation, muscle incoordination and anemia (Underwood and Suttle, 1999).Reaching toxic levels is much less likely to occur than a cobalt deficiency because toxic levels appear to be at least 300 times the requirement (Miller et al., 1988). Work in poultry also suggests that sulfur amino acids, especially cysteine, may form complexes with cobalt reducing its absorption (NRC, 2005).

COPPER

Function and Metabolism

Copper is one of the oldest known minerals to humans, being shown in 1928 as required for growth and hemoglobin formation in rats (Underwood and Suttle, 1999). Copper is a component of many important metalloenzymes such as (1) cytochrome oxidase, involved in electron transport during aerobic respiration; (2) lysyl oxidase, aids in formation of cross links in collagen and elastin; (3) ceruloplasmin, which has a role in iron absorption and transport for hemoglobin synthesis; (4) Tyrosinase, involved in melanin production, and (5) superoxide dismutase, which acts as an anti-oxidant in cells and plays a role in phagocytic cell function (Andrieu, 2008). Absorption of Cu declines as a ruminant matures declining from nearly 75% in young pre-ruminants to less than 10% in mature ruminants (Underwood and Suttle, 1999). The drastic reduction in Cu

absorption in mature ruminants is believed to be related to interactions among Cu, sulfur, and molybdenum in the rumen (NRC, 2005). Copper is absorbed along the entire gastro-intestinal tract with the duodenum being a major site. Absorption is regulated at the mucosal level of the intestine, but the exact mechanism is not completely understood. Absorbed Cu binds to albumin in the portal circulation and is transported primarily to the liver where it is synthesized to ceruloplasmin, the predominant form of copper in plasma, or other copper metalloenzymes (NRC, 2005). Liver is the major storage site for Cu with concentrations in ruminants ranging from 100 to 400 mg Cu per kg DM (Underwood and Suttle, 1999). Liver is also the best indicator of Cu status in live cattle. Copper is secreted from the liver bound to ceruloplasmin for uptake and use by tissues in the body, however, the exact form of Cu transport to other tissues is unknown. Bile is the method of Cu excretion from the gastro-intestinal tract and is the mechanism of homeostatic control of Cu absorption, but is much less effective in ruminants than nonruminants (NRC, 2005). In ruminants, feces are the primary route of Cu excretion from the body (Miller et al., 1988).

Underwood and Suttle (1999) reported copper requirements for ruminants may vary from 4 to over 20 mg Cu per kg of diet, depending on concentrations of dietary antagonists present. These values are higher than NRC (2001) requirements which range from 12 to 15 mg per kg of diet for a 300 kg heifer and a 650 kg cow producing 40 kg of milk per day, respectively. Abdelrahman and Kincaid (1993) measured Cu levels in fetal tissue at different stages of pregnancy in dairy cattle and found that stage of gestation had no effect on Cu levels in fetal liver or kidney tissue. Researchers in Canada observed that

Cu concentrations in bovine fetal liver were higher than those present in the dam (Gooneratne and Christensen, 1989).

Deficiency and Toxicity

One of the earliest signs of Cu deficiency in cows is a decline in fertility (Hidiroglou, 1979). Other classic symptoms include reduced growth and milk production, severe diarrhea, stiff joints, loss of hair coat color, hair loss, fragile or broken bones, anemia, neonatal ataxia, cardiovascular disorders and impaired immune function (Miller et al., 1988 and NRC, 2005). A Cu deficiency may also include early embryonic death, resorption of the embryo, increased incidence of retained placentas and necrosis of the placenta all of which affect the reproductive performance of the animal (Miller et al., 1988). These symptoms may vary slightly by species and most cases of deficiency in ruminants are due to presence of antagonists such as Mo, S, and Fe interfering with Cu absorption (NRC, 2005). An example of such an interaction is the formation of thiomolybdates in the rumen from Mo and S, which react with Cu and make it unavailable to intestinal absorption. When high dietary iron and sulfur react, the inhibition of Cu absorption is more pronounced (Underwood and Suttle, 1999). Sulfur also appears to affect Cu absorption when they react to form cupric sulfate which is relatively insoluble. Water containing elevated levels of Fe may be responsible for creating a Cu deficiency, but how Fe affects Cu absorption is not well known (NRC, 2001). Work by Ivan and Grieve (1976) with Holstein bull calves, found that while supplemental manganese may improve Cu absorption, increased zinc supplementation appears to inhibit Cu absorption. Cadmium is also an antagonist to Cu absorption and interferes with tissue metabolism of Cu in the liver and kidneys (Underwood and Suttle,

1999; NRC, 2001). High levels of zinc (Zn) result in higher levels of metallothionen along the lumen of the small intestine; the increased metallothionen binds Cu affecting its absorption. It has been postulated that the antagonism of Cu absorption by Mo and S could be prevented if Cu or Mo supplements are delivered to a site other than the rumen. Research has shown that complexed sources of Cu may indeed be protected from chemical reactions within the gastro-intestinal tract and therefore avoiding the effects of antagonists (Ashmead, 1993). Work by Ashmead and Ashmead (2004) in Holstein bull calves supported the earlier research as they observed an AA chelate of Cu overcame the effects of antagonists and was more readily available for absorption.

Species differences in Cu toxicity are well documented and include nausea, vomiting, salivation, abdominal pain, convulsion, paralysis and death. Cattle are moderately susceptible while sheep are very susceptible to Cu toxicity. Cattle breeds exhibit differences in thresholds to Cu toxicity as Du et al., (1996) found Cu accumulated more rapidly in liver of Jerseys than in Holsteins. Age of cattle also has an affect with young cattle being more susceptible to Cu toxicity than older cattle due to higher absorption ability in younger animals. Maximum safe dietary levels of Cu are 50 ppm Cu for calves and 80-100 ppm Cu for adult cows (NRC, 2005). The susceptibility of ruminants to Cu toxicity is due to accumulation of Cu in the liver as Cu intake increases above required amounts. Ruminants have very little control of Cu absorption and liver storage (Miller et al., 1988) and the factors that regulate liver Cu in cattle are poorly understood. More research is needed to understand Cu excretion in bile and the factors that regulate it. Copper toxicity often results because of improperly formulated supplements or diets, excessive dose of Cu to correct a deficiency, exposure to Cu-

containing fertilizers or insecticides in pasture, or consumption of feedstuffs or compounds with very high Cu content by mistake, like drinking from a footbath containing Cu for example. Copper toxicity is most often treated with an intravenous dose of thiomolybdate, for reasons described earlier.

MANGANESE

Function and Metabolism

Research by Orent and McCollum (1931) in rats was the first to classify manganese (Mn) as an essential trace mineral for reproduction. Manganese is involved in activation of enzymes for synthesis of proteoglycans, lipid and carbohydrate metabolism, bone formation and growth, reproduction, and central nervous system function (Underwood and Suttle, 1999 and NRC, 2005). Manganese is also essential for cholesterol synthesis, the precursor of estrogen, progesterone, and testosterone (Underwood and Suttle, 1999). Insufficient production of these steroid hormones would result in a decline in reproductive performance. Manganese is present in the body in very small amounts (2.5 ppm DM basis) and it is unevenly distributed in the body, being concentrated in mitochondria and mitochondria rich tissues such as liver (Miller et al., 1988). Absorption of Mn is relatively constant and occurs all along the small intestine. While there is no known homeostatic control of Mn absorption, it is proposed that regulation is by Mn source and dietary antagonists. Absorption of Mn by ruminants is low, less than 1%, (Miller et al., 1988) and is hypothesized this is a reflection of the substantial surplus of Mn provided by most practical rations (Underwood and Suttle, 1999). While the NRC (2001) does not provide absorption coefficients for organic sources of Mn, Henry et al. (1992) reported increased Mn tissue concentrations in sheep

supplemented with organic Mn. A study by Weiss and Socha (2005) observed no difference between sources, organic or inorganic, for Mn absorption, but apparent retention was greater for cows supplemented with Mn compared to those receiving Mn from the basal diet only. Interactions between Mn and other elements have been observed, but the reasons behind them are not well understood and conflicting evidence exists. Iron and Mn at high amounts interfere with absorption of each other (Underwood and Suttle, 1999). Dietary excesses of calcium or potassium result in increased fecal losses of Mn and excess dietary Mn will result in increased Zn and decreased Cu content in liver (Miller et al., 1988).

Deficiency and Toxicity

While Mn deficiencies in cattle may arise in certain situations, it is very unlikely to occur due to widespread availability of Mn in feedstuffs. Forages on average contain higher Mn levels than grains, but variability with individual forages may be as high as ten-fold (Miller et al., 1988). The Mn requirement for dairy cattle varies by maturity from 24 ppm for a 300 kg heifer, 17.8 ppm for a 650 kg cow at 270 days of gestation, and 16.7 ppm for a 650 kg cow producing 40 kg milk per day (NRC, 2001). While the Mn requirement for dairy cattle varies by maturity or stage of life, the Mn requirement is more the reflection of animal's DMI rather than age. Symptoms of deficiency include poor growth and impaired reproduction, characterized by testicular atrophy in males (Hurley and Doane, 1989) and slower exhibition of estrus and increased services per conception in females (Hidiroglou and Knipfel, 1981). In calves born from Mn-deficient dams, low birth weights, reduced gain, and general weakness were reported (Miller et al.,

1988). A Mn deficiency during gestation may cause biologically important alterations in utilization of certain minerals by both the dam and fetus (Hidiroglou and Knipfel, 1981).

Manganese is considered to be one of the least toxic of the essential minerals (NRC, 2001). The maximum tolerable level (MTL) of Mn for dairy cattle in feed is 1000 ppm (NRC, 2005). Jenkins and Hidiroglou (1991) reported decreased feed efficiency and weight gains when calves were fed milk replacer containing 1000 ppm Mn for 5 weeks and 100% calf mortality at 5000 ppm. However, both of these levels are well above NRC (2001) recommendations. Dietary Mn greater than 1,000 ppm in pre-ruminant calves greatly increases plasma lipids and appears to impair essential fatty acid metabolism in the liver (NRC, 2005). A study was also conducted by Raeth-Knight et al. (2005) to evaluate Mn content in drinking water on animal performance. The authors concluded that manganese carbonate added in water up to 0.75 ppm Mn, did not significantly impact calf performance or health. Manganese content in water and the effect it may have on performance is of growing concern, as elevated levels of Mn in water have been reported on a national basis**.** More research is needed to determine the effect of excess Mn on the performance of dairy cattle.

ZINC

Function and Metabolism

Zinc is one of the most studied trace minerals but it wasn't until 1960 that research proved it was a required nutrient for ruminants (Miller et al., 1988).
Zinc is a component of over 200 proteins and enzymes that are involved in many metabolic processes. These processes include protein synthesis, carbohydrate metabolism, and DNA and RNA replication and repair (Zn finger proteins) (Miller et al.,

1988; Underwood and Suttle, 1999; NRC, 2001). Zinc is a component of metalloenzymes such as copper-zinc superoxide dismutase, carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase, and RNA polymerase, which affects carbohydrate, protein, lipid, and nucleic acid metabolism (NRC, 2001; NRC, 2005). Zinc also has a role in thymosin, a hormone produced in the thymus that helps with regulation of cell-mediated immunity (Underwood and Suttle, 1999; NRC, 2001). Organic forms of Zn may also improve foot hardness, decrease sole abscesses, and reduce somatic cell count (**SCC**) (Ashmead, 1993).

Zinc is second only to Fe in total body concentration with the highest amount of Zn found in the liver and skeletal muscle. However, Zn in general is poorly stored in body tissues (Miller et al., 1988). The small intestine is the primary site of Zn absorption although there is a small amount that is absorbed from the rumen (Underwood and Suttle, 1999; NRC, 2005). Zinc absorption occurs via two processes: 1) a nonmediated (non-saturable) process that is not affected by dietary Zn intake and 2) a mediated (saturable) process that is stimulated by Zn depletion. The result is Zn absorption is inversely related to dietary Zn levels (Solomon and Cousins, 1984; Cousins 1996; NRC 2005), however, the understanding of these two intricate processes is very limited. Once Zn is absorbed it is carried into portal circulation by albumin. In Zn deficient animals, Zn readily enters the enterocytes and is transported across the cell by a cysteine rich intestinal protein (**CRIP**) and released into portal circulation to be carried by transferrin and albumen (NRC, 2001). However, in normal animals metallothionein, present in mucosal cells, competes with CRIP for Zn. Metallothionen within the mucosal enterocytes appears to be the regulatory factor controlling the amount of Zn available in circulation (Miller et al., 1988; NRC,

2005). Metallothionein regulates Zn by binding it and holding it in the enterocytes until it dies and is sloughed, once the enterocytes dies the Zn will then be released and excreted in the feces (NRC, 2001). How Zn status regulates metallothionein levels in the intestine is unknown, but it is known that it takes several days to weeks for metallothionein to adjust to low dietary levels of Zn (Taylor et al., 1991). The primary route of Zn excretion is in the feces and includes dietary Zn that was not absorbed as well as endogenous losses (Miller et al., 1988; NRC, 2005).

The NRC (2001) established a coefficient of 15% for Zn absorption, however, organic chelates can increase the efficiency of Zn absorption, by forming soluble zinc complexes within the small intestine allowing Zn to be better absorbed (NRC, 2001). A study conducted by Wright and Spears (2004) showed 500 mg of Zn per kg of diet as Zn AA complex was absorbed and or retained to a greater extent than was Zn sulfate. At 20 ppm however, there was no difference between Zn AA and Zn sulfate in Zn absorption or retention which the authors attributed to normal homeostatic control mechanisms for zinc absorption.

The dietary Zn requirements for cattle vary from a minimum of 22.8 ppm of diet DM for a 650 kg cow at 270 d of gestation to 63 ppm of dietary DM for a cow of the same size producing 40 kg of milk per day. In the past, 40 ppm of Zn was recommended for all classes of dairy cattle which might have underestimated the levels required for lactation (NRC, 2001). While colostrum contains 3-4 times more Zn than milk, milk is still a major demand for Zn despite a relatively low Zn content because of the large volume of milk produced by dairy cows (Miller et al., 1988). Zinc demands in the gestating cow are also quite significant as Zn levels in the bovine fetus increase by 13

times between the first and second trimesters of pregnancy and by another 7 times in the final trimester (Miller et al., 1988). Total Zn accumulated by 270 d averages 135mg in placenta, 3 mg in placental fluids, and 551 mg in the fetus (Miller et al., 1988),

While phytate and calcium are well known antagonists of Zn in swine and poultry diets, they do not have the same effect on Zn in ruminant diets. Miller et al. (1998) demonstrated rumen microbes can hydrolyze phytate and prevent complexes from forming. Zinc and Cu are antagonistic to each other and in most cases excess Zn interferes with Cu absorption and metabolism (Miller et al., 1988; NRC, 2001). However, with very high dietary levels of Cu, Zn absorption is reduced (NRC, 2001). Other minerals inhibiting Zn absorption or interfering with Zn function are lead and cadmium (White et al., 1985, Underwood and Suttle, 1999; NRC, 2001).

Deficiency and Toxicity

A deficiency of Zn impairs thymus activity, neutrophil, lymphocyte, and natural killer cell functions and antibody-dependent, cell-mediated immune response (Miller et al., 1988; Underwood and Suttle, 1999). Animals deficient in Zn will quickly exhibit reduced feed intake, reduced rate of growth, lethargy, increased susceptibility to infection and prolonged deficiency will result in weak hoof horns, reduced testes growth in males, perakeratosis of skin on the legs, head, nostrils, and neck (Miller, 1970; Miller et al., 1988; NRC, 2001). In Zn deficient animals, many of the vital organs and other soft tissues have an increased affinity for Zn relative to the same tissues of normal animals (Miller, 1970). The most reliable indicator of a Zn deficiency is when Zn levels in the liver drop below 100 mg/kg on a DM basis (NRC, 2001).

Zinc toxicity in adult cattle is uncommon. Excess dietary Zn will result in increased Zn levels in liver, a moderate increase in Zn levels in some tissues and milk, but no change in others including red muscle (Miller, 1970). The maximum tolerable concentration of Zn is 500 mg/kg (NRC, 2005). Research by Jenkins and Hidiroglou (1991) supports this level as they saw no effects on calf performance when fed 500 mg/kg for five weeks, but with 700 mg/kg they observed reduced gain and feed intake. However, the maximum tolerance for mature cows may be higher as Miller et al. (1988) noted that lactating cows have tolerated diets up to 1,300 ppm Zn without reduced performance. One exception to the rule may be due to a genetic defect that greatly reduces the ability of Zn absorption in Black Pied and Dutch-Friesan breed. These cattle become severely Zn deficient unless fed extremely high levels of dietary Zn (Miller et al., 1988; NRC, 2001). Other sources of Zn known to cause toxicity are consumption of galvanized coating off of gates and feed pans, contact with batteries, fungicides and certain automotive parts cattle may have been exposed to while grazing in pastures (NRC, 2005). Initial signs of Zn toxicosis are similar to Zn deficiency symptoms and include reduced feed intake and gain, as well as secondary deficiencies of other minerals (NRC, 2005).

TRACE MINERAL SUPPLEMENTATION

Effect on animal performance

Cobalt, Cu, Mn, and Zn have important roles in maintaining fertility, health and production of dairy cattle (Miller et al., 1988 and NRC, 2001). Research has shown specific amino acid complexes of trace minerals are more bioavailable and are better retained in the body than inorganic sources of trace minerals (Ashmead, 1970; Wedekind

et al., 1992; Nockels et al., 1993). The focus of this review will be to evaluate the effect of organic complexes of Co, Cu, Mn, and Zinc on production and reproductive performance in cattle. The reason Co, Cu, Mn, and Zn are often supplemented together is due to the fact they are the trace minerals found to be deficient most often in cattle.

Production and SCC

The increased bioavailability and retention of Cu, Mn and Zn AA complexes compared to inorganic sources of the minerals has resulted in improved animal performance in numerous studies. Kellogg et al. (2004) in a summary of 12 trials found feeding dairy cattle Zn AA complexes resulted in increased milk yield and reduced somatic cell count (**SCC)** compared to cows fed inorganic Zn. Siciliano-Jones et al. (2008) reported that cows fed AA complexes of Cu, Mn, and Zn and Co glucoheptonate (**AATM**) had increased yields of milk protein and solids and tended to produce more milk than cows fed minerals in sulfate form. In the study by Siciliano-Jones et al. (2008), approximately 21, 14, 37 and 100% of the supplemental Zn, Mn, Cu, and Co, respectively, were supplied by AATM with the remainder of the supplemental mineral supplied by sulfate minerals. Similar increases in milk yield were reported when AATM were added to dairy cow diets (Campbell et al., 1999; Kellogg et al., 2003; Griffiths et al., 2007) or when portions of the Zn, Mn, Cu, and Co from inorganic sources were replaced with AATM (Ballantine et al., 2002; Kincaid and Socha, 2004; Kinal et al., 2005; Nocek et al., 2006; Toni et al., 2007).

Griffiths et al. (2007) attributed the improvement in lactational performance to a result of improved Cu and vitamin B_{12} reserves as well as increased Zn as their intensively grazed pastures contained low levels of Cu and Zn. Ballantine et al. (2002)

noted a greater response in milk and milk component yields at peak production and mid to late lactation for cows fed AATM compared to those fed inorganic mineral sources. The authors attributed the response to the cows being heat stressed during that phase of lactation. Kincaid and Socha (2004) observed cows fed AATM (approximately 26, 18, 45, and 100% of supplemental Zn, Mn, Cu, and Co from AATM, respectively) produced more milk and milk components at peak lactation and less milk and components in mid-lactation. The authors attribute this to a time by treatment interaction, whereas milk and milk component yield will decrease as the days in milk increase. Nocek et al. (2006) reported that fortification of trace minerals with inorganic and complexed sources together at or above NRC requirements improved production performance. Nocek et al. (2006) also noted that supplementation of 75% of NRC recommendations with AATM allows for the same performance as inorganic sources fed at 100% of NRC levels. In a trial where AATM was fed to first calf heifers, increased milk yield (34.65 vs. 32.94 kg/d), and milk component yield was reported compared to heifers fed diets containing inorganic sources of minerals (Ashmead et al., 2004). A study on the effect of AATM supplementation on three successive lactations in Holstein dairy cows revealed that feeding AATM improved milk and milk component production each lactation, compared to cows supplemented with inorganic mineral sources (Ashmead and Samford, 2004). This is in contrast to work done by Campbell et al., (1999), Ferguson et al., (2004a), and Uchida et al. (2001) where there was no effect on milk yield, with supplementation of AATM. Work by Formigoni et al. (1993) reported no effect of supplementation with 100% AATM beginning 10 d postpartum through 221 d on milk yield or components.

Increases in milk yield and components have not been observed in studies by Formigoni et al. 1993 and Uchida et al., 2001. Formigoni et al., (1993) did not elaborate as to why no effect of AATM was observed. However, Uchida et al., (2001) attributed their lack of a response to AATM to the high mineral content of their basal diets. However, (Kellogg et al., 2003; Ferguson et al., 2004a; Kincaid and Socha, 2004; Toni et al. 2007) reported greater milk component content for cows fed diets containing AATM compared to those fed inorganic sources of trace minerals. A study by Kinal et al. (2007) where 20 or 30% of daily mineral sources came from AATM also observed no effect on milk yield or basal milk components, but they did observe a drop in SCC with AATM supplementation (22 and 34% decline with 20% and 30% AATM replacement, respectively). Studies by Kinal et al. (2005) and Toni et al. (2007) also reported a reduction in SCC for cows fed diets containing AATM compared to those fed diets containing inorganic sources of trace minerals. Kellogg et al. (2003) in a summary of eight dairy trials feeding metal specific amino acids observed that cows fed organic complexed trace minerals tended to have a reduced SCC compared to cows fed inorganic sources of trace minerals (301,000 vs. 343,000, respectively).

Research has shown that adding AATM to diets of dairy cattle can improve claw health and integrity (Nocek et al., 200; Drendel et al., 2005). Drendel et al. (2005) observed that supplementing dairy heifers with 360 mg Zn, 200 mg Mn, 125 mg Cu, and 12 mg from AATM from 12 months of age to 1 month postpartum decreased claw disorders present at 2 months postpartum and helped alleviate the effects of claw disorders on milk production during first lactation. Siciliano-Jones et al., (2008) reported that replacing sulfate minerals with AATM resulted in improved claw integrity. These

results are similar to those reported by Ballantine et al. (2002) where replacement of sulfate minerals with AATM, at 21 d prepartum, decreased the incidence and severity of white line disease at both 75 and 250 d postpartum, severity of sole ulcers at 250 d postpartum, and severity of heel erosion at 75 d postpartum. Ferguson et al. (2004b) also observed a reduction in sole ulcers and lesions when AATM replaced inorganic mineral sources. Nocek et al. (2006) saw a reduction in white line separation and heel erosion with feeding AATM compared to inorganic sources, but saw no effect on solar hemorrhage, sole ulcers or digital dermatitis. However, work by Toni et al. (2007) noted no improvements in claw integrity when inorganic mineral sources were replaced with AATM. Toni et al. (2007) speculated that there treatment of 9 months may have not been sufficient time to detect improvements in claw integrity and that based on their measurements 12 to 30 months is a more realistic time period to observe an affect if one was to exist.

Reproductive performance

The role of minerals in improving fertility is a topic that is of great interest to the dairy industry. Economic loss due to poor reproductive performance is second only to mastitis (Manspeaker and Robl, 1993). There have been numerous studies designed to study the effect of mineral supplementation and source on reproductive performance during lactation and the months prior to insemination. Increased display of estrus and conception rates have been observed when trace minerals were added to deficient diets (Kropp, 1993). Even when dietary levels were adequate in trace mineral content, the supplementation of diets with AATM have resulted in improved reproductive

performance in beef cattle (Kropp, 1993) and dairy heifers (Manspeaker and Robl, 1993; Ashmead et al., 2004).

Studies by Toni et al. (2007) and Siciliano-Jones et al. (2008) observed minimal effects of replacing inorganic minerals with AATM in dairy cow diets on fertility, but herd culling rate was decreased with feeding AATM. The authors attributed the decreased culling rate to better overall animal health with AATM supplementation. Previous research has shown cows fed AATM, either in addition to inorganic minerals or in substitution for inorganic sources, exhibited fewer days to first estrus (Campbell et al., 1999; Nocek et al., 2006), fewer services per conception (Uchida et al, 2001), reduced days to conception (Uchida et al., 2001; Ballantine et al., 2002; Kellogg et al. 2003; Bosseboeuf et al., 2006), and increased pregnancy rate (Ballantine et al., 2002; Ferguson et al., 2004a; Bosseboeuf et al., 2006; Nocek et al., 2006).

While Campbell et al. (1999) observed that cows given an additional 359 mg Zn, 199 mg, Mn, 125 mg Cu, and 26 mg Co from AATM sources had reduced days to first estrus, there was no effect on days to first service, days open, days from first service to conception and services per conception. The authors believe this may be more of an effect of management than treatment due to their 60 d voluntary waiting period. Campbell et al. (1999) did note that cows given additional AATM that had a retained placenta had fewer days to first estrus, days to first luteal activity and days to first corpus luteum than control cows that had a retained placenta. Formigoni et al. (1993) observed a similar response in days open, in which cows fed AATM had similar days to first service as those fed inorganic minerals.

Studies in beef cattle have exhibited inconsistent results with feeding AATM on reproductive performance as well. One study by Ahola et al. (2004) observed that supplementing beef cows with AATM over a two-year period resulted in increased pregnancy rates to timed A.I. if observed in estrus (77%, 65% and 58% for AATM, inorganic minerals and no supplementation, respectively). In the same study, kilograms of calf weaned per cow exposed was increased for cows supplemented with AATM. DiCostanzo et al., (1986) observed that supplementing a low Mn diet, with a chelated form of Mn, decreased the number of services required to achieve conception.

A study by Floyd et al. (1995) noted no improvement in reproductive performance of beef cows with AATM supplementation. Similarly, Olson et al. (1999) observed that AATM supplementation 60 d prior to breeding had a reduction in reproductive performance compared to cows with inorganic or no supplementation. However, Olson et al. (1999) fed the AATM at twice the recommended levels and they believe that this may have caused a mineral imbalance or subclinical toxicosis that lead to the decrease in reproductive performance. However, improvements in reproductive performance and fertility in response to increased bioavailability of Cu, Mn, and Zn are of biological significance.

Health and Immunity

Adequate trace mineral nutrition is essential for good health. Only recently have researchers began to study trace minerals for their affect on immune system function. A review by Failla (2003) stated these conclusions on the relationship between trace mineral status and immune function: 1) an inadequate supply of essential trace minerals is associated with suppression of cell activity in both the innate and acquired branches of

the immune system; 2) the observed alterations in immune function reflect either decreased activity of immune cells, a reduction in the total number of effector cells in one or more tissues, or a combination of fewer cells with each cell having decreased capacity; 3) the extent of immune system impairment due to trace mineral deficiency can lead to increased morbidity and mortality due to infection; and 4) reversal of the trace mineral deficiency restores immune function. Most of the current research evaluating the relationship between, trace minerals and immune function in cattle has been conducted using beef cattle or beef calves under stressed conditions. The primary trace minerals investigated in the relationship with immune function have been Cu, Fe, Se, and Zn. For the purposes of this discussion the focus will be on the specific roles of Cu and Zn.

Very limited research on the impact of Co and Mn on immune function has been conducted. It has been suggested that a Co deficiency may impact neutrophil function and resistance to parasites (Spears, 2000). While work by Smialowicz et al. (1985) observed that Mn-chloride helped to enhance the immune response in mice by increasing the functional activity of macrophages. Research with these two trace minerals is very limited but may be of interest to researchers in the future.

Copper and zinc have been studied primarily for their role as required components of superoxide dismutase, an enzyme which protects cells from reactive oxygen species formed by neutrophils in order to destroy pathogens (Spears, 2000). Research by Fraker et al. (1977) noted atrophy of the thymus and an increase in leukocyte count with a reduced number of active lymphocytes in a Zn deficient state. Because the thymus is important in T-cell formation a Zn deficiency could have a pronounced effect on this part of the immune system. Zinc has also been studied intensively because of its roles in cell

division processes, and would appear probable to play an important role in lymphocyte functions which are dependent on rapid cell division in response to antigen stimulation (Chirase et al., 1991). As stated earlier, Cu has been studied with great detail because of its role in the copper/zinc-superoxide dismutase enzyme complex. However, the role of Cu in immune response is very complex due to the many interactions that occur between Cu and other minerals (Spears, 2000). While research by Xin et al. (1991) suggests that a decreased Cu status may reduce neutrophil killing ability, most studies on the effect of Cu on immune response in cattle suggest very little or no effect (Stabel et al., 1993; Arthington et al., 1995; Ward et al., 1997; Ward and Spears, 1999).

In a review of cattle health/immunity and nutrition by Galyean et al. (1999), the authors noted that while supplemental Cu and Zn have altered the immune response and decreased respiratory disease under field conditions, the results have been inconsistent. Improvements in immune response were observed when Zn (Kegley et al., 2001) or AATM was fed above requirements (Ahola et al., 2005a; Ahola et al., 2005b; George et al., 1997; Stanton et al., 2001) compared to those cattle fed inorganic sources of minerals. George et al. (1997) and Chirase et al. (1991) observed that calves fed elevated AATM levels had a reduction in incidence and reoccurrence of respiratory disease.

In contrast, a study in weanling pigs by van Heugten et al. (2003) noted that Zn at recommended dietary levels was sufficient for optimal immune response and additional supplemental Zn above dietary requirements, from organic sources did not improve immune response. Spears and Kegley (2002) also observed no improvement in cell mediated or humoral immune response in beef cows supplemented with additional Zn from organic or inorganic sources.

The exact amounts of dietary trace minerals required to optimize immune system function are not known and may depend on level of deficiency, antagonist levels and physiological state of the animal. It is well understood that animals fed a diet balanced in all nutrients is more likely to be resistant to infections. Feeding a balanced diet will allow for normal body function, maintenance of skin integrity and other body tissues, normal antibody production and normal blood flow among many other factors. Acknowledging that trace mineral requirements are determined by maintenance, growth, animal performance, and reproductive stress, more work needs to be done to determine how immune challenges may affect requirement levels. However, we must keep in mind that mineral balance is important for proper function of the immune system and supplementation at very high levels of an individual mineral may reduce the efficacy of the immune system just as would be observed in a deficiency.

Effect on intestinal integrity

Intake of colostrum and the minerals, nutrients, and biologically active substances it contains are essential for proper calf nutrition, and acquisition of passive immunity (Bush and Staley, 1980; Kume and Tanabe, 1993; Quigley and Drewry, 1996; Quigley et al., 2001; Wheeler et al., 2007). Colostrum intake is also needed to stimulate development and function of the gastrointestinal tract in calves (Buhler et al., 1998; Blattler et al., 2001; David et al., 2003; Norrman et al., 2003; Roffler et al., 2003) and in pigs (Odle et al., 1996). Recently there has been interest in feeding high supplemental Zn (pharmacological levels) and its effect on intestinal development. The interest in Zn is related to its role in cell division as described earlier with specific interest to supplementation from organic sources. Research by Baldwin et al. (2004) noted that

intestinal mass and metabolism in pre and postweaned calves respond to dietary changes just like the rumen as the calf transitions from an all milk diet to a grain based diet. The authors also noted that amino acid use by the intestinal tissue is high as the tissue continues to develop. This may help to explain the interest in using AA complexes of trace minerals. Caine et al. (2001) supplemented gestating sows with a 250 ppm diet Zn AA complex from d 80 of gestation until farrowing and observed increased villus height (258 vs. 225 μm), lower crypt depth (78 vs. 85 μm), and a higher villus height to crypt depth ratio (3.7 vs. 2.9) in the jejunum than those piglets supplemented with no additional Zn. Intra-epithelial lymphocyte counts per 250 μm length of villus epithelium were higher in duodenum, jejunum and ileum of piglets from sows fed Zn AA complex (5.5, 3.6, and 4.0, respectively) compared to (4.2, 2.8, and 2.9, respectively) those supplemented with no additional Zn.

Similar results were observed by Payne et al. (2006) where weanling pigs from sows given 100 ppm supplemental Zn in sulfate or organic forms, in addition to 120 ppm of dietary Zn, had increased jejunum villus height compared to those sows not supplemented with Zn. Payne et al. (2006) also noted that regardless of Zn form, pigs weaned from sows given supplemental Zn had increased villus height compared to control sows. The authors concluded, however, that supplementing the additional 100 ppm of Zn in organic form in the sow diets better provided for the fetuses during times of important development resulting in increased pigs born and weaned per litter compared to those supplemented with the sulfate form of Zn.

Takeo et al. (2005) injected Zn AA complex into the amniotic fluid of un-hatched chicks and noted enhanced intestinal development and functionality as well as

increased jejunal villus surface area. This research in chicks (Takeo et al., 2005) and baby pigs (Caine et al., 2001 and Payne et al., 2006) indicates supplementation of zinc during the gestation period can increase intestinal villi growth and enhance nutrient absorption during the first few weeks of life. More research is needed to investigate if trace mineral form and the feeding of chelated zinc above requirements, have a similar affect on intestinal villi development and nutrient absorption in other species.

Effect on Colostrum and Immunoglobulins

According to the USDA, mortality and morbidity rates for pre-weaned dairy calves are 7.8% and 31.4%, respectively (National Animal Health Monitoring Service, 2007). Costs associated with the morbidity and mortality in 2002 during the pre-weaned period are estimated between 90 and 180 million dollars, with approximately 1.3 million man-hours dedicated to the care of sick calves (National Animal Health Monitoring Service, 2002). The biggest impact on neonatal calf health is consumption of quality colostrum and sufficient absorption of IgG.

Quigley and Drewry (1996) suggest providing the transition cow with sufficient levels of trace minerals to minimize the negative effects of stress on the animal, allowing for improved lactation performance and producing high quality colostrum. The goal of trace mineral supplementation to transition cows is to improve the immune status of the cow and the calf by providing adequate minerals for placental transfer and mineral content of colostrum. The supplementation of trace minerals may also help to prevent a decline in animal performance that may exist in high stress transition cows. For approximately the first three weeks of its life, the calf relies primarily on the antibodies acquired from colostrum to protect itself against pathogens (Franklin, 2004), because

virtually no immunoglobulins are transferred *in utero* (Smith and Foster, 2007). In the past, feeding strategies for newborn calves were to feed high quality colostrum to provide at least 100 g of IgG within 1 to 2 h after birth (Quigley et al., 2001). Recent research using colostrum replacers suggests that the amount of IgG fed to newborn calves may need to be increased to 150 to 200 g of IgG within 1 to 2 h of birth (Foster et. al 2006 and Godden et al. 2009).

Kinal et al. (2005) supplemented organic trace minerals (**TM**) from 6 wks prior to calving through 305 DIM, at 315 mg for Zn and Mn and 63 mg of Cu in daily rations for dairy cows and observed increased Zn and Cu serum levels in cows, which increased immunoglobulin content in colostrum and in blood serum of calves. Kinal et al. (2005) also noted improved daily weight gain in calves for the first 3 months of life. Earlier research by Kincaid and Socha (2004) fed organic TM from 21 d prepartum to 150 DIM and found a similar response in IgG levels; however, there was no effect on IgM, or mineral content of colostrum. Besides the difference in supplementation length the Kincaid et al. (2004) and Kinal et al. (2005) studies differed in TM levels too as, Kincaid and Socha (2004) supplemented with organic Co and lower levels of Cu and Mn.

While there are numerous studies evaluating colostrum for content of immunoglobulins and its affect on calf health and performance, there is very little research evaluating the effect of trace mineral supplementation to gestating dairy cows on colostrum quality. While trace mineral supplementation to cows during the dry period is important (NRC, 2001), future research should focus on the effect trace mineral supplementation has on the fat, protein, mineral and immunoglobulin content in colostrum. This research would provide data regarding the role of trace minerals in

enhancing the immune status of dairy calves and the impact of feeding trace minerals on the composition of colostrum.

CONCLUSIONS AND IMPLICATIONS

In 1996, according to Quigley and Drewry, the management of gestating heifers and cows on dairy farms was minimal and producers largely believed the nutrient requirements of these animals were low and could be met with pasture or fair to poor quality hay. Over the last 10-15 years considerable progress has been made towards understanding and meeting the nutritional requirements of transitioning cows from the dry period into lactation (Overton and Waldron, 2004). Most recently, researchers have begun to investigate the relationship between the dams' nutritional status and calf health and performance. There is little research however, that has determined the impact of the cow's trace mineral status on calf performance and health. Improved understanding of this relationship would enable producers to better provide optimum trace mineral levels to the cow during the dry period.

Trace minerals are generally recognized to have the following functions within the body: structural, physiological, catalytic, and regulatory (Underwood and Suttle, 1999). While much is known about trace minerals and their function within the body of animals there is not a complete understanding of how they function on a cellular level. Improved knowledge in this area may be the key to explaining how chemical form of dietary trace minerals affects immunity, and if relative trace element availability within cells is a factor as suggested by Kincaid and Socha (2004).

Impact of dietary trace mineral source and zinc supplementation during the dry period on the subsequent lactation and reproductive performance.

OVERVIEW

The objective of this study was to investigate the effects of supplemental dietary trace mineral source and zinc concentration during the non-lactating period of approximately 60 d prepartum on dairy cow trace mineral status and performance through 150 DIM. Fifty-two Holstein and cross-bred cows were utilized at the University of Minnesota (U of MN) St. Paul dairy (**STP**) and 62 Holstein cows at the U of MN Northwest Research and Outreach Center (**NWROC,** Crookston, MN) dairy. Cows were blocked by breed (STP) or parity and season of expected calving (NWROC), and then randomly assigned to one of three trace mineral treatments. Treatments were: 1) 100% inorganic trace mineral supplementation (**CON**); 2) organic trace mineral supplemented at the following rate: 40% of supplemented zinc (Zn), 24% of supplemented manganese (Mn), 69% of supplemented copper (Cu) and 100% of supplemented cobalt (Co) supplied by 4-Plex.(Zinpro Corp., Eden Prairie, MN; **OTM**) and 3) OTM with additional Zn from Zn-methionine (**OTMZ**). Dietary trace mineral supplements were formulated to provide similar amounts of Co, Cu and Mn. Dietary Zn supplemental amounts were similar for CON and OTM (75 mg/d), but approximately two times higher for OTMZ. Under conditions of the current study, trace mineral source or amount of Zn supplemented during the dry period did not have an effect on serum vitamin B_{12} concentrations at parturition, averaging 248.3 pg/mL. Milk yield was unaffected by mineral treatment averaging 40.0 kg/d at STP and 44.9 kg/d and 31.5 kg/d for multiparous and primiparous cows at NWROC, respectively. Number of pregnant multiparous cows at NWROC fed

OTMZ tended ($P = 0.09$) to be greater at 150 days in milk (**DIM**) compared to CON and OTM.

(**Key Words:** transition cows, organic trace minerals, performance)

INTRODUCTION

Trace minerals such as cobalt (**Co**), copper (**Cu**), manganese (**Mn**), and zinc (**Zn**) have important roles in physiological processes related to growth, reproduction, and health. Studies have shown feeding amino acid (AA) complexes of trace minerals pre- and postpartum may result in improved milk yield and immune function, and decreased incidence of hoof health issues and mastitis in dairy cows (Uchida et al., 2001; Ballantine et al., 2002; Kellogg et al., 2003, 2004; Kincaid and Socha, 2004; Nocek et al., 2006; Siciliano-Jones et al., 2008). However, there is limited research evaluating the effect of organic or inorganic trace mineral supplementation or Zn amount during the dry period on the nutrient and immunoglobulin content in colostrum (Kincaid and Socha, 2004; Kinal et al., 2007). The effect of trace mineral source and Zn amount during the dry period on colostrum nutrient and immunoglobulin content and calf performance will be discussed further in a companion paper (Golombeski et al., 2011).

The objective of this study was to investigate the effects of supplemental dietary trace mineral source and Zn amount fed during the non-lactating period of approximately 60 d prepartum on dairy cow trace mineral status and performance following parturition, as measured by milk yield, milk composition, and percent of cows pregnant at 150 days in milk (**DIM**).

MATERIALS AND METHODS

Location, Cows, and Treatments

All animal care and usage was according to a protocol approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC # 0603A83108). Cows were housed at the University of Minnesota (U of MN) St. Paul dairy (**STP**) and the U of MN Northwest Research and Outreach Center dairy (**NWROC,** Crookston, MN). Fifty-two Holstein and cross-bred cows at STP (multiparous) and 62 Holstein cows at NWROC (multiparous and primiparous) were utilized. Cows were blocked by breed in STP and by parity and season of expected calving at NWROC, and then randomly assigned to one of three trace mineral treatments.

Treatments were supplemental trace minerals fed as: 1) 100% inorganic trace mineral (**CON**); 2) organic trace minerals supplemented at the following rate: 40% of supplemented zinc (Zn), 24% of supplemented manganese (Mn), 69% of supplemented copper (Cu) and 100% of supplemented cobalt (Co) supplied by 4-Plex(Zinpro Corp., Eden Prairie, MN) (**OTM**) and 3) OTM with additional Zn from Zn-methionine (Zinpro Corp., Eden Prairie, MN); (**OTMZ**). Dietary trace mineral supplements were formulated to provide similar amounts of Co, Cu and Mn, however OTMZ contained approximately twice as much Zn as compared to CON and OTM. Trace mineral supplements contained dried distillers grains as a carrier and were top-dressed on the total mixed ration (**TMR**) at 0.422 kg of DM per head daily starting approximately 60 d pre-partum until calving.. To ensure the same trace mineral mix was fed at STP and NWROC, the trace mineral supplements were manufactured in a single batch per treatment and then split between STP and NWROC for feeding.

The analyzed trace mineral concentration of the supplements are presented in Table 1. While all three trace mineral treatments were formulated to contain similar amounts of Co, the OTMZ contained less than half the amount contained in CON and OTM. The concentration of Cu was about one-third less and Mn concentration about one-fifth less for OTMZ compared to CON and OTM. The reason for differences in Co, Cu, and Mn in the OTMZ treatment is unknown as mixes were manufactured according to formulation specifications. Zinc concentration of OTMZ was 56% greater than CON or OTM which was similar to the formulated amount. Although OTMZ was lower in concentrations of Co, Cu and Mn than CON or OTM it still met or exceeded NRC (2001) requirements for those minerals.

At STP, cows were housed in a tie stall barn and individually fed throughout the study. At NWROC, cows were loose housed and group fed outside from 60 to 30 d prior to expected calving date and then moved to a tie stall barn and individually fed the final 30 d before expected calving date. Within each location, a common basal **far-off** diet was fed 60 to 30 d prepartum and a **close-up diet** beginning 30 d prior to expected due date. Ingredient composition of the far-off and close-up basal diets varied slightly by location, however, nutrient composition of diets were formulated to be similar across the two locations for both the far-off and close-up periods (STP, Table 2; NWROC, Table 3, respectively). Feedstuffs at each location were analyzed for nutrient content prior to formulation of far-off and close-up diets. Diets were formulated based on NRC (2001) recommendations and a projected daily DM intake (**DMI**) of 12.7 kg/d, however cows were fed to ad libitum intake. Samples of the far-off and close-up diets at both the STP and NWROC were evaluated for particle length using the Penn State particle separator

(Tables 2 and, 3 respectively). The recommended range for material on the top screen is 2 to 8%, while both the far-off and close-up diets at STP were slightly above that, the cows did not appear to sort the diets. As far as the recommendations on the 2nd and 3rd sieve both diets at STP and NWROC were within the recommendations of 30-50% of material on the 2nd and 3rd sieve. The goal is to have less than 20% of the total in the bottom pan and diets at both locations achieved the goal. Following parturition, trace mineral treatments were discontinued and all cows received the same TMR within location. The lactation diet was formulated to support 40.8 kg/d of milk and cows were supplemented to NRC (2001) recommendations with inorganic trace minerals. Animal performance was followed until 150 DIM.

Data Collection – Dry Period. Daily DMI was recorded from 30 d prepartum to parturition. Samples of feed ingredients were taken weekly and composited by month. Total mixed ration samples were collected every other week and frozen at -20°C until analysis. Blood samples and liver biopsies were taken from each cow at 60±7 d pre-partum and prior to feeding the assigned trace mineral treatment. Blood was sampled from a coccygeal vein using evacuated tubes for serum and serum trace mineral with no additive (Vacutainer, Becton Dickinson and Co., Franklin Lakes, NJ). Blood samples were stored on ice for transport to the laboratory where they were centrifuged followed by serum transfer to a polypropylene tube and frozen at −20°C until analysis. Liver biopsies were obtained from the right side of the cow at approximately the intersection of a line running from the tuber coxae to the shoulder joint. Samples were taken between the ninth or tenth intercostal space using a custom 10-gauge biopsy instrument. Samples

were rinsed in saline and immediately placed on ice packs, brought to the laboratory, and stored at −20°C until analysis. All calving and cow health events were recorded.

Data Collection - Lactating Period. Blood samples were collected, via coccygeal venipuncture, from each cow immediately following parturition and centrifuged to collect serum. Liver biopsies were collected within 2 days of parturition as previous described. Cow health events were recorded. Samples of the lactation TMR were taken every two weeks and frozen until analysis. Cows were milked twice daily with individual milk weights recorded at each milking. Individual metered milk samples were taken every two weeks during the a.m. and p.m. milkings for component analysis. Milk yield and composition as well as reproductive performance data were collected through 150 DIM.

Laboratory Analysis

Liver and serum samples. Liver tissue was analyzed for mineral concentration by the University of Minnesota Soil Testing and Research Analytical Laboratory. Liver samples were prepped with 0.5 mL of nitric acid and 2 mL of 30% hydrogen peroxide. Twelve vessels at a time were heated in the microwave at 33% power for 6 min. After the initial reaction, the microwave was programmed to use full power to heat the samples to 165°C for 8 min. After cooling, the samples were transferred to centrifuge tubes, adjusted to 10 mL with deionized water and 1 ppm yttrium as an internal standard during analysis. The analysis was done on a dual viewing Perkin Elmer 3000DV Inductively Coupled Plasma - Atomic Emission Spectrometer(ICP-AES). Potassium, Mg, and Na were read using radial viewing of the plasma and the other elements were read using axial viewing. Serum vitamin B_{12} concentration was determined by Washington State University using RIA (Dualcount®, Diagnostic Products Co., Los Angeles, CA). The analysis was

modified so proteins were denatured by heating serum samples in a boiling water bath for 15 min instead of incubating at 37°C for 30 min.

Feed Samples. All dry and lactation feed sample composites were dried for 48 h in a 60°C forced air oven (model DC-246-E; Blue M Electric, Watertown, WI) to determine DM and ground to pass through a 1-mm screen (Wiley mill; Swedesboro, NJ). Subsamples of feed composites were dried at 105°C for 24 h to correct to 100% DM. Organic matter was determined by ashing the samples in a muffle furnace at 500°C (AOAC, 2000). Samples were analyzed for CP (LECO, Tru-Spec N, St. Joseph, MI; AOAC, 2000), and ether extract (EE; AOAC, 2000). The Ankom^{200} fiber system (Ankom Technology Corporation, Fairport, NY) was used for sequential analysis of NDF and ADF (Hintz et al., 1996). Samples were analyzed for NDF using sodium sulfite and α-amylase (Sigma no.A3306; Sigma Chemical Co., St. Louis, MO). Neutral detergent insoluble CP and acid detergent insoluble CP were determined by Kjeldahl analysis (AOAC, 2000) using the NDF or sequential ADF residue, respectively. Minerals were analyzed by the University of Minnesota Soil Testing and Research Analytical Laboratory using a Perkin Elmer 3000DV ICP-AES. Cobalt was analyzed using ICP by Minnesota Valley Testing Labs, Inc. (New Ulm, MN). Samples of TMR from the far-off and close-up diets were analyzed for particle size using the Penn State Particle Separator.

Milk samples. Milk composition analysis was conducted by Minnesota DHIA Laboratory (Sauk Center, MN and Zumbrota, MN) for true protein, fat, and lactose using near-infrared spectroscopy (Bentley 200 Infrared Milk Analyzer; Bentley Instruments, Chaska, MN), milk urea nitrogen (MUN) using chemical methodology based on a modified Berthelot reaction (ChemSpec 150 Analyzer; Bentley Instruments), and somatic

cell count (**SCC**) using a flow cytometer laser (Somacount 500; Bentley Instruments). The Gaines formula (NRC, 2001) was used to calculate 4% fat corrected milk (4% FCM, kg/d = 0.4 x milk, kg/d + 15 x fat, kg/d). Energy-corrected milk was calculated as follows (Orth, 1992): ECM (kg/d) = [(0.327 × kg of milk) + (12.95 × kg of fat) + (7.2 × kg of protein)].

Statistical Analysis

St. Paul

Data were analyzed as a randomized complete block. Cows were blocked by breed and randomly assigned to treatment within block. Weekly means of pre-partum DM, milk yield and milk composition were analyzed as repeated measures. The data were analyzed using the MIXED procedures of SAS (SAS Institute, 2001). Pre-partum DM intake, serum vitamin B_{12} concentrations, liver mineral concentrations, milk yield and composition data were analyzed with the following model:

$$Y_{ijk} = \mu + B_i + T_j + W_k + (B_i \times T_j) + (T_i \times W_k) + e_{ijk}$$

where μ = overall mean, B_i = effect of breed (i = 1 to 2), T_j = effect of treatment (j = 1 to 3), W_k = effect of week (k = 1 to 21), $(B_i \times T_j)$ = interaction of B_i and T_j, $(T_j \times W_k)$ = interaction of T_j and W_k and e_{ij} = random residual error. Interactions that were not significant were dropped from the model.

The LOGISTIC procedure of SAS was used to determine risk for pregnancy at 150 DIM. Cows were omitted from the data set if they died, were placed on the "do not breed" (**DNB**) list, or culled prior to 150 DIM. Significance was declared at $P < 0.05$, and a tendency at $P < 0.10$.

NWROC

Data were analyzed as a randomized complete block. Cows were blocked by season of calving and randomly assigned to treatment within block. Block 1 = cows that calved in August, September, October, and November and Block 2 = cows that calved in December, January, February, and March. Weekly means of DMI and milk yield and composition were analyzed as repeated measures. Primiparous and multiparous cows were both utilized at the NWROC location but the data were analyzed separately. Data from the primiparous and multiparous cows were analyzed separately due to unbalanced numbers across parity and block. The data were analyzed using the MIXED procedures of SAS (SAS Institute, 2001). Pre-partum DMI, liver mineral concentrations, milk yield and composition data were analyzed with the following model:

$Y_{ijk} = \mu + T_i + S_j + W_k + (T_i \times S_j) + (T_i \times W_k) + e_{ijk}$

where μ = overall mean, T_i = effect of treatment (j = 1 to 3), S_j = effect of season of calving (m = 1 to 2), W_k = effect of week (k = 1 to 21), $(T_i \times S_j)$ = interaction of T_i and S_j, $(T_i \times W_k)$ = interaction of T_i and W_k and e_{ijk} = random residual error. Interactions that were not significant were dropped from the model.

The LOGISTIC procedure of SAS was used to determine risk for pregnancy at 150 DIM. Cows were omitted from the data set if they died, were placed on the DNB list, or culled prior to 150 DIM. Significance was declared at $P < 0.05$, and a tendency at $P < 0.10$.

RESULTS AND DISCUSSION

Location and Cows

At STP, 52 cows started on trial however 5 cows were removed from the study for health reasons (Table 4). No treatment associated reasons were apparent and no treatment had more than 2 cows removed. One Holstein cow aborted after only 20 d on treatment, one cow died 13 DIM and the other 3 cows were removed from the study due to severe mastitis starting at parturition. At the NWROC, 62 cows started on trial, and 9 cows were removed from the study (Table 4). Treatment OTM had the most cows removed (n=4) for a variety of health reasons and complications following displaced abomasum (**DA**) surgery. Three cows were removed from the CON treatment: two cows were euthanized within 30 DIM, one was a downer cow and the other had complications from DA surgery, and one cow was sold due to complications after DA surgery. Two cows were removed from OTMZ, one cow died within 30 DIM from ketosis following DA surgery and the other cow was sold due to mastitis after 100 DIM. Overall, no health disorders could be directly associated with treatment, as cow numbers per treatment were inadequate to test for health disorder differences and similar disorders occurred across all 3 treatments.

Dry Matter Intake

There was no significant differences in prepartum **DMI** ($P = 0.80$) at STP (Figure 1) or within the multiparous (Figure 2) and primiparous (Figure 3) cows at NWROC due to treatment. Weekly DMI for multiparous cows in STP averaged 13.0 kg/d from 60 d to 30 d prepartum. Holstein cows at STP tended ($P = 0.08$) to have a higher DMI than the Crossbred cows (12.7 vs. 11.9 kg/d, respectively). Trace mineral treatment had no effect

($P = 0.95$) on prepartum DMI for multiparous cows at NWROC, with a daily average DMI of 12.7 kg/d. Multiparous cows at STP and NWROC had similar DMI the last 30 d prepartum. Prepartum DMI for NWROC primiparous cows averaged 8.91 kg/d and was not affected ($P = 0.56$) by trace mineral source. The DMI of multiparous cows at both locations and primiparous at NWROC was very close to predicted DMI reported in the NRC (2001). At NWROC, since multiparous and primiparous cows were loose housed together by treatment and group fed from approximately 60 to 30 d prior to expected calving date, only average pen DMI was available for the far-off diet. Pen DMI at NWROC during the far-off period averaged 10.3 kg/d.

Treatments provided the following supplemental trace minerals amounts during the dry period: CON = 25.5, 192.8, 409.6 and 1375.0 mg/d of Co, Cu, Mn, and Zn respectively; OTM = 23.7, 204.6, 405.6, and 1337.0 mg/d respectively; and OTMZ = 10.3, 133.5, 335.8, and 3092.1 mg/d respectively. Total intake of Co, Cu, Mn, and Zn, basal diet plus treatment mineral mix, for both the far-off and close-up periods by location are listed in Table 5. Using the NRC (2001) required dietary concentrations Co, Cu, Mn and Zn for gestating dry cows and a predicted DMI of 12.7 kg/d, the daily requirement amounts were calculated for Co (1.4 mg/d), Cu (174.0 mg/d), Mn (226.1 mg/d) and Zn (289.6 mg/d). Cobalt amounts consumed in this study (25 mg/d) were approximately 18 times greater than NRC recommendations, but similar to amounts fed by Kincaid et al. (2003). Increased levels of Co was fed to provide sufficient Co for vitamin B_{12} synthesis and thereby meet the demand for vitamin B_{12} in early lactation cows. Dietary Cu was fed at amounts 1 to 1.5 above NRC (2001) requirements. Total dietary Mn in the current study was slightly higher than the amount fed by Weiss and

Socha (2005). The purpose of supplementing above NRC (2001) requirements is to provide adequate Mn to achieve a positive Mn balance, and allow for fetal Mn tissue accretion. For the dam, adequate Mn is required for good reproductive performance (Nocek et al. 2006), but very few studies have assessed the effects of feeding different forms of Mn during the dry period and what carry-over affects, if any, on reproductive performance during the subsequent lactation. Dietary Zn levels for CON and OTM were similar to amounts fed by Campbell and Miller (1998) to late gestating dry cows to improve fertility and health post calving. Total dietary Zn intake for OTMZ was approximately twice the amount of CON and OTM and about 10 times above NRC (2001) recommendations. Recently, supplementation of organic Zn sources during gestation in swine (Caine et al. 2001 and Payne et al. 2006) and pre-hatch poultry (Takeo et al. 2005) was shown to promote intestinal villi development and increase nutrient absorption in the offspring. Results from this study on intestinal morphology and nutrient absorption by the calf to feeding high amounts of organic Zn during the gestation period are reported in the following chapter.

Serum Vitamin B_{12} Concentration

Vitamin B_{12} concentrations at dry-off averaged 136 pg/mL for cows assigned to the CON and OTM and 110 pg/mL for cows assigned to the OTMZ treatment (Table 6). There was no significant difference in serum B_{12} concentrations among treatments. Our serum B_{12} concentrations at 60 d prepartum were lower than the 576 pg/mL reported by Kincaid and Socha (2007) at 55 d prepartum when feeding 0.15, 0.89 or 1.71 mg/kg of Co in the diet. At parturition, serum concentration of vitamin B_{12} was approximately twice the concentration observed at 60 d prepartum (128 vs. 248 pg/mL). Our

concentrations are less than the 325 pg/mL vitamin B_{12} observed at parturition by Girard and Matte (2005) with weekly injections of 10 mg of B_{12} but slightly higher than those reported by Kincaid and Socha (2007) at 20 d prepartum (230 pg/mL) and 7 d postpartum (205 pg/mL). Maternal reserves of vitamin B_{12} were reported to drop with increased fetal growth during late gestation and milk synthesis in early lactation (Kincaid and Socha, 2007). A decrease in DMI, and therefore Co intake, just prior to parturition also reduces the ruminal synthesis of vitamin B_{12} contributing a lower serum vitamin B_{12} concentration. In contrast to Kincaid and Socha (2007), serum vitamin B_{12} concentrations in the current study increased an average of 125 pg/mL from 55 d prepartum to parturition. This indicates even our lowest supplement level for Co (24 mg/kg) was adequate to increase serum B_{12} concentrations during the third trimester of gestation.

Liver Mineral Concentrations

Source of trace minerals had no affect on liver macro (Table 7 & 8) or micro (Table 9 & 10) mineral concentrations at dry-off and parturition at STP or NWROC. At 60 d prepartum, cows at STP differed slightly in liver Ca concentration and all cows would be considered marginal to deficient (< 40 mg/kg, wet weight basis) in liver Ca concentration according to Puls (1994). Liver concentrations of Mg and K for STP cows were approximately half those for the multiparous cows at NWROC. Based on values reported by Puls (1994), cows at STP would be defined as deficient (< 1,400 mg/kg) in liver K.

Micro minerals dry-off

Zinc liver concentrations at dry-off for cows at STP were about half the amount observed for cows at NWROC. Minimum concentration of Zn to be deemed adequate is

25 mg/kg (Puls, 1994) and cows at STP averaged 17.2 mg/kg. At dry-off or prior to feeding the TM treatments, cows at STP had a tendency ($P = 0.06$) to differ in Fe concentration. Normal liver iron concentrations during gestation are 43 to 200 mg/kg at 100 d of gestation and between 62 and 100 mg/kg at 280 d of gestation (Puls, 1994). Based on the values reported by Puls (1994), all cows at STP and the OTMZ multiparous cows at NWROC were below adequate liver concentrations for Fe prior to beginning treatments or approximately 220 d of gestation, but were not in a deficiency as defined (< 30 mg/kg) by Puls (1994). Copper concentrations at dry-off were highest for the STP cows, the NWROC multiparous cows were intermediate, with the primiparous cows being deemed less than adequate (<25 mg/kg Cu; Puls, 1994). Prior to beginning trace mineral treatments, all cows except the primiparous cows on the CON and OTMZ treatments were deemed marginal for liver Mn concentration (<2.5 mg/kg Mn; Puls, 1994).

The change in trace mineral concentration from 60 d prepartum to parturition was different between locations and parity. In STP, cows on all treatments either gained or remained constant in liver mineral concentrations liver during the supplementation period. Even though liver K concentrations increased for cows at STP, cows fed OTM and OTMZ were still defined as less than adequate concentrations. Multiparous cows at Crookston increased concentration of all trace minerals during the supplementation period, whereas primiparous cows increased liver mineral concentration of Cu, Fe and Zn, but decreased Mn liver concentrations during the feeding period. Cows at STP and NWROC fed OTMZ had numerically lower liver Zn concentrations than both the CON and OTM cows at the end of the 60 d supplementation period. While the cows fed OTMZ

at STP increased liver Zn concentrations from dry-off to parturition, the concentration was still less than adequate (<25 mg/kg Zn; Puls, 1994). The explanation for this is unknown.

Liver mineral concentrations of primiparous cows at NWROC tended to be higher in general than the multiparous cows at either location. Primiparous cows at NWROC tended to increase Cu and Zn during the 60 d trace mineral feeding period while Mn decreased. Manganese liver concentrations at parturition for all cows except the NWROC multiparous cows fed CON and OTMZ was defined as being marginal (< 2.5 mg/kg Mn). The exact reason for the marginal level of liver Mn is unknown and was not expected as total dietary Mn levels were fed above NRC (2001) requirements. However, these results do support the work of Weiss and Socha (2005) where they found that 580 mg/d Mn resulted in the average cow being at zero Mn balance, but there were cows on study consuming up to 1000 mg Mn daily and still in negative Mn balance. Similar liver trace mineral concentrations for cows supplemented with inorganic or organic trace minerals during the dry period, when adequate levels of Cu, Mn and Zn have been fed, have been reported by a number of authors (Ballantine et al., 2002; Ferguson et al., 2004a; Nocek et al., 2006; Siciliano-Jones et al., 2008).

Milk Production and Composition

Milk production and milk composition of cows at STP and NWROC were not affected by trace mineral supplementation during the dry period (Table 11 for STP and Tables 12 and 13, respectively for NWROC multiparous and primiparous cows). Multiparous cows at NWROC produced the most milk, averaging 44.9 kg/d followed by multiparous cows at STP (40.0 kg/d) with primiparous cows at NWROC having the lowest production

average (31.5 kg/d). There was a significant breed effect on milk yield at STP as Holsteins produced approximately 5 kg/d more than the crossbreds (42.3 vs. 37.7 kg/d). Our milk production results agree with other studies that have reported source of trace mineral supplementation had no effect on milk production (Campbell et al., 1999; Uchida et al. 2001; Ferguson et al., 2004a; and Kinal et al., 2007), but are in disagreement with studies finding an effect on milk production (Ballantine et al., 2002; Kincaid and Socha, 2004; Kinal et al., 2005; Nocek et al., 2006; Griffiths et al., 2007; Toni et al., 2007 and Siciliano-Jones et al. 2008). The difference between previous research and the current study is our trace mineral supplementation treatments were discontinued at parturition. Cows were supplemented to NRC (2001) recommendations during lactation with inorganic trace minerals, but there was no carry over effect from supplementing organic trace minerals during the dry period on milk production.

There was a trend for cows in STP fed the OTM to have an increased fat percent however, this trend was not detected for the multiparous or primiparous at NWROC. At STP, Crossbred cows had a higher percentage of fat (4.00 vs. 3.72%) and true protein (3.09 vs. 2.95%) in milk than the Holsteins. Yield of true milk protein trended higher for cows in STP on the OTMZ treatment, but this trend was not observed for cows at NWROC. These results are consistent with others (Formigoni et al. 1993; Uchida et al., 2001 and Kinal et al., 2007) who reported no differences in milk composition when feeding different sources of trace minerals. There was no effect of trace mineral treatment on energy corrected milk (**ECM**) yield at either STP or NWROC. However, at STP there was a significant effect of breed ($P \leq 0.01$) as Holsteins produced more ECM than crossbred cows (39.4 vs. 37.0 kg/d). Milk urea N was higher in milk from cows at

STP than NWROC cows and significantly higher for cows fed the CON and OTMZ treatments than the OTM in STP. The higher MUN in STP may be a result of higher dietary CP in the lactating diet than at NWROC (17.4 vs. 15.0% CP, respectively), however reasons for the treatment difference are unknown.

The inclusion of AA complexes of trace minerals in lactation diets has been shown to decrease SCC or somatic cell score (**SCS**) (Kellogg et al., 2003; Kinal et al. 2005; Kinal et al., 2007; and Toni et al. 2007). This response has been attributed to the role of Cu, Mn and Zn in maintaining immune function (Miller, 1988). Kinal et al., (2007) and increased keratin formation in the teat canal when cows were supplemented with organic Zn. These results are in contrast to the current study, as multiparous cows fed OTM at NWROC tended ($P = 0.07$) to have increased linear SCC compared to cows fed CON and OTMZ. The same tendency was not observed for cows at STP or the primiparous cows at NWROC. However, all of the previous research studies with AA trace mineral complexes continued supplementation into lactation while our study discontinued the organic form of trace mineral supplementation at parturition.

Previous work in lactating dairy cows has evaluated the impact of feeding different Zn sources (Kellogg et al., 2004 and Cope et al., 2009) and high levels of inorganic Zn (Miller et al., 1989) on milk yield and quality. In a 12 study summary, Kellogg et al. (2004) noted that cows fed organic Zn (Zn-methionine) produced more milk, had greater milk fat and protein yields and reduced SCC compared to cows fed inorganic Zn. The authors suggested feeding organic Zn reduces SCC resulting in a healthier udder and thereby improving milk yield. Cope et al. (2009) noted a similar benefit in milk yield when feeding organic Zn, however, no improvement in milk

component yield was observed. Miller et al. (1989) reported that supplementing lactating dairy cows with 1000 ppm inorganic Zn had no adverse affects on milk or milk component yield, but 12 weeks of supplementation with 2000 ppm inorganic Zn, reduced milk and milk protein yields. Cope et al. (2009) observed that supplementing Zn regardless of form reduced SCC in milk. In the current study, a difference in milk yield or quality due to organic Zn or increased feeding rate of organic Zn during the dry period was not observed.

Reproduction

Average days to first service for STP and NWROC were not affected by trace mineral supplementation source during the dry period (Table 14). Our results are similar to those reported by Campbell et al. (1999) however in this study a voluntary waiting period of 60 DIM was observed and timed A.I. programs were implemented, which negated some of the reproduction measures.

Odds ratios were calculated for cows at STP and NWROC to determine risk for pregnancy at 150 DIM (Table 15). There was no effect of treatment for risk of pregnancy ($P = 0.66$) for cows at STP. however there was a significant effect ($P = 0.004$) of breed on risk for pregnancy at 150 DIM. Crossbred cows were 7.80 times more likely to become pregnant by 150 DIM than the Holstein cows. There was also no effect of treatment on risk of pregnancy by 150 DIM for multiparous ($P = 0.45$) or primiparous ($P = 0.48$) cows at NWROC.. A lack of improvement in fertility seen in our study when cows were fed AA complexes of trace minerals during lactation is in contrast to previously reported studies (Ballantine et al., 2002; Ferguson et al., 2004a, Bosseboeuf et al., 2006; Nocek et al., 2006), However, increased cow numbers are generally required to

determine statistical differences in reproductive measures. Using statistical power analysis, we would have needed approximately 450 cows per treatment in STP to detect treatment differences in reproduction. To detect differences in reproduction at NWROC approximately 100 and 1500 cows per treatment would have been needed for primiparous and multiparous cows per treatment, respectively.

CONCLUSIONS

Under conditions of the current study, trace mineral source or amount of Zn supplemented during the dry period did not have an effect on serum vitamin B_{12} concentration and concentrations of liver trace minerals. Prepartum DMI, lactation performance, and days to first service were not different for cows at STP and NWROC regardless of trace mineral source or amount. There was no improvement in percent of cows pregnant at 150 days in milk with organic trace mineral supplementation.

Table 1. Micro mineral composition of treatment mineral mixes[1].

	Treatments[2]		
Item	CON	OTM	OTMZ
Co, mg/kg	60.4	56.3	24.4
Cu, mg/kg	459.1	487.1	317.8
Mn, mg/kg	975.3	965.7	799.4
Zn, mg/kg	3273.9	3183.3	7362.1

[1]Trace mineral mix was top-dressed onto diet TMR at 0.42 kg/head per day.
[2]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.

Table 2. Ingredient and nutrient composition of far-off and close-up diets fed at St. Paul[1].

Item	Far-Off diet	Close-up diet
Ingredient	----------(g/100g of DM)----------	
Corn silage	59.84	43.54
Alfalfa hay	12.86	15.60
Wheat straw	16.64	16.15
Corn, ground	0.00	9.98
Soybean meal, 48%	6.66	8.07
Molasses, beet	2.88	3.49
Limestone	0.00	2.24
Salt	0.37	0.00
Mg oxide	0.19	0.27
di-Ca phosphate	0.56	0.66
Nutrient composition		
DM, %	50.0	56.7
CP, %	10.8	11.7
ADF, %	24.5	22.2
NDF, %	39.2	35.2
NFC[2]	40.2	41.6
ADICP[3], %	0.50	0.52
NDICP[4], %	0.82	0.84
EE[5], %	2.47	2.43
Ash, %	7.26	9.12
Ca, %	0.53	1.30
P, %	0.35	0.38
K, %	1.27	1.33
Mg, %	0.37	0.45
Fe, mg/kg	216.2	280.1
Na, mg/kg	2015.7	680.6
Mn, mg/kg	27.9	26.6
Zn, mg/kg	22.7	24.5
Cu, mg/kg	4.54	5.22
Co, mg/kg	0.39	1.18
Particle size distribution[6]	----------% of total----------	
Top sieve	12.3	14.1
Second sieve	37.9	32.0
Third sieve	39.3	41.2
Pan	10.4	12.5

[1]All cows received the same far-off and close up diets, fed as a total mixed ration, with respective treatment mineral mixes top-dressed.
[2]Non-fiber carbohydrate (NFC) = 100 – (%NDF + %CP + % ether extract + % ash).
[3]Acid detergent insoluble crude protein.
[4]Neutral detergent insoluble crude protein.
[5]Ether Extract.
[6]Diets were particle-sized using Penn State particle separator.

Table 3. Ingredient and nutrient composition of far-off and close-up diets fed at NWROC[1].

Item	Far-Off diet	Close-up diet
Ingredient	----------(g/100g of DM)----------	
Corn silage	54.3	39.2
Alfalfa silage	20.0	19.5
Wheat straw	13.6	18.7
Corn, high moisture	5.70	10.4
Soybean meal, 48%	4.10	9.03
Limestone	1.10	2.25
Salt	0.40	0.00
Mg oxide	0.20	0.22
di-Ca phosphate	0.60	0.67
Nutrient composition		
DM, %	45.2	50.6
CP, %	9.93	12.0
ADF, %	27.0	26.4
NDF, %	41.3	39.9
NFC[2]	38.7	36.7
ADICP[3], %	0.57	0.67
NDICP[4], %	0.83	0.87
EE[5], %	2.49	2.40
Ash, %	7.53	9.01
Ca, %	0.93	1.32
P, %	0.34	0.39
K, %	1.13	1.20
Mg, %	0.43	0.46
Fe, mg/kg	224.8	268.2
Na, mg/kg	1649.4	218.8
Mn, mg/kg	29.1	28.8
Zn, mg/kg	22.2	22.8
Cu, mg/kg	4.18	4.74
Co, mg/kg	0.26	0.26
Particle size distribution[6]	----------% of total----------	
Top sieve	11.1	5.90
Second sieve	53.6	41.1
Third sieve	33.5	49.5
Pan	1.80	3.50

[1]All cows received the same far-off and close up diets, fed as a total mixed ration, with respective treatment mineral mixes top-dressed.
[2]Non-fiber carbohydrate (NFC) = 100 – (%NDF + %CP + % ether extract + % ash).
[3]Acid detergent insoluble crude protein.
[4]Neutral detergent insoluble crude protein.
[5]Ether Extract.
[6]Diets were particle-sized using Penn State particle separator.

Table 4. Cows assigned and removed from study at St. Paul and NWROC.

	Treatments[1]		
Item	**CON**	**OTM**	**OTMZ**
STP			
Cows assigned to study			
Holstein	10	8	10
Crossbred	8	8	8
Total	18	16	18
Cows removed from study[2]			
Holstein	1	0	1
Crossbred	1	1	1
NWROC			
Cows assigned to study			
Multiparous	9	9	9
Primiparous	11	12	12
Cows removed from study[3]			
Multiparous	1	1	0
Primiparous	2	3	2

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]One cow aborted and 4 cows had severe mastitis starting at parturition.
[3]One cow was a downer cow, 1 cow had severe mastitis and 7 cows were removed for ketosis, displaced abomasums, and other metabolic disorders.

Table 5. Total micro mineral intake during the far-off and close-up periods at St. Paul and NWROC.

	Treatments[1]		
Item	CON	OTM	OTMZ
STP			
Far-off intake[2]			
Co, mg/d	30.4	28.5	15.1
Cu, mg/d	249.6	259.5	189.3
Mn, mg/d	758.4	743.2	678.9
Zn, mg/d	1658.8	1611.7	3371.3
Close-up intake[3]			
Co, mg/d	40.2	38.0	24.8
Cu, mg/d	258.1	267.8	197.7
Mn, mg/d	742.1	727.4	662.9
Zn, mg/d	1681.3	1633.5	3393.5
NWROC			
Far-off intake[4], all cows			
Co, mg/d	28.1	26.4	12.9
Cu, mg/d	235.7	247.2	176.3
Mn, mg/d	707.9	703.9	634.0
Zn, mg/d	1602.6	1564.6	3319.7
Close-up intake[3], multiparous			
Co, mg/d	28.8	27.1	13.6
Cu, mg/d	252.6	265.3	193.2
Mn, mg/d	772.5	774.2	698.6
Zn, mg/d	1662.3	1628.8	3379.4
Close-up intake[3], primiparous			
Co, mg/d	27.8	26.1	12.6
Cu, mg/d	235.3	246.9	175.8
Mn, mg/d	668.0	662.8	592.9
Zn, mg/d	1579.5	1540.6	3295.7

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.

[2]Micro mineral intake during the far-off period -60 to -30 d (calculated using actual DM intake of basal diet + treatment mineral mix).

[3]Micro mineral intake during the close-up period -30 d to parturition (calculated using actual DM intake of basal diet + treatment mineral mix).

[4]Average pen micro mineral intake during the far-off period -60 to -30 d, as all animals were group housed outside (calculated using average pen DM intake of basal diet + treatment mineral mix).

Table 6. Serum vitamin B_{12} concentrations of dairy cows at dry-off and at parturition at St. Paul.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Number	16	15	16	-	-
Serum vitamin B_{12}					
Dry-off, pg/mL[2]	137.3	136.5	110.1	19.2	0.52
Parturition, pg/mL[3]	274.0	226.3	244.7	33.6	0.61
Percent increase	154.3	115.1	136.2	36.9	0.76

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Baseline measure taken prior to beginning trace mineral treatments.
[3]Taken at parturition after approximately 60 days of being fed respective trace mineral treatment.

Table 7. Least square means for liver macro mineral concentration of dairy cows at St. Paul and NWROC at dry-off (mg/kg wet wt).

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
	---------- Dry-off[2] ----------				
STP					
Number	16	15	16	--	--
Ca, mg/kg	36.1[ab]	32.1[a]	39.9[b]	2.02	0.03
P, mg/kg	1950.9	1826.7	2016.2	81.4	0.24
Mg, mg/kg	58.3	50.6	59.9	5.13	0.39
Na, mg/kg	2553.6	2678.1	2681.8	104.0	0.63
K, mg/kg	907.0	859.6	934.4	79.4	0.79
NWROC					
Multiparous					
Number	9	9	9	--	--
Ca, mg/kg	50.5	51.7	43.5	8.49	0.76
P, mg/kg	3247.2	2857.1	2873.9	303.1	0.58
Mg, mg/kg	125.0	108.2	102.8	19.1	0.68
Na, mg/kg	3055.0	2962.0	3170.3	316.5	0.90
K, mg/kg	1890.5	1658.9	1615.0	271.6	0.73
Primiparous					
Number	11	10	11	--	--
Ca, mg/kg	67.8	60.8	80.1	12.5	0.55
P, mg/kg	3971.4	3271.6	3990.2	548.8	0.55
Mg, mg/kg	171.4	132.2	174.2	28.9	0.52
Na, mg/kg	3749.5	2977.2	4015.1	631.9	0.49
K, mg/kg	2723.6	2124.4	2390.8	380.2	0.55

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Baseline measures taken prior to beginning trace mineral treatments.
[a,b]Means in the same row with differing superscripts differ.

Table 8. Least square means for liver macro mineral concentration of dairy cows at St. Paul and NWROC at parturition (mg/kg wet wt).

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
	---------- Parturition[2] ----------				
STP					
Number	16	15	16	--	--
Ca, mg/kg	47.7	50.8	43.6	3.80	0.40
P, mg/kg	2783.2	2484.7	2429.8	158.9	0.26
Mg, mg/kg	101.2	78.4	79.5	8.78	0.13
Na, mg/kg	2764.2	3088.2	2943.6	207.6	0.55
K, mg/kg	1440.7	1052.8	1150.1	123.2	0.08
NWROC					
Multiparous					
Number	8	7	9	--	--
Ca, mg/kg	63.8	62.3	64.8	7.04	0.97
P, mg/kg	3209.5	2477.7	2750.1	330.5	0.32
Mg, mg/kg	138.5	101.7	114.2	19.4	0.42
Na, mg/kg	3744.9	3324.0	3517.8	333.1	0.69
K, mg/kg	1896.7	1432.4	1596.0	285.2	0.52
Primiparous					
Number	11	11	11	--	--
Ca, mg/kg	67.1	63.3	63.8	6.27	0.89
P, mg/kg	3106.9	2722.2	2713.8	214.1	0.35
Mg, mg/kg	145.2	118.5	117.7	14.1	0.31
Na, mg/kg	2869.9	3469.5	3340.8	228.9	0.17
K, mg/kg	1833.2	1556.0	1574.6	197.5	0.55

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Taken at parturition after approximately 60 days of being fed respective trace mineral treatment.
[a,b]Means in the same row with differing superscripts differ.

Table 9. Least square means for liver micro mineral concentration of dairy cows at St. Paul and NWROC at dry-off (mg/kg wet wt).

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
	---------- Dry-off[2] ----------				
STP					
Number	16	15	16	--	--
Zn, mg/kg	16.7	17.3	17.6	1.02	0.83
Fe, mg/kg	34.1	29.9	43.5	4.12	0.06
Cu, mg/kg	102.4	118.1	129.9	10.8	0.22
Mn, mg/kg	1.48	1.33	1.53	0.20	0.74
NWROC					
Multiparous					
Number	9	9	9	--	--
Zn, mg/kg	33.3	35.5	27.6	5.57	0.60
Fe, mg/kg	67.1	91.8	53.7	19.2	0.40
Cu, mg/kg	75.2	71.5	83.0	12.0	0.79
Mn, mg/kg	2.23	2.05	2.18	0.23	0.86
Primiparous					
Number	11	10	11	--	--
Zn, mg/kg	33.0	32.2	39.6	5.21	0.54
Fe, mg/kg	87.4	63.9	75.2	13.8	0.49
Cu, mg/kg	12.9	17.1	21.2	4.23	0.38
Mn, mg/kg	2.59	2.19	2.75	0.42	0.63

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Baseline measures taken prior to beginning trace mineral treatments.
[a,b]Means in the same row with differing superscripts differ.

Table 10. Least square means for liver micro mineral concentration of dairy cows at St. Paul and NWROC at parturition (mg/kg wet wt).

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
	---------- Parturition[2] ----------				
STP					
Number	16	15	16	--	--
Zn, mg/kg	27.9	30.0	23.9	2.30	0.17
Fe, mg/kg	51.2	50.1	48.0	4.73	0.89
Cu, mg/kg	111.3	138.7	123.4	12.3	0.31
Mn, mg/kg	1.93	2.05	1.95	0.15	0.84
NWROC					
Multiparous					
Number	8	7	9	--	--
Zn, mg/kg	49.7	55.5	42.3	7.56	0.49
Fe, mg/kg	100.2	103.1	87.8	14.0	0.71
Cu, mg/kg	80.7	89.6	96.2	18.2	0.81
Mn, mg/kg	3.14	2.23	2.68	0.46	0.42
Primiparous					
Number	11	11	11	--	--
Zn, mg/kg	87.1	70.4	70.9	11.2	0.49
Fe, mg/kg	92.7	90.7	93.4	17.5	0.99
Cu, mg/kg	34.5	47.8	31.9	6.82	0.22
Mn, mg/kg	2.37	2.03	2.04	0.19	0.35

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Taken at parturition after approximately 60 days of being fed respective trace mineral treatment.
[a,b]Means in the same row with differing superscripts differ.

Table 11. Least square means for milk yield and composition of dairy cows at St. Paul.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Number	16	15	16	-	-
Milk[2], kg/d	40.0	38.6	41.4	0.99	0.15
4% FCM, kg/d	39.1	38.2	39.7	0.71	0.34
ECM, kg/d	38.4	37.3	38.8	0.66	0.25
Milk fat, %	3.88	3.98	3.73	0.08	0.07
Milk fat, kg/d	1.54	1.51	1.54	0.03	0.76
Milk true protein, %	3.04	3.02	3.00	0.03	0.76
Milk true protein, kg/d	1.20	1.15	1.23	0.02	0.07
Milk lactose, %	4.86	4.84	4.79	0.33	0.31
Milk lactose, kg/d	1.94	1.87	1.98	0.05	0.35
Other solids, %	5.63	5.61	5.56	0.03	0.21
Other solids, kg/d	2.25	2.16	2.30	0.06	0.30
Solids non-fat, %	8.53	8.49	8.43	0.05	0.40
Solids non-fat, kg/d	3.38	3.26	3.46	0.08	0.24
SCS, linear	4.99	5.05	5.06	0.13	0.90
Milk urea N, mg/dL	16.6[a]	15.3[b]	16.2[a]	0.33	0.01

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Milk and milk component yield was followed through 150 DIM.
[a,b]Means in the same row with differing superscripts differ.

Table 12. Least square means for milk yield and composition of multiparous cows at NWROC.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Number	8	8	9	-	-
Milk[2], kg/d	46.0	42.6	46.0	1.68	0.30
4% FCM, kg/d	43.0	39.1	44.5	2.45	0.31
ECM, kg/d	42.3	38.8	43.7	2.23	0.32
Milk fat, %	3.56	3.37	3.63	0.18	0.60
Milk fat, kg/d	1.63	1.45	1.73	0.14	0.40
Milk true protein, %	2.95	3.01	2.98	0.03	0.45
Milk true protein, kg/d	1.37	1.30	1.38	0.04	0.41
Milk lactose, %	4.81	4.78	4.84	0.04	0.64
Milk lactose, kg/d	2.23	2.09	2.25	0.08	0.40
Other solids, %	5.72	5.69	5.74	0.04	0.67
Other solids, kg/d	2.65	2.49	2.67	0.10	0.39
Solids non-fat, %	8.66	8.71	8.73	0.05	0.51
Solids non-fat, kg/d	4.01	3.80	4.04	0.14	0.46
SCS, linear	4.82	5.33	4.99	0.15	0.07
Milk urea N, mg/dL	11.4	11.9	12.1	0.46	0.48

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Milk and milk component yield was followed through 150 DIM.
[a,b]Means in the same row with differing superscripts differ.

Table 13. Least square means for milk yield and composition of primiparous cows at NWROC.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Number	9	9	10	-	-
Milk[2], kg/d	31.2	31.9	31.5	0.95	0.87
4% FCM, kg/d	29.8	30.6	29.8	1.21	0.88
ECM, kg/d	29.5	30.1	29.3	1.11	0.89
Milk fat, %	3.61	3.64	3.53	0.09	0.82
Milk fat, kg/d	1.14	1.18	1.14	0.06	0.88
Milk true protein, %	2.93	2.94	2.87	0.03	0.15
Milk true protein, kg/d	0.96	0.95	0.93	0.03	0.77
Milk lactose, %	4.89	4.90	4.92	0.02	0.59
Milk lactose, kg/d	1.58	1.58	1.59	0.05	0.98
Other solids, %	5.79	5.82	5.83	0.02	0.44
Other solids, kg/d	1.87	1.88	1.89	0.06	0.97
Solids non-fat, %	8.77	8.75	8.70	0.04	0.38
Solids non-fat, kg/d	2.82	2.83	2.82	0.08	0.99
SCS, linear	4.95	4.96	5.13	0.13	0.53
Milk urea N, mg/dL	10.1	10.8	10.9	0.33	0.17

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Milk and milk component yield was followed through 150 DIM.
[a,b]Means in the same row with differing superscripts differ.

Table 14. Average days to first service of cows at St. Paul and NWROC.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
STP					
Number	15	16	15	--	--
Days to 1st service	73.5	78.4	81.1	--	--
NWROC					
Multiparous cows					
Number	9	6	8	--	--
Days to 1st service	86.8	88.8	84.1	--	--
Primiparous cows					
Number	9	8	10	--	--
Days to 1st service	90.5	89.3	92.9	--	--

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[a,b]Means in the same row with differing superscripts differ.

Table 15. Percent of cows pregnant at 150 days in milk by treatment at St. Paul and NWROC[1].

	% Pregnant (n of n)	Odds ratio (CI)	*P*-value
STP[2]			
CON	53.3 (8/15)	0.84 (0.16, 4.22)	0.69
OTM	62.5 (10/16)	0.49 (0.10, 2.43)	
OTMZ	46.7 (7/15)	.	
Breed			
Holstein	34.6 (9/26)	7.80 (1.96, 31.1)	0.004
Crossbred	80.0 (16/20)	.	
NWROC			
Multiparous cows[3]			
CON	44.9 (4/9)	1.24 (0.18, 8.60)	0.45
OTM	16.7 (1/6)	5.01 (0.39, 64.6)	
OTMZ	50.0 (4/8)	.	
Primiparous cows[4]			
CON	44.4 (4/9)	0.30 (0.04, 2.35)	0.48
OTM	25.0 (2/8)	0.77 (0.08, 7.22)	
OTMZ	20.0 (2/10)	.	

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Cows removed: CON = 1 cow died and 1 cow was marked do not breed (DNB); OTM = 1 cow marked DNB; and OTMZ = 1cow aborted and 1cow was marked DNB.
[3]Cows removed: CON = 1 cow died; OTM = 2 cows marked DNB; and OTMZ = 1cow had incorrect breeding date.
[4]Cows removed: CON = 2 cows were sold; OTM = 1 cow marked DNB, 1 cow was an ET donor, and 2 cows were sold; and OTMZ = 2 cows were sold.

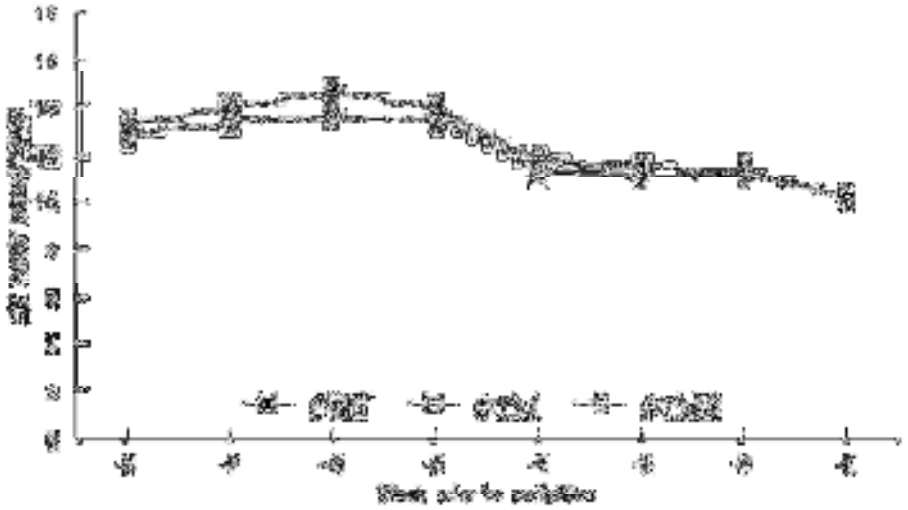

Figure 1. Effect of trace mineral source and zinc amount on prepartum dry matter intake of cows at St. Paul. Treatments were: CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100. Error bars = SEM.

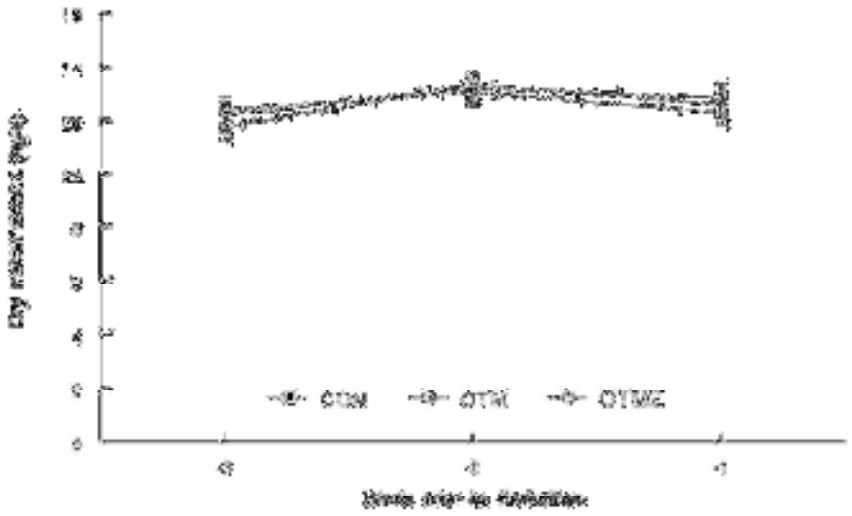

Figure 2. Effect of trace mineral source and zinc amount on prepartum dry matter intake of multiparous cows at Crookston. Treatments were: CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100. Error bars = SEM.

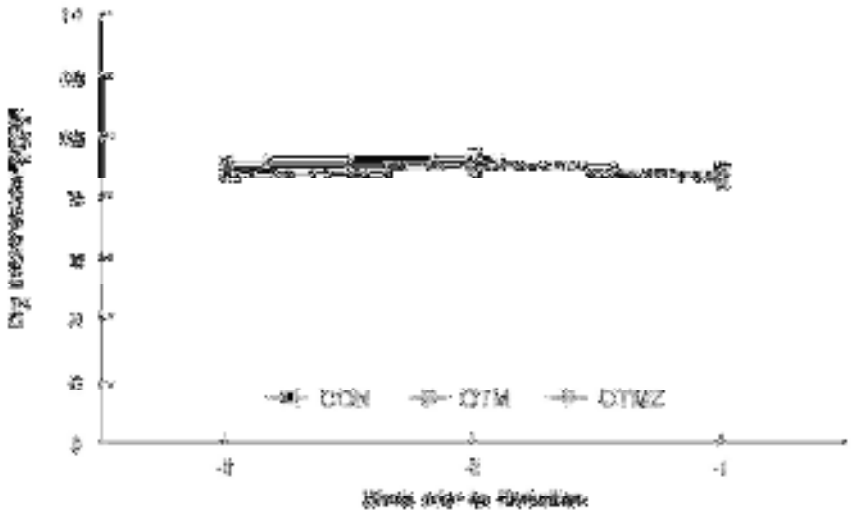

Figure 3. Effect of trace mineral source and zinc amount on prepartum dry matter intake of primiparous cows at Crookston. Treatments were: CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100. Error bars = SEM.

Impact of dietary trace mineral source and zinc supplementation during the dry period on colostrum nutrient profile and calf performance.

OVERVIEW

The objective of this study was to evaluate the effect of trace mineral (**TM**) feeding during late gestation on composition of colostrum and calf performance. Fifty-two Holstein and cross-bred cows at the University of Minnesota (U of MN), St. Paul dairy (**STP**) and 62 Holstein cows at the U of MN Northwest Research and Outreach Center dairy (**NWROC**) were blocked by breed (STP) or parity (NWROC) and randomly assigned to 1 of 3 treatments. Treatments were: 1) 100% inorganic trace mineral supplementation (**CON**); 2) organic trace mineral supplemented at the following rate: 40% of supplemented zinc (Zn), 24% of supplemented manganese (Mn), 69% of supplemented copper (Cu) and 100% of supplemented cobalt (Co) supplied by 4-Plex.(Zinpro Corp., Eden Prairie, MN; **OTM**) and 3) OTM with additional Zn from Zn-methionine (**OTMZ**). Dietary trace mineral supplements were formulated to provide similar amounts of Co, Cu and Mn. Dietary Zn supplemented amounts were similar for CON and OTM (75 mg/d), but approximately two times higher for OTMZ. Heifer calves born from the cows at STP and all calves born at NWROC were evaluated for feed intake and growth performance through 56 d of age. Bull calves born at STP were used in a study to evaluate the affect of additional Zn from zinc-methionine on intestinal development. There were no effects of TM source or dietary Zn concentration on colostrum protein or immunoglobulin concentration at either location. For STP and primiparous cows at NWROC, treatments did not significantly affect the fat content of colostrum which averaged 5.88% in STP and 8.63% at NWROC across treatments.

Treatment did affect colostrum fat concentration from multiparous cows at NWROC with cows fed OTMZ having a higher concentration of fat in colostrum (7.63%) than cows fed CON (3.38%). Daily gain of calves through 56 days of life was not affected by TM source or Zn amount fed to the dam prepartum. No effect of TM treatment was observed on liver TM concentration, hematology profile, and intestinal villus height or crypt depth under the conditions of this study.

(**Key Words:** colostrum, organic trace mineral, dairy calves)

INTRODUCTION

Trace minerals such as cobalt (**Co**), copper (**Cu**), manganese (**Mn**), and zinc (**Zn**) have important roles in physiological processes related to growth, reproduction, and health. The goal of trace mineral (**TM**) supplementation during late gestation and the dry period of dairy cows is to improve the immune status of the cow and subsequently to the calf through placental transfer and mineral content of colostrum. However, there is limited research evaluating the effect of TM supplementation during the dry period on the nutrient and immunoglobulin (Ig) content in colostrum (Kincaid and Socha, 2004; Kinal et al., 2007). Kincaid and Socha (2004) started TM supplementation 42 d pre-partum and continued supplementation to 150 DIM. Kinal et al. (2007) started mineral supplementation 60 d pre-partum, but supplemented no Co and a higher level of Cu than the Kincaid and Socha (2004) study. The studies by both Kincaid and Socha (2004) and Kinal et al. (2007) provide only a limited colostrum nutrient profile, and they did not follow performance of the calves after being fed colostrum. Other than a review by and Foley and Otterby in 1978 and a more recent survey by Kehoe et al. (2007), there is limited comprehensive data on the nutrient profile of colostrum. Quigley and Drewry

(1996) suggested providing late gestation dry cows with sufficient amounts of TM would help minimize the negative effects of stress on the animal and increase production of high quality colostrum.

The most important factor for health of the neonatal calf is consumption of adequate amounts of high quality colostrum and sufficient absorption of immunoglobulins. Colostrum intake also stimulates development and function of the gastrointestinal tract in calves (Buhler et al., 1998; Blattler et al., 2001). Research in other species found feeding high levels of Zn from organic sources to pregnant animals or injecting Zn *in ovo* improved gut development and health of offspring. Caine et al. (2001) observed increased villus height (258 vs. 225 μm), lower crypt depth (78 vs. 85 μm), and a higher villus height to crypt depth ratio (3.7 vs. 2.9) in the jejunum of piglets from sows supplemented with 250 ppm of a Zn amino acid (**AA)** complex from d 80 of gestation until farrowing compared to piglets from sows not supplemented with Zn. Similar results were observed by Payne et al. (2006) where weanling pigs from sows fed 100 ppm supplemental Zn in sulfate or organic form, in addition to the basal diet of 120 ppm of Zn, had increased jejunum villus height compared to piglets from sows not supplemented with Zn. While both the inorganic and organic form of supplemented Zn increased villus height in piglets compared to piglets from sows given no supplemental Zn, they did observe supplementing the additional 100 ppm of Zn in organic form to sows increased pigs born and weaned per litter compared to those supplemented with the sulfate form of Zn. Takeo et al. (2005) injected Zn AA complex into the amniotic fluid of un-hatched chicks and found enhanced intestinal development and functionality as well as increased jejunal villus surface area compared to chicks receiving no additional Zn. The research in

chicks and baby pigs indicates supplementation of Zn during the gestation period can increase intestinal villi growth and enhance nutrient absorption during the first few weeks of life, however it is unknown if increasing the amount of organic Zn supplemented to gestating dairy cattle has the potential to improve villi development and nutrient absorption of young calves. The objectives of the current study were to investigate the effects of supplemental dietary TM source and Zn amount fed 60 d pre-partum to dairy cows on: 1) colostrum nutrient profile and Ig content; 2) dry matter (DM) intake and average daily gain (ADG) of calves during the first 56 days of life and 3) intestinal development in neonatal calves.

MATERIALS AND METHODS

Study 1 (Progeny Performance Study)

Location, Calves and Treatments. Calves born to cows assigned to the University of Minnesota (**U of MN**) St. Paul Dairy barn (**STP**) and the U of MN Northwest Research and Outreach Center (**NWROC**) cow study reported in a companion paper (Golombeski et al., 2010) were evaluated for health and performance during the first 56 d of life. Prepartum treatments fed to dams were: 1) 100% inorganic trace mineral supplementation (**CON**); 2) organic trace mineral supplemented at the following rate: 40% of supplemented zinc (Zn), 24% of supplemented manganese (Mn), 69% of supplemented copper (Cu) and 100% of supplemented cobalt (Co) supplied by 4-Plex.(Zinpro Corp., Eden Prairie, MN; **OTM**) and 3) OTM with additional Zn from Zn-methionine (**OTMZ**). Dietary trace mineral supplements were formulated to provide similar amounts of Co, Cu and Mn. Dietary Zn supplemented amounts were similar for CON and OTM (75 mg/d), but approximately two times higher for OTMZ.

At STP, only heifer calves were evaluated for DM intake and ADG for the first 56 d of life. One cow fed OTM gave birth to twins, one calf on OTMZ died from unknown causes and another was removed from CON due to an ulcer at the omasal/abomasal junction. Bull calves born at STP (n = 21) were used in an intestinal morphology study. At NWROC, heifer and bull calves were evaluated for DM intake and ADG during the first 56 d of life. There were two cows from both the CON and OTM treatments that gave birth to twins. Two calves born to cows fed OTM and one calf each from CON and OTMZ were stillborn. All calves born at NWROC were Holstein while calves born at STP were Holstein or crosses of Jersey-Montbeliarde-Holstein or Montbeliarde-Jersey-Holstein. Animal care and usage for this study was approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC # 0603A83108).

All calves received a composite sample of their dam's colostrum for the first three feedings after birth (1.89 L per feeding) and were individually housed either in a climate controlled room (NWROC) or in outside individual calf hutches (STP). Calves at STP were housed the first 3 d of age in a climate controlled room and then moved to hutches. Calves at both locations were fed the same medicated (20% CP: 20% fat) milk replacer twice daily from d 2 of life until weaning at 42 d. At STP, calves were fed 0.34 kg milk replacer powder (as-fed) in 1.89 L warm water (15.2 % solids) twice per d in two equal feedings. Calves at NWROC were fed 0.34 kg milk replacer powder (as-fed) in 2.25 L warm water (13.0 % solids) twice per d in two equal feedings. At 3 d of age, calves at both locations had ad libitum access to water and were fed a common texturized calf starter that contained decoquinate (1.23 mg/kg). Nutrient composition of the milk replacer and calf starter are in Table 1.

Data Collection. Milk replacer intake was recorded from d 1 to weaning at 42 d of age and starter intake was recorded daily from d 1 to 56 d of age. Body weights were recorded at birth, d 42, and d 56. All health and treatment data were recorded, but . calves were relatively healthy with very few treatments administered resulting in insufficient data to report on health incidences given our limited sample size and ability to detect differences due to a lack of statistical power. Blood samples were collected at birth and approximately 30 h of age via jugular venipuncture using serum and trace mineral free tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Whole blood was centrifuged, serum was removed and stored in 5-mL polyethylene tubes and frozen at −20°C until analysis for serum total proteins, serum IgG concentration and serum IgM concentration.

Apparent efficiency of absorption (**AEA**) for colostrum IgG at 30 h was calculated using the formula [serum IgG (grams per liter) × plasma volume (liters) × 100]/IgG intake grams, taken from Quigley et al., (1998a). Plasma volume was calculated using the following regression equation: plasma volume (milliliters) = -2393.1 + 68.09 × BW (kilograms) + breed (1 = Holstein) + 127.3 × age (hours) at sampling (Quigley et al., 1998a). The following assumptions were made: 1) all calves received two feedings of colostrum before the blood sample was taken at 30 h of age; and 2) the first and second feeding of colostrum were similar in nutrient and Ig content as we only conducted analysis on the first colostrum feeding of colostrum. Given these assumptions, we are likely overestimating the IgG content of colostrum in the second feeding which would result in a. higher than actual amount of Ig being fed, lowering the efficiency of absorption. Therefore our calculations would error on the conservative side of the apparent efficiency of absorption estimate.

Study 2 (Intestinal morphology study)

Location, Calves and Treatments. Bull calves born from STP study cows were used to study intestinal morphology at two different ages. Animal care and usage was approved as previously described (IACUC # 0603A83108). Calves were euthanized at two different ages depending on the d of the week they were born. Calves born Monday through Wednesday were euthanized at birth and calves born Thursday through Sunday were fed for approximately 14 d then euthanized. Calves euthanized at birth were not fed colostrum prior to euthanasia. Calves euthanized at 2 wk of age received colostrum, milk replacer and starter as described for the calf performance study at STP.

Data Collection. Milk replacer and starter intake were recorded daily along with health and treatment data. Body weights were recorded at birth and d of euthanasia. Following euthanasia, intestinal and ruminal tissue were collected immediately post-mortem. The intestinal tract was removed and the small intestine was separated from the large intestine at the ileal-cecal junction. Tissue samples were collected from three different sites along the small intestine. The entire section of ileum and duodenum were collected and a sample of jejunum was taken at approximately 120 cm from the ileal-jejunal junction. Tissue was also collected from the ventral side of the rumen to evaluate papillae development. The tissues were then cut longitudinally, flushed with 10% neutral buffered formalin (**NBF**) to remove digesta, and then fixed in 10% NBF for 3 d to insure adequate fixation. Additionally, segments of each intestinal section were tied-off at the ends with string and injected with approximately 10 mL of 10% NBF and fixed as previously described. Selected tissues were then cut into cross-sections and processed for routine histology. For calves euthanized at 2 wk of age, a full necropsy was performed.

Blood samples were taken from all bull calves at birth, approximately 30 h of age, and time of euthanasia for calves sacrificed at 2 wk of age. Blood samples were collected and processed as previously described. An additional blood sample was taken at birth and 2 wk of age using an evacuated tube containing 7.5% EDTA for a hematology profile (University of Minnesota Clinical Pathology Laboratory, St. Paul, MN). Liver samples were collected following euthanasia from the right rear lobe and frozen at −20°C until analyzed for TM content.

Laboratory Analysis – (Study 1 and Study 2)

Colostrum. Fat analysis was conducted at Land O' Lakes Inc. (Ft. Dodge, IA) by alkaline hydrolysis and CP was determined using Kjeldahl analysis (AOAC, 2000). Samples were dried at 105°C for 24 h to determine DM. Organic matter was determined by ashing the samples in a muffle furnace at 500°C (AOAC, 2000). Colostrum mineral analysis was conducted by the University of Minnesota Soil Testing and Research Analytical Laboratory. The colostrum samples were prepped with 10 mL of concentrated nitric acid in sealed Teflon vessels. Nine vessels at a time were heated in the microwave at full power until a temperature of 177°C was reached. The microwave (CEM MDS 2100 with a peak power output of 930 watts) was programmed to hold this temperature for four and a half minutes by regulating the power. After the vessels cooled, the digested samples were transferred to centrifuge tubes and adjusted to 40 mL with deionized water. These samples were analyzed for mineral concentration by ICP (PE Optima 3000) which is a radial view ICP. Colostrum IgG and IgM content was determined using radial immunodiffusion (RID) and insulin-like growth factor (IGF-I) content was determined by

ELISA (Global Beta Health, Ames IA). Four samples were lost during preparation due to over pressurization of the microwave vessels.

Feed Samples. Samples of milk replacer and starter for both study 1 and 2 were taken weekly and composited by month. All composited samples were dried for 48 h in a 60°C forced air oven (model DC-246-E; Blue M Electric, Watertown, WI) to determine DM content. Starter samples were ground to pass through a 1-mm screen (Wiley mill; Swedesboro, NJ). Subsamples of feed composites were dried at 105°C for 24 h to correct to 100% DM. Organic matter was determined by ashing the samples in a muffle furnace at 500°C (AOAC, 2000). Samples were analyzed for CP (LECO, Tru-Spec N, St. Joseph, MI; AOAC, 2000), and ether extract (EE; AOAC, 2000). The Ankom^{200} fiber system (Ankom Technology Corporation, Fairport, NY) was used for sequential analysis of NDF and ADF (Hintz et al., 1996). Samples were analyzed for NDF using sodium sulfite and α-amylase (Sigma no. A3306; Sigma Chemical Co., St. Louis, MO). Neutral detergent insoluble crude protein and acid detergent insoluble crude protein were determined by Kjeldahl analysis (AOAC, 2000) using the NDF or sequential ADF residue, respectively. Minerals were analyzed by the University of Minnesota Soil Testing and Research Analytical Laboratory using a Perkin Elmer Optima 3000 ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectrometer). Cobalt was analyzed using ICP by Minnesota Valley Testing Labs, Inc. (New Ulm, MN).

Liver and serum samples. Liver tissue was analyzed for mineral concentration by the University of Minnesota Soil Testing and Research Analytical Laboratory. Liver samples were prepped with 0.5 mL of nitric acid and 2 mL of 30% hydrogen peroxide. Twelve vessels at a time were heated in the microwave at 33% power for 6 minutes. After the

initial reaction, the microwave was programmed to use full power to heat the samples to 165 degrees C for 8 minutes. After cooling, the samples were transferred to centrifuge tubes and adjusted to 10 mL with deionized water. The samples were spiked with 1 ppm yttrium which was used as an internal standard during analysis. The analysis was done on a Perkin Elmer 3000DV (Inductively Coupled Plasma - Atomic Emission Spectrometer) which is a dual viewing ICP. Potassium, Mg, and Na were read using radial viewing of the plasma and the other elements were read using axial viewing. Analysis of calf serum for IgG and IgM content was determined using radial immunodiffusion (RID) and insulin-like growth factor (IGF-I) content was determined by ELISA (Global Beta Health, Ames IA). Serum protein was measured using a portable refractometer (Kernco Instruments Co., Inc, El Paso, TX).

Histology. Paraffin-embedded tissue blocks were serial sectioned at 5-µm, and stained with hematoxylin and eosin. The sections were examined histologically to determine villus height (µm) and crypt depth (µm). Measurements were taken using a 4X objective, in order to allow all villi to be included in the field of view. Three slides per calf were prepared from each of the following: ileum, jejunum, and duodenum (n=9 total slides per calf). A total of twenty measurements per calf of villus height and crypt depth were taken from the jejunum. These measurements were then averaged for each section respectively. Slides were viewed on an Olympus BX40 microscope (Olympus Optical Co., LTD; Japan) equipped with Spot, InSight Color Model 3.20 digital camera and imaging software (Diagnostic Instruments Inc., Sterling Heights, MI).

Statistical Analysis

Study 1 (Progeny performance study)

St. Paul and NWROC progeny performance data were analyzed separately due to differences in location management of calves, calf housing, and the inclusion of breed at STP and calf sex at NWROC.

St. Paul

Colostrum nutrient and Ig content data were analyzed as a randomized complete block. The data were analyzed using the MIXED procedures of SAS (SAS Institute, 2001). Data were analyzed with the model:

$Y_{ij} = \mu + B_i + T_j + e_{ij}$

where μ = overall mean, B_i = effect of breed (i = 1 to 2), T_j = effect of treatment (j = 1 to 3) and e_{ij} = random residual error. Significance was declared at $P \leq 0.05$, and a tendency at $P \leq 0.10$.

Progeny serum total protein, serum IgG and IgM were analyzed as a randomized complete block. Treatment and breed were included in the model as fixed effects. Dry matter intake and growth measurements taken over time were analyzed as repeated measures. The data were analyzed using the MIXED procedures of SAS (SAS Institute, 2001). Data were analyzed with the model:

$Y_{ijk} = \mu + B_i + T_j + W_k + e_{ijk}$

where μ = overall mean, B_i = effect of breed (i = 1 to 2), T_j = effect of treatment (j = 1 to 3), W_k = effect of week (k = 1 to 8) and e_{ijk} = random residual error. Significance was declared at $P \leq 0.05$, and a tendency at $P \leq 0.10$.

NWROC

Colostrum nutrient and Ig content data were analyzed as a randomized complete block. Cows were blocked by season of calving and randomly assigned to treatment

within block. Block 1 = cows that calved in August, September, October, and November and Block 2 = cows that calved in December, January, February, and March. Both primiparous and multiparous cows were utilized at the NWROC location, but the data were analyzed separately. The data were analyzed using the MIXED procedures of SAS (SAS Institute, 2001) with the model:

$Y_{ij} = \mu + T_i + Pj + S_k + e_{ijk}$

where μ = overall mean, T_i = effect of treatment (j = 1 to 3), P_j = effect of sex (j = 1 to 2), S_k = effect of season of calving (m = 1 to 2) and e_{ij} = random residual error. Significance was declared at $P \leq 0.05$, and a tendency at $P \leq 0.10$.

Progeny serum total proteins were analyzed as a randomized complete block with treatment and sex as fixed effects. Intake and growth measurements taken over time were analyzed as repeated measures. The data were analyzed using the MIXED procedures of SAS (SAS Institute, 2001) with the model:

$Y_{ijk} = \mu + T_i + P_j + e_{ij}$

where μ = overall mean, T_i = effect of treatment (j = 1 to 3), P_j = effect of sex (m = 1 to 2), and e_{ijk} = random residual error. Significance was declared at $P \leq 0.05$, and a tendency at $P \leq 0.10$.

Study 2 (Intestinal morphology study)

Intestinal morphology

Data were analyzed using the PROC MIXED procedures of SAS© with the model:

$Y_i = \mu + T_i + e_i$

where μ = overall mean, T_i = effect of treatment (j = 1 to 3) and e_i = random residual error. Breed was not included in the model as only 3 Holstein calves were euthanized at

birth and 3 crossbreds at 2 wks of age. Response variables included villus height (microns) and crypt depth (microns) for the jejunal intestinal segments. Significance was declared at $P \leq 0.05$, and a tendency at $P \leq 0.10$.

RESULTS AND DISCUSSION

Study 1 (Progeny performance)

Colostrum nutrient profile

Colostrum nutrient profile and Ig concentrations are in Table 2 for STP and Table 3 and 4 for multiparous and primiparous cows at NWROC, respectively. There was no effect of TM source or dietary Zn concentration on colostrum CP concentration at either location averaging 14.2% CP in STP and 14.0% CP at NWROC. The CP content of colostrum reported in the current study is similar to the 14.9% CP reported by Kehoe et al., (2007) and 14.0% CP reported by Foley and Otterby (1978). The CP content of colostrum of primiparous cows at NWROC was lower than the 15% reported by Kume and Tanabe (1993). For cows at STP, mineral treatments did not affect the fat content of colostrum which averaged 5.88% across treatments. At NWROC, treatment did not affect fat content of colostrum from primiparous cows (average 8.63%), but fat content of colostrum did differ by treatment in multiparous cows. Cows receiving OTMZ produced colostrum higher in fat than those receiving the CON (7.63 vs. 3.38%). The fat content of colostrum reported by Kehoe et al. (2007) and Foley and Otterby (1978) was 6.70%. This value is higher than the fat content of colostrum for all cows at STP and multiparous cows fed CON and OTM at NWROC. Multiparous cows fed OTM and all primiparous cows had a colostrum fat value higher than the one reported by Kehoe et al. (2007) and Foley and Otterby (1978). One of the reasons for the difference may be related to

procedure. In the current study, fat content was determined by alkaline hydrolysis and the previous two studies used the Babcock procedure.

Cows at STP averaged 59.4 mg/mL of IgG compared to 66.8 mg/mL for multiparous and 61.2 mg/mL for primiparous cows at NWROC. Trace mineral source or amount of Zn fed did not affect IgG concentration in colostrum at either location. The reason for the lower IgG content of colostrum from cows in STP compared to the cows at NWROC is unknown. Both IgG and IgM concentration in colostrum averaged across all treatments slightly higher for multiparous cows at NWROC compared to primiparous (66.8 vs. 61.3 mg/mL for IgG and 6.63 vs. 5.89 mg/mL of IgM). The higher average IgG content of colostrum from multiparous cows compared to primiparous appears to be partially due to a higher IgG content in CON multiparous cow colostrum than CON primiparous colostrum as colostrum IgG content of multiparous and primiparous cows on the other two treatments was similar. Parity has been shown to affect the nutrient and Ig content of colostrum (Pritchett et al., 1991; Kume and Tanabe 1993; Weaver et al., 2000) with first lactation or primiparous cows usually being lower in Ig content than multiparous cows.

Kehoe et al. (2007) reported a concentration of 34.9 mg/mL for IgG_1in colostrum samples collected from 55 dairy farms in PA compared to 48.2 mg/mL of IgG_1 in colostrum reported by Pritchett et al. (1991). According to Kehoe et al. (2007) approximately 85% of the total IgG concentration is IgG_1. This equates to a total IgG contration of 41.0 mg/mL in the Kehoe et al. study (2007) and 56.7 mg/mL in the Pritchard et al. (1991). In the current study, the average colostrum IgG concentration across locations and parities was higher (61.8 mg/mL) than those reported by Foley and

Otterby (1978) (32.0 mg/mL) and Kehoe et al. (2007), but similar to the value reported by Pritchett et al. (1991). Reasons for differences in colostrum IgG content have been related to TM status, dry period length, parity, volume of colostrum produced among many other factors (Weaver et al., 2000). However, in our study TM source or amount of Zn fed did not affect IgG content of colostrum.

Cows at STP had an average IgM concentration of 4.59 mg/mL while the multiparous and primiparous cows at NWROC had an average concentration of 6.63 and 5.87 mg/mL, respectively. Colostrum IgM concentration was not affected by TM treatment which is in agreement with the study by Kincaid et al. (2004) where organic TM were fed from 21 d prepartum to 150 d postpartum. The average colostrum IgM concentration reported by Kehoe et al. (2007) was 4.32 mg/mL which is slightly lower than the values observed in our study, but well within the range (3 to 12 mg/mL) reported by Kehoe et al. (2007). Similar to IgG, the cows at NWROC had a higher average IgM concentration than the cows at STP. Multiparous cows at NWROC also had a numerically higher IgM concentration than the primiparous cows.

Cows fed CON at STP tended ($P = 0.06$) to produce colostrum with higher ash content compared to OTM and OTMZ. At NWROC both the multiparous and primiparous cows fed CON produced colostrum with more ash than OTM or OTMZ. Ash content of colostrum across all treatments, locations and parities averaged 1.33 % which is considerable higher than the 0.05% reported by Kehoe et al., (2007). However, that was a survey study and the mineral supplementation level and other background data of those cows are unknown. There was no effect of TM source or dietary zinc concentration on Zn, Cu or Mn in colostrum of cows at STP (Table 3). At NWROC,

treatment did not affect the TM content of colostrum from multiparous cows, but Mn concentrations were lower ($P<0.04$) in CON and OTMZ fed cows compared to OTM fed cows. Our findings are in general agreement with those of Kincaid et al. (2004) where feeding organic trace minerals prepartum had no affect on mineral content of colostrum. The reason for the difference in Mn content of primiparous cows fed OTM compared to CON and OTMZ is unknown and probably not of biological significance at a concentration of only 0.01 mg/kg difference. The Mn values observed in the current study are similar to the average values reported by Foley and Otterby (1978) but are lower than values reported by Kehoe et al. (2007). Most all mineral values reported in the current study are within the range of values reported by Kume and Tanabe (1993) and Kehoe et al. (2007). It is interesting to note that cows in STP produced colostrum that contained 4 to 8 times more Cu than cows at NWROC. The reason for this is unknown as diets at both locations were similar in supplemental and total Cu content.

Serum Parameters. Successful passive transfer of Ig from colostrum into a calf is qualitatively defined by total protein in the serum of a calf less than 30 h of age. Serum total protein has a high correlation (approximately 0.71, (Quigley, 2001) with IgG in the blood as the major source of protein consumed by a calf in the first 24 hours of life is colostrum protein and IgG. A value greater than 5.5 g/dL of protein in serum is the threshold target to declare successful passive transfer of Ig with moderate passive transfer defined at a concentration between 5.0 and 5.4 g/dL (Quigley, 2001). For calves born in STP, serum protein levels were not affected by treatment and averaged 4.37 mg/dL at birth before colostrum feeding and 6.27 mg/dL at approximately 30 h of age (Table 5). At NWROC, serum protein concentration in calves was unaffected by treatment and

averaged similar to STP calves at birth (4.35 mg/dL), but slightly lower (5.63 mg/dL) at approximately 30 h of age (Table 6). However, calves at both locations would be defined as having achieved passive transfer of Ig.

In the past, feeding strategies for newborn calves were to feed high quality colostrum to provide at least 100 g of IgG within the first 1 to 2 h after birth (Quigley et al., 2001). Recent research using colostrum replacers suggests that the amount of IgG fed to newborn calves may need to be increased to 150 to 200 g of IgG within1 to 2 h after birth (Foster et al., 2006 and Godden et al., 2009). Failure of passive transfer is defined when calves have a serum IgG concentration of less than 10 mg/mL at 24 h of age. Immunoglobulin concentration in serum was not measured at NWROC, but in STP there was no significant effect of treatment on serum IgG concentration at birth or at approximately 30 h of age (Table 5). All calves achieved passive transfer as serum IgG concentrations averaged 18.9 mg/mL across all treatments.

Apparent efficiency of absorption (AEA) of IgG was calculated for the heifer calves at STP (Table 5). Calves from cows fed CON and OTM averaged 40.7% AEA while calves from OTMZ fed cows averaged 30.8%. Differences were not significant. The AEA values in the current study are higher than the 25% and 24% AEA values for maternal colostrum reported by Quigley et al. (1998b) and the 23% reported by Arthington et al. (2000). Quigley et al. (1998b) obtained single colostrum samples randomly from cows within the herd for measuring IgG whereas Arthington et al. (2000) used pooled colostrum.

Serum IgM concentration at birth for calves in STP was not affected by TM treatment of dams and averaged 0.18 mg/mL (Table 5). However, calves born from cows

fed OTM tended ($P = 0.10$) to have a lower serum IgM than calves born from cows fed CON or OTMZ at 30 h of age. While we observed no treatment affects of TM source or Zn amount on colostrum IgG or IgM concentration studies by Kinal et al. (2005) and Kincaid et al. (2004) reported a 30% increase in serum Ig concentration in calves born to dams receiving organic TM compared to inorganic TM.

Milk replacer and starter intake. Milk replacer was limit fed at a fixed amount of (0.68 kg/d) to calves and therefore, no differences in milk replacer intake were anticipated or found averaging 0.65 kg/d for calves in STP and 0.63 kg/d for calves in NWROC from 3 d of age to weaning (Table 5 and 6). In STP, calves from dams fed the CON treatment had increased starter intake compared to OTM treatment born calves with OTMZ born calves intermediate (Table 5). As a result of the increased starter intake, total DM intake for the CON calves tended to be higher than the OTM calves. At NWROC, source of TM or amount of Zn fed to the dam prior to parturition had no affect on starter intake or total DM intake. The reason for the difference in starter intake at STP is unknown. The daily starter intake of calves at STP was almost twice the amount consumed by calves at NWROC. The difference in starter intake by calves at the two locations may be attributed to differences in housing types as the STP calves were housed outside during winter months while the calves at NWROC were housed in a climate controlled room. The difference in starter intake may also be a reflection of the difference in serum protein concentration and the effect it may have on animal performance. Kinal et al. (2005) noted that calves with a high serum IgG concentration had improved daily weight gain the first 3 months of life.

Calf performance. Calf body weight at birth, 42 d and 56 d were not affected by treatment at either STP or NWROC locations (Table 5 and 6). At STP, there was a significant effect of breed for 56 d weight as Holsteins had greater body weight than the crossbred calves (77.0 vs. 71.8 kg, respectively). At NWROC, there was a significant effect of sex on 56 d body weight with bull calves weighing more than the heifer calves (73.5 vs. 67.4 kg, respectively). The calves born at NWROC weighed on average about 2 kg more at birth than those at STP (42 vs. 40 kg, respectively), but at 56 d of age the body weights at STP were numerically greater. The difference in body weight at birth between STP and NWROC may be due to the fact that Jersey crossbred cows were used in STP and only Holsteins at NWROC. While bull calves were included in the study at NWROC and not in STP, there was no effect of sex on birth weight or DM intake. The reason for the higher 56 d body weight of calves at STP compared to NWROC is likely due to the higher starter intake.

Calves born in STP from cows fed OTM had a lower ($P<0.02$) ADG from 1 to 42 d compared to calves from CON fed cows and intermediate to calves from OTMZ fed cows (Table 5). Overall ADG for calves in STP from 1 to 56 d of age was not affected by TM source or Zn amount fed to the dam However, there was a breed effect as Holsteins tended ($P = 0.09$) to gain more than the cross-bred calves (0.94 vs. 0.80 kg/d, respectively) from 43 to 56 d and as a result there was a trend ($P = 0.06$) for Holstein heifers to be heavier than crossbred heifers (77.0 vs. 71.8 kg, respectively) at 56 d of age.

At NWROC, d 1 to 42 ADG was not affected by treatment and averaged 0.43 kg/d. Average daily gain from 43 to 56 d tended ($P<0.09$) to be higher for calves from OTMZ fed cows than calves from either CON or OTM treatment fed cows (0.91 vs. 0.66

and 0.67 kg/d, respectively). Heifer calves had a significantly lower ($P = 0.04$) 43 to 56 d ADG than bull calves (0.64 vs. 0.86 kg/d, respectively). Overall, ADG of calves from d 1 to 56 was not affected by treatment and averaged 0.51 kg/d.

Study 2 (Intestinal morphology)

Serum parameters. Serum protein and Ig concentrations for bull calves used in the intestinal morphology study in STP are in Table 7. Bull calves fed colostrum achieved passive transfer as measured by total serum proteins of 5.61 mg/mL or greater and IgG concentrations of 11.0 mg/mL or greater. However, bulls born from cows fed OTM tended ($P = 0.10$) to have lower serum protein concentration at 30 h of age than bulls born to CON or OTMZ fed cows and had lower serum IgG concentration than bull calves born to CON fed cows with bull calves from OTM fed cows intermediate. . The reason for these differences is not known since there was no significant affect of treatment on colostrum immunoglobulin content and these differences were not observed in the heifer calves in STP. No effect of TM source or zinc concentration was observed for serum IgM concentrations at birth, 30 h of age or at 2 wk of age (Table 7).

Trace mineral form or zinc amount fed to the dam during the dry period had no affect on the blood hematology profile of bull calves at birth or two weeks of age (Table 8). Red blood cell count ($P = 0.12$) and hemoglobin concentration ($P = 0.11$) tended to be slightly higher at birth in bull calves born from cows fed OTMZ than bull calves from CON or OTM fed cows. Kume and Tanabe (1993) reported values of 10.2 g/dL and 34.5 % for hemoglobin and hematocrit for calves at birth, respectively. While these values are similar to ours, it should be noted that all bull calves in the current study were born to multiparous cows and blood samples were taken prior to feeding colostrum whereas

calves in the Kume and Tanabe (1993) study were from primiparous cows and they did not specify if blood samples were collected prior to or after feeding colostrum.

Dry matter intake. Milk replacer intake, calf starter intake and total DM intake were not affected by TM treatment (Table 7). No differences in DM intake were expected as all calves were healthy and most of the DM consumed came from milk replacer which was fed in an equal amount across treatments.

Liver mineral concentrations. Mineral concentrations in the liver of bull calves at birth were not affected by treatment, however liver phosphorus content tended ($P = 0.10$) to be higher for CON compared to the OTM calves (Table 9). The difference in liver P was not expected, as macro and TM feeding to dams on all treatments was similar and contained the same amount and source of P. No differences in liver mineral concentrations were observed at 2 wk.

Intestinal measures

There was no significant effect of TM treatment feeding to the dam on calf intestinal crypt depth or villus height at birth or 2 wk of age (Table 10). In the current study, jejunal crypt depth and villus height at birth averaged 199.7 and 952.5 μm and 278.6 and 575.3 μm at 2 wk of age, respectively. These values are much higher than the 35.2 for crypt depth and the 56 μm villus height reported by Buhler et al. (1998) in calves euthanized at 8 d of age and fed only colostrum or milk replacer. Crypt depth and villus height of 142 and 722 μm, respectively, were found in nursing lambs 1 wk of age (Attaix and Meslin, 1991) and are much closer to the values reported here for bull calves at birth than those of Buhler et al. (1998). At 2 wks of age, our values for both crypt depth and villus height are greater than those reported in suckling lambs. This would be expected

as a bull calf is much larger than a lamb and they were twice as old as the lambs at the time of measurement. As the intestinal tissue develops, the villus would increase in length and in turn the crypt depth would increase to support the longer villus. The exact reason for the great difference in intestinal crypt depth and villus length between the current study and Buhler et al. (1998) is unknown.

It is unfortunate our sample size was too small to detect differences. However, our bull calf numbers were limited by the number of cows in the STP herd and we had no prior research data on measurement variation in which to estimate sample size needed. . A post-study power analysis to detect a difference in jejunum villus height at birth indicated we would have needed 10 calves per treatment at birth and 14 per treatment at 2 wk of age. Crypt depth would have required even more calves per treatment to detect a difference with 68 calves per treatment required at birth and 82 calves per treatment at 2 wk of age.

Previous studies in swine and poultry have shown differences in intestinal morphology. With an increase in villus height and crypt depth, theoretically there is more surface area inside the intestine. Greater surface area would allow for increased nutrient absorption by the progeny both initially from colostrum and throughout the milk feeding period as the animal ages. While research in nonruminants (chicks and baby pigs) has shown supplementation of zinc during the gestation period can increase intestinal villi growth and enhance nutrient absorption in the offspring during the first few weeks of life (Caine et al., 2001; Caine et al., 2009; Payne et al., 2006; Takeo et al., 2005) , we were unable to find similar effects in calves from dams fed supplemental Zn from an organic source.

CONCLUSIONS

There was no effect of TM supplementation source or amount of Zn supplemented on colostrum protein or Ig concentration at either STP or NWROC location. For cows at STP, mineral treatments did not significantly affect the fat content of colostrum. At NWROC, treatment did not affect fat concentration of colostrum from primiparous cows, but fat content of colostrum did differ by treatment in multiparous cows. Cows receiving OTMZ produced colostrum higher in fat than those receiving CON and colostrum from cows fed OTM was intermediate in fat content. Ash content of colostrum from both primiparous and multiparous cows fed CON at NWROC was higher than OTM or OTMZ fed cows. Calf serum proteins and Ig concentration were not affected by treatment. At STP, starter intake for calves born from cows fed CON was greater than OTM or OTMZ. Day 1 to 42 ADG for calves from CON cows was greater than calves from OTM cows with calves from OTMZ cows being intermediate. Daily gain of calves through 56 days of life was not affected by TM source or zinc amount fed to dams prepartum.

In the intestinal morphology study, serum IgG concentrations at 30 h of age were higher for bull calves from CON cows compared to calves from OTM, with OTMZ being intermediate. At 2 weeks of age, bull calves from CON and OTMZ cows had greater concentrations of serum IgG than OTM calves. Serum IGF-I concentrations at birth and 30 h of age were not affected by TM treatment feeding to the dam. However, at 2 wks of age OTMZ calves had significantly higher serum IGF-I concentrations than CON or OTM calves. Trace mineral feeding to the dam of bull calves had no effect on serum IgM

or liver TM concentrations, hematology profile, intestinal villus height or intestinal crypt depth.

Table 1. Nutrient composition of milk replacer and calf starter fed to calves at St. Paul and Crookston-Study 1 and 2.

Item	Milk replacer	Calf starter
DM, %	97.3	88.3
	---------- (g/100g DM)----------	
CP, %	20.3	20.1
ADF, %	0.75	8.59
NDF, %	0.86	16.7
ADICP[1], %	0.15	0.60
NDICP[2], %	0.02	1.07
EE[3], %	20.3	4.58
Ash, %	8.78	9.10
Ca, mg/kg	9351.7	12592.1
P, mg/kg	7025.5	7831.6
K, mg/kg	18796.1	13929.3
Mg, mg/kg	1301.5	3953.1
Na, mg/kg	7039.6	2663.5
Fe, mg/kg	20.5	244.7
Mn, mg/kg	15.1	136.5
Zn, mg/kg	69.7	251.9
Cu, mg/kg	17.6	37.5

[1]Acid detergent insoluble crude protein.
[2]Neutral detergent insoluble crude protein.
[3]Ether Extract.

Table 2. Effect of trace mineral source and zinc amount on colostrum composition at St. Paul-Study 1 and 2.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Number	16	15	13	-	-
DM, %	24.9	23.0	24.4	1.06	0.42
Fat, %	5.64	6.12	5.87	0.78	0.91
CP, %	15.1	13.2	14.4	0.90	0.28
IgG, mg/mL	59.7	56.5	62.2	5.95	0.79
IgM, mg/mL	4.28	4.37	5.13	0.69	0.64
Ash, %	1.33	1.23	1.27	0.03	0.06
Ca, mg/kg	2429.9	2155.0	2176.8	144.1	0.34
P, mg/kg	2368.3	2091.2	2110.3	111.9	0.17
Mg, mg/kg	382.7	337.3	340.6	23.2	0.33
Na, mg/kg	527.3	542.2	506.2	27.2	0.64
K, mg/kg	1747.9	1717.7	1713.0	46.1	0.85
Zn, mg/kg	24.2	19.2	20.9	1.99	0.21
Fe, mg/kg	0.96	0.64	0.59	0.24	0.52
Cu, mg/kg	0.16	0.16	0.14	0.01	0.96
Mn, mg/kg	0.06	0.09	0.06	0.03	0.80

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.

Table 3. Effect of trace mineral source and zinc amount on colostrum composition of multiparous cows at Crookston-Study 1.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Number	8	8	9	-	-
DM, %	23.8	24.0	25.1	0.73	0.36
Fat, %	3.38[a]	5.96[ab]	7.63[b]	1.04	0.02
CP, %	16.1	14.0	13.8	1.49	0.48
IgG, mg/mL	77.8	58.5	64.1	10.5	0.43
IgM, mg/mL	6.95	6.31	6.63	1.25	0.94
Ash, %	1.45[a]	1.33[b]	1.31[b]	0.02	<0.001
Ca, mg/kg	2659.5	2215.9	2439.9	208.0	0.35
P, mg/kg	2496.2	2233.3	2471.4	172.9	0.52
Mg, mg/kg	404.6	338.6	382.0	34.6	0.42
Na, mg/kg	517.9	536.0	492.3	36.3	0.71
K, mg/kg	1933.3	1781.7	1757.8	135.2	0.61
Zn, mg/kg	22.9	24.6	19.4	2.31	0.28
Fe, mg/kg	1.89	1.75	1.60	0.78	0.96
Cu, mg/kg	0.02	0.04	0.03	0.02	0.49
Mn, mg/kg	0.001	0.004	0.02	0.007	0.22

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[a,b]Means in the same row with differing superscripts differ.

Table 4. Effect of trace mineral source and zinc amount on colostrum composition of primiparous cows at Crookston-Study 1.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Number	11	12	11	-	-
DM, %	26.6	26.1	25.7	1.68	0.93
Fat, %	8.36	8.56	8.97	1.10	0.92
CP, %	14.1	13.5	12.6	1.12	0.66
IgG, mg/mL	57.7	66.8	59.0	8.18	0.69
IgM, mg/mL	6.11	6.44	5.06	0.79	0.44
Ash, %	1.46[a]	1.34[b]	1.22[b]	0.06	0.04
Ca, mg/kg	2365.0	2540.0	2343.3	237.1	0.80
P, mg/kg	2245.7	2290.6	2207.4	193.1	0.95
Mg, mg/kg	319.5	325.4	322.4	35.7	0.99
Na, mg/kg	661.8	753.9	631.9	81.5	0.53
K, mg/kg	1401.4	1415.7	1596.5	82.7	0.20
Zn, mg/kg	21.9	27.4	23.9	3.69	0.57
Fe, mg/kg	0.84	1.21	0.97	0.14	0.19
Cu, mg/kg	0.02	0.04	0.06	0.02	0.45
Mn, mg/kg	0.02[a]	0.03[b]	0.007[a]	0.007	0.04

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[a,b]Means in the same row with differing superscripts differ.

Table 5. Effect of trace mineral source and zinc amount during dry-period on heifer calf serum protein, immunoglobulin concentration and performance at St. Paul-Study 1.

	Treatments[1]				
Item	**CON**	**OTM**	**OTMZ**	SEM	*P*-value
Number[2]	10	10	9	-	-
Serum protein, mg/dl					
Birth	4.19	4.58	4.34	0.17	0.29
~30 h of age	6.41	5.95	6.45	0.30	0.44
Serum IgG, mg/mL					
Birth	0.64	0.99	1.16	0.45	0.69
~30 h of age	20.7	16.2	19.7	1.84	0.21
IgG absorption[3,4], %	40.6	40.8	30.8	4.72	0.29
Serum IgM, mg/mL					
Birth	0.19	0.17	0.19	0.08	0.96
~30 h of age	2.20	1.40	2.13	0.28	0.10
Dry matter intake, kg/d					
Milk replacer	0.66	0.65	0.65	0.003	0.75
Starter	0.89[a]	0.70[b]	0.78[ab]	0.03	0.03
Total	1.37	1.26	1.27	0.05	0.07
Body weight, kg					
Birth	39.9	40.1	38.9	1.74	0.87
42 d	64.1	60.3	61.8	2.18	0.39
56 d	76.4	72.6	74.1	2.25	0.48
Average daily gain, kg/d					
d 1 to 42	0.59[a]	0.45[b]	0.54[ab]	0.03	0.02
d 43 to 56	0.85	0.88	0.88	0.07	0.95
d 1 to 56	0.65	0.56	0.62	0.03	0.17

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]One cow aborted and 1 cow had a set of twins.
[3]Apparent efficiency of IgG absorption calculated as [plasma IgG (g/L) × plasma volume (L) × 100]/IgG intake (g) according to Quigley et al. 1998.
[4]Number of animals analyzed per treatment is as follows: CON = 9; OTM = 8; and OTMZ = 6.
[a,b]Means in the same row with differing superscripts differ.

Table 6. Effect of trace mineral source and zinc amount during dry-period on calf serum protein, immunoglobulin concentration and performance at Crookston-Study 1.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Number of calves[2]					
Heifers	12	13	9	-	-
Bulls	9	8	10	-	-
Total	21	21	19	-	-
Serum protein, mg/mL					
Birth[3]	4.31	4.52	4.21	0.16	0.41
~30 h of age[4]	5.94	5.39	5.56	0.24	0.25
Dry matter intake, kg/d					
Milk replacer	0.63	0.63	0.64	0.004	0.16
Starter	0.39	0.39	0.44	0.04	0.36
Total	0.86	0.86	0.92	0.03	0.21
Body weight, kg					
Birth	41.0	43.0	41.2	1.87	0.71
42 d	59.4	60.6	59.9	2.09	0.91
56 d	68.6	69.9	72.6	2.38	0.49
Average daily gain, kg/d					
d 1 to 42	0.44	0.42	0.44	0.03	0.83
d 43 to 56	0.66	0.67	0.91	0.09	0.08
d 1 to 56	0.50	0.48	0.56	0.32	0.26

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]One cow aborted, four calves were dead at birth and there were 4 sets of twins.
[3]Number of animals analyzed by treatment CON = 21; OTM = 19; and OTMZ = 19.
[4]Number of animals analyzed by treatment CON = 19; OTM = 14; and OTMZ = 16.
[a,b]Means in the same row with differing superscripts differ.

Table 7. Effect of trace mineral source and zinc amount during dry-period on bull calf serum protein, immunoglobulin concentration and performance at St. Paul-Study 2.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Serum protein, mg/dl					
Birth[2]	4.19	4.46	4.25	0.22	0.66
~30 h of age[3]	6.26	5.61	6.50	0.28	0.10
~2 wk of age[3]	5.12	4.82	4.78	0.49	0.80
Serum IgG, mg/mL					
Birth[2]	0.14	0.52	0.00	0.34	0.32
~30 h of age[3]	19.9[a]	11.0[b]	15.2[ab]	1.84	0.04
~2 wk of age[3]	24.8[a]	14.3[b]	22.9[a]	1.81	0.008
Serum IgM, mg/mL					
Birth[2]	0.09	0.09	0.02	0.10	0.80
~30 h of age[3]	0.51	0.50	0.57	0.08	0.85
~2 wk of age[3]	2.08	1.54	2.19	0.41	0.50
Serum IGF-I, mg/mL					
Birth[2]	117.4	108.5	108.5	18.3	0.92
~30 h of age[3]	60.5	76.0	87.3	17.3	0.57
~2 wk of age[3]	104.3[a]	104.0[a]	164.3[b]	13.8	0.03
Dry matter intake[3], kg/d					
Milk replacer intake	0.65	0.65	0.65	0.01	0.74
Starter intake	0.10	0.20	0.10	0.05	0.23
Total DMI	0.75	0.85	0.74	0.05	0.27

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]Number of animals analyzed by treatment is CON = 7; OTM = 7; OTMZ = 7.
[3]Number of animals analyzed by treatment is CON = 4; OTM = 4; OTMZ = 3.
[a,b]Means in the same row with differing superscripts differ.

Table 8. Effect of trace mineral source and zinc amount during the dry period on blood hematology profile of bull calves euthanized at two time points from cows at St. Paul-Study 2.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Birth					
Number	7	7	7	-	-
WBC[2], 10^3/μL	7.30	7.76	8.47	0.74	0.54
NeutSegs, 10^3/μL	4.88	4.61	5.14	0.85	0.91
Lymph, 10^3/μL	2.01	2.60	2.70	0.44	0.50
Monocyte, 10^3/μL	0.36	0.45	0.59	0.09	0.22
Eosinophil, 10^3/μL	0.05	0.11	0.04	0.03	0.23
RBC, 10^6/μL	7.37	7.90	8.59	0.40	0.12
HGB, g/dL	9.89	10.5	11.5	0.53	0.11
HCT, %	35.0	32.3	35.8	3.21	0.73
MCHC, g/dL	32.2	32.3	32.2	0.34	0.90
PLT, 10^3/μL	515.3	556.3	561.3	43.9	0.70
TPP, g/dL	5.79	5.53	5.13	0.36	0.44
2 wk of age					
Number	4	4	3	-	-
WBC, 10^3/μL	9.95	8.73	8.90	0.93	0.60
NeutSegs, 10^3/μL	4.68	3.83	3.72	0.61	0.48
Lympho, 10^3/μL	4.42	4.37	4.37	0.65	1.00
Monocyte, 10^3/μL	0.80	0.53	0.67	0.18	0.56
Eosinophil, 10^3/μL	0.06	0.00	0.14	0.06	0.33
RBC, 10^6/μL	8.26	9.38	9.53	0.73	0.41
HGB, g/dL	10.9	12.0	13.2	0.83	0.20
HCT, %	32.9	35.9	39.2	2.36	0.22
MCHC, g/dl	33.2	33.4	33.7	0.24	0.39
PLT, 10^3/μL	830.0	939.0	760.0	54.2	0.15
TPP, g/dL	5.88	5.67	5.67	0.25	0.79

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.
[2]WBC = white blood cells; RBC = red blood cells; NuetSegs = segmented neutrophils; Lymph = lymphocytes; Monocyte = monocytes; Eosinophil = eosinophils; HGB = hemoglobin; HCT = hematocrit; MCHC = mean corpuscular hemoglobin concentration; PLT = platelets; and TPP = total plasma protein.

Table 9. Effect of trace mineral source and zinc amount during the dry period on liver mineral concentration (wet wt basis) of bull calves euthanized at two time points from cows at St. Paul-Study 2.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Birth					
Number	3	3	4	-	-
Ca, mg/kg	60.8	52.6	60.6	5.48	0.53
P, mg/kg	2270.0	2230.0	2409.0	232.2	0.84
Mg, mg/kg	125.2	113.9	133.5	18.2	0.75
Na, mg/kg	1680.2	1767.8	1896.4	140.2	0.56
K, mg/kg	2043.7	2010.3	2051.5	222.1	0.99
Zn, mg/kg	159.2	86.2	115.8	36.9	0.45
Fe, mg/kg	59.0	23.4	31.8	18.2	0.43
Cu, mg/kg	128.6	135.9	201.8	26.0	0.14
Mn, mg/kg	1.21	1.02	1.20	0.19	0.74
2 wk of age					
Number	4	4	3	-	-
Ca, mg/kg	43.1	44.0	45.8	3.63	0.88
P, mg/kg	2376.0	1885.5	2023.0	149.5	0.10
Mg, mg/kg	104.8	79.7	89.3	8.55	0.15
Na, mg/kg	1706.5	1868.4	1627.6	151.7	0.55
K, mg/kg	2297.8	1560.3	1731.2	255.7	0.15
Zn, mg/kg	51.4	71.5	56.2	17.4	0.68
Fe, mg/kg	69.0	113.6	270.8	72.4	0.20
Cu, mg/kg	166.0	175.5	124.3	26.5	0.42
Mn, mg/kg	2.16	1.68	2.02	0.30	0.50

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.

Table 10. Effect of trace mineral source and zinc amount during the dry period on jejunum intestinal measurements of bull calves from cows at St. Paul-Study 2.

	Treatments[1]				
Item	CON	OTM	OTMZ	SEM	*P*-value
Birth					
Number	3	3	4	-	-
Crypth depth, μm	217.9	193.0	188.1	29.9	0.76
Villus height, μm	845.7	1064.2	947.7	98.5	0.39
2 wk of age					
Number	4	4	3	-	-
Crypth depth, μm	279.1	301.9	254.9	25.0	0.41
Villus height, μm	638.8	443.1	644.0	90.2	0.19

[1]CON = inorganic trace mineral supplementation; OTM = 1/3 organic TM and 2/3 inorganic TM supplementation; and OTMZ = OTM with additional Zn from Zinpro 100.

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