DISSERTATION

EFFECTS OF TRACE MINERAL SUPPLEMENTATION, TRACE MINERAL SOURCE, GROWTH IMPLANTS, AND INDUCED MORBIDITY ON PERFORMANCE, TRACE MINERAL STATUS, IMMUNE FUNCTION, CARCASS CHARACTERISTICS, AND LIPID METABOLISM IN STEERS

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY KRISTY LEIGH DORTON ENTITLED "EFFECTS OF TRACE MINERALS, IMPLANTS, AND INDUCED MORBIDITY ON PERFORMANCE, TRACE MINERAL STATUS, IMMUNE FUNCTION, CARCASS CHARACTERISTICS, AND LIPID METABOLISM IN STEERS" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

EFFECTS OF TRACE MINERAL SUPPLEMENTATION AND SOURCE, GROWTH IMPLANTS, AND MORBIDITY ON PERFORMANCE, TRACE MINERAL STATUS, IMMUNE FUNCTION, CARCASS CHARACTERISTICS, AND LIPID METABOLISM IN STEERS

Steers were utilized to determine the effects of trace mineral (TM) supplementation and source, growth implants, and morbidity on performance, TM status, immune function, carcass characteristics, and lipid metabolism. In experiment 1, steers were backgrounded at their respective ranches for 30 d post weaning on one of the following treatments: 1) control (no supplemental TM), 2) inorganic TM, and 3) iso-amounts of organic TM. After the 30 d backgrounding period, steers were transported to the feedlot where half of the steers per treatment were implanted and the remaining half were not implanted. In experiment 2, the first group of steers was inoculated intranasally with IBRV four months prior to weaning. The second group was inoculated with IBRV on arrival to the feedlot and the third group was not inoculated with IBRV. Once at the feedlot, calves were sorted to treatments, which consisted of 1) organic TM and 2) iso-amounts of inorganic TM. During the backgrounding phase of experiment 1, calves supplemented with organic TM had a greater ADG and a lower incidence of morbidity than calves supplemented with inorganic TM. Steers supplemented with organic TM had

greater concentration of total IgM during the growing phase and greater concentrations of antibody titer specific to ovalbumin than steers supplemented with inorganic TM. Steers that were implanted had a greater ADFI, REA, HCW, dressing percentage, and acetyl CoA carboxylase activity, and a lower KPH than non-implanted steers. In experiment 2, liver Mn and Zn concentrations were decreased in IBRV steers, whereas, Cu concentrations were unaffected. Inoculated steers had lower oleic acid (18:1 n-9) and monounsaturated fatty acids. Steers that were inoculated with IBRV prior to arrival at the feedlot had lower concentrations of myristic acid (14:0) and linoleic acid (18:2 n-6).
Key Words: Steers, trace minerals, growth implants, induced morbidity, performance, immunity, carcass characteristics, lipid metabolism

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LITERATURE REVIEW

Chemical Properties of Trace Minerals

Copper:

Copper (Cu) is the 29th element found in group IB on the periodic table. Copper has a lustrous reddish brown color. Copper's relative atomic mass is 63.546 (Lide and Frederikse, 1993; McDowell, 1992); and it has a melting point of 1083.4°C, a boiling point of 2567°C, and is insoluble in water (Lide and Frederikse, 1993). Copper is malleable, a good thermal conductor, and is an excellent electrical conductor (Dameron and Howe, 1998). Copper has four oxidation states: metallic Cu (Cu^{o}), cuprous ion (Cu(I) or Cu^{+}), cupric ion (Cu(II) or Cu^{2+}), and trivalent ion (Cu^{3+}). Copper can form organometallic compounds and is found in a variety of mineral salts and organic compounds in the elemental or metallic forms (Dameron and Howe, 1998). The metallic form is stable in dry air at low temperatures, but in moist air a slow reaction occurs. During this reaction, hydrocarbonate or hydrosulfate is formed which causes a greenish gray film to develop that protects the metal underneath from further attack by oxygen (Dameron and Howe, 1998). Cotton and Wilkinson (1989) stated that Cu can be dissolved in highly acidic solutions and in certain basic solutions.

The chemical properties of Cu depend on the oxidative state that Cu resides. The ease at which Cu changes oxidation state gives Cu redox properties that can be both beneficial and deleterious for biological systems (Dameron and Howe, 1998). Copper(I) and Cu(II) are the most common oxidation states with Cu(I) being quickly oxidized by

oxidizing reagents and Cu(II) being the most important oxidation state in a natural, aqueous environment (Dameron and Howe, 1998). Chemical behaviors determine the metabolism of Cu because these chemical behaviors determine how Cu will interact with other minerals and compounds during absorption, transport, and distribution of Cu to body tissues (Dameron and Howe, 1998).

Zinc:

Zinc (Zn) is the fourth most predominant metal in use trailing only aluminum, copper, and iron (Wikipedia, 2005). Zinc is the 30th element found in group IIB on the periodic table. Zinc has a bluish white color and is a relatively soft metal (density = 7.133 g/cc; Assembly of Life Sciences, 1979; McDowell, 1992). Zinc's relative atomic mass is 65.37 (Assembly of Life Sciences, 1979 McDowell, 1992); and it has a melting point of 419.5°C, a boiling point of 907°C, and is insoluble in water (Assembly of Life Sciences, 1979; Lide and Frederikse, 1993; Wikipedia, 2005). Zinc is a brittle compound until heated to 120°C where it becomes easy to shape (Assembly of Life Sciences, 1979). Zinc has five stable isotopes: Zn-64, Zn-66, Zn-67, Zn-68, and Zn-70; and 6 radioactive isotopes: Zn-62, Zn-63, Zn-65, Zn-69, Zn-72, and Zn-73, of which Zn-65 and Zn-69 are the most widely used with a half-life of 244 d and 58 minutes, respectively (Assembly of Life Sciences, 1979). Zinc is stable in dry air; however, as temperature and/or moisture increase the corrosion rate, or chemical reactions that occur, increases rapidly (Assembly of Life Sciences,1979). The addition of carbon dioxide accelerates the process. During this reaction, hydrated basic carbonate is produced (light gray film) and adheres tightly to the surface preventing further corrosion (Assembly of Life Sciences, 1979). This property makes Zn useful in protecting other metals, such as iron and steel, from

corrosion by being used as a galvanizing agent (Assembly of Life Sciences, 1979). Zinc also combines with many other compounds. One such category of compounds is mineral acids, which easily attack Zn. The Assembly of Life Sciences (1979) stated that Zn "displays a vigorous reducing power". Zn releases hydrogen from compounds, such as sulfuric acid or hydrochloric acid. Zinc is also found in association with lead, copper, cadmium, and iron (Miller et al., 1979).

Manganese:

Manganese (Mn), the 25th element found in group VIIA of the periodic table, is a hard brittle metal that is grayish-white or silver in color (NSC, 2004a; Helmenstine, 2005a). Manganese has a relative atomic mass of 54.94, a melting point of 1244°C, and a boiling point of 1962°C (Helmenstine, 2005a; Winter, 2003). Manganese does not break down or disappear in the environment; however, certain compounds containing Mn dissolve in water or dilute mineral acids (NSC, 2004a; Helmenstine, 2005a). Manganese has four molecular structural forms: two of which are the alpha and gamma forms (Helmenstine, 2005a). The alpha form is stable at room temperature; and the gamma form can change into the alpha form at room temperature. The gamma form is also softer and more flexible than the alpha form, which makes it easier to cut and shape (Helmenstine, 2005a). Manganese is often utilized as an alloy agent to add strength, toughness, wear resistance, and hardenability to metals (Helmenstine, 2005a). It is utilized to remove the green color or to add an amethyst color to glass and is the natural coloring agent of amethyst stones (Helmenstine, 2005a).

Manganese is a chemically reactive element (Helmenstine, 2005a) and is not normally found in the environment as a pure metal (NSC, 2004a). It is often found

combined with up to 100 different compounds, such as sulfides, oxides, carbonate, and phosphates (NSC, 2004a). The National Safety Council (NCS, 2004a) stated that the most important compounds containing Mn are manganous chloride ($MnCl_2$), manganous sulfate ($MnSO_4$), manganese tetroxide (Mn_3O_4), manganese dioxide (MnO_2), and potassium permanganate ($KMnO_4$). Manganous chloride and $MnSO_4$ are often utilized in animal feed as a nutritional supplement for Mn.

Cobalt:

Cobalt (Co) is the 27th element found in group VIII (the first of the transition series) on the periodic table (NSC, 2004b; Helmenstine, 2005b). Cobalt is a hard brittle metal that, in its pure form, is steel gray to black in color (NSC, 2004b; Helmenstine, 2005b). Cobalt has a relative atomic mass of 58.93, a melting point of 1495°C, and a boiling point of 2870°C (Helmenstine, 2005b). Pure cobalt does not dissolve in water but will dissolve in acids (NSC, 2004b). Cobalt can also stay suspended in the air for several days; however, cobalt will remain in water and soil for years (NSC, 2004b). Cobalt has two molecular structural forms: beta and alpha. The beta form is dominant at temperatures less than 400°C, whereas, the alpha form is dominant at higher temperatures (Helmenstine, 2005b).

Cobalt is often found in association with copper, nickel, manganese, and arsenic. It is often combined with iron and nickel to make alloys that provide great magnetic strength and to make super alloys that maintain strength at higher temperatures (NSC, 2004b; Helmenstine, 2005b). Cobalt salts are utilized to add a blue color to glass, whereas, other forms of cobalt are utilized to remove color from glass (Helmenstine,

2005b). Cobalt is also an important component of vitamin B12, which makes it important in nutrition of animals.

Metabolism: Absorption, Transport, Storage, Excretion

Absorption:

Copper: Copper absorption is regulated by metabolic need of an animal (McDowell, 1992). Intestinal absorption is influenced by the chemical form of Cu and by interactions that Cu has with other components in the diet (McDowell, 1992). McDowell (1992) stated that Cu is poorly absorbed in most species. Ruminants absorb only one to three percent of Cu, while non-ruminants are able to absorb five to ten percent (McDowell, 1992). Absorption occurs primarily in the upper portion of the small intestine (Allen and Solomon, 1984; Linder, 1991; McDowell, 1992; Linder et al., 1999). Absorption can occur in other parts of the GI tract as well, but the amount absorbed is much lower than that absorbed in the upper small intestine (Dameron and Howe, 1998).

Copper is absorbed across the intestinal mucosa in a two step process (Crampton et al., 1965). First, Cu is transported across the mucosal membrane. Then, it is transported across the cell. Copper can be absorbed through one of two mechanisms: saturable (active transport) or unsaturable (simple diffusion; Allen and Solomons, 1984; Bronnerd and Yost, 1985; Dameron and Howe, 1998). On the basolateral side of the intestinal cell, transport into the blood stream seems to be energy dependent and may limit the rate of Cu transfer to albumin (Allen and Solomons, 1984; Dameron and Howe, 1998). If high levels of Cu are consumed or if body stores are adequate, other energy dependent mechanisms are activated to bring Cu into the intestinal cell (Linder, 1991).

Copper absorption may be regulated by Cu status of the body. Copper absorption is increased when lower levels of Cu are present in the diet (Strickland et al., 1972; Giltin et al., 1960). Copper absorption may also be regulated or more likely restricted by metallothionine (Allen and Solomons, 1984). Metallothionine has a high affinity for Cu and will bind Cu very readily and keep it from being absorbed. Copper transfer into the blood may be regulated by cystolic protein that enhances or blocks the transfer of Cu into the blood (Allen and Solomons, 1984).

Zinc: The amount of Zn absorbed by ruminants seems to be determined by the metabolic need of the animal (Suttle et al., 1982). It has been reported that Zn absorption occurs in the abomasum (Miller and Cragle, 1965) and rumen of in cattle and the rumen of sheep (McDowell, 1992) with the greatest absorption via the small intestine (Miller and Cragle, 1965). In monogastric animals, absorption occurs mostly in the small intestine (McDowell, 1992) primarily in the duodenum (Naveh et al., 1988).

Although the mechanisms of Zn absorption are not fully understood, Cousins (1982) stated that there are four phases of absorption. The first phase of Zn absorption is the uptake of Zn from the lumen of the intestine into the intestinal cells. Zinc is transferred, often in chelated form (Solomon and Cousins, 1984), across the brush border of the intestine by an active, saturable, carrier mediated process (Davies, 1980; Cousins, 1982; McDowell, 1992). Zinc then becomes detached from dietary ligands within the lumen of the small intestine (Cousins, 1982) where it then becomes attached to a carrier ligand. The carrier can be either a high molecular weight protein that requires ATP for transport of Zn through the intestinal cell wall (Davies et al., 1968; Kowarsky et al., 1974) or a low molecular weight protein, such as citrate, EDTA, or amino acids, that may

not require ATP (passive transport; Oestreicher and Cousins, 1982; Seal and Heaton, 1987; Hambidge et al., 1986). This attachment makes Zn available to the microvilli for absorption into the mucosal cell (Hambidge et al., 1986).

Zinc within the mucosal cell includes what is absorbed from the rumen as well as what is reabsorbed from the blood making Zn absorption bidirectional (Hambidge et al., 1986). However, Zn is absorbed into the mucosal cell primarily from the lumen (Hambidge et al., 1986). The mechanisms associated with intracellular transport of Zn from the lumen to the blood and from the blood to the lumen are unknown. Hempe and Cousins (1991) reported that once Zn is absorbed into the mucosal cell it becomes bound to one of three proteins: nonspecific binding protein (NSB); metallothionine (MT; synthesized by the intestinal mucosal cell; McDowell, 1992); or a cysteine rich intestinal rich binding protein (CRIP). If Zn concentrations are lower in the blood, Zn will bind with a CRIP. Cysteine rich intestinal rich binding protein is a "diffusible intracellular Zn transport protein", which moves Zn from the brush border, or the lumen side of the cell, to the basolateral membrane (Hempe and Cousins, 1991). Once on the basolateral side of the cell, Zn will be transported out of the cell, become attached to a carrier molecule, and then transported throughout the body (Hempe and Cousins, 1991).

Hambidge et al. (1986) indicated that when lower concentrations of Zn are present in the blood, more Zn will be absorbed and enter the blood stream; however, when higher concentrations of Zn are present in the blood, Zn is retained in the intestinal cell. If Zn concentrations in the blood are adequate or high, Zn will become attached to MT in the intestinal cell. Metallothionine inhibits Zn absorption by limiting Zn ability to bind to CRIP within the intestinal cell. Zinc that is attached to MT cannot be absorbed

into the blood stream (Hambidge et al., 1986; Hempe and Cousins, 1991) which aids in regulating Zn concentrations in the body.

Absorption of Zn into the blood is a slower process than absorption and accumulation of Zn within the mucosal cell (Hambidge et a., 1986). Davies (1980) reported that during the first 30 minutes post dosing with Zn, a rapid transfer of Zn into the blood stream occurs; however, over the next 5.5 hours Zn absorption is slower. This has been stated to be the rate limiting step of Zn absorption in both rats (Davies, 1980; Smith and Cousins, 1980) and ruminants (Miller 1969). Once absorbed into the blood, Zn becomes bound to albumin, which transports Zn through the circulatory system (Hambidge et al., 1986).

Manganese: Manganese is poorly absorbed in animals of all species (Underwood, 1981b; McDonald, 1992; Kies, 1987). Of the total concentration of Mn in the diet, rats absorb 3-4 % (Greenberg and Campbell, 1940), avian absorb less than 1 % (Turk et al., 1982), cattle absorb 1-4 % (Samsom et al., 1976; Abrams et al, 1977; Sullivan et al, 1979), and humans absorb 3-4 % (Hurley and Keen, 1986). Absorption is higher in younger animals as reported in calves (Howes and Dyer, 1971) and young rats (absorbing 20% of dietary Mn; Keen et al., 1986) and decreases with age.

Underwood and Suttle (1999) indicated that Mn absorption is initially regulated by "the limits set by Mn source and dietary antagonist. When dietary concentrations are low, the percent of Mn absorbed increases. When dietary concentrations are high, the percent of Mn absorbed decreases. This can be better termed as the efficiency of absorption. Weigand et al. (1986) and World Health Organization (1996) stated that the efficiency of Mn absorption increases as dietary concentration and antagonist

concentration decreases in the diet. High concentrations of iron decrease Mn absorption (World Health Organization, 1996; Gruden, 1987; Ho et al., 1984). This effect is though to be caused by Mn competing with Fe, as well as with cobalt, for binding sites on the mucosal cell and carrier proteins within the intestines (McDowell, 1992). When dietary Fe is high, Fe will bind to these binding sites decreasing the binding sites available for Mn. Calcium also has an inhibitory effect on Mn absorption (Davies and Nightingale, 1975; McDermott and Kies, 1987; McDowell 1992). However, the profound negative effect on Mn absorption and bioavailability is caused by fiber (Johnson and Neilson, 1990). Fiber is negatively charged and will tightly bind Mn. Therefore, Mn absorption is reduced.

Manganese absorption can occur through one of two process: 1) high affinity, low capacity, saturable active transport system possibly with a membrane receptor (Garcia-Aranda et al., 1983; Lonnderdal et al., 1987) or 2) non-saturable, passive transport (diffusion allowing increased uptake of Mn; Lonnderdal et al., 1987). Absorption occurs through a two step process (Thomson and Valberg, 1971; Thomson et al., 1971; McDowell, 1992). Manganese is absorbed from the lumen of the small intestine through the brush border membrane (Lonnderdal et al., 1987). This step is followed by transfer across mucosal cells. These two steps are thought to occur simultaneously (Thomson et al., 1971; Thomson and Valberg, 1971) and to be enhanced by low MW ligands such as L-histidine and citrate (Hurley and Keen, 1986).

Cobalt: Cobalt is absorbed more slowly and less completely in ruminants than in monogastric species (Rothery et al., 1953; Smith and Marston, 1970) with greater absorption rates when dietary concentrations are elevated (Hedrich et al., 1973).

McDowell (1992) indicated that the loss of some of the dietary Co may be due to Co being utilized to make non-B_{12} compounds that can not be absorbed or utilized by the animal (McDowell, 1992). Also, poor absorption of Co may be due to rapid binding of Co by rumen microbes for vitamin B_{12} synthesis (McDowell, 1992). Up to 3 % of dietary Co is converted to vitamin B_{12} in the rumen (Smith and Marston 1970). Of the total vitamin B_{12} that is produced, only 1 to 3 % is absorbed (McDowell, 1992). Underwood and Suttle (1999) indicated that the remainder of vitamin B_{12} is lost during passage through the digestive tract.

Absorption of Co is dependent on many factors, or compounds. McDowell (1992) stated that these compounds are both intrinsic and non intrinsic carrier compounds. The following five compounds are required for Co absorption (McDowell, 1989): 1) adequate quantities of vitamin B_{12}, 2) normal stomach function for breakdown of food proteins to release dietary vitamin B_{12}, 3) normal stomach function for production of intrinsic factor for absorption of B_{12} through ileum, 4) normal pancreas function (trypsin) required for release of bound vitamin B_{12} before B12 binds to the intrinsic factor, and 5) normal ileum with receptor and absorption sites.

During absorption, corrinoids become bound to R proteins from saliva or other sources (Underwood and Suttle, 1999). These complexes are digested by pancreatic enzymes in the stomach (Underwood and Suttle, 1999). Cobalamin becomes bound by intrinsic factor, which is produced by parietal cells in the abomasum in ruminants (McKay and McLeary, 1981). The cobalamin-intrinsic factor complex will travel through the small intestine and be absorbed in the ileum (McDowell, 1992). The cobalamin-intrinsic factor complex enters the mucosal cell through receptor mediated endocytosis

(Underwood and Suttle, 1999). Smith (1997) reported that bile salts are needed for this complex to bind to receptors in the brush border of the ileum. Little is know about how cobalamin is released from intrinsic factor within the cell (Smith, 1997) or how it is transported across the cell to portal circulation.

Transport:

Copper: Once Cu is absorbed across the small intestine into the blood stream, Cu becomes attached to albumin, amino acids (such as histidine), or transcuprein (Lau et al., 1974; Schmitt et al., 1983; McArdle et al., 1999). Copper in the blood is primarily bound to albumin (Peters and Hawn, 1967), which has specific and non-specific binding sites for Cu (McArdle et al., 1999; Linder et al., 1999). Small amounts are bound in plasma to amino acids (Anon, 1981). Transcuprein also binds Cu. Linder et al. (1999) reported that transcuprein has an N-terminal amino acid that is like the high affinity binding site on albumin. Transcuprein has a higher affinity for Cu than albumin and readily exchanges Cu(II) with albumin (Linder et al., 1999).

Once bound, Cu is carried through the blood stream and is transported to the liver and kidney (Linder et al., 1999). The liver is very important in the metabolism of Cu (McArdle et al., 1999) and is the primary organ for Cu storage and distribution (Dameron and Howe, 1998). Uptake across the hepatic membrane is believed to be carrier mediated (Ettinger et al., 1986; McArdle et al., 1999) and is not dependent on cell energy or coupled to the sodium (Na) gradient (McArdle et al., 1999). Hepatocytes remove Cu from the high affinity sites on albumin; however, copper can be transported across the hepatic membranes from any Cu transporting complex (McArdle et al., 1990). The transporter that brings Cu into the hepatic cell membrane is a dimer attached to two

disulfide bridges (McArdle et al., 1999). One of the bridges is on the outside of the hepatic membrane, while the second is on the inside of the membrane (McArdle et al., 1999). McArdle et al. (1999) indicated that little is truly known about how Cu is transported across the hepatic cell membrane; however, it has been reported that Cu(II) if first reduced to Cu(I) by NADH oxidase and then transported into the hepatocyte. Copper(I) then enters the cell through the disulfide bridge and is reoxidized. It is not known whether Cu(I) is reoxidized to Cu(II) before or after Cu enters the Golgi apparatus in the hepatic cell. Copper is transported across the Golgi apparatus by ATP7B, which is energy dependent (McArdle et al., 1999).

Zinc: Once Zn enters the blood stream, it becomes attached to one of two molecules: albumin or α_2-macroglobulin. Two thirds of plasma Zn becomes loosely bound to plasma albumin (Smith et al., 1979; Underwood and Suttle, 1999). The majority of the remainder of plasma Zn becomes attached to the α_2-macroglobulin (Underwood and Suttle, 1999). Also, Zn may become bound to amino acids or non-protein ligands as metallo-ligand complexes to be transported throughout the body (McDowell, 1992). Zinc attached to albumin is readily taken up into tissues (McDowell, 1992). The most rapid accumulation is in the pancreas, liver, kidney, and spleen (McKenny et al., 1962). Uptake of Zn by the CNS and bone is slow. However, once incorporated, Zn is firmly bound and less available to be utilized when a deficiency is present (Hambidge et al., 1986; McDowell, 1992)

Stacey and Klaasen (1981) and Starcher et al. (1980) described two phases of entry of Zn into cells. The first phase is an early rapid uptake that is saturable and probably carrier mediated. The second phase is a slower phase and is apparently passive.

Hepatic metallothionine is believed to play a key role in removing Zn from plasma and partitioning it between different pathways in the body (McDowell, 1992). Uptake of Zn beyond the liver is not well understood (Cousins 1996); however, 30 to 40 % of Zn that enters the hepatic venous supply is subsequently released back into the blood stream (Aamodt et al., 1979; McDowell, 1992).

Manganese: Once in the blood, Mn remains free or is rapidly bound to α_2-microglobulin (Hurley and Keen, 1986). Manganese is transported to the liver where it is removed from the α_2-microglobulin and remains in the liver or enters the portal circulation (Hurley and Keen, 1986). Once in the portal circulation, Mn is oxidized to the manganic state (Mn^{3+}) and becomes bound to transferrin (Samson et al., 1976; Davidsson et al., 1989). Davidsson et al. (1989) stated that transferrin is the major plasma carrier protein for Mn. Transferrin bound Mn is then transported to extrahepatic tissues (Gibbons et al., 1976).

Excess Mn is removed from circulation rapidly. Cotzias et al. (1978) reported that within 10 minutes of injection with Mn that only 1% of the total Mn injected remained in the plasma. Borg and Cotzias (1958) indicated that excess Mn is cleared from blood in three phases. The first phase, which is the most rapid phase, includes a normal transcapillary movement such as seen with smaller ions. In the second phase, Mn is moved into the mitochondria of the cell. Kato (1963) stated that Mn tends to accumulate and have preference for organs that are rich in mitochondria. The final phase (and slowest phase) includes accumulation of Mn into the nucleus of the cell. However, more Mn accumulates in the mitochondria of the cell rather than the nucleus (Hurley and Keen, 1986).

Cobalt: Cobalamin, once absorbed into the blood stream, becomes bound to plasma proteins called transcobalamins (Underwood and Suttle, 1999). There are three forms of transcobalamins (0, I, and II), which are distributed differently in diverse species and have different binding properties.

Storage and Excretion:

Copper: Copper resides in various pools within the liver. These pools consist of transit, storage, and excretion pools (Owen, 1980). Copper within the transit pool is used or incorporated into ceruloplasm and other Cu dependent enzymes such as Cu-Zn superoxide dismutase (SOD). Ceruloplasm binds six Cu atoms tightly and one Cu atom loosely near the surface of the molecule (Zaitseva et al., 1996). Sixty to ninety percent of Cu in plasma is bound to ceruloplasm with the rest being bound to albumin, transcuprein, and ligands (McArdle et al., 1999). Ceruloplasm and bound Cu are transported to specific tissues throughout the body (Linder, 1991; Weiss et al., 1985, McDowell, 1992) utilizing specific receptors and transporters for these molecules (Linder et al., 1999).

Copper that is placed into the storage pool is stored in the liver until it is needed. The liver is the major storage organ for Cu, and Cu is stored in the form of mitochondrial cuprein (McDowell, 1992). The liver has the capacity to store approximately 20 % of the body's Cu supply. Muscle tissue (40 %), brain (20 %), connective tissue (8 %), blood (8 %) and kidneys (8 %) can store and utilize Cu (Dameron and Howe, 1998); however the liver is the true storage organ for the body. Copper in the liver can be mobilized and shipped to extrahepatic tissues when the body is in a negative Cu balance (Dameron and Howe, 1998), while tissues and blood utilize Cu and do not supply other tissues with appreciable amounts of Cu. The amount of Cu stored is dependent on Cu intake and Cu

status of an animal (McDowell, 1992). Metallothionine may be essential or may play a role in the storage of Cu (McArdle et al., 1999). Metallothionine (a class of low molecular weight binding proteins) can bind up to twelve Cu(I) atoms. This aids in Cu storage as well as protecting the body from Cu toxicities (Bremner, 1987). Copper can be moved from the storage pool to the transit pool if necessary when the body is in a negative Cu balance.

Copper that is placed into the excretion pool is excreted out of the body. Copper is excreted primary in feces (McDowell, 1992) and the amount is dependent on Cu absorbed from the diet (Underwood, 1977). Excretion occurs mostly through bile (Committee on Animal Nutrition et al., 1980; Allen and Solomons, 1984; McDowell, 1992; Dameron and Howe, 1998). Eighty percent of Cu excreted from the body is excreted through bile (Winge and Mehra, 1990). Copper can complex with bile salts in the canaliculi (McArdle et al., 1999) or through bilary excretion of glutathione (Freedman et al., 1989). Copper can also be excreted in urine (Underwood, 1977; Harris, 1991; Linder et al., 1999), milk, sloughed off intestinal cells, and through perspiration, but the amount excreted via these mechanisms is small (Underwood, 1977).

Zinc: Little is understood about how Zn is stored in the body (Underwood and Suttle, 1999). Zinc is widely distributed throughout the body; however, there is a low capacity for storage that can be readily utilized by the body when needed (McDowell, 1992). The highest concentration of Zn is found in soft tissues such as the pancreas, liver, pituitary gland, kidney, and the adrenal gland (McDowell, 1992). Within the liver, metallothionine is the major storage form of Zn (Richards and Cousins, 1976). This form of Zn can be mobilized easily when needed (Richards and Cousins, 1976). Zinc is also

found within muscle and bone (Underwood and Suttle, 1999); however, Zn in bone is not readily available to the rest of the body during times of Zn deficiency.

Zinc is predominantly excreted via pancreatic secretions and feces (Underwood and Suttle, 1999) with fecal excretion being the major route of excretion (McDowell, 1992). Fecal excretion not only includes dietary losses but also includes endogenous losses. Endogenous loss in the feces come from one of three places: gastrointestinal, pancreatic, and bilary secretions (McDowell, 1992); however, Underwood and Suttle (1999) stated that there is little loss of Zn through endogenous losses. Also, only a small amount of Zn is lost in the urine (Miller, 1969; Miller, 1970; Underwood and Suttle, 1999).

Manganese: Manganese is widely distributed in the body; however, Mn is distributed in low concentrations (McDowell, 1992). Manganese is found in the lowest concentrations of all trace elements within tissues (Underwood and Suttle, 1999). In sheep and calves, whole body concentrations of Mn ranged from 0.5 to 3.9 mg/kg DM (Suttle, 1979; and Grace, 1983). Higher concentrations are found in the bone, liver, kidney, and pancreas than in skeletal muscle (McDowell, 1992). Within a cell, more Mn is located in the cytosol than in the mitochondria (McDowell, 1992). Although Mn is found within the liver, the liver has limited storage capacity of Mn when compared to copper, iron, and selenium (McDowell, 1992).

The majority of the Mn in the body is found within the skeleton. The skeleton contains one fourth of total body Mn; however, this source of Mn is not readily available to be released during a deficiency (McDowell, 1992; Underwood and Suttle, 1999). Although Mn is found within tissues and other parts of the body, there is really no storage

of Mn. Underwood and Suttle (1999) stated that if you find storage of Mn in tissues there is unsuccessful homeostasis within the body (Underwood and Suttle, 1999).

The main route of excretion of surplus Mn is through bile (Papavasiliou et al., 1966; Hurley and Keen, 1986; Kies, 1987; Underwood and Suttle, 1999). Hurley and Keen, 1986 stated that when Mn enters the liver it will enter the mitochondria and the lysosomal compartments of the cell. The Mn complex from the lysosomal compartment will then enter bile and be excreted. This seems to be a rapid process. In rats that were injected with Mn, 50 % of the injected Mn was excreted within 24 hours, with 17 % more excreted within the next 24 hours and majority of the remaining Zn is excreted by the 3rd day (Klassen and Watkins, 1984). It is believed that the addition of Mn into bile is an active process requiring energy (Klassen and Watkins, 1984). Ligands within the liver and plasma have a higher affinity for Mn (Hurley and Keen, 1986), therefore, energy would be needed to remove the Mn from the liver and plasma and add it to bile.

Excretion through bile is not the only route of excretion for Mn. Manganese is excreted into the intestines via pancreatic juice (Kies, 1987; McDowell, 1992). This route of excretion increases when high concentrations of Mn are present in the diet or when the bilary is pathway blocked (Hurley and Keen, 1986). Manganese has been reported to be excreted through the intestinal walls (Hurley and Keen, 1986) into the lumen of the intestine. Urine is not a good excretory route for Mn. It has been reported that little Mn is excreted in the urine (Hurley and Keen, 1986) with the percentage of total Mn excreted being 0.1 to 3 % (Thomas, 1970).

Cobalt: Although little evidence has been reported (Marston, 1970), it is "customary to refer to excess vitamin B_{12} as being stored in the liver" (Underwood and

Suttle, 1999). However, vitamin B_{12}, like other water soluble vitamins, is poorly stored in the body (Underwood and Suttle, 1999). Forty three percent of body "stores" are found in the muscle and 14 % is in the bone (Underwood 1977). The remainder of Co/vitamin B_{12} in the body is found in the tissues such as the kidney and liver (McDowell, 1992). Within the liver, Co/vitamin B_{12} concentrations vary with the status of the animal (McDowell, 1992).

Underwood and Suttle (1999) stated that little know about vitamin B_{12} excretion in ruminants (Underwood and Suttle, 1999); however, McDowell (1992) stated that Co/vitamin B_{12} is mainly excreted in the feces. Also, there are variable amounts of Co/vitamin B_{12} excreted in the urine (Smith and Marston, 1970). In humans, the major excretory route is in the urine (McDowell, 1992).

Physiological Functions of Trace Minerals

Underwood (1981) stated that there are three basic functions of trace minerals: 1) they can be structural components of body organs and tissues, 2) minerals can be constituents of body fluids and tissues as electrolytes involved with acid-base balance, membrane permeability, and tissue irritability, and 3) they can also be catalysts in enzymes and hormone systems.

Copper:

Copper is an essential trace mineral that is required by the body in order for the body to function normally. Copper plays a role in activating a number of enzymes (Committee on Animal Nutrition et al., 1980; Dameron and Howe, 1998). Copper is also incorporated into a vast amount of enzymes and structural proteins. Copper is an essential component of these metalloenzymes (McDowell, 1992; Dameron and Howe,

1998; Baker and Ammerman, 1995). It is second only to Zn in the number of enzymes that it functions within (Committee on Animal Nutrition et al., 1980). Cytochrome C oxidase, lysyl oxidase, superoxide dismutase, dopamine β hydroxylase, and tyrosine are the major Cu dependent enzymes that have been extensively researched.

Due to the vast array of enzymes that Cu activates and is incorporated into, Cu has many functions throughout the body. Copper plays a role in iron (Fe) metabolism. Copper is important for Fe absorption and mobilization. Ceruloplasm, which contains Cu, is necessary for oxidation of Fe (McDowell, 1992). Ceruloplasm catalyzes the conversion of Fe^{2+} (ferrous form) to Fe^{3+} (ferric form), which is necessary for Fe to be transported as transferrin throughout the body (McDowell, 1992). Copper is necessary for hemoglobin synthesis, serving as a catalyst before the body can utilize Fe for hemoglobin formation (McDowell, 1992), and in erythropoiesis (Committee on Animal Nutrition et al., 1980; Baker and Ammerman, 1995; Dameron and Howe, 1998).

Copper has been shown to be essential in cellular respiration. Copper is a component of cytrochrome C oxidase (Allen and Solomons, 1984; Dameron and Howe, 1998; Linder et al., 1999). Cytochrome C oxidase is the terminal oxidase in cellular respiration chain (McDowell, 1992; Baker and Ammerman, 1995). It catalyzes the reduction of oxygen (O_2) to water (H_2O; McDowell, 1992).

Copper is also an important component in the cross-linking of connective tissues. Lysyl oxidase is a Cu containing enzyme that is a key component in the formation of cross linkages in collagen and elastin (Allen and Solomons, 1984; McDowell, 1992; Linder et al., 1999). Collagen and elastin are key components in connective tissue, blood vessels, and gives strength and stability to bones (Committee on Animal Nutrition et al.,

1980). Lysyl oxidase is also important in reproduction, bone development, and growth (Committee on Animal Nutrition et al., 1980; Dameron and Howe, 1998)

Pigmentation and keratinization of hair and wool are dependent on Cu. Copper is a component of tyrosinase, which is the enzyme that catalyzes the conversion of tyrosine to melanin (Committee on Animal Nutrition et al., 1980; Allen and Solomons, 1984; McDowell, 1992). Melanin is responsible for the pigmentation of hair and wool. Copper is also required for the formation and/or is incorporated into disulfide groups in keratin synthesis (McDowell, 1992). These disulfide groups provide cross linkages or bonding of keratin.

The central nervous system is dependent on Cu for proper function. Cytochrome C oxidase is the key enzyme in this system because this enzyme is essential in the myelination of nerve fibers. Cytochrome C oxidase is important in the production of phospholipids in the liver, which make up a large portion of myelin (Zimmerman et al., 1976; McDowell, 1992).

Copper also provides protection against oxidants. Impaired Fe metabolism can promote free radical generation (Committee on Animal Nutrition, 1980; Dameron and Howe, 1998). Copper causes the activation of a Fe containing heme enzyme catalase (Committee on Animal Nutrition et al., 1980). This catalase protects tissues from hydrogen peroxide (H_2O_2) and hydroperoxide damage (Golden and Ramdath, 1987). Ceruloplasm is believed to aid in antioxidant defense as well. Saenko et al. (1994) reported that ceruloplasm is believed to scavenge Fe radicals and other free radicals in the body that could cause tissue damage. Copper-zinc superoxide dismutase (Cu-Zn SOD) is another Cu containing enzyme that aids in antioxidant defense (Committee on Animal

Nutrition et al., 1980). This enzyme is responsible for ridding the body of superoxide radicals (O_2) that cause tissue degradation. Copper-Zn SOD will be discussed in more depth below.

There are many more functions that Cu has in the body. Copper plays a role in reproduction and lipid metabolism. It also is important for proper function of the immune system. Without Cu, the enzymes listed above would not be activated or produced and the systems that the enzymes are working within would not be able to function properly.

Zinc:

As with Cu, Zn has many functions. Zinc has structure and functional roles in biomembranes and plasma membranes (Hambidge et al., 1986). Bettger et al. (1978) reported that during a Zn deficiency oxidative damage occurs to membranes and results in structural strains within the membrane. Functions of membranes have also been shown to be altered during a deficiency. During a Zn deficiency, the function of specific receptors and nutrient absorption sites, the activity of membrane bound enzymes, the function of permeability channels, and the function of carrier and transport proteins within the membrane are altered.

There are over 200 enzymes and proteins that have been identified that require Zn for activity (McDowell, 1992). Zinc serves in both catalytic and structural capacities within enzymes, as well as regulatory roles (Hambidge et al. 1986; McDowell, 1992). Zinc was first identified to play a role in enzyme activity in 1940 when Keilen and Mann determined that Zn was a component of carbonic anhydrase (which converts carbon dioxide and water to bicarbonate). Carbonic anhydrase contains about 0.3% Zn

(McDowell, 1992). Vallee and Neurath (1954) determined that Zn was essential in the activity of carboxypeptidase A, which is required for protein digestion in the intestine. Alcohol dehydrogenase utilizes Zn for both catalytic and structural roles. Hambidge et al. (1986) reported that alcohol dehydrogenase contains 4 g atoms of Zn/mole; two of which are utilized for catalytic activity and the remaining 2 g atoms of Zn is utilized in the structure of this enzyme (Hambidge et al. 1986). Zinc is utilized to stabilize the quaternary structure of the enzyme superoxide dismutase (SOD), which will be discussed more in depth in a later section (Hambidge et al. 1986; McDowell, 1992). Other enzymes that require zinc are alkaline phosphatase, glutamate dehydrogenase (utilized in protein metabolism), malate dehydrogenase (utilized in the TCA cycle of metabolism), and lactate dehydrogenase (converts lactate into pyruvate). Also, some of the enzymes that require Zn include those involved in cell replication and differentiation (Hambidge et al., 1986), such as RNA sythetase, reverse transcriptase, nucleoside phosphorylase, and DNA polymerase.

Besides being important for enzyme activity in cell replication and differentiation and gene expression, Zn is a structural component of DNA. Within DNA, there is a tetrahedral coordination of Zn to cysteine and histidine residues. This coordination creates Zn finger domains in DNA binding proteins (Berg, 1990) and influences transcription and cell replication (Chesters, 1992). Chesters (1992) reported that the involvement of Zn in gene expression may be how Zn helps to regulate digestion, glycolysis, DNA synthesis, nucleic acid synthesis, and protein metabolism and not from a direct effect on these pathways.

Zinc is thought to affect glucose and lipid metabolism. Scott (1934) stated that there are considerable quantities of Zn in crystalline insulin. Zinc may affect the solubility of insulin and may play a role in insulin release (Hambidge et al., 1986). If Zn concentrations are low, it is possible that insulin concentrations will be low as well, therefore, decreasing glucose uptake. A decrease in glucose utilization has been shown to increase lipid catabolism in Zn deficient animals (Hambidge et al. 1986). Lipids will be oxidized to make energy that is lost from the lack of glucose utilization. Zinc has also been shown to be important in the elongation-desturation pathway in essential fatty acid metabolism (Chvapil et al, 1975).

Manganese:

Underwood and Suttle (1999) stated that the functions of Mn are linked to metalloenzymes that are activated by the element. Manganese will bind to a substrate, such as ATP, or to the protein directly, and cause conformational change that leads to the activation of the enzyme (Hurley and Keen, 1986). Manganese serves as a constituent of metalloproteins (Hurley and Keen 1986; McDowell, 1992). Manganese also serves as an integral part of certain enzymes. The list includes arginase, pyruvate carboxylase, and Mn superoxide dismutase (Mn SOD; Underwood and Suttle, 1999). Arginase contains 4 moles of Mn^{2+} per mole of enzyme and is important in the formation of urea (Hirsch-Kolb et al., 1971). Pyruvate carboxylase – contains 4 moles Mn(II) per mole enzyme (Hurley and Keen, 1986). Pyruvate carboxylase is important in converting pyruvate into oxaloacetate in the TCA cycle (Murray et al., 2000). Manganese SOD is composed of two subunits of equal size and contains one Mn^{2+} per molecule of the enzyme (Keele et al., 1970). Superoxide dismutase is a Mn-containing enzyme that protects cells from

damage by the free oxygen radical superoxide (O_2^-; Underwood and Suttle, 1999) which is produced during normal cellular functions by converting it to H_2O_2 and O_2 (Gregory and Fridovich1974). Within the mitochondria, 60 % of cellular oxygen consumption occurs (Underwood and Suttle, 1999). Leach and Harris (1997) indicated that this makes the mitochondria vulnerable to free radical damage. Manganese SOD is necessary within the mitochondria to aid in hindering this free radical damage.

Another function of Mn is in bone growth and cartilage development (McDowell, 1992). Manganese is needed for glycosyl transferase activity for synthesis of mucopolysaccharides, which are vital structural components of cartilage (Leach 1971; Leach, 1988). Mucopolysaccharides are essential for the development of the organic matrix of bone (Leach 1971). Without Mn, bone formation and/or growth may be stunted. Glycosyl transferases, and therefore Mn, are also important in blood clotting. Underwood and Suttle (1999) stated that glycosyl transferase activation is important in the formation of prothrombin. Prothrombin will then be converted to thrombin that in turn converts fibrinogen to fibrin (Murray et al., 2000). Fibrin is essential the formation of blood cells.

Cobalt:

Cobalt functions as a component of vitamin B_{12}, or cobalamin, which is the only known function of Co (McDowell, 1992). Cobalamin is utilized by a number of enzyme systems (McDowell, 1992). Cyanocobalamin is converted in cells to two active forms of cobalamin: methylcobalamin (a coenzyme for methyltransferase) or adenosylcobalamin (the coenzyme for mutase) (McDowell, 1992). Adenosylcobalamin is important in energy metabolism by facilitation of the formation of glucose by assisting the conversion

of propionate to succinate via methylmalonyl-coenzyme A (CoA) mutase (Underwood and Suttle, 1999). Ruminal microorganisms are also dependent on this mutase in the rumen for the same reaction (Babior, 1975).

The second active form of cobalamin is methylcobalamin (McDowell, 1992). Methylcobalamin is important in the transfer of methyl groups from one molecule to another. Methylcobalamin assists methyltransferase enzymes by donating methyl groups (Underwood and Suttle, 1999). Methylcobalamin is also important in one carbon metabolism, or the building of carbon chains (Underwood and Suttle, 1999). Microbes also need methylcobalamin for the production of methane, acetate, and methionine in the rumen (Poston and Stadman, 1975)

McDowell (1989) stated that there are four functions of cobalamin enzymes, two of which are mentioned above (transfer of methyl groups and adenosylcobalamin as a coenzyme for mutase). Cobalamin enzymes are also involved in purine and pyrimadine synthesis as well as the formation of proteins from amino groups (McDowell, 1989). Cobalamin has also been reported to be important for red blood cell synthesis and nervous system integrity (McDowell, 1992).

Immune System and Trace Mineral Relationship

Immunity is a reaction by the body to a foreign substance, such as microbes and/or a virus (Abbas et al., 1991). Immunity is divided into two categories: innate and acquired immunity. Innate immunity is the basic resistance to disease, which includes both the physical and chemical barriers to disease (Abbas et al., 1991). Kuby (1994) stated that innate immunity is composed of four barriers: 1) anatomic (skin), 2) physiologic (temperature or pH), 3) phagocytic (ingestion of macromolecules by

macrophages and neutrophils), and 4) inflammatory (vasodilation and capillary permeability). Acquired immunity is stimulated by exposure to an antigen through vaccination or natural exposure (Abbas et al., 1991; Kuby, 1994). Acquired immunity can be divided into two categories: humoral and cell-mediated immunity. Humoral immune response is mediated by B-cells that respond to antigens to become antibody producing cells and memory cells (Galyean et al., 1999). B-cells and antibodies provide defense against extracellular infections (Galyean et al., 1999) via antibodies binding to the antigen outside of the cell, neutralizing them and causing the antigens' elimination by other immune cells (Kuby, 1994). Cell-mediated immune response is mediated by T-cells and their associated cytokines (Galyean et al., 1999). Galyean et al. (1999) reported that cell-mediated immunity provides a defense against intracellular pathogens and tumor cells.

According to Kuby (1994), an immune response occurs as follows. Immune cells, such as macrophages, roam the body and engulf (sample) cells and/or proteins. They sample both self (body) and non-self (foreign bodies) cells because macrophage are unable to tell the difference between self and non-self. The macrophage will engulf cells and break the cells apart. Pieces of the cells are presented on the outside of the macrophage. T-cells will attach to the presented piece of the cell. T-cells have the ability to distinguish between self and non-self structures. If the presented cell is a self-cell, the T-cell will detach and move on to the next macrophage. If the presented cell is an antigen, the T-cell will bind strongly to the antigen and the macrophage and cause a cascade of events to occur. Cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor (TNF), will be released from the T-cell and macrophage upon recognition of a

foreign pathogen presented by a macrophage. Cytokines activate T-cells and B-cells to proliferate or divide into more T-cells and B-cells and also into memory T-cells and B-cells. The activated T-cells bind to more antigen presented by the macrophage and cause more cytokines to be released. B-cells will produce and release antibodies that will bind to the free antigens in the infected area. When bound, the antibodies mark the antigen for destruction by the complement system. Macrophage will bind to the antibodies on the antigen, which allows the macrophage to engulf the antigen more efficiently. Cytokines also "recruit" other macrophage and killer T-cells, whose purpose is to destroy infected body cells that cannot be saved once infected with an antigen. This proliferation and recruitment of more immune cells increases the immune response. When the antigen is eradicated from the body, suppressor T-cells will come into the previously infected area and decrease the immune response. If the same antigen enters the body again, the memory T-cells and B-cells will be activated to rid the body of the antigen. Memory T-cells and B-cells will cause the secondary immune response to be faster at responding to an antigen that has previously infected the host animal (Kuby, 1994).

Research on trace minerals (TM) effects on the immune system has shown inconsistent results. Results seem to vary between species, breed, environment, and the segment of the immune system being studied. Discussed below are a few aspects of the immune system and there relationship with TM.

Antibodies (Immunoglobulin G (IgG) and Immunoglobulin M (IgM)): Immunoglobulin are antibodies that aid in defense against foreign proteins, or antigens that enter the body. Antibodies are produced by B-cells when B-cells are activated by cytokines. Kuby (1994) stated that antibodies, once secreted, will circulate in the blood

and search for antigen. Immunoglobulins will attach themselves to the antigen or the infected cell which in turn marks the antigen for destruction (Kuby, 1994). This is part of the humoral immune response.

There are various isotypes of antibodies that have different functions. Two of these isotypes are IgG and IgM. Immunoglobulin G is the most abundant isotype in serum (Kuby, 1994). Immunoglobulin G constitutes 80 % of the total serum immunobulins (Kuby, 1994). There are four subclasses of IgG that play roles in protecting the developing fetus, activating complement, and binding to antigen and phagocytic cells to initiate phagocytosis (Kuby, 1994). Immunoglobulin M makes up 5 to 10 % of total serum immunoglobulin (Kuby, 1994). Immunoglobulin M is always found in the serum in a pentameter form (five IgM antibodies bound together). During a primary immune response, IgM is the first antibody produced and seems to be more efficient than IgG at binding to antigen. It takes a smaller amount (100 to 1000 times less) of IgM molecules to perform the same tasks as IgG molecules (Kuby, 1994). Immunoglobulin M has similar roles as IgG; however, because of its larger size, it does not diffuse well in the blood stream, which results in lower concentrations in the body.

There have been minimal studies that have examined TM effects on total IgM and IgG as an indicator of overall immune response. Stabel et al. (1993) conducted a study using fourteen Holstein steers. Steers received a milk replacer diet containing a mineral mix then were fed a starter diet containing cottonseed hulls, cornstarch, and urea. The basal diet contained 1.5 mg of Cu/kg DM. Steers were then placed on one of two treatments: 1) no supplemental Cu or 2) 10 mg of Cu/kg DM from $CuSO_4$. They reported

that total IgM and IgG were not affected by Cu status in Cu-adequate and Cu-deficient Holstein calves (Stable et al., 1993).

Ward et al. (1997) examined IgM and IgG in cattle. They utilized forty, 2-year-old Angus, Simmental, and Charolais heifers that were depleted of Cu for 17 d using 20 mg Mo/kg DM. The basal diet was a corn silage based diets that met or exceeded the NRC recommendations for all TM for pregnant heifers, with the exception of Cu. The basal diets contained the following Cu concentrations: control and gestation diets contained 4 mg Cu/kg DM and the lactation diet contained 4.5 mg Cu/kg DM. Calves were bled 7 d after birth. They found that treatment had no effect on total IgG concentration in the serum of calves. However, 17 d may not be adequate in depletion of Cu. It may take a longer period of time to deplete the body of Cu to the point of having an effect on the immune system.

In contrast to the above mentioned studies, Huerta et al. (2002) reported a TM source effect on IgG concentrations in heifers fed a barley-corn-pea hay based basal diet (64 mg Zn/kg DM) supplemented with 200 mg Zn/kg DM from either $ZnSO_4$ or Zn methionine. They reported that heifers supplemented with inorganic Zn tended to have a lower ($P < 0.10$) concentration of IgG than steers supplemented with organic Zn. Heifers supplemented with Zn had similar IgG concentrations to control heifers.

Superoxide Dismutase (SOD): When phagocytic cells ingest and breakdown antigens or other cells, superoxide radicals (anions) are produced (Stabel and Spears, 1990). Superoxide anions (O_2^-) can cause oxidative damage to cellular membranes (Stabel and Spears, 1990). These anions break down tissues in the body. A Cu

containing enzyme, Cu-Zn SOD, is important in inhibiting the harmful effects of O_2^-. Superoxide dismutase is a key enzyme in the following reaction:

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2 \rightarrow H_2O_2 + 2GSH \xrightarrow{GSH\text{-}PX} GSSG + 2H_2O$$

Superoxide dismutase converts O_2^- to hydrogen peroxide (H_2O_2) and oxygen (O_2). Glutathione peroxidase (GSH-PX) then reduces H_2O_2 to water (H_2O) decreasing the harmful effects of the O_2^- (Murray et al., 2000). Superoxide dismutase contains 2 atoms of Cu and 2 atoms of Zn, and Cu contained within the enzyme is important for catalytic activity (Paytner, 1987). When Cu is sufficient in the diet, SOD activity seems to be unaffected. In an experiment by Ward et al. (1993; diet and treatments mentioned above), blood samples were taken from 72 of 126 Angus and Angus x Hereford steers on d 21, 42, 63, and 84 of the study. Samples were centrifuged and the red blood cells (RBC) were lysed. The lysed RBC were analyzed for SOD activity using a procedure described by Jones and Suttle (1981). No differences were reported among treatments regarding SOD activity when supplementing $CuSO_4$ or CuLys verses no supplemental Cu. Torre et al. (1995) conducted a study using 10 primigravid Holstein heifers that were approximately 2-years of age. The heifers received no supplemental Cu from 16 to 20 weeks of age. When the heifers reached approximately 84 d prior to parturition, they were randomly assigned to receive the basal diet or the basal diet supplemented with 20 mg Cu/kg DM from $CuSO_4$. The basal diet was composed of alfalfa silage, corn silage, and cottonseed and contained 6 to 7 mg Cu/kg DM. Superoxide dismutase activity was measured in lysed whole blood. The results showed that there were no differences in SOD activity between treatments.

In the above studies, RBC SOD was unaffected and Cu deficiency was not present. Ward et al. (1993) stated that they believed that if they had continued their experiment for more that 98 d, SOD activity may have been decreased in steers supplemented with Mo and S because Cu deficiency may have occurred.

It is believed that RBC SOD activity is only affected during a severe, prolonged Cu deficiency (Paytner, 1987). Copper deficiency is reached when liver Cu concentration are less than 20 mg Cu/kg DM and when plasma Cu concentrations are less than 0.6 mg Cu/L (Mills, 1987). A severe, prolonged deficiency would be when liver and plasma Cu concentrations become less than these levels for a prolonged periods of time. Paynter (1987) reported that SOD in RBC is one of the last enzymes that is affected by Cu deficiency. He stated that RBC SOD activity has a longer half-life than other enzymes, such as plasma ceruloplasmin. RBC have a longer half-life, which could explain why RBC SOD is affected when Cu deficiency is more severe (Paytner, 1987). Paytner (1987) also reported that RBC SOD activity is closely related with liver Cu status.

Superoxide dismutase activity differences have been reported in experiments where Cu deficiency is observed. Andrewartha and Caple (1980) conducted a studying using 7 nine-month old Corriedale rams. Four rams were fed a commercial sheep diet containing 10 to 12 mg Cu/kg DM supplemented with 20 mg Mo per day from sodium molybdate and 6 g sulfate/d from ammonium sulphate. The remaining 3 rams were fed only the commercial sheep diet. Blood samples were taken prior to initiation of the depletion diet and 90 d post supplementation. The RBC were lysed and analyzed for SOD activity using a method described by Beauchamp and Fridovich (1971). Rams in

this study that were depleted of Cu by adding molybdenum and sulfur to the diet had RBC SOD activities that were 40 % lower than the control rams (Andrewartha and Caple, 1980). In a separate study using deficient lambs that grazed a pasture known to be deficient in Cu, SOD activity increased when lambs were fed the commercial sheep diet containing 10 to 12 mg Cu/kg DM (Andrewartha and Caple, 1980). Jones and Suttle (1981) utilized eight Scottish Blackface ewes that were fed a Cu deficient diet and six Scottish Blackface cross Border Leicester ewes that were fed a complete diet containing an adequate amount of Cu (controls). Blood samples for SOD activity were taken and the RBC were lysed and analyzed using optical density readings. Sheep had plasma Cu concentrations that were less than 0.5 mg/L, which is considered deficient according to the levels mentioned above (< 0.6 mg/L; Mills, 1987). The deficient sheep had lower SOD activity than control sheep (Jones and Suttle, 1981). In another study by Xin et al. (1991), twelve Holstein steers were supplemented with 20 mg Cu/kg DM from $CuSO_4$ or 10 mg Mo/kg DM from ammonium molybdate to a basal diet containing alfalfa haylage and concentrate for the first 5 months or fescue hay and concentrate for the last 3 months that contained 18 to 30 mg Cu/kg DM. Blood samples were taken prior to the initiation of the experimental diets and once a month post initiation of the experimental diets through the end of the trial. Red blood cells were lysed and analyzed using a modified technique described by Jones and Suttle (1981). Deficient steers had plasma Cu concentrations less than 0.8 mg/L and liver Cu concentrations less than 19 mg/kg, which are considered deficient or almost deficient as mentioned above (< 0.6 mg/L and < 20 mg/kg; Mills, 1987). Superoxide dismutase activity was lower ($P < 0.01$) in Cu deficient steers than Cu supplemented cattle (Xin et al, 1991).

According to above mentioned studies, it appears that an animal needs to be Cu deficient in order for a reduction in SOD activity to be observed. Without this severe deficiency, SOD activity would remain within normal limits.

Primary and Secondary Humoral Immune Response to Pig red blood cells (PRBC) and ovalbumin: Kuby (1994) defines humoral immunity as the host's defenses that are mediated by antibody present in the plasma, lymph, and tissue fluids. B-cells interact with antigen present in the body. The B-cells will proliferate and differentiate into antibody secreting plasma cells and memory B-cells (Kuby, 1994). These plasma cells will produce and secrete antibodies specific to that antigen. The antibodies will then attach themselves to the antigen, which labels the antigen as foreign. Macrophage and other immune cells will then recognize the antigen as foreign and will phagocytose the antigen and destroy it (Kuby, 1994).

A primary immune response is when the body is first introduced to an antigen (Kuby, 1994). The body has to produce T-cells, B-cells, antibodies, and other immune cells to respond to a specific antigen. Memory T-cells and B-cells are also produced and remain dormant until the same antigen re-infects the body. When the same antigen reenters the body, a secondary immune response occurs where the response to the antigen will be rapid and heightened because memory immune cells are already present in the body to combat the antigen and do not have to be produced (Kuby, 1994). During a primary immune response, IgM is the first immunoglobulin that is produced to combat an antigen and less IgM is needed to neutralize a virus than IgG (Kuby, 1994). During a secondary immune response, IgG is released first (Kuby, 1994). The antibodies produced during a secondary immune response have a higher affinity for the antigen and antibody

levels are often 100 to 1000 times higher than in the primary immune response (Kuby, 1994).

Antigens are often injected into animals in order to elicit an immune response to observe how humoral immunity is affected by nutrients such as minerals or protein. Pig red blood cells (PRBC) are often utilized as an antigen in cattle. When injected, the body will immunologically respond to the foreign antigen. Pig red blood cells are often diluted in phosphate buffered saline (PBS) in varying concentrations (examples: 20 % and 25 % PRBC). The solution is injected into the neck muscle of the animal and blood samples are usually taken prior to injection and 7, 14, and 21 d post-injection. The serum is then analyzed for antibody titers specific for PRBC. This technique is often used to investigate the humoral immune response.

Results from studies where PRBC were injected have shown varying outcomes. In 1997, Ward et al. conducted a study utilizing calves whose mothers (40, 2-year old Angus, Simmental, and Charolais heifers) were supplemented with 600 mg Fe/kg DM from $FeCO_3$, 5 mg Mo/kg DM from $NaMoO_4$, 10 mg Cu/kg DM from $CuSO_4$, or had no supplemental minerals added to a basal diet containing 4 mg Cu/kg DM during gestation and 4.5 mg Cu/kg DM during lactation. The calves where injected with 5 mL of a 20 % PRBC solution on d 169 of the study when the calves were approximately 70 d old (primary immune response) and then again on d 246 when the calves were approximately 147 d old (secondary immune response). Blood samples were taken prior to injection and 7, 14, 21, and 28 d post injection. The serum was then analyzed for total antibody titers specific for PRBC using a procedure described by Droke et al. (1993). There was no treatment effect observed for a primary immune response; however, calves supplemented

with Cu had less antibody titers than control steers 14 d ($P < 0.07$) and 21 d ($P < 0.09$) post-secondary injection (Ward et al., 1997). In another study, Ward and Spears (1999) supplemented 42 Angus bull calves with 0 or 5 mg Cu/kg DM from $CuSO_4$, or 0 or 5 mg Mo/kg DM from Na_2MoO_4 to a diet containing 5.2 mg Cu/kg DM. Calves were injected with 10 mL of 25 % PRBC solution on d 133 (primary immune response). Blood samples were collected prior to injection and 7, 14, 21,and 28 d post injection. Calves that were supplemented with Cu had less ($P < 0.07$) antibody titers to PBRC than control steers (Ward and Spears, 1999).

Droke et al. (1989) found no effect of TM supplementation or TM source (25 mg Zn/kg DM from Zn oxide or Zn methionine) on antibody titer concentrations to PRBC in lambs fed a basal diet containing 27.6 mg Zn/kg DM. Droke et al. (1993) conducted another study in lambs fed a cottonseed hulls basal diet that was severely deficient in Zn (3.7 mg Zn/kg DM). The lambs received the basal diet alone or were supplemented with 5 mg Zn/kg DM from Zn oxide (marginal concentrations of Zn) or 40 mg Zn/kg DM from Zn oxide. Droke et al. (1993) reported no treatment differences in concentrations of antibody titers to PRBC in the lambs. In two different studies, there was also no effect of TM supplementation on antibody titer concentrations to PRBC in steers supplemented with inorganic or organic TM (Engle et al., 1999; Engle et al., 1999b). Contrasting results were reported in a study by Dorton et al. (2003) where steers that were supplemented with organic TM had lower antibody titer concentrations to PRBC than steers that were supplemented with inorganic TM.

Another antigen that is injected into animals to elicit a humoral immune response is ovalbumin (OVA). Ovalbumin is a phosphorylated-glycoprotein that is the major

protein constituent of egg whites. Ovalbumin is used as a carrier protein to conjugate to synthetic peptides for use as an immunogen. Ovalbumin is not very antigenic antigen when it is presented to the body alone. Ovalbumin is often combined into a solution containing PBS and Freund's incomplete adjuvant (FIA). Freund's Incomplete Adjuvant is a water-in-oil emulsion made from non-metabolizable oils (paraffin oil and mannide monooleate). Freund's incomplete adjuvant enhances the immune response when an antigen has low immunogenicity, prolongs antigen persistence from days to weeks, and induces granuloma formation that may be responsible for facilitating antigen processing and presentation and T helper cell activation (Kuby 1994). In short, it causes a heightened and prolonged immune response.

Studies where OVA was injected into animals to determine treatment differences in humoral immunity have also shown varying results. In the study mentioned above by Ward and Spears (1999), four milliliters of solution containing 4 mg of OVA in PBS and FIA was injected subcutaneously into the calves on d 133. Blood samples were taken prior to injection and 7, 14, 21, and 28 d post injection. Serum was analyzed for antibody titer concentrations specific to OVA using a modified procedure of Droke and Loerch (1989). Ward and Spears (1999) reported that immune response to OVA was greater in Angus bull calves supplemented with Cu than in control steers 7, 14, 21, and 28 d post injection ($P < 0.06$). Ward et al. (1993) reported opposite results when conducting a study using 126 Angus and Angus x Hereford crossbred steers. The steers were assigned to one of 6 dietary treatments (control diet containing no supplemental minerals, 5 mg Mo/kg DM plus 0.2% S from MoS, 5 mg Cu/kg DM from $CuSO_4$, 5 mg Cu/kg DM from $CuSO_4$ plus 5 mg Mo and 0.2% S from MoS, 5 mg Cu/kg DM from CuLys, or 5 mg

Cu/kg DM from CuLys plus 5 mg Mo and 0.2% S from MoS) to a basal diet containing 6.2 mg Cu/kg DM. Four milliliters of a solution containing 4 mg OVA in PBS and FIA was injected subcutaneously on d 7 and 77 of the study. Different animals per pen were injected on each day. Blood samples were taken prior to injection and 7, 14, and 21 d post injection. Serum samples were analyzed for antibody titers specific to OVA using an Elisa procedure described by Droke and Loerch (1989). They reported that treatment had no effect on antibody titer production specific for OVA on either day. Contrasting results were reported in a study by Dorton et al. (2003) where steers that were supplemented with organic TM had greater antibody titer concentrations to ovalbumin antigen and lower antibody titer concentrations to PRBC than steers that were supplemented with inorganic TM.

In some of the above mentioned studies, TM had no effect on antibody titer concentrations specific to PRBC and OVA. It is unknown why there was no effect. It may be because an animal may need to be deficient or have excess amounts of TM before an immunological effect is observed.

Trace Mineral Deficiency and Toxicity

Trace mineral deficiency and toxicity can result from many factors (i.e. mineral antagonist, low/high concentrations of TM in forage or feed, bioavailability of TM in forage or feed). The effects of these factors can depend on the species and breed of the animal. For example, sheep are more susceptible to Cu toxicity than cattle and pigs because they cannot excrete Cu in bile as efficiently as the two other species (Dameron and Howe, 1998). Deficiencies are more widespread and occur naturally in grazing ruminants (Underwood, 1981; McDowell, 1992). This may be caused by poor uptake of

TM by plants (McDowell, 1992), which in turn can be caused by a lack of TM in the soil or other antagonistic minerals that will bind and prevent absorption of certain trace minerals by the plant (Underwood, 1981).

Another factor that can cause deficiencies is the chemical form of TM. Different forms of TM are more available to the animal. For example, Cu carbonate ($CuCO_3$) is more available than Cu sulfate ($CuSO_4$) and $CuSO_4$ is more available that Cu oxide (CuO) (Allen and Solomons, 1984). If CuO were the only Cu source available to swine, they may become Cu deficient because CuO is poorly available to swine (Cromwell et al., 1989).

The availability of TM sources to the animal may also be affected by interactions with other TM or with other substances. Certain TM can have antagonistic effects on other TM if they are not fed in proper ratios. Two antagonistic minerals to Cu are molybdenum (Mo) and sulfur (S). These minerals by themselves if fed in high amounts can cause a Cu deficiency, but high S fed along with high Mo can exaggerate the interaction between Mo and Cu (McDowell, 1992). Ruminants are more susceptible to this interaction than non-ruminants because of the reducing rumen environment (McDowell, 1992). When sulfate enters the rumen, the microorganisms convert the sulfate to sulfide (Dick et al., 1975; Suttle, 1975; Allen and Solomons, 1984; McDowell, 1992). The sulfide will bind to Mo and form a thiomolybdate (Allen and Gawthorne, 1987, McDowell, 1992; Baker and Ammerman, 1995), which then binds to Cu. Tetrathiomolybdates (MoS_4^{2-}) are excreted from the body because they cannot be absorbed, while mono-, di-, and trithiomolybdates are absorbed into the blood stream and bind to albumin. Thiomolybdates then become tightly bound to albumin in the plasma

where they can bind Cu and prevent Cu from being released from albumin (Dick et al., 1975; McDowell, 1992; Baker and Ammerman, 1995). Copper deficiency results because the Cu within thiomolybdates is highly insoluble and non-utilizable (McDowell, 1992; Dameron and Howe, 1998) and cannot be used by body tissues (McDowell, 1992).

Cu deficiency can also result when other antagonistic minerals, such as Fe and Zn (Allen and Solomons, 1984), cadmium (Hill and Starcher, 1965), silver (Hill et al. 1964), and lead (Klauder and Petering, 1977), are present in high concentrations within the diet. Also, high ascorbic acid (Allen and Solomons, 1984), high fiber (Allen and Solomons, 1984; Dameron and Howe, 1998), and high protein (McCall and David, 1961; Reinhold et al., 1967) in the diet can cause TM deficiencies.

Copper:

Since Cu is incorporated into many enzymes in the body that result in Cu being important in many physiological functions, problems can occur in the body when Cu is deficient. Anemia is one of these problems (Underwood, 1981; McDowell, 1992; Baker and Ammerman, 1995). There is an impairment of Fe mobilization (Baker and Ammerman, 1995) because ceruloplasm production is decreased in the liver (Owen, 1965; Harris and Disilvestro, 1981). Plasma Cu levels can be as low as 0.1 to 0.2 mg/ml.

Bone disorders can result from Cu deficiency (Underwood, 1981; McDowell, 1992; Dameron and Howe, 1998). Decreased Cu has been shown to cause bone disorders in cattle (Mills et al, 1976) and rats (Alfaro and Heaton, 1973) as well as in other species, although the occurrence of bone disorders is low in cattle (Underwood, 1981). Collagen and lysyl oxidase (Cu dependent enzymes) are both important in providing strength and stability for bone (Committee on Animal Nutrition et al., 1980; Underwood, 1981). Bone

disorders can occur because of a decrease in lysyl oxidase and collagen production (each contain Cu; Committee on Animal Nutrition et al., 1980; Underwood, 1981). During Cu deficiency, collagen fails to undergo cross-linking and maturation (Harris and Odell, 1974). Lysyl oxidase is important in the conversion of lysine to desmosine, which is important in forming this cross-linking (Committee on Animal Nutrition et al., 1980; Underwood, 1981). Without crosslinking, the stability and strength of collagen is decreased which causes a decrease in bone strength (Committee of Animal Nutrition et al., 1980). Lameness can result from lack of cross-linking during Cu deficiency. Copper deficient cattle have been seen to have swollen fetlocks/pasterns (Becker et al., 1965; Underwood, 1981).

Cardiovascular disorders can occur during Cu deficiency. Copper is important for elastin synthesis (Underwood, 1981). Elastin allows the veins and arteries to expand and contract without tearing. Lysyl oxidase is involved in the conversion of lysine to desmosine, which is needed for elastin to undergo cross-linking (Committee on Animal Nutrition et al., 1980; Underwood, 1981). This allows arteries and veins to be elastic. Problems can occur if the arteries cannot expand and contract. Because of this reduced elasticity (Committee on Animal Nutrition et al, 1980), aortic ruptures have occurred during a severe Cu deficiency in pigs (Underwood, 1981; Dameron and Howe, 1998).

Falling disease is a result of Cu deficiency in cattle (Bennett and Hall, 1939; Underwood,1981; McDowell, 1992; Dameron and Howe, 1998) when cattle have plasma Cu concentrations as low as 0.01 to 0.02 mg/dl and liver Cu concentrations around 2 mg Cu/kg DM (Underwood, 1981). Falling disease occurs when cattle die suddenly because of an acute heart failure (McDowell, 1992). Small lesions that form on the heart (Mills et

al., 1976; Underwood, 1981; McDowell, 1992) cause a slow but progressive degeneration of the myocardium with the replacement rigid fiber compounds (Underwood, 1977). Cardiovascular lesions have also been seen in mice (Rowe et al., 1977), rats (Petering et al., 1986), rabbits (Hunt and Carlton, 1965), and pigs (Ganezer et al., 1976).

Copper deficiency has been shown to cause achromotrichia or discoloration of hair and wool (Underwood, 1981; Blakely and Hamilton, 1985; McDowell, 1992) because of a decrease in tyrosinase (McDowell, 1992). Tyrosinase is important in converting tyrosine to melanin. Sheep are very sensitive to Cu deficiency, which results in bands of varying colors being produced easily in wool (Underwood, 1981). Underwood (1981) stated that in black wool the synthesis of the black color is inhibited after only two days of deficiency. Keratinization of hair and wool is also affected by Cu deficiency as well (Underwood, 1981; Blakely and Hamiliton, 1985; McDowell, 1992). Problems occur because Cu is important for the formation or for incorporation into disulfide groups that provide cross-linkages or bonding of keratin (McDowell, 1992). There is a decrease in growth and appearance of wool (Suttle and Angus, 1976). Tensile strength and elastin production are decreased because of a decrease in lysyl oxidase, a Cu-dependent enzyme (Underwood, 1981). The result is a thin, harsh coat appearance (Dameron and Howe, 1998). Wool becomes straight, steely, stringy, and has no crimp (Underwood, 1981).

Copper deficiency can cause swayback or neonatal ataxia in lambs (Bennett and Chapman, 1937; Committee on Animal Nutrition, 1980; Underwood, 1981; McDowell, 1992). Swayback results in spastic paralysis, an incoordination of the hind legs where the animal walks with a stiff gait (Underwood, 1981; McDowell, 1992). They also exhibit an

exaggerated swaying of the hind quarters (NRC, 1985). This central nervous system (CNS) disorder is believed to be linked to a maternal Cu status deficiency during the development of the CNS (Underwood, 1981; McDowell, 1992). Some lambs are paralyzed at birth and die immediately, while other lambs are born weak, are unable to nurse, and eventually die (McDowell, 1992). This disease is believed to be caused by incomplete myelin formation or degeneration of the myelin sheath of nerve fibers (myelin aplasia; Underwood, 1981; McDowell, 1992). It is believed that not enough energy is being produced to make phospholipids because cytochrome C oxidase (a Cu dependent enzyme important in energy production) is deficient in the brain (Howell and Davison, 1959) and spinal cord (Barlow, 1963). Myelin aplasia will result from a lack of myelin (Mills and Williams, 1962) and will also result in degeneration of motor neurons by the brain and spinal cord (Underwood, 1981).

Copper deficiency has been shown to cause reproductive problems (Underwood, 1981; McDowell, 1992). Estrus can be delayed or depressed in animals (McDowell, 1992). Calving difficulties and retained placentas have been shown to occur during deficiency (McDowell, 1992). The fetus can also be born dead or calves can be born with congenital rickets because the mother was Cu deficient during the calf's development (McDowell, 1992).

The immune system has also been shown to be affected by Cu deficiency. During a Cu deficiency, T-cell, B-cell, neutrophils, and macrophage production is decreased (McDowell, 1992). In mice, antibody producing cells have been shown to be decreased during a severe Cu deficiency (Prohaska et al., 1983). Sheep tend to have a decreased resistance to infection (Woolliams et al., 1986).

Copper toxicity occurs when tissue levels of Cu are elevated. Ruminants are most sensitive to Cu toxicity (McDowell, 1992). In cattle, a concentration of 100 mg Cu/kg DM in the diet is the tolerance level with concentrations between 100 and 400 mg Cu/kg DM being toxic (McDowell, 1992). Sheep are more susceptible to Cu toxicity than cattle (Underwood, 1981). Toxicity concentrations in sheep are approximately 40 to 50 mg Cu/kg in the diet (NRC, 1985). In sheep, excretion of Cu in bile is minimal and may be why sheep can only tolerate low levels of Cu in the diet before toxicity occurs (Dameron and Howe, 1998).

Copper poisoning can result because other minerals (antagonists) that can bind Cu, such as Mo, are low in the diet (Dick and Bull, 1945). There are many symptoms of Cu toxicity. They can either be acute or chronic symptoms. Animals can suffer nausea and vomiting (Allen and Solomons, 1984; McDowell, 1992). McDowell (1992) stated that other symptoms of Cu poisoning are increased salivation, abdominal pain, convulsions, and collapse. Anemia, muscular dystrophy, decreased growth, and impaired reproduction can result due to Cu because of toxicity (NRC, 1980), as well as jaundice, hepatic necrosis, and intravascular hemolysis which can be fatal (Allen and Solomons, 1984). If liver Cu concentrations reach 1000 mg Cu/kg DM, Cu may be released into the blood and increase blood values 10 times that of normal values, which results in hemolytic crisis and ultimately death (Underwood, 1981).

Cu toxicity can be prevented by providing proper ratios of minerals need to be maintained in the diet. For example, if Cu is increased significantly in the diet without increasing Zn, toxicity could result in the animal because there are fewer substances available to bind excess Cu. Zinc can be used to protect against Cu toxicity. Zinc

decreases storage of Cu in the liver (Pope, 1971), but this is seen mostly in monogastrics (McDowell, 1992). Metallothionine, a zinc-containing molecule, can also help prevent Cu toxicity. Metallothionine has a higher affinity for Cu than for Zn, but the synthesis of the metallothionine protein in the intestinal cell is induced by high dietary Zn concentrations. Copper can displace Zn on metallothionine and bind up to 12 Cu molecules (Dameron and Howe, 1998) decreasing the likelihood of toxicity.

Zinc:

Blackmon et al. (1960) stated that there are twelve clinical signs of Zn deficiency. A few of these include a rough hair coat, cracks of skin of coronary bands around hooves that may become deep fissures as Zn deficiency intensifies, dry scaly skin on ears, bowing of hind legs, and loss of hair or wool. Zn deficient animals have a loss of appetite, which is seen in all species (Underwood and Suttle, 1999).

Zinc deficiency has been shown to lead to a decrease in the activity of certain enzymes. A decrease in enzymes that utilize Zn leads to a decline in nucleic acid metabolism and protein synthesis (McDowell, 1992). Protein, DNA, and RNA concentrations will decline leading to a decline of cellular division, growth, and repair (McDowell, 1992). Im et al. (1975) stated that six enzymes involved in glycolysis were also decreased up to 30 to 50 % during a Zn deficiency in rats, signifying that carbohydrate metabolism is affected by Zn deficiency as well.

Zinc deficiency causes many abnormalities in the skin. One skin abnormality is parakeratosis. Parakeratosis is seen in all species resulting from a severe Zn deficiency (Underwood and Suttle, 1999). With parakeratosis, there will be a thickening and hardening of the skin and fissures or cracks will form (McDowell, 1992; Underwood and

Suttle, 1999). McDowell (1992) stated that this is a result of failure of complete nuclear degeneration of the cells in this area. In calves, the most affected areas are the muzzle, neck, ears, scrotum and back of hind limbs (Underwood and Suttle, 1999). In lambs, there will be open lesions around the eyes, above the hooves, and on the scrotum (Ott et al., 1966).

When animals are Zn deficient, wound healing is slowed. This may be the result of impaired cellular replication and differentiation that occurs during tissue repair, as well as impaired tensile strength of the collagen matrix (Sandstead et al, 1967). Zinc deficiency results in a loss of the normal ring structure in hooves of lambs (Underwood and Suttle, 1999). Horns often shed leaving "a soft spongy outgrowth that often hemorrhages" (Mills et al., 1976). Also, wool will loose its crimp, then become loose and eventually fall out (McDowell, 1992; Underwood and Suttle, 1999). Underwood and Somers (1969) stated that the wool will re-grow when Zn is added back into the diet.

Zinc deficiency also results in an increased susceptibility to infection (World Health Organization, 1996). During a Zn deficiency, the thymus atrophies (McDowell, 1992). There is a decreased production of thymulin, which is involved in the differentiation of T-cells (Prasad, 1988). Without T-cells, antigen infected cells will not be recognized and therefore will not be destroyed by the immune system. Zinc deficiency has been reported to decrease macrophage (James et al., 1987) and cytokine function (Shi et al., 1998) and reduce antibody production (Haas et al., 1976). However, immune function seems to be less sensitive than other factors during a Zn deficiency. Beach et al. (1981) stated that impaired immunity is secondary to loss of appetite. Droke

and Spears (1993) reported that loss of appetite, poor growth, and skin lesions occur before susceptibility to infection increases in lambs.

Zinc toxicity occurs when Zn concentrations reach approximately 1000 mg of Zn/kg DM (NRC, 1980). However, tolerances may be increased or decrease depending on species and the composition of the diet (amount of antagonistic TM such as Ca, Cu, Fe, or Cd that is present in the diet; Underwood, 1981; Underwood and Suttle, 1999). Zinc toxicity has been seen to affect performance. Ott et al. (1966) reported that lambs that were supplemented with 1000 mg Zn/kg DM had decreased weight gains and feed efficiency. When the dietary concentration reached 1500 mg Zn/kg DM, feed intakes decreased and continued to decrease as concentrations continued to rise. Dairy cattle have been reported to have decreased milk yields and feed intake when Zn concentrations were 2000 mg/kg DM in the diet (Miller et al., 1979). Ott et al. (1966) stated that metabolism in ruminants may be affected because of the affect of high Zn concentrations on microbes in the rumen. Also, toxic concentrations of Zn may result in Cu deficiency. Zinc competes with Cu for absorption sites in the intestines and blood transport proteins (Cunnane, 1981)

Manganese:

Manganese deficiency had not been reported to a large extent in grazing sheep and cattle (McDowell, 1992). Underwood and Suttle (1999) stated that there is doubt that Mn deficiency occurs in a natural setting for these species and seems to be confined to avian species. However, Mn deficiency in ruminants has been shown to decreased bone strength and cause abnormal bone shape, ataxia, muscular weakness, and excess accumulation of body fat (Thomas, 1970; Hidiroglou, 1980).

Manganese deficiency has been reported to cause skeletal abnormalities. One such abnormality is chondrodystrophy, or dwarfism (Liu et al., 1994). Manganese deficiency causes a decrease in growth of the endochondral bone and results in dwarfism. In pigs, lameness, enlarged hock joints, and crooked and shortened legs have been reported (Underwood and Suttle, 1999). Calves and sheep have difficulty standing and have pain in their joints. They also have poor locomotion and balance (Underwood and Suttle, 1999). Perosis is another skeletal abnormality that results from manganese deficiency. With perosis, there will be an enlargement and malformation of tibiometatarsal joint, twisting and bending of the tibia, thickening and shortening of long bones and slipping of tendons from the condyles (Underwood and Suttle, 1999). Also, laying hens that are deficient in Mn will produce embryos that have short thick wings and legs with a "parrot beak", in which the mandible is shorter (Underwood and Suttle, 1999)

Manganese deficiency also results in ataxia, or a loss of the ability to coordinate muscular movement (Underwood and Suttle, 1999; McDowell, 1992). Ataxia is a consequence of Mn deficiency during late gestation (McDowell, 1992). With ataxia, comes a loss of equilibrium, which seems to be a result of impaired vestibular function due to a structural defect in the inner ear (Underwood and Suttle, 1999). This defect may be the result of impaired mucopolysaccharides synthesis (Hurley et al, 1960). Also, when an animal is ataxia, there will be retraction tremors of the head, increased susceptibility to stimuli, and delayed development of body righting reflexes (Hurley and Keen, 1986).

Manganese appears to be one of the least toxic elements (McDowell, 1992; Hurley and Keen, 1986). In sheep and cattle, toxicity concentrations are above 1000 mg Mn/kg DM (NRC, 1980). When Mn concentrations reach concentrations greater than

1000 mg Mn/kg DM, problems can occur. McDowell (1992) stated that at concentrations above 2000 mg Mn/kg DM resulted in growth retardation, anemia, gastrointestinal lesions, and neurological signs. Also, high Mn concentrations have antagonistic effects on Fe. When Mn concentrations are high, there are some reductions in heart and plasma Fe (Grace, 1983). Matrone et al. (1959) stated that Mn and Fe compete for binding sites during absorption.

Cobalt:

Cobalt deficiency has been reported to lead to failure of propionate metabolism. In ruminants there will be a decreased appetite that is not seen in non-ruminants (Underwood and Suttle (1999). This difference is thought to be the result of the differences in energy sources among ruminants (volatile fatty acids) and non-ruminants (glucose; Underwood and Suttle, 1999). It is believed that the rate and synthesis of propionate is normal, but the clearance of propionate from the blood decreases which leads to a buildup of intermediates within this pathway. Also, Somers (1969) stated that an increase in propionate in the blood may also lead to a decrease in acetate clearance from the blood.

During a Co deficiency, there is a failure of methylation that leads to an increase in homocysteine and a decrease in methionine because the methyl group cannot be added to homocysteine to make methionine. This buildup of homocysteine may lead to the product of oxidants within the cell and leads to oxidative damage of the mitochondria (Kennedy et al, 1997). Altered lipid metabolism can result from this failure of methylation. During Co deficiency, decreased appetite occurs, which results in an increase of fat mobilization and free fatty acids for energy production, to make up for the

lack of energy that would normally come from the diet (Underwood and Suttle, 1999). Normally, these free fatty acids will enter β-oxidation. However, there is a build up of methylmalonic acid CoA which inhibits β-oxidation of fatty acids (Underwood and Suttle, 1999). Also, these free fatty acids normally are utilized to make triglycerides that are transported by VLDL to parts of the body. However, VLDL synthesis requires methylcobalamin (Underwood and Suttle, 1999). Since these free fatty acids are not being utilized, they will travel to the liver and may result in fatty liver disease (Underwood and Suttle, 1999).

Cobalt toxicity is normally thought to be low. However, cobalt is only surpassed by copper, selenium, and iodine in toxicity (Dickson and Bond, 1974). It was reported that greater than 4 mg Co/kg DM live weight is toxic to sheep and greater than 1 mg/kg DM live weight is toxic for young cattle (Agricultural Research Council, 1980). It is recommended that diets should contain no more than 30 mg Co/kg DM. If concentrations of Co become too high, it may lead to pancreatic fibrosis and hepatic necrosis (Diaz et al., 1994).

Trace Mineral Supplementation

Underwood and Suttle (1999) stated that farm animals derive the majority of their trace minerals from the feed that they consume. However, there is often great variability in the mineral composition of feed. Feeds and forages that are grown on mineral deficient soils or that have inadequate mineral absorption capabilities will be deficient in minerals. When feeds are deficient in minerals, minerals are often supplemented. However, this is not the only reason minerals are supplemented. In the swine industry, minerals are often supplemented above recommend concentrations because of the

positive effects on performance and health that have been reported. Nonetheless, these affects have not always been consistent.

Performance:

Trace mineral supplementation has been reported to have varying affects on performance. Hatfield et al. (2001) conducted a study utilizing 6-yr old ewes. The ewes were sorted to treatments consisting of no supplemental Zn or Cu (basal diet contained 12.2 mg Zn/kg DM and 5.37 mg Cu/kg DM), Zn-amino acid complex, Zn-amino acid complex plus Cu-amino acid complex, $ZnSO_4$, and $ZnSO_4$ plus $CuSO_4$ to provide 102.2 mg Zn/kg DM and 15.4 mg Cu/kg DM in the total diet (3 times the Zn and 1.5 times the Cu concentrations recommended by the NRC. They reported a tendency for an increase ($P = 0.09$) in final BW in ewes that were supplemented with Zn (Hatfield et al., 2001). In steers fed a corn silage, corn, and soybean meal based diet (33 mg Zn/kg DM and 16 mg Cu/kg DM), ADG was increased when the steers were supplemented with 25 mg Zn/kg DM as Zn oxide or Zn proteinate (Spears and Kegley, 2002). However, steers supplemented with Zn (25 mg Zn/kg DM Zn oxide or Zn proteinate containing either 10 or 15 % Zn) had greater ADG than control steers during the growing phase (Spears and Kegley, 2002).

In other experiments, TM supplementation has been reported to have no effect on performance (George et al., 1997; Droke et al., 1998; Spears et al., 1991; Green et al., 1988. Due to the variability reported among studies, it is difficult to determine the true effects of TM supplementation on performance. It is possible that other factors such as species, breed, other dietary components, and location of the experiments may play a factor in the outcome of the experiments.

Liver and plasma trace mineral concentrations:

Trace mineral supplementation has been reported to increase liver TM concentrations (Dorton et al., 2003). In trials supplementing 2 year old beef heifers ($CuSO_4$; Gengelbach et al., 1994), Simmental, Charolais, and Angus calves ($CuSO_4$ and CuLys; Kegley and Spears, 1994), Holstein bull calves ($CuSO_4$; Ward et al., 1997), Holstein heifers ($CuSO_4$; Torre et al., 1995), and gestating beef cattle ($CuSO_4$ and $CuCO_3$; Niederman et al., 1994), animals supplemented with Cu had greater ($P < 0.05$) plasma Cu concentrations overall than control animals Furthermore, Engle and Spears (2001) reported similar results in a study supplementing $CuSO_4$ to Simmental steers.

Liver Zn concentrations have been reported to be increased in TM supplemented wethers (Rojas et al., 1995) and steers (Huerta et al., 2002). In a study utilizing ewes, Zn supplemented as either $ZnSO_4$ or a Zn amino acid complex resulted in greater ($P = 0.04$) liver Zn concentrations than reported in control ewes not supplemented with Zn (Hatfield et al., 2001). This was also reported in cows (Ahola et al., 2004).

Trace mineral supplementation had no effect on TM status in heifers (Huerta et al., 2002), steers (Mullis et al., 2003), and lactating Holstein cows (Kellogg et al., 1989) supplemented with 200, 250, and 360 mg TM/kg DM, respectively. Ward et al. (1993), when supplementing $CuSO_4$ and CuLys, found no differences in plasma Cu concentrations among supplemented and unsupplemented steers. Furthermore, Kegley and Spears (1994) reported no differences in plasma Cu concentrations in steers supplemented with Cu oxide (CuO) and $CuSO_4$. In other studies, similar liver Cu concentrations were seen among control and Zn supplemented heifers (Kincaid et al,

1997), steers (Huerta et al., 2002; Mullis et al., 2003), and sheep (Rojas et al., 1995; Hatfield et al., 2001).

Immune function and health:

Ward et al. (1993) reported no difference among treatments in SOD activity when supplementing $CuSO_4$ or $CuCO_3$. This was also reported in steers (Dorton et al., 2003), Holstein heifers (Torre et al., 1995), and gestating beef heifers (Niederman et al., 1994). Supplementation had no effect on total immunoglobulin G (IgG) and total immunoglobulin M (IgM) concentrations in serum of growing and finishing steers (Dorton et al., 2003). Stabel et al. (1993) found total IgM and IgG were not affected by Cu status in Cu-adequate and Cu-deficient Holstein calves. Ward et al. (1997) also reported that supplementing Cu ($CuSO_4$) to a diet deficient in Cu fed to heifers had no effect on total IgG concentration in the serum of their calves. Antibody titer production to pig red blood cells as well as ovalbumin have also been reported to be unaffected by TM supplementation (Ward et al, 1993; Droke et al., 1998; Droke et al., 1993; Engle et al., 1999a; Engle et al., 1999b).

Immunoglobulin M antibody titers specific for PRBC have been reported to be greater ($P < 0.09$) in steers supplemented with Cu than controls. Similar results have been reported by Ward and Spears (1999) when supplementing 5 mg Cu/kg DM from $CuSO_4$ to a diet containing 5.2 mg Cu/kg DM. Immune response to OVA was greater in Angus bull calves supplemented with Cu than in control steers 7, 14, 21, and 28 d post injection ($P < 0.06$). Holstein bull calves supplemented with 10 mg Cu/kg from $CuSO_4$ had a greater ($P < 0.06$) immune response to PHA than did control steers at 12 h and 24 h post injection (Ward et al., 1997). However, in another study, Ward et al. (1997)

reported that supplementing 10 mg Cu/kg DM from $CuSO_4$ to a diet containing 4.5 mg Cu/kg DM to 2-year-old pregnant heifers, Cu supplementation produced a lower skin swelling response to PHA in the calves of the heifers. Dorton et al. (2003) reported that during the finishing phase, IgG antibody titers to pig red blood cells were greater ($P <$ 0.03) in control steers than Cu supplemented steers (Dorton et al., 2003). When 70 d old calves were supplemented with 10 mg Cu/kg DM from $CuSO_4$ to a diet containing 4.5 mg Cu/kg DM, calves supplemented with Cu had less antibody titers than control steers 14 d ($P < 0.07$) and 21 d ($P < 0.09$) post-secondary injection (Ward et al., 1997). Also, Angus bull calves supplemented with 5 mg Cu/kg DM from $CuSO_4$ to a diet containing 5.2 mg Cu/kg DM had less ($P < 0.07$) antibody titers to PBRC than control steers (Ward and Spears, 1999).

Carcass characteristics:

Trace mineral supplementation has been reported to have no effect on carcass characteristics in steers fed a corn-alfalfa based basal diet (3 mg Cu/kg DM, 20.1 mg Zn/kg DM, and 9.6 mg Mn/kg DM) supplemented with organic Cu, Zn, Mn, and Co (1x or 2x NRC recommended concentrations in an amino acid complexed form) or $CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$ (3x or 6x NRC recommended concentrations; Rhoads et al., 2003) and wether lambs fed a basal diet of barley and alfalfa supplemented with Zn methionine (0, 0.396, or 0.791 g/head/day; Hatfield et al., 1992). Backfat has been reported to be greater ($P < 0.05$) in control steers than in steers supplemented with $CuSO_4$ (Engle and Spears, 2000; Engle et al., 2000a; Engle and Spears, 2001) and tended to be lower in steers supplemented with inorganic or organic sources of Cu (Engle et al., 2000b). When supplementing Zn (200 mg Zn/kg DM from $ZnSO_4$ or Zn methionine) to a

basal diet containing 64 mg Zn/kg DM and 30 mg Cu/kg DM, Huerta et al. (2002) reported that backfat thickness ($P < 0.06$) and adjusted backfat thickness tended ($P < 0.08$) to be greater in control heifers than in supplemented heifers. Maturity has been reported to be lower ($P < 0.01$) in heifers supplemented with Zn than control heifers (Huerta et al., 2002). In contrast, Ward and Spears (1997) reported that steers supplemented with $CuSO_4$ had lower yield grade ($P < 0.04$) and REA ($P < 0.09$) than control steers. Hot carcass weight has been reported to be greater ($P < 0.05$) in control steers than steers supplemented with TM (Engle et al., 2000b). Also, heifers supplemented with TM had a lower ($P < 0.07$) dressing percentage than control heifers (Huerta et al., 2002), whereas, steers supplemented with TM had a greater ($P < 0.10$) dressing percentage than control steers (Spears and Kegley, 2002). However in steers, backfat was lower in control steers than steers supplemented with inorganic or organic sources of Zn (Green et al., 1988; Spears and Kegley, 2002). Furthermore, Spears and Kegley (2002) reported that supplementing steers with Zn oxide or Zn proteinate (10 or 15 % Zn) resulted in a greater marbling score ($P < 0.05$) and higher yield grade ($P < 0.10$), but had no effect on kidney, pelvic, and heart fat.

Lipid metabolism:

Copper supplemented steers have been reported to have lower cholesterol concentrations in serum than control steers (Engle and Spears, 2000). In other studies, cholesterol concentrations in steers have been unaffected by TM supplementation (Engle et al., 2000b; Engle and Spears, 2001). Engle and Spears (2000) reported that steers supplemented with 10 or 20 mg Cu/kg DM from $CuSO_4$ to a corn-soybean meal-corn silage basal diet containing 4.9 mg Cu/kg DM and 51 mg Zn/kg DM had lower ($P < 0.05$)

concentrations of myristoleic acid (14:1 n-5) and greater ($P < 0.05$) concentrations of linoleic acid (18:2 n-6), linolenic acid (18:3 n-3), unsaturated fatty acids (USFA), and polyunsaturated fatty acids (PUFA) than control steers. Steers supplemented with 20 mg $CuSO_4$ to a corn-soybean-meal-corn silage basal diet (5.2 mg Cu/kg DM and 52.1 mg Zn/kg DM) had similar fatty acid profiles as control steers, except stearic acid (18:0) concentrations tended ($P < 0.10$) to be lower in supplemented steers than control steers (Engle et al., 2000a). Engle et al. (2000b) supplemented steers with 20 or 40 mg Cu/kg DM from $CuSO_4$ or 20 mg Cu/kg DM from Cu citrate, Cu proteinate, or tribasic Cu chloride to a corn silage-soybean meal basal diet (10.2 mg Cu/kg DM and 54.8 mg Zn/kg DM). They reported that steers supplemented with Cu had lower 18:2 n-6, 18:3 n-3, and saturated fatty acids (SFA) and greater PUFA than control steers. Trace mineral supplementation has been reported to have no effect on percent lipid in longissimus muscle tissue (Foutz et al., 1997; Engle et al., 2000a; Engle and Spears, 2000).

Organic verses Inorganic Sources of Trace Minerals

There have been debates over whether organic or inorganic sources of TM are more bioavailable to the animal. Spears (1996) stated that there is a theory that organic sources of minerals were more bioavailable than inorganic sources because the organic sources are more similar to biologically active forms of minerals in the body and in feed. Organic forms of minerals may prevent binding of antagonists or other molecules in the body because they are already bound to a molecule and have less or no binding sites available. Also, animals fed inorganic sources of minerals have to first convert these minerals to biologically active forms before they can be utilized by the body (Spears, 1996).

Traditionally, TM are supplemented in inorganic forms and are fed in the form of mineral salts. Some forms of inorganic sources of Cu are Cu sulfate ($CuSO_4$), Cu chloride ($CuCl_2$), cupric oxide (Cu_2O), Cu oxide (CuO), and Cu carbonate ($CuCO_3$). Copper sulfate is the most widely used inorganic form of Cu in diets of animals. Furthermore, Zn is often supplemented as Zn sulfate ($ZnSO_4$) or Zn oxide (ZnO). Manganese is supplemented as Mn sulfate ($MnSO_4$) or Mn chloride ($MnCl_2$) and Co carbonate is the predominant inorganic form of Co supplemented to livestock.

Spears (1996) reported that in recent years there has been increased interest in using organic forms of TM because of improved growth, reproduction, and health reported in various studies. Organic forms of TM are often bound to an amino acid or protein in various types of complexes, chelates, or proteinates (Spears, 1996). Complexes are formed when a ligand (amino acids, peptides, polypeptides) binds to a metal ion. An example of a complex is Cu lysine (CuLys) or Zn methionine. Chelates are complexes between a ligand and a metal ion. Kratzer and Vohra (1996) stated that to be classified as a chelate the ligand must contain a minimum of two functional groups (oxygen, nitrogen, amino, or hydroxyl) that can donate a pair of electrons to be able to bind to the metal ion. The ligand must also form a heterocyclic ring structure with the metal. Chelates are often composed of a variety of minerals rather than just one type of metal. Proteinates are metals that are chelated to amino acids and/or partially hydrolyzed proteins. An example of a proteinate is Cu proteinate (CuProt).

Results tend to vary among experiments in the availability of TM between organic and inorganic forms. In studies using rats, Kirchgessner and Grassmann (1970) fed rats a low Cu diet for 3 to 4 weeks. The rats were then supplemented with 3.18 mg of Cu/kg

DM. Copper was supplemented as either $CuSO_4$ (inorganic Cu source) or Cu-amino acid, Cu peptide or Cu polypeptide complex (organic Cu sources) for 14 d. The results showed that absorption rate was greater in rats fed the organic forms of Cu compared to rats fed $CuSO_4$ (Kirchgessner and Grassmann, 1970). Kincaid et al. (1986) conducted a study using 30 heifer and 15 bull Holstein calves. The calves were fed a basal diet composed of barley and hay. The calves were supplemented with Cu proteinate (CuProt) or $CuSO_4$ in a pellet form or given no supplemental Cu. The pellet included either CuProt or $CuSO_4$ at 19 mg of Cu/kg DM. The un-supplemented concentrate mix contained 5 mg Cu/kg DM and the hay contained 1 mg Cu/kg DM. Liver and plasma Cu concentrations were measured. Kincaid et al. (1986) reported that the organic source of Cu (CuProt) was more available than the inorganic Cu source ($CuSO_4$).

Other studies have shown that organic sources of Cu were not more available than inorganic sources of Cu. Baker et al. (1991) fed 180 New Hampshire x Columbian crossbred male chicks a basal diet composed of corn and soybeans that was fortified with 275 mg Cu/kg DM from $CuSO_4$ (total dietary Cu was 290 mg Cu/kg DM). The chicks were supplemented with 75 or 150 mg Cu/kg DM from $CuSO_4$, Cu-lysine (CuLys), cupric oxide (Cu_2O), or Cu oxide (CuO) or received no supplemental Cu. They reported that the availabilities of Cu from organic sources were similar to that of inorganic sources of Cu. Similar results were shown by Aoyogi and Baker (1993). Eighty, one day old New Hampshire x Columbian crossbred male chicks were fed a Cu deficient casein-soy concentrate basal diet that contain 0.56 mg Cu/kg DM. The chicks were supplemented with 0, 0.5, and 1 mg Cu/kg DM from $CuSO_4$ or 0.5 and 1 mg Cu/kg DM from CuLys. At the end of the study, chicks were harvested and bile was removed from the gall

bladder and analyzed for Cu. Results showed that organic sources of Cu were no more available than inorganic sources of Cu.

Performance: Previous studies evaluating TM supplementation to growing diets fed to ruminants have yielded inconsistent results on performance between organic or inorganic sources of TM. Ward et al. (1993) supplemented 5 mg Mo/kg DM plus 0.2% S from MoS, 5 mg Cu/kg DM from $CuSO_4$, 5 mg Cu/kg DM from $CuSO_4$ plus 5 mg Mo and 0.2% S from MoS, 5 mg Cu/kg DM from CuLys, or 5 mg Cu/kg DM from CuLys plus 5 mg Mo and 0.2% S from MoS, or no supplement (the basal diet contained 6.2 mg Cu/kg DM) to 126 Angus and Angus x Hereford crossbred steers. They reported that steers supplemented with an inorganic Cu source ($CuSO_4$) had greater ($P < 0.01$) gains and tended to have greater ($P < 0.10$) feed efficiency (FE) than steers supplemented with an organic Cu source (CuLys; Ward et al., 1993). Kegley et al. (2001) reported an increase in ADG in steers supplemented with organic Zn (Zn amino acid complex) compared to steers supplemented with inorganic Zn ($ZnSO_4$) during the last 15 days of their study. This was also reported by Spears (1989), in which growing heifers supplemented with 25 mg Zn/kg DM from Zn methionine tended to have greater ADG ($P < 0.13$) than heifers supplemented with iso-amounts of Zn oxide (basal diet contained 24 mg Zn/kg DM). In 2000, Engle and Spears conducted a study where they fed 36 Angus and 24 Hereford-Angus crossbred steers a corn silage based growing diet that contained 10.2 mg Cu/kg DM. They supplemented the steers 20 or 40 mg Cu/kg DM from $CuSO_4$ or 20 mg Cu/kg DM from Cu citrate, Cu Prot, or tribasic Cu chloride, or gave no supplemental Cu. They reported no significant differences in performance among steers supplemented with either organic or inorganic Cu sources. During the finishing phase of

this same trial where a high concentrate diet containing 4.9 mg Cu/kg DM was fed, Engle and Spears (2000) supplemented the same steers with the same treatments. Performance in the finishing phase was also not affected by Cu source. Similar results were reported in a study by Rabiansky et al. (1999) where they supplemented 40 Brahman x Hereford crossbred heifers with 8 or 16 mg Cu/kg DM from $CuSO_4$ or CuLys or no supplemental Cu to a basal diet containing 28 mg Cu/d. Copper source had no effect on performance. Similar results were reported by Kegley and Spears (1994), Droke et al. (1998) and Kegley et al. (2001). It is unclear as to why these studies had varying results and makes it difficult to determine the effects of TM source on performance. Other factors such as species, breed, other dietary components, and location of the experiments may play a factor in the outcome of the experiments.

Liver/Plasma Cu status: Organic and inorganic TM source effects on liver and plasma TM concentrations seem to vary. Kincaid et al. (1986) conducted a study using 30 heifer and 15 bull Holstein calves. The calves were fed a basal diet composed of barley and hay and were supplemented with 19 mg Cu/kg DM from either Cu proteinate (CuProt) or $CuSO_4$ in a pellet form or given no supplemental Cu. The un-supplemented concentrate mix contained 5 mg Cu/kg DM and the hay contained 1 mg Cu/kg DM. The calves that were supplemented with the organic Cu source (CuProt) had greater liver Cu concentrations than calves supplemented with the inorganic Cu source ($CuSO_4$). Plasma Cu concentrations were also greater ($P < 0.05$) in calves supplemented with the organic Cu source compared to the inorganic Cu source (Kincaid et al., 1986). Engle and Spears (2000) reported that steers supplemented with the organic Cu source (Cu proteinate) tended to have greater liver Cu concentrations than steers supplemented with the

inorganic Cu source ($CuSO_4$). Hatfield et al. (2001) reported a TM source effect in ewes. Ewes that were supplemented with organic TM tended to have greater (P = 0.06) liver Zn concentrations than ewes supplemented with inorganic TM. This effect was also seen in cows (Ahola et al, 2004).

Similar results have been reported in rats. Du et al. (1996) conducted a study using 60 male Sprague-Dawley rats. The rats were supplemented with 0 or 1000 mg Zn/kg DM or 5 or 15 mg Cu/kg DM from $CuSO_4$, CuProt, or CuLys to a basal diet containing 0.81 mg Cu/kg DM. Rats supplemented with an organic Cu source had greater ($P < 0.05$) liver Cu concentrations than rats supplemented with inorganic Cu ($CuSO_4$; Du et al., 1996). In a second experiment, Du et al. (1996) utilized the same number and type of rats, the same diet and treatments, except Zn was replaced with Fe. They reported that overall liver Cu concentrations were again greater ($P< 0.05$) when rats were supplemented with an organic Cu source than when supplemented with an inorganic Cu source ($CuSO_4$).

In contrast to the above mentioned studies, Stanton et al. (1998) reported higher ($P < 0.05$) liver Cu concentrations in steers that were supplemented with $CuSO_4$ relative to steers supplemented with iso-amounts of an organic Cu source. Huerta et al. (2002) reported a TM source effect in feedlot heifers. Heifers supplemented with 200 mg Zn/kg DM from $ZnSO_4$ had greater liver Zn concentrations than heifers supplemented with 200 mg Zn/kg DM from Zn methionine. Also, plasma Cu concentrations were unaffected by Cu source in an experiment conducted by Engle and Spears, (2000). The reason for the discrepancies in results reported in these studies is unknown. The difference could

possibly be due to the type of animals utilized in each study (steers vs. ewes vs. heifers) or possibly could be the result of the total amount of TM being supplemented.

Immune function and health: Hutcheson (1989) and Spears (2001) indicated that organic Cu sources might be more available when an animal is stressed (for example by transport, illness, and other factors). Illness can put tremendous amount of stress on the body and cause the body to respond immunologically to an antigen. Increased excretion of minerals in urine during stress has been reported (Orr et al., 1990). Nockels et al. (1993) reported that when supplementing CuLys or $CuSO_4$ to eight Charolais crossbred steer calves, upon feed and water removal (stressor), Cu form CuLys was retained to a greater extent than $CuSO_4$. This could explain why organic sources of Cu are believed to be more available than inorganic sources of Cu during a stressed period.

Results from immunological-trace mineral source experiments have varied. Nockels (1991) supplemented Cu Prot and $CuSO_4$ to steers and reported that antibody titers to IBR were higher in cattle that were fed the organic Cu when compared to cattle fed inorganic Cu. Huerta et al. (2002) reported a TM source effect on IgG concentrations rather than IgM concentrations in heifers fed a barley-corn-pea hay based basal diet (64 mg Zn/kg DM) supplemented with 200 mg Zn/kg DM from either $ZnSO_4$ or Zn methionine. They reported that heifers supplemented with organic Zn tended to have a greater ($P < 0.10$) concentration of IgG than steers supplemented with inorganic Zn. Steers supplemented with organic TM had greater antibody titer concentrations to ovalbumin antigen and lower antibody titer concentrations to PRBC than steers that were supplemented with inorganic TM (Dorton et al. (2003). In contrast, Ward et al. (1993) reported that steers supplemented with 5 mg/kg Cu from either $CuSO_4$ or CuLys to a diet

containing 6.2 mg Cu/kg DM had similar antibody titer concentrations specific to OVA when compared to controls. Trace mineral source had no effect on antibody titer concentrations to PRBC in steers fed a corn silage-soybean meal basal diet (Engle et al., 1999a; Engle et al., 1999b). Droke et al. (1993) also reported no effects of TM source on antibody titer concentrations to PRBC in lambs supplemented with either 25 mg Zn/kg DM from Zn oxide or Zn methionine to a basal diet containing 27.6 mg Zn/kg DM. Chirase and Green (2001) reported that calves that were supplemented with 50 mg Zn methionine and Mn methionine/kg DM had similar morbidity rates to calves supplemented with Zn oxide and Mn oxide. Other studies have also reported no differences in morbidity rates (Engle et al., 1999b; Kegley et al., 2001). The differences in results between studies is unknown but could be caused by breed differences, environmental differences, source differences, or even the portion of the immune system being studied.

Carcass characteristics: Green et al. (1988) and Malcolm-Callis et al. (2000) reported that steers supplemented with organic TM had greater kidney, pelvic, heart fat than steers supplemented with inorganic TM. Green et al. (1988) and Huerta et al. (2002) reported that steers that were supplemented with Zn methionine had greater marbling scores (MARB) ($P < 0.05$ and $P < 0.07$, respectively) than steers supplemented with Zn oxide or $ZnSO_4$. Steers that were supplemented with Cu proteinate had greater ($P < 0.05$) MARB than steers supplemented with $CuSO_4$ (Engle et al., 2000). Backfat thickness has been reported to be lower ($P < 0.10$) in steers supplemented with $ZnSO_4$ than steers supplemented with Zn methionine, whereas, dressing percentage, ribeye area, MARB, and yield grade were unaffected by TM source (Malcolm-Callis et al., 2000). Also, hot

carcass weight has been reported to be lower ($P < 0.05$; Spears and Kegley, 2002) and dressing percentage has been reported to be greater ($P < 0.02$; Huerta et al., 2002) in steers and heifers supplemented with inorganic TM verses organic TM.

Lipid metabolism: Engle et al. (2000b) reported that TM source had no effect on fatty acid composition in longissimus muscle tissue, except steers supplemented with 20 mg Cu/kg DM from $CuSO_4$ had lower ($P < 0.05$) concentrations of C22:1 than steers supplemented with 20 mg Cu/kg DM from Cu proteinate. Also, percent lipid in longissimus muscles of steers was unaffected by TM source, inoculation, or time of inoculation (Engle et al., 2000a; Engle and Spears, 2000).

Summary

Because of their incorporation into many enzymes, TM are part of many physiological functions in the body. Some of these physiological functions are metabolism, wound healing, growth, and immune function. If TM were not present in the diet, deficiencies would occur. Trace mineral deficiencies can cause depigmentation of hair and wool, bone problems, cardiac lesions, and even death. High amounts of TM in the diet can cause TM toxicities that can lead to death. Many studies have been conducted testing the effects of TM on the body under many different scenarios. Recently, research has begun to focus on whether inorganic or organic sources of TM are more available to animals. Because results have been inconsistent, more research is needed in order to determine the effects of TM on the body.

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Chapter II

EFFECTS OF TRACE MINERAL SUPPLEMENTATION FOR 28 DAYS POST-WEANING ON PERFORMANCE OF RECEIVING CATTLE

ABSTRACT

Three hundred and seventy-three steer calves (approximately 7 mo of age and 248.7 ± 19.4 kg) were utilized to determine the effects of trace mineral (TM) supplementation and source on performance of calves during the backgrounding and receiving phases. At their respective ranches, calves were stratified by body weight into six groups that were assigned to six pens. Pens were then randomly assigned to treatments. The treatments consisted of: 1) control (no supplemental trace minerals), 2) inorganic trace mineral ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace mineral (iso-amounts of organic Cu, Zn, Mn, and Co). Mineral treatments were fed in alfalfa pellets with controls receiving alfalfa pellets with no additional Cu, Zn, Mn, or Co. Calves were allowed free access to grass hay throughout the 30 d backgrounding phase. On day 30 post-weaning, calves were weighed and transported to the Agricultural Research, Development, and Education Center (ARDEC) facility in Fort Collins, CO. Steers were blocked by ranch, stratified by initial body weight, and randomly assigned to one of 36 pens (9 – 12 head per pen). Pens within a block were then randomly assigned to treatments (12 pens per treatment). Treatment consisted of: 1) control (no

supplemental Cu, Zn, Mn, and Co), 2) inorganic trace minerals (CuSO4, ZnSO4, MnSO4, and CoCO3), and 3) organic trace minerals (iso-amounts of organic Cu, Zn, Mn, and Co). Steers were fed a corn silage-based growing diet throughout the 28 d receiving period. There was no effect of TM supplementation on performance of calves. Steers that were supplemented with organic TM had greater ($P < 0.05$) ADG than steers supplemented with inorganic TM by the end of the receiving phase. The results indicate that TM source may have an effect on performance of backgrounded calves.
Key words: Calves, Trace Minerals, Backgrounding, Performance, Morbidity

INTRODUCTION

Feeder cattle that have been shipped are subjected to various degrees of stress. Stress associated with shipping results in increased susceptibility to respiratory tract and other infectious diseases. Morbidity and mortality rates are often high in these animals, despite vaccination against respiratory diseases. Feed intake can also be greatly reduced in cattle that have been stressed by shipping. This results in reduced quantities of trace elements being ingested, unless dietary concentrations are increased during the stress period. Some attention has been focused on the role of trace mineral nutrition in the immunological adaptation of beef cattle to stress. However, little focus has been placed on mineral supplementation to weaned calves prior to transport to the feedlot.

Zinc (Zn) and copper (Cu), and to some extent manganese (Mn), and cobalt (Co), have been shown to play a role in the immune response of cattle (Galyean et al., 1999; Spears, 2000). Therefore, supplementing these trace minerals to weaned calves prior to shipping may allow for an enhanced immune response when the calves are exposed to stressors such as transport and the new feedlot environment. Supplementing trace

minerals prior to transport of newly weaned calves to the feedlot could also help to compensate for the decrease in dry matter intakes normally observed in newly received calves (Hutcheson and Cole, 1986). With the observed reduction in dry matter intakes of newly received cattle, trace minerals are typically increased in the receiving ration to compensate for the reduced feed intake. Therefore, supplementing trace minerals prior to transport and increasing the trace mineral concentrations in the receiving ration of stressed calves may reduce the number of incidences of morbidity and mortality in newly received cattle. Therefore the objective of this experiment was to determine the effects of trace mineral supplementation for 30 days post weaning on performance of receiving cattle.

MATERIALS AND METHODS

The Colorado State University Animal Care and Use Committee approved care, handling, and sampling of the animals defined in this experiment prior to the initiation of this study.

Three hundred and seventy three steer calves (approximately 7 mo of age and 248.7 (1.1 kg) were utilized in this experiment. Calves were obtained from 3 different Colorado State University Research facilities (127 Hereford x Angus calves from Maxwell Ranch in Livermore, CO; 135 crossbred calves from the San Juan Basin Research Center in Hesperus, CO; and 111 black Angus calves from the Colorado State University Beef Improvement Center in Saratoga, WY).

Calves were backgrounded at their respective ranch locations. Post-weaning calves were weighed on two consecutive days and stratified by body weight into six groups. Groups were then randomly assigned to one of six group pens equipped with

bunk feeders and automatic waterers. Pens were then randomly assigned to treatments. The treatments consisted of: 1) control (no supplemental trace minerals), 2) inorganic trace mineral ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace mineral (iso-amounts of organic Cu, Zn, Mn, and Co). Mineral treatments were fed in alfalfa pellets with control calves receiving alfalfa pellets with no additional Cu, Zn, Mn, or Co. Calves were allowed free access to grass hay throughout the 30 d backgrounding phase. On day 30 post-weaning, calves were weighed and transported to the Agricultural Research, Development, and Education Center (ARDEC) facility in Fort Collins, CO. Transport time varied between ranches. Calves from the Maxwell Ranch traveled approximately 48 km. Calves from the San Juan Basin Research Center in Hesperus, CO, traveled approximately 656 km and calves from the Colorado State University Beef Improvement Center traveled approximately 230 km.

Receiving phase:

Upon arrival, all steers were weighed (on two consecutive days), vaccinated with Ultrabac®7/Somubac and Bovishield™ 4+L5, and dewormed with Decomax (Pfizer Animal Health, Exton, PA). Calves were blocked by backgrounding treatment within ranch, stratified by initial body weight, and sorted into one of 36 pens (9-12 head per pen; 12 pens per treatment) equipped with automatic waterers. Calves remained on the same trace mineral treatments that they received during the on-farm backgrounding phase. Treatments consisted of: 1) control (no supplemental trace minerals), 2) inorganic trace minerals ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace minerals (iso amounts of organic Cu, Zn, Mn, and Co). Half of the steers from each treatment (6 pens per treatment) were implanted with 200 mg progesterone and 20 mg estradiol benzoate at

the initiation of the experiment and the remaining steers (6 pens per treatment) received no implant. Steers were fed a corn silage-based growing diet (Table 1) throughout the 28 d receiving phase. Diets were fed once daily in the morning in amounts adequate to allow ad libitum access to feed throughout the day. Calves were weighed 28 d post arrival at the feedlot. Morbidity and mortality were monitored throughout the receiving phase.

Statistics:

Statistical analysis of data was performed using Proc Mixed procedure of SAS (2001). The study was a completely randomized design. The model included fixed effects of treatment. Morbidity data were analyzed using the chi-squared analysis of SAS (2001). P-values for morbidity were determined and the number of times calves were retreated for an illness were analyzed using Proc GLM procedure of SAS (2001). The contrast statements were: 1) inorganic trace mineral vs. organic trace mineral treatments.

RESULTS AND DISCUSSION

In the beef cattle industry, disease can often hinder profitability by increasing cost of production and decreasing returns. The most costly disease to the cattle industry is bovine respiratory disease (BRD). Bovine respiratory disease accounts for approximately 75 % of feedlot morbidity and 50 % of feedlot mortalities (Martin et al., 1984; Edwards, 1996; Chirase et al., 2001) and is thought to be the primary disease of newly weaned and received calves (Roth, 1986). Death loss, reduced feed efficiency, and therapeutic costs results in an economic loss of 800 to 900 million dollars a year to the cattle industry (Chirase and Green, 2000) and increased processing to treat sick cattle results in a loss of 110 million dollars per year (Smith et al., 1991).

Susceptibility to disease is influenced by many factors. One of these factors seems to be stress. Cattle face many stressors throughout life. Hoerlein and Marsh (1957) stated that stressors are related to weaning, water and feed deprivation during transport, overcrowding, and infectious agents. Other stressors are environmental changes, castration, dehorning, and processing (Hutcheson and Cole, 1986). Newly received cattle stressors result in altered endocrine responses, altered metabolism, changes in appetite and growth rate, and may alter rumen function, which seems to result in immunosuppression and therefore increased susceptibility to disease (Loerch and Fluharty, 1999; Speer et al., 2001).

Producers often utilize preconditioning programs to try to diminish the effects of stress on calves in order to decrease incidence of disease. Preconditioning programs are broken into three categories: 1) vaccination (prior to weaning and after arrival to the feedlot), 2) surgery (i.e. castration and dehorning), and 3) feeding or bunk training (Cole, 1985; Speer et al., 2001). Vaccinating prior to weaning and after arrival at the feedlot has been reported to decrease the incidence of morbidity in calves (Kreikemeier et al., 1997). Feeding has had varying results on decreasing incidence of disease and is often the most costly of preconditioning programs (increased management/labor costs; Galyean et al., 1999; Speer et al., 2001). Feeding consists of factors such as training calves to eat from a bunk. The goal of bunk training is to prohibit a decrease in dry matter intake (DMI) once the calves are in the feedlot as well as to prohibit a decrease in performance due to the decrease in DMI.

A decrease in DMI in calves entering the feedlot is common. Nutrient concentrations, such as trace minerals, are often increased in a ration to compensate for

the decrease in DMI in order to meet the nutrient needs of the animal. Maintaining mineral concentrations in the body is crucial to maintaining optimal health. It has been reported that a copper deficiency resulted in reduced immune function and an increased susceptibility to disease (Stable and Spears, 1990). Engle et al. (1995) reported that heifers fed a Zn-deficient diet had a lower immune response to phytohemagglutinin injections intradermally than heifers fed a Zn-adequate diet. These studies indicate that TM may be needed to maintain a functional immune system to maintain optimal health.

Another feeding preconditioning program that has been studied is supplementing TM post weaning prior to shipping calves to the feedlot, as was utilized in the present study. Supplementing TM to calves prior to transport may build up the animal's stores of TM to compensate for the lower intake of TM after arrival to the feedlot when DMI is lower. Supplementing TM has been reported to reduce the incidence of a decrease in DMI. Chirase et al. (1991) reported that steers that were supplemented with trace minerals had a lower decrease in DMI and returned to normal DMI quicker than steers not supplemented with TM in three different experiments, when steers were subjected to stress. With DMI being decreased to a lesser extent by stress, performance and subsequently health and carcass quality may be less impacted.

As mentioned previously, in the present study, calves were supplemented with inorganic and organic sources of TM post weaning 30 d prior to transport to the feedlot. During the backgrounding phase, average daily gain (ADG) was similar across treatments (Table 2). However, organic supplemented calves had higher ($P < 0.05$) ADG than inorganic and control cattle at the end of the 28 d feedlot receiving phase. Chirase et al. (2001) reported that calves supplemented with Zn methionine plus Mn methionine or Zn

oxide plus Mn oxide post weaning had similar body weights. By d 28 after arrival to the feedlot body weights and ADG were greater ($P < 0.05$) in calves supplemented with organic TM than calves supplemented with inorganic TM (Chirase et al., 2001). Kegley et al. (2001) reported that ADG of steer and heifer calves were unaffected by TM supplementation (360 mg Zn/kg DM from $ZnSO_4$ or a Zn amino acid complex). Similar results were reported by Spears et al. (1991) in receiving cattle supplemented with Zn oxide or Zn methionine. Also, Kegley et al (2001) demonstrated that during the last 14 d of the 28 d receiving period, steer calves supplemented with organic TM had a greater ADG than steers supplemented with inorganic Zn .

There was no effect of TM supplementation on morbidity or the number of times a calf was retreated for an illness (RETRT) throughout the receiving phase (Table 3). However, there was a tendency for an effect of TM source on morbidity. Calves supplemented with organic TM tended ($P < 0.09$) to have a lower incidence of morbidity than calves supplemented with inorganic TM. This may be the reason why calves supplemented with organic TM had a greater ADG by the end of the receiving phase than steers supplemented with inorganic TM. In calves supplemented with inorganic or organic Zn and Mn, morbidity was unaffected by TM source (Chirase et al., 2001). Similar results were reported in steer calves (Kegley et al., 2001) and heifers (George et al., 1997). In contrast to theses studies and similar to the present study, Johnson et al. (1988) reported a lower incidence of morbidity in calves supplemented with Zn methionine. A lower incidence of morbidity was also seen in newly weaned steers supplemented with 35 or 70 mg Zn/kg DM from Zn methionine or 70 mg Zn/kg DM from $ZnSO_4$ (Galyean et al., 1995).

It is unclear why calves supplemented with organic TM had greater ADG and lower incidence of morbidity. Hutcheson (1989) indicated that animals supplemented with organic TM respond better to "stressors", such as infection or adverse environmental conditions, than animals supplemented with inorganic TM. Also, it is believed that organic sources of minerals have a greater bioavailability than inorganic TM sources. Spears (1996) stated a theory that organic sources of minerals were more bioavailable than inorganic sources because the organic sources are more similar to biologically active forms of minerals in the body and in feed. Organic forms of minerals may prevent binding of antagonists or other molecules in the body because they are already bound to a molecule and have less or no binding sites available. Possibly through a combination of having a greater bioavailability and aiding in a better response to stressors, steers supplemented with organic TM preformed better.

CONCLUSION

During the backgrounding phase, TM supplementation had no effect on performance. Calves supplemented with organic TM had a greater ADG and a lower incidence of morbidity than calves supplemented with inorganic TM. It is easy to speculate that supplementing organic TM to calves prior to weaning improves performance and the health of the calves. Since performance is improved, carcass quality may be improved as well in the long run. However, little research has been conducted in this area. Therefore, further research is needed to determine the effects of TM supplementation post weaning and prior to transport to the feedlot on performance and health of calves.

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Table 1: Ingredient composition of basal diets

Ingredient	Backgrounding	Receiving
	----------%[a]----------	
Corn Silage	-----	17.80
Alfalfa Hay	94.90	17.32
Flaked Corn	-----	55.96
Protein Supplement	5.10	8.92
Protein Supplement Composition		
Cottonseed Meal	-----	46.00
Soybean Hulls	38.17	-----
Soybean Meal	-----	20.00
Soybean Oil	0.62	-----
Sunflower Meal 32 %	10.60	4.52
Wheat Midds	40.00	-----
Molasses Cane Blend	3.50	-----
Urea	-----	6.98
Rumensin 80[b]	-----	0.19
Bentonite	1.00	-----
Biofos[c]	0.82	3.90
Cobalt Carbonate	-----	-----
Copper Sulfate	-----	-----
Dyna-K	1.47	3.93
Limestone	3.27	11.75
Manganese Sulfate	-----	-----
Iodine	0.02	0.06
Salt	0.45	0.90
Selenium	0.02	0.08
Sulfur Flower	-----	0.04
Vitamin A 30/0	-----	0.07
Vitamin A & D 30/10	0.03	-----
Vitamin E 125	0.02	0.05
Chemical Composition		
DM, %	91.10	68.82
OM, %	92.27	94.51
CP, %	14.28	13.60
NDF, %	64.18	27.72
Ash, %	7.73	5.49
Ca, %	1.23	0.91
P, %	0.30	0.31
K, %	1.43	0.78
Mg, %	0.27	0.21
Na, %	0.10	0.06
S, %	0.24	0.21
Fe, ppm	270.16	188.45
Mn, ppm	33.08	27.89
Zn, ppm	45.17	50.68
Cu, ppm	16.17	15.56
Mo, ppm	0.11	0.19

[a] Dry matter basis

[b] Provided 33 mg of monensin/kg DM.

Table 2. Effects of trace mineral supplementation and source on backgrounding and receiving performance of newly weaned calves.

Item	Dietary Treatments			SEM
	Control	Inorganic	Organic	
No. of animals/treatment	125	126	124	---
Backgrounding Phase 0-30d				
Initial Body Weight, kg	238.8	239.2	238.9	4.1
ADG, kg	0.57	0.57	0.61	0.03
Receiving Phase, 0-28d				
Body Weights, kg				
Initial	249.0	249.9	250.9	3.0
Final	290.8	290.3	296.6	4.2
ADG, kg	1.49[b]	1.44[b]	1.63[a]	0.05

Means within a row with differing superscripts differ ($P < 0.05$).

Table 3: Effects of trace mineral supplementation and source on morbidity of receiving steers.

Item	Dietary Treatments			P-value	
	Control	Inorganic TM	Organic TM	TM	TM Source[b]
	-[c]	-[c]	-[c]		
Steers, no.	124	124	125	——	——
Morbidity, %	13/124 (10 %)	17/124 (14 %)	9/125 (7 %)	0.99	0.09
Treated per morbid	1.15	1.06	1.00	0.19	0.60

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with Synovex-S[R.] in the growing phase and Revalor IS in the finishing phase.

Chapter III

EFFECTS OF TRACE MINERAL SUPPLEMENTATION, TRACE MINERAL SOURCE, AND GROWTH IMPLANTS ON PERFORMANCE AND TRACE MINERAL STATUS OF GROWING AND FINISHING FEEDLOT STEERS

ABSTRACT

Three hundred and seventy-three steer calves (approximately 7 mo of age and 247 ± 19.4 kg) were utilized to determine the effects of trace mineral (TM) source and growth implants on performance and trace mineral status. Steers were blocked by ranch, stratified by initial body weight, and randomly assigned to one of 36 pens (9 – 12 head/pen). Pens within a block were then randomly assigned to treatments (12 pens/treatment). Treatment consisted of: 1) control (no supplemental Cu, Zn, Mn, and Co), 2) inorganic trace minerals ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace minerals (iso-amounts of organic Cu, Zn, Mn, and Co). Six pens of steers per treatment received a growth implant (200 mg progesterone and 20 mg estradiol benzoate) at the beginning of the experiment and were re-implanted with 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase. The remaining steers received no growth implant. Steers were fed a corn silage-based growing diet for 56 d then were gradually switched to a high concentrate finishing diet. During the finishing phase, all minerals, excluding Zn, were fed at the recommended concentrations in inorganic form.

Treatments during the finishing phase consisted of: 1) control (no supplemental Zn); 2) inorganic Zn (30 mg of Zn/kg DM from $ZnSO_4$); and 3) organic Zn (iso-amounts of organic Zn). Implanted steers had greater ($P = 0.04$) average daily feed intake than non-implanted steers in the growing phase. On d 56 of the growing phase and d 84 of the finishing phase, implanted steers had greater ($P < 0.01$) plasma Cu concentrations than non-implanted steers. During the growing phase, liver Cu concentrations ($P < 0.01$) and plasma Zn concentrations ($P < 0.02$) were greater in steers that were supplemented with TM compared to control steers. Steers that were supplemented with inorganic minerals had greater liver Cu concentrations than steers supplemented with organic minerals at the beginning ($P < 0.01$) and end ($P = 0.02$) of the growing phase. During both the growing ($P = 0.02$) and finishing phases ($P = 0.05$), non-implanted control steers had greater plasma Cu concentrations than non-implanted steers supplemented with TM, whereas, implanted control steers had similar plasma Cu concentrations than implanted steers supplemented with TM. Non-implanted steers that received inorganic TM had lower plasma Cu concentrations ($P = 0.03$) during the growing phase and ceruloplasm activity ($P < 0.04$) during the finishing phase than non-implanted steers that received organic TM, whereas, implanted steers supplemented with either organic or inorganic TM had similar plasma Cu concentrations. These results indicate that TM supplementation and growth implants may impact performance and mineral status of steers.

Key words: Steers, Performance, Trace Minerals, Growth Implants

INTRODUCTION

The recommended concentration of zinc (Zn) in beef cattle diets is 30 mg of Zn/kg DM (NRC, 1996). Recent reports indicate that supplementing Zn during the

finishing period improves carcass quality and average daily gain (Spears and Kegley, 2002). However, little controlled research has been conducted in this area.

Growth implants are often utilized in cattle production in order to increase gain and feed efficiency (Apple et al., 1991; Duckett et al., 1999). This increase is thought to occur by an increase in protein accretion, which in turn may increase trace mineral requirements. Huerta et al. (2002) reported a decrease in liver Zn concentrations in implanted heifers and steers that were fed a control diet containing 64 mg Zn/kg DM and 84 mg Zn/kg DM, respectively. This decrease in liver Zn concentrations may be due to the importance of Zn in protein metabolism. Zinc forms a tetrahedral complex with cysteine and histidine residues to form Zn finger domains in DNA binding proteins (Berg, 1963), which aid in transcription of messenger RNA and cell replication (Chester, 1978). Increasing protein accretion with the use of growth implants may increase the Zn requirement of an animal. Therefore the objective of this experiment was to determine the effects of trace mineral source and growth implants on performance and trace mineral status of steers.

MATERIALS AND METHODS

Prior to the initiation of this experiment, the Colorado State University Animal Care and Use Committee approved care, handling, and sampling of the animals defined herein.

Three hundred and seventy three steer calves (approximately 7 mo of age and 247 ± 19.4 kg) were utilized in this experiment. Calves were obtained from 3 different Colorado State University Research facilities (127 Hereford x Angus calves from the Maxwell Ranch in Livermore, CO; 135 crossbred calves from the San Juan Basin

Research Center in Hesperus, CO; and 111 Black Angus calves from the Colorado State University Beef Improvement Center in Saratoga, WY).

Prior to the initiation of the study, calves were backgrounded at their respective ranch locations for 30 d. Post-weaning, at each ranch location, calves were weighed on two consecutive days and stratified by body weight into six groups. Groups were then randomly assigned to one of six group pens equipped with bunk feeders and automatic waterers. Pens were then randomly assigned to treatments. Treatments consisted of: 1) control (no supplemental trace minerals), 2) inorganic trace mineral ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace mineral (iso-amounts of organic Cu, Zn, Mn, and Co). Mineral treatments were fed in alfalfa pellets with control calves receiving alfalfa pellets with no supplemental Cu, Zn, Mn, or Co. Calves were allowed free access to grass hay in round bale feeders throughout the 30 d backgrounding phase. On day 28 post-weaning, calves were weighed and transported to the Agricultural Research, Development, and Education Center (ARDEC) feedlot facility in Fort Collins, CO. Transport time varied between ranches. Calves from the Maxwell Ranch traveled approximately 48 km. Calves from the San Juan Basin Research Center in Hesperus, CO, traveled approximately 656 km, and calves from the Colorado State University Beef Improvement Center traveled approximately 230 km. Pre-conditioning data is reported elsewhere.

Receiving/growing phase:

Upon arrival, all calves were weighed (on two consecutive days), vaccinated with Ultrabac®7/Somubac and Bovishield™ 4+L5, and dewormed with Dectomax (Pfizer Animal Health, Exton, PA). Calves were blocked by ranch and stratified by initial body

weight and backgrounding treatment and were sorted into one of thirty-six pens (9-12 head per pen) equipped with automatic waterers. Calves remained on the same trace mineral treatments that they received during the on-farm backgrounding phase. Treatments consisted of: 1) control (no supplemental trace minerals), 2) inorganic trace minerals ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace minerals (iso amounts of organic Cu, Zn, Mn, and Co). Half of the steers from each treatment (6 pens per treatment) were implanted with 200 mg progesterone and 20 mg estradiol benzoate at the initiation of the experiment and the remaining steers (6 pens per treatment) received no implant. Steers were fed a corn silage-based growing diet (Table 1) for 56 days. Diets were formulated to meet or exceed NRC (1996) requirements for energy, protein, macro- and micro-minerals with the exception of Cu, Zn, Mn, and Co. Diets were fed once daily in the morning in amounts adequate to allow ad libitum access to feed throughout the day. Daily feed offerings were recorded and feed refusal was measured every 28 d. Calves were weighed every 28 d and average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (FE) were determined.

Liver biopsies samples were obtained from 3 calves per pen on d 0 and d 56 of the growing phase using the true-cut technique described by Pearson and Craig (1980), as modified by Engle and Spears (2000). Briefly, a 10 cm x 10 cm area on the right side of the steer between the 11^{th} and 12^{th} ribs was clipped of hair and scrubbed three times with betadine alternating with 70 % alcohol. Five milliliters of a two percent lidocaine hydrochloride solution (Abbott Laboratories, Chicago, IL) were injected via a 20-gauge x 2.5 cm needle between the 11^{th} and 12^{th} rib on a line from the tubercoxae to the tip of the shoulder. A small incision (approximately 1.0 cm) was made using a #11 scalpel blade.

A core sample of liver was collected using a modified Jam Shide bone marrow punch (0.5 cm x 14 cm; Sherwood Medical, St. Louis, MO). Following collection, samples were immediately rinsed with 0.154 M phosphate buffered saline (PBS) solution (pH 7.4) and placed into an acid washed polyethylene tube, capped, and stored on ice and transported to the laboratory. Samples were then stored at –20°C until analyzed for trace minerals.

Blood samples were collected in hepranized trace mineral free vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) every 28 d from the same 3 steers per pen that were biopsied for analysis of plasma trace mineral content. On d 0 and d 56, plasma samples were also analyzed for ceruloplasm activity.

Finishing phase:

At the beginning of the finishing phase, steers that had received an implant in the growing phase were re-implanted with 80 mg trenbolone acetate and 16 mg estradiol. The finishing diet was fed until the steers reached a finished weight of approximately 540 kg. Diets were formulated to meet or exceed NRC (1996) requirements for energy, protein, macro- and micro-minerals with the exception of Zn. Steers were fed and offerings and feed refusal were measured as described in the receiving phase. Trace mineral treatments during the finishing phase were: 1) control (no supplemental Zn); 2) inorganic Zn (30 mg Zn/mg DM from $ZnSO_4$); and 3) iso-amounts of organic Zn. Every 28 d during the finishing phase, all calves were weighed and ADG, ADFI, and FE were determined. On day 112, a liver biopsy was collected as previously described from the same 3 calves per pen that were biopsied during the growing phase.

During the finishing phase, blood samples were obtained every 28 d in trace mineral free hepranized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) from the same three calves per pen that were biopsied and analyzed for trace mineral concentrations. On d 84 of the finishing phase, plasma samples were analyzed for ceruloplasm activity.

Analytical Procedures

Blood preparation. All blood samples were centrifuged at 1200 x *g* for 25 min at room temperature. Plasma from blood samples collected in trace mineral free vacutainer tubes was harvested and stored in an acid washed polyethylene tubes until analyzed for plasma trace mineral concentrations and ceruloplasm activity.

Plasma and Liver mineral analysis. Plasma and liver samples were analyzed via inductively coupled plasma-atomic emission spectroscopy (ICP-AES) methods (Braselton et al., 1997 as described by Ahola et al. (2004) for Zn, Cu, Mo, Mn, S, and Fe concentrations. Briefly, samples were thawed and dried for 4 hours at 95°C, then allowed to cool to room temperature. Samples were weighed and then combined with 2 mL of 3.6N nitric acid. The mixture was allowed to digest overnight at 95°C and then cooled to room temperature. Samples were diluted in dH_2O to fit within a linear range of a standard curve generated by linear regression of known TM concentrations. Multielemental analysis was then carried out by the simultaneous/sequential ICP-AES, with cross flow nebulization, procedure.

Plasma samples were prepared for analysis as follows. One mL of 10% trichloroacetic acid (TCA) was added to one mL of plasma or standard and was mixed vigorously. The mixture was placed in a -20°C freezer for 30 minutes to aid in

precipitation and then centrifuged at 1200 x g for 10 minutes. The supernatant was removed and placed into a clean tube and was diluted in dH_20 to fit within a linear range of a standard curve generated by linear regression of known Cu concentrations. Multielemental analysis was also carried out by the simultaneous/sequential ICP-AES, with cross flow nebulization, procedure.

Ceruloplasm activity. Ceruloplasm activity was determined using a procedure described by Houchin (1958). Briefly, 100 □l of plasma were incubated at 37°C for 20 minutes. One milliliter of a 1% para-phenylenediamine (PPD) solution was added. This solution was incubated for 30 minutes at 37°C and five milliliters of 0.02% sodium azide solution was then added. The sample was then read at 525 nm using a spectrophotometer (Spectronic Genesis 5, Spectronic Instruments, Rochester, NY). The results were recorded as absorbency values.

Statistical analysis.

Statistical analysis of data was performed using Proc Mixed procedure of SAS (2001) for a 2 X 3 factorial arrangement in a completely randomized design. The model included the fixed effects of treatment, implant, time, ranch, and all possible interactions. The random effect of pen within treatment × implant was included in the model. When an interaction was not significant, it was removed from the model and the reduced model was re-ran. Initial weights were used as a covariate for body weights and ADFI. Differences among means were determined using preplanned single degree of freedom contrasts. The contrast statements were: 1) inorganic trace mineral vs, organic trace mineral treatments.

RESULTS AND DISCUSSION

Performance:

Trace mineral supplementation and TM source had no effect on performance in the growing (Table 2) and finishing phases (Table 3). Effects of TM supplementation on performance have varied among studies. George et al. (1997) conducted a study utilizing heifer calves fed an alfalfa hay-grass hay-corn diet containing 22.3 mg Zn/kg DM, 9.5 mg Cu/kg DM, 22.2 mg Mn/kg DM, and 0.122 mg Co/kg DM. The heifers were supplemented with one of the following treatments: 1) inorganic TM (106 mg Zn/kg DM from $ZnSO_4$, 37 mg Cu/kg DM $CuSO_4$, 58 mg Mn/kg DM from Mn oxide, and 7 mg Co/kg DM from $CoCO_3$), 2) organic TM (106 mg Zn/kg DM from Zn methionine, 37 mg Cu/kg DM from Cu lysine, 58 mg Mn/kg DM from Mn methionine, and 7 mg Co/kg DM from Co glucoheptonate), or 3) 3 times organic TM (318 mg Zn/kg DM from Zn methionine, 111 mg Cu/kg DM from Cu lysine, 174 mg Mn/kg DM from Mn methionine, and 21 mg Co/kg DM from Co glucoheptonate for 14 d then treatment 2 for the remainder of the study). They reported that TM supplementation and concentration had no effect on DMI, ADG, and FE over the entire 42 d feeding period, but ADG and FE were increased ($P < 0.05$) with the higher concentration of TM from d 29 to d 42 (George et al., 1997). Hatfield et al. (2001) conducted a study utilizing 6-yr old ewes. The ewes were sorted to treatments consisting of no supplemental Zn or Cu (basal diet contained 12.2 mg Zn/kg DM and 5.37 mg Cu/kg DM), Zn-amino acid complex, Zn-amino acid complex plus Cu-amino acid complex, $ZnSO_4$, and $ZnSO_4$ plus $CuSO_4$ to provide 102.2 mg Zn/kg DM and 15.4 mg Cu/kg DM in the total diet (3 times the Zn and 1.5 times the Cu concentrations recommended by the NRC). They reported a tendency for an increase

($P = 0.09$) in final BW in ewes that were supplemented with Zn (Hatfield et al., 2001). Similar to the present study, Droke et al. (1998) and Spears et al. (1991) reported no effect of TM supplementation on growth in lambs and steers supplemented with 25 mg Zn/kg DM from Zn oxide or Zn methionine to a basal diet containing 27.6 and 26.4 mg Zn/kg DM. Droke et al. (1998), Green et al. (1988), and Spears and Kegley (2002) reported that TM supplementation had no effect on ADFI and FE in steers supplemented with 25 mg Zn/kg DM from Zn oxide or Zn methionine, 360 mg Zn/kg DM from Zn oxide or Zn methionine, and 25 mg Zn/kg DM from Zn oxide or Zn proteinate to a basal diet containing 27.6, 82, and approximately 30 mg Zn/kg DM, respectively. In steers fed a corn silage-corn-and soybean meal-based diet (33 mg Zn/kg DM and 16 mg Cu/kg DM), ADG was increased when the steers were supplemented with 25 mg Zn/kg DM as Zn oxide or Zn proteinate (Spears and Kegley, 2002). In contrast, Spears et al. (1991) observed no effect of Zn supplementation on ADG when steers were fed a diet containing 26.4 mg Zn/kg DM. Huerta et al. (2002) found similar results in a study utilizing heifers fed a high concentrate barley diet (64 mg Zn/kg DM), where control heifers and heifers supplemented with Zn (200 mg Zn/kg DM from $ZnSO_4$ or Zn methionine) had similar ADG. However, steers supplemented with Zn (25 mg Zn/kg DM Zn oxide or Zn proteinate containing either 10 or 15 % Zn) had greater ADG than control steers during the growing phase (Spears and Kegley, 2002). Due to the variability reported among studies, it is difficult to determine the direct effects of the effects of TM supplementation on performance. It is possible that other factors such as species, breed, other dietary components, and location of the experiments may play a factor in the outcome of the experiments.

Effects of TM source on performance also vary among studies. Kegley and Spears (1995) reported no effect of TM source (25 mg Zn/kg DM from Zn oxide or Zn methionine) on body weights of ewes. They also reported no effect of TM source on ADG over the 111 d study. Similar results were reported by Droke et al. (1998), Kegley and Spears (1995) and Kegley et al. (2001)where ADFI and FE were also not affected by trace mineral source. In contrast to these studies and the present study, Kegley et al. (2001) reported an increase in ADG in steers supplemented with organic Zn (Zn amino acid complex) compared to steers supplemented with inorganic Zn ($ZnSO_4$) during the last 15 days of their study. This was also reported by Spears (1989), in which growing heifers supplemented with 25 mg Zn/kg DM from Zn methionine tended to have greater ADG ($P < 0.13$) than heifers supplemented with iso-amounts of Zn oxide to a basal diet containing 24 mg Zn/kg DM. It is unclear as to why these studies had varying results and makes it difficult to determine the effects of TM source on performance.

In the present study, implanted steers tended to have greater final body weights ($P = 0.07$) and FE ($P < 0.08$) than non-implanted steers in the growing phase (Table 2). A greater final body weight was also reported in Holstein steers (Ainslie et al., 1992), cross bred steers (Dehaan et al., 1990; Perry et al., 1991, Mader et al., 1994), and heifers (Dehaan et al., 1990), where as, Lemieux et al. (1988), Foutz et al. (1997), and Paisley et al. (1999) reported no change in body weights of implanted verses non-implanted steers. Effects of growth implants on FE have varied. In studies utilizing steers and heifers, FE has been reported to be greater for implanted cattle compared to non-implanted cattle (Dehaan et al., 1990). Guiroy et al. (2002) reported similar results in 7 out 8 trials where implanted heifers had a greater FE compared to non-implanted cattle. In other studies,

FE has been lower in implanted steers than in non-implanted steers (Perry et al., 1991; Ainslie et al., 1992; Johnson et al., 1996). It has also been reported that growth implants have had no effect on FE in heifers, beef steers, and Holstein steers (Mader et al., 1994; Apple et al., 1991). Results may be dependent on the type of implant utilized in the study.

Implanted steers had greater ($P = 0.04$) ADFI than non-implanted steers in the growing phase, but growth implants tended to decrease ($P = 0.06$) ADFI in the finishing phase. Growth implants have been reported to increase ADFI in steers (Dehaan et al., 1990; Perry et al., 1991; Mader et al., 1994; Samber et al., 1996), heifers (Dehaan et al., 1990), and Holstein steers (Ainslie et al., 1992). Johnson et al. (1996) reported a decrease in ADFI in implanted steers from d 41 to d 115 but reported no difference in ADFI from d 0 to d 40 or from d 116 to d 143. In other studies it has been reported that growth implants had no effect on ADFI (Dehaan et al., 1990; Apple et al., 1991; Foutz et al., 1997).

In the present experiment, growth implants had no effect on ADG in the growing and finishing phase. Similar results were reported in steers (Dehaan et al., 1990; Mader et al., 1994; Foutz et al., 1997). In contrast to the present experiment, Huerta et al. (2002) reported that steers had greater ($P < 0.04$) ADG than non-implanted steers. Growth implants also increased ADG in Holstein steers (Apple et al., 1991; Ainslie et al., 1992), heifers (Dehaan et al., 1990; Mader et al., 1994) and feedlot steers (Lemieux et al., 1988, Perry et al., 1991; Johnson et al., 1996).

It is unknown why there was a difference in the effects of growth implants on performance between the trials mentioned above. Different types of implants used in the different experiments may be one reason for these discrepancies. Loy et al. (1988)

reported that steers implanted with 100 mg progesterone and 10 mg estradiol benzoate gained faster than steers that were implanted with 36 mg zeranol. Apple et al. (1991) implanted Holstein steers with 36 mg zeranol, 20 mg estradiol benzoate plus 200 mg progesterone (EP), 140 mg TBA (TBA), TBA plus zeranol, and TBA plus EP. Overall, steers that were implanted with TBA + EP had a greater ADG than steers that were implanted with TBA plus Z, TBA, and Z. The authors stated that combination implants (TBA plus an estrogenic implant) seem to yield greater effects on performance than single compound implants (Apple et al., 1991). However, a combination implant of TBA plus an estrogenic implant affected performance similarly in several other studies as well (Galbraith and Coelho, 1978; Heitzman et al., 1981; Fisher et al., 1986).

Another possible reason why implants have varying effects on performance may be due in part to time of initial implantation and repeated implants over the finishing phase. Scheffler et al. (2003) reported that when Holstein steers were implanted on d 0, d 112, and d 224, d 112 and d 224, or d 224 that implanting the steers multiple times resulted in greater ADG than implanting the steers only once. Scheffler et al. (2003) also indicated that steers that received an initial implant later in the trial resulted in an improved gain:feed. In re-implanted steers, gain:feed was decreased in steers initially implanted with zeranol before the finishing phase (Mader et al., 1985; Simms et al., 1988). Rumsey et al. (1992) conducted 2 trials in which Hereford steers were implanted on d 0 (120 d from slaughter) and re-implanted 60 d prior to slaughter, implanted on d 30 (90 d prior to slaughter), or implanted on d 0 only. Feed intake was greater in steers that were implanted earlier in the trial and in steers that were re-implanted.

There was an implant x TM interaction for FE ($P = 0.03$) during the growing phase. Non-implanted control steers had a lower FE than non-implanted steers supplemented with TM, whereas, implanted control steers had a greater FE than implanted steers supplemented with TM. Huerta et al. (2002) reported an implant x Zn interaction ($P < 0.001$) on performance. Control heifers that received an implant had similar ADG to implanted heifers supplemented with TM, whereas, non-implanted heifers that were supplemented with TM ($ZnSO_4$ and Zn methionine) had greater ADG than non-implanted control heifers. The interaction in the present study seems to be due to a function of the relationship between gain and feed intake in determining FE. Although not significantly different, non-implanted steers supplemented with TM had a greater ADG and a lower ADFI than non-implanted control steers. This increase in ADG and decrease in ADFI would result in a higher FE. Implanted control steers and steers supplemented with TM had similar ADG, however, ADFI was numerically lower for implanted control steers.

There was also a tendency ($P < 0.06$) for a TM source x implant interaction on FE during the growing phase. Non-implanted steers supplemented with inorganic TM tended ($P < 0.06$) to have greater FE than non-implanted steers supplemented with organic TM, whereas, implanted steers supplemented with inorganic TM tended to have lower FE than implanted steers supplemented with organic TM. This difference seems again to be a result of ADG and ADFI. Non-implanted steers supplemented with inorganic TM had numerically greater ADG and numerically lower ADFI than non-implanted steers supplemented with organic TM, which would result in a higher FE.

Implanted steers supplemented with inorganic TM had numerically lower ADG than implanted steers supplemented with organic TM, both having similar ADFI.

Mineral Status:

Liver mineral concentrations. Liver mineral concentrations are shown in Table 4 and Table 5 for the growing and finishing phases, respectively. Liver mineral concentrations were unaffected by implant during the growing phase; and liver Zn and Cu concentrations were unaffected by implant during the finishing phase. Steers that were implanted tended to have greater liver Mn concentrations ($P = 0.06$) than non-implanted steers during the finishing phase. Research investigating the effects of growth implants on liver mineral concentrations is limited. Huerta et al. (2002) reported that non-implanted heifers had greater ($P = 0.08$) liver Zn and Cu concentrations than implanted heifers on d 50 of the study but the effect was not observed on d 120. They also reported no significant difference in liver Zn and Cu concentrations among implanted and non-implanted steers.

Trace mineral supplementation had no effect on liver Zn and Mn concentration in either the growing or finishing phases. Similar results were reported in studies utilizing heifers (Huerta et al., 2002), steers (Mullis et al., 2003), and lactating Holstein cows (Kellogg et al., 1989) supplemented with 200, 25, and 360 mg Zn/kg DM, respectively. Liver Fe concentrations were not affected by TM supplementation in lactating Holstein cows (Kellogg et al., 1989), heifer calves (Kincaid et al., 1997), and steers (Mullis et al., 2003). In contrast to the present study, liver Zn concentrations have been reported to be increased in TM supplemented wethers (Rojas et al., 1995) and steers (Huerta et al., 2002). In a study utilizing ewes, Zn supplemented as either $ZnSO_4$ or a Zn amino acid

complex resulted in greater ($P = 0.04$) liver Zn concentrations than reported in control ewes not supplemented with Zn (Hatfield et al., 2001). Ahola et al. (2004) observed varying results in a two year cow study. The cows received no supplemental Cu, Zn, or Mn or were supplemented with iso-amounts of inorganic TM or organic TM (50 % organic Cu, Zn, and Mn and 50% inorganic Cu, Zn, and Mn) in free choice trace mineral feeders. The basal diet contained native pasture consisting of blue grama, prairie sandreed, and needle-and-thread grass (13.1 mg Cu/kg DM, 16.1 mg Zn/kg DM, and 36.6 mg Mn/kg DM) and millet hay (19.6 mg Cu/kg DM, 32.1 mg Zn/kg DM, and 52.2 mg Mn/kg DM). In year 1, they reported that trace mineral supplementation increased liver Mn concentrations. However, in year 2, trace mineral supplementation decreased liver Mn concentrations, both of which were in contrast to the present study. Ahola et al. (2004) explained that this difference may be due, in part, to liver Mn concentrations not being a good indicator of Mn status in the body. Ivan and Hidiroglou (1980) and Watson et al. (1973) reported that liver Mn only increased 3 to 4 fold when dietary Mn was increase 130 to 140 fold. The reason why liver Zn and Mn concentrations were unaffected by trace mineral supplementation in the present study may be similar. Furthermore, Zn concentrations in tissues shows little change when dietary Zn changes (Miller et al., 1968) because there is little readily available Zn in tissues (McDowell, 1992). Bone may be a better indicator of Zn status than liver (Underwood and Suttle, 1999) but again Zn in bone is not readily available for use by the body (McDowell, 1992). Therefore, the liver may not be the best tissue to examine in order to determine the mineral status of an animal for these minerals.

In the present study, initial liver Cu concentrations were greater ($P < 0.0001$) in TM supplemented steers than controls at the beginning of the growing phase, which may be the result of TM supplementation during the background phase at the ranch. By the end of the growing phase, this difference was still apparent ($P = 0.0002$). Similar results were reported in a study in which steers that were supplemented with Zn, Cu, Mn, and Co (Stanton et al., 1988) and cows that were supplemented with either inorganic or organic TM (Ahola et al., 2004). Huerta et al. (2002) reported contrasting results where control steers tended ($P < 0.14$) to have greater liver Cu concentrations than Zn supplemented steers. In other studies, similar liver Cu concentrations were seen among control and Zn supplemented heifers (Kincaid et al, 1997), steers (Huerta et al., 2002; Mullis et al., 2003), and sheep (Rojas et al., 1995; Hatfield et al., 2001). Trace mineral supplementation had no effect on liver Cu concentration in the finishing phase. The reason that liver Cu concentrations were affected by TM supplementation and Zn, Co, and Mn were not may be due to the livers ability to store and repartition Cu to the rest of the body (Owen, 1980; McDowell, 1992). There was no effect of TM supplementation on liver TM concentrations during the finishing phase.

In the present study, TM source had no effect on liver Zn and Mn concentrations in the growing phase and liver Zn in the finishing phase. In contrast to the present study, Hatfield et al. (2001) reported a TM source effect in ewes. Ewes that were supplemented with organic TM tended ($P = 0.06$) to have greater liver Zn concentrations than ewes supplemented with inorganic TM. Huerta et al. (2002) also reported a TM source effect in feedlot heifers. Heifers supplemented with 200 mg Zn/kg DM from $ZnSO_4$ had greater liver Zn concentrations than heifers supplemented with 200 mg Zn/kg DM from Zn

methionine. The reason for the discrepancies in results reported in these two studies and the present study is unknown. The difference could possibly be due to the type of animals utilized in each study (steers vs. ewes vs. heifers) or possibly could be the result of the total amount of TM being supplemented.

Trace mineral source had an effect on liver Cu concentrations. Steers that were supplemented with inorganic minerals had greater liver Cu concentrations than steers supplemented with organic minerals at the beginning ($P < 0.0001$) and end ($P = 0.02$) of the growing phase. However, this difference was not present at the end of the finishing phase. In contrast to the present study, ewes supplemented with organic TM (Zn amino acid complex) tended to have greater liver Cu concentrations than ewes supplemented with $ZnSO_4$ (Hatfield et al., 2001). This effect was also seen in cows (Ahola et al, 2004).

Steers supplemented with organic TM tended to have greater ($P < 0.07$) liver Mn concentrations than steers supplemented with $ZnSO_4$ in the finishing phase. Spears (1996) proposed a theory that organic sources of minerals were more bioavailable than inorganic sources because the organic sources are more similar to biologically active forms of minerals in the body and in feed. Organic forms of minerals may prevent binding of antagonists or other molecules in the body because they are already bound to a molecule and have less or no binding sites available. Animals fed inorganic sources of minerals have to first convert the minerals to biologically active forms before they can be utilized (Spears, 1996). This may be why the steers had greater liver Mn concentrations when supplemented with organic TM. The reason that liver Cu concentrations were greater when supplemented with inorganic TM is unclear.

There was a TM x implant interaction ($P = 0.0026$) for liver Cu concentrations at the end of the growing phase. Non-implanted control steers had lower liver Cu concentrations than non-implanted steers supplemented with TM. This difference was also observed between implanted control and implanted steers supplemented with TM, where the implanted control steers had lower liver Cu concentrations than steers supplemented with TM, but the difference was more prominent in the non-implanted cattle.

At the end of the growing phase, there was a tendency for a TM source x implant interaction ($P < 0.10$) for liver Zn concentrations. Non-implanted steers that were supplemented with inorganic TM tended to have lower liver Zn concentrations than non-implanted steers supplemented with organic TM, whereas, implanted steers supplemented with inorganic TM had similar liver Zn concentrations than implanted steers supplemented with organic Zn. This interaction was not observed by Huerta et al. (2002).

Plasma mineral concentrations. Growth implants had no effect on plasma Zn concentrations during the growing and finishing phase (Table 6 and 7, respectively). By the end of the growing phase, implanted steers had greater ($P < 0.01$) plasma Cu concentrations than non-implanted steers. This effect was also observed during the finishing phase ($P = 0.0069$). In contrast, Huerta et al. (2002) found no effect of growth implants on serum Cu concentration in heifers and steers, but implanted heifers had greater serum Zn concentration than non-implanted heifers.

Trace mineral supplementation had no effect on plasma Cu concentrations during the growing or the finishing phases. At the beginning of the growing phase, steers that

were supplemented with TM had greater ($P < 0.02$) plasma Zn concentrations than control steers, but were similar throughout the remainder of the study. Plasma and serum mineral concentrations have varied in studies in which cattle and sheep have received a TM supplement vs. a non-supplemented control diet. Mayland et al. (1980) reported that cows and calves that were supplemented with TM had a greater plasma Zn concentration than cows and calves fed the non-supplemented control diet. Steers and heifers supplemented with either $ZnSO_4$ or an organic Zn source had a greater serum Zn concentration than non-supplemented steers and heifers. However, serum Cu concentration were unaffected by TM supplementation in this study (Huerta et al., 2002). Spears et al. (1991) observed no effect of TM supplementation on serum Zn and Cu concentrations when supplementing steers with Zn oxide or Zn Methionine. Various studies have reported contrasting results where Zn supplementation had no effect on serum Zn and Cu concentrations in sheep (Droke et al., 1988) and steers (Chirase et al., 1991; Malcolm and Callis, 2002; Spears and Kegley, 2002). Plasma Zn and Cu concentrations have also been reported to be unaffected by TM supplementation in sheep (Spears, 1989; Kegley and Spears, 1994) and steers (Chirase et al., 1994).

The initial difference in plasma Zn concentrations may be due to the animals having been backgrounded on TM before arriving at the feedlot. Plasma mineral concentrations are stabilized or at adequate concentrations by the mobilization of these minerals form storage depots from the liver when plasma mineral concentrations decrease below adequate levels. In the present study, plasma Zn and Cu concentrations were adequate (Zn < 0.4 mg/L; Cu < 0.6 mg/L; Mills, 1987; Pulz, 1994). Plasma mineral

concentrations may have been unaffected by TM supplementation because plasma mineral concentrations were at adequate concentrations (Mills, 1987).

Trace mineral source had no effect on plasma mineral concentrations during the growing phase and the finishing phase. Serum Zn and Cu concentrations were unaffected by TM source ($ZnSO_4$ and $CuSO_4$ or organic Zn and Cu) in steers (Mullis et al., 2003). In contrast to the present study, Chirase et al (1994) reported an increase in plasma Zn concentrations in steers supplemented with Zn methionine rather than Zn oxide. Heifers supplemented with Zn methionine had a greater serum Zn concentration than heifers supplemented with $ZnSO_4$ (Huerta et al., 2002). Contrasting results were reported in lambs. Lambs supplemented with Zn oxide had greater serum Cu concentrations than lambs supplemented with Zn methionine (Kegley and Spears, 1995). The reason that plasma mineral concentrations were unaffected by TM source in this study is unknown but may be due to plasma mineral concentrations being at adequate concentrations.

There was a TM x implant interaction during the growing phase ($P < 0.02$) and the finishing phase ($P < 0.05$) for plasma Cu concentrations. Non-implanted control steers had greater plasma Cu concentrations than non-implanted steers supplemented with TM, whereas, implanted control steers had similar plasma Cu concentrations than implanted steers supplemented with TM. Huerta et al. (2002) reported a TM x implant interaction ($P = 0.02$) for serum Cu concentrations. Non-implanted control heifers had lower serum Cu concentrations than non-implanted heifers supplemented with TM, whereas, implanted control steers had greater serum Cu concentrations than implanted heifers supplemented with TM. This effect was not observed in steers (Huerta et al., 2002).

There was also a TM source x implant interaction($P = 0.03$) during the growing phase and a tendency ($P < 0.10$) for an interaction during the finishing phase. Non-implanted steers that received inorganic TM had lower plasma Cu concentrations than non-implanted steers that received organic TM, whereas, implanted steers supplemented with either organic or inorganic TM had similar plasma Cu concentrations. Huerta et al. (2002) reported no TM source x implant interaction in neither heifers nor steers.

Ceruloplasm activity. Growth implant, TM supplementation, and TM source had no effect on ceruloplasm activity during the growing finishing phases (Table 6). Ceruloplasm, an enzyme that contains Cu, is necessary for oxidation of Fe (McDowell, 1992). Ceruloplasm catalyzes the conversion of Fe^{2+} (ferrous Fe) to Fe^{3+} (ferric Fe), which is necessary for Fe to be transported as transferrin throughout the body or for Fe to be mobilized from ferritin, the storage form of Fe, and utilized by the body (McDowell, 1992). In the finishing phase of the present study, there was a TM source x implant interaction ($P < 0.04$) for ceruloplasm activity. Implanted steers supplemented with organic TM had greater ceruloplasm activity than implanted steers supplemented with inorganic minerals, whereas, non-implanted steers supplemented with organic TM had similar ceruloplasm concentrations than non-implanted steers supplemented with inorganic TM. Trace mineral supplementation and source had no effect on ceruloplasm activity in a study utilizing steers that were fed a control diet that contained no supplemental TM or that were supplemented with TM (Arthington et al., 2003). In a study utilizing heifers, TM supplementation increased ceruloplasm concentrations, but TM source had no effect on ceruloplasm concentrations (Arthington et al., 2003). Mullis et al. (2003) also reported that TM source (inorganic vs. organic) had no effect on

ceruloplasm concentrations. It seems that TM supplementation and source have little or no effect on ceruloplasm activity. The effects of implants on ceruloplasm activity are unknown because the present study is the first experiment to investigate the effects of implants on ceruloplasm activity. From the present study, it seems ceruloplasm activity is unaffected by growth implants or TM supplementation alone, but an interaction between implants and TM supplementation did exist.

CONCLUSION

The effects of TM supplementation and source on performance and TM concentrations in the body are variable. In the present study, TM supplementation and source had no effect on performance, liver Zn and Mn concentrations, plasma mineral concentrations, and ceruloplasm activity were also unaffected by TM supplementation and source. As mentioned previously, the liver is not always a good indicator of trace mineral status because the liver is not the main storage vessel for all minerals. Plasma trace mineral concentrations are often very stable unless there is a severe deficiency in a certain mineral because the trace minerals will be released from storage to maintain plasma trace mineral concentration at adequate levels. This may be the reason why TM concentrations in plasma were unaffected. Trace mineral supplementation increased liver Cu concentrations. Since the liver is where the majority of Cu is stored and is a good indicator of Cu status, this is probably why an increase in liver Cu was detected. Growth implants tended to increase body weights and FE and increased ADFI in the growing phase and tended to increase ADFI in the finishing phase. Implantation is has often been observed to increase body weights of cattle; and it has been shown that feed intake increases with the use of implants. Growth implants had little effect on liver mineral and

ceruloplasm activity, but increased plasma Cu concentrations. The effect of growth implants on mineral concentrations has not been widely studied and it is unclear how growth implants impact trace mineral status in the body. Further research is needed to determine the effects of growth implants and TM supplementation and source on performance and trace mineral status of beef cattle.

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Table 1: Ingredient composition of basal diets

Ingredient	Backgrounding	Growing	Finishing
		----------%[a]----------	
Corn Silage	-----	17.80	9.31
Alfalfa Hay	94.90	17.32	7.95
Flaked Corn	-----	55.96	78.68
Protein Supplement	5.10	8.92	4.06
Protein Supplement Composition			
Cottonseed Meal	-----	46.00	43.59
Soybean Hulls	38.17	-----	-----
Soybean Meal	-----	20.00	20.16
Soybean Oil	0.62	-----	-----
Sunflower Meal 32 %	10.60	4.52	7.89
Wheat Midds	40.00	-----	-----
Molasses Cane Blend	3.50	-----	-----
Urea	-----	6.98	7.03
Rumensin 80[b]	-----	+	+
Bentonite	1.00	-----	-----
Monocalcium Phosphate	0.82	3.90	3.91
Cobalt Carbonate	-----	-----	< 0.01
Copper Sulfate	-----	-----	0.05
Dyna-K[c]	1.47	3.93	3.96
Limestone	3.27	11.75	11.84
Manganese Sulfate	-----	-----	0.08
Iodine 20 GM	0.02	0.06	0.06
Salt	0.45	0.90	0.91
Selenium	0.02	0.08	0.08
Sulfur Flower	-----	0.04	0.01
Vitamin A 30/0	-----	0.07	0.07
Vitamin A & D 30/10	0.03	-----	-----
Vitamin E 125	0.02	0.05	0.05
Chemical Composition			
DM, %	91.10	68.82	75.62
OM, %	92.27	94.51	96.51
CP, %	14.28	13.60	10.65
NDF, %	64.18	27.72	18.64
Ash, %	7.73	5.49	3.49
Ca, %	1.23	0.91	0.56
P, %	0.30	0.31	0.24
K, %	1.43	0.78	0.51
Mg, %	0.27	0.21	0.14
Na, %	0.10	0.06	0.04
S, %	0.24	0.21	0.15
Fe, ppm	270.16	188.45	126.63
Mn, ppm	33.08	27.89	19.74
Zn, ppm	45.17	50.68	48.42
Cu, ppm	16.17	15.56	14.95
Mo, ppm	0.11	0.19	0.09

[a] Dry matter basis

[b] Provided 20 and 33 mg of monensin/kg DM in the growing and finishing ration, respectively.

Table 2: Effects of TM supplementation, TM source, and growth implants on performance of steers during the growing phase.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Body Weights, kg												
Initial	246.51	252.81	244.09	253.07	243.35	252.63	3.90	—	0.72	—	0.91	—
Final	318.48	330.67	327.32	328.03	323.87	332.10	4.38	0.07	0.39	0.15	0.96	0.70
ADG, kg												
Overall	1.28	1.39	1.47	1.34	1.44	1.41	0.69	0.73	0.22	0.22	0.80	0.44
ADFI, kg												
Overall	10.04	10.34	9.46	10.65	9.90	10.75	0.34	0.04	1.00	0.11	0.39	0.34
FE												
Overall	0.12	0.15	0.14	0.14	0.13	0.15	0.01	0.08	0.55	0.03	0.73	0.06

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate.

Table 3: Effects of TM supplementation, TM source, and growth implants on performance of steers during the finishing phase.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Body Weights, kg												
Initial	318.63	330.48	327.48	327.87	327.87	331.94	5.52	0.07	0.39	0.15	0.96	0.70
Final	520.28	559.20	529.94	560.01	524.65	554.64	9.01	0.67	0.39	0.60	0.52	0.94
ADG, kg												
Overall	1.50	1.44	1.53	1.52	1.51	1.46	0.05	0.45	0.42	0.72	0.35	0.77
ADFI, kg												
Overall	11.09	10.64	11.06	11.48	10.85	10.51	0.30	0.06	0.58	0.28	0.75	0.43
FE												
Overall	0.14	0.14	0.14	0.14	0.14	0.14	0.004	0.79	0.19	0.55	0.52	0.60

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 80 mg trenbolone acetate and 16 mg estradiol.

Table 4: Effects of TM supplementation, TM source, and growth implants on liver and plasma mineral concentrations of steers during the growing phase.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Liver Zn												
Initial	90.19	91.52	101.84	89.79	113.72	98.26	8.28	—	0.14	—	0.17	—
Final	105.21	105.79	82.04	113.03	113.28	106.03	8.28	0.31	0.76	0.68	0.22	0.10
Liver Cu												
Initial	49.63	62.38	197.67	190.19	134.42	122.90	37.51	—	<0.0001	—	<0.0001	—
Final	192.62	225.90	299.36	306.40	248.64	263.44	37.51	0.26	0.0002	0.0026	0.02	0.20
Liver Mn												
Initial	6.88	6.30	7.12	6.74	6.58	7.04	0.73	—	0.37	—	0.69	—
Final	7.32	7.11	7.67	7.32	7.59	7.18	0.73	0.39	0.56	0.78	0.81	0.89
Plasma Zn												
Initial	0.84	0.78	0.89	0.94	0.97	0.99	0.066	—	0.02	—	0.30	—
Final	1.01	0.96	1.02	0.95	1.10	1.07	0.065	0.36	0.36	0.66	0.14	0.41
Plasma Cu												
Initial	1.16	1.30	1.18	1.25	1.17	1.26	0.073	—	0.84	—	0.94	—
Final	1.01	1.18	0.92	1.30	1.03	1.21	0.092	0.0029	0.86	0.02	0.92	0.03

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate.

Table 5: Effects of TM supplementation, TM source, and growth implants on liver and plasma mineral concentrations of steers during the finishing

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Liver Zn												
Initial	105.21	105.79	82.04	113.03	113.28	106.03	8.28	0.31	0.76	0.68	0.22	0.10
Final	96.61	88.55	95.09	108.97	95.60	90.72	8.57	0.96	0.55	0.82	0.31	0.50
Liver Cu												
Initial	192.62	225.90	299.36	306.40	248.64	263.44	37.51	0.26	0.0002	0.0026	0.02	0.20
Final	231.90	267.13	318.86	220.46	255.26	239.00	40.68	0.66	0.82	0.82	0.76	0.84
Liver Mn												
Initial	7.32	7.11	7.67	7.32	7.59	7.18	0.73	0.39	0.56	0.78	0.81	0.89
Final	7.99	9.76	7.16	7.84	8.31	10.88	0.75	0.06	0.71	0.36	0.07	0.18
Plasma Zn												
Initial	1.01	0.96	1.02	0.95	1.10	1.07	0.065	0.36	0.36	0.66	0.14	0.41
Final	1.10	1.14	1.16	1.10	1.18	1.06	0.061	0.36	0.93	0.51	0.89	0.51
Plasma Cu												
Initial	1.01	1.18	0.92	1.30	1.03	1.21	0.092	0.0029	0.86	0.02	0.92	0.03
Final	1.02	1.15	0.91	1.11	1.04	1.13	0.060	0.0069	0.45	0.05	0.21	0.10

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 80 mg trenbolone acetate and 16 mg estradiol.

Table 6: Effects of TM supplementation, TM source, and growth implants on ceruloplasm activity (abs) of steers during the growing and finishing phase.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Growing												
Day 0	0.178	0.224	0.261	0.230	0.178	0.230	0.033	—	0.38	—	0.19	—
Day 56	0.223	0.211	0.206	0.274	0.155	0.242	0.033	0.18	0.97	0.30	0.32	0.18
Finishing												
Day 84	0.176	0.114	0.111	0.105	0.099	0.190	0.033	0.69	0.38	0.12	0.15	0.04

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate in the growing phase and 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase.

Chapter IV

EFFECTS OF TRACE MINERAL SUPPLEMENTATION, TRACE MINERAL SOURCE, AND GROWTH IMPLANTS ON HEALTH OF GROWING AND FINISHING FEEDLOT STEERS

ABSTRACT

Three hundred and seventy-three steers were utilized to determine the effects of growth implants and trace mineral (TM) supplementation and source on TM status and immune response. Steers were stratified by initial body weight and were randomly assigned to one of 36 pens, which were then randomly assigned to treatments. Treatment consisted of: 1) control (no supplemental Cu, Zn, Mn, and Co), 2) inorganic TM ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic TM (iso-amounts of ORG Cu, Zn, Mn, and Co). On d 28 of the experiment, steers from 6 pens per treatment received a growth implant containing 200 mg progesterone and 20 mg estradiol benzoate, while the other half of the steers within the same treatment (6 pens) did not receive a growth implant. Steers were fed a corn silage-based growing diet for 56 d and were then gradually switched to a high-concentrate finishing diet. At the beginning of the finishing phase, only steers receiving growth implants during the growing phase were re-implanted with 80 mg trenbolone acetate and 16 mg estradiol. During the finishing phase (approximately 140 d), all minerals excluding Zn, were fed at NRC recommended concentrations in INORG form. Treatments during the finishing phase consisted of: 1) control (no

supplemental Zn), 2) inorganic Zn (30 mg Zn/kg DM from $ZnSO_4$), and 3) organic Zn (iso-amounts). At the end of the growing phase, implanted steers had greater ($P < 0.01$) plasma Cu concentrations than non-implanted steers and steers receiving supplemental TM had higher liver Cu ($P < 0.01$) and plasma Zn concentrations ($P < 0.02$) than controls. Steers receiving organic TM had greater ($P < 0.02$) total IgM concentrations than inorganic-supplemented steers. On d 56 of the growing phase, implanted steers tended ($P < 0.07$) to have higher IgG antibody titer concentrations specific for pig red blood cells than non-implanted steers. In the finishing phase, steers supplemented with organic TM had higher ($P = 0.04$) ovalbumin antibody titer concentrations than steers supplemented with inorganic TM. These results indicate that TM supplementation, source, and growth implants may impact TM status and immune response in steers.

Key Words: Steer, trace minerals, immune response, growth implants

INTRODUCTION

Stress induced by weaning and transport of feeder calves has been shown to decrease the ability of the animal to respond immunologically to antigens that they may encounter (Kelly, 1980). Despite vaccinations against respiratory disease, morbidity and mortality rates are usually higher and dry matter intakes lower in newly received calves to the feedlot (Hutcheson and Cole, 1986). Typically, trace minerals are fed in excess to compensate for the reduction in feed intake observed in weaned calves arriving to the feedlot. This results in excess mineral excretion by the animal. In order to minimize excretory losses of metals, sources providing better utilization to the animal must be found. To provide better mineral utilization, criteria of mineral adequacy and availability must be established using immunological, hormonal, and biochemical indices.

Although the role of Zn in the immune system is not completely clear, Zn has been shown to be essential to the integrity of the immune system (Hambridge et al., 1986). Zinc is a structural component in superoxide dismutase enzyme (SOD), which aids in destroying free radicals produced during an immune response (Murray et al., 2000). During a Zn deficiency, it has been reported that immune function is impaired through a decrease in T-cell function as well as a decrease in function of many other key components of the immune system (i.e. thymus, natural killer T-cell, neutrophils; Hambridge et al., 1986). Growth implants have been reported to decrease liver Zn concentrations in implanted heifers and steers that were fed a control diet containing 64 mg Zn/kg DM and 84 mg Zn/kg DM, respectively Huerta et al. (2002). This decrease in liver Zn concentrations may be due to zinc's importance in protein metabolism (protein accretion; McDowell, 1992), which seems to be increased by growth implants. If Zn is being utilized for protein accretion and stores are being depleted, less Zn may be available to be utilized by the immune system.

Growth implants are often utilized in cattle production in order to increase gain and feed efficiency (Apple et al., 1991; Duckett et al., 1999). Because of wide use of growth implants by the beef industry and potential effect on Zn status in the body, metabolism may be altered. Therefore the objective of this experiment was to determine the effects of trace mineral source and growth implants on immune function of steers.

MATERIALS AND METHODS

Prior to the initiation of this experiment, the Colorado State University Animal Care and Use Committee approved care, handling, and sampling of the animals defined herein.

Three hundred and seventy three steer calves (approximately 7 mo of age and 247 ± 19.4 kg) were utilized in this experiment. Calves were obtained from 3 different Colorado State University Research facilities (127 Hereford x Angus calves from the Maxwell Ranch in Livermore, CO; 135 crossbred calves from the San Juan Basin Research Center in Hesperus, CO; and 111 Black Angus calves from the Colorado State University Beef Improvement Center in Saratoga, WY).

Prior to the initiation of the study, calves were backgrounded at their respective ranch locations for 30 d. Post-weaning, at each ranch location, calves were weighed on two consecutive days and stratified by body weight into six groups. Groups were then randomly assigned to one of six group pens equipped with bunk feeders and automatic waterers. Pens were then randomly assigned to treatments. Treatments consisted of: 1) control (no supplemental trace minerals), 2) inorganic trace mineral ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace mineral (iso-amounts of organic Cu, Zn, Mn, and Co). Mineral treatments were fed in alfalfa pellets with control calves receiving alfalfa pellets with no supplemental Cu, Zn, Mn, or Co. Calves were allowed free access to grass hay in round bale feeders throughout the 30 d backgrounding phase. On day 28 post-weaning, calves were weighed and transported to the Agricultural Research, Development, and Education Center (ARDEC) feedlot facility in Fort Collins, CO. Transport time varied between ranches. Calves from the Maxwell Ranch traveled approximately 48 km. Calves from the San Juan Basin Research Center in Hesperus, CO, traveled approximately 656 km, and calves from the Colorado State University Beef Improvement Center traveled approximately 230 km. Pre-conditioning data is reported elsewhere.

Receiving/growing phase:

Upon arrival, all calves were weighed (on two consecutive days), vaccinated with Ultrabac®7/Somubac and Bovishield™ 4+L5, and dewormed with Dectomax (Pfizer Animal Health, Exton, PA). Calves were blocked by ranch and stratified by initial body weight and backgrounding treatment and were sorted into one of thirty-six pens (9-12 head per pen) equipped with automatic waterers. Pens within ranch were then assigned to treatments. Treatments consisted of: 1) control (no supplemental trace minerals), 2) inorganic trace minerals ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace minerals (iso amounts of organic Cu, Zn, Mn, and Co). Calves remained on the same trace mineral treatments that they received during the on-farm backgrounding phase. Half of the steers from each treatment (6 pens per treatment) were implanted with 200 mg progesterone and 20 mg estradiol benzoate at the initiation of the experiment and the remaining steers (6 pens per treatment) received no implant. Steers were fed a corn silage-based growing diet (Table 1) for 56 days. Diets were formulated to meet or exceed NRC (1996) requirements for energy, protein, macro- and micro-minerals with the exception of Cu, Zn, Mn, and Co. Diets were fed once daily in the morning in amounts adequate to allow ad libitum access to feed throughout the day.

Upon arrival to the feedlot, blood samples were obtained in non-hepranized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) on days 0, 7, 14, and 21 from 2 to 4 calves per pen and analyzed for infectious bovine rhinotracheitis virus (IBRV) antibody titers. Blood samples were collected in hepranized trace mineral free vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) on d 0, d 28, and d 56 d from the 3 steers per pen to determine superoxide dismutase (SOD) activity. On d 0 and

d 56, plasma samples were collected for the determination of interferon gamma (INF-γ concentrations. Blood samples were also obtained in non-hepranized trace mineral free vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) on d 0 and d 56 for total immunoglobulin G (IgG) and immunoglobulin M (IgM) analysis.

On d 28 of the growing phase, three steers per pen were injected (i.m.) in the neck muscle with 5 ml of a 25% pig red blood cell (PRBC) solution (Engle et al., 1999). Blood samples were obtained in non-hepranized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) prior to injection and 7, 14, and 21 d post injection. All blood samples were stored on ice until transported to the laboratory for processing. At the end of the receiving phase, the animals were gradually switched to a finishing diet over a two week period (Table 1).

Finishing phase:

At the beginning of the finishing phase, steers that had received an implant in the growing phase were re-implanted with 80 mg trenbolone acetate and 16 mg estradiol. The finishing diet was fed until the steers reached a finished weight of approximately 540 kg. Diets were formulated to meet or exceed NRC (1996) requirements for energy, protein, macro- and micro-minerals with the exception of Zn. Steers were fed and offerings and feed refusal were measured as described in the receiving phase. Trace mineral treatments during the finishing phase were: 1) control (no supplemental Zn); 2) inorganic Zn (30 mg Zn/mg DM from $ZnSO_4$); and 3) iso-amounts of organic Zn.

Blood samples were also obtained on d 84 in trace mineral free hepranized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) from the same three calves per pen that were bled in the growing phase to determine SOD activity and INF-γ

concentrations. Another blood sample was taken on d 84 in a non-hepranized trace mineral free vacutainer tube (Becton Dickenson Co., Franklin Lakes, NJ) and serum was analyzed for total IgG and IgM concentrations.

On d 98, three steers per pen in six of the pens per treatment (3 pens receiving growth implants and 3 pens not receiving growth implants) that were injected with PRBC in the growing phase were re-injected with 5ml of a 25 % PRBC solution (i.m.) in the neck muscle to elicit a secondary humoral immune response to PRBC. Blood samples were collected via jugular venapuncture in non-hepranized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) prior to injection and 7, 14, and 21 d post-injection.

Three steers per pen in the remaining 6 pens per treatment (3 pens receiving growth implants and 3 pens not receiving growth implants) that were injected with PRBC in the growing phase were injected with a second antigen to elicit a primary immune response to a second antigen according to a procedure described by Ward et al. (1993) with a few modifications. Briefly, two milliliters of a solution containing 160 mg of ovalbumin (OVA; Sigma A5503), 60 mL Freund's Incomplete Adjuvant (FIA; Sigma F-5506), and 60 mL of sterile phosphate buffered saline (PBS) were injected subcutaneously and one milliliter was injected intradermally to give a total injection of 4000 □g of OVA/animal. Blood samples were collected via jugular venapuncture in non-hepranized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) prior to injection and 7, 14, and 21 d post injection.

Analytical Procedures

Blood preparation. All blood samples were centrifuged at 1200 x *g* for 25 min at room temperature. Plasma and serum from blood samples collected in trace mineral free

vacutainer tubes were harvested and stored in an acid washed polyethylene tubes until analyzed for INF-γ concentrations and serum total IgG and IgM concentrations. One milliliter of the red blood cells (RBC) from the hepranized vacutainer tubes was lysed in 4 mL of cold dH_2O in an acid washed polyethylene tube for the determination of SOD activity. Serum samples for PRBC antibody titer analysis were stored in polystyrene tubes. Serum samples for OVA antibody titer analysis were divided equally into three microcentrifuge tubes and were heated for 30 min at 56°C. All samples, except plasma for INF-γ determination, were stored at -20°C. Plasma for INF-γ was store at –70°C.

Superoxide dismutase enzyme activity. Lysed RBC were analyzed for SOD activity using SOD 525™ Assay Kit (Biotech® 21010; Oxis Health Products, Inc., Portland, OR). Briefly, 250 µL of sample was vortexed with 400 µl of a chloroform/ethanol solution (62.5/37.5 v/v) to remove hemoglobin interference. The mixture was centrifuged at 4°C for 5 minutes and the upper aqueous layer was collected (extracted sample) and placed into a clean microcentrifuge tube. Nine hundred microliters of buffer (50 mM 2-amino-2methyl-1,3-propanediol containing 3.3 mM boric acid and 0.11 mM diethylenetriaminepentaacetic acid (DTPA); pH 8.8), 40 µL of extracted sample, and 30 µL of reagent 2 (33.3 mM 1,4,6-trimethyl-2-vinylpyridinium trifluoromethanesulfonate in 1mM HCl) were mixed together by vortexing. The mixture was incubated at 37°C for 1 min. Thirty microliters of reagent 1 (0.66 mM 5,5,5a,11b tetrahydro-3,9,10-trihydroxybenzo[c]flourene in 32 mM HCl containing 0.5 mM DTPA and 2.5% ethanol) was added to this mixture. The mixture was immediately read at 525 nm using a spectrophotometer (Spectronic Genesis 5, Spectronic Instruments, Rochester, NY). Superoxide dismutase activity was expressed as SOD activity per milligram

hemoglobin. Hemoglobin concentrations was determined using a Total Hemoglobin Assay Kit (Sigma 525-A). Twenty microliters of whole blood was mixed with 5 mL of Drabkin's solution (sodium bicarbonate, potassium ferricyanide, and potassium cyanide; 100 parts:20 parts:5 parts, respectively). The solution set at room temperature for 15 min and was read at 540 nm using a spectrophotometer (Spectronic Genesis 5, Spectronic Instruments, Rochester, NY).

Total serum IgG and IgM. Total IgG and IgM concentrations were determined using single radioimmunodiffusion assay kits (VMRD 240-30 and 246-30; Pullman, WA) as described by Stable et al. (1993). Briefly, three microliters of serum were added to a well on a single radial immunodiffusion (SRID) plate that contained a monospecific antisera specific for either IgG or IgM. The plates were covered and left undisturbed for approximately 18 hours at room temperature. When the serum diffused into the gel containing the antisera, a ring of precipitation formed that was proportional to the concentration of IgG or IgM in the serum. The diameter of the rings that formed was measured with a ruler in millimeters. These measurements were then compared to standards of known concentrations.

Interferon gamma. Plasma samples were analyzed for INF-γ concentration using an ELISA assay (Biosource KBC1231, Biosource International, Inc., Camarillo, CA). The assays was designed to be a qualitative assay. The assay for this study was modified into a quantitative assay. Positive control that was supplied with the kit was diluted with negative control to make standards. The standards consisted of an undiluted positive control, and positive control diluted to $1/4^{th}$, $1/16^{th}$, and $1/64^{th}$ of the positive controls original concentration. Linear regression coefficient analysis of sample absorbency

verses the expected absorbency yielded a correlation coefficient of 0.997 showing that a semi-quantitative assay could be constructed. Samples were reported as $\log_{10}$.

Pig red blood cell antibody titers. Antibody titers specific for PRBC were measured using a microtiter hemagglutination assay to determine total immunoglobulin (Ig), immunoglobulin G (IgG), and immunoglobulin M (IgM) concentrations specific for PRBC (Ferket and Qureshi, 1992). Serum was heat inactivated in H_2O bath for 30 min at 56°C. A set of 96 well (V-bottom) microtiter plates was prepared. Plate one was for determination of total immunoglobulin (total Ig) concentration. Plate two was for determination of IgG concentration. Into the plate one, 25 µl of 0.1 M PBS (pH 7.4) was pipetted into the first row of wells. Into the second plate, 12.5 µl of 0.1 M PBS (pH 7.4) and 12.5 µL of 0.2 M Mercaptoethanol solution (ME) was pipetted into the first row of wells (ME destroys IgM antibody titers). In duplicate on each plate, 25 µL of serum was added. The plates were covered and incubated for 30 min at 37°C. Once incubated, 25 µL of PBS was added to the remaining wells. Using a microdiluter, a serial dilution of the solution in row one was conducted (part of the solution in row one was diluted into row two; part of the solution in row two was diluted into row 3; etc). Once the dilution was completed, 25 µL of 2.0% PRBC solution was added to each well. The plate was covered and incubated for 30 min at 37°C. After sitting at room temperature, the plates were read. The plates were read by tilting the plate slightly and determining at which dilution (row) agglutination of RBC to the antibody titers was last noticeable (the last well where a small ball was formed). The duplicates were averaged for each sample on each plate. Immunoglobulin M was determined by subtracting the values determined on

the second plate (IgG) from the values determined on the first plate (total Ig). Samples were reported as $\log_2$.

Ovalbumin antibody titers. Serum samples were analyzed for antibody titers specific to ovalbumin using an ELISA procedure described by Engvall and Perlmann (1972). Prior to initiation of the assay, each well of a 96 well (flat bottom) microtiter plates was coated with 100 μL of an Ovalbumin/sodium carbonate-bicarbonate buffer solution (50 μg Ovalbumin/mL). The plates were incubated at 4°C for 18 hours. Plates were washed 3 times with a PBS-0.1% Tween solution. To each well, 100μL of PBS-1% gelatin solution was added and the plates were incubated for 30 min at 39°C. Plates were washed 3 times with PBS-0.1% Tween solution. One hundred microliters of serum (diluted 1:16 with dH_2O) and positive and negative controls were added to the wells in duplicate. The plates were incubated for 1 hour at 39°C and washed 3 times with PBS-0.1% Tween. One hundred microliters of an anti-bovine IgG-peroxidase conjugate (Sigma A5295; diluted 1:20,000 with dH_2O) was added to each well. The plates were incubated for 1 hour at 39°C and were washed 3 times with PBS-0.01% Tween. To each well, 100 μL of substrate solution (containing Citric acid buffer, 2,2`-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma A1888), and 30% hydrogen peroxide) was added. The plates were incubated at room temperature for 15 min and were read at 405 nm on a microtiter plate reader. Samples were reported as $\log_{10}$.

Statistical analysis.

Statistical analysis of data was performed using Proc Mixed procedure of SAS (2001) for a 2 x 3 factorial arrangement in a completely randomized design. The model

included the fixed effects of treatment, implant, time, ranch, and all possible interactions. The random effect of pen within treatment × implant was included in the model. When an interaction was not significant, it was removed from the model and the reduced model was re-ran. Initial weights were used as a covariate for body weights and ADFI. Differences among means were determined using preplanned single degree of freedom contrasts. The contrast statements were: 1) inorganic trace mineral vs, organic trace mineral treatments.

RESULTS AND DISCUSSION

Infectious bovine rhinotracheitis virus antibody titer concentrations:

The effects of growth implants on IBRV antibody titer concentrations were not determined in this study because at the time of serum collection the steers were not implanted. There was no effect of TM supplementation or source on IBRV antibody titer concentrations (data not shown). Similar results were reported in a study utilizing steers pastured on Bermudagrass Hay that were not supplemented with TM or supplemented with $ZnSO_4$ or a Zn amino acid complex to provide 360 mg Zn/d (Kegley et al., 2001). Rhoads et al. (2003) also reported that TM supplementation had no effect on antibody titer concentrations to IBRV in steers fed a corn-alfalfa based basal diet (3 mg Cu/kg DM, 20.1 mg Zn/kg DM, and 9.6 mg Mn/kg DM) supplemented with organic Cu, Zn, Mn, and Co (1x or 2x NRC recommended concentrations in an amino acid complexed form) or $CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$ (3x or 6x NRC recommended concentrations). In contrast to these studies and the present study, George et al. (1997) reported a treatment x time interaction ($P < 0.01$) for IBRV antibody titer concentration in Hereford and Angus-Gelbveih crossbred heifers fed a corn-alfalfa-grass hay basal diet

(22.3 mg Zn/kg DM, 9.5 mg Cu/kg DM, 22.2 mg Mn/kg DM, and 0.12 mg Co/kg DM) that were vaccinated for IBRV. The heifers were supplemented with one of the following treatments: 1) inorganic elements (Cu, Zn, Mn, Co) at NRC recommended concentrations (INORG-1x); 2) organic elemental complexes iso-elemental to treatment 1 at NRC recommended concentrations (ORG-1x); and 3) organic elemental complex at 3x the NRC recommended concentrations for 14 d then ORG-1x for the remaining 42 d of the study (3x/1x). At d 14 post vaccination, steers that were supplemented with ORG-1x had a greater ($P < 0.01$) concentration of antibody titers to IBRV than steers that were supplemented with INORG-1x. By d 28 post-injection, steers that were supplemented with ORG-1x had a greater ($P < 0.01$) concentration of antibody titers to IBRV than steers that were supplemented with INORG-1x and 3x/1x (George et al., 1997).

Superoxide dismutase activity:

Superoxide dismutase (SOD) contains 2 atoms of Cu and 2 atoms of Zn (Murray et al., 2000). Copper is important for catalytic activity (Paytner, 1987) and Zn plays a structural role in the enzyme. Superoxide dismutase aids in antioxidant defense (Committee on Animal Nutrition et al., 1980) by ridding the body of superoxide radicals (O_2^-) produced during a normal immune response that lead to tissue degradation by causing oxidative damage to cellular membranes (Stabel and Spears, 1990; Cerone et al., 2000). Superoxide dismutase converts O_2^- to hydrogen peroxide (H_2O_2) and oxygen (O_2). Glutathione peroxidase (GSH-PX) then reduces H_2O_2 to water (H_2O) decreasing the harmful effects of the O_2^- (Murray et al., 2000).

In the present study, SOD activity was unaffected by growth implants, TM, or TM source (Table 2). When TM are sufficient in the diet, SOD activity seems to be

unaffected. In a trial by Ward et al. (1993), SOD activity was unaffected in Angus and Angus x Hereford steers when fed a corn silage based basal diet containing 6.2 mg Cu/kg DM supplemented with either $CuSO_4$ or Cu lysine. Torre et al. (1995) conducted a study using 10 primigravid Holstein heifers that were approximately 2-years of age. The heifers were fed a basal diet composed of alfalfa silage, corn silage, and cottonseed and contained 6 to 7 mg Cu/kg DM. The treatments consisted of no supplemental Cu or 20 mg Cu/kg DM from $CuSO_4$. There were no differences in SOD activity between treatments (Torre et al., 1995).

Superoxide dismutase activity in RBC seems to be only affected during a severe, prolonged Cu deficiency (less than 10 mg Cu/kg DM and 20 mg Zn/kg DM in the liver and less than 0.6 mg Cu/L and 0.4 mg Zn/L in plasma for extended periods of time; Paytner, 1987; Mills, 1987). Superoxide dismutase in RBC has a longer half-life than other enzymes, such as plasma ceruloplasm, because the half-life of a RBC is approximately 3 months (Murray et al., 2000). The steers in the present study were not deficient in either Cu or Zn (data shown elsewhere). This is most likely the reason why no difference in SOD activity was observed between TM treatments.

Total Immunoglobulin G and Total Immunoglobulin M concentrations:

Immunoglobulin G constitutes 80% of the total serum immunobulins (Kuby, 1994). There are 4 subclasses of IgG that assists in protecting the developing fetus, activating complement system, and binding to antigen and phagocytic cells to initiate phagocytosis (Kuby, 1994). Immunoglobulin M makes up 5 to 10 % of total serum immunoglobulin (Kuby, 1994). Immunoglobulin M is always found in the serum in a pentameter form (5 IgM bound together). During a primary immune response, IgM is the

first antibody produced. Immunoglobulin M seems to be more efficient than IgG. It takes a smaller concentrations (100 to 1000 times less) of IgM molecules to perform the same tasks as IgG molecules (Kuby, 1994).

In the present study, total immunoglobulin G and total IgM concentrations were defined as not being specific for any antigen. This analysis was used to determine the total IgM and IgG concentrations already present in the body. Growth implants and TM supplementation had no effect on total IgG and total IgM concentrations in both the growing and finishing phases (Table 3). At the end of the growing phase, there was a TM source effect on total IgM concentrations. Steers that were supplemented with organic TM had a greater ($P < 0.02$) concentration of IgM than steers supplemented with inorganic TM. This difference had disappeared by d 84 of the finishing phase. Total IgG concentrations were not affected by TM source in either the growing or the finishing phase. Huerta et al. (2002) reported a TM source effect on IgG concentrations in heifers fed a barley-corn-pea hay based basal diet (64 mg Zn/kg DM) supplemented with 200 mg Zn/kg DM from either $ZnSO_4$ or Zn methionine. Heifers supplemented with inorganic Zn tended to have a lower ($P < 0.10$) concentration of IgG than steers supplemented with organic Zn. Immunoglobulin M concentrations were not measured in this study.

At the end of the growing phase, there was an implant x TM interaction for both total IgG and total IgM concentrations. Non-implanted control steers had a greater concentration of IgM ($P = 0.03$) and tended to have a greater concentration of IgG ($P < 0.10$) than non-implanted steers supplemented with TM, whereas, implanted control steers had a lower concentration of IgM and tended to have a lower concentration of IgG than implanted steers supplemented with TM.

There was also a TM source x implant interaction at the end of the growing phase for total IgM concentrations. Non-implanted steers supplemented with inorganic TM had a lower (P = 0.05) concentration of IgM than non-implanted steers supplemented with organic TM. Implanted steers supplemented with inorganic TM also had a lower concentration of IgM than implanted steers supplemented with organic TM but the difference was not as large as that observed for the non-implanted steers.

Interferon gamma concentrations.

There was no effect of implant, TM supplementation, and TM source on INF-γ concentrations in both the growing and finishing phase (Table 4). Interferon gamma is a cytokine that is released from cytotoxic T cells (important in lysing infected cells), T helper cells (responsible for cell mediated functions), and natural killer cells (important in tumor cell lysis; Kuby, 1994; Janeway and Travers, 1996). Interferon gamma blocks viral replication, increases the production of Major Histocompatibility Complex I (MHC I; important in T cell recognition of infected cells), activates macrophage, and aids in B-cell differentiation (Kuby, 1994; Janeway and Travers, 1996). Interferon gamma also works as a positive feedback mechanism on cells to increase the activity of cytotoxic T cells, macrophage, and natural killer cells (Kuby, 1994; Janeway and Travers, 1996).

The reason there was no affect of TM source or growth implants on INF-γ in the present study is unclear and little research has been conducted in this area. As with SOD activity, INF-γ may only be affected during a TM deficiency. Because of the importance of INF-γ in helping to facilitate an immune response to an antigen, it is possible that TM may be preferentially utilized from other parts of the body by the immune system. In the

present study, based on TM status, all treatments were adequate in TM status (data reported elsewhere).

Humoral immunity.

Kuby (1994) defines humoral immunity as the host's defenses that are mediated by antibody present in the plasma, lymph, and tissue fluids. B-cells interact with antigen present in the body. B-cells will proliferate and differentiate into plasma cells that secrete antibodies specific to the antigen that the B-cell interacted with. The antibodies will then attach themselves to the antigen, which labels the antigen as foreign. Macrophage and other immune cells will then recognize the antigen as foreign and will phagocytose the antigen and destroy it (Kuby, 1994). In the present study, the humoral branch of the immune system was challenged with two antigens: ovalbumin and pig red blood cells.

Antibody titers to pig red blood cells. Pig red blood cells are often utilized as an antigen in cattle. In the present study, steers were exposed to PRBC twice to illicit a primary and a secondary immune response to PRBC. The primary immune response (the first injection during the growing phase) exposes the animal to the antigen. When the animal is initially exposed to the antigen, B-cells will be exposed to the antigen and initiate proliferation and differentiation to become antibody secreting plasma cells (Kuby, 1994). The antibodies produced will be specific to the PRBC antigen. Memory B-cells are also produced. If the antigen re-infects the body, antibodies to the antigen are already available and the body does not have to go through the long process of re-producing the antibodies (Kuby, 1994). The secondary immune response (the second injection during the finishing phase) utilizes these memory B-cells in order to combat the antigen. The

body recognizes that it has seen this antigen previously and activates the memory B-cells from their "hibernation" state. The memory B-cells will then produce antibodies to the antigen. When the body is subjected to an antigen for the second time, the immune response should be quicker because the antibodies are already present in the body.

In the present study, within the serum samples collected prior to the initial injection during the growing phase, antibody titers to PRBC were detected (Table 5). This signifies that the steers may have been exposed to PRBC previously. It is unknown if the steers were previously exposed to PRBC; and it is unknown why antibody titers were present prior to the injection. One potential reason why antibody titers were detected may be the result of the sensitivity of the assay. As mentioned previously, the results of this assay are determined by tilting the plate slightly and determining at which dilution (row) agglutination of RBC to the antibody titers was last noticeable (the last well where a small ball was formed). The results determined on one plate may vary between "readers". One person may see agglutination where another person may not. This may be why agglutination was seen on d 0 of the growing phase. The reader may have mistaken a well to have agglutination of antibody titers to PRBC when agglutination was not present.

During the growing phase, there were no differences in total immunoglobulin antibody titer concentrations and IgM antibody titer concentration to PRBC. Overall, steers that were implanted tended to have greater ($P < 0.07$) IgG antibody titer concentrations to PRBC than non-implanted steers. During the finishing phase, there were no significant differences in total Ig, IgG, and IgM antibody titer concentrations to PRBC (Table 6). Similar to the present study, Droke et al. (1998) reported no effect of

TM supplementation or TM source (25 mg Zn/kg DM from Zn oxide or Zn methionine) on antibody titer concentrations to PRBC in lambs fed a basal diet containing 27.6 mg Zn/kg DM. Droke et al. (1993) conducted another study in lambs fed a cottonseed hull based diet that was severely deficient in Zn (3.7 mg Zn/kg DM). The lambs received the basal diet alone or were supplemented with 5 mg Zn/kg DM from Zn oxide (marginal concentrations of Zn) or 40 mg Zn/kg DM from Zn oxide). Droke et al. (1993) reported no differences in concentrations of antibody titers to PRBC in the lambs. In two different studies, there was also no effect of TM supplementation on antibody titer concentrations to PRBC in steers supplemented with inorganic or organic TM to a corn silage-soybean meal basal diet (Engle et al., 1999; Engle et al., 1999b).

Antibody titers to ovalbumin. Ovalbumin is a phosphorylated-glycoprotein that is the major protein constituent of egg whites (Product information sheet, Sigma A5503). Ovalbumin is used as a carrier protein to conjugate to synthetic peptides for use as an immunogen. Ovalbumin is not very antigenic antigen when it is presented to the body alone. Ovalbumin is often combined with Freund's Incomplete Adjuvant (FIA) to make it more antigenic. Freund's Incomplete Adjuvant is a water-in-oil emulsion made from non-metabolizable oils (paraffin oil and mannide monooleate; Product information sheet, Sigma 5506). Freund's incomplete adjuvant is designed to provide continuous release of antigens, which provides a strong, persistent immune response.

In the present study, growth implants had no effect on antibody titer concentration to ovalbumin antigen. There was a TM source and a tendency for a TM supplementation effect on antibody titer concentrations to ovalbumin antigen overall (Table 7). Steers that were supplemented with TM tended ($P < 0.10$) to have greater antibody titer

concentrations to ovalbumin antigen than control steers, with steers supplemented with organic TM having greater (P = 0.04) antibody titer concentrations to ovalbumin antigen than steers supplemented with inorganic TM.

It is unclear why there is a difference in the effects of growth implants and TM supplementation and source between antigens utilized in this study. Growth implants tend to increase body growth quickly. The stress associated with an increase in growth rates may come from the body utilizing a vast amount of nutrients for growth at the expense of other functions. In the present study, implanted steers produced a greater concentration of IgG antibody titers to PRBC and, although not significant, a numerically greater concentration of total antibody titers to PRBC than non-implanted steers.

In the present study, steers that were supplemented with organic Zn had greater concentration of antibody titers to ovalbumin than steers supplemented with inorganic Zn, whereas, TM source had no effect on antibody titer concentration to PRBC. Although supplementing Cu instead of Zn, similar results were reported in a study by Dorton et al. (2002) where steers that were supplemented with organic Cu had greater antibody titer concentrations to ovalbumin antigen and lower antibody titer concentrations to PRBC than steers that were supplemented with inorganic Cu. Hutcheson (1989) indicated that animals supplemented with organic Cu sources respond better to "stressors", such as infection or adverse environmental conditions. This concept may hold true for organic Zn sources as well. Animals supplemented with organic Zn sources may respond better to "stressors". The "stressor" in this study would be the Freund's incomplete adjuvant that was mixed with the ovalbumin antigen. As mentioned above, Freund's incomplete adjuvant is often used to take a less antigenic antigen (such

as OVA) and make it more antigenic (Kuby, 1994). Freund's incomplete adjuvant also prolongs the antigenic persistence from days to weeks and induces granuloma formation that may be responsible for facilitating antigen processing and presentation and T-cell activation (Kuby, 1994). Thus, Freund's incomplete adjuvant induces not only the humoral branch of the immune system, but also the cell-mediated immune system (T-cell mediated immune response).

CONCLUSION

Growth implants, TM supplementation, and TM source had varying effects on the immune system. Growth implants, TM supplementation, and TM source had no effect on total IgG concentrations, SOD activity, and INF-γ concentrations throughout the entire trial. Trace mineral supplementation tended to increase antibody titer concentrations to ovalbumin. When organic TM were supplemented to the steers, there was a greater concentration of total IgM during the growing phase and greater concentrations of antibody titer specific to ovalbumin. This signifies that when an antigen is more harmful to the body it seems that steers that are supplemented with organic TM have a greater immune response to the antigen.

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Table 1: Ingredient composition of basal diets

Ingredient	Backgrounding	Growing	Finishing
		----------%[a]----------	
Corn Silage	-----	17.80	9.31
Alfalfa Hay	94.90	17.32	7.95
Flaked Corn	-----	55.96	78.68
Protein Supplement	5.10	8.92	4.06
Protein Supplement Composition			
Cottonseed Meal	-----	46.00	43.59
Soybean Hulls	38.17	-----	-----
Soybean Meal	-----	20.00	20.16
Soybean Oil	0.62	-----	-----
Sunflower Meal 32 %	10.60	4.52	7.89
Wheat Midds	40.00	-----	-----
Molasses Cane Blend	3.50	-----	-----
Urea	-----	6.98	7.03
Rumensin 80[b]	-----	0.19	0.32
Bentonite	1.00	-----	-----
Biofos[c]	0.82	3.90	3.91
Cobalt Carbonate	-----	-----	< 0.01
Copper Sulfate	-----	-----	0.05
Dyna-K	1.47	3.93	3.96
Limestone	3.27	11.75	11.84
Manganese Sulfate	-----	-----	0.08
Iodine	0.02	0.06	0.06
Salt	0.45	0.90	0.91
Selenium	0.02	0.08	0.08
Sulfur Flower	-----	0.04	0.01
Vitamin A 30/0	-----	0.07	0.07
Vitamin A & D 30/10	0.03	-----	-----
Vitamin E 125	0.02	0.05	0.05
Chemical Composition			
DM, %	91.10	68.82	75.62
OM, %	92.27	94.51	96.51
CP, %	14.28	13.60	10.65
NDF, %	64.18	27.72	18.64
Ash, %	7.73	5.49	3.49
Ca, %	1.23	0.91	0.56
P, %	0.30	0.31	0.24
K, %	1.43	0.78	0.51
Mg, %	0.27	0.21	0.14
Na, %	0.10	0.06	0.04
S, %	0.24	0.21	0.15
Fe, ppm	270.16	188.45	126.63
Mn, ppm	33.08	27.89	19.74
Zn, ppm	45.17	50.68	48.42
Cu, ppm	16.17	15.56	14.95
Mo, ppm	0.11	0.19	0.09

[a] Dry matter basis

[b] Provided 33 mg of monensin/kg DM.

Table 2: Effects of TM supplementation, TM source, and growth implants on superoxide dismutase activity in steers in the growing and finishing, phase, U/mg hemoglobin.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Growing												
Day 0	0.425	0.484	0.373	0.452	0.389	0.423	0.100	—	0.38	—	0.90	—
Day 56	0.489	0.525	0.478	0.482	0.436	0.440	0.100	0.68	0.21	0.59	0.35	0.77
Finishing												
Day 140	0.819	0.755	0.667	1.093	0.739	0.772	0.100	0.30	0.82	0.50	0.42	0.34

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate in the growing phase and 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase.

Table 3: Effects of TM supplementation, TM source, and growth implants on total IgG and total IgM concentrations in growing and finishing steers, mg/dl.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
IgG												
Growing												
Day 0	2150.00	1600.00	1966.67	1766.67	1758.33	2083.33	162.88	-----	0.92	-----	0.80	-----
Day 56	1933.33	1500.00	1658.33	1716.67	1758.33	1925.00	126.92	0.51	0.66	0.10	0.23	0.45
Finishing												
Day 84	1558.33	1666.67	1933.33	1658.33	1666.67	1516.67	142.45	0.37	0.52	0.42	0.16	0.35
IgM												
Growing												
Day 0	274.82	177.77	193.03	207.63	155.53	243.58	42.11	-----	0.57	-----	0.99	-----
Day 56	227.07	133.85	118.58	162.32	185.40	210.40	22.56	0.66	0.57	0.03	0.02	0.05
Finishing												
Day 84	169.10	201.58	149.12	127.43	126.58	127.43	45.17	0.92	0.19	0.53	0.80	0.79

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate in the growing phase and 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase.

Table 4: Effects of TM supplementation, TM source, and growth implants on INF-γ concentrations of steers in the growing and finishing phase,

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Growing												
Day 0	0.152	0.208	0.168	0.179	0.180	0.160	0.033	-----	0.68	-----	0.88	-----
Day 56	0.085	0.167	0.111	0.114	0.138	0.113	0.033	0.51	0.83	0.44	0.73	0.86
Finishing												
Day 84	0.113	0.180	0.106	0.145	0.102	0.107	0.033	0.23	0.33	0.40	0.57	0.34

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate in the growing phase and 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase.

Table 5: Effects of TM supplementation, TM source, and growth implants on antibody titer concentration specific for PRBC of steers in the growing phase, log_2.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Total Ig												
d 0	0.06	0	0.05	0.07	0.11	0.06	0.27	—	0.36	—	0.59	—
Overall	1.85	2.02	1.71	2.03	2.06	2.15	0.17	0.15	0.75	0.92	0.14	0.83
IgG												
d 0	0.22	0.22	0.33	0.20	0.11	0.28	0.25	—	0.88	—	0.44	—
Overall	1.85	1.96	1.73	1.97	1.83	2.12	0.15	0.07	0.96	0.86	0.38	0.84
IgM												
d 0	-0.17	-0.22	-0.22	-0.22	0	-0.22	0.20	—	0.77	—	0.33	—
Overall	-0.001	0.057	0.028	-0.009	0.222	0.028	0.117	0.53	0.30	0.75	0.69	0.58

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate in the growing phase.

Table 6: Effects of TM supplementation, TM source, and growth implants on antibody titer concentration specific for PRBC of steers in the finishing phase, log_2.

	Dietary Treatments						SEM[a]	P- value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Total Ig												
Day 0	0.78	0.89	0.83	1.17	1.39	1.44	0.32	0.50	0.17	0.18	0.53	0.58
Overall	3.13	3.04	3.08	3.08	3.27	3.33	0.25	0.99	0.47	0.88	0.27	0.81
IgG												
Day 0	1.00	1.00	0.89	0.83	0.67	0.89	0.17	0.73	0.12	0.49	0.22	0.66
Overall	3.26	3.13	2.91	3.15	3.00	3.33	0.28	0.55	0.23	0.56	0.79	0.65
IgM												
Day 0	-0.22	-0.11	-0.06	0.33	0.72	0.55	0.41	0.73	0.12	0.49	0.22	0.66
Overall	-0.13	-0.08	0.17	-0.07	0.17	0	0.21	0.50	0.37	0.41	0.87	0.52

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase

Table 7: Effects of TM supplementation, TM source, and growth implants on antibody titer concentration specific for ovalbumin antigen of steers in the finishing phase, log_{10}.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant x TM	TM Source[b]	TM Source x Implant
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Day 0	0.014	0	0.006	0.004	0.022	0.008	0.012	0.27	0.77	0.70	0.37	0.73
Day 7	0.017	0.028	0.036	0.020	0.019	0.022	0.014	0.95	0.91	0.88	0.56	0.82
Day 14	0.067	0.010	0.049	0.042	0.065	0.081	0.025	0.44	0.35	0.33	0.29	0.76
Day 21	0.007	0.011	0.009	0.010	0.012	0.013	0.003	0.57	0.46	0.80	0.27	0.83
Overall	0.027	0.012	0.025	0.019	0.030	0.031	0.006	0.72	0.10	0.71	0.04	0.57

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase.

Chapter V

EFFECTS OF TRACE MINERAL SUPPLEMENTATION, TRACE MINERAL SOURCE, AND GROWTH IMPLANTS ON CARCASS CHARACTERISTICS AND LIPID METABOLISM OF GROWING AND FINISHING FEEDLOT STEERS

ABSTRACT

Three hundred and seventy three steer calves (approximately 7 mo of age and 247 (19.4 kg) were utilized to determine the effects of trace mineral (TM) source and growth implants on carcass characteristics, lipid metabolism, and fatty acid enzyme activity. Steers were blocked by backgrounding treatment within ranch, stratified by initial body weight, and randomly assigned to one of 36 pens (9 – 12 head per pen). Pens within a block were then randomly assigned to treatments (12 pens per treatment). Treatment consisted of: 1) control (no supplemental Cu, Zn, Mn, and Co), 2) inorganic trace minerals ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace minerals (iso-amounts of organic Cu, Zn, Mn, and Co). On d 28 of the growing phase, six pens of steers per treatment received a growth implant (200 mg progesterone and 20 mg estradiol benzoate) at the beginning of the experiment and were re-implanted with 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase. The remaining steers received no growth implant. Steers were fed a corn silage-based growing diet for 56 d then were gradually switched to a high concentrate finishing diet. At the beginning of the

finishing phase, steers that were implanted during the growing phase were re-implanted with 80 mg trenbolone acetate and 16 mg estradiol. During the finishing phase, all minerals, excluding Zn, were fed at the recommended concentrations in inorganic form. Treatments during the finishing phase consisted of: 1) control (no supplemental Zn); 2) inorganic Zn (30 mg of Zn/kg DM from ZnSO4); and 3) organic Zn (iso-amounts of organic Zn). Equal numbers of steers per treatment were slaughtered after receiving the finishing diet for 132 or 158 d. Steers that were implanted had greater bone maturity (P = 0.0066), lean maturity $P < 0.05$), overall maturity ($P = 0.0095$), and ribeye area ($P <$ 0.04), and lower kidney, pelvic, and heart fat (KPH; P = 0.0004) and marbling score ($P =$ 0.0003) than non-implanted steers. There was a treatment × time effect ($P < 0.02$) for hot carcass weight. On d 133, implanted steers had a greater ($P = 0.04$) HCW than non-implanted steers. Steers supplemented with trace mineral tended to have a greater ($P <$ 0.08) HCW than control steers, with steers supplemented with inorganic trace minerals having greater ($P < 0.04$) HCW than steers supplemented with inorganic trace minerals. On d 159, implanted steers also had a greater ($P = 0.0069$) HCW than non-implanted steers, as was seen on d 133 although the difference on d 159 seemed to be greater. Overall, implanted steers had a greater ($P < 0.03$) HCW than non-implanted steers. There was also a treatment*time effect ($P = 0.01$) for dressing percentage. On d 133, implanted steers had a greater ($P = 0.04$) dressing percentage than non-implanted steers. Steers supplemented with inorganic trace minerals had greater ($P < 0.02$) dressing percentage than steers supplemented with inorganic trace minerals. On d 159, implanted steers also had a greater ($P = 0.0054$) dressing percentage than non-implanted steers. Overall, implanted steers had a greater ($P < 0.02$) dressing percentage than non-implanted

steers. Non-implanted steers had greater concentrations of oleic acid (18:1 n-9; $P = 0.02$), monounsaturated fatty acids (MUFA; $P = 0.0021$), and unsaturated fatty acids (USFA; $P < 0.04$) than implanted cattle. Non-implanted cattle had lower concentrations of linoleic acid (18:2 n-6; $P = 0.0005$), docosahexaenoic acid (22:6 n-3; $P < 0.0001$), saturated fatty acids (SFA; $P < 0.04$), and polyunsaturated fatty acids (PUFA; $P = 0.0012$) than implanted steers. Implanted steers had a greater ($P < 0.04$) ratio of saturated fatty acids SFA:USFA and also a greater ($P = 0.04$) ratio of PUFA:SFA than non-implanted cattle. Steers that were supplemented with inorganic trace minerals had lower concentrations of 18:2 n-6 ($P = 0.0005$) and 22:6 n-3 ($P = 0.04$) than steers that were supplemented with organic trace minerals. Steers that were supplemented with inorganic trace minerals also had a lower ($P = 0.03$) ratio of PUFA:SFA than steers that were supplemented with organic trace minerals. Subcutaneous adipose lipolytic enzyme activities were similar across treatments. These results indicate that TM supplementation and source and growth implants may have an effect on carcass characteristics and lipid metabolism.

Key Words: Carcass characteristics, trace minerals, growth implants, lipid metabolism, enzyme activity.

INTRODUCTION

Growth implants are often utilized in cattle production in order to increase gain and feed efficiency (Apple et al., 1991; Duckett et al., 1999). This increase in gain and feed efficiency is accomplished by retaining nitrogen (Hutcheson et al., 1997) and increasing skeletal muscle accretion in growing cattle (Galbraith and Topps, 1981). Although, growth implants are beneficial to the beef industry, studies have shown a

reduction of marbling in implanted cattle (Vanderwert et al, 1985; Platter et al., 2003; Reiling et al., 2003). Growth implants have also been reported to decrease zinc (Zn) concentrations in the body (Huerta et al., 2002). This decrease in liver Zn concentrations may be due to the role of Zn in protein accretion (McDowell, 1992).

The recommended concentration of Zn in beef cattle diets is 30 mg of Zn/kg DM (NRC, 1996). Recent reports have surfaced that indicate that Zn supplementation above NRC (1996) requirements to finishing cattle improves carcass quality. However, little controlled research has been conducted in this area. Recently, Spears and Kegley (2002) reported that Zn supplementation (25 mg Zn/kg DM) to finishing steer diets (basal diet contained 26 mg of Zn/kg DM) increased quality grade and marbling score ($P < 0.05$) compared to non-Zn supplemented steers. Furthermore, the addition of Zn (20,100, or 200 mg of Zn/kg DM from $ZnSO_4$) to a finishing diet resulted in a quadratic increase in fat thickness (Malcolm-Callis et al., 2000). From these data it is apparent that Zn supplementation can alter lipid metabolism in finishing steers when supplemented at NRC (1996) recommended concentrations. However, the specific metabolic interaction of zinc and lipogenesis and lipolysis has not been identified. Zinc has been reported to potentate the signaling of insulin (Kiss et al., 1997), potentate lipogenesis in rat epididymal adipocytes (Coulston and Dandona, 1980) and increase glucose transport into rat epididymal adipocytes (May and Contoreggi, 1982). Furthermore, adipose tissue lipogenesis (measured via the incorporation of acetate) in steers receiving 60 to 120 mg of Zn/kg DM tended ($P < 0.11$) to be higher as insulin concentrations in the incubation medium increased compared to steers supplemented with 20, 240, or 280 mg of Zn/kg DM (Archibeque et al., 2001). Therefore, the objective of this experiment was to

determine the effects of trace mineral supplementation and source and growth implants on carcass characteristics and lipid metabolism in steers.

MATERIALS AND METHODS

Prior to the initiation of this experiment, the Colorado State University Animal Care and Use Committee approved care, handling, and sampling of the animals defined herein.

Three hundred and seventy three steer calves (approximately 7 mo of age and 247 ± 19.4 kg) were utilized in this experiment. Calves were obtained from 3 different Colorado State University Research facilities (127 Hereford x Angus calves from the Maxwell Ranch in Livermore, CO; 135 crossbred calves from the San Juan Basin Research Center in Hesperus, CO; and 111 Black Angus calves from the Colorado State University Beef Improvement Center in Saratoga, WY).

Prior to the initiation of the study, calves were backgrounded at their respective ranch locations for 30 d. Post-weaning, at each ranch location, calves were weighed on two consecutive days and stratified by body weight into six groups. Groups were then randomly assigned to one of six group pens equipped with bunk feeders and automatic waterers. Pens were then randomly assigned to treatments. Treatments consisted of: 1) control (no supplemental trace minerals), 2) inorganic trace mineral ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace mineral (iso-amounts of organic Cu, Zn, Mn, and Co). Mineral treatments were fed in alfalfa pellets with control calves receiving alfalfa pellets with no supplemental Cu, Zn, Mn, or Co. Calves were allowed free access to grass hay in round bale feeders throughout the 30 d backgrounding phase. On day 28 post-weaning, calves were weighed and transported to the Agricultural Research,

Development, and Education Center (ARDEC) feedlot facility in Fort Collins, CO. Transport time varied between ranches. Calves from the Maxwell Ranch traveled approximately 48 km. Calves from the San Juan Basin Research Center in Hesperus, CO, traveled approximately 656 km, and calves from the Colorado State University Beef Improvement Center traveled approximately 230 km. Pre-conditioning data is reported elsewhere.

Receiving/growing phase:

Upon arrival, all calves were weighed (on two consecutive days), vaccinated with Ultrabac®7/Somubac and Bovishield™ 4+L5, and dewormed with Dectomax (Pfizer Animal Health, Exton, PA). Calves were blocked by ranch and stratified by initial body weight and backgrounding treatment and were sorted into one of thirty-six pens (9-12 head per pen) equipped with automatic waterers. Calves remained on the same trace mineral treatments that they received during the on-farm backgrounding phase. Treatments consisted of: 1) control (no supplemental trace minerals), 2) inorganic trace minerals ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 3) organic trace minerals (iso amounts of organic Cu, Zn, Mn, and Co). Half of the steers from each treatment (6 pens per treatment) were implanted with 200 mg progesterone and 20 mg estradiol benzoate at the initiation of the experiment and the remaining steers (6 pens per treatment) received no implant. Steers were fed a corn silage-based growing diet (Table 1) for 56 days. Diets were formulated to meet or exceed NRC (1996) requirements for energy, protein, macro- and micro-minerals with the exception of Cu, Zn, Mn, and Co. Diets were fed once daily in the morning in amounts adequate to allow ad libitum access to feed throughout the day. Daily feed offerings were recorded and feed refusal was measured every 28 d.

Blood samples were collected in non-hepranized trace mineral free vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) on d 0 and d 56 to be analyzed for total cholesterol concentrations.

Finishing phase:

At the beginning of the finishing phase, steers that had received an implant in the growing phase were re-implanted with 80 mg trenbolone acetate and 16 mg estradiol. Steers were gradually switched to a high concentrate finishing diet (Table 1), which was fed as described in the growing phase. The finishing diet was fed until the steers reached a finished weight of approximately 540 kg. Trace mineral treatments during the finishing phase were: 1) control (no supplemental Zn); 2) inorganic Zn (30 mg Zn/mg DM from $ZnSO_4$); and 3) iso-amounts of organic Zn. All other trace minerals (Cu, Mn, and Co) were supplemented in the inorganic form at NRC (1996) recommended concentrations. A blood sample was taken on d 84 in non-hepranized trace mineral free vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) and analyzed for total cholesterol concentrations.

On d 119, adipose tissue biopsies were taken from 18 steers (3 implanted and 3 non-implanted steers per treatment). Briefly, the tail head (pone) area on the right side of the steer was clipped of hair and scrubbed three times with betadine alternating with 70 % alcohol. The incision site was anaesthetized with 10 ml of a two percent lidocaine hydrochloride solution (Abbott Laboratories, Chicago, IL). An incision (2.5 to 3.5 cm in length) was made between the tail head and the tuber ischii using a # 20 scalpel blade. Approximately 5 g of adipose tissue was excised from this incision. The incision was then sutured with # 3 Vetafil suture material. The adipose tissue was rinsed in phosphate

buffered saline (PBS), wrapped in foil, and flash frozen in liquid nitrogen. Upon returning to the laboratory, the frozen adipose tissue was stored at -70°C until analyzed for fatty acid synthase (FAS), acetyl Co-A carboxylase (ACC), and lipoprotein lipase (LPL) enzyme activity.

On day 132 and 158 of the finishing phase, equal numbers of animals from each treatment were transported to a slaughter plant where carcass data was collected. Carcass data collected included kidney, pelvic, and heart fat (KPH), bone maturity (BMAT), lean maturity (LMAT), overall maturity (OMAT), mean fat thickness (MFT), adjusted fat thickness (AFT), ribeye area (REA), final yield grad (FYG), hot carcass weight (HCW), marbling score (MARB), and dressing percentage (DRESS). A longissimus muscle tissue sample was obtained postmortem and analyzed for fatty acid composition. These samples were stored on ice and transported back to the laboratory where they were stored at –20°C until analyzed.

Analytical Procedures

Total cholesterol concentrations. Blood samples were centrifuged at 1200 × *g* for 25 min. Serum was harvested and stored in an acid washed polyethylene tubes until analyzed for total cholesterol concentrations. Total cholesterol was analyzed using an Infinity Cholesterol Reagent Kit (Sigma Catalog number 40125P). Briefly, serum was added to the infinity reagent (contains cholesterol oxidase, cholesterol esterase, peroxidase, 4-aminoantipyrine, and hydroxybenzoic acid) in a 1:100 ratio. This mixture was then incubated at 37°C for 5 min and analyzed at 500 nm on a spectrophotometer. Samples were reported as mg/dl.

Fatty acid profile. External fat was removed from the longissimus muscle samples and the samples were ground in a food processor. One gram of ground sample was weighed into a tin-weighing dish used for lipid extraction (wet weight). Lipid extraction of longissimus subsamples were accomplished via the Soxlette method. Briefly, the sample was dried overnight at 100°C. The sample was then reweighed to determine dry matter, folded within filter paper, and placed into the Soxlette apparatus for 10 hours. The samples were then removed from the Soxlette apparatus and allowed to air-dry overnight. Once air-dry, the samples were dried in an oven at 100°C overnight and weighed. Percent fat was determined by dividing the weight of fat that was extracted from the sample by the wet weight of the sample prior to drying and extraction.

A second one-gram sample was weighed into a 20 mL scintillation vial to be analyzed for fatty acid composition. The samples were placed into a freeze-drier for approximately 36 hours. Methyl ester derivatives of the longissimus samples were prepared using a combination of $NaOCH_3$ followed by HCl/CH_3OH as described by Kramer et al. (1997). Fatty acid compositions were determined by gas chromatography using an Agilent 6890 Series gas chromatograph (Wilmington, DE) fixed with a 6B90 series injector and flame ionization detector. The instrument was equipped with a 100-m × 0.25mm (i.d.) fused silica capillary column (SP™-2560 Supelco Inc. Bellefonte, PA). Fatty acid methyl ester preparations were injected (1 μL) using the split mode. The carrier gas was helium, and the split ratio was 100:1 at 180°C. The oven temperature was programmed from an initial temperature of 140°C (0 min) to a final temperature of 225°C at a rate of 2.8°C/min. The final temperature was held for 18 min. Chromatograms were recorded with a computing integrator (ChemStation *Plus* chromatograph manager,

Agilent Technologies, Wilmington, DE). Recovery of total fatty acids was quantified by the incorporation of an internal standard. Standard fatty acid methyl ester mixtures were used to calibrate the gas chromatograph system using reference standards KEL-FIM-FAME-5 (Matreya, Pleasant Gap, PA). Identification of the fatty acids was made by comparing the relative retention times of fatty acid methyl ester peaks with those of the standards. Fatty acids were calculated as normalized area percentages of fatty acids.

Enzyme Activity. Fatty acid synthase enzyme was extracted from adipose tissue as described by Moibi et al. (2000). Briefly, frozen adipose tissue was ground in liquid nitrogen using a mortar and pestle. Approximately 2 g of ground tissue was weighed was homogenized for 30 s at 4°C in 3 volumes of phosphate bicarbonate buffer (70 m*M* $KHCO_3$, 85 m*M* K_2HPO4, 9 m*M* KH_2PO_4, 1 m*M* DTT, pH 8). The homogenate was centrifuged at 10,000 × *g* for 10 min. The supernatant was removed and centrifuged at 105,000 × *g* for 60 min at 4°C to obtain adipose tissue cytosol. Supernatant was brought to saturation with an ammonium sulfate solution (3 m*M* EDTA and 1 m*M* β-mercaptoethanol), stirred for 60 min on ice, and centrifuged at 105,000 × *g* for 60 min. After removing and discarding the supernatant, the pellet was dissolved in 5 % of original volume of homogenate buffer and centrifuged briefly in a microcentrifuge to remove insoluble protein. Protein content of the supernatant was assayed according to Bradford (1976).

Fatty acid synthase activity was determined in duplicate according to the method of Nepokroeff et al. (1975) by measuring the malonyl-CoA-and acetyl-CoA-dependent oxidation of NADPH using a UV-visible automated spectrophotometer equipped with a temperature controller set at 30°C. For each assay, reference (blank) and sample cuvettes

were measured simultaneously; and the decrease in absorbance at 340 nm was recorded. The change in concentration of NADPH during oxidation was calculated using the following equation: $\Delta C = \Delta A/E$, where ΔC = change in concentration of NADPH, ΔA = change in absorbance, and E = extinction coefficient of NADPH (E340 = 6.33 $mM^{-1} \cdot cm^{-1}$). The change in FAS activity was expressed in nmol NADPH oxidized$\cdot min^{-1} \cdot$mg $protein^{-1}$.

Acetyl-CoA carboxylase enzyme was extracted from adipose tissue as described by Moibi et al. (2000). Frozen subcutaneous adipose tissue was pulverized under liquid nitrogen. Approximately 1 g of ground tissue was homogenized in a "homogenizing" buffer (50 m*M* Tris-HCL (pH 7.5 at 4°C), 50 m*M* NaF, 0.25 *M* mannitol, 1 m*M* EDTA, 1 m*M* ethylene glycol-bis, 1 m*M* dithiotheritol, 5 m*M* sodium pyrophosphate, 1 m*M* PMSF, 1 m*M* benzamidine, and 4 μg/mL each of soybean trypsin inhibitor, aprotinin, leupeptin, and pepstatin A). The homogenized sample was centrifuged at 14,000 × *g* for 20 min at 4°C. The supernatant was removed, made to a final concentration of 2 % PEG, stirred for 10 min at 4°C, and centrifuged at 10,000 × *g* for 10 min at 4°C. Acetyl-CoA carboxylase protein was precipitated from the supernatant in a 10 % PEG solution, stirred on ice for 10 min, and centrifuged at 10,000 × *g* for 10 min at 4°C. The precipitate was collected, washed with 10 % PEG/homogenizing buffer, and centrifuged at 10,000 × *g* for 10 min at 4°C. The pellet was resuspended in buffer (100 m*M* Tris-HCl (pH 7.5 at 4°C), 1 m*M* EDTA; 1 m*M* EGTA, 1 m*M* DTT, 50 m*M* NaF, 5 m*M* sodium pyrophosphate, 10 % glycerol, 0.02 % sodium azide, 4 μg/mL each of soybean trypsin inhibitor, aprotinin, leupeptin, and pepstatin A, and 1 m*M* benzamidine). The protein content of the resuspended enzyme was determined using the Bradford method (Bradford, 1976).

Acetyl-CoA carboxylase activity was determined as described by Lopaschuck et al. (1994), with slight modifications. The HPLC procedure was replaced by measuring the rate of incorporation of [^{14}C] bicarbonate into malonyl-CoA (an acid-stable compound; Thampy and Wakil, 1985). Briefly, 3 parts of enzyme extract was preincubated for 5 min at 37°C in one part buffer (0.24 *M* Tris-acetate, 4 mg/mL BSA, 20 m*M* Mg-acetate, 40 m*M* citrate, and 5.2 m*M* β-mercaptoethanol). The reaction was initiated by adding 10 μL of preincubated enzyme in a final assay mixture of 165 μL (60.6 m*M* Tris-acetate, 2.12 m*M* ATP; 1.31 m*M* β-mercaptoethanol, 5.0 m*M* Mg-acetate, 10 m*M* potassium citrate, 1.06 m*M* acetyl-CoA, 18.18 m*M* $NaHCO_3$, 0.33 μCi/μmol $Na^{14}CO_3$, and 1 mg/mL fatty acid free-BSA (pH 7.5)). After incubating for 4 min at 37°C in a shaking water bath, the reaction was stopped by adding 25 μL of 10 % perchloric acid. Reaction tubes were placed in a dessicator under vacuum to remove unreacted label as $^{14}CO_2$. Tubes were then centrifuged at 2,900 × *g* for 20 min. The supernatant (approximately 160 μL) was removed to a glass scintillation vial and evaporated to dryness at 80°C under vacuum. The residue was dissolved in 100 μL of H_2O and mixed with 4 mL of scintillation fluid for determination of radioactivity. Acetyl-CoA carboxylase activity will be expressed as nanomoles of 14C-bicarbonate into malonyl CoA·min^{-1}·mg protein^{-1}.

Lipoprotein lipase enzyme activity was determined at 37°C for 1 h as described by McNamara et al. (1982; obtained form Sprinkle et al., 1998) in 0.25 mL of final volume containing 4 m*M* triolein, 0.8 μCi glycerol tri-[9-10-^{3}H]oleate, 0.11 mg phosphatidylcholine, 130 μL 0.156 *M* Tris base (pH 8.6), 0.705 mg BSA in 20 μL 0.156 M Tris base, 50 μL homogenate, and 50 μL of heat-inactivated (60 °C for 10 min) rat

serum. Samples were preincubated for 30 min with rat serum before adding enzyme (or Tris base for blanks). After the 1-h incubation, free fatty acids (FFA) were extracted as described by Belfrage and Vaughn (1996) and McNamara et al. (1982). The FFA were vortexed and centrifuged at 1,500 × *g* for 30 min. One milliliter of the upper phase was aliquoted to 10 mL of scintillation fluid for counting. Enzyme activity will be reported as nanomoles of FFA released·hour^{-1}·milligram of adipose tissue cystolic protein^{-1}.

Statistical analysis.

Statistical analysis of data was performed using Proc Mixed procedure of SAS (2001). The study was a 2 × 3 factorial arrangement in a completely randomized design. The model included fixed effects of treatment, implant, time, and all possible interactions and a random effect of pen(treatment × imp). When an interaction was not significant, it was removed and the analysis re-ran. Differences among means were determined using preplanned single degree of freedom contrasts. The contrast statement was Inorganic mineral vs. Organic mineral treatments.

RESULTS AND DISCUSSION

Carcass Characteristics:

The effects of growth implants, trace mineral supplementation, and trace mineral source on carcass characteristics are shown in Table 2. Trace mineral supplementation and trace mineral source had no effect on KPH, BMAT, LMAT, OMAT, MFT, AFT, REA, FYG, and MARB. Similar results were reported in steers fed a corn-alfalfa based basal diet (3 mg Cu/kg DM, 20.1 mg Zn/kg DM, and 9.6 mg Mn/kg DM) supplemented with organic Cu, Zn, Mn, and Co (1x or 2x NRC recommended concentrations in an amino acid complexed form) or inorganic Cu, Zn, Mn, and Co (3x or 6x NRC

recommended concentrations; Rhoads et al., 2003) and wether lambs fed a basal diet of barley and alfalfa supplemented with Zn methionine (0, 0.396, or 0.791 g/head/day; Hatfield et al., 1992). Backfat has been reported to be greater ($P < 0.05$) in control steers than in steers supplemented with $CuSO_4$ (Engle and Spears, 2000; Engle et al., 2000a; Engle and Spears, 2001) and tended to be lower in steers supplemented with inorganic or organic sources of Cu (Engle et al., 2000b). When supplementing Zn (200 mg Zn/kg DM from $ZnSO_4$ or Zn methionine) to a basal diet containing 64 mg Zn/kg DM and 30 mg Cu/kg DM, Huerta et al. (2002) reported that backfat thickness ($P < 0.06$) and adjusted backfat thickness tended ($P < 0.08$) to be greater in control heifers than in supplemented heifers, but this difference was not reported in steers. In contrast to these studies and to the present study, backfat was lower in control steers than steers supplemented with inorganic or organic sources of Zn (Green et al., 1998; Spears and Kegley, 2002). The reason there are discrepancies between experiments relating to the effects of TM supplementation on backfat measurements is unknown. It may be possible that different minerals have different affects on subcutaneous adipose tissue metabolism. In the aforementioned experiments, it seems that supplementing Cu decreases backfat, where as, Zn supplementation yields varying results on backfat thickness. In the present study, both Cu and Zn were supplemented at the beginning of the study. This may be why no effect on backfat was observed.

Overall maturity has been reported to be lower ($P < 0.01$) in heifers supplemented with Zn than control heifers (Huerta et al., 2002). Spears and Kegley (2002) reported that supplementing steers with Zn oxide or Zn proteinate (10 or 15 % Zn) resulted in more MARB ($P < 0.05$) and higher yield grade ($P < 0.10$), but had no effect on KPH. In

contrast, Ward and Spears (1997) reported that steers supplemented with $CuSO_4$ had lower yield grade ($P < 0.04$) and REA ($P < 0.09$) than control steers.

Source of TM also affected carcass quality in various studies. Kidney, pelvic, heart fat was greater in steers supplemented with organic TM than steers supplemented with inorganic TM (Green et al., 1988; and Malcolm Callis et al., 2000). Green et al. (1988) and Huerta et al. (2002) reported that steers that were supplemented with Zn methionine had greater MARB ($P < 0.05$ and $P < 0.07$, respectively) than steers supplemented with Zn oxide or $ZnSO_4$. In contrast, steers that were supplemented with Cu proteinate had greater ($P < 0.05$) MARB than steers supplemented with $CuSO_4$ (Engle et al., 2000). Backfat thickness has been reported to be lower ($P < 0.10$) in steers supplemented with $ZnSO_4$ than steers supplemented with Zn methionine, whereas, DRESS, REA, MARB, and yield grade were unaffected by TM source (Malcolm-Callis et al., 2000).

Growth implants had no effect on MFT, AFT, and FYG. Steers that were implanted had greater BMAT ($P = 0.0066$), LMAT ($P < 0.05$), OMAT ($P = 0.0095$), and REA ($P < 0.04$). Similar results were reported in steers where BMAT, LMAT, and OMAT were greater in implanted steers (Foutz et al., 1992; Paisley et al., 1999; Huerta et al., 2002; Reiling and Johnson, 2003). Ribeye area was also reported to be greater in implanted Holstein steers (Apple et al., 1991; Ainslie et al., 1992), beef steers (McCaan et al., 1991; Duckett et al., 1997; Reiling and Johnson, 2003), and heifers (Mader et al., 1994). In contrast to the present study and to these studies, growth implants had no effect on REA in heifers and steers (Dehaan et al., 1990; Perry et al., 1991; Paisley et al., 1999).

In the present study, steers that were implanted had and lower KPH ($P = 0.0004$) and MARB ($P = 0.0003$) than non-implanted steers. Kidney, pelvic, and heart fat has been reported to be lower in implanted heifers and steers (Dehaan et al., 1990; Platter et al., 2003; Reiling and Johnson, 2003). Marbling scores have also been reported to be lower in implanted Holstein steers (Ainslie et al., 1992), beef steers (Dehaan et al., 1990; Duckett et al., 1992; Mader et al., 1994), and heifers Mader et al., 1994). In contrast to the present study and these studies, Foutz et al. (1992), Johnson et al. (1996), and Dehaan et al. (1990) reported no effect on growth implants on MARB in steers and heifers

There was an implant × trace mineral interaction for KPH ($P = 0.0021$) and marbling score ($P = 0.0049$). Implanted steers that were supplemented with trace minerals has greater KPH and marbling score than implanted control steers, whereas, non-implanted steers that were supplemented with trace minerals or that were not supplemented with trace minerals (control steers) had similar KPH and marbling score.

There was a trace mineral source × implant interaction for marbling score ($P < 0.02$) and KPH ($P = 0.09$). Non-implanted steers that were supplemented with organic trace minerals had a greater marbling score and tended to have a greater KPH than non-implanted steers supplemented with inorganic trace minerals. Implanted steers that were supplemented with either organic or inorganic trace minerals had similar marbling scores and KPH. Huerta et al. (2002) reported a trace mineral source × implant interaction for KPH, BFT, and AFT in heifers. Implanted heifers that were supplemented with organic TM had lower KPH ($P < 0.08$), BFT ($P < 0.06$), and AFT ($P < 0.05$) than implanted heifers supplemented with inorganic TM, whereas, non-implanted heifers supplemented with organic TM had greater KPH ($P < 0.08$), BFT ($P < 0.06$), and AFT ($P < 0.05$) than

non-implanted heifers supplemented with inorganic TM. This is similar to what was observed in the present study for KPH.

There was a trend for an implant × time effect for MFT ($P < 0.07$) and AFT ($P < 0.10$). Non-implanted steers that were harvested on d159 of the finishing phase tended to have greater MFT and AFT than non-implanted steers that were harvested on d 133. There was no difference in harvest dates among the implanted steers.

There was a treatment × implant × time interaction for REA ($P < 0.03$). On d133, non-implanted steers supplemented with inorganic trace minerals had greater REA than implanted steers supplemented with organic trace minerals. On d 159, a similar result was observed within the non-implanted steers except that steers supplemented with inorganic trace minerals also had a greater REA than control steers. Within the implanted steers, there was also differences noted. Steers supplemented with inorganic trace minerals had greater REA than both steers supplemented with organic trace minerals and control steers on d 133. Steers supplemented with organic trace minerals also had greater REA than control steers on d 133. On d 159, control steers had greater REA than steers supplemented with organic trace minerals. The reason that this interaction occurred is unknown.

There was a treatment × time effect ($P < 0.02$) for hot carcass weight (HCW; Table 2). On d 133, implanted steers had a greater ($P = 0.04$) HCW than non-implanted steers. Steers supplemented with trace mineral tended to have a greater ($P < 0.08$) HCW than control steers, with steers supplemented with inorganic trace minerals having greater ($P < 0.04$) HCW than steers supplemented with inorganic trace minerals. There was a trace mineral source × implant interaction ($P < 0.04$) for HCW. Within the implanted

steers, steers supplemented with inorganic trace minerals had a greater HCW than steers supplemented with organic trace minerals. This difference was also observed among the non-implanted steers.

On d 159, implanted steers also had a greater (P = 0.0069) HCW than non-implanted steers, as was seen on d 133 although the difference on d 159 seemed to be greater. The trace mineral and trace mineral source effects were not present on d 159. There was an implant*trace mineral interaction (P = 0.03) for HCW. Non-implanted steers supplemented with trace minerals had a greater HCW than non-implanted control steers. There was no significant difference among the implanted steers. There was a tendency for a trace mineral source × implant interaction for HCW (P = 0.06). Implanted steers that were supplemented with organic trace minerals tended (P = 0.06) to have a greater HCW than implanted steers that were supplemented with inorganic trace minerals, where as, there was no difference among the non-implanted steers. This is the opposite of what was observed on d 133.

Trace mineral supplementation and source had no effect on HCW overall. Similar results were reported in steers (Green et al., 1988; Engle and Spears, 2000; Engle et al., 2000a; Rhoads et al., 2003). However, HCW was reported to be greater (P < 0.05) in control steers than steers supplemented with TM (Engle et al., 2000b). Hot carcass weight has been reported to be affected by TM source. Spears and Kegley (2002) reported a lower (P < 0.05) HCW in steers supplemented with inorganic TM than in steers supplemented with organic TM. In the present study, overall, implanted steers had a greater (P < 0.03) HCW than non-implanted steers. Hot carcass weight has been reported to be greater in implanted Holstein steers (Apple et al., 1991), heifers (Dehaan et

al., 1990, Mader et al., 1994), and beef steers (McCaan et al., 1991; Reiling and Johnson, 2003), whereas, in other studies, implant had no effect on HCW in Holstein and beef steers (Ainslie et al., 1992; Foutz et al., 1997).

There was a trace mineral source × implant interaction for HCW ($P < 0.05$). Non-implanted steers that were supplemented with inorganic trace minerals had a greater HCW than non-implanted steers supplemented with organic trace minerals. This difference was also seen among the implanted steers although the HCW of these steers was not as great as the difference seen among the non-implanted steers. There was a tendency for an implant × trace mineral interaction for HCW ($P < 0.06$). Non-implanted steers supplemented with trace minerals had a greater HCW than non-implanted control steers, whereas, implanted control steers and implanted steers supplemented with trace minerals had similar HCW.

There was also a treatment × implant × time interaction ($P = 0.01$) for HCW overall. On d1 33, non-implanted steers supplemented with inorganic trace minerals had greater HCW than implanted control steers. On d 159, non-implanted steers supplemented with inorganic trace minerals had greater HCW than steers supplemented with organic trace minerals. Within the implanted steers, steers supplemented with inorganic trace minerals had greater HCW than both steers supplemented with organic trace minerals and control steers on d 133. Steers supplemented with organic trace minerals also had greater HCW than control steers on d 133. On d 159, steers supplemented with inorganic trace minerals had a lower HCW than control steers and steers supplemented with organic trace minerals. The reason that this interaction occurred is unknown.

There was also a treatment × time effect (P = 0.01) for DRESS. On d 133, implanted steers had a greater (P = 0.04) DRESS than non-implanted steers. Steers supplemented with inorganic trace minerals had a greater (P < 0.02) DRESS than steers supplemented with inorganic trace minerals. There was also a tendency for a trace mineral effect on d 133. Steers supplemented with trace mineral tended to have a greater (P < 0.08) DRESS than control steers.

On d 159, implanted steers also had a greater (P = 0.0054) DRESS than non-implanted steers, as was seen on d 133 although the difference on d 159 seemed to be greater. There was an implant × trace mineral interaction (P < 0.03) for DRESS. Non-implanted steers supplemented with trace minerals had a greater DRESS than control steers. There was no difference among the implanted steers.

Trace mineral supplementation and source had no effect on DRESS overall. The effects of TM supplementation and source on DRESS varied among studies. Similar to the present study, steers supplemented with Zn had similar DRESS as control steers. Heifers supplemented with TM had lower (P < 0.07) DRESS than control heifers (Huerta et al., 2002), whereas, steers supplemented with TM had greater (P < 0.10) DRESS than control steers (Spears and Kegley, 2002). Huerta et al. (2002) reported a greater (P < 0.02) DRESS in heifers in steers supplemented with $ZnSO_4$ than heifers and steers supplemented with Zn methionine.

In the present study, overall, implanted steers had a greater (P < 0.02) DRESS than non-implanted steers. Non-implanted heifers had a greater (P < 0.10) DRESS than non-implanted heifers (Huerta et al., 2002), which is contrast to the present study. There was an implant × trace mineral interaction for DRESS (P = 0.05). Implanted steers

supplemented with trace minerals had a greater DRESS than implanted control steers, whereas non-implanted control steers and non-implanted steers supplemented with trace minerals had a similar DRESS. Huerta et al. (2002) reported an implant × TM interaction on DRESS in heifers. As reported in the present study, implanted control heifers had a lower (P = 0.005) DRESS than implanted heifers supplemented with TM, whereas, non-implanted control heifers had greater (P = 0.005) DRESS than non-implanted steers supplemented with TM.

Also, in the present study, there was a trace mineral source × implant interaction for DRESS (P = 0.03). Non-implanted steers that were supplemented with inorganic trace minerals had a greater DRESS than non-implanted steers supplemented with organic trace minerals. This difference was also observed among the implanted steers although the DRESS of these steers was not as great as the difference observed among the non-implanted steers.

There was also a treatment × implant × time interaction (P = 0.01) for DRESS overall. On d133, there was no difference in treatments among non-implanted steers. On d 159, non-implanted steers supplemented with inorganic trace minerals had a greater DRESS than control steers. Within the implanted steers, steers supplemented with inorganic trace minerals had a greater DRESS than both steers supplemented with organic trace minerals and control steers on d 133. Steers supplemented with organic trace minerals also had a greater DRESS than control steers on d 133. On d 159, steers supplemented with inorganic trace minerals had a greater DRESS than steers supplemented with organic trace minerals. The reason that this interaction occurred is unknown.

Lipid Metabolism:

Total cholesterol concentrations. There was no effect of implant or trace mineral source on total cholesterol concentrations throughout this study (Table 3). On d 84 of the finishing phase, steers supplemented with TM tended ($P < 0.06$) to have greater total cholesterol than control steers. Although it is unclear as to which TM supplemented in the present study may have affected total cholesterol concentrations, Cu supplemented steers have been reported to have lower cholesterol concentrations in serum than control steers (Engle and Spears, 2000). In other studies, cholesterol concentrations in steers have been unaffected by TM supplementation (Engle et al., 2000b; Engle and Spears, 2001).

Percent lipid in longissimus muscle tissue. There was no effect of implant, trace mineral supplementation, or trace mineral source on percent lipid in longissimus muscle tissue throughout this study (Table 4). Similar results were reported in steers (Foutz et al., 1997; Engle et al., 2000a; Engle and Spears, 2000). Growth implants have also been shown to have no effect on percent lipid in longissimus muscle tissue of steers (Johnson et al., 1996; Duckett et al., 1997). There was also an implant × time effect ($P = 0.0061$) for percent lipid in longissimus muscle. On d 133, implanted steers had a greater percent lipid concentration in longissimus muscle tissue than non-implanted steers. On d 159, implanted steers had a lower percent lipid concentration in longissimus muscle tissue than non-implanted steers.

Fatty acid composition of longissimus muscle tissue. Trace mineral supplementation had no effect on fatty acid composition of longissimus muscle tissue (Table 5). In a study utilizing steers, supplementing 10 or 40 mg Cu/kg DM from $CuSO_4$

to a soybean meal-corn silage basal diet (9.8 mg Cu/kg DM, 52.4 mg Zn/kg DM) had no effect on fatty acid composition of longissimus muscle tissue, which is in agreement with the present study. However, Engle and Spears (2000) reported that steers supplemented with 10 or 20 mg Cu/kg DM from $CuSO_4$ to a corn-soybean meal-corn silage basal diet containing 4.9 mg Cu/kg DM and 51 mg Zn/kg DM had lower ($P < 0.05$) concentrations of myristoleic acid (14:1 n-5) and greater ($P < 0.05$) concentrations of linoleic acid (18:2 n-6), linolenic acid (18:3 n-3), unsaturated fatty acids (USFA), and polyunsaturated fatty acids (PUFA) than control steers. Steers supplemented with 20 mg $CuSO_4$ to a corn-soybean-meal-corn silage basal diet (5.2 mg Cu/kg DM and 52.1 mg Zn/kg DM) had similar fatty acid profiles as control steers, except stearic acid (18:0) concentrations tended ($P < 0.10$) to be lower in supplemented steers than control steers (Engle et al., 2000a). Engle et al. (2000b) supplemented steers with 20 or 40 mg Cu/kg DM from $CuSO_4$ or 20 mg Cu/kg DM from Cu citrate, Cu proteinate, or tribasic Cu chloride to a corn silage-soybean meal basal diet (10.2 mg Cu/kg DM and 54.8 mg Zn/kg DM). They reported that steers supplemented with Cu had lower 18:2 n-6, 18:3 n-3, and saturated fatty acids (SFA) and greater PUFA than control steers.

Myristic acid (14:0), 14:1 n-5, palmitic acid (16:0), conjugated linoleic acid (CLA), 18:3 n-3, eicosatrienoic acid (20:3 n-6), arachidonic acid (20:4 n-6), and eicosapentanoic acid (20:5 n-3) concentrations in longissimus muscle tissue were also unaffected by trace mineral source and growth implants. Steers that were supplemented with inorganic trace minerals tended to have a greater ($P < 0.07$) concentration of 18:0 than steers that were supplemented with organic trace minerals. Steers that were supplemented with inorganic trace minerals had lower concentrations of 18:2 n-6 ($P =$

0.0005) and docosahexaenoic acid (22:6 n-3; P = 0.04) and tended (P < 0.07) to have a lower concentration of PUFA than steers that were supplemented with organic trace minerals. Steers that were supplemented with inorganic trace minerals also had a lower (P = 0.03) ratio of PUFA:SFA than steers that were supplemented with organic trace minerals. Engle et al. (2000b) reported that TM source had no effect on fatty acid composition in longissimus muscle tissue, except steers supplemented with 20 mg Cu/kg DM from $CuSO_4$ had lower (P < 0.05) concentrations of 22:1 (not determined in the present study) than steers supplemented with 20 mg Cu/kg DM from Cu proteinate.

Duckett et al. (1997) reported that implanting cattle had no effect on fatty acid concentrations in longissimus muscle. However, in the present study, non-implanted steers had greater concentrations of oleic acid (18:1 n-9; *P* = 0.02), monounsaturated fatty acids (MUFA; P = 0.0021), and USFA (P < 0.04), and tended to have greater (P < 0.08) concentrations of palmitoleic acid (16:1 n-7; P = 0.05) than implanted cattle. Non-implanted cattle had lower concentrations of 18:2 n-6 (P = 0.0005), 22:6 n-3 (P < 0.0001), SFA (P < 0.04), and PUFA (P = 0.0012) than implanted steers. Implanted steers had a greater (P < 0.04) ratio of saturated fatty acids SFA:USFA and also a greater (P = 0.04) ratio of PUFA:SFA than non-implanted cattle.

There was an implant × trace mineral interaction for fatty acid composition in longissimus muscle. Non-implanted control steers had lower concentrations of 18:2 n-6 (P = 0.0045), MUFA (P =0.01), and PUFA (P = 0.01), greater (P = 0.0005) concentrations of 22:6 n-3 (P =0.0005), and tended to have lower concentrations of 18:1 n-9 than non-implanted steers supplemented with trace minerals. Implanted control steers had greater concentrations of MUFA (P = 0.01 and tended (P = 0.09) to have

greater concentrations of 18:1 n-9 than implanted steers supplemented with TM. There was no difference between concentrations of 18:2 n-6, PUFA, and 22:6 n-3 in implanted control steers and implanted steers supplemented with trace minerals.

There was a trace mineral source × implant interaction for 22:6 n-3 and MUFA. Implanted steers supplemented with organic TM had greater ($P = 0.0012$) concentrations of 22:6 n-3 and tended ($P < 0.08$) to have greater concentrations of MUFA than implanted steers supplemented with inorganic TM. Non-implanted steers supplemented with organic TM had greater concentrations of 22:6 n-3 (as observed with the implanted cattle although not as prominent; $P = 0.012$) and tended ($P < 0.08$) to have lower concentrations of MUFA than non-implanted steers supplemented with inorganic TM.

There was an implant × time interaction for 18:0 and 18:2 n-9 concentrations. On d 133, implanted steers had a greater concentrations of 18:0 ($P = 0.04$) and lower ($P < 0.04$) concentration of 18:2 n-9 than non-implanted steers. There was no difference in concentration of 18:0 and 18:2 n-9 between implanted and non-implanted cattle on d 156.

Subcutaneous adipose lipolytic enzyme activity. Growth implants and TM supplementation had no effect on lipoprotein lipase (LPL) and fatty acid synthase (FAS) concentrations in adipose tissue. However, implanted steers tended ($P < 0.06$) to have greater acetyl CoA carboxylase (ACC) concentrations than non-implanted steers (4.80 and 4.03 nmol HCO_3/min/mg protein, respectively). There was an implant × TM effect on FAS concentrations in the adipose tissue. Non-implanted control steers tended ($P < 0.07$) to have greater FAS concentrations than non-implanted steers supplemented with TM, whereas, control steers that were implanted tended ($P < 0.07$) to have lower FAS concentrations than implanted steers supplemented with TM.

CONCLUSION

Trace mineral supplementation and source had little effect on carcass characteristics and lipid metabolism in steers. Implanted steers had a greater overall maturity, REA, HCW, and DRESS and a lower KPH. Acetyl CoA carboxylase activity was increased by growth implants. However, FAS and LPL were unaffected by growth implants or TM supplementation. The effects of TM supplementation on carcass characteristics and lipid metabolism varies between studies, and their effects on enzyme activity in adipose tissue has been examined very little. Therefore, more research needs to be conducted to determine the effects of TM supplementation and growth implants on carcass characteristics and lipid metabolism in steers.

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Table 1: Ingredient composition of basal diets

Ingredient	Backgrounding	Growing	Finishing
		----------%[a]----------	
Corn Silage	-----	17.80	9.31
Alfalfa Hay	94.90	17.32	7.95
Flaked Corn	-----	55.96	78.68
Protein Supplement	5.10	8.92	4.06
Protein Supplement Composition			
Cottonseed Meal	-----	46.00	43.59
Soybean Hulls	38.17	-----	-----
Soybean Meal	-----	20.00	20.16
Soybean Oil	0.62	-----	-----
Sunflower Meal 32 %	10.60	4.52	7.89
Wheat Midds	40.00	-----	-----
Molasses Cane Blend	3.50	-----	-----
Urea	-----	6.98	7.03
Rumensin 80[b]	-----	0.19	0.32
Bentonite	1.00	-----	-----
Biofos[c]	0.82	3.90	3.91
Cobalt Carbonate	-----	-----	< 0.01
Copper Sulfate	-----	-----	0.05
Dyna-K[c]	1.47	3.93	3.96
Limestone	3.27	11.75	11.84
Manganese Sulfate	-----	-----	0.08
Iodine	0.02	0.06	0.06
Salt	0.45	0.90	0.91
Selenium	0.02	0.08	0.08
Sulfur Flower	-----	0.04	0.01
Vitamin A 30/0	-----	0.07	0.07
Vitamin A & D 30/10	0.03	-----	-----
Vitamin E 125	0.02	0.05	0.05
Chemical Composition			
DM, %	91.10	68.82	75.62
OM, %	92.27	94.51	96.51
CP, %	14.28	13.60	10.65
NDF, %	64.18	27.72	18.64
Ash, %	7.73	5.49	3.49
Ca, %	1.23	0.91	0.56
P, %	0.30	0.31	0.24
K, %	1.43	0.78	0.51
Mg, %	0.27	0.21	0.14
Na, %	0.10	0.06	0.04
S, %	0.24	0.21	0.15
Fe, ppm	270.16	188.45	126.63
Mn, ppm	33.08	27.89	19.74
Zn, ppm	45.17	50.68	48.42
Cu, ppm	16.17	15.56	14.95
Mo, ppm	0.11	0.19	0.09

[a] Dry matter basis

[b] Provided 33 mg of monensin/kg DM.

Table 2: Effects of TM supplementation, TM source, and growth implants on carcass characteristics of steers.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant	TM	TM Source
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]				× TM	Source[b]	× Implant
KPH	1.94	1.67	1.88	1.78	1.92	1.76	0.05	0.0004	0.75	0.0021	0.73	0.09
BMAT	49.10	59.39	51.71	65.41	52.35	58.38	3.31	0.0066	0.32	0.89	0.34	0.58
LMAT	55.74	64.97	56.70	61.47	59.60	64.55	2.82	0.05	0.79	0.81	0.34	0.72
OMAT	53.34	61.63	54.40	62.57	55.76	60.62	2.41	0.0095	0.59	0.82	0.85	0.83
MFT	1.17	1.19	1.31	1.28	1.18	1.24	0.09	0.78	0.37	0.81	0.35	0.71
AFT	1.38	1.40	1.52	1.46	1.38	1.46	0.08	0.84	0.34	0.73	0.35	0.67
REA	73.32	76.28	75.35	78.93	73.67	76.63	1.37	0.04	0.24	0.85	0.13	0.81
FYG	3.23	3.21	3.30	3.19	3.21	3.27	0.13	0.74	0.75	0.64	0.97	0.67
Marbling	435.40	382.73	425.40	394.19	453.07	390.90	14.58	0.0003	0.79	0.0049	0.31	0.02
HCW												
d 133	316.43	320.10	319.85	332.48	315.71	323.29	2.73	0.04	0.08	0.12	0.03	0.04
d 159	321.50	329.59	326.31	326.65	323.54	329.95	2.65	0.0069	0.73	0.03	0.72	0.06
Overall	318.96	324.85	323.08	329.56	319.63	326.62	2.38	0.02	0.15	0.06	0.16	0.05
Dressing %												
d 133	58.46	59.05	59.03	61.31	58.23	59.67	0.50	0.04	0.08	0.14	0.02	0.23
d 159	59.40	60.85	60.22	60.37	59.77	60.95	0.48	0.0054	0.75	0.03	0.74	0.58
Overall	58.93	59.95	59.63	60.84	59.00	60.31	0.44	0.02	0.16	0.05	0.16	0.03

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate in the growing phase and 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase.

Table 3: Effects of TM supplementation, TM source, and growth implants on serum cholesterol concentration of steers.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant × TM	TM Source[b]	TM Source × Implant
	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Growing												
Day 0	72.13	69.04	67.21	66.92	83.29	84.47	8.01	----	0.46	----	0.03	----
Day 56	88.61	104.67	96.96	106.79	106.08	93.88	7.31	0.45	0.50	0.43	0.80	0.56
Finishing												
Day 84	139.57	160.01	189.15	165.22	172.95	176.14	14.71	0.97	0.06	0.18	0.90	0.81

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate in the growing phase and 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase.

Table 4: Effects of TM supplementation, TM source, and growth implants on percent lipid in longissimus muscles of steers.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant × TM	TM Source[b]	TM Source × Implant
	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]						
Overall	7.48	7.24	7.63	6.79	7.63	6.84	0.41	0.11	0.70	0.64	0.95	0.79

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate in the growing phase and 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase.

Table 5: Effects of TM supplementation, TM source, and growth implants on fatty acid composition in longissimus muscles of steers.

	Dietary Treatments						SEM[a]	P-value				
	Control		Inorganic TM		Organic TM			Implant	TM	Implant	TM	TM Source
Item	-[c]	+[d]	-[c]	+[d]	-[c]	+[d]				× TM	Source[b]	× Implant
14:0	2.87	3.27	3.11	3.36	3.34	3.81	0.43	0.48	0.14	0.37	0.46	0.74
14:1	0.98	0.84	0.89	0.91	0.95	1.00	0.07	0.82	0.73	0.94	0.32	0.78
16:0	34.31	33.79	33.94	34.24	33.94	34.53	0.71	0.55	0.76	0.92	0.74	0.94
16:1	5.79	5.47	5.95	5.20	5.98	5.69	0.27	0.08	0.46	0.29	0.30	0.37
18:0	11.30	11.17	10.61	12.11	10.03	10.68	0.50	0.14	0.13	0.12	0.07	0.14
18:1	41.53	41.14	42.06	40.19	41.76	39.83	0.83	0.02	0.77	0.09	0.48	0.17
18:2	2.31	3.31	2.49	3.04	2.96	3.32	0.21	0.0005	0.23	0.0045	0.05	0.11
CLA	0.28	0.32	0.29	0.27	0.27	0.31	0.01	0.20	0.76	0.60	0.53	0.40
18:3	0.31	0.34	0.30	0.31	0.34	0.31	0.02	0.79	0.56	0.71	0.20	0.35
20:3	0.08	0.08	0.08	0.08	0.09	0.10	0.01	0.70	0.19	0.50	0.12	0.55
20:4	0.22	0.20	0.23	0.25	0.26	0.28	0.03	0.60	0.11	0.35	0.15	0.63
20:5 (EPA)	0.029	0.031	0.032	0.032	0.037	0.036	0.005	0.79	0.12	0.31	0.18	0.64
22:6 (DHA)	0.034	0.048	0.029	0.043	0.035	0.055	0.004	<0.0001	0.69	0.0005	0.04	0.0012
SFA	48.46	48.22	47.66	49.67	47.31	49.07	0.82	0.04	0.73	0.12	0.74	0.23
MUFA	48.28	47.46	48.89	46.32	48.69	46.51	0.79	0.0021	0.96	0.01	0.79	0.08
PUFA	3.25	4.32	3.45	4.02	4.01	4.42	0.25	0.0013	0.33	0.02	0.07	0.14
USFA	51.54	51.78	52.34	50.33	52.70	50.93	0.82	0.04	0.73	0.12	0.74	0.23
SFA:USFA	1.14	1.13	1.10	1.18	1.10	1.20	0.04	0.04	0.86	0.16	0.54	0.21
PUFA:SFA	0.08	0.10	0.09	0.10	0.11	0.11	0.01	0.04	0.14	0.16	0.03	0.37

[a]Standard error of the mean.

[b]Inorganic TM vs. Organic TM.

[c]Non-implanted.

[d]Implanted with 200 mg progesterone and 20 mg estradiol benzoate in the growing phase and 80 mg trenbolone acetate and 16 mg estradiol in the finishing phase.

Chapter VI

INDUCED MORBIDITY IN PRE-WEANED CALVES AND RECEIVING CATTLE: SUBSEQUENT EFFECT OF FEEDLOT RATION TRACE MINERAL SOURCE ON FEEDLOT PERFORMANCE, IMMUNE FUNCTION, AND CARCASS CHARACTERISTICS OF STEERS

ABSTRACT

Three hundred and eighty eight steer calves (approximately 7 mo of age and 221 kg) were utilized to determine the effects of time of respiratory disease challenge (pre-weaning vs. feedlot) and trace mineral source on feedlot performance and carcass characteristics. One-third of the steer calves from each ranch were inoculated intranasally with 0.005-mL/kg body weight of infectious bovine rhinotraecheitis virus (IBRV) containing a mean cell culture infective dose ($CCID_{50}$) of 2.0×10^8 viral units/mL 4 mo prior to weaning at the ranch. One-third of the calves not inoculated at the ranch were inoculated upon arrival at the feedlot. The remaining one-third of the caves from each ranch were not inoculated. Calves were blocked by ranch and inoculation status, stratified by bodyweight (2 d mean weight), sorted into pens (approximately 10 calves per pen) and randomly assigned to treatments. Receiving phase treatments consisted of 1) Availa-4 (organic forms of Zn, Cu, and Mn) and 2) iso-amounts of inorganic minerals ($ZnSO_4$, $CuSO_4$, and $MnSO_4$). Finishing phase treatments consisted of: 1) organic Zn (Availa Zn) and 2) iso-amounts of inorganic Zn ($ZnSO_4$). All other

trace minerals were fed at NRC levels in the inorganic form. On d 105, d140, and d 168 of the finishing phase, equal numbers of animals from each treatment were transported to a slaughter plant where carcass and lung lesion data were collected. During the growing phase, steers that were inoculated at the ranch had lower ($P = 0.04$) ADG than steers that were inoculated at the feedlot. During the finishing phase, steers that were inoculated tended to have lower ($P < 0.09$) ADFI than steers that were not inoculated with IBRV; and steers that were inoculated at the ranch tended to have greater ($P < 0.07$) ADG than steers that were inoculated at the feedlot. Steers that were supplemented with inorganic TM had greater ($P = 0.05$) concentration of total IgG than steers supplemented with organic TM. Morbidity tended ($P < 0.08$) to be greater in steers that were inoculated. Inoculated animals also tended ($P = 0.07$) to be retreated more often than steers that were not inoculated with IBRV. Steers that were not inoculated with IBRV tended to have greater final weights ($P = 0.06$) and hot carcass weights ($P = 0.09$) than inoculated steers. These results indicate that TM source may have an effect on immunity and that performance, health, and carcass characteristics may be affected by disease challenge.

INTRODUCTION

Feeder cattle that have been shipped are subjected to various degrees of stress. Stress associated with shipping results in increased susceptibility to respiratory tract and other infectious diseases. Morbidity and often mortality rates are high in these animals, despite vaccination against respiratory diseases. Costs associated with morbidity are the most important determinants of profitability of feedlot cattle (Gardner et al., 1996). It has been reported that approximately ca. 8% of all production costs can be attributed to treatment costs (medication and labor) of morbid feedlot cattle and the associated costs of

mortality without consideration of losses resulting from decreased performance (Griffin et al., 1995). Several researchers monitoring ranch-to-rail programs have reported net return differences among cattle that have remained healthy throughout the feeding period vs. morbid cattle McNeill, 1994-1998; Griffin et al., 1995; Gardner et al., 1996; Roeber et al., 2001). However, the majority of reports have only monitored morbidity during the feedlot phase of cattle production. Little information is available relative to how cow/calf morbidity affects feedlot performance and profitability.

As indicated in previous literature, trace minerals such as copper (Cu), zinc (Zn), manganese (Mn) and cobalt (Co) are required for proper growth, immune function and reproduction in cattle (Underwood, 1971). Typically these minerals are fed in excess to compensate for the decrease in feed intake observed in weaned calves arriving to the feedlot, thus, resulting in excess mineral excretion by the animal. Mineral contamination of soil and ground water in areas of livestock concentrations are currently or will soon be monitored. The government will set contamination limits and regulate the amounts of these minerals being fed to animals. The allowable amounts of these elements may not necessarily be determined by the animals needs, but by waste content. Therefore, in order to minimize excretory losses of minerals, sources providing better utilization to the animal must be found. In order to do this, criteria of mineral adequacy and availability must be established using immunological, hormonal, and biochemical indices. Therefore, the objective of this experiment was to determine the effects of time of respiratory disease challenge (pre-weaning vs. feedlot) and trace mineral source (inorganic vs. organic) on feedlot performance, immune status, and carcass characteristics of steers.

MATERIALS AND METHODS

The Colorado State University Animal Care and Use Committee approved care, handling, and sampling of the animals defined in this experiment prior to the initiation of this study.

Three hundred and eighty eight steer calves (approximately 7 mo of age and 221 kg) were utilized in this experiment. Calves were obtained from 3 different Colorado State University Research facilities (133 composite calves from the San Juan Basin Research Center in Hesperus, CO; 139 Hereford x Angus calves from Maxwell Ranch in Livermore, CO; and 116 black Angus calves from the Colorado State University Beef Improvement Center in Saratoga, WY).

Ranch phase:

Four months prior to weaning, calves from all ranches were allotted to one of three groups based on body weight, age, and health records. One-third of the steers calves from each ranch were then inoculated intranasally with 0.005-mL/kg body weight of infectious bovine rhinotraecheitis virus (IBRV) containing a mean cell culture infective dose ($CCDI_{50}$) of 2.0 x108 viral units/mL. Post-inoculation, inoculated and non-inoculated calves and their respective dams were maintained in separate pastures for 4 weeks to prevent cross inoculation of non-inoculated calves. After weaning (approximately the first week in October), all calves were weighed and transported to the ARDEC feedlot facility, Fort Collins, CO.

Receiving phase:

Upon entry into the feedlot, one-third of the calves from each ranch not receiving IBRV inoculation prior to weaning, were inoculated with IBRV (0.005-mL/kg body

weight). Feedlot inoculated calves were separated from the other calves for 2 weeks. Post-inoculation, all calves were weighed, bled, implanted with 200 mg progesterone and 20 mg estradiol, and vaccinated. Calves were then blocked by ranch and inoculation status, stratified by bodyweight (2 d mean weight) and previous allotment group, sorted into pens (approximately 10 calves per pen) and randomly assigned to TM treatments. Receiving trace mineral treatments consisted of: 1) inorganic trace minerals ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 2) organic trace minerals (iso-amounts of organic Cu, Zn, Mn, and Co). Calves were fed the growing diet (Table 1) for 45 d or until they reached an approximate weight of 345 kg. Diets were fed once daily in the morning in amounts adequate to allow ad libitum access to feed throughout the day. Daily feed offerings were recorded and feed refusal were measured every 28 d. Calves were weighed on d 0, 28, and 45 of the growing phase and average daily gain, feed intake, and efficiency were determined.

At the beginning and end of the growing phase, blood samples were collected in hepranized trace mineral free vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) from 3 steers per pen to determine superoxide dismutase (SOD) activity and interferon gamma concentrations (INF-γ). Blood samples were also obtained in non-hepranized trace mineral free vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) on d 0 and d 56 for total immunoglobulin G (IgG) and immunoglobulin M (IgM) analysis.

On d 0 and 56 of the growing phase, three steers per pen were injected (i.m.) in the neck muscle with 5 ml of a 25% pig red blood cell (PRBC) solution (Engle et al., 1999). Blood samples were obtained in non-hepranized vacutainer tubes (Becton

Dickenson Co., Franklin Lakes, NJ) prior to injection and 7, 14, and 21 d post injection. All blood samples were stored on ice until transported to the laboratory for processing.

Morbidity and mortality were monitored throughout the 45 d receiving phase. At the end of the receiving phase, the same 3 calves/pen that were initially liver biopsied were biopsied again and all steers were gradually switched to a finishing diet. The transition to the finishing diet was completed by d 56 and all receiving data is recorded through d 56.

Finishing phase:

At the beginning of the finishing phase, steers were re-implanted with 80 mg trenbolone acetate and 16 mg estradiol and all steers were gradually switched to a finishing diet (Table 1) and fed until they reach a finished weight of approximately 580 kg. Steers were fed and offerings and feed refusal measured as described in the receiving phase. Trace mineral treatments during the finishing phase were: 1) inorganic Zn (30 mg Zn/mg DM from $ZnSO_4$) and 2) iso-amounts of organic Zn. All other trace minerals (Cu, Mn, and Co) were supplemented in the inorganic form at NRC (1996) recommended concentrations. Every 28 d throughout the finishing phase, all calves were weighed and ADG, ADFI, and FE were determined.

Blood samples were also obtained on d 84 in trace mineral free hepranized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) from the same three calves per pen that were bled in the growing phase to determine SOD activity and INF-γ concentrations. A blood sample was taken on d 84 in a non-hepranized trace mineral free vacutainer tube (Becton Dickenson Co., Franklin Lakes, NJ) and serum was analyzed for total IgG and IgM concentrations.

On d 84, three steers per pen in half of the pens per treatment that were injected with PRBC in the growing phase were re-injected with 5ml of a 25 % PRBC solution (i.m.) in the neck muscle to elicit a secondary humoral immune response to PRBC. Blood samples were collected via jugular venapuncture in non-hepranized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) prior to injection and 7, 14, and 21 d post-injection.

Three steers per pen in the remaining pens per treatment that were injected with PRBC in the growing phase were injected with a second antigen to elicit a primary immune response to a second antigen according to a procedure described by Ward et al. (1993) with a few modifications. Two milliliters of a solution containing 160 mg of ovalbumin (OVA; Sigma A5503), 60 mL Freund's Incomplete Adjuvant (FIA; Sigma F-5506), and 60 mL of phosphate buffered saline (PBS) were injected subcutaneously and one milliliter was injected intradermally to give a total injection of 4000 μg of OVA/animal. Blood samples were collected via jugular venapuncture in non-hepranized vacutainer tubes (Becton Dickenson Co., Franklin Lakes, NJ) prior to injection and 7, 14, and 21 d post injection.

On d 105, d 140, and d 168 of the finishing phase, equal numbers of animals from each treatment were transported to a slaughter plant where carcass data was collected. Carcass data collected included kidney, pelvic, and heart fat (KPH), bone maturity (BMAT), lean maturity (LMAT), overall maturity (OMAT), mean fat thickness (MFT), adjusted fat thickness (AFT), ribeye area (REA), final yield grad (FYG), hot carcass weight (HCW), marbling score (MARB), and dressing percentage (DRESS).

Analytical Procedures:

Blood preparation. All blood samples were centrifuged at 1200 x *g* for 25 min at room temperature. Plasma and serum from blood samples collected in trace mineral free vacutainer tubes were harvested and stored in an acid washed polyethylene tubes until analyzed for INF-γ, concentrations and serum total IgG and IgM concentrations. One milliliter of the red blood cells (RBC) from the hepranized vacutainer tubes was lysed in 4 mL of cold dH_2O in an acid washed polyethylene tube for the determination of SOD activity. Serum samples for PRBC antibody titer analysis were stored in polystyrene tubes. Serum samples for OVA antibody titer analysis were divided equally into three microcentrifuge tubes and were heated for 30 min at 56°C. All samples, except plasma for INF-γ determination, were stored at -20°C. Plasma for INF-γ was store at –70°C.

Superoxide dismutase enzyme activity. Lysed RBC were analyzed for SOD activity using SOD 525™ Assay Kit (Biotech® 21010; Oxis Health Products, Inc., Portland, OR). Briefly, 250 µL of sample was vortexed with 400 µl of a chloroform/ethanol solution (62.5/37.5 v/v) to remove hemoglobin interference. The mixture was centrifuged at 4°C for 5 minutes and the upper aqueous layer was collected (extracted sample) and placed into a clean microcentrifuge tube. Nine hundred microliters of buffer (50 mM 2-amino-2methyl-1,3-propanediol containing 3.3 mM boric acid and 0.11 mM diethylenetriaminepentaacetic acid (DTPA); pH 8.8), 40 µL of extracted sample, and 30 µL of reagent 2 (33.3 mM 1,4,6-trimethyl-2-vinylpyridinium trifluoromethanesulfonate in 1mM HCl) were mixed together by vortexing. The mixture was incubated at 37°C for 1 min. Thirty microliters of reagent 1 (0.66 mM 5,5,5a,11b tetrahydro-3,9,10-trihydroxybenzo[c]flourene in 32 mM HCl containing 0.5 mM DTPA and 2.5% ethanol) was added to this mixture. The mixture was immediately read at 525

nm using a spectrophotometer (Spectronic Genesis 5, Spectronic Instruments, Rochester, NY). Superoxide dismutase activity was expressed as SOD activity per milligram hemoglobin. Hemoglobin concentrations was determined using a Total Hemoglobin Assay Kit (Sigma 525-A). Twenty microliters of whole blood was mixed with 5 mL of Drabkin's solution (sodium bicarbonate, potassium ferricyanide, and potassium cyanide; 100 parts:20 parts:5 parts, respectively). The solution set at room temperature for 15 min and was read at 540 nm using a spectrophotometer (Spectronic Genesis 5, Spectronic Instruments, Rochester, NY).

Total serum IgG and IgM. Total IgG and IgM concentrations were determined using single radioimmunodiffusion assay kits (VMRD 240-30 and 246-30; Pullman, WA) as described by Stable et al. (1993). Briefly, three microliters of serum were added to a well on a single radial immunodiffusion (SRID) plate that contained a monospecific antisera specific for either IgG or IgM. The plates were covered and left undisturbed for approximately 18 hours at room temperature. When the serum diffused into the gel containing the antisera, a ring of precipitation formed that was proportional to the concentration of IgG or IgM in the serum. The diameter of the rings that formed was measured with a ruler in millimeters. These measurements were then compared to standards of known concentrations.

Interferon gamma. Plasma samples were analyzed for INF-γ concentration using an ELISA assay (Biosource KBC1231, Biosource International, Inc., Camarillo, CA). The assays was designed to be a qualitative assay. The assay for this study was modified into a quantitative assay. Positive control that was supplied with the kit was diluted with negative control to make standards. The standards consisted of an undiluted positive

control, and positive control diluted to $1/4^{th}$, $1/16^{th}$, and $1/64^{th}$ of the positive controls original concentration. Linear regression coefficient analysis of sample absorbency verses the expected absorbency yielded a correlation coefficient of 0.997 showing that a semi-quantitative assay could be constructed. Samples were reported as $\log_{10}$.

Pig red blood cell antibody titers. Antibody titers specific for PRBC were measured using a microtiter hemagglutination assay to determine total immunoglobulin (Ig), immunoglobulin G (IgG), and immunoglobulin M (IgM) concentrations specific for PRBC (Ferket and Qureshi, 1992). Serum was heat inactivated in H_2O bath for 30 min at 56°C. A set of 96 well (V-bottom) microtiter plates was prepared. Plate one was for determination of total immunoglobulin (total Ig) concentration. Plate two was for determination of IgG concentration. Into the plate one, 25 µl of 0.1 M PBS (pH 7.4) was pipetted into the first row of wells. Into the second plate, 12.5 µl of 0.1 M PBS (pH 7.4) and 12.5 µL of 0.2 M Mercaptoethanol solution (ME) was pipetted into the first row of wells (ME destroys IgM antibody titers). In duplicate on each plate, 25 µL of serum was added. The plates were covered and incubated for 30 min at 37°C. Once incubated, 25 µL of PBS was added to the remaining wells. Using a microdiluter, a serial dilution of the solution in row one was conducted (part of the solution in row one was diluted into row two; part of the solution in row two was diluted into row 3; etc). Once the dilution was completed, 25 µL of 2.0% PRBC solution was added to each well. The plate was covered and incubated for 30 min at 37°C. After sitting at room temperature, the plates were read. The plates were read by tilting the plate slightly and determining at which dilution (row) agglutination of RBC to the antibody titers was last noticeable (the last well where a small ball was formed). The duplicates were averaged for each sample on

each plate. Immunoglobulin M was determined by subtracting the values determined on the second plate (IgG) from the values determined on the first plate (total Ig). Samples were reported as $\log_2$.

Ovalbumin antibody titers. Serum samples were analyzed for antibody titers specific to ovalbumin using an ELISA procedure described by Engvall and Perlmann (1972). Prior to initiation of the assay, each well of a 96 well (flat bottom) microtiter plates was coated with 100 μL of an Ovalbumin/sodium carbonate-bicarbonate buffer solution (50 μg Ovalbumin/mL). The plates were incubated at 4°C for 18 hours. Plates were washed 3 times with a PBS-0.1% Tween solution. To each well, 100μL of PBS-1% gelatin solution was added and the plates were incubated for 30 min at 39°C. Plates were washed 3 times with PBS-0.1% Tween solution. One hundred microliters of serum (diluted 1:16 with dH_2O) and positive and negative controls were added to the wells in duplicate. The plates were incubated for 1 hour at 39°C and washed 3 times with PBS-0.1% Tween. One hundred microliters of an anti-bovine IgG-peroxidase conjugate (Sigma A5295; diluted 1:20,000 with dH_2O) was added to each well. The plates were incubated for 1 hour at 39°C and were washed 3 times with PBS-0.01% Tween. To each well, 100 □L of substrate solution (containing Citric acid buffer, 2,2`-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma A1888), and 30% hydrogen peroxide) was added. The plates were incubated at room temperature for 15 min and were read at 405 nm on a microtiter plate reader. Samples were reported as $\log_{10}$.

Statistical Analysis:

Statistical analysis of data was performed using Proc Mixed procedure of SAS (2001). The model included treatment, inoculation, time, ranch, and all possible interactions with pen being the experimental unit. When an interaction was not significant, it was removed and the analysis re-ran.

RESULTS AND DISCUSSION

Morbidity in cattle can be detrimental to the feedlot industry. It is estimated that morbidity costs the industry $500 million dollars per year (NASS, 1996). These costs are incurred not only through medical costs but also through the effects of morbidity on performance and carcass characteristics. In a ranch-to-rail study that was conducted 1992 to 2001 by Texas A& M University (McNeill, 1992-2001) these effects were reported. Over the 9 year period, 14,618 cattle were utilized, of which 3616 were determined to be morbid during some point within the study. Morbid cattle were reported to have lower ADG (2.68 lb/d vs. 2.96 lb/d) and lower carcass quality grades. Carcasses of morbid cattle had a lower percent choice (27 % vs. 38 %) and a greater percentage of select (64 % vs. 57 %) and standard (8 % vs. 5 %) verses healthy cattle.

The profitability of steers was also reported to be affected by morbidity. Total cost of gain was increased by approximately 16 % when cattle were morbid (an average of $11 dollars more per head); and treatment costs averaged approximately $30 per head for sick cattle. Morbid cattle had a lower profit, or net return, than healthy cattle, averaging approximately $91 less per head than healthy cattle. McNeill (1992-2001) also reported the difference in price per cwt of morbid cattle verses healthy (never treated) cattle upon arrival to the feedlot. The difference in price per cwt was calculated by taking the difference in profitability between healthy and morbid cattle then dividing by

average weight of calves upon entry into the feedlot divided by 100. For this ranch to rail study, it was reported that morbid calves are worth $15.78 less per cwt upon arrival to the feedlot than healthy calves. Lost value was also calculated in this study. Lost value is calculated from the difference in profitability, or net return, between healthy steers and morbid steers minus medical costs. The lost value over the 9 year study was approximately $61 per steer. This calculates to be a loss of $220,576 in profit for the 3616 morbid steers. In the United States alone, 25 to 30 million cattle are slaughtered each year. If morbidity rates within the United States are similar to what was reported in the ranch to rail study (25 % morbidity), 7.5 million cattle will become morbid. This will amount to $457.5 million dollars in lost profit, not including medical costs, to the cattle industry because of the effects of morbidity on performance and carcass characteristics. In the present study, the effects of induced morbidity as well as TM supplementation on performance, immunity, and carcass characteristics were examined.

Performance:

There was no effect of TM source on performance during both the growing phase and finishing phase (Table 2 and 3). Effects of TM source on performance vary among studies. As reported in the present study, Kegley and Spears (1995) reported no effect of TM source (25 mg Zn/kg DM from Zn oxide or Zn methionine) on body weights and ADG of ewes. Similar results were reported by Droke et al. (1998) and Kegley et al. (2001) overall. Trace mineral source has also been reported to have no effect on ADFI and FE in ewes (Kegley and Spears 1995). In contrast in a second experiment, Kegley et al. (2001) reported an increase in average daily gain in steers supplemented with organic Zn (Zn amino acid complex) compared to steers supplemented with inorganic Zn

($ZnSO_4$) during the last 15 days of their study. This was also reported by Spears (1989), in which growing heifers supplemented with 25 mg Zn/kg DM from Zn methionine tended ($P < 0.13$) to have greater ADG than heifers supplemented with iso-amounts of Zn oxide to a basal diet containing 24 mg Zn/kg DM.

There was no effect of inoculation and time of inoculation on body weights, ADFI, and FE during the growing phase and body weights and FE in the finishing phase (Table 2 and 3). Steers that were inoculated with IBRV had similar ADG than steers that were not inoculated. In 1999, Garner et al. conducted a study utilizing 22 Charolais steer calves. The calves were monitored daily for clinical signs of respiratory infection throughout the 151 d trial. They reported that ADG was lower in steers that were treated for respiratory disease than cattle that were not treated; however, final body weights were similar for morbid and healthy animals. Similar results have been reported by Van Donkersgoed et al. (1993) and Wittum and Perino (1995), however, Jim et al. (1993) and Wittum et al. (1996) reported no effect of treating for respiratory disease on ADG (as reported by Garner et al., 1999). Garner et al. (1999) stated that these differences may be due to differences in diagnosing the disease (rectal temperatures verses clinical diagnosis) or in the type of organism that led to the disease (bacterial verses viral).

During the growing phase, steers that were inoculated at the ranch had lower ($P = 0.04$) ADG than steers that were inoculated at the feedlot. During the finishing phase, steers that were inoculated at the ranch tended to have greater ($P < 0.07$) ADG than steers that were inoculated at the feedlot. It is unclear why steers that were inoculated at the feedlot had greater ADG during the finishing phase but lower ADG during the growing phase. Also, during the finishing phase, steers that were inoculated tended to have lower

($P < 0.09$) ADFI than steers that were not inoculated with IBRV. This decrease in ADG and ADFI may be due in part to animals that are in poor health spending less time eating than healthy animals (Sowell et al., 1993).

Immune function and health:

Superoxide dismutase. Superoxide dismutase was unaffected by TM source, inoculation, and time of inoculation (Table 4). Superoxide dismutase is an important antioxidant. During normal immune function, superoxide radicals (O_2^-) are produced that can damage cellular membranes (Stable and Spears, 1990; Cerone et al., 2000). Superoxide dismutase will aid in neutralizing O_2^- by converting to hydrogen peroxide and oxygen, which will eventually be neutralized further to water (Murray et al., 2000).

Since SOD contains Cu and Zn, its activity may be affected if mineral status is not adequate, but SOD activity seems to be unaffected if TM are sufficient in the diet. In the present study, steers were not deficient in TM (data shown elsewhere) and may explain why SOD activity was unaffected in the present study. Also, since SOD activity was measured in RBC, SOD activity will have a similar lifespan as RBC. The half-life of RBC is approximately 3 months (Murray et al., 2000). If a decrease in SOD activity were to be detected, the steers would have to have been deficient in TM for an extended period of time in order for the decrease in activity to be detected. A animal would be deficient when there is less than 10 mg Cu/kg DM and 20 mg Zn/kg DM in the liver and less than 0.6 mg Cu/L and 0.4 mg Zn/L in plasma and this deficiency would have to be for an extend period of time (Paytner, 1987; Mills, 1987).

There is limited data on the effects of inoculation with IBRV on SOD activity and it is unclear why inoculation with IBRV had no effect on SOD activity in RBC. It is

possible that measuring SOD activity in RBC is not the correct place to determine the change in activity of SOD activity caused by induced morbidity. Since IBRV affects the respiratory system more so than the circulatory system, measuring SOD activity in samples collected from the respiratory system (i.e. cells from the lungs) may have been more appropriate.

Total IgG and total IgM concentrations. Immunoglobulin G and M are antibodies that aid in defense against foreign proteins, or antigens, that enter the body. Immunoglobulins will attach themselves to the antigen or the infected cell and marks the antigen for destruction (Kuby, 1994). Immunoglobulin M is of higher concentration than IgG in the body, is smaller in size, and is the first antibody produced in response to an antigen (Kuby, 1994).

In the present study, total IgG and total IgM concentrations were analyzed to determine concentrations of these antibodies that are present normally in the body and were not specific to any antigen. Total IgM was unaffected by TM source (Table 5). However, steers that were supplemented with inorganic TM had a greater ($P = 0.05$) concentration of total IgG than steers supplemented with organic TM by the end of the growing phase. Contrasting results were reported in heifers. Heifers supplemented with 200 mg Zn/kg DM from $ZnSO_4$ to a barley-corn-pea hay based basal diet that containing 64 mg Zn/kg DM tended to have a lower ($P < 0.10$) concentration of IgG than steers supplemented with 200 mg Zn/kg DM from Zn methionine (Huerta et al., 2002). It is unclear why there was a difference in results among this study and the present study.

Total IgM and IgG were unaffected by inoculation, and time of inoculation (Table 6). It would seem that steers that were inoculated with IBRV would have more

antibodies within their blood stream than steers that were not inoculated with IBRV because their immune system would be producing antibodies to rid the body of the antigen. In the present study, blood samples were collected on d 0 and 56 of the growing phase and d 84 of the finishing phase. The steers were inoculated with IBRV 4 months prior to arrival at the feedlot and on d 0 of the growing phase. By the time of blood collection on d 0 of the growing phase, antibody concentrations most likely returned to normal in steers that were inoculated 4 months prior and may be why differences caused by induced morbidity were undetectable. This statement would be similar for steers inoculated with IBRV on d 0 of the growing phase. The antibodies would not be produced yet for that inoculation and may be back to normal proximity for the subsequent measurements.

Interferon gamma. Interferon gamma is a cytokine that is important in hindering viral replication, activating macrophage, and aids in B-cell differentiation (Kuby 1994; Janeway and Travers, 1996). It increases the production of Major Histocompatibility Complex I (MHC I), which is important in T-cell recognition of infected cells (Kuby, 1994). Interferon gamma also increases the activity of cytotoxic T-cells, macrophage, and natural killer cells (Kuby, 1994; Janeway and Travers, 1996).

In the present study, INF-γ concentrations were unaffected by TM source, inoculation, and time of inoculation (Table 6). In a study conducted in our laboratory where similar TM treatments were utilized, INF-γ concentrations were also unaffected by TM source (in press). It is unclear why INF-γ concentrations were unaffected in both of these trials. It is possible that INF-γ concentrations are only affected during a deficiency of TM as seen with SOD activity. In the present study, steers were not deficient in TM.

It may also be possible that the TM utilized in these studies may not be associated with INF-γ. Since INF-γ has not been studied extensively it is difficult to come to this conclusion and needs to be studied further.

Interferon gamma concentrations were also unaffected by inoculation and time of inoculation (Table 6). As with Total IgG and IgM concentrations INF-γ concentrations were determined on d 0 and 56 of the growing phase and d 84 of the finishing phase. It is possible that INF-γ concentrations were affected by inoculation with IBRV, however, measurements may have been obtained after the INF-γ concentrations had return back to normal post infection. Also, as with SOD activity, measuring INF-γ concentrations in the same tissue or fluid collected from the respiratory system may have been more appropriate in determining the effects of morbidity on INF-γ concentrations since IBRV affects the respiratory system more so than the circulatory system.

Antibody titer concentration specific to pig red blood cells and ovalbumin. Pig red blood cells and ovalbumin are often utilized as antigens in cattle. When utilized, effects on the humoral immunity, the host's defenses against antigens that are mediated by antibodies (Kuby, 1994), are being determined. During the present study, steers were exposed to PRBC on three different occasions: at the beginning and end of the growing phase and mid-way through the finishing phase. During the initial exposure, a primary immune response will occur. The immune system will produce B-cells and antibodies that are specific for that antigen. Memory B-cells are also produced during a primary immune response. Memory B-cells are important during a second exposure to the antigen, as well as subsequent exposures. If the same antigen infects an animal, the immune system will recognize that it has exposed to this antigen previously. The

memory B-cells will be activated from their resting state and will produce antibodies to the antigen. Since these memory B-cells are already produced, the immune system does not have to go through the process of producing cells specific for the antigen. Therefore, the secondary immune response will occur more rapidly than the primary immune response.

During the present study, antibody titers concentrations specific to PRBC were unaffected by TM source, inoculation, and time of inoculation in both the growing and finishing phases (Table 7, 8, and 9). Similar to the present study, TM source had no effect on antibody titer concentrations to PRBC in steers fed a corn silage-soybean meal basal diet (Engle et al., 1999; Engle et al., 1999b). Droke et al (1993) also reported no effects of TM source on antibody titer concentrations specific to PRBC in lambs supplemented with either 25 mg Zn/kg DM from Zn oxide or Zn methionine to a basal diet containing 27.6 mg Zn/kg DM. Contrasting results were seen in a study by Dorton et al. (2003) where steers that were supplemented with organic TM had lower antibody titer concentrations specific to PRBC than steers that were supplemented with inorganic TM. It is unclear why inoculation with IBRV had no affect on antibody titer concentrations to PRBC but may be due to timing of challenge with PRBC.

A second antigen, ovalbumin, was also injected into the steers to illicit a primary immune response to a second antigen. Ovalbumin is a phosphorylated-glycoprotein that is a major constituent of egg whites. Ovalbumin is used as a carrier protein to conjugate to synthetic peptides for use s an immunogen. If injected alone, ovalbumin is not very antigenic. To make ovalbumin more antigenic, Freund's Incomplete Adjuvant is often added. Freund's Incomplete Adjuvant is a water-in-oil emulsion made from non-

metabolizable oils (paraffin oil and mannide monooleate: Product information sheet, Sigma 5506). Freund's incomplete adjuvant is designed to provide a continuous release of antigens. Therefore, Freund's Incomplete Adjuvant will provide a strong, persistent immune response.

In the present study, antibody titer concentration specific to ovalbumin were unaffected by TM source, inoculation, and time of inoculation (Table 10). Although supplementing Cu instead of Zn, Ward et al. (1993) reported that steers supplemented with 5 mg/kg Cu from either $CuSO_4$ or CuLys to a diet containing 6.2 mg Cu/kg DM had similar antibody titer concentrations specific to OVA when compared to controls. Contrasting results were reported in a study by Dorton et al. (2003) where steers that were supplemented with organic TM had greater antibody titer concentrations to ovalbumin antigen and lower antibody titer concentrations to PRBC than steers that were supplemented with inorganic TM. Again, it is unclear why inoculation with IBRV had no affect on antibody titer concentrations to PRBC but may be due to timing of challenge with ovalbumin antigen.

Morbidity and Mortality. Five animals died during this experiment (1 % mortality): 4 of which were inoculated with IBRV at the ranch (3 from the inorganic mineral treatment group) and 1 of which was inoculated at the feedlot (inorganic mineral treatment group). No steers died that were in the group that was not inoculated. Mortality was unaffected by TM source, inoculation, and time of inoculation (Table 11). However, morbidity was unaffected by TM source. Chirase and Green (2001) reported similar results in which calves that were supplemented with 50 mg of Zn methionine and Mn methionine/kg DM had similar morbidity rates to calves supplemented with Zn oxide

and Mn oxide. Other studies have also reported no differences in morbidity rates (Engle et al., 1999b; Kegley et al., 2001). In the present study, morbidity tended ($P < 0.08$) to be greater in steers that were inoculated (Table 12). Inoculated animals also tended ($P = 0.07$) to be retreated more often than steers that were not inoculated with IBRV. This was as to be expected.

Carcass characteristics:

The effects of TM source and inoculation with IBRV on carcass data are reported in Table 12. Trace mineral source and time of inoculation had no effect on carcass characteristics.

Trace mineral source has been reported to have varying effects on carcass characteristics. Green et al. (1988) and Malcolm Callis et al. (2000) reported that steers supplemented with organic TM had greater kidney, pelvic, heart fat than steers supplemented with inorganic TM. Green et al. (1988) and Huerta et al. (2002) reported that steers that were supplemented with Zn methionine had greater MARB ($P < 0.05$ and $P < 0.07$, respectively) than steers supplemented with Zn oxide or $ZnSO_4$. Steers that were supplemented with organic Cu had greater ($P < 0.05$) MARB than steers supplemented with $CuSO_4$ (Engle et al., 2000). Backfat thickness has been reported to be lower ($P < 0.10$) in steers supplemented with $ZnSO_4$ than steers supplemented with Zn methionine, whereas, DRESS, REA, MARB, and yield grade were unaffected by TM source (Malcolm-Callis et al., 2000). Also, HCW has been reported to be lower ($P < 0.05$; Spears and Kegley, 2002) and dressing percentage has been reported to be greater ($P < 0.02$; Huerta et al., 2002) in steers and heifers supplemented with inorganic TM verses organic TM.

In the present study, steers that were not inoculated with IBRV tended to have greater final weights ($P = 0.06$) and hot carcass weights ($P = 0.09$) than inoculated steers. The remaining carcass characteristics were unaffected by inoculation with IBRV. Similar to the present study, Gardner et al. (1999) and Roeber et al. (2001) reported lower carcass weights in morbid cattle ($P < 0.01$). Morbid cattle had lower backfat, KPH fat, carcass quality (although not significantly different), yield grade and marbling scores (Gardner et al., 1999). Lower percent choice carcasses were also reported by McNeill et al. (1996). As expected, morbidity has unfavorable effects on carcass quality.

The percent of lung lesions and the percent of discoloration of the lung were also examined in the present study. Inoculation with IBRV, as well as TM source, had no effect on the percent of lung lesions and discoloration of the lungs. It is unclear why inoculated animals had similar percentage of lung lesions relative to non-inoculated animals. Gardner et al. (1999) reported that lung lesions were found in cattle that were not treated for morbidity. In animals that were not treated, 37 % had lung lesions. Similar findings were reported by Wittum et al. (1996), in which 68 % of untreated steers had lesions on the lungs signifying that untreated animals were infected with an antigen that resulted in lung lesions. This may be the reason why inoculated steers had a similar percentage of lung lesions to steers that were not inoculated with IBRV. Steers that were not inoculated, may have become infected with IBRV or some other pathogen that resulted in lung lesions.

There was a time × inoculation effect for final body weights, marbling score, and final yield grade. There was a tendency ($P < 0.06$) for inoculated steers that were harvested on d 105 of the finishing phase to have a lower final weight than steers

harvested on d 140 of the finishing phase. Steers that were not inoculated and harvested on d 105 also tended ($P < 0.06$) to have lower final weights but the difference was not as prominent as seen among the inoculated steers. Non-inoculated steers that were harvested on d 105 had lower ($P < 0.04$) marbling scores than steers harvested on d 140 and d 168 of the finishing phase. The same trend was observed among the inoculated steers except steers harvested on d 168 had a greater marbling score than steers harvested on d 140. Also, steers that were not inoculated that were harvested on d 105 had a greater ($P < 0.03$) final yield grade than non-inoculated steers harvested on d 140, whereas, inoculated steers harvested on d 105 and d 140 had similar final yield grades.

There was a time × treatment × inoculation effect for preliminary yield grade. On d 133, steers supplemented with inorganic trace minerals that were not inoculated had greater ($P < 0.0064$) preliminary yield grade than steers supplemented with inorganic trace minerals that were inoculated with IBRV. Similar results were seen among the steers supplemented with organic trace minerals but the difference was not as prominent. On d 140, steers supplemented with organic trace minerals that were not inoculated had lower ($P < 0.0064$) preliminary yield grade than steers supplemented with organic trace minerals that were inoculated with IBRV, whereas, steers supplemented with organic trace minerals that were either inoculated or not inoculated had a similar preliminary yield grade. On d 168, steers supplemented with inorganic trace minerals that were not inoculated had greater ($P < 0.0064$) preliminary yield grade than steers supplemented with inorganic trace minerals that were inoculated with IBRV, whereas, steers supplemented with organic trace minerals that were not inoculated had a lower ($P <$

0.0064) preliminary yield grade than steers supplemented with organic trace minerals that were inoculated with IBRV.

There was a time × treatment × inoculation effect for mean fat thickness. On d 133, steers supplemented with inorganic trace minerals that were not inoculated had a greater ($P < 0.0011$) mean fat thickness than steers supplemented with inorganic trace minerals that were inoculated with IBRV. Among the steers supplemented with organic trace minerals, mean fat thickness was similar. On d 140, steers supplemented with organic trace minerals that were not inoculated had greater ($P < 0.0011$) mean fat thickness than steers supplemented with organic trace minerals that were inoculated with IBRV, whereas, steers supplemented with organic trace minerals that were either inoculated or not inoculated had a similar mean fat thickness. On d 168, steers supplemented with inorganic trace minerals that were not inoculated had a greater ($P < 0.0011$) mean fat thickness than steers supplemented with inorganic trace minerals that were inoculated with IBRV, whereas, steers supplemented with organic trace minerals that were not inoculated had a lower ($P < 0.0011$) preliminary yield grade than steers supplemented with organic trace minerals that were inoculated with IBRV.

There was also a tendency for a time × treatment × inoculation effect for KPH. On d 133, steers supplemented with inorganic trace minerals that were not inoculated tended ($P < 0.06$) to have a greater preliminary yield grade than steers supplemented with inorganic trace minerals that were inoculated with IBRV. Among the steers supplemented with organic trace minerals, inoculated and non-inoculated steers had similar KPH. On d 140, steers supplemented with organic trace minerals that were not inoculated tended to have a greater ($P < 0.06$) KPH than steers supplemented with

organic trace minerals that were inoculated with IBRV, whereas, steers supplemented with inorganic trace minerals that were not inoculated tended to have a lower ($P < 0.06$) KPH than steers supplemented with inorganic trace minerals that were inoculated with IBRV. On d 168, steers supplemented with inorganic trace minerals that were not inoculated tended to have a greater ($P < 0.06$) KPH than steers supplemented with inorganic trace minerals that were inoculated with IBRV, whereas, the steers supplemented with organic trace minerals that were either inoculated or not inoculated had similar KPH.

IMPLICATIONS

Although TM supplementation and inoculation with IBRV did not have a significant impact on immune function, the health of cattle seems to be affected though an increase in morbidity. Although not significantly different, morbidity in turn decreased performance through a decrease in ADG and a decrease in ADFI. Morbidity also affected carcass characteristics by decreasing final body weight and HCW, however, carcass quality was not affected as has been reported in other studies. Time of inoculation seemed to have little effect on health and carcass characteristics, as well as little effect on performance. It seems that effects on these parameters are dependent on whether or not the animal becomes morbid and not when the animal becomes morbid. However, little research has been conducted in this area. Further research is needed to determine the role of time of inoculation and trace minerals on performance, immunity, and carcass characteristics in beef cattle.

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Table 1: Ingredient composition of basal diets

Ingredient	Growing	Finishing
	----------%[a]----------	
Corn silage	67.05	80.45
Alfalfa hay	12.42	8.04
Steam flaked corn	13.68	7.67
Protein Supplement	6.84	3.84
Protein Supplement Composition		
Cotton seed meal	40.95	41.67
Soybean meal (46.5%)	20.0	20.0
Limestone	11.75	11.75
Sunflower meal (32%)	11.18	10.20
Urea	6.98	6.97
Dyna-K	3.93	3.92
Biofos	3.85	3.87
Salt	0.90	0.90
Rumensin 80 [b]	0.19	0.31
Selenium (0.16)	0.08	0.08
Vitamin A 30/0	0.07	0.07
Iodine	0.06	0.06
Vitamin E 125	0.05	0.05
Sulfur flowers	0.03	0.01
Cobalt carbonate	0.0003	0.0002
Chemical Composition		
DM, %	72.35	77.44
OM, %	95.31	96.61
CP, %	12.61	10.97
Crude Fat, %	3.88	4.06
TDN, %	81.5	84.35
NEg, Mcal/kg	1.29	1.38
NDF, %	16.65	13.33
ADF, %	7.67	5.43
Ash, %	4.68	3.39
Ca, %	0.59	0.34
P, %	0.42	0.32
K, %	0.91	0.68
Mg, %	0.17	0.14
Na, %	0.06	0.04
S, %	0.16	0.15
Fe, ppm	88.25	64.33
Mn, ppm	14.73	11.44
Zn, ppm	7.57	4.48
Cu, ppm	10.88	6.16

[a] Dry matter basis

Table 2. Effects of TM source and IBRV inoculation on performance of steers in the growing phase.

	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs	TM	Inoculated	Time of Inoculation
Item	Control	Ranch	Feedlot	Control	Ranch	Feedlot		Inoculated	Source	× TM Source	(Ranch vs. Feedlot)
Body Weights, kg											
Initial	237.20	222.19	218.03	236.05	215.27	212.81	17.93	0.20	0.81	0.87	0.85
Final	311.07	279.66	295.61	308.53	285.04	292.75	21.40	0.24	0.97	0.92	0.57
Overall	274.29	250.76	257.44	272.16	251.17	253.01	19.49	0.23	0.68	0.66	0.98
ADG, kg											
Overall	1.32	1.17	1.39	1.30	1.25	1.43	0.09	1.00	0.83	0.64	0.04
ADFI, kg											
Overall	7.03	6.22	6.84	6.84	6.60	6.43	0.52	0.35	0.82	0.84	0.65
FE											
Overall	0.19	0.20	0.20	0.19	0.19	0.22	0.02	0.27	0.66	0.77	0.31

[a]Standard error of the mean.

Table 3. Effects of TM source and IBRV inoculation on performance of steers in the finishing phase.

	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs	TM	Inoculated	Time of Inoculation
Item	Control	Ranch	Feedlot	Control	Ranch	Feedlot		Inoculated	Source	× TM Source	(Ranch vs. Feedlot)
Body Weights, kg											
Initial	311.07	279.66	295.61	308.53	285.04	292.75	20.06	0.24	0.97	0.92	0.57
Final	529.35	507.45	510.65	524.47	507.41	501.77	20.06	0.14	0.96	0.69	0.45
Overall	429.38	395.55	409.47	425.10	402.55	402.67	18.52	0.16	0.87	0.93	0.87
ADG, kg											
Overall	1.33	1.35	1.24	1.30	1.32	1.18	0.07	0.44	0.49	0.83	0.07
ADFI, kg											
Overall	12.15	10.15	10.85	11.48	10.93	10.60	0.80	0.09	0.76	0.49	0.80
FE											
Overall	0.11	0.14	0.11	0.12	0.12	0.11	0.01	0.53	0.88	0.41	0.21

[a]Standard error of the mean.

Table 4. Effects of TM source and IBRV inoculation on Superoxide dismutase activity in growing and finishing steers, U/mg hemoglobin.

	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs	TM	Inoculated	Time of Inoculation
Item	Control	Ranch	Feedlot	Control	Ranch	Feedlot		Inoculated	Source	× TM Source	(Ranch vs. Feedlot)
Day 0	0.34	0.32	0.36	0.34	0.37	0.36	0.031	0.51	0.47	0.53	0.65
Day 56	0.52	0.48	0.55	0.52	0.57	0.57	0.031	0.51	0.48	0.52	0.65
Day 140	0.42	0.39	0.44	0.42	0.46	0.45	0.031	0.51	0.47	0.53	0.66

[a]Standard error of the mean.

Table 5. Effects of TM source and IBRV inoculation on total IgG and total IgM concentrations in growing and finishing steers, mg/dl

	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs	TM	Inoculated.	Time of Inoculation
Item	Control	Ranch	Feedlot	Control	Ranch	Feedlot		Inoculated	Source	× TM Source	(Ranch vs. Feedlot)
IgG											
Day 0	1834.67	1795.00	1815.20	1804.75	1803.92	1800.80	12.31	0.16	0.12	0.23	0.47
Day 56	1863.83	1827.00	1848.40	1832.25	1834.50	1830.20	12.20	0.17	0.05	0.16	0.51
Day 140	1860.00	1827.50	1848.20	1832.50	1834.67	1834.20	16.10	0.49	0.25	0.41	0.49
IgM											
Day 0	178.17	184.25	177.20	183.50	178.50	181.80	3.36	0.81	0.39	0.33	0.72
Day 56	181.00	187.50	180.20	186.75	182.00	184.60	3.36	0.85	0.37	0.32	0.62
Day 140	182.67	189.50	182.00	188.75	188.75	183.67	3.36	0.86	0.36	0.30	0.65

[a]Standard error of the mean.

Table 6. Effects of TM source and IBRV inoculation on INF-γ concentrations in growing and finishing steers, log_{10}.

	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs Inoculated	TM Source	Inoculated. × TM Source	Time of Inoculation (Ranch vs. Feedlot)
Item	Control	Ranch	Feedlot	Control	Ranch	Feedlot					
Day 0	0.13	0.15	0.13	0.14	0.14	0.15	0.011	0.87	0.36	0.66	0.84
Day 56	0.14	0.16	0.14	0.16	0.15	0.16	0.012	1.00	0.42	0.63	0.78
Day 140	0.15	0.17	0.15	0.17	0.16	0.17	0.012	0.97	0.41	0.67	0.73

[a]Standard error of the mean.

Table 7. Effects of TM source and IBRV inoculation on antibody titer concentration specific for PRBC in growing steers (d 0), log_2.

Item	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs Inoculated	TM Source	Inoculated. × TM Source	Time of Inoculation (Ranch vs. Feedlot)
	Control	Ranch	Feedlot	Control	Ranch	Feedlot					
Total Ig											
Day 0	0	0	0	0	0.028	0.048	0.026	0.19	0.19	0.19	0.74
Day 7	0.70	0.67	0.69	0.71	0.69	0.70	0.034	0.59	0.78	0.99	0.69
Day 14	1.92	1.85	1.90	1.95	1.89	1.92	0.067	0.52	0.69	0.98	0.70
Day 21	2.13	2.05	2.11	2.17	2.10	2.13	0.072	0.53	0.68	1.00	0.68
Overall	1.19	1.14	1.18	1.20	1.18	1.20	0.045	0.26	0.52	0.41	0.73
IgG											
Day 0	0	0	0	0	0	0	0.055	----	----	----	----
Day 7	0.49	0.47	0.48	0.50	0.48	0.49	0.055	0.50	0.61	0.99	0.70
Day 14	1.54	1.48	1.52	1.56	1.52	1.54	0.055	0.51	0.66	0.99	0.69
Day 21	1.92	1.85	1.90	1.95	1.89	1.92	0.055	0.52	0.69	0.98	0.70
Overall	0.99	0.95	0.97	1.00	0.97	0.99	0.050	0.59	0.74	0.99	0.95
IgM											
Day 0	0	0	0	0	-0.028	-0.048	0.013	0.19	0.19	0.19	0.74
Day 7	0.21	0.20	0.21	0.21	0.21	0.21	0.013	0.63	0.80	0.80	0.92
Day 14	0.39	0.37	0.38	0.39	0.38	0.38	0.013	0.54	0.80	0.94	0.74
Day 21	0.21	0.21	0.21	0.22	0.21	0.21	0.013	0.63	0.62	0.79	0.55
Overall	0.20	0.19	0.20	0.20	0.19	0.19	0.0095	0.24	0.85	0.41	0.97

[a]Standard error of the mean.

Table 8. Effects of TM source and IBRV inoculation on antibody titer concentration specific for PRBC in growing steers (d 56), log_2.

Item	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs Inoculated	TM Source	Inoculated. × TM Source	Time of Inoculation (Ranch vs. Feedlot)
	Control	Ranch	Feedlot	Control	Ranch	Feedlot					
Total Ig											
Day 0	0	0	0	0	0.11	0.14	0.082	0.16	0.16	0.16	0.89
Day 7	0.78	0.75	0.77	0.79	0.77	0.78	0.082	0.53	0.70	0.93	0.70
Day 14	2.16	2.07	2.13	2.19	2.13	2.15	0.082	0.51	0.69	0.97	0.71
Day 21	2.67	2.57	2.64	2.70	2.63	2.66	0.082	0.51	0.71	0.96	0.71
Overall	1.40	1.35	1.38	1.42	1.41	1.43	0.073	0.93	0.48	0.65	0.95
IgG											
Day 0	0	0	0	0	0	0	0.068	----	----	----	----
Day 7	0.54	0.52	0.54	0.55	0.54	0.54	0.068	0.58	0.73	0.90	0.69
Day 14	1.73	1.66	1.71	1.75	1.70	1.72	0.068	0.52	0.69	0.98	0.71
Day 21	2.40	2.31	2.38	2.44	2.37	2.40	0.068	0.52	0.69	0.98	0.69
Overall	1.17	1.12	1.16	1.18	1.15	1.17	0.062	0.60	0.75	0.98	0.95
IgM											
Day 0	0	0	0	0	-0.11	-0.14	0.028	0.16	0.16	0.16	0.89
Day 7	0.23	0.22	0.23	0.24	0.23	0.23	0.028	0.70	0.55	0.79	0.55
Day 14	0.43	0.41	0.43	0.44	0.43	0.43	0.028	0.54	0.70	1.00	0.65
Day 21	0.27	0.26	0.26	0.27	0.27	0.27	0.028	0.50	0.71	0.84	0.75
Overall	0.23	0.22	0.23	0.24	0.20	0.20	0.017	0.14	0.47	0.23	0.97

[a]Standard error of the mean.

Table 9. Effects of TM source and IBRV inoculation on antibody titer concentration specific for PRBC in finishing steers (d 84), log_2.

Item	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs Inoculated	TM Source	Inoculated. × TM Source	Time of Inoculation (Ranch vs. Feedlot)
	Control	Ranch	Feedlot	Control	Ranch	Feedlot					
Total Ig											
Day 0	0	0	0	0	0	0	0.11	----	----	----	----
Day 7	1.72	1.65	1.64	1.74	1.69	1.71	0.11	0.39	0.53	0.78	0.98
Day 14	3.43	3.30	3.39	3.48	3.38	3.42	0.11	0.52	0.69	0.98	0.71
Day 21	3.81	3.66	3.77	3.87	3.76	3.81	0.11	0.51	0.69	0.98	0.70
Overall	2.24	2.15	2.20	2.27	2.21	2.24	0.10	0.58	0.73	0.97	0.95
IgG											
Day 0	0	0	0	0	0	0	0.11	----	----	----	----
Day 7	1.43	1.38	1.36	1.45	1.41	1.42	0.11	0.38	0.53	0.80	0.98
Day 14	3.12	3.00	3.09	3.16	3.07	3.11	0.11	0.53	0.70	0.98	0.70
Day 21	3.63	3.49	3.59	3.68	3.58	3.62	0.11	0.52	0.71	0.97	0.70
Overall	2.05	1.96	2.01	2.07	2.01	2.04	0.10	0.59	0.75	0.97	0.95
IgM											
Day 0	0	0	0	0	0	0	0.01	----	----	----	----
Day 7	0.29	0.28	0.27	0.29	0.28	0.29	0.01	0.50	0.59	0.76	0.94
Day 14	0.31	0.30	0.30	0.32	0.31	0.31	0.01	0.38	0.58	1.00	0.83
Day 21	0.18	0.18	0.18	0.19	0.18	0.18	0.01	0.45	0.37	0.80	0.63
Overall	0.19	0.19	0.19	0.20	0.19	0.20	0.01	0.47	0.51	0.98	0.93

[a]Standard error of the mean.

Table 10. Effects of TM source and IBRV inoculation on antibody titer concentrations specific for ovalbumin antigen in finishing steers, log_{10}.

Item	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs Inoculated	TM Source	Inoculated × TM Source	Time of Inoculation (Ranch vs. Feedlot)
	Control	Ranch	Feedlot	Control	Ranch	Feedlot					
Day 0	0	0	0	0	0	0	0.0022	----	----	----	----
Day 7	0	0.0008	0	0.0003	0.0005	0	0.0022	0.58	0.76	0.63	0.15
Day 14	0.049	0.052	0.049	0.052	0.051	0.052	0.0022	0.94	0.26	0.64	0.65
Day 21	0.061	0.065	0.061	0.065	0.064	0.065	0.0022	0.91	0.27	0.63	0.66
Overall	0.027	0.029	0.027	0.029	0.029	0.029	0.0018	0.92	0.33	0.67	0.93

aStandard error of the mean.

Table 11. Effects of TM source and IBRV inoculation on morbidity and mortality of steers.

Item	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs Inoculated	TM Source	Inoculated × TM Source	Time of Inoculation (Ranch vs. Feedlot)
	Control	Ranch	Feedlot	Control	Ranch	Feedlot					
Steers, no.	66	65	66	66	65	65	----	----	----	----	----
Morbidity, %	22/66 (33%)	20/65 (31%)	13/66 (20%)	21/66 (32%)	15/65 (23%)	15/65 (23%)	0.055	0.08	0.70	0.95	0.30
Treated per morbid	0.48	0.49	0.26	0.44	0.29	0.25	0.09	0.07	0.34	0.70	0.11
Mortality, %	0 % (0/66)	5 % (3/65)	2 % (1/66)	0 % (0/66)	2 % (1/65)	0 % (0/65)	0.014	0.11	0.34	0.34	0.17

[a]Standard error of the mean.

Table 12. Effects of TM source and IBRV inoculation on carcass characteristics of steers

Item	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs Inoculated	TM Source	Inoculated. × TM Source	Time of Inoculation (Ranch vs. Feedlot)
	Control	Ranch	Feedlot	Control	Ranch	Feedlot					
Final Weight	535.82	516.15	507.41	530.32	522.18	505.08	12.87	0.06	0.80	0.69	0.59
HCW	321.03	313.12	307.23	319.58	315.84	306.80	7.58	0.09	0.97	0.74	0.66
Dressing %	59.73	59.77	59.51	59.83	59.30	58.12	0.65	0.33	0.55	0.43	0.54
REA	73.97	73.52	72.40	74.27	73.99	70.13	2.05	0.23	0.67	0.81	0.22
Marbling	388.71	437.21	405.19	406.37	416.52	409.68	16.38	0.19	0.88	0.67	0.46
KPH	2.19	2.16	2.05	2.13	2.12	2.05	0.06	0.24	0.53	0.72	0.28
MFT	1.46	1.33	1.22	1.28	1.39	1.23	0.09	0.36	0.45	0.16	0.22
AFT	1.45	1.46	1.37	1.48	1.46	1.28	0.07	0.26	0.96	0.64	0.13
PYG	3.43	3.31	3.20	3.26	3.37	3.24	0.09	0.39	0.48	0.12	0.28
APYG	3.43	3.43	3.35	3.45	3.44	3.25	0.07	0.27	0.93	0.65	0.13
YG	2.94	2.82	2.83	2.71	2.77	2.57	0.13	0.47	0.17	0.94	0.66
FYG	3.30	3.35	3.24	3.35	3.22	3.07	0.11	0.29	0.65	0.32	0.43
Lung Score	1.88	2.04	3.53	2.43	0.65	1.12	1.25	0.77	0.53	0.26	0.45
Lung Score (Chi Square)	14/52 (27%)	11/37 (30%)	9/44 (20%)	16/44 (36%)	5/39 (13%)	12/43 (28%)	0.07	0.63	0.12	0.24	0.64
% Discoloration of lung score	5.73	6.66	16.88	7.27	6.46	5.86	3.65	0.49	0.56	0.28	0.45

[a]Standard error of the mean.

Chapter VII

INDUCED MORBIDITY IN PRE-WEANED CALVES AND RECEIVING CATTLE: SUBSEQUENT EFFECTS OF FEEDLOT RATION TRACE MINERAL SOURCE ON TRACE MINERAL STATUS AND LIPID METABOLISM OF STEERS

ABSTRACT

Three hundred and eighty eight steer calves (approximately 7 mo of age and 221 kg) were utilized to determine the effects of time of respiratory disease challenge (pre-weaning vs. feedlot) and trace mineral source on feedlot performance and carcass characteristics. One-third of the steer calves from each ranch were inoculated intranasally with 0.005-mL/kg body weight of infectious bovine rhinotraecheitis virus (IBRV) containing a mean cell culture infective dose ($CCID_{50}$) of 2.0×10^8 viral units/mL 4 mo prior to weaning at the ranch. One-third of the calves not inoculated at the ranch were inoculated upon arrival at the feedlot. The remaining one-third of the caves from each ranch were not inoculated. Calves were blocked by ranch and inoculation status, stratified by bodyweight (2 d mean weight), sorted into pens (approximately 10 calves per pen) and randomly assigned to treatments. Receiving phase treatments consisted of 1) Availa-4 (organic forms of Zn, Cu, and Mn) and 2) iso-amounts of inorganic minerals ($ZnSO_4$, $CuSO_4$, and $MnSO_4$). Finishing phase treatments consisted

of: 1) organic Zn (Availa Zn) and 2) iso-amounts of inorganic Zn ($ZnSO_4$). All other trace minerals were fed at NRC levels in the inorganic form. On d 105, d140, and d 168 of the finishing phase, equal numbers of animals from each treatment were transported to a slaughter plant where longissimus muscle samples were collected to be analyzed for fatty acid concentrations. Trace mineral source had no effect on Zn concentrations in the liver. At the end of the growing phase, liver Cu concentrations tended ($P = 0.07$) to be lower in steers supplemented with inorganic TM. At the end of the finishing phase liver Cu concentrations were lower ($P < 0.02$) in inorganic verses organic supplemented steers. Time of inoculation had no effect on TM concentrations. On d 0 of the growing phase, steers that were inoculated tended to have lower ($P = 0.06$) liver Mn than steers that were not inoculated. On d 56 of the growing phase, inoculated steers tended to have greater ($P = 0.07$) liver Zn concentrations than steers that were not inoculated. There was an inoculation × source effect on liver Cu and Mo concentrations. During the finishing phases, inoculated steers supplemented with inorganic TM tended to have greater liver Cu concentrations ($P < 0.08$) and liver Mo concentrations ($P = 0.09$) than non-inoculated steers supplemented with inorganic TM, whereas, steers supplemented with organic TM tended to have lower liver Cu concentrations ($P < 0.08$) and liver Mo concentrations ($P = 0.09$) than non-inoculated steers supplemented with organic TM. There was a time × inoculation effect for liver Fe concentrations. Within the inoculated steers, liver Fe concentrations increased ($P = 0.02$) by the end of the growing phase but decreased below initial liver Fe concentrations by the end of the finishing phase. Within the steers that were not inoculated, liver Fe concentrations remained similar throughout the growing phase but increased ($P = 0.02$) by the end of the finishing phase. Trace mineral source

had no effect on fatty acid concentrations in longissimus muscle. Inoculated steers tended ($P = 0.06$) to have lower concentrations of oleic acid (18:1 n-9) and monounsaturated fatty acids (MUFA). Steers that were inoculated at the ranch had lower concentrations of myristic acid (14:0; $P < 0.04$) and linoleic acid (18:2 n-6; $P < 0.02$) and tended ($P = 0.06$) to have lower concentrations of arachodonic acid (20:4 n-6) than steers inoculated at the feedlot. These results indicate that TM source and inoculation with IBRV may impact mineral status of steers; however, fatty acid concentrations seemed unaffected by TM source.

Key words: Steers, Trace Minerals, Induced Morbidity, IBRV, Lipid Metabolism

INTRODUCTION

Trace mineral supplementation has been shown to enhance trace mineral status (TM; Huerta et al., 2002; Dorton et al., 2003) and alter lipid metabolism in cattle (Engle et al., 2000a). Furthermore, Spears (1996) stated is a theory that organic sources of minerals were more bioavailable than inorganic sources because the organic sources are more similar to biologically active forms of minerals in the body and in feed. This would allow the body to utilize these minerals more efficiently. Nevertheless, studies examining the effects of TM source have reported varying results on TM status (Kincaid et al., 1986; Stanton et al. 1998) and lipid metabolism in steers (Engle et al., 2000a; Engle et al. 2000b).

Morbidity rates in the feedlot industry are high, despite vaccination against respiratory disease. Morbidity has been reported to affect TM status of cattle by decreasing Zn concentrations in the plasma (Orr et al., 1991; Chirase et al., 1991) to decrease growth of infectious agents and increase Cu concentrations in the plasma

(Gengelbach et al., 1997). Little is known about the effects of morbidity on lipid metabolism in cattle. Therefore, the objective of this experiment was to determine the effects of time of respiratory disease challenge (pre-weaning vs. feedlot) and trace mineral source (inorganic vs. organic) on trace mineral (TM) status and lipid metabolism in steers.

MATERIALS AND METHODS

The Colorado State University Animal Care and Use Committee approved care, handling, and sampling of the animals defined in this experiment prior to the initiation of this study.

Three hundred and eighty eight steer calves (approximately 7 mo of age and 221 kg) were utilized in this experiment. Calves were obtained from 3 different Colorado State University Research facilities (133 composite calves from the San Juan Basin Research Center in Hesperus, CO; 139 Hereford x Angus calves from Maxwell Ranch in Livermore, CO; and 116 black Angus calves from the Colorado State University Beef Improvement Center in Saratoga, WY).

Ranch phase:

Four months prior to weaning, calves from all ranches were allotted to one of three groups based on body weight, age, and health records. One-third of the steers calves from each ranch were then inoculated intranasally with 0.005-mL/kg body weight of infectious bovine rhinotraecheitis virus (IBRV) containing a mean cell culture infective dose ($CCID_{50}$) of 2.0 x108 viral units/mL. Post-inoculation, inoculated and non-inoculated calves and their respective dams were maintained in separate pastures for 4 weeks to prevent cross inoculation of non-inoculated calves. After weaning

(approximately the first week in October), all calves were weighed and transported to the ARDEC feedlot facility, Fort Collins, CO.

Receiving phase:

Upon entry into the feedlot, one-third of the calves from each ranch not receiving IBRV inoculation prior to weaning, were inoculated with IBRV (0.005-mL/kg body weight). Feedlot inoculated calves were separated from the other calves for 2 weeks. Post-inoculation, all calves were weighed, bled, implanted with 200 mg progesterone and 20 mg estradiol, and vaccinated. Calves were then blocked by ranch and inoculation status, stratified by bodyweight (2 d mean weight) and previous allotment group, sorted into pens (approximately 10 calves per pen) and randomly assigned to TM treatments. Receiving trace mineral treatments consisted of: 1) inorganic trace minerals ($CuSO_4$, $ZnSO_4$, $MnSO_4$, and $CoCO_3$), and 2) organic trace minerals (iso-amounts of organic Cu, Zn, Mn, and Co). Calves were fed the growing diet (Table 1) for 45 d or until they reached an approximate weight of 345 kg. Diets were fed once daily in the morning in amounts adequate to allow ad libitum access to feed throughout the day.

Liver biopsies samples were obtained from 3 calves per pen on d 0 and d 56 of the growing phase using the true-cut technique described by Pearson and Craig (1980), as modified by Engle and Spears (2000). Briefly, a 10 cm x 10 cm area on the right side of the steer between the 11^{th} and 12^{th} ribs was clipped of hair and scrubbed three times with betadine alternating with 70 % alcohol. Approximately five milliliters of a two percent lidocaine hydrochloride solution (Abbott Laboratories, Chicago, IL) was injected via a 20-gauge x 2.5 cm needle between the 11^{th} and 12^{th} rib on a line made from the tubercoxae to the tip of the shoulder. A small incision (approximately 1.0 cm) was made

using a #11 scalpel blade. A core sample of liver was collected using a modified Jam Shide bone marrow punch (0.5 cm x 14 cm; Sherwood Medical, St. Louis, MO). Following collection, samples were immediately rinsed with 0.01 M phosphate buffered saline (PBS) solution (pH 7.4) and placed into an acid washed polyethylene tube, capped, and stored on ice until transported to the laboratory. Samples were then stored at –20°C until analyzed for trace minerals.

Finishing phase:

At the beginning of the finishing phase, steers were re-implanted and all steers were gradually switched to a finishing diet (Table 1) and fed until they reach a finished weight of approximately 580 kg. Trace mineral treatments during the finishing phase were: 1) inorganic Zn (30 mg Zn/mg DM from $ZnSO_4$) and 2) iso-amounts of organic Zn. All other trace minerals (Cu, Mn, and Co) were supplemented in the inorganic form at NRC (1996) recommended concentrations. On day 84, a liver biopsy was collected as previously described from the same 3 calves per pen that were biopsied during the growing phase.

On d 105, d 140, and d 168 of the finishing phase, equal numbers of animals from each treatment were transported to a slaughter plant where carcass data was collected. A longissimus muscle tissue sample was obtained postmortem to be analyzed for fatty acid profile. Samples were stored on ice and transported back to the laboratory where they were stored at –20°C.

Analytical Procedures

Liver samples were analyzed via inductively coupled plasma-atomic emission spectroscopy (ICP-AES) methods (Braselton et al., 1997 as described by Ahola et al.

(2004) for Zn, Cu, Mo, Mn, S, and Fe concentrations. Briefly, samples were thawed and dried for 4 hours at 95°C, then allowed to cool to room temperature. Samples were weighed and then combined with 2 mL of 3.6N nitric acid. The mixture was allowed to digest overnight at 95°C and then cooled to room temperature. Samples were diluted in dH_2O to fit within a linear range of a standard curve generated by linear regression of known TM concentrations. Multielemental analysis was then carried out by the simultaneous/sequential ICP-AES, with cross flow nebulization, procedure.

Fatty acid profile. External fat was removed from the longissimus muscle samples and the samples were ground in a food processor. One gram of ground sample was weighed into a tin-weighing dish used for lipid extraction (wet weight). Lipid extraction of longissimus subsamples were accomplished via the Soxlette method. Briefly, the sample was dried overnight at 100°C. The sample was then reweighed to determine dry matter, folded within filter paper, and placed into the Soxlette apparatus for 10 hours. The samples were then removed from the Soxlette apparatus and allowed to air-dry overnight. Once air-dry, the samples were dried in an oven at 100°C overnight and weighed. Percent fat was determined by dividing the weight of fat that was extracted from the sample by the wet weight of the sample prior to drying and extraction.

A second one-gram sample was weighed into a 20 mL scintillation vial to be analyzed for fatty acid composition. The samples were placed into a freeze-drier for approximately 36 hours. Methyl ester derivatives of the longissimus samples were prepared using a combination of $NaOCH_3$ followed by HCl/CH_3OH as described by Kramer et al. (1997). Fatty acid compositions were determined by gas chromatography using an Agilent 6890 Series gas chromatograph (Wilmington, DE) fixed with a 6B90

series injector and flame ionization detector. The instrument was equipped with a 100-m × 0.25mm (i.d.) fused silica capillary column (SP™-2560 Supelco Inc. Bellefonte, PA). Fatty acid methyl ester preparations were injected (1 μL) using the split mode. The carrier gas was helium, and the split ratio was 100:1 at 180°C. The oven temperature was programmed from an initial temperature of 140°C (0 min) to a final temperature of 225°C at a rate of 2.8°C/min. The final temperature was held for 18 min. Chromatograms were recorded with a computing integrator (ChemStation Plus chromatograph manager, Agilent Technologies, Wilmington, DE). Recovery of total fatty acids was quantified by the incorporation of an internal standard. Standard fatty acid methyl ester mixtures were used to calibrate the gas chromatograph system using reference standards KEL-FIM-FAME-5 (Matreya, Pleasant Gap, PA). Identification of the fatty acids was made by comparing the relative retention times of fatty acid methyl ester peaks with those of the standards. Fatty acids were calculated as normalized area percentages of fatty acids.

Statistical Analysis

Statistical analysis of data was performed using Proc Mixed procedure of SAS (2001). The model included treatment, inoculation, time, and all possible interactions with pen being the experimental unit. When an interaction was not significant, it was removed and the analysis re-ran.

RESULTS AND DISCUSSION

Trace mineral status:

The effects of TM source, inoculation, and time of inoculation are reported in Table 2. Trace mineral source had no significant effect on Zn concentrations in the liver; however, steers supplemented with organic TM had numerically great liver Zn

concentrations than steers supplemented with inorganic TM. Huerta et al. (2002) reported a TM source effect in feedlot heifers. Heifers supplemented with 200 mg Zn/kg DM from $ZnSO_4$ had greater liver Zn concentrations than heifers supplemented with 200 mg Zn/kg DM from Zn methionine. In contrast to the present study, ewes that were supplemented with organic TM tended to have greater ($P = 0.06$) liver Zn concentrations than ewes supplemented with inorganic TM (Huerta et al., 2002).

On d 0 of the growing phase, steers supplemented with inorganic TM had lower ($P = 0.0001$) liver Cu concentrations, tended to have lower ($P < 0.07$) liver Mn concentrations than steers supplemented with organic TM. Trace mineral supplementation could not have had an affect d 0 liver TM concentrations since the steers did not receive treatments until after samples were collected on d 0. Normally, a covariate would be utilized to account for and adjust for differences seen at the beginning of an experiment; however, in this case, it was not appropriate to do so. Since the experiment was initiated at the ranch (inoculation of one third of the calves at each ranch), utilizing a covariate would have removed this effect.

Liver Cu concentrations tended ($P = 0.07$) to remain lower in steers supplemented with inorganic TM throughout the growing phase as well as at the end of the finishing phase ($P < 0.02$). In contrast to the present study, ewes supplemented with organic TM (Zn amino acid complex) tended to have greater liver Cu concentrations than ewes supplemented with ZnSO4 (Hatfield et al., 2001). This effect was also reported in cows (Ahola et al, 2004).

In the present study, time of inoculation had no effect on liver Zn, Cu, and Mn concentrations; and inoculation of steers with IBRV had no effect on liver Cu

concentrations. On d 0 of the growing phase, steers that were inoculated tended to have lower (P = 0.06) liver Mn than steers that were not inoculated. On d 56 of the growing phase, inoculated steers tended to have greater (P = 0.07) liver Zn concentrations than steers that were not inoculated. There was no effect of inoculation during the finishing phase.

Although in the present study effects of inoculation with IBRV on plasma mineral concentrations were not examined, studies that have examined these effects on plasma mineral concentrations add incite into the results found in the present study regarding liver mineral concentrations. Gengelbach et al. (1997) reported that post IBRV inoculation plasma Cu concentrations increased in calves, which they explained as an association of Cu with an acute phase immune response due to interleukin-1. In the present study, liver Cu concentrations were unaffected by inoculation with IBRV. Also, ceruloplasm is synthesized by hepatocytes during an infection (Underwood, 1977). Ninety percent of serum Cu is bound to ceruloplasm (Underwood, 1977). If the excess Cu being utilized by the immune system is being pulled from liver storage then it would seem that liver Cu concentrations would be decreased, however, liver Cu was similar between steers that were inoculated and steers that were not inoculated.

Plasma Zn concentrations have been reported to be decreased during an infection (Orr et al., 1991; Chirase et al., 1991; Chirase et al., 1994). It has been suggested that Zn is removed from the blood stream (Henken, 1974) possibly through renal clearance (Southern and Baker, 1983). This may be the body's natural defense mechanism to decrease plasma zinc in order to make Zn unavailable to be utilized by infectious agents for growth (Young, 1981). In the present study, liver Zn concentrations were increased

on d 56 in steers that were inoculated with IBRV. It may be possible that Zn is taken out of the bloodstream and stored with in the liver to make Zn unavailable to infectious agents.

There was also an inoculation × source effect on liver Cu concentrations. During the finishing phases, inoculated steers supplemented with inorganic TM tended to have greater liver Cu concentrations ($P < 0.08$) than non-inoculated steers supplemented with inorganic TM, whereas, steers supplemented with organic TM tended to have lower liver Cu concentrations ($P < 0.08$) than non-inoculated steers supplemented with organic TM.

Lipid metabolism:

Percent lipid in longissimus muscle tissue. Percent lipid in longissimus muscles of steers was unaffected by TM source, inoculation, or time of inoculation (Table 3). Similar results were reported in steers (Engle et al., 2000a; Engle and Spears, 2000).

Fatty acid composition of longissimus muscle tissue. Trace mineral source also had no effect on fatty acid concentrations in longissimus muscle (Table 3). Engle et al. (2000b) reported that TM source had no effect on fatty acid composition in longissimus muscle tissue, except that steers supplemented with 20 mg Cu/kg DM from $CuSO_4$ had lower ($P < 0.05$) concentrations of 22:1 (not determined in the present study) than steers supplemented with 20 mg Cu/kg DM from Cu proteinate. In a previous study conducted in our laboratory, steers that were supplemented with inorganic trace minerals tended to have a greater ($P < 0.07$) concentration of 18:0, lower concentrations of 18:2 n-6 ($P = 0.0005$) and docosahexaenoic acid (22:6 n-3; $P = 0.04$), tended ($P < 0.07$) to have a lower concentration of PUFA than steers that were supplemented with organic trace minerals. Also, steers that were supplemented with inorganic trace minerals also had a lower ($P =$

0.03) ratio of PUFA:SFA than steers that were supplemented with organic trace minerals. The reason for the difference in results among studies is unknown

In the present study, inoculated steers tended (P = 0.06) to have lower concentrations of oleic acid (18:1 n-9) and monounsaturated fatty acids (MUFA). Steers that were inoculated at the ranch had lower concentrations of myristic acid (14:0; P < 0.04) and linoleic acid (18:2 n-6; P < 0.02) and tended (P = 0.06) to have lower concentrations of arachodonic acid (20:4 n-6) than steers inoculated at the feedlot. Little research has been conducted into the effects of morbidity on fatty acid composition of steers. It is possible that when animals become morbid that they shift production of fatty acids to those that are utilized by the immune system, however, the reason for the results reported here are unclear.

CONCLUSIONS

Steers supplemented with inorganic TM had lower concentrations of Cu and Mn in the liver than steers supplemented with organic TM; however Zn concentrations in the liver and fatty acid concentrations were unaffected by TM source. Induced morbidity had varying effects on TM status and fatty acid concentrations. Manganese and Zn concentrations were decreased in the liver of inoculated steers. Inoculated steers had lower oleic acid (18:1 n-9) and monounsaturated fatty acids (MUFA); however, other fatty acids examined were unaffected by inoculation with IBRV. Time of inoculation had no effect on TM status, but fatty acid concentration was affected. Steers that were inoculated prior to arrival at the feedlot had lower concentrations of myristic acid (14:0) and linoleic acid (18:2 n-6). This suggests that calves that become morbid prior to arrival to the feedlot may have metabolically altered lipid metabolism. These results indicate

that TM source and inoculation with IBRV may impact mineral status of steers; however, fatty acid concentrations seemed unaffected by TM source.

It seems that effects on these parameters, especially TM status, is more dependent on whether or not the animal becomes morbid and not when the animal becomes morbid. However, little research has been conducted in this area. Further research is needed to determine the role of time of inoculation and trace minerals on performance, immunity, and carcass characteristics in beef cattle.

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Table 1: Ingredient composition of basal diets

Ingredient	Growing	Finishing
	----------%[a]----------	
Corn silage	67.05	80.45
Alfalfa hay	12.42	8.04
Steam flaked corn	13.68	7.67
Protein Supplement	6.84	3.84
Protein Supplement Composition		
Cotton seed meal	40.95	41.67
Soybean meal (46.5%)	20.0	20.0
Limestone	11.75	11.75
Sunflower meal (32%)	11.18	10.20
Urea	6.98	6.97
Dyna-K	3.93	3.92
Biofos	3.85	3.87
Salt	0.90	0.90
Rumensin 80[b]	0.19	0.31
Selenium (0.16)	0.08	0.08
Vitamin A 30/0	0.07	0.07
Iodine	0.06	0.06
Vitamin E 125	0.05	0.05
Sulfur flowers	0.03	0.01
Cobalt carbonate	0.0003	0.0002
Chemical Composition		
DM, %	72.35	77.44
OM, %	95.31	96.61
CP, %	12.61	10.97
Crude Fat, %	3.88	4.06
TDN, %	81.5	84.35
NEg, Mcal/kg	1.29	1.38
NDF, %	16.65	13.33
ADF, %	7.67	5.43
Ash, %	4.68	3.39
Ca, %	0.59	0.34
P, %	0.42	0.32
K, %	0.91	0.68
Mg, %	0.17	0.14
Na, %	0.06	0.04
S, %	0.16	0.15
Fe, ppm	88.25	64.33
Mn, ppm	14.73	11.44
Zn, ppm	7.57	4.48
Cu, ppm	10.88	6.16

[a] Dry matter basis

Table 2. Effects of TM source and IBRV inoculation on liver mineral concentrations in growing and finishing steers.

Item	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs Inoculated	TM Source	Inoculated × TM Source	Time of Inoculation (Ranch vs. Feedlot)
	Control	Ranch	Feedlot	Control	Ranch	Feedlot					
Liver Zn											
Day 0	96.31	91.68	87.54	88.93	97.04	96.25	5.92	0.92	0.97	0.16	0.68
Day 56	89.06	87.80	110.75	83.50	108.17	99.37	8.98	0.07	0.95	0.54	0.52
Day 168	89.71	70.64	83.57	89.96	90.15	86.68	10.68	0.43	0.52	0.54	0.55
Liver Cu											
Day 0	228.60	244.00	243.20	342.90	379.30	362.10	31.60	0.43	0.0001	0.81	0.84
Day 56	384.80	382.10	358.40	417.20	439.70	456.50	34.92	0.78	0.07	0.44	0.92
Day 168	114.84	199.90	246.80	301.22	305.40	204.96	48.23	0.47	0.02	0.08	0.60
Liver Mn											
Day 0	6.81	7.55	7.24	7.39	8.43	7.87	0.41	0.06	0.07	0.80	0.34
Day 56	8.10	7.98	7.30	7.72	7.76	8.10	0.46	0.75	0.91	0.40	0.73
Day 168	3.00	5.53	6.13	5.96	6.88	4.61	1.08	0.18	0.14	0.12	0.44

[a]Standard error of the mean.

Table 3. Effects of TM source and IBRV inoculation on percent lipid and fatty acid concentrations in longissimus muscles of steers.

Item	Dietary Treatments						SEM[a]	P-value			
	Inorganic TM			Organic TM				Control vs Inoculated	TM Source	Inoculated × TM Source	Time of Inoculation (Ranch vs. Feedlot)
	Control	Ranch	Feedlot	Control	Ranch	Feedlot					
% Lipids	4.43	5.68	4.15	4.17	4.10	2.79	0.61	0.18	0.82	0.37	0.13
14:0	4.43	4.09	4.89	4.64	4.26	4.56	0.22	0.89	0.76	0.40	0.04
16:0	28.68	29.03	30.23	29.18	30.90	28.35	1.16	0.42	0.77	0.84	0.53
18:0	14.85	14.29	14.62	14.61	15.41	14.14	0.69	0.92	0.90	0.60	0.51
18:1	46.97	43.91	44.40	46.06	44.62	45.24	1.25	0.06	0.92	0.42	0.74
18:2	3.74	3.41	4.44	4.27	3.65	5.87	0.44	0.54	0.26	0.94	0.02
CLA	0.36	4.25	0.38	0.35	0.35	0.33	1.27	0.47	0.46	0.47	0.34
18:3	0.43	0.58	0.37	0.36	0.34	0.43	0.10	0.79	0.39	0.90	0.70
20:4	0.51	0.40	0.62	0.47	0.39	0.96	0.16	0.55	0.82	0.63	0.06
20:5	0.05	0.05	0.06	0.06	0.09	0.13	0.04	0.44	0.39	0.52	0.77
22:6	0	0	0.0025	0.0075	0	0	0.004	0.31	0.31	0.14	0.34
SFA	47.96	47.41	49.74	48.43	58.56	47.05	1.13	0.52	0.72	0.94	0.62
MUFA	46.97	43.91	44.40	46.06	44.62	45.24	1.25	0.06	0.92	0.43	0.74
PUFA	5.08	8.68	5.87	5.52	4.81	7.71	1.25	0.28	0.80	0.53	0.91
USFA	52.05	52.59	50.26	51.58	49.44	52.95	1.13	0.52	0.72	0.94	0.62
SFA:USFA	1.67	1.23	1.42	1.44	1.78	1.47	0.26	0.77	0.86	0.22	0.70
PUFA:SFA	0.16	0.25	0.18	0.18	0.13	0.29	0.04	0.42	0.87	0.72	0.41

[a]Standard error of the mean.