DISSERTATION

COPPER, ZINC, AND MANGANESE IN BEEF CATTLE PRODUCTION: EFFECTS OF SUPPLEMENTATION AND SOURCE ON REPRODUCTION, MINERAL STATUS, FEEDLOT PERFORMANCE, IMMUNITY, AND CARCASS CHARACTERISTICS

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JASON KEE AHOLA ENTITLED "COPPER, ZINC, AND MANGANESE IN BEEF CATTLE PRODUCTION: EFFECTS OF SUPPLEMENTATION AND SOURCE ON REPRODUCTION, MINERAL STATUS, FEEDLOT PERFORMANCE, IMMUNITY, AND CARCASS CHARACTERISTICS" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

COPPER, ZINC, AND MANGANESE IN BEEF CATTLE PRODUCTION: EFFECTS OF SUPPLEMENTATION AND SOURCE ON REPRODUCTION, MINERAL STATUS, FEEDLOT PERFORMANCE, IMMUNITY, AND CARCASS CHARACTERISTICS

Over a two-year period, crossbred mature beef cows (n = 178, Year 1; n = 148, Year 2) and young females (n = 43 nulliparous heifers, Year 1; n = 37 primiparous cows, Year 2) grazing in eastern Colorado were used to evaluate the effects of Cu, Zn, and Mn supplementation and source on reproduction, mineral status, immunity, and cow and calf performance. Cow treatments included: 1) control (no supplemental Cu, Zn, or Mn); 2) organic (50% organic and 50% inorganic Cu, Zn, and Mn); and 3) inorganic (100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$) trace minerals. Heifer treatments included: 1) organic, or 2) inorganic trace minerals. Free-choice mineral feeders were used to provide current NRC-recommended concentrations of Cu, Zn, and Mn from 54 and 82 d (Year 1, heifers and cows, respectively) and 81 d (Year 2) prior to the average calving date of the herd through 110 and 119 d (Year 1, cows and heifers, respectively) and 135 d (Year 2) post-calving. Terminal steer and heifer calves from each year's calf crop were maintained on their appropriate pasture trace mineral treatments and had exclusive access

to mineral treatments via creep feeders from approximately 95 d of age until weaning. After weaning, calves were grown and finished in a feedlot on the same pre-weaning trace mineral treatments. Performance, immune response, mortality, morbidity, mineral status, carcass traits, and longissimus dorsi fatty acid profiles were evaluated.

In the grazing portion of the experiment, results indicate that trace mineral supplementation in cows and source in cows and heifers affected trace mineral status. Reproductive results were variable in heifers; however, in cows trace mineral supplementation improved pregnancy rate to AI compared to cows not supplemented with Cu, Zn, or Mn for more than 1 yr. Calf performance was greater in non-supplemented control calves vs. supplemented calves in both years, while source also affected calf performance but not consistently in both years. Trace mineral source did not affect calf performance in young grazing females. During the feedlot phase in Year 1, gain to feed ratio was greater in Inorganic vs. Organic calves in both the growing and finishing phases and greater in non-supplemented control calves vs. supplemented calves only during the finishing phase; however, gain to feed ratios were not affected by either supplementation or source in Year 2. Liver Cu and Mn concentrations were affected by supplementation, however immune response, morbidity, carcass traits, and longissimus dorsi fatty acid profiles were not different across treatments.

Based on the reduced reproductive performance in non-supplemented cows, as well as literature indicating that Cu affects luteinizing hormone (LH) release, the effect of Cu status, supplementation and source on pituitary responsiveness to gonadotropin releasing hormone (GnRH) were evaluated using 12 multiparous, non-pregnant, non-suckling, ovariectomized Angus cows. After receiving 5 mg Mo/kg diet and 0.3% S during a 216-

d Cu depletion phase, nine cows were considered Cu deficient (liver Cu concentrations < 30 mg Cu/kg) and were stratified based on age, BW, BCS, and liver Cu concentration and randomly assigned to treatments. Treatments included: 1) control (deficient Cu status; no supplemental Cu), 2) organic (adequate Cu status; 100% organic Cu, 10 mg Cu/kg diet), and 3) inorganic (adequate Cu status; 100% inorganic $CuSO_4$, 10 mg Cu/kg diet). By d 77 of the 159-d repletion phase all supplemented cows had adequate liver Cu, and liver Cu concentrations were greater in supplemented cows vs. non-supplemented controls, and this was maintained throughout the repletion phase. Beginning on d 99, cows were catheterized every fifth day and blood samples were collected at 15 min. intervals for 1 h prior to, and 4 h after, GnRH administration at low (0, 3, and 9 ug; experiment one) and high doses (0, 27, and 81 ug; experiment two) and analyzed for LH concentration. Copper status, supplementation, and source had no effect on pituitary sensitivity to GnRH (based on basal, peak, and total LH released at varying GnRH doses) or pituitary stores of LH.

Key words: Beef cattle, copper, immunity, manganese, performance, reproduction, zinc

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DEDICATION

This dissertation is dedicated to Sara Colson – my best friend and partner. Your never-ending love, support, and belief in my abilities gave me the strength and courage to complete this dissertation and my Ph.D.

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CHAPTER I

REVIEW OF LITERATURE

Section I: Trace Mineral Metabolism

Micro or "trace" minerals differ from macro minerals based strictly on the amount required in the diet, depending on metabolic needs (NRC, 1996). Trace minerals are required at concentrations less than 100 mg/kg diet while macro minerals are required at concentrations greater than 100 mg/kg diet (McDowell, 1992). According to the NRC (1996), 15 trace minerals are essential, 10 of which are considered essential for beef cattle, including chromium (Cr), cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn). Additional trace minerals, including arsenic (As), boron (B), lead (Pb), silicon (Si), and vanadium (V), have been identified as essential; however, evidence of any nutritional importance in beef cattle is lacking (NRC, 1996). Other researchers have identified a similar but different list of essential trace minerals (for virtually all animal species) and have increased the number of essential trace minerals to 18 due to the inclusion of fluorine (F), lithium, and tin (Sn; McDowell, 1992) or exchange of Sn and F for B and Pb (Underwood and Suttle, 1999).

Although differences exist about which trace minerals are considered essential, trace minerals can easily be broken down into a subset shown to be important in livestock nutrition – Fe, I, Zn, Cu, Mn, Se, and Co – and a subset that have not yet been observed as practically important in livestock nutrition – Mo, Cr, Sn, V, F, Si, Ni, and As (Underwood and Suttle, 1999). Interestingly, of the 3.5% of an animal's body weight (BW) that is comprised of all the macro minerals, trace minerals constitute less than 0.3% (McDowell, 1992).

Physiological Functions of Trace Minerals

General Functions. To promote normal tissue growth, homeostasis, enzyme function, and cell regulation, it is imperative that the essential trace minerals be maintained within narrow concentrations in the body (Underwood and Suttle, 1999). If proper trace mineral homeostasis is maintained, optimum growth, health, and productivity of domestic livestock can be ensured (Underwood and Suttle, 1999). When trace mineral homeostasis is not successful, situations of toxicity or deficiency can result (McDowell, 1992), possibly resulting in depressed productivity, health, and(or) growth and development.

Trace mineral deficiencies can occur as either a primary or secondary deficiency. A primary deficiency is due to inadequate amount, availability, and(or) intake of a trace mineral from the environment (i.e. forage), while a secondary deficiency is caused by the presence of one or more antagonists that reduce the availability of a trace mineral. Most of the essential trace minerals are present in adequate quantities in commonly used feedstuffs (NRC, 1996); however, mineral deficiencies often result due to an animal's

inability to absorb and utilize a trace mineral due to the presence of antagonists in the gastrointestinal tract and the formation of insoluble and usually unabsorbable complexes.

It is generally agreed upon that there are four general functions of macro and micro minerals, which include: 1) structural component of organs, tissues, molecules, and(or) membranes, 2) maintenance of physiological function for homeostasis, 3) catalyst or component of enzyme and hormonal systems, and 4) regulation of cell replication and differentiation (Underwood and Suttle, 1999). The three trace minerals that will be the focus of this review – Cu, Zn, and Mn – are primarily involved in the regulation of enzyme activity (as either an activator or a structural component of a metalloenzyme) and cell function (McDowell, 1992).

Copper. Copper is important for a wide range of body functions, including hemoglobin formation, growth, prevention of pathological disorders, and most importantly for the activities of metal-activated enzymes and metalloenzymes that are essential for normal reproduction, bone and connective tissue development, growth, and hair pigmentation (Underwood, 1977; Underwood and Suttle, 1999). Enzymes dependent on Cu include tyrosinase (converts tyrosine to melanin for hair pigmentation), lysyl oxidase (cross-links connective tissue for bone and blood vessel strength), ceruloplasmin (mobilizes Fe for hemoglobin synthesis via transferrin formation), Cu-Zn superoxide dismutase (Cu-Zn SOD; protects cells from oxidative stress), cytochrome oxidase (electron transport chain function and nerve myelination via phospholipid synthesis), peptidylglycine α-amidating monooxygenase (brain function), and liver desaturase (fatty acid metabolism; Underwood, 1977; Underwood and Suttle, 1999). These effects of Cu status on enzyme activity have been well documented; however,

directly associating specific changes in performance or pathology due to changes in enzyme activity caused by a trace mineral can be difficult (Baker and Ammerman, 1995a).

Zinc. The activities of over 200 enzymes depend on Zn, of which the metalloenzyme carbonic anhydrase was the first to be identified (McDowell, 1992). Zinc-activated enzymes include plasma alkaline phosphatase, alcohol dehydrogenase, kinases, pancreatic carboxypeptidase, and RNA-polymerase (McDowell, 1992). In addition to enzyme activation, Zn is important for the normal functioning of several enzyme systems, including nucleic acid metabolism, protein synthesis, carbohydrate metabolism, glycolytic pathways, and transcription and translation (McDowell, 1992).

Along with enzyme activity, Zn has been associated with normal growth, possibly via nucleic acid biosynthesis and(or) feed intake (Hambidge et al., 1986), skeletal development (Hidiroglou, 1980), stabilization of RNA, DNA, and ribosomes (Prask and Plocke, 1971), cell replication and differentiation (Hambidge et al., 1986), carbohydrate metabolism (Im et al., 1975), synthesis and activity of hormones, and skin and wound healing (McDowell, 1992).

Manganese. In beef cattle production systems, Mn probably has much more influence than previously realized (Corah and Arthington, 1993). Unlike Cu and Zn, Mn is very widely distributed in cells and tissues of the body, but at very low concentrations. Manganese is vital for growth, bone development, and reproductive function (Underwood and Suttle, 1999). Most of the functions associated with Mn are based primarily on research in poultry, since monogastrics are more prone to Mn deficiency than ruminants (McDowell, 1992).

The primary function of Mn in the body involves enzyme regulation, including metalloenzymes – arginase, pyruvate carboxylase (for lipid metabolism), Mn-SOD (cellular protection from oxidative stress; Hurley and Keen, 1987) – and Mn-activated enzymes such as hydrolases, kinases, decarboxylases, and transferases (McDowell, 1992). Glycosyltransferase, the most widely studied of the Mn-activated enzymes, is involved in the synthesis of cartilage mucopolysaccharides, which provide the matrix for bone (Hidiroglou, 1980) and the formation of prothrombin (McDowell, 1992), which is necessary for normal blood clotting.

Although the mechanisms of action are not known, Mn is important for immune and central nervous system function (Hurley and Keen, 1987), cholesterol synthesis (Davis et al., 1990), steroid hormone production and carbohydrate metabolism (McDowell, 1992), and function of the corpus luteum (Hidiroglou, 1975).

Mechanisms of Absorption

Since minute quantities of trace minerals are required, the range between deficiency and toxicity is narrow; however, effective regulatory mechanisms necessary to control absorption are limited (Sharma and Sharma, 1997). In most species, dietary trace minerals are poorly absorbed, particularly in adult ruminants (Underwood, 1977). The efficiency of Cu absorption from normal feeds, for instance, ranges from approximately 1 to 15% across all species, but appears to be higher in non- and young ruminants compared to adult ruminants (Hemken et al., 1993). Two mechanisms are involved in the absorption process: 1) an active transport process (at low concentrations), and 2) simple diffusion (typically only at high concentrations; Bronner and Yost, 1985). The rate of

absorption is probably regulated at the level of the intestine (the site of absorption), but probably only at low dietary concentrations (Underwood, 1977), which involves homeostatic mechanisms including metallothionein in the mucosal cells (Underwood and Suttle, 1999). In addition, metal-binding components appear to be involved in the absorption of trace minerals, a possible reason why the presence of some minerals (e.g. Fe) may be antagonistic to others (e.g. Cu) due to competition for these metal binding sites (Underwood, 1977).

Copper. In ruminants, the relatively low rate of Cu absorption is unique compared to non-ruminants primarily due to the presence of known antagonists sulfur (S) and Mo and the conversion of sulfate to sulfide in the rumen (Underwood, 1977; Underwood and Suttle, 1999). Other Cu antagonists reduce Cu absorption and cause Cu deficiency, including Fe, calcium (Ca), cadmium (Cd), Zn, and silver (Ag; Underwood and Suttle, 1999; Underwood, 1977). For instance, the concentration of Zn in the lumen of the intestine negatively affects Cu uptake into mucosal cells of the small intestine (Oestreicher and Cousins, 1985). Additionally, when S is consumed as either sulfate (SO_4^{2-}) or via an S-containing amino acid, it is converted to sulfide (S^{2-}) in the rumen (Ward, 1978). Rumen microbes use sulfide as a source of S for bacterial synthesis of S-containing amino acids; however, sulfide readily combines with Mo (if present) to form a thiomolybdate (MoS_4^{-2}), which binds Cu and forms $CuMoS_4$ (Suttle, 1991). When Cu is bound to either sulfide or molybdate, it is almost completely unabsorbed by ruminants (Huisingh and Matrone, 1976). It is not clear if the Cu-Mo-S complex can be absorbed; however, this complex would be completely unavailable for metabolism by the animal if absorbed (Ward, 1978). Mills and others (1977) evaluated the effects of increasing

dietary Mo concentration at either low or high dietary concentrations of S and increasing dietary S concentration at either low or high dietary concentrations of Mo. The authors reported reductions in liver Cu retention when Mo was increased when S concentrations were high and low, and when S was increased at while Mo was at a high concentration; however, liver Cu retention was not affected when dietary S concentration was increased at the same time Mo concentration was low.

The primary reason for Cu deficiency in ruminants is due to the poor rate of absorption of Cu from the diet (Suttle, 1994). Copper deficiencies in ruminants can be caused by one or more of four types of feed: 1) High Mo (over 100 mg Mo/kg diet), 2) low Cu:Mo ration (2:1 or less), 3) inadequate Cu (less than 5 mg Cu/kg diet), and(or) 4) high protein (20 to 30% protein; Ward, 1978). Most Cu deficiencies are caused by feeds with inadequate Cu, while Cu deficiencies due to a high protein feed are least common (Ward, 1978).

Zinc. Zinc is absorbed via the small intestine (Cousins, 1985), which is controlled via absorption mechanisms (Miller, 1975). Zinc absorption begins with carrier-mediated transport (Solomons and Cousins, 1984) via ligands that take Zn across the brush border (Hambidge et al., 1986). The transfer of Zn across an enterocyte is regulated by metallothionein, which in turn is regulated by dietary and plasma Zn concentrations (Cousins, 1978). Antagonists can affect Zn absorption, such as phytate or Ca (only when phytate is present; NRC, 1996) in non-ruminants, and fiber, phosphorus (P), Cu, Cd, and Cr (McDowell, 1992; NRC, 1996). Copper appears to inhibit the transfer of Zn out of the mucosal cell into the circulation when Cu is present at high concentrations (Oestreicher and Cousins, 1985). The absorption of Zn can be increased via the presence of chelating

agents such as ethylene diamine tetraacetic acid (EDTA; Vohra and Kratzer, 1964), but the rate of Zn absorption is affected most by the amount of Zn in the diet (McDowell, 1992). In cattle fed a low Zn diet, the rate of absorption was approximately 50% (Stake et al., 1975), however the range of Zn absorption has been reported to vary from 10% from a high Zn diet (McDowell, 1992) up to 80% in calves orally dosed with Zn (Miller, 1970).

Manganese. The absorption of Mn is very low (possibly only 3 or 4%; Henry, 1995) in nearly all species, making the presence or absence of a Mn deficiency dependent on differences in availability among feeds or supplementation sources (Underwood and Suttle, 1999; McDowell, 1992). Manganese antagonists that have been identified include phytate and fiber (which can substantially limit availability; Wedekind et al., 1991), P, Ca, Fe, and Co (McDowell, 1992). Most Mn absorption research has been done with poultry (McDowell, 1992), while dietary factors that impact the rate of absorption of Mn in ruminants are not well understood (NRC, 1996). Since the most common Mn antagonist, phytate, is broken down in the rumen, it is believed that Mn absorption is probably higher (approximately 10 to 20%) in ruminants, especially when Mn intake is low since absorption rates appear to be closely linked to Mn availability in the feed (Underwood and Suttle, 1999). However, in one experiment the availability of Mn in cattle was only about 1% (Abrams et al., 1977).

Homeostasis of Mn in ruminants appears to be managed via Mn absorption and fecal excretion via bile (NRC, 1996; Watson et al., 1973). In calves consuming either 32 (normal) or 1,000 (high) mg Mn/kg diet, when ^{54}Mn was infused (via both i.v. and duodenal) average tissue concentrations of ^{54}Mn and Mn absorption were lower in the

high Mn calves, indicating that circulating and stored concentrations of Mn may not play a major role in Mn absorption (Abrams et al., 1977). Similarly, lambs supplemented with 22, 300, or 3,000 mg Mn/kg diet and infused with ^{54}Mn rumenally had declining Mn absorption as dietary Mn increased (Ivan and Hidiroglou, 1980).

Trace Mineral Transport and Storage

Once absorbed at the site of the small intestine, trace minerals are loosely bound to albumin and amino acids in the serum (Underwood, 1977). Serum albumin and amino acids can also bind trace minerals when released by tissues based on physiological needs elsewhere in the body (Underwood, 1977). Once a trace mineral reaches the liver (via absorption from the diet or tissue release) it is either stored in the liver typically via the incorporation into mitochondria, microsomes, nuclei, and(or) parenchymal cells, released for immediate incorporation into metalloenzymes, or excreted from the body usually via bile (Underwood, 1977). Once absorbed, if not bound to albumin it is believed that trace minerals bind tightly to alpha-2 macroglobulin, with some additional metals becoming oxidized in the circulation and binding transferrin (Hambidge et al., 1986; Hidiroglou, 1979).

Storage of trace minerals in body tissues can be affected by species, age, diet composition, and disease condition, while distribution of trace minerals in the body appears to only be affected by species, age, and trace mineral status (Underwood, 1977). Interestingly, sex of the animal has little impact on the storage of trace minerals, yet animal-to-animal variation can be high (Underwood, 1977).

Copper. In the liver, adults of most species contain about 10 to 50 mg Cu/kg, with the majority of animals containing 15 to 30 mg Cu/kg (Underwood, 1977). Adult ruminants store about 100 to 400 mg Cu/kg in the liver, possibly due to an improved capacity for sheep and cattle to bind Cu in the liver (Underwood, 1977). Since liver Cu concentration can be sensitive to low Cu diets, diagnosing Cu deficiency is commonly done via analysis of liver Cu concentration (Underwood and Suttle 1999). Although concentrations of Cu are among the highest in the liver, over half of total body Cu is contained in muscle and bone (Sharma and Sharma, 1997), with additional Cu stored in blood, heart, kidney, brain, lung, and skin (Underwood, 1977). Tissues including endocrine glands (which are typically low in Cu concentration, including the pituitary, thyroid, thymus, and ovary), muscles, and the heart are less sensitive to body Cu needs, and therefore Cu from these body tissue is less available for use by other tissues (Underwood, 1977).

Zinc. Most mammalian tissues contain 30 to 250 mg Zn/kg, while whole-body Zn concentration in cattle ranges from 20 to 30 mg Zn/kg (Hambidge et al., 1986). Once Zn is absorbed and reaches the liver, a substantial portion is sent back to the blood and incorporated into various tissues such as bone or central nervous tissue, which are not very available for use by other tissue (McDowell, 1992). Additionally, Zn is stored in the liver, pancreas, kidney, and spleen (McKenney et al., 1962; Feaster et al., 1954). The ability of stored Zn to be readily available for use by other tissues is limited in most species (Underwood, 1977). Zinc that is intended for immediate use in cases of low intake is stored via metallothionein (Richards and Cousins, 1976) or SOD (McDowell, 1992).

Manganese. Compared to Cu and Zn, concentrations of Mn in tissues of livestock are low, typically ranging from 0.5 to 3.9 mg Mn/kg in sheep and cattle carcasses (Underwood and Suttle, 1999). Manganese is commonly found in liver, bone (which has a low concentration of Mn but contains approximately 25% of the total body Mn), pancreas, and kidney tissue, while very little Mn is in muscle. It is believed that most Mn in the body is stored in the mitochondria, supported by evidence that tissues rich in mitochondria (liver and kidney) had greatest retention rates of Mn in growing lambs (Watson et al., 1973). Although bone reserves are the largest in the body, Mn from bone is not readily available for mobilization if intake is low (McDowell, 1992). Interestingly, storage capacity for Mn in the liver appears to be limited, compared to other trace minerals such as Cu (McDowell, 1992). There is also evidence that liver Mn concentration responds very little (concentration may only double, at most) or not at all to Mn supplementation (Carter et al., 1974), except in calves (Howes and Dyer, 1971).

Evaluating Trace Mineral Status

To determine an animal's trace mineral status or to diagnose a trace mineral deficiency or toxicity, blood is commonly used due to ease of collection (Bull, 1980). However, trace mineral-dependent enzymes (e.g. ceruloplasmin, a Cu-dependent enzyme with activity proportional to Cu concentration) have also been analyzed to determine trace mineral status since collection is easy, and possible Cu contamination can be avoided (Bull, 1980). The most reliable method of diagnosing a mineral deficiency is to monitor an animal's response to the supplementation of a particular trace mineral (McDowell, 1992) by monitoring health and(or) production after supplementation, since

conventional indices of trace mineral status (blood or liver concentrations) are only approximate measurements (Suttle, 1994). However, due to the significant cost and time constraints of such experiments, the analysis of animal tissue(s) for trace mineral concentration is the most ideal indicator of trace mineral status (McDowell, 1992).

Copper. Substantial storage of Cu in the liver is possible (NRC, 1996), and therefore analysis of liver for Cu concentration is considered the best method of classifying Cu status and to document changes in Cu status (Hemken et al., 1993). However, determination of Cu status via the analysis of Cu-dependent enzymes including ceruloplasmin and SOD is also common. Analysis of serum Cu concentrations to estimate mineral status is done, but the minimum liver Cu concentration necessary to maintain normal plasma Cu concentrations in ruminants is approximately 40 mg Cu/kg (Underwood, 1977), making serum evaluation a less valuable method to classify Cu status, particularly if cattle are sub-clinically deficient. Analysis of blood samples alone for diagnosis of Cu status can be misleading, and therefore should be accompanied by liver and forage analyses for Cu concentration (Corah and Arthington, 1993).

Zinc. Using plasma Zn concentration as a method of classifying Zn status may be inaccurate if cattle are not truly Zn deficient (Hambidge et al., 1986). In sheep, less than 0.5% of total body Zn is in the blood, of which over 80% is in the red blood cells for carbonic anhydrase activity (Hambidge et al., 1986). However, the analysis of liver tissue for Zn concentration to predict Zn status may not be the best indicator either (McDowell, 1992), since soft tissue Zn concentration varies little with Zn status. Zinc concentrations in plasma and bone have been shown to reflect Zn-deficient diets (Baker and Ammerman, 1995b). Severe Zn deficiency can be diagnosed easily due to the

observation of obvious clinical symptoms; however, since sensitive methods to analyze Zn status are not available, diagnosis of sub-clinical Zn deficiency can be difficult (McDowell, 1992). As with any trace mineral, observation of production responses (such as feed intake or growth with Zn, specifically) due to supplementation is the best way to diagnose a deficiency (Miller, 1970). Plasma Zn is the most commonly used method to evaluate Zn status (McDowell, 1992); however, using a combination of plasma and forage Zn concentrations may be more acceptable, particularly if large numbers of ruminants are being evaluated (McDowell, 1992). However, in cases when Zn deficiency was severe, changes in Zn concentration in the liver and blood were limited (Miller, 1970).

Manganese. In ruminants, the liver contains the most mobilizable Mn (Hidiroglou, 1979); however, liver Mn concentration does not respond substantially to Mn supplementation, even at extreme dietary concentrations (Underwood and Suttle, 1999; Miller, 1979). When dietary Mn concentration was increased 130- to 140-fold, only a fourfold increase in liver Mn was detected (Ivan and Hidiroglou, 1980; Watson et al., 1973). However, when the distribution of Mn was evaluated in sheep, concentrations were highest in the liver, which were greater than the pancreas and kidney (Underwood and Suttle, 1999). The ability of the liver to store Mn for long periods of time is thought to be limited (Hidiroglou, 1979). Bone can store a large amount of Mn; however, a small increase in Mn across the entire skeletal system could be unnoticed (possibly undetectable with modern laboratory techniques), even if a substantial change in the total amount of stored Mn occurred (Underwood and Suttle, 1999). Although using blood is easy for evaluating Mn status, blood Mn concentrations decline slowly only at extremely

low Mn intakes (Hidiroglou, 1979). The best indicator of Mn status may involve the use of a compartmental model, which evaluates Mn concentrations in several tissues (i.e. liver, kidney, and bone) to determine Mn status (Henry et al., 1992).

Effects of Trace Mineral Supplementation

Copper. A large amount of research has evaluated the ability of trace mineral supplementation to affect trace mineral status. The most common methods of supplementing Cu to grazing beef cattle are via mineral mixes or boluses, or the uncommonly used injections (Corah and Arthington, 1993). In grazing cattle, liver Cu concentrations increased with mineral supplementation compared with non-supplemented controls (Olson et al., 1999). In feedlot cattle, plasma Cu concentrations were not affected during the growing phase due to Cu supplementation (Ward et al., 1993; Engle et al., 2000c). In contrast, plasma Cu concentrations were greater due to Cu supplementation at the end of the growing phase and throughout both the growing and finishing phases (Ward and Spears, 1997), at the end of the finishing phase (Engle et al., 2000c), and beginning on d 7 after the initiation of Cu supplementation in beef calves on a Cu-deficient diet since birth (Kegley and Spears, 1994) compared to non-supplemented controls. Similarly, liver Cu concentrations were greater in Cu-supplemented vs. non-supplemented control cattle at the end of the finishing phase (Engle and Spears, 2000a) and at the end of the growing phase (Engle and Spears, 2001; Engle et al., 2000c). Based on these results, it is clear that Cu supplementation commonly leads to increased Cu status (based on both plasma and liver concentrations) in all classes of beef cattle.

Zinc. Since classification of Zn status is more difficult due to the limited ability of cattle to increase plasma and liver Zn stores, results of Zn supplementation in beef cattle have been variable. In feedlot heifers, plasma Zn concentrations tended to be higher due to Zn supplementation in the growing phase, yet throughout the entire feeding period plasma Zn concentrations were not different (Spears, 1989). In contrast, however, Zn supplementation did not affect plasma Zn concentration in feedlot cattle (Spears and Samsell, 1984; Greene et al., 1988; Spears and Kegley, 2002). In poultry, Zn supplementation at high concentrations (0, 250, 500, or 750 mg Zn/kg diet) compared to non-supplemented controls resulted in no differences in liver Zn concentration across treatments. However, Zn concentrations in the tibia were greater in Zn-supplemented chicks compared to non-supplemented controls (Wedekind et al., 1992), providing evidence that using blood and(or) liver tissue to determine Zn status may not be the most accurate prediction of Zn status.

Manganese. Results of Mn supplementation experiments have been limited, and inconsistent. In a recent experiment with two breeds of beef cattle, liver Mn concentrations were greater in Simmental heifers receiving 30 or 50 mg Mn/kg diet than non-supplemented controls; however, there was no difference in liver Mn concentrations in Angus heifers (Hansen et al., 2004). Although difficult to evaluate due to very low liver concentrations (i.e. 8 to 12 mg Mn/kg), additional research is needed to address the effects of Mn supplementation on Mn status.

Factors Affecting Trace Mineral Requirements

Dietary requirements for cattle are a function of metabolic requirements, endogenous losses, and the efficiency of mineral absorption (Spears, 2002). Although the true metabolic requirement for trace minerals such as Cu, Mn, and Zn in beef cattle can never be known, recommendations for concentrations of trace minerals that should be included in diets based on estimated endogenous losses and availability are reported and updated regularly (NRC, 1996) and are based on widespread research. If recommendations inadvertently underestimate true needs, deficiencies and reductions in performance may result due to inadequate supplementation and status. However, if recommendations are overestimated and in excess of requirements, supplementation may lead to a loss of trace minerals into the environment via cattle waste and possible environmental contamination (NRC, 1996). Besides the effect of species on trace mineral requirements, the true requirement for a trace mineral can be impacted by many factors, including age, sex, physiological status, breed, mineral interactions in the gastrointestinal tract, environment, trace mineral source and availability, concentration, stress, and duration of supplementation. To briefly, but fairly, address this topic, only well-documented factors will be discussed.

Age. Age can substantially affect the rate of absorption of particular trace minerals. During early life, calf growth and mineral deposition can be intensive (Annenkov, 1981). The rate of Zn absorption decreases with age (McDowell, 1992), partially due to a slowing of growth and protein deposition (Stake et al., 1973), a primary function of Zn. In addition to a change from growth to maintenance associated with age, trace mineral requirements can also be affected by changes in the gastrointestinal tract due to age.

Absorption rates of Zn decreased with age (Suttle, 1979), possibly due to development of the rumen. Liver Mn concentrations are very non-responsive to Mn supplementation (McDowell, 1992); however, in calves liver Mn concentrations responded substantially to increased Mn intake (Howes and Dyer, 1971), suggesting that the metabolism of Mn in a newborn calf differs from that in an older animal.

Breed. In beef cattle, several experiments have reported differences in absorption rates of trace minerals between breeds, with Cu in particular. In an experiment using nine breeds of beef cattle that compared liver Cu, Zn, and Fe and serum Cu, Zn, Ca, and magnesium (Mg) concentrations across breeds, Cu metabolism was affected by breed based on greater liver Cu concentrations in Limousin cattle compared to all other breeds except Angus, leading the authors to conclude that Limousin cattle may be more able to maintain liver Cu concentrations on reduced intake of Cu (Littledike et al., 1995). Another breed effect was noted in beef cattle when Cu excretion via bile was evaluated, and biliary excretion of Cu was greater in Simmental vs. Angus cattle (Gooneratne et al., 1994). Similarly, both Simmental and Charolais cow/calf pairs were more susceptible to Cu deficiency compared to Angus cow/calf pairs (Ward et al., 1995).

Interactions. Interactions among minerals and between minerals and other dietary compounds can affect trace mineral absorption and therefore requirements. Common mineral interactions have been summarized (Puls, 1994). Probably the best example of an interaction that reduces availability is the interaction of Cu with molybdate and sulfide in the rumen, which combine to form a thiomolybdate (Suttle, 1991), an insoluble and practically unabsorbable complex (NRC, 1996), as discussed earlier. Elevated concentrations of dietary Fe have also caused Cu deficiency in young calves (Phillippo et

al., 1987) and elevated dietary Zn depressed Cu absorption in cattle (Davis and Mertz, 1987). These interactions typically reduce or prevent absorption of the mineral, since the trace mineral becomes part of a large and insoluble complex. Absorption of thiomolybdates has been detected, but since they most likely bind albumin in the blood, the Cu component is unavailable and unusable to the body (Gooneratne et al., 1989). Ward (1978) predicted that problems with marginal mineral deficiencies and interactions will continue to increase in prevalence as the trend for intensified animal agriculture and forage production continues.

Physiological Status. Physiological status of an animal (i.e. growing, gestating, lactating, etc.) can have a major effect on trace mineral requirements (Annenkov, 1981). This can clearly be seen by the two-fold increase in Mn recommended in beef cattle diets from feedlot cattle (20 mg Mn/kg diet) to gestating and early lactating cattle (40 mg Mn/kg diet) by the NRC (1996). This increase is based on research indicating that reproductive performance requires more Mn than simply growth and development (Rojas et al., 1965). During late pregnancy, fetal weight and mineralization of tissue occurs, in addition to maternal mineral deposition for use in early lactation (Annenkov, 1981). When the apparent absorption and retention of Cu and Zn from an alfalfa-based diet were evaluated in pregnant (third trimester) and non-pregnant Angus cows and Suffolk ewes, in the absence of supplemental Cu, apparent absorption and apparent retention of Cu and Zn were greater in pregnant than non-pregnant cows, while apparent absorption and retention of Zn was greater in pregnant vs. non-pregnant ewes (Vierboom, 2002), indicating that pregnancy can affect absorption of Cu and Zn, likely due to increased physiological need. In an experiment where maternal and fetal liver Cu concentrations

were measured at the same time, and classified based on stage of gestation, Cu concentration in the fetal liver increased and maternal liver decreased during gestation, particularly during the third trimester (Gooneratne and Christensen, 1985).

Stress. Stress in cattle (caused by weaning, parturition, disease, environment, etc.) can affect absorption and requirements of trace minerals. When adrenocorticotropic hormone was administered to beef calves every 8 h over a 3 d period at the same time as feed and water restriction in order to simulate stressful conditions, urinary excretion of Cu and Zn decreased and affected the rate of trace mineral retention (Nockels et al., 1993).

NRC (1996) Recommended Concentrations

Copper. Recommendations for the concentrations that Cu, Zn, and Mn should be incorporated into beef cattle diets have been reported (NRC, 1996). Based on known antagonisms between Cu, S, and Mo, recommended Cu concentrations depend heavily on the concentrations of Mo and S in the diet (NRC, 1996), unique to ruminants. The current recommendation for Cu is 10 mg Cu/kg diet for all physiological classes of beef cattle, based on a diet with low concentrations of antagonists (less than 0.25% S and 2 mg Mo/kg diet; NRC, 1996). However, since Cu is more available in high concentrate feedlot cattle diets when antagonists are not elevated, the true Cu requirement may be lower (NRC, 1996).

Zinc. Factors affecting Zn requirements in ruminants are not well known (McDowell, 1992). However, based on research that indicates increased growth does not result from elevated concentrations of dietary Zn, inclusion of Zn in the diet has been recommended at 30 mg Zn/kg diet (NRC, 1996). The recommended concentration of Zn that should be

included in the diet for gestating and early lactating cows is the same as feedlot cattle; however, the effects of Zn on reproduction are not well known (NRC, 1996).

Manganese. For beef cattle, the recommended concentration of Mn that should be included in the diet is 20 mg Mn/kg diet for feedlot cattle (during the growing and finishing phases) and 40 mg Mn/kg diet for gestating or early lactating cows (NRC, 1996). The doubling of the recommendation is based on research indicating that more Mn is needed for the reproductive performance of grazing cattle compared to growth and skeletal development (Rojas et al., 1965; Underwood and Suttle, 1999). The true requirement of Mn in ruminant diets can also be affected by the presence of elevated P concentrations (NRC, 1996).

Trace Mineral Sources and Availability

The number of experiments that have compared the availability of trace minerals from different sources is substantial. However, results of availability differences have been fairly inconclusive. It has been stated that differences in availability based on chemical

ıust occur since Cu from fresh forage is less effective at increasing Cu status than cured hay, even when the concentration of Cu is the same in both forages (Underwood, 1977).

Historically, trace minerals were only available for supplementation to cattle as inorganic salts (Spears, 1996), where the trace mineral was usually bound to a sulfate, carbonate, oxide, or chloride group. Once a process was developed that enabled the replacement of an inorganic group with one or several amino acids, several trace minerals (Cu, Zn, Mn, Co, and Se) also became commercially available in an organic form. Five

types of organic minerals have been defined by the Association of American Feed Control Officials: 1) 57.151 metal specific-amino acid complex (complexing of a metal salt with a specific amino acid; e.g. Zn-methionine), 2) 57.150 metal amino acid complex (complexing of a metal salt with one ore more nonspecific amino acids), 3) 57.142 metal amino acid chelate (the complexing of a metal ion from a soluble salt with amino acids at a specific molar ratio), 4) 57.23 metal proteinate [chelation (a specific type of complexing process) of a metal from a soluble salt with amino acids and(or) hydrolyzed protein], or 5) 57.29 metal polysaccharide complex (complexing of a soluble salt with a polysaccharide solution; USDA, 2002). Interest in the use of organic trace minerals has increased in recent years, due to reports of improved growth, reproduction, and health in ruminants receiving organic trace minerals (Spears, 1996); however, performance results have been inconsistent, and reasons for observed differences in performance have not been identified.

The benefits of organic trace minerals over inorganic trace minerals is based on the theory that organic trace minerals are more available since they are more similar to physiological forms that occur in the body (Spears, 1996). It is thought that differences in availability may be due to differences in absorption if organic trace minerals are able to remain intact until the site of absorption; however, no data has indicated that this occurs (Spears, 1996). Based on computer modeling, it has been reported that an organic complex such as Cu-lysine would be degraded in the gastrointestinal tract and not reach the site of absorption, or be absorbed, intact (Reid and Attaelmannan, 1998). In addition, based on *in vivo* evaluations of the solubility and structural integrity of organic trace minerals with gel filtration, at low pH a metal that was once bound to a proteinaceous

ligand became dissociated (Brown and Zeringue, 1994). The authors therefore hypothesized that it is unlikely that organic trace minerals are absorbed differently from inorganic trace minerals in the gastrointestinal tract.

Early research with Cu compared the rate of absorption of different sources of inorganic Cu in beef cattle via the use of radioactive Cu (^{64}Cu; Chapman and Bell, 1963). After measuring the relative amounts of ^{64}Cu in the blood, these researchers reported that the highest amount of Cu was absorbed from $CuCO_3$ followed by $Cu(NO_3)_2$, $CuSO_4$, CuCl, Cu_2O, CuO (powder), CuO (needles), and the least absorbed Cu was from wire. Today, commonly used Cu sources for supplementation of Cu to livestock are $CuSO_4$, CuO, and $CuCO_3$ (Hemken et al., 1993; NRC, 1996). However, Cu from CuO is very unavailable to ruminants, based on lower plasma Cu and ceruloplasmin concentrations in calves supplemented with 30 mg Cu/d from CuO vs. $CuSO_4$, and no difference between cattle receiving 8 mg Cu/kg diet from CuO and non-supplemented controls when Mo and(or) Fe were present as antagonists (Kegley and Spears, 1994). The availability of $ZnSO_4$ and ZnO has been reported to be similar in ruminants (Kincaid, 1979; NRC, 1996). However, when Mn availability was evaluated in lambs, $MnSO_4$ was more available than MnO (Henry et al., 1992). Common Zn sources used to supplement beef cattle include $ZnSO_4$, ZnO, and organic Zn (Zn-methionine and Zn-proteinate; NRC, 1996). Similarly, Mn is typically supplemented to beef cattle diets as $MnSO_4$, MnO, or organic Mn (Mn-methionine, Mn-proteinate, Mn-polysaccharide, or Mn-amino acid chelate; NRC, 1996).

Although most research has been variable, Suttle (1994) concluded that Cu chelates, as a Cu supplement, were not superior to $CuSO_4$ in availability. In agreement, $CuSO_4$

was as available as Cu-lysine in growing calves receiving 8 mg Cu/kg diet in the presence of antagonists (Kegley and Spears, 1994). However, organic Cu sources were equally, if not more, available than $CuSO_4$ in rats in both the absence and presence of known antagonists (Fe and Zn), possibly due to different absorption mechanisms between organic and inorganic Cu (Du et al., 1996), while Cu-lysine was more available than $CuSO_4$ in feedlot cattle (Nockels et al., 1993). In Cu depleted heifers supplemented with 50 mg Cu/d, plasma Cu was not different between $CuCO_3$, $CuSO_4$, and two different Cu-proteinate treatments; however, in the presence of 0.15% additional S and 5 mg Mo/kg diet, supplementation of Cu (5 mg Cu/kg diet) from a Cu-proteinate source had a greater availability than $CuSO_4$ (Ward et al., 1996). When elevated concentrations of Mo were fed for 84 d (via high Mo hay), organic Cu (proteinate) was more available than $CuSO_4$, although means for liver Cu concentrations in both treatments were relatively high (above 200 mg Cu/kg; Kincaid et al., 1986). Based on these experiments, it appears that trace mineral status may be impacted by trace mineral source, possibly due to differences in availability. However, no differences in Cu availability were detected in cattle between organic and inorganic Cu sources in the presence of supplemental Mo and S (Ward et al., 1993), when Cu-deficient steers received high dietary concentrations of Mo (Wittenberg et al., 1990), or when Cu-deficient heifers received Cu during a 55-d repletion period (Corah and Arthington, 1993). In other species, when eight organic Zn products were compared to $ZnSO_4$ using chicks and lambs at several different Zn concentrations (0, 200, 400, and 600 mg Zn/kg diet in chicks, and 0, 700, 1,400, and 2,100 mg Zn/kg diet in lambs), only one organic Zn product was more available than $ZnSO_4$ (Cao et al., 2000). Also with Zn, organic Zn sources (lysine or methionine) were equally or more available

than $ZnSO_4$, based on concentrations of Zn, Cu, and metallothionein in various tissue (Rojas et al., 1995). Although not evaluated in many experiments, the availability of Mn from an organic Mn source (Mn-methionine) was reported to be 120% that of $MnSO_4$ (Henry et al., 1992). Differences in availability of Cu, Zn, and Mn sources have not been consistent. However, evidence does suggest that trace mineral status can be impacted by trace mineral source, possibly due to differences in the metabolism of different sources, as evidenced by differences in the metabolism of ZnO and Zn-methionine, even when the availability between sources was not different (Spears, 1989).

In practical feedlot settings, when plasma Cu was used to evaluated status, Cu source did not impact plasma Cu concentration (Kegley and Spears, 1994; Ward et al., 1993; Engle and Spears, 2000b). However, greater plasma Cu concentrations were detected at the end of the growing and finishing phases in cattle supplemented with organic Cu compared to inorganic Cu (Dorton et al., 2003). When liver Cu concentrations were evaluated, cattle supplemented with organic Cu had greater liver Cu concentrations at the end of the finishing phase (Dorton et al., 2003) and tended to have greater liver Cu concentrations than those supplemented with an inorganic form at the end of the feeding period (Engle and Spears, 2000b). When Zn from an inorganic and organic source was compared, no effect of Zn source on plasma (Greene et al., 1988; Spears, 1989; Spears and Kegley, 2002) or liver Zn concentration during a repletion phase in growing heifers (Engle et al., 1997) was observed.

In grazing beef cows, multiple experiments have evaluated the effect of several trace minerals simultaneously on trace mineral status. In a season-long experiment using grazing Angus cows, Cu, Zn, Mn, and Co were supplemented at low or high

concentrations (high concentrations were 2.1, 1.44, 1.44, and 10 times higher than low concentrations for Cu, Zn, Mn, and Co, respectively) in either an organic or inorganic form (Stanton et al., 2000). The authors reported that liver Cu and Mn concentrations increased numerically; however, liver Cu, Zn, and Mn concentrations were not affected by source at either the mid-point or end of the supplementation period (Stanton et al., 2000). When older beef cows and their calves received either organic, inorganic (at twice the concentration of organic), or no trace minerals (Cu, Zn, Mn, and Co), liver Cu and Zn concentrations were not affected by trace mineral source; however, liver Mn concentrations were greater in cows receiving inorganic trace minerals compared to organic trace minerals (Grotelueschen et al., 2001). No effect of Cu, Zn, or Mn source on liver or serum concentrations was observed when both liver and serum trace mineral concentrations were evaluated in young cows receiving organic, inorganic, or no Cu, Zn, Mn, and Co at twice the NRC (1996) recommended concentrations over a two-year period (Olson et al., 1999).

Although results of availability comparisons between different sources of trace minerals have been variable in beef cattle (based on liver and plasma trace mineral concentrations), differences in performance due to trace mineral source have been reported, indicating that differences in availability may actually exist. Regardless, due to reports of enhanced cattle performance and increased availability associated with organic trace minerals, some cow/calf and feedlot operators have begun to include organic trace minerals into grazing cow and calf, receiving cattle, and feedlot cattle mineral supplements (Spears, 1996).

Section II: Trace Minerals and Beef Cattle Production

Supplementation of trace minerals to beef cattle is a common practice. However, problems associated with supplementation strategies may be due to: 1) poorly defined requirements, 2) extreme variability in forages across the U.S., which are poorly documented, 3) variable and poorly described trace mineral concentrations in commercially available supplements, and 4) the fact that specific effects of trace minerals on reproductive, growth, and immune function are not well known (Corah and Arthington, 1993). Widespread analyses of forage trace mineral concentrations have been done, and researchers have concluded that typical forage diets for cow/calf operations across the U.S. may not be adequate in Zn, and likely are marginal in Cu, with low availability of Cu due to elevated concentrations of Fe and Mo (Corah et al., 1996; Mortimer et al., 1999). Manganese was adequate in 76.0% of samples; however Zn was considered adequate in only 2.5% of the forage samples, and 49.7% of samples were marginal in Cu, while 9.2% of samples were very high in Mo and 11.7% of samples were very high in Fe (Corah et al., 1996).

Reproduction

Overall Reproductive Performance. In beef cattle, Cu, Zn, and Mn supplementation caused lower 60-d pregnancy rates compared with non-supplemented controls, when supplementation was at two times NRC (1996) recommended concentrations (Olson et al., 1999). When Cu alone was evaluated, no difference at the end of a 60-d breeding season due to Cu supplementation was detected (Arthington et al., 1995; Muehlenbein et al., 2001). In dairy cows, improved fertility resulted from supplementation with both Cu

and Mg, but not due to supplementation with Cu or Mg alone (Ingraham et al., 1987). In dairy cows receiving bovine somatotropin, Campbell et al. (1999) reported enhanced fertility in cows receiving organic Cu, Zn, Mn, and Co compared to non-supplemented controls.

Trace mineral source (organic vs. inorganic) has also been linked to reproductive performance. An enhanced pregnancy rate to synchronized AI was reported in cows receiving supplemental Cu, Zn, and Mn at high concentrations in an organic form compared to an inorganic form; however, overall pregnancy rate at the end of the 60-d breeding season was not affected by trace mineral source (Stanton et al., 2000). However, based on reported mean liver Cu concentrations (Stanton et al., 2000), on average cows were deficient in Cu (liver Cu < 30 mg Cu/kg; Mills, 1987). In two-year old beef cows, no difference in pregnancy rate was observed in cows supplemented with organic vs. inorganic forms of trace minerals, although cows receiving inorganic trace minerals conceived earlier than cows receiving organic trace minerals in the first year of the two-year experiment (Olson et al., 1999). Other researchers have associated organic trace minerals with reduced postpartum interval to breeding, compared to inorganic trace minerals (Swenson et al., 1998), and an improved pregnancy rate within the first 30 d of the breeding season when Cu was supplied in an organic form compared with non-supplemented controls (Muehlenbein et al., 2001).

The challenge when interpreting reproductive data from trace mineral experiments is that few trials have evaluated only one trace mineral at a time, confounding the results. Additionally, since most reproductive data is binomial, and relatively limited numbers of animals have been used, detecting an effect of trace mineral treatment on reproductive

performance is unlikely. Furthermore, little has been reported concerning likely physiological mechanisms of trace mineral effects on reproductive performance.

The effects of Cu on reproduction have been more widely studied than those of other trace minerals, including research with several different species. In cattle, data have indicated that Cu can be important (Muehlenbein et al., 2001; Kropp, 1993; Phillippo et al., 1982; Underwood, 1977) or not important (Phillippo et al., 1982, 1987) for reproductive performance or physiological events involved in reproduction (Xin et al., 1993). In cattle fed Mo or Fe to cause Cu deficiency, elevated dietary Mo and not low Cu appeared to be the cause of delayed puberty (possibly due to depressed basal LH release, affecting follicular estradiol production), reduced conception rate, and failure to ovulate (Phillippo et al., 1987). In other species including sheep, guinea pigs, rats, and rabbits, researchers have linked Cu and reproduction (Howell, 1969; Hall and Howell, 1969; Howell and Hall, 1969, 1970; Henkin, 1980; Suzuki and Bialy, 1964; Underwood, 1977).

The actual mechanism(s) by which Cu impacts female reproduction is not known. Possible mechanisms include an effect of Cu on ovarian activity (Xin et al., 1993), uterine tissue repair after parturition (Manspeaker et al., 1993), enzyme activity in the steroidogenic pathway causing differences in steroid hormone production (Henkin, 1980) and(or) within the hypothalamic-pituitary axis (Kochman et al., 1997; Phillippo et al., 1987).

Endocrinology. Extensive literature has addressed the effect of Cu on GnRH release, leading to widespread agreement there is a link between Cu and the hypothalamic-pituitary axis. This was first identified by Fevold and others (1936) when Cu salts

induced ovulation in rabbits, which has been repeated (Suzuki and Bialy, 1964; Pau and Spies, 1986; Tsou et al., 1977). The effect of Cu on ovulation may be due to increased secretion of GnRH from the hypothalamus (Tsou et al., 1977; Burrows and Barnea, 1982; Barnea and Cho, 1984) possibly mediated by the presence of ovarian hormones (Pau and Spies, 1986) or prostaglandin E_2 (Barnea et al., 1985), which may enable extracellular Cu to influence hypothalamic granules and cause an increase in GnRH release. Recent research has also identified a Cu-dependent monooxydase, peptidylglycine (an α-amidating enzyme that amidates bioactive peptides at the carboxy terminus to allow for full biological activity), which amidates GnRH within the hypothalamic granules (Kochman et al., 1997), supporting the theory that some GnRH in the portal blood may be complexed with Cu.

Due to the important role played by GnRH in the regulation and release of LH and FSH, which directly impact gonadal and reproductive activity, more recent research has examined the impact of Cu on the pituitary gland. Several researchers have evaluated the effect of GnRH when complexed with a metal (e.g. Cu, Zn, or Ni) on the release of LH from the pituitary. Kochman et al. (1992) documented that GnRH, when complexed with Cu, increased the release of both LH and FSH from the pituitary in ovariectomized rats. Also using rats, Hazum (1983) reported that Cu ions have a direct effect on the pituitary, which resulted in increased levels of LH release, dependent on Ca. Similarly, Schvartz and Hazum (1986) saw a stimulatory effect of Cu on GnRH release as well as a desensitization of pituitary cells, which they theorized as being mediated through the GnRH receptor. Kochman and others (1997), based on previous literature and their results using rat pituitaries *in vitro*, theorized that Cu ions probably directly modify the

GnRH receptor and plasma membranes, in addition to indirectly affecting the modification of low-density lipoproteins. In one of few studies using cattle to examine the impact of Cu on the pituitary, Xin et al. (1993) used dairy steers to determine whether low Cu status caused by Mo supplementation affected circulating LH concentrations due to endogenous or exogenous GnRH. The authors reported that LH concentrations were lower in pituitary samples of Cu-deficient animals, but LH concentrations did not differ in the circulation, even if induced by exogenous GnRH. The appropriateness of the dairy steer as a model for evaluating pituitary responsiveness in female cattle was questioned, as well as the confounding effects of Mo supplementation on Cu deficiency status (Xin et al., 1993). An earlier study by LaBella et al. (1973), which used hypothalamic tissue from cattle to evaluate pituitary response *in vitro*, found that Cu was in the highest concentration within hypothalamic extracts, compared to Ni and Zn, and that it stimulated the release of all pituitary hormones including LH. Although female cattle have not been studied directly, based on these previous experiments it appears that the action of the pituitary gland, including the synthesis and subsequent GnRH-triggered release of LH, is probably affected by the presence of Cu ions, implying that Cu status and supplementation probably have an effect at the pituitary cell level, possibly via GnRH receptors.

Uterine and Ovarian Function. An effect of trace mineral source on uterine function in first-calf dairy heifers receiving organic trace mineral supplements compared to inorganic trace minerals has been reported (Manspeaker et al., 1987). The authors observed lower periglandular fibrosis (evidence of more rapid endometrial tissue regeneration post-calving) due to organic trace minerals supplementation from 30 d after

parturition through confirmed pregnancy, which theoretically could lead to a shorter postpartum interval. However, trace mineral concentrations in the organic and inorganic treatments supplemented to females were not equal (Manspeaker et al., 1987).

Performance

A large percentage of forages in the U.S. are deficient or marginally adequate in Cu and(or) Zn, as well as marginal or high in Cu antagonists such as Fe and Mo (Corah et al., 1996; Mortimer et al., 1999). Based on this fact, extensive research has evaluated the effect of trace minerals on cattle performance, including research in grazing cattle (cows and suckling calves) and feedlot cattle.

Grazing Cow and Calf. Neither trace mineral supplementation nor source affected cow BW or body condition score (Olson et al., 1999; Stanton et al., 2000; Muehlenbein et al., 2001). When the effect of Cu bolus administration to calves before weaning was examined, weaning weights were heavier in bull calves and tended to be heavier in heifer calves that received supplemental Cu compared with non-supplemented controls (Arthington et al., 1995). The average daily gain (ADG) of calves prior to weaning improved when Zn was supplemented from the time of bull removal until weaning (Mayland et al., 1980). In addition, calves from cows receiving organic trace minerals had greater calf weaning weights when cows received supplemental Cu, Zn, Mg, Mn, and potassium in an organic form compared to calves from cows receiving inorganic trace minerals (Kropp, 1990). In contrast, others have reported no effect of trace mineral source on calf performance (Muehlenbein et al., 2001; Olson et al., 1999). Calf ADG was not affected by Cu, Co, Mn, and Zn supplementation compared with non-

supplemented controls (Olson et al., 1999), and calf ADG was not affected by supplementation with organic Cu, inorganic Cu, or an organic Cu/Zn combination (Muehlenbein et al., 2001). Pre-weaning ADG was greater in calves that had access to a high level of supplemental Cu, Zn, and Mn in an organic form compared with calves receiving high or low levels of inorganic Cu, Zn, and Mn (Stanton et al., 2000).

In the feedlot, post-weaning performance has been positively, negatively, and not affected by trace mineral supplementation. During the receiving, growing, and finishing phases, trace mineral supplementation and source can impact performance (Ward and Spears, 1997; Engle et al., 2000c; Spears and Kegley, 2002), although several experiments have reported no effect of Cu supplementation on the performance of growing or finishing steers (Engle and Spears, 2000a, 2001). In contrast, Cu decreased feed intake, gain-to-feed ration (G:F), and ADG when supplemented at 20 or 40 mg of Cu/kg of diet (Engle and Spears, 2000b), and Cu supplementation at 10 or 40 mg of Cu/kg of diet improved ADG and daily feed intake (Engle et al., 2000a).

Growing Phase Performance. The majority of trace mineral experiments in feedlots have focused on the supplementation of a single trace mineral (i.e. Cu, Zn, or Mn) only during the growing and(or) finishing phases. Researchers have concluded that Cu supplementation does not affect performance during the growing phase (Ward et al., 1993; Engle and Spears, 2000b; Engle and Spears, 2001; Engle et al., 2000c; Lee et al., 2002). Compared to non-supplemented controls, Cu supplementation had no affect on BW, ADG, or G:F in growing heifers receiving Cu supplementation at concentrations two- or five-times above NRC (1996) recommended concentrations in the presence of an antagonist (Bailey et al., 2001). However, in a long growing phase (196-d), greater dry

matter intake (DMI) and a tendency for lower G:F have been observed when Cu was provided for a short time prior to weaning versus non-supplemented controls, but ADG was not affected by Cu supplementation (Ward and Spears, 1997).

Reported effects of Zn supplementation on growing phase performance are more variable than Cu. Spears and Kegley (2002) observed no effect of Zn supplementation on growing phase BW, DMI, or G:F compared to non-supplemented controls, but ADG was greater in Zn-supplemented cattle compared to non-supplemented controls. Greater ADG was reported due to Zn supplementation (25 mg Zn/kg diet above basal), in addition to greater G:F and no difference in DMI in growing lambs and heifers supplemented with Zn (Zn-methionine) during a 56-d growing phase, but no effect of Zn supplementation on performance (Spears, 1989). During the first 56 d of a 118- or 128-d feeding period, Rust and Schlegel (1993) observed greater ADG and DMI in Zn-supplemented steers, while negative effects of Zn supplementation occurred when Zn was supplemented to growing heifers at an elevated concentration (50 mg Zn/kg diet; Spears and Samsell, 1984).

Source of Cu had no effect on growing phase BW, ADG, or DMI, or G:F (Engle and Spears, 2000b; Lee et al., 2002), but Ward et al. (1993) reported that $CuSO_4$ supplemented steers had greater G:F during the first 21 d of a 98-d growing phase than steers receiving supplemental Cu-lysine. Several researchers have reported no effect of Zn source on growing phase BW, ADG, DMI, or G:F (Spears and Kegley, 2002; Engle et al., 1997; Spears, 1989).

When the combined effects of Cu, Zn, Mn, and Co supplementation and source on weaned heifer calf performance were evaluated during a 42-d growing phase, DMI, ADG, and G:F were not impacted by either trace mineral supplementation or source;

however, when these minerals were provided in the organic form at three-times the NRC (1996) recommended concentration for the first 14 d of the feeding period, heifers had greater ADG and G:F compared to those receiving either inorganic or organic minerals at NRC (1996) recommended concentrations (George et al., 1997).

Finishing Phase Performance. Compared to growing phase performance, effects of Cu supplementation on finishing phase performance have been much more variable. Greater finishing phase ADG and G:F, but not DMI, in Cu-supplemented cattle compared to non-supplemented controls (Ward and Spears, 1997) and greater final BW, ADG, and DMI, but not G:F in Cu-supplemented cattle vs. non-supplemented controls (Engle et al., 2000c) have been reported. In contrast, compared to non-supplemented controls Cu-supplemented cattle had lower ADG, DMI, and G:F (Engle and Spears, 2000b) and no differences in finishing phase BW, ADG, DMI, or G:F (Engle and Spears, 2000a; Engle nd Spears, 2001; Engle et al., 2000b).

Zinc supplemented cattle tended to have a greater ADG than non-supplemented controls throughout a 118- or 128-d feeding period, but were not different in G:F or DMI (Rust and Schlegel, 1993). Other researchers have reported that a linear decrease in DMI occurred as Zn concentration increased, but there was no effect of Zn supplementation on overall BW, ADG, or G:F during the finishing phase (Malcolm-Callis et al., 2000), and no effect of Zn supplementation on finishing phase ADG, DMI, or G:F (Spears and Kegley, 2002).

Steers receiving organic Cu had greater final BW and tended to have greater ADG compared to steers receiving inorganic Cu, but DMI and G:F did not differ (Lee et al., 2002). No effect of Cu source on finishing phase performance has also been observed

(Engle and Spears, 2000b). There was a tendency for ADG and G:F to be greater in cattle supplemented with organic Zn compared to inorganic Zn, while BW and DMI were not impacted during an 84- or 112-d feeding period (Spears and Kegley, 2002). Furthermore, ADG was greater in cattle receiving $ZnSO_4$ compared to Zn-methionine, yet DMI and G:F were not affected (Nunnery et al., 1996), while Zn source did not impact finishing phase performance in several other experiments (Rust and Schlegel, 1993; Greene et al., 1988; Malcolm-Callis et al., 2000).

Carcass Characteristics. Carcass traits, including backfat thickness (BFAT) and fatty acid profile have been impacted by trace mineral supplementation, likely due to changes in lipid metabolism due to Cu (Ward and Spears, 1997; Engle et al., 2000a) and Zn (Spears and Kegley, 2002; Rust and Schlegel, 1993).

Engle and Spears (2001) reported no effect of Cu supplementation on BFAT, marbling (MARB), dressing percent (DP), hot carcass weight (HCW), kidney pelvic and heart (KPH) fat, yield grade (YG), ribeye area (REA), or quality grade (QG) in feedlot cattle. However, Cu supplementation during the feeding period caused a decrease in BFAT but did not affect MARB (Engle and Spears, 2000a; Ward and Spears, 1997; Engle et al. 2000a; Engle et al., 2000c; Engle et al., 2000b). Lower DP and YG along with greater REA, with no impact on HCW, KPH, or MARB, occurred due to Cu supplementation (Ward and Spears, 1997), as well as decreased HCW (Engle et al., 2000a) and increased HCW (Engle et al., 2000c) compared to non-supplemented controls. When Cu was supplemented at either 10 or 20 mg Cu/kg diet, organic Cu increased HCW and DP compared to inorganic Cu, and there was a tendency for increased HCW, DP, and REA in cattle receiving organic Cu compared to inorganic Cu, but only when Cu was

supplemented at a concentration of 10 mg Cu/kg diet (Johnson, 2002). Fatty acid composition of longissimus muscle samples was not affected by Cu supplementation in Simmental steers (Engle and Spears, 2001), but in Angus-based steers, Cu supplementation increased the weight percentage of polyunsaturated fatty acids 18:2 and 18:3 (Engle et al., 2000c), and tended to decrease saturated fatty acids (Engle et al., 2000a; Engle and Spears, 2000a) compared to non-supplemented controls. In addition, Cu-supplemented cattle had lower 18:1t concentrations (Engle et al., 2000a) and increased weight percentages of total unsaturated fatty acids and decreased fatty acid 14:1 (Engle and Spears, 2000a) compared to non-supplemented controls.

Neither Zn supplementation nor concentration impacted any carcass characteristics (Galyean et al., 1995) or quality traits (Nunnery et al., 1996). However, increased QG and MARB and a tendency ($P < 0.10$) for greater DP, YG and BFAT were reported in Zn-supplemented cattle compared to non-supplemented controls, with no difference in REA, HCW, or KPH (Spears and Kegley, 2002). Steers receiving supplemental Zn had greater BFAT and YG with lower REA compared to non-supplemented controls, but Zn supplementation had no effect on HCW, REA, or QG (Rust and Schlegel, 1993).

Steers supplemented with inorganic Cu had higher MARB than steers receiving organic Cu; however, Cu source did not impact USDA QG or any other carcass characteristic, including DP, HCW, BFAT, KPH, YG, and REA (Engle et al., 2000a). When fatty acids were evaluated, cattle receiving organic Cu had a higher weight percentage of fatty acid 22:1 compared to steers receiving inorganic Cu (Engle et al., 2000a); however, Cu source did not impact fatty acid profile in steers supplemented with Cu (Johnson, 2002). Zinc source had no impact on any carcass characteristics (Rust and

Schlegel, 1993) or quality traits (Nunnery et al., 1996). In another study, Zn source did affect carcass characteristics, including greater HCW, REA, and DP in cattle supplemented with Zn from one of two organic sources compared to an inorganic source, but QG, YG, MARB, KPH, and BFAT were not different (Spears and Kegley, 2002). Greater KPH, QG, and MARB were reported in steers provided an organic Zn supplement (Zn-methionine) compared to inorganic Zn (ZnO), but BFAT, REA, HCW, and YG were not affected after a 112-d feeding period (Greene et al., 1988). There was a tendency for greater BFAT and lower KPH in steers that received Zn via an amino acid- or a polysaccharide-complex compared to steers receiving Zn as $ZnSO_4$, but no effect of Zn source on HCW, DP, REA, MARB, or YG (Malcolm-Callis et al., 2000).

Immune Response

Morbidity. During the receiving, growing, and finishing phases, trace mineral supplementation and source can impact health status (Spears, 2000; Galyean et al., 1999). Zinc concentration and source affected feedlot performance, based on differences in morbidity distribution in steers during a 28-d receiving period and in a 21-d step-up period in which twice as many steers were treated in the basal and low organic Zn treatments compared to the high organic and inorganic Zn treatments (Galyean et al., 1995).

When the effect of lifetime supplementation of trace minerals (Cu, Zn, Mn, and Co) on calf morbidity was examined, fewer organic and non-supplemented calves were treated during the first 28-d compared to the inorganic treatment, fewer organic calves were treated throughout the feeding period compared to inorganic calves, and organic

calves were re-treated less than inorganic and non-supplemented control calves during the first 28-d period and throughout the entire feeding period (Grotelueschen et al., 2001).

Humoral Immune Response. The systemic immune response that involves antibody production is known as humoral immune response (Parham, 2000). This immune response (either primary or secondary) can be an important indicator of the immune status of an animal, since the response involves the secretion of antibodies that bind and neutralize antigens in the extracellular space (Goldsby et al., 2003). Immune responses to conventional viruses [e.g. infectious bovine rhinotracheitis virus (IBRV)] have been evaluated in cattle, as well as responses to foreign proteins introduced into animals, including pig red blood cells (PRBC) and ovalbumin (OVA) which serve as antigens. Understanding how trace minerals can affect an immune response is important to beef cattle production, particularly in young cattle arriving into a feedlot while under the substantial physiological stresses due to weaning, transport, vaccination, and reduced intake of water and feed.

In feedlot heifers, IBRV antibody titer response against a modified live vaccine was enhanced in heifers at d 14 and 28 after injection when heifers received supplemental Cu, Zn, Mn and Co at NRC (1984) recommended concentrations in an organic form compared to an inorganic form during a 42-d growing phase (George et al., 1997). Copper supplementation had no effect on primary immune response to PRBC in 70 d old calves (Ward et al., 1997). Lower antibody titers to PRBC occurred in non-stressed steers supplemented with Cu versus non-supplemented controls, while in stressed steers Cu supplementation led to increased antibody titers to PRBC (Ward and Spears, 1999). Greater antibody titer concentrations to PRBC occurred in calves receiving 20 mg Cu/kg

diet of supplemental Cu compared with calves receiving 10 mg supplemental Cu/kg diet (Dorton et al., 2003). Copper supplemented cattle did not respond differently than non-supplemented controls to an injection of OVA (Ward et al., 1993). A greater immune response to OVA was found in Cu-supplemented cattle vs. non-supplemented controls (Dorton et al., 2003; Ward and Spears, 1999). Reduced secondary immune response to PRBC occurred in cattle supplemented with Cu compared to non-supplemented controls on d 14 and 21 after injection (Ward et al., 1997). Concentrations of immunoglobulin (Ig) G were greater on d 14 in cattle receiving 20 mg supplemental Cu/kg diet compared to those receiving 10 mg Cu/kg diet, but on d 21 IgG concentrations were greater in non-supplemented controls than Cu-supplemented cattle, and IgM concentrations tended to be greater in Cu-supplemented cattle than non-supplemented controls (Dorton et al., 2003).

When organic trace minerals were supplemented to dams, suckling calves had enhanced immune response compared to calves whose dams received inorganic trace minerals (Ward and Spears, 1999; George et al., 1997). Concentrations of IgG and total Ig in response to PRBC were greater during the growing phase in calves receiving inorganic Cu vs. organic Cu (Dorton et al., 2003). Greater primary antibody titers to OVA occurred in calves receiving organic Cu compared to inorganic Cu on d 14 and 21 after injection with OVA (Dorton et al., 2003). In response to PRBC, concentrations of IgG and total Ig were greater in cattle receiving inorganic Cu than organic Cu (Dorton et al., 2003).

Consistent effects of Cu deficiency on humoral immune response have not been found in previous research (Spears, 2000). The effect of a Cu deficiency or marginal Zn

deficiency on a secondary humoral immune response has not been consistently observed in cattle, in contrast to humans and rodents (Spears, 2000).

Cell-mediated Immune Response. In addition to activity of the humoral immune system, which responds to foreign bodies in extracellular spaces, the body's cell-mediated immune (CMI) response is present in order to find and eliminate cells with intracellular pathogens (Goldsby et al., 2003). Since a CMI response leads to an inflammatory response, administration of phytohemagglutinin (PHA) intradermally has been incorporated into beef cattle experiments to evaluate the effect of trace minerals on CMI response. A swelling response commonly occurs immediately after PHA injection, which can be evaluated in cattle easily by measurement and documentation of changes in skin-fold thickness over time (usually 48 h) beginning immediately post-injection.

Ward and Spears (1999) reported no effect of Cu supplementation on CMI response to PHA in calves at the beginning of the receiving phase after injection with 90 mg of Cu 28 d prior to weaning (Ward and Spears, 1999). Similarly, no effect of Cu supplementation on CMI response to PHA was observed in feedlot cattle, but Cu concentration did impact CMI response, as seen by greater skin-fold thickness, in cattle supplemented with 20 vs. 10 mg Cu/kg diet (Dorton et al., 2003). A lower CMI response in 70 d old beef calves receiving supplemental Cu was detected compared to non-supplemented controls at 4, 8, 12, 24, and 48 h after the PHA injection in one experiment, while a greater CMI response was found in young Holstein calves receiving supplemental Cu compared to non-supplemented control calves at 12 and 24 h after PHA injection in a second experiment (Ward et al., 1997).

Zinc supplementation did not impact CMI response to PHA in steers during the growing phase (Spears and Kegley, 2002), although during a 21-d depletion phase, calves receiving no supplemental Zn had lower skin-fold thickness measurements at 8 and 12 h after PHA injection compared to calves supplemented with Zn (Engle et al., 1997). Heifers supplemented with Zn had a greater CMI response at 24 h after PHA injection compared to non-supplemented control heifers on d 71 of the growing phase (Kegley et al., 2001). Although evidence exists that trace minerals affect CMI, it has not been reliably shown that a Cu deficiency or a marginal Zn deficiency in cattle will affect CMI response, in contrast to results in humans and laboratory animals (Spears, 2000).

Trace mineral source did not impact CMI response to PHA, as seen by no differences in skin-fold thickness between cattle receiving an organic or inorganic Cu source (Dorton et al., 2003). Calves receiving organic Zn had a lower CMI response than inorganic Zn treatments at 8 h after PHA; however, calves receiving inorganic Zn or no supplemental Zn had a lower CMI response at 24 h after the PHA injection than calves receiving organic Zn (Engle et al., 1997).

When Cu, Zn, Mn, and Co were combined and supplemented to stressed weaned heifer calves, no effect of concentration or source of trace minerals on CMI response at 12 or 24 h was reported after PHA injection on d 7 after arrival to the feedlot; however, a lower CMI response at 48 h after PHA injection occurred in calves supplemented with the organic form of Cu, Zn, Mn, and Co at three-times the NRC (1984) recommendation compared to calves receiving the inorganic form at NRC (1984) recommended concentrations (George et al., 1997). In this same experiment on d 21 after arrival to the feedlot, greater and longer CMI responses to PHA at 12, 24, and 48 h were observed in

calves supplemented with organic trace minerals at three-times the NRC (1984) recommended concentrations versus calves receiving the trace minerals in an organic or inorganic form at NRC (1984) recommended concentrations. Furthermore, of the calves receiving Cu, Zn, Mn, and Co at NRC (1984) recommended concentrations, calves receiving the organic form had a greater CMI response to PHA than the inorganic form, but only at 48 h post PHA injection (George et al., 1997).

Superoxide Dismutase. The activity of SOD, a trace mineral-dependent enzyme responsible for protecting cells from oxidative stress, in red blood cells has also been evaluated in several trace mineral experiments with feedlot cattle. In feedlot cattle, no effect of Cu supplementation or source on SOD activity was detected during either the growing or finishing phases (Dorton et al., 2003; Ward et al., 1993). However, greater SOD activity in Cu-supplemented cattle on d 0, 28, and 56 of the growing phase, but not after d 56 (Ward and Spears, 1997), has been reported. Additionally, Cu supplementation prevented a decrease in SOD activity late in the growing phase (d 84) when Mo was present as an antagonist (Ward and Spears, 1997). When high concentrations of Mo (10 mg Mo/kg diet) were present, SOD activity in red blood cells decreased, and neutrophil bactericidal function was depressed in Cu depleted Holstein steers after eight months of a depletion phase, compared to Cu-supplemented steers (Xin et al., 1991). Although SOD activity has been linked with trace mineral supplementation and status, a reduction in SOD activity typically only occurs during extended Cu deficiency (Paynter, 1987).

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CHAPTER II

EFFECTS OF COPPER, ZINC, AND MANGANESE SUPPLEMENTATION AND SOURCE ON REPRODUCTION, MINERAL STATUS, AND PERFORMANCE IN GRAZING BEEF CATTLE OVER A TWO-YEAR PERIOD

ABSTRACT

Crossbred, multiparous beef cows (n = 178 in Year 1; n = 148 in Year 2) were used to evaluate the effects of Cu, Zn, and Mn supplementation and source on reproduction, mineral status, and performance in grazing cattle in eastern Colorado over a 2-yr period. Cows were stratified by expected calving date, age, BW, BCS, and liver mineral status and assigned to the following treatments: 1) control (no supplemental Cu, Zn, or Mn); 2) organic (ORG; 50% organic and 50% inorganic Cu, Zn, and Mn); and 3) inorganic (ING; 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$) trace minerals. Free-choice mineral feeders were used to provide current NRC-recommended concentrations of Cu, Zn, and Mn from 82 d (Year 1) and 81 d (Year 2) before the average calving date of the herd through 110 d (Year 1) and 135 d (Year 2) after calving. At the end of Year 1, supplemented cows had greater liver Cu ($P < 0.01$), Zn ($P < 0.05$), and Mn ($P < 0.01$) concentrations than controls, whereas liver Cu concentration was greater ($P < 0.01$) in ORG vs. ING cows. At the end of Year 2, supplemented cows had greater ($P < 0.01$) liver Cu concentrations

relative to controls, whereas control cows had greater ($P < 0.02$) liver Mn concentration than supplemented cows. In Year 1, pregnancy rate to AI in control cows did not differ ($P = 0.47$) from supplemented cows, but there was a trend ($P < 0.08$) for pregnancy rate to be higher for ORG than ING cows. In Year 2, supplemented cows had a higher ($P < 0.02$) pregnancy rate to AI than controls. In both years, when cows were inseminated after an observed estrus, supplemented cows had a higher ($P < 0.04$) pregnancy rate than controls. Also, for both years, overall 60-d pregnancy rate tended ($P = 0.10$) to be higher for supplemented cows than for controls. In Year 1, kilograms of calf weaned per cow exposed was greater ($P < 0.02$) in controls than in supplemented cows, and kilograms of calf weaned per cow exposed was greater ($P < 0.01$) in ING than ORG treatments. However, in Year 2, kilograms of calf weaned per cow exposed was greater ($P < 0.02$) in controls than supplemented cows, and tended ($P = 0.09$) to be greater in ORG than ING treatments. Results indicate that supplementation and source of trace minerals affected mineral status and kilograms of calf weaned per cow exposed in grazing beef cows. Supplementation also improved pregnancy rate to AI compared with cows not supplemented with Cu, Zn, or Mn for more than 1 yr. Furthermore, mineral source may influence pregnancy rate to AI.

INTRODUCTION

Responses in reproduction and performance to copper (**Cu**), zinc (**Zn**), and manganese (**Mn**) supplementation in ruminants have been variable (Underwood and Suttle, 1999). Olson et al. (1999) reported no difference in reproductive performance in cows supplemented with organic vs. inorganic forms of trace minerals. However,

supplemented cows had lower pregnancy rates than controls, most likely because supplementation levels were two times NRC (1996) recommendations. In contrast, Stanton et al. (2000) reported that cows receiving organic trace minerals exhibited higher pregnancy rates to AI than those receiving inorganic trace minerals. Improved reproductive performance has been reported in dairy cows receiving organic mineral supplements (Manspeaker et al., 1987), which was attributed to improved repair of damaged uterine tissue following calving.

The increase in performance associated with organic trace minerals in several species may be due to increased bioavailability. Du et al. (1996) and Kegley and Spears (1994) suggested that organic Cu sources are equally, if not more, bioavailable than $CuSO_4$ in rats and growing calves, respectively. Conversely, Cao et al. (2000) compared eight organic Zn products in chicks and lambs and found that only one was more bioavailable than $ZnSO_4$.

There has been tremendous variability in the concentration and source of trace mineral supplementation used in different studies, making interpretation of data and ability to reach a concise conclusion challenging. We hypothesized that not supplementing trace minerals would be detrimental to reproductive performance of cows and that source of trace mineral supplementation may have different effects on reproductive performance of cows and on calf performance. The objective of this study was to determine the effect of the supplementation and source of Cu, Zn, and Mn at levels recommended by NRC (1996) on reproductive performance, mineral status, and performance in grazing beef cattle.

MATERIALS AND METHODS

Experimental Design

Before starting this study, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee. Twelve days before the start of this experiment (January 17, 2001), 178 crossbred (Red Angus-based), multiparous beef cows at the Eastern Colorado Research Center (Akron, CO) were stratified based on age, expected calving date, BW, BCS, and liver mineral status (Table 2.1) and randomly assigned to one of nine replicates. The replicates were then assigned to one of three treatments (n = 19 to 20 cows per replicate), resulting in three replicates per treatment in each year. Treatments were as follows: 1) control (no supplemental Cu, Zn, or Mn; n = 59); 2) organic (**ORG**; 50% organic and 50% inorganic Cu, Zn, and Mn; n = 60); and 3) inorganic (**ING**; 100% inorganic Cu, Zn, and Mn; n = 59) trace minerals.

All procedures described below were repeated over two consecutive years, except where noted. Cows remained in the same treatment for both years. Inorganic trace minerals were supplemented as $CuSO_4$, $ZnSO_4$, and $MnSO_4$, whereas organic trace minerals were provided from a commercially available mineral proteinate source (Bioplex^{TM}, Alltech Inc., Nicholasville, KY). Salt was added to the supplements to limit consumption of Cu, Zn, and Mn to approximately NRC (1996) recommended levels. Vitamins A, D, and E were added to meet NRC (1996) requirements and mixed thoroughly by hand at the time of mineral delivery to trace mineral feeders. Ingredient composition and laboratory analysis of the basal supplement and trace mineral treatments are shown in Table 2.2. Basal forage and water trace mineral concentrations were

determined using samples collected from pasture, stored hay, and water sources. Trace mineral concentrations were as follows: pasture = 13.1 ppm Cu, 16.1 ppm Zn, and 36.6 ppm Mn; stored hay = 19.6 ppm Cu, 32.1 ppm Zn, and 52.2 ppm Mn; and water <0.01 ppm Cu, <0.01 ppm Zn, and 0.08 ppm Mn.

After replicates and treatments were assigned, animals were housed by replicate in nine separate pastures. Cows were maintained on native pastures that consisted primarily of blue grama (*Bouteloua gracilis*), prairie sandreed (*Calamovilfa longifolia*), and needle-and-thread grass (*Stipa comata*). Early in the first year of the study (December through March), supplemental millet hay and range cubes were provided to compensate for poor winter forage quality. Estimated intakes of millet hay and range cubes were similar across treatments. Forage and range cube supplementation were discontinued as range quality increased during early spring.

Mineral treatments were provided at a single location in each pasture in free-choice mineral feeders beginning 82 (d –82; Year 1) and 81 d (d –81; Year 2) before the average expected calving date (d 0) of the cowherd. Mineral treatments remained available for ad libitum consumption until 110 (d +110; Year 1) and 135 d (d +135; Year 2) after the average calving date of the cowherd. Mineral treatments were also made available exclusively to the calves via creep feeders in each pasture when calves within the pasture averaged 90 (Year 1) and 99 d (Year 2) of age. All calves received the same respective mineral treatment as their dam. In Year 1, on d +110 after the average calving date, treatments were discontinued and all cows within a treatment were combined and given access to the control (basal) mineral supplement, which did not contain any supplemental Cu, Zn, or Mn (Table 2.2) for a period of 160 d. Calves continued to have access to their

respective mineral treatments until weaning at an age of 185 and 164 d in Years 1 and 2, respectively. Beginning 81 d before the average expected calving date of the cowherd in Year 2, cows were sorted into the same respective treatment groups as in Year 1, assigned to new replicates, and maintained on treatments until d +135 of Year 2. At the beginning of Year 2, there were fewer cows in the experiment (n = 148) than at the beginning of Year 1 (n = 178) because cows that were not pregnant after the final pregnancy rate was determined in Year 1 were removed from the experiment.

Replicates were rotated among pastures approximately every 28 d in order to minimize pasture effects. In conjunction with pasture rotations, mineral weigh-backs were performed at each rotation in order to calculate mineral disappearance. Replicates were pooled but remained within treatments for approximately 30 d around the time of calving and 60 d during the time of breeding to allow for more intensive management of the cattle. Mineral treatments continued to be available at these times, although mineral disappearance within replicates could not be monitored.

Mineral Status. Mineral status of the cows was measured using two methods. The first method involved the collection of a liver biopsy sample from every cow before the start of the experiment and then from a subgroup of animals (five per replicate) at the end of the supplementation period in Year 1 and at the beginning and end of the supplementation period in Year 2. This was accomplished by using the true-cut technique described by Pearson and Craig (1980), as modified by Engle and Spears (2000b). Following collection, samples were immediately rinsed with 0.01 *M* PBS, placed in an acid-washed polypropylene tube, capped, and placed on ice for 5 h before storing at –20°C. Liver

samples were analyzed for trace mineral concentrations as described by Engle et al. (1997).

The second method used to determine mineral status involved the collection of whole blood via jugular venipuncture in heparinized, trace-mineral-free Vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) at the beginning, midpoint, and end of mineral supplementation from a subgroup of animals (five per replicate), the same animals from whom liver biopsies were obtained. Once collected, samples were placed on ice for 5 h before being centrifuged at 2,000 × *g* for 15 min. Plasma was then transferred to acid-washed storage vials and stored at –20°C.

Plasma Cu and Zn concentrations were measured after the samples were thawed at room temperature for 3 to 4 h. For Cu analysis, 1 mL of a 10% (wt/vol) trichloroacetic acid solution was added to 1 mL of plasma or standard and then vortexed vigorously for 20 s. To aid in precipitation, the sample was placed in a –20°C freezer for 30 min, and then centrifuged at 1,200 × *g* for 10 min at room temperature. The supernatant was removed and diluted in deionized water to concentrations that fit within a linear range of a standard curve generated by linear regression of known concentrations. Plasma Cu concentrations were read at 324.7 nm using a flame atomic absorption spectrophotometer (model 1275, Varian, Walnut Creek, CA). Plasma Zn concentrations were analyzed in a similar manner as described above. Five hundred microliters of plasma was diluted to a 1:5 ratio (plasma:deionized water). This dilution was then read at 213.9 nm using the same atomic absorption spectrophotometer previously mentioned. Only samples from Year 1 were analyzed for Cu and Zn concentrations.

Cow Performance. To determine the effects of trace mineral supplementation and source on reproductive performance, every cow was inseminated once following a modified Select-Synch (Geary et al., 2000) estrus synchronization protocol. Beginning on d +55 (Year 1) and d +69 (Year 2) after the average calving date of the cowherd, all cows were injected with 100 μg of GnRH (Fertagyl, Intervet, Millsboro, DE; i.m.), and then injected with 25 mg of $PGF_{2\alpha}$ 7 d later (Lutalyse, Pharmacia & Upjohn, Kalamazoo, MI; i.m.). Calves were removed for 72 h from all cows at the time of $PGF_{2\alpha}$ administration in an effort to increase estrous response. For management reasons, all cows were pooled and treatments were withheld during the 72-h synchronized AI breeding period. Cattle were observed for signs of behavioral estrus from 12 h before $PGF_{2\alpha}$ injection through 72 h after $PGF_{2\alpha}$ injection. Detection of estrus was accomplished by visual observation for a minimum of 1 h at dawn, noon, and dusk. Animals observed in behavioral estrus were inseminated approximately 12 h after first detected in estrus. At 72 h after $PGF_{2\alpha}$, all females that had not been observed in estrus were given a second injection of GnRH (100 μg; i.m.) and mass inseminated. Calves were returned to their dams following AI, and all cattle were sorted back into treatments and returned to appropriate pastures after mass insemination.

To minimize variation in breeding measurements, an effort was made to decrease confounding variables. Two AI technicians performed the inseminations, equally represented within each replicate, and one technician thawed semen. Semen from one purebred Charolais sire that had been acquired from a single collection was used to avoid a sire effect. To allow for the accurate differentiation between pregnancy to AI vs. pregnancy to natural service, cows were not exposed to bulls until 14 d after mass

insemination. Six Red Angus-based composite bulls that had previously passed a breeding soundness evaluation were exposed to the cows for 46 d (two bulls per treatment).

In Year 2 only, immediately before the initiation of the synchronization protocol, two blood samples were collected via jugular venipuncture at 10-d intervals and progesterone concentrations were determined to identify cows that had cycled since parturition. Cows were identified as cycling if at least one blood sample had a serum progesterone concentration greater than 1.0 ng/mL, indicating the presence of a functional corpus luteum. Serum was harvested and stored at –20°C until samples were evaluated in duplicate for progesterone concentration by solid-phase RIA (Coat-a-Count kit; Diagnostic Products Corp., Los Angeles, CA), as described by Bellows et al. (1991). The intra- and interassay CV for the two assays were 5.9 and 2.2%, respectively. The sensitivity of the assay was 0.04 ng/mL of serum.

To determine pregnancy rate to AI, cattle were examined via rectal ultrasonography (Aloka 500V equipped with 5.0-MHz linear array transducer, Corometrics Medical Systems, Wallingford, CT) by a state-licensed veterinarian 40 d after mass mating. Cows with fetuses that were approximately 40 d old were classified as pregnant to AI, whereas all other cattle were classified as not pregnant to AI. Final pregnancy rates following the 60-d breeding season were determined via palpation per rectum with the aid of ultrasonography approximately 40 d after bull removal.

Cow performance was also monitored by collection of BW and BCS data at approximately 56-d intervals throughout the duration of the supplementation period in each year. The BCS measurements collected were on a scale of 1 to 9 (1 = emaciated, 9 =

obese; Richards et al., 1986) and were assigned by the same technician throughout the study.

Calf Performance. Calf performance was measured in both years based on kilograms of calf weaned per cow exposed. Weaning weights were collected on each calf before shipment to the feedlot. At the end of Years 1 and 2, calves averaged 185 and 164 d of age, respectively.

Statistical Analysis

Cow performance, mineral status, and calf performance data (including BCS; BW; liver Cu, Zn, and Mn concentrations; plasma Cu and Zn concentrations; and weaning weight) were assessed using a restricted maximum likelihood-based, mixed-effects model, repeat-measures analysis (PROC MIXED; SAS Inst., Inc., Cary, NC). Initial cow performance and mineral status models contained fixed effects of treatment, time, and treatment × time interaction. Initial calf performance models included fixed effects of treatment, year, age of dam, age of calf, sex of calf, and all relevant two- and three-way interactions. A spatial power covariance structure was used in the analysis and the containment approximation was used to calculate denominator degrees of freedom. Pasture was used as the experimental unit. Reproductive response data (including estrous cyclicity, estrous response, pregnancy to a synchronized AI, and final pregnancy throughout the 60-d breeding season) were analyzed using logistic regression (PROC GENMOD of SAS). Initial models for reproductive response contained fixed effects of treatment, postpartum interval, BCS, BW, year, and AI technician, in addition to all relevant two- and three-way interactions. When an interaction was not significant, it was

removed from the model. If the interaction of year × treatment was not significant, data were pooled across years; otherwise, data were reported for each year separately. Differences among means were determined using single-df contrasts; comparisons made were as follows: 1) control vs. supplemented and 2) ORG vs. ING.

RESULTS AND DISCUSSION

Mineral Consumption

Average mineral disappearance for the cows during Year 1 was 0.12 ± 0.06, 0.11 ± 0.07, and 0.12 ± 0.07 kg·cow^{-1}·d^{-1} for the ORG, ING, and control treatments, respectively. Average mineral disappearance during Year 2 was 0.13 ± 0.06, 0.12 ± 0.06, and 0.13 ± 0.06 kg·cow^{-1}·d^{-1} for the ORG, ING, and control treatments, respectively. In both years, the amount of daily mineral disappearance indicated that the ORG and ING supplemented cattle consumed enough Cu, Zn, and Mn to meet their NRC-recommended requirements of 10 mg of Cu/kg of DM, 30 mg of Zn/kg of DM, and 40 mg of Mn/kg of DM (NRC, 1996; estimated DMI = 10.9 kg). Because forage basal trace minerals were at low concentrations relative to NRC (1996) recommendations, no effects on performance were attributed to excess supplementation of Cu, Zn, or Mn.

Liver Mineral Status

Liver mineral status for cows in this experiment was affected ($P < 0.05$) by trace mineral supplementation and source (Table 2.3). A year × treatment interaction was detected ($P = 0.03$) for liver Cu concentration. At the end of both years, liver Cu concentration was greater ($P < 0.01$) in supplemented vs. control cows; however, liver Cu

concentration in control cows decreased in Year 2. In addition, at the end of Year 1, ORG cows had greater ($P < 0.01$) liver Cu concentration than did ING cows, whereas at the end of Year 2, liver Cu concentration did not differ ($P > 0.38$) between ORG and ING cows. Liver Cu results are consistent with those reported by other researchers (Du et al., 1996; Olson et al., 1999), wherein liver Cu concentrations increased with mineral supplementation compared with controls. Although an increase in liver Cu concentration is often observed in conjunction with trace mineral supplementation, and in this case with source in Year 1, it is not clear as to the positive or negative effects of liver Cu retention on reproduction and/or calf performance.

For liver Mn concentration there was a year × treatment interaction ($P = 0.01$). Liver Mn concentration was greater ($P < 0.01$) in supplemented vs. control cows at the end of Year 1; however, at the end of Year 2, control cows had greater ($P < 0.02$) liver Mn concentration than supplemented cows (Table 2.3). Liver Mn concentration did not differ ($P > 0.51$ and $P > 0.24$, Years 1 and 2, respectively) between ORG and ING treatments. The data are difficult to interpret as to why liver Mn concentrations were greater in non-supplemented control cows than in supplemented cows in Year 2. An animal's true Mn status may not be correctly evaluated when using a liver biopsy sample to determine Mn concentration. In ruminants, liver Mn concentration does not respond substantially to Mn supplementation, even at extreme dietary concentrations (Underwood and Suttle, 1999), as evidenced by only a fourfold increase in liver Mn when dietary Mn increased 130- to 140-fold (Ivan and Hidiroglou, 1980; Watson et al., 1973). Furthermore, the elevated liver Mn concentration observed in the control cows in Year 2 may not be biologically significant.

There was no year × treatment interaction ($P = 0.11$) for liver Zn concentration. Liver Zn concentration was greater ($P < 0.05$) in supplemented than in control cows at the end of Year 1; however, liver Zn concentration did not differ ($P = 0.56$) between supplemented and control cows at the end of Year 2. Liver Zn concentration did not differ ($P = 0.86$) between ORG and ING treatments at the end of Year 1 (Table 3.2), but ING cows tended ($P < 0.07$) to have greater liver Zn concentrations than ORG cows at the end of Year 2.

Plasma Mineral Status

Plasma trace mineral concentrations were impacted by trace mineral supplementation, but not by source. Plasma Cu concentrations at the end of the supplementation period in Year 1 tended ($P = 0.08$) to be greater in supplemented vs. control cows, and plasma Zn concentrations were greater ($P < 0.01$) in the supplemented cows vs. controls (Appendix A).

Cow Performance

Mean BW and BCS did not differ among treatments throughout the 2-yr experiment (Appendix A). However, there was a main effect of time ($P < 0.05$). Both BW and BCS declined following calving but returned to precalving levels by mid-summer. Similarly, previous experiments have reported that neither trace mineral supplementation nor source affected cow BW or BCS (Olson et al., 1999; Stanton et al., 2000; Muehlenbein et al., 2001).

Reproductive performance data throughout the experiment are reported in Table 2.4. There were no differences between control and supplemented cows, or between ORG and ING supplemented cows for the rate of estrous cyclicity at the start of the breeding season (Year 2 only) or percentage of cows exhibiting behavioral estrus in response to the synchronization protocol in either year. There was a year × treatment interaction ($P < 0.02$) for pregnancy rate to a synchronized AI, and therefore data were reported individually for each year. In Year 1, pregnancy rate to AI did not differ between control and supplemented cows; however, ORG-supplemented cows tended ($P < 0.08$) to have a higher pregnancy rate to AI than did ING-supplemented cows. Unlike Year 1, control cows had a lower ($P < 0.02$) pregnancy rate to AI than did supplemented cows in Year 2. Additionally, ORG- and ING-supplemented cows did not differ in pregnancy rate to AI in Year 2. To further evaluate the pregnancy response to AI data, time of AI was classified as being administered either 12 h after an observed estrus (**EAI**) or via mass insemination at 72 h after $PGF_{2\alpha}$ injection. Within the EAI group, supplemented cows had a higher ($P < 0.04$) pregnancy rate to AI than did control cows, and ORG-supplemented cows tended ($P = 0.13$) to have a higher pregnancy rate compared with ING-supplemented cows. Conversely, no differences were observed within the mass-insemination group; however, a much smaller number of cows were mass inseminated due to the high estrous response (approximately 80%) that occurred in both years.

The lack of a difference in pregnancy rate to AI between control and supplemented cows in Year 1, and the presence of a difference in Year 2, suggests that supplemented cows in Year 1 may not have benefited from ORG or ING supplementation. However, the lower pregnancy rate to AI in the control cows in Year 2 seems to have been caused by

the removal of supplemental Cu, Zn, and Mn for over 1 yr. The tendencies for ORG-supplemented cows to have a higher pregnancy rate to AI than ING-supplemented cows in Year 1, and when AI was based on an observed estrus in Year 2, are supported by results from Stanton et al. (2000), who observed a higher pregnancy rate to AI in cows receiving organic Cu, Zn, and Mn vs. the inorganic forms. However, many of the cows used by Stanton et al. (2000) had deficient liver Cu concentrations (defined as less than 20 to 30 ppm Cu; Mills, 1987) compared with the cows used in this study. Improved early pregnancy rates (pregnancies within the first 30 d of the breeding season) have also been reported by Muehlenbein et al. (2001) when Cu was supplied in an organic form compared with controls. Further research is needed to determine the likely physiological mechanisms that seem to enable trace minerals to affect pregnancy rates early in the breeding season.

Pregnancy rates collected at the end of the 60-d breeding season included pregnancies to both the synchronized AI and natural service. Supplemented cows tended ($P = 0.10$) to have a higher pregnancy rate than control cows; however, there was no difference between ORG- and ING-supplemented cows. To our knowledge, this is the first study to report that trace mineral supplementation may affect season-long reproductive performance in beef cattle. Trace mineral supplementation affected pregnancy rates to AI (as reported above) and possibly also natural service pregnancy rates during the 46-d period of bull exposure. Earlier studies have reported no differences at the end of a 60-d breeding season due to trace mineral supplementation (Arthington et al., 1995; Muehlenbein et al., 2001). However, Olson et al. (1999) reported a depressed 60-d pregnancy rate in cows that received supplemental Cu, Zn, and Mn in organic or

inorganic forms at twice the NRC (1996) recommended levels compared with non-supplemented controls. The beneficial reproductive performance observed in the current study, when supplementation was provided at NRC (1996) recommended levels for 2 yr, suggests that trace minerals impact reproductive performance positively when supplementation follows NRC recommendations.

Calf Performance

From d +90 until weaning in Year 1, mineral disappearance from calf creep feeders was 0.010 ± 0.003, 0.014 ± 0.004, and 0.011 ± 0.004 kg·calf^{-1}·d^{-1} for ORG, ING, and control treatments, respectively. Conversely, from d +99 until weaning in Year 2, mineral disappearance from creep feeders of calves was 0.048 ± 0.012, 0.042 ± 0.12, and 0.061 ± 0.018 kg·calf^{-1}·d^{-1} for ORG, ING, and control treatments, respectively. In Year 2, mineral disappearance from creep feeders was greater ($P < 0.01$) than in Year 1. It is not clear why calves consumed more supplement in the second year of this experiment.

There was a year × treatment interaction ($P < 0.05$) for calf performance, as measured by kilograms of calf weaned per cow exposed, and therefore data were reported separately for each year (Table 2.5). In both years, trace mineral supplementation and source affected kilograms of calf weaned per cow exposed. In Year 1, there were more ($P < 0.02$) kilograms of calf weaned per cow exposed in the control treatment than in supplemented cows, and more ($P < 0.01$) kilograms of calf weaned per cow exposed in the ING treatment than in ORG. Year 2 results for the effect of supplementation were similar to Year 1 because controls had more ($P < 0.02$) kilograms of calf weaned per cow

exposed vs. supplemented; however, unlike Year 1, the ORG treatment tended ($P = 0.09$) to have more kilograms of calf weaned per cow exposed than ING.

It is not clear why calf performance was higher in the control treatment vs. the supplemented treatment in both years, nor is it clear why calf performance was affected by trace mineral source differently in Year 1 than Year 2. Conflicting results have been reported in the literature as to the effects of trace minerals on calf performance in both preweaning (grazing) and postweaning (feedlot) situations. Preweaning calf ADG improved when Zn was supplemented from the time of bull removal until weaning (Mayland et al., 1980). Alternatively, calf gain was not affected by Cu, Co, Mn, and Zn supplementation compared with unsupplemented controls (Olson et al., 1999) or when calves were supplemented with organic Cu, inorganic Cu, or an organic Cu/Zn combination (Muehlenbein et al., 2001). In a study that evaluated the effect of Cu bolus administration before weaning, weaning weights were heavier in bull calves and tended to be heavier in heifer calves that received supplemental Cu compared with non-supplemented controls (Arthington et al., 1995). Studies have also reported that trace mineral source can impact calf performance. Stanton et al. (2000) reported increased preweaning ADG in calves that had access to a high level of supplemental Cu, Zn, and Mn in an organic form compared with calves receiving high or low levels of inorganic Cu, Zn, and Mn.

The effect of Cu on postweaning feedlot calf performance in the growing and finishing phases has been evaluated in several experiments. However, much like preweaning performance, postweaning performance has been shown to be positively affected, negatively affected, and also not affected by trace mineral supplementation. It has been

reported that Cu supplementation does not impact the performance of growing or finishing steers (Engle and Spears, 2000a, 2001). However other experiments have shown both positive and negative effects of Cu on feedlot performance during the finishing phase. Copper decreased feed intake, feed efficiency, and ADG when supplemented at 20 or 40 mg of Cu/kg of DM (Engle and Spears, 2000b). Conversely, Cu supplementation at 10 or 40 mg of Cu/kg of DM improved ADG and daily feed intake (Engle et al., 2000).

It seems that Cu, Zn, and Mn supplementation and source affect calf performance; however, based on the highly variable results of other studies, it is not known why this occurs. Further research into the effect of trace mineral supplementation and source on calf performance is needed.

IMPLICATIONS

Both supplementation (at concentrations recommended by the NRC) and source of copper, zinc, and manganese affected the concentration of these minerals in the liver and plasma of multiparous beef cows, although the effect of these differing body mineral concentrations on reproduction or calf performance is unclear. After 1 yr without supplemental copper, zinc, and manganese, pregnancy rate to a synchronized artificial insemination may be decreased, and season-long reproductive performance also may be affected. Additionally, trace mineral source may affect pregnancy rate to a synchronized artificial insemination, particularly if the insemination was administered based on an observed estrus. Finally, under certain conditions, kilograms of calf weaned per cow exposed may decrease due to trace mineral supplementation, less so with an organic than

inorganic source. However, these effects are not well understood and deserve further attention in future research.

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Table 2.1. Least squares means for initial body weight, body condition score, age, and liver mineral status of multiparous cows

Item	Treatments			SEM	Contrasts ($P <$)	
	Control	ORG[a]	ING[b]		Cont vs. Suppl.	ORG vs. ING
No. of cows	59	60	59	–	–	–
Age, yr	5.83	6.17	5.71	0.30	0.77	0.28
BW, kg	619.4	616.1	615.3	6.8	0.67	0.94
BCS[c]	5.91	5.97	5.97	0.06	0.51	0.99
Initial liver mineral status:						
Cu, mg/kg of DM	55.1	42.8	66.5	13.4	0.97	0.19
Zn, mg/kg of DM	99.7	98.2	99.2	4.6	0.88	0.90
Mn, mg/kg of DM	7.2	6.7	7.2	0.3	0.66	0.31

[a]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[b]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[c]Body condition score; 1=emaciated, 9=obese (Richards et al., 1986).

Table 2.2. Ingredient composition and laboratory analysis of the three trace mineral treatments

	Trace Mineral Treatments		
Item	Control	ORG[a]	ING[b]
Ca_2PO_4, %	52.0	52.0	52.0
NaCl, %	21.5	21.5	21.5
Dried distillers grain, %	15.6	15.6	15.6
MgO (52%), %	4.9	4.9	4.9
Soybean oil, %	4.0	4.0	4.0
Se (0.16%), %	1.9	1.9	1.9
Anise-fenugreek dry, %	0.11	0.11	0.11
EDDI (79.6%), %[c]	0.009	0.009	0.009
Chemical analyses:			
Ca, %	10.7	10.7	10.7
P, %	11.4	11.4	11.4
Cu, mg/kg of DM	6.2	1,038	1,087
Zn, mg/kg of DM	17.1	3,173	3,241
Mn, mg/kg of DM	15.2	2,921	2,895

[a]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[b]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[c]EDDI = Ethylenediamine dihydroiodide.

Table 2.3. Least squares means for liver mineral concentrations of cows supplemented with different trace mineral treatments[a]

Item	Treatments			SEM	Contrasts ($P <$)	
	Control	ORG[b]	ING[c]		Cont. vs. Suppl.	ORG vs. ING
Cu, mg/kg of DM						
d –82 yr 1[d]	55.1	42.8	66.5	13.4	0.97	0.19
d +110 yr 1	50.3	150.6	97.3	12.9	0.01	0.01
d –81 yr 2	62.8	105.4	107.8	11.2	0.01	0.88
d +135 yr 2	43.7	156.1	141.8	10.9	0.01	0.39
Zn, mg/kg of DM						
d –82 yr 1[d]	99.7	98.2	99.2	4.6	0.88	0.90
d +110 yr 1	89.1	106.3	105.3	4.4	0.05	0.87
d –81 yr 2	90.6	87.3	130.5	15.6	0.32	0.07
d +135 yr 2	91.5	85.0	93.9	3.2	0.57	0.07
Mn, mg/kg of DM						
d –82 yr 1[d]	7.2	6.7	7.2	0.3	0.66	0.31
d +110 yr 1	8.0	8.9	9.2	0.3	0.01	0.52
d –81 yr 2	6.7	6.6	6.4	0.3	0.46	0.60
d +135 yr 2	9.5	8.9	8.3	0.3	0.02	0.25

[a]Day is relative to average calving date (d 0) of the cow herd.
[b]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[c]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[d]Initial (d –82) liver values indicate concentrations measured before supplementation, which began on January 17, 2001. Day is relative to average calving date (d 0) of the cowherd.

Table 2.4. Reproductive performance of cows supplemented with different trace mineral treatments[a]

Item	Treatments			Contrasts ($P <$)	
	Control	ORG[b]	ING[c]	Cont. vs. Suppl.	ORG vs. ING
Estrous cyclicity[d]	26% (11/43)[i]	28% (13/46)	23% (11/47)	0.95	0.61
Estrus observed within 72 h of $PGF_{2\alpha}$	83% (80/96)	77% (78/101)	77% (79/103)	0.19	0.93
Pregnancy rate to AI (yr 1)[e]	65% (34/52)	67% (36/54)	52% (29/56)	0.47	0.08
Pregnancy rate to AI (yr 2)[e]	34% (15/44)	57% (26/46)	58% (25/43)	0.02	0.80
Pregnancy rate to AI if observed in estrus[f]	58% (46/80)	77% (59/77)	65% (49/75)	0.04	0.13
Pregnancy rate to AI if mass inseminated[g]	19% (3/16)	13% (3/23)	21% (5/24)	0.51	0.66
Overall pregnancy rate after 60-d season[h]	89% (85/96)	93% (94/101)	95% (98/103)	0.10	0.54

[a]Values reported are raw percentages, with raw ratios in parentheses.
[b]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[c]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[d]Percentage of estrous cyclicity at the beginning of the breeding season was evaluated in Year 2 only via the collection of two blood samples at 10-d intervals. Cows were identified as "cycling" if at least one blood sample had a serum progesterone concentration greater than 1.0 ng/mL, indicating the presence of a functional corpus luteum.
[e]There was a year × treatment interaction ($P < 0.02$) for pregnancy to AI, so data could not be pooled across years. For each year, the value includes all cows in the study because all cows were inseminated once (either based on an observation of estrus within 72 h after $PGF_{2\alpha}$ administration or via mass-insemination at 72 h after $PGF_{2\alpha}$ administration).
[f]Values reported include only cows observed in estrus within 72 h of $PGF_{2\alpha}$ administration and inseminated based solely on this observation of behavioral estrus.
[g]Values reported include only cows never observed in estrus and subsequently mass-inseminated at 72 h post $PGF_{2\alpha}$.
[h]Overall 60-d pregnancy rate includes data from both Years 1 and 2.
[i]Number of animals observed/number of animals evaluated.

Table 2.5. Performance of calves from multiparous cows supplemented with different trace mineral treatments for Years 1 and 2

Item	Treatments			SEM	Contrasts ($P <$)	
	Control	ORG[a]	ING[b]		Cont vs. Suppl.	ORG vs. ING
Year 1						
Actual weaning wt., kg[c,d]	212.7	200.0	209.3	3.0	0.04	0.01
205-d adjusted weaning weight, kg[c,e]	266.5	247.7	262.9	4.2	0.04	0.01
Exposed cows that weaned a calf, %[f]	94.9%	93.4%	95.3%	0.7	0.49	0.29
Kilograms of calf weaned per cow exposed[c,h]	200.3	184.7	198.8	2.9	0.02	0.01
Year 2						
Actual weaning wt., kg[c,d]	197.1	184.3	178.6	9.3	0.01	0.19
205-d adjusted weaning weight, kg[c,e]	239.2	224.8	217.6	4.6	0.01	0.28
Exposed cows that weaned a calf, %[g]	78.0%	81.0%	79.0%	3.8	0.34	0.92
Kilograms of calf weaned per cow exposed[c,h]	151.1	148.4	139.2	2.8	0.02	0.09

[a]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[b]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[c]Least squares means.
[d]Actual (unadjusted) weaning weight taken at weaning on October 2, 2001 (Year 1) and September 6, 2002 (Year 2), with age of dam, sex of calf, and age of calf included in the model statement.
[e]Weaning weight adjusted to 205 d of age using Beef Improvement Federation adjustments for age of dam and sex of calf.
[f]Percentage of cows pregnant at the start of the experiment that weaned a calf on October 2, 2001.
[g]Percentage of cows pregnant at Year 1 final pregnancy check and weaned a calf on September 6, 2002.
[h]Based on the actual (unadjusted) weaning weight taken on October 2, 2001 (Year 1) and September 6, 2002 (Year 2) and the "exposed cows that weaned a calf" percent.

CHAPTER III

EFFECT OF COPPER, ZINC, AND MANGANESE SOURCE ON MINERAL STATUS, REPRODUCTIVE PERFORMANCE, IMMUNE RESPONSE, AND CALF PERFORMANCE IN YOUNG GRAZING BEEF FEMALES OVER A TWO-YEAR PERIOD

ABSTRACT

Crossbred beef females (n = 43 nulliparous heifers, Year 1; n = 37 primiparous cows, Year 2) were used to evaluate the effects of copper (Cu), zinc (Zn), and manganese (Mn) source on mineral status, reproductive performance, immune response and calf performance in young grazing cattle in Eastern Colorado over a 2-yr period. Pregnant nulliparous heifers in the last trimester of gestation were stratified by expected calving date, BW, BCS, and liver mineral status and assigned to treatments. Treatments included: 1) organic (ORG; 50% organic and 50% inorganic Cu, Zn, and Mn); and 2) inorganic (ING; 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$) trace minerals. Treatments were supplemented as free-choice trace minerals in mineral feeders and were formulated to provide current NRC recommended concentrations at a targeted consumption rate of 115 $g \cdot head^{-1} \cdot d^{-1}$ via ad libitum access from 54 (Year 1) and 81 d (Year 2) prior to the average calving date through 119 (Year 1) and 135 d (Year 2) post-calving. There were

no treatment × yr interactions ($P > 0.10$) for any of the responses evaluated. Final liver Cu concentrations were greater ($P < 0.05$) and final liver Mn concentrations tended to be greater ($P < 0.06$) in ORG vs. ING females. Plasma Cu and Zn and liver Zn concentrations were similar across treatments ($P > 0.10$). Estrous cyclicity tended ($P < 0.08$) to be greater in ORG vs. ING females, yet rates of behavioral estrus and pregnancy to a synchronized AI were similar ($P > 0.10$) across treatments. At the end of the 60-d breeding season, there was a tendency ($P < 0.07$) for higher pregnancy rate in ING vs. ORG females. Primary humoral immune response was measured in Year 1 only by injecting (i.m.) a 20% porcine red blood cell (PRBC) solution into pregnant heifers and collecting blood samples immediately pre-injection (d 0) and on d 7, 14, and 21 post-injection. Concentrations of immunoglobulin (Ig) M antibody titers specific for PRBC were similar across treatments; however, IgG titers specific for PRBC were greater ($P < 0.03$) in ORG than ING heifers on d 14, and total Ig titers specific for PRBC were greater ($P < 0.05$) in ORG than ING heifers on d 21. Kilograms of calf weaned per female exposed were not affected ($P > 0.10$) by trace mineral source. These results indicate that ORG trace minerals can improve liver Cu and Mn status and humoral immune response; however, plasma Cu or Zn concentrations, liver Mn concentration, and calf performance were not affected by trace mineral source. Additionally, the effects of trace mineral source on reproductive parameters are variable.

INTRODUCTION

Source (organic vs. inorganic) of trace minerals such as copper (**Cu**), zinc (**Zn**), and manganese (**Mn**), has been shown to affect the performance of grazing beef cattle.

Improved pregnancy rate to AI (Stanton et al., 2000), reduced postpartum interval to breeding (Swenson et al., 1998), and enhanced repair of uterine tissue following parturition (Manspeaker et al., 1987) have been associated with organic trace mineral supplementation. When organic trace minerals were supplemented to dams, suckling calves had enhanced performance (Stanton et al., 2000; Kropp, 1990) and immune response (Ward and Spears, 1999; George et al., 1997) compared to calves whose dams received inorganic trace minerals. Other experiments, however, showed no effect of trace mineral source on reproductive performance (Olson et al., 1999) or calf performance (Muehlenbein et al., 2001; Olson et al., 1999).

Due to reports of enhanced cattle performance and increased bioavailability associated with organic trace minerals, some cow/calf producers have begun to include organic trace minerals into grazing cow and calf mineral supplements (Spears, 1996). Comparisons of inorganic and organic trace mineral supplements on grazing cattle performance at NRC (1996) recommended concentrations for more than one year are limited, particularly in young cows. Therefore, the objectives of this study were to determine the effects of Cu, Zn and Mn source (at current NRC recommendations) on mineral status, reproductive performance, immune response, and calf performance over a two-year period in nulli- (Year 1) and primiparous (Year 2) grazing beef females.

MATERIALS AND METHODS

Prior to the initiation of this experiment, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee.

Experimental Design. Over a two-year period, 80 crossbred (Red Angus-based) beef females (n = 43 nulliparous heifers, Year 1; n = 37 primiparous cows, Year 2) at the Eastern Colorado Research Center (Akron, CO) were utilized for this experiment. Beginning 12 d prior to the initiation of the experiment in Year 1 (January 2001), 43 pregnant nulliparous heifers in the last trimester of gestation (22 mo, 474.1 ± 6.3 kg, BCS 5.7 ± 0.08) were stratified based on expected calving date, BW, BCS, and liver mineral status and randomly assigned to one of six replicates (Table 3.1). Replicates were then assigned to one of two trace mineral treatments (n = 6 to 8 heifers per replicate). Treatments consisted of: 1) organic (**ORG**; 50% organic and 50% inorganic Cu, Zn, and Mn; n = 21), and 2) inorganic (**ING**; 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$; n = 22) trace minerals.

All procedures described below were repeated over two consecutive years, except where noted. All females remained on the same treatment for both years. Inorganic trace minerals were supplemented as $CuSO_4$, $ZnSO_4$, and $MnSO_4$, whereas organic trace minerals were provided from a commercially available mineral proteinate source (Bioplex™ trace minerals Alltech Inc., Nicholasville, KY). Salt (NaCl) was added to the supplements to limit consumption of Cu, Zn, and Mn to approximately NRC (1996) recommended concentrations. Vitamins A, D, and E were added in order to meet NRC (1996) recommendations, and were mixed thoroughly by hand at the time of mineral delivery to free-choice trace mineral feeders. Ingredient composition and laboratory analysis of the trace mineral treatments are listed in Table 3.2. Basal forage and water trace mineral concentrations were determined using samples collected from pasture (freshly consumed forage was collected via two fisulated mature cows; samples were

collected after rumens were evacuated and cows were allowed to graze for approximately 30 min), stored hay, and water sources. Trace mineral concentrations were as follows: pasture = 13.1 ppm Cu, 16.1 ppm Zn, and 36.6 ppm Mn; stored hay = 19.6 ppm Cu, 32.1 ppm Zn, and 52.2 ppm Mn; and water < 0.01 ppm Cu, <0.01 ppm Zn, and 0.08 ppm Mn.

After replicates and treatments were assigned, animals were housed by replicate in six separate native pastures consisting primarily of blue grama (*Bouteloua gracilis*), prairie sandreed (*Calamovilfa longifolia*), and needle and thread grass (*Stipa comata*). Early in Year 1 of the experiment (December through March) supplemental millet hay and range cubes were provided to compensate for poor winter forage quality. Estimated intakes of millet hay and range cubes were similar across treatments. Forage and range cube supplementation were discontinued as range quality increased during early spring.

Mineral treatments were provided at a single location in each pasture in free-choice mineral feeders beginning 54 (d –54; Year 1) and 81 d (d –81; Year 2) before the average calving date (d 0) of the females. Mineral treatments remained available for ad libitum consumption for 119 (d +119; Year 1) and 135 d (d +135; Year 2) after the average calving date. Mineral treatments were also made available exclusively to the calves via creep feeders in each pasture when calves within the pasture averaged 98 (Year 1) and 111 d (Year 2) of age. All calves received the same respective trace mineral treatment as their dam. In Year 1, on d +119 after the average calving date, treatments to cows were discontinued, and all cows within a treatment were combined and given access to a basal trace mineral supplement that did not contain any supplemental Cu, Zn, or Mn for a period of 160 d. Calves continued to have access to their respective mineral treatments via creep feeders until weaning at an average age of 185 (Year 1) and 176 d (Year 2). On

d –81 in Year 2, cows were sorted back into the same respective treatment groups as in Year 1, assigned to new replicates, and maintained on treatments until d +135 of Year 2. At the beginning of Year 2, there were six fewer females in the experiment (n = 37) compared to the beginning of Year 1 (n = 43) because some females were culled from the herd and removed from the experiment because they were not pregnant at the final pregnancy diagnosis at the end of Year 1 or for other reasons unrelated to this experiment. Of the six females removed, four were from the ORG treatment and two were from the ING treatment.

During times of supplementation in both years, replicates were rotated among pastures every 28 d in order to minimize pasture effects. In conjunction with pasture rotations, mineral weigh-backs were performed at each rotation in order to calculate mineral disappearance. Replicates were pooled, but remained within treatments, for approximately 30 d during the time of calving and 60 d during the time of breeding to allow for more intensive cattle management. Trace mineral treatments continued to be available during these times, although mineral disappearance within replicates was not monitored.

Mineral Status. To determine trace mineral status of females, a liver biopsy was collected from every heifer before the start of the experiment in Year 1 and then from a subgroup of animals (five per replicate) at the end of the supplementation period in Year 1 and at the beginning and end of the supplementation period in Year 2. This was accomplished using the true-cut technique described by Pearson and Craig (1980), as modified by Engle and Spears (2000). Immediately post-collection, samples were rinsed with 0.01 M PBS (pH 7.4), placed into acid washed polypropylene tubes, capped, placed

on ice for approximately 5 h, transported back to the laboratory, and stored at –20°C. Liver samples were analyzed for concentrations of Cu, Zn, and Mn as described by Engle et al. (1997).

Blood was collected via jugular venipuncture into heparinized, trace mineral-free vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) at the beginning, midpoint, and end of the trace mineral supplementation period from five animals per replicate (the same animals from which liver biopsies were obtained). Once collected, samples were placed on ice for approximately 5 h and transported back to the laboratory. Blood samples were then centrifuged at 2000 × *g* for 15 min at room temperature, and plasma was transferred to acid-washed storage vials and frozen at –20°C. Plasma Cu and Zn concentrations were determined after samples thawed at room temperature for approximately 3 h, as described by Ahola et al. (2004).

Cow and Calf Performance. Cow performance was monitored by collection of BW and BCS data at approximately 56-d intervals throughout the supplementation period in each year. The BCS data collected were on a scale of 1 to 9 (1 = emaciated, 9 = obese; Richards et al., 1986) and were assigned by the same technician throughout the experiment.

To determine the effects of trace mineral source on reproductive performance, every female was artificially inseminated once following a modified Select-Synch (Geary et al., 2000) estrous synchronization protocol. Beginning on d +83 (Year 1) and d +69 (Year 2) after the average calving date, all females were injected with 100 μg of GnRH (Fertagyl, Intervet, Millsboro, DE; i.m.), and 7 d later injected with 25 mg of $PGF_{2\alpha}$ (Lutalyse, Pharmacia and UpJohn Kalamazoo, MI; i.m.). Calves were removed for 72 h from all

females at the time of $PGF_{2\alpha}$ administration in an effort to increase estrous response (Williams, 1990). For management reasons, all females were pooled and treatments were withheld during the 72-h AI period. Cattle were observed for signs of behavioral estrus from 12 h prior to $PGF_{2\alpha}$ injection through 72 h after $PGF_{2\alpha}$ injection. Detection of estrus was accomplished by visual observation for a minimum of 1 h at dawn, noon, and dusk. Animals observed in behavioral estrus were inseminated approximately 12 h after first detected in estrus. At 72 h post $PGF_{2\alpha}$, all females that had not been previously observed in estrus were given a second injection of GnRH (100 μg; i.m.) and were mass inseminated. Calves were returned to their dams immediately following AI, and all cattle were sorted back into treatments and returned to the appropriate pastures after mass insemination.

To minimize variation in reproductive measurements, an effort was made to decrease confounding variables. Two AI technicians performed the inseminations, equally represented within each replicate, and one technician thawed semen. Semen from one proven purebred Charolais sire that had been acquired from a single collection was used. To allow for the accurate differentiation between pregnancy to AI vs. pregnancy to natural service, females were not exposed to bulls until 14 d after mass insemination. Four composite (Red Angus-based) bulls that had previously passed a breeding soundness evaluation were exposed to the females for 46 d (two bulls per treatment).

In Year 2 only, immediately before the initiation of the synchronization protocol, two blood samples were collected via jugular venipuncture at 10-d intervals, and progesterone concentrations were determined to identify cows that had cycled at least once since parturition. Cows were identified as cycling if at least one blood sample had a serum

progesterone concentration greater than 1.0 ng/mL, indicating the presence of a functional corpus luteum. Serum was harvested and stored at –20°C until samples were evaluated in duplicate for progesterone concentration by solid-phase RIA (Coat-a-Count kit; Diagnostic Products Corp., Los Angeles, CA), as described by Bellows et al. (1991). The intra- and interassay CV for the two assays were 5.9 and 2.2%, respectively. The sensitivity of the assay was 0.04 ng/mL of serum.

To determine pregnancy rate to AI, cattle were examined via rectal ultrasonography (Aloka 500V equipped with a 5.0-MHz linear array transducer, Corometrics Medical Systems, Wallingford, CT) by a state-licensed veterinarian 40 d after the mass insemination. Cows with fetuses that were approximately 40 d of age were classified as pregnant to AI, whereas all other cows were classified as not pregnant to AI. Final pregnancy rates following the 60-d breeding season were determined via palpation per rectum with the aid of ultrasonography approximately 40 d after bull removal.

Calf performance was measured in both years based on kilograms of calf weaned per female exposed. A weaning weight was collected on each calf before shipment to the feedlot. At the end of Years 1 and 2, calves averaged 185 and 176 d of age, respectively.

Immune Response. In Year 1 only, primary humoral immune response was evaluated in the pregnant nulliparous heifers using porcine red blood cells (PRBC; Droke et al., 1993; Ferket and Qureshi, 1992). A subgroup of heifers (five per replicate, different from the liver biopsy subgroup) were randomly selected and injected (i.m.) in the neck with 5 mL of a 20% PRBC solution (Sigma-Aldrich, St. Louis, MO) on d 21 prior to the average calving date. On the day prior to injection, whole pig blood was collected into filter-sterilized Alsever solution (1:1 ratio blood:Alsever solution; Kuhlman et al., 1988)

and PRBC were washed three times in 0.01 M autoclaved PBS (pH 7.4) and re-suspended in PBS prior to injection. Blood was collected from heifers via jugular venipuncture into non-heparinized vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) from injected animals on d 0 (immediately prior to PRBC injection) and d 7, 14 and 21 post-PRBC injection. Samples were stored on ice for approximately 2 h prior to being centrifuged at 2,000 × *g* for 15 min at room temperature. Serum was harvested and stored in polyethylene tubes at –80°C until analyzed. Samples were thawed, and antibody titers to PRBC were measured using a microtiter hemagglutination assay (Ferket and Qureshi, 1992). Concentrations of total immunoglobulin (**Ig**), immunoglobulin G (**IgG**), and immunoglobulin M (**IgM**) specific for PRBC were determined.

Statistical Analysis. Mineral status (liver Cu, Zn, and Mn concentrations and plasma Cu and Zn concentrations), cow performance (change in BW and BCS), calf performance, and immune response data were assessed using a restricted maximum likelihood-based, mixed-effects model, repeated measures analysis (PROC MIXED, SAS Inst. Inc., Cary, NC). Initial models for mineral status and female performance contained the fixed effects of treatment, time, and a treatment × time interaction. Initial models for calf performance included the fixed effects of treatment, yr, age of dam, age of calf, sex of calf, and all relevant two- and three-way interactions. A spatial power covariance structure was used in the analysis and the containment approximation was used to calculate denominator degrees of freedom. Pasture was used as the experimental unit. Reproductive response data (including estrous cyclicity, estrous response, pregnancy to a synchronized AI, and final pregnancy throughout the 60-d breeding season) were analyzed using logistic regression (PROC GENMOD; SAS Inst. Inc., Cary, NC). Initial

models for reproductive response contained the fixed effects of treatment, postpartum interval, BCS, BW, AI technician, and year in addition to all relevant two- and three-way interactions. When an interaction was not significant, it was removed from the model. If a yr × treatment interaction was not significant, data were pooled across years.

RESULTS AND DISCUSSION

No treatment × yr interaction was present ($P = 0.45$) for mineral disappearance for the nulli- and primiparous females. In Year 1, average mineral disappearance for the females was 0.146 ± 0.028 kg·female^{-1}·d^{-1} and 0.164 ± 0.028 kg·female^{-1}·d^{-1} for the ORG and ING treatments, respectively. Average mineral disappearance during Year 2 was 0.132 ± 0.016 kg· female^{-1}·d^{-1} and 0.116 ± 0.016 kg· female^{-1}·d^{-1} for the ORG and ING treatments, respectively. Throughout the experiment, mineral disappearance was similar ($P > 0.10$) across treatments. In both years the amount of daily mineral disappearance indicated that females consumed enough Cu, Zn, and Mn to meet NRC (1996) recommended concentrations (estimated daily DMI was 10.9 kg). Since forage basal trace mineral concentrations were low relative to NRC (1996) recommendations, any differences in overall performance across treatments were not attributed to trace minerals available from the forage.

Plasma concentrations of Cu and Zn in Year 1 were similar ($P > 0.10$) for both treatments at the mid-point and end of supplementation in Year 1 (Appendix A). No treatment × yr interactions were present for liver concentrations of Cu ($P = 0.87$), Zn ($P = 0.52$), or Mn ($P = 0.40$). Initial liver Cu, Zn, and Mn concentrations were similar ($P > 0.10$) across trace mineral treatments throughout the two-year experiment (Table 3.3);

however, final liver Cu concentrations were greater ($P < 0.05$) and liver Mn concentration tended ($P < 0.06$) to be greater in ORG than ING females. Additionally, liver trace mineral concentrations increased over the course of the experiment, most likely due to supplementation. In the current experiment, plasma concentrations of Cu and Zn and liver concentrations of Cu, Zn, and Mn in the females were maintained above levels considered deficient (Puls, 1994; Mills, 1987).

In agreement with the current experiment, in a 209-d experiment with 300 grazing Angus cows in southern Wyoming, Stanton et al. (2000) reported that liver Cu and Mn concentrations increased numerically when Cu, Zn, Mn, and cobalt (**Co**) were supplemented at high levels in either the organic or inorganic form, which consisted of concentrations 2.1, 1.44, 1.44, and 10 times higher than the low trace mineral treatment (for Cu, Zn, Mn, and Co, respectively). However, in contrast, the authors also reported that liver Cu, Zn, and Mn concentrations were not affected by source at either the mid-point or end of the supplementation period. Similarly, when 641 older spring-calving cows and their calves grazing in western Nebraska received either organic, inorganic (at twice the concentration of organic), or no trace minerals (Cu, Zn, Mn, and Co) from June through September, liver Cu and Zn concentrations were not impacted by trace mineral source (Grotelueschen et al., 2001). The authors also reported that liver Mn concentrations were greater in cows receiving inorganic trace minerals compared to organic trace minerals. Olson et al. (1999) reported no effect of Cu, Zn, or Mn source on liver or serum concentrations when both liver and serum trace mineral concentrations were evaluated in young cows receiving organic, inorganic, or no Cu, Zn, Mn, and Co at twice NRC (1996) recommendations over two consecutive years. The impact of trace

mineral source on final Cu and Mn concentrations observed in the current experiment may have been due to the length of the current experiment (two years), which was longer than both of the experiments discussed above.

A time effect was present for both BW ($P < 0.01$) and BCS ($P < 0.01$), since both variables declined during late spring/early summer (due to parturition and early lactation) and returned to initial levels by mid-summer in both years. However, no treatment × time interactions were present for either BW ($P = 0.31$) or BCS ($P = 0.20$). Trace mineral source did not impact mean BW ($P = 0.63$) or BCS ($P = 0.57$) throughout the experiment (Appendix A).

All data relative to reproductive performance of females are reported in Table 3.4. No treatment × yr interactions were present ($P > 0.10$) for any of the reproduction variables evaluated. Estrous cyclicity, as measured by the presence of one blood progesterone concentration above 1 ng/mL (in Year 2 only), tended ($P < 0.08$) to be greater in ORG than ING cows. However, the rate of behavioral estrus within 72 h of $PGF_{2\alpha}$ was not affected ($P = 0.84$) by trace mineral source. Additionally, the overall percent of females cycling based on progesterone concentration appeared to be low (only 2 of 35 females cycling), yet the percent that responded to the estrous synchronization protocol and displayed behavioral estrus appeared high. The reason for this apparent discrepancy is not known.

Trace mineral source had no impact ($P = 0.34$) on pregnancy rate to AI. Furthermore, trace mineral source did not impact pregnancy rate when the insemination was based on an observed estrus ($P = 0.45$) or simply via mass-insemination 72 h after $PGF_{2\alpha}$ administration ($P = 0.19$). However, overall pregnancy rate at the end of the 60-d

breeding season tended ($P < 0.07$) to be greater in ING females than ORG females. Interpretation of the reproductive data is difficult due to the fact that all data used to characterize reproduction were categorical, and low numbers of cattle were evaluated, even when data from two consecutive years was incorporated into one experiment.

These data support previous research by Olson et al. (1999), where no effect of trace mineral source on pregnancy rate was observed in two-year old beef cows, although cows receiving inorganic trace minerals conceived earlier than cows receiving organic trace minerals in the first year of the two-year experiment. In contrast to the results of the current experiment, Stanton et al. (2000) reported a greater pregnancy rate to mass-insemination in cows supplemented with organic trace minerals than cows receiving the inorganic form; however, pregnancy rate at the end of the 60-d breeding season was not affected by trace mineral source. However, based on liver Cu concentrations reported by Stanton et al. (2000), cows appeared to be deficient in Cu compared to cattle in the current experiment. Females in the current experiment maintained liver Cu concentrations within the range considered to be adequate for normal physiological function (Puls, 1994; Mills, 1987).

Trace mineral source affected primary humoral immune response specific for PRBC in pregnant heifers in Year 1 (Table 3.5). Following PRBC injection, total Ig concentrations in ORG heifers tended ($P < 0.10$) to be greater on d 14 and were greater ($P < 0.05$) on d 21 than ING heifers. Similarly, IgG concentrations were greater ($P < 0.03$) on d 14 and tended to be greater ($P < 0.11$) on d 21 after PRBC injection in ORG vs. ING heifers. These findings support research previously reported by George et al. (1997) in feedlot heifers where IBRV antibody titer response against a modified live

vaccine was enhanced in heifers at d 14 and 28 after injection when heifers received supplemental Cu, Zn, Mn and Co at NRC (1984) recommended concentrations in an organic form compared to an inorganic form during a 42-d growing phase.

No treatment × yr interaction was present ($P = 0.67$) for mineral disappearance from calf creep feeders; however, there was a yr effect on mineral disappearance ($P < 0.05$). In Year 1, creep feeder mineral disappearance was 0.023 ± 0.008 kg·calf^{-1}·d^{-1} and 0.023 ± 0.008 kg·calf^{-1}·d^{-1} for the ORG and ING treatments, respectively. In Year 2 mineral disappearance was 0.048 ± 0.005 kg·calf^{-1}·d^{-1} and 0.042 ± 0.005 kg·calf^{-1}·d^{-1} for the ORG and ING treatments, respectively. It is unclear why mineral disappearance from creep feeders was greater in Year 2.

No treatment × yr interactions were present ($P > 0.10$) for any of the calf performance variables evaluated. Calf performance, as analyzed by kg of calf weaned per female exposed, was not affected ($P > 0.10$) by trace mineral source (Table 3.6). Kilogram of calf weaned per female exposed did not differ ($P > 0.10$) across treatments.

These results are similar to those reported by Grotelueschen et al. (2001) and Olson et al. (1999). In contrast, however, other studies have reported that calves from cows receiving organic trace minerals had greater pre-weaning ADG (Stanton et al., 2000) and greater calf weaning weights when cows received supplemental Cu, Zn, magnesium, Mn, and potassium in an organic form (Kropp, 1990) compared to calves from cows receiving inorganic trace minerals. It is not known why the effects of trace mineral source on pre-weaning calf performance are so variable. Different dietary trace mineral antagonists, differences in trace mineral metabolism or immune response between breeds, and(or) environmental differences may contribute to the variation observed in the literature

pertaining to the impact of different trace mineral sources on performance and immune response in cattle.

IMPLICATIONS

Source of copper, zinc, and manganese did not affect plasma copper or zinc concentrations; however, young grazing females receiving organic trace minerals had Greater final liver copper concentrations and tended to have greater final liver manganese concentrations than inorganic supplemented females, indicating a potential difference in the availability and(or) metabolism of trace mineral sources. Pregnancy rate to a synchronized AI was not affected by trace mineral source; however, estrous cyclicity tended to be higher in organic supplemented females, and season-long reproductive performance tended to be greater in females receiving inorganic trace minerals. The reproductive data were not very informative since data were categorical, and low numbers of cattle were evaluated. Primary humoral immune response to a foreign antigen was higher in young grazing females receiving organic versus inorganic trace minerals. Although morbidity was extremely low in this experiment, an enhanced ability to respond to a foreign antigen may be beneficial if animals encounter a stressor or are exposed to disease. Calf performance, as measured by kilograms of calf weaned per female exposed, was not affected by trace mineral source.

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Table 3.1. Initial age, body weight, and body condition score of pregnant nulliparous heifers at the initiation of the experiment

Item	Treatments		SEM	($P <$)
	ORG[a]	ING[b]		
No. of nulliparous heifers	21	22	---	---
Age, mo	22	22	---	---
BW, kg[c]	475.7	475.0	8.9	0.80
BCS[c,d]	5.73	5.80	0.11	0.66

[a]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[b]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[c]Least squares means.
[d]BCS: 1 = emaciated, 9 = obese (Richards et al., 1986).

Table 3.2. Ingredient composition and laboratory analysis of the trace mineral treatments

Item	Treatments	
	ORG[a]	ING[b]
Ca_2PO_4, %	52.0	52.0
NaCl, %	21.5	21.5
Dried distillers grain, %	15.6	15.6
MgO (52%), %	4.9	4.9
Soybean oil, %	4.0	4.0
Se (0.16%), %	1.9	1.9
Anise-fenugreek (dry), %	0.11	0.11
EDDI (79.6%), %[c]	0.009	0.009
Chemical analyses:		
Ca, %	10.7	10.7
P, %	11.4	11.4
Cu, mg/kg of DM	1,038	1,087
Zn, mg/kg of DM	3,173	3,241
Mn, mg/kg of DM	2,921	2,895

[a]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[b]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[c]EDDI = Ethylenediamine dihydroiodide.

Table 3.3. Least squares means for liver trace mineral concentrations in beef females[a]

Item	Treatments		SEM	($P <$)
	ORG[b]	ING[c]		
Cu, mg/kg of DM				
Initial[d]	80.2	65.6	17.15	0.57
Final[d]	188.0	130.6	17.15	0.05
Zn, mg/kg of DM				
Initial	88.5	87.4	6.18	0.91
Final	90.9	101.7	5.90	0.22
Mn, mg/kg of DM				
Initial	7.13	6.77	0.33	0.45
Final	8.58	7.60	0.33	0.06

[a]Due to the absence of a yr × treatment interaction ($P > 0.10$), data from Year 1 and Year 2 were combined.
[b]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[c]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[d]Initial (d –54, Year 1; d –81, Year 2) and final (d +119, Year 1; d +135, Year 2) liver values indicate concentrations determined from samples collected prior to, and at the end of, the supplementation period in each year, respectively.

Table 3.4. Reproductive performance of beef females supplemented with different trace mineral sources[a,b]

Item	Treatments		
	ORG[c]	ING[d]	($P <$)
Estrous cyclicity[e]	12.5% (2/16)[j]	0% (0/19)	0.08
Estrus observed within 72 h of $PGF_{2\alpha}$ administration	78.9% (30/38)	78.6% (33/42)	0.84
Pregnancy rate to AI[f]	62.2 % (23/37)	50% (21/42)	0.34
Pregnancy rate to AI if observed in estrus[g]	72.4% (21/29)	60.6% (20/33)	0.45
Pregnancy rate to AI if mass inseminated[h]	25.0% (2/8)	11.1% (1/9)	0.20
Overall pregnancy rate after 60-d season[i]	86.5% (32/37)	97.6% (41/42)	0.07

[a]Values are reported as percentages with raw ratios in brackets.
[b]Values reported include data from both Year 1 and Year 2 with the exception of 'estrous cyclicity', which was only evaluated in Year 2.
[c]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[d]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[e]Percent estrous cyclicity at the beginning of the breeding season was evaluated only in Year 2 via the collection of two blood samples at 10-d intervals. Cows were identified as "cycling" if at least one blood sample had a serum progesterone concentration greater than 1.0 ng/ml, indicating the presence of a functional corpus luteum.
[f]Values reported include all females in the experiment since all females were inseminated once (either based on an observation of estrus within 72 h of $PGF_{2\alpha}$ administration or via mass insemination at 72 h after $PGF_{2\alpha}$ administration).
[g]Values reported only include females observed in estrus within 72 h of $PGF_{2\alpha}$ administration and inseminated approximately 12 h later based solely on this observation of behavioral estrus.
[h]Values reported only include females never observed in estrus and subsequently mass-inseminated at 72 h after $PGF_{2\alpha}$.
[i]Overall 60-d pregnancy rate includes pregnancies from both AI and natural service.
[j]Number of animals observed/number of animals evaluated.

Table 3.5. Least squares means for antibody titer response specific for porcine red blood cells in pregnant heifers[a]

Item	Treatments		SEM	($P <$)
	ORG[b]	ING[c]		
Total Ig antibody titer concentration, log_2				
d 0	0.0[d]	0.0[d]	---	---
d 7	6.68	6.38	0.274	0.46
d 14	6.14	5.46	0.274	0.10
d 21	4.32	3.48	0.274	0.05
IgM antibody titer concentration, log_2[e]				
d 0	0.0[d]	0.0[d]	---	---
d 7	4.99	4.92	0.254	0.86
d 14	4.53	4.70	0.254	0.64
d 21	3.24	2.98	0.254	0.48
IgG antibody titer concentration, log_2[f]				
d 0	0.0[d]	0.0[d]	---	---
d 7	1.69	1.46	0.229	0.49
d 14	1.62	0.76	0.229	0.03
d 21	1.07	0.50	0.229	0.11

[a]Primiparous heifers were injected with a 20% porcine red blood cell solution intramuscularly 21 d prior to their average calving date, and blood samples were collected on d 0 (prior to injection), 7, 14 and 21 post injection to determine primary humoral immune response specific for this antigen.

[b]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.

[c]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.

[d]Total, IgM, and IgG antibody titer concentrations were undetectable at d 0.

[e]IgM = immunoglobulin M.

[f]IgG = immunoglobulin G.

Table 3.6. Performance of calves from beef females supplemented with different trace mineral treatments

Item	Treatments		SEM	($P <$)
	ORG[a]	ING[b]		
Actual weaning weight, kg[c,d]	190.1	183.1	3.61	0.28
205 d adjusted weaning weight, kg[c,e]	208.6	207.6	3.18	0.85
Exposed females that weaned a calf, %[f]	85.4%	88.2%	7.43	0.80
Kilograms of calf weaned per female exposed, kg[c,g]	159.6	165.1	13.81	0.79

[a]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[b]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[c]Least squares means.
[d]Mean actual (unadjusted) weaning weight taken at weaning time each year, with age of dam, sex of calf, and age of calf included in the model statement.
[e]Actual weaning weight adjusted to 205 d of age using the Beef Improvement Federation standard adjustment factors for age of dam and sex of calf.
[f]Percentage of females pregnant at the start of the experiment (Year 1) and exposed for breeding by AI and natural service (Year 2) that also weaned a calf that same year.
[g]Based on the actual (unadjusted) weaning weight taken at weaning in each year and the 'exposed females that weaned a calf' percent.

CHAPTER IV

EFFECT OF LIFETIME COPPER, ZINC, AND MANGANESE SUPPLEMENTATION AND SOURCE ON PERFORMANCE, MINERAL STATUS, IMMUNITY, AND CARCASS CHARACTERISTICS OF FEEDLOT CATTLE

ABSTRACT

Two consecutive calf crops consisting of 170 steers (n = 90, Year 1; n = 80, Year 2) and 100 heifers (n = 50, Year 1; n = 50, Year 2) from the same cowherd were used to evaluate the effects of lifetime trace mineral [copper (Cu), zinc (Zn), and manganese (Mn)] supplementation and source on performance, mineral status, immunity, and carcass traits of feedlot cattle. Calves' dams were supplemented with one of three free-choice trace mineral treatments from approximately 80 d pre-calving through 120 d post-calving. Treatments consisted of: 1) Control (no supplemental Cu, Zn, or Mn); 2) Organic (ORG; 50% organic and 50% inorganic Cu, Zn, and Mn); or 3) Inorganic (ING; 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$) trace minerals. The ORG and ING free-choice trace mineral treatments were formulated to supply 10 ppm Cu, 30 ppm Zn, and 40 ppm Mn. Calves were also given exclusive access to the same trace mineral treatments as their dams via creep feeders from approximately 95 d of age until weaning. Upon arrival to

the feedlot, calves were maintained on the same pre-weaning trace mineral treatments, except that the ORG treatment was reformulated to contain 33% organic and 67% inorganic Cu, Zn, and Mn, and both ORG and ING treatments were formulated to supply 10 ppm Cu, 30 ppm Zn, and 20 ppm Mn. Cattle were fed a growing ration for 56 d and a high concentrate finishing diet for 140 d (Year 1) and 145 or 181 d (Year 2) prior to harvest. Cattle performance, immune response, mortality, morbidity, mineral status, carcass traits, and longissimus dorsi fatty acid profiles were evaluated. Initial and final BW, ADG, and DMI were similar across treatments for both years. There was a tendency for a yr × treatment interaction ($P < 0.10$) for gain to feed ratio (G:F). In Year 1, ORG-supplemented cattle had a greater ($P < 0.05$) G:F than ING-supplemented cattle throughout the entire feeding period while non-supplemented control cattle had a greater ($P < 0.01$) G:F than supplemented cattle during the finishing phase. In Year 2, G:F were similar across treatments. Supplemented cattle had greater ($P < 0.01$) liver Cu concentrations than non-supplemented controls throughout the entire feeding period, and liver Mn concentrations were greater ($P < 0.02$) in supplemented cattle than non-supplemented controls at the end of the finishing phase. Immune response, morbidity, carcass traits, and longissimus dorsi fatty acid profiles were not affected ($P > 0.10$) by either trace mineral supplementation or source.

INTRODUCTION

Trace minerals, such as copper (**Cu**), zinc (**Zn**), and manganese (**Mn**), are required for normal tissue growth, homeostasis, enzyme function, and cell regulation and must be maintained within narrow concentrations in the body to ensure optimal growth, health,

and performance in domestic livestock (Underwood and Suttle, 1999). Feedlot diets are typically fortified with trace minerals because feedstuffs utilized in feedlot rations normally contain low concentrations of essential trace minerals and(or) may contain high concentrations of known trace mineral antagonists [i.e. molybdenum (**Mo**), iron (**Fe**), sulfur (**S**), etc.]. The chemical form of a supplemented trace mineral (oxide, sulfate, organic, etc.) has been reported to impact trace mineral status, possibly due to differences in availability (Du et al., 1996), absorption (Du et al., 1996; Kegley and Spears, 1994) and(or) metabolism (Spears, 1989; Nockels et al., 1993). Furthermore, physiological concentrations of Cu and Zn supplemented to feedlot cattle diets have been reported to decrease backfat thickness (Cu; Ward and Spears, 1997), increase longissimus muscle polyunsaturated fatty acids (Cu; Engle et al., 2000a), and increase carcass yield (Zn; Rust and Schlegel, 1993) and quality grade (Zn; Spears and Kegley, 2002).

Several experiments have focused on trace mineral supplementation during the cow/calf (Olson et al., 1999; Stanton et al., 2000) or feedlot (Rhoads et al., 2003; Ward and Spears, 1997; Engle et al., 2000c; Spears and Kegley, 2002) phases. However, the effects of lifetime (pre-natal through harvest) trace mineral supplementation and source on feedlot performance have not been well addressed. Since a large percentage of forages grazed by beef cattle in the U.S. are deficient or marginally adequate in Cu and(or) Zn, as well as marginal or high in Cu antagonists such as S, Fe, and Mo (Mortimer et al., 1999), it is possible for calves to enter the feedlot with a marginal trace mineral status. Therefore, the objectives of this experiment were to evaluate the effects of lifetime supplementation (at current NRC recommendations) and source (organic vs. inorganic) of Cu, Zn, and Mn on feedlot performance, mineral status, immunity, carcass

traits, and longissimus dorsi fatty acid profile in beef cattle during the growing and finishing phases.

MATERIALS AND METHODS

Animal Procedures. Prior to the initiation of this experiment all care, handling, and sampling of the animals herein was approved by the Colorado State University Animal Care and Use Committee. The effects of Cu, Zn, and Mn supplementation and source on the performance, immunity, and carcass traits of feedlot cattle were evaluated using two calf crops from the same cowherd over two consecutive years. Calf crops consisted of crossbred calves (predominantly Red Angus × Charolais F1 hybrids) that originated from the Colorado State University (**CSU**) Eastern Colorado Research Center (**ECRC**; Akron, CO). In total, 270 calves were transported to, and fed until harvest, at the CSU Agricultural Research and Development Education Center (**ARDEC**) Research Feedlot (Ft. Collins, CO), consisting of 140 head in Year 1 (219.5 ± 5.3 kg; 90 steers, 50 heifers) and 130 head in Year 2 (211.2 ± 5.3 kg; 80 steers, 50 heifers).

Prior to arrival to the feedlot in each year, calves and their dams were part of a two-year experiment evaluating the effects of trace mineral supplementation and source on grazing beef cow and calf performance (Ahola et al., 2004). In that experiment, beginning approximately 80 d prior to the average calving date of the cowherd in each year, dams were assigned to one of three treatments: 1) Control (no supplemental Cu, Zn, or Mn); 2) Organic (50% organic and 50% inorganic Cu, Zn, and Mn); and 3) Inorganic (100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$) trace minerals. The ORG and ING free-choice trace mineral treatments were formulated to supply 10 ppm Cu, 30 ppm

Zn, and 40 ppm Mn daily. In both years, treatments were provided ad libitum in mineral feeders from 82 d (Year 1) and 81 d (Year 2) prior to the average calving date of the cowherd through 110 d (Year 1) and 135 d (Year 2) post-calving. When calves reached an average age of 90 d (Year 1) and 99 d (Year 2), calves were provided access to the same respective mineral treatments as their dams via creep feeders in each pasture. Calves continued to have access to mineral treatments exclusively until weaning at an average age of 185 d (Year 1) and 164 d (Year 2).

In both the cow/calf and feedlot phases of this two-year experiment, inorganic trace minerals were supplemented as $CuSO_4$, $ZnSO_4$ and $MnSO_4$, while organic trace minerals were provided from a commercially available mineral proteinate source (Bioplex^{TM} trace minerals Alltech Inc., Nicholasville, KY). All supplemented minerals were formulated to meet NRC (1996) recommended daily concentrations.

Growing and Finishing Phases. After shipment from ECRC to ARDEC (200 km) in both years, calves were individually weighed on two consecutive days, vaccinated (Cattle Master 4 and Bovishield; Pfizer, Exton, PA), dewormed (Dectomax; Pfizer, Exton, PA), and blocked by gender, BW, and previous pasture trace mineral treatment and allotted to one of 15 pens (7 m × 40 m). All calves received the same respective treatments that they and their dam received during the cow/calf phase of the experiment. Feedlot treatments were: 1) Control (no supplemental Cu, Zn, or Mn); 2) Organic (**ORG**; 33% organic and 67% inorganic Cu, Zn, and Mn); and 3) Inorganic (**ING**; 100% inorganic $CuSO_4$, Zn SO_4, and Mn SO_4) trace minerals.

Diets fed during the growing and finishing phases were formulated to meet or exceed NRC (1996) recommendations for all nutrients except Cu, Zn, and Mn (Table 4.1). The

ORG and ING treatments were formulated to supply 10 ppm Cu, 30 ppm Zn, and 20 ppm Mn daily. The mineral analyses of the three trace mineral treatments fed during the feeding period are listed in Tables 4.2 and 4.3 for the growing and finishing phases, respectively. During the initial 56-d growing phase, calves were fed a corn silage-based ration. This was followed by a finishing phase where calves were gradually (over a 10-d period) switched over to a high concentrate diet and fed for an additional period of 140 d (Year 1) and 145 or 181 d (Year 2). In Year 2 equal numbers of cattle per treatment were harvested after receiving the finishing diet for 145 d, and the remaining cattle were harvested after 181 d on the finishing diet. Cattle were fed once daily in the morning in amounts necessary to allow ad libitum access to feed.

Throughout both the growing and finishing phases of the feeding period, daily feed offerings were recorded, and feed refusal was measured every 28 d. From these data, average daily gain (**ADG**), dry matter intake (**DMI**), and a gain-to-feed ratio (**G:F**) were determined for each pen during both the growing and finishing phases. A final weight at the end of the experiment was collected over two consecutive days immediately prior to harvest.

On d 28 of the growing phase in both years, all cattle were implanted [steers: Progesterone (200 mg) and estradiol benzoate (20 mg), Synovex-S, Fort Dodge Animal Health, Fort Dodge, IA; heifers: Trenbolone acetate (200 mg); Finaplix-H; Intervet, Inc. Millsboro, DE]. All cattle were re-implanted on d 28 (Year 1) and d 56 (Year 2) of the finishing phase [steers: Trenbolone acetate (80 mg), and estradiol benzoate (16 mg), Revalor-IS; heifers: Trenbalone acetate (200 mg), Finaplix-H, Intervet, Inc. Millsboro, DE].

Mineral Status. In order to compare mineral status across treatments at different time points and throughout the entire feeding period, blood and liver samples were collected from a sub-group of randomly selected animals (three per pen). Blood was collected from the same animals every 28 d throughout the entire feeding phase via jugular venipuncture into heparinized trace mineral-free vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ). Once collected, samples were placed on ice for approximately 2 h, transported to the laboratory, and centrifuged at 2,000 × *g* for 15 min at room temperature. Plasma was removed and transferred to acid-washed polyethylene storage vials and stored at –20°C. Plasma was collected in both years; however, plasma concentrations of Cu and Zn were determined for Year 1 only. Plasma samples were analyzed for Cu and Zn concentrations as described by Ahola et al. (2004).

Liver biopsy samples were collected (from the same animals that were used for blood collection) on d 0 and 56 of the growing phase, and d 112 (Year 1) and 140 (Year 2) of the finishing phase using the true-cut technique described by Pearson and Craig (1980), as modified by Engle and Spears (2000b). Briefly, a 10 cm × 10 cm area was clipped of hair on the right side of each animal between the eleventh and twelfth ribs and scrubbed three times with iodine and 70% alcohol. Approximately 5 ml of 2% lidocaine hydrochloride (Abbott Laboratories, North Chicago, IL) was injected via a 20-gauge × 2.5 cm needle between the eleventh and twelfth ribs on a line from the hip to the point of the shoulder. A small incision (approximately 1.0 cm) was made using a scalpel blade (number 11) and a core sample of liver tissue was collected using a modified Jan Shide bone marrow biopsy punch (0.5 cm × 14 cm; Sherwood Medical, St. Louis, MO). Following collection, samples were immediately rinsed with 0.01 *M* PBS (pH 7.4) and

placed into acid-washed polyethylene tubes, capped, placed on ice for approximately 4 h, and stored at –20°C. Liver samples were analyzed for Cu, Zn, and Mn concentrations as described by Ahola et al. (2004).

Immune Response and Health Status. An *in vivo* evaluation of cell-mediated immune (**CMI**) response was performed via the use of phytohemagglutinin (**PHA**; Sigma-Aldrich, St. Louis, MO) to stimulate an immune response (Fritz et al., 1990) using a select subset of animals (three per pen). On d 33 (Year 1) and 43 (Year 2) of the finishing phase, each animal was restrained in a squeeze chute and a square 10 cm × 10 cm area of hair was clipped on the animal's left side immediately posterior to the scapula. Within the clipped area, in each of two separate sites approximately 2.5 cm apart, 0.1 ml of a PHA solution (150 μg PHA in 0.1 ml PBS) was injected intradermally with a one ml tuberculin syringe and 26-gauge needle (Becton Dickinson CO., Franklin Lakes, NJ). The skin-fold thickness was measured (in mm) at both injection sites using skin-fold calipers (Vernier Type 6914; Scienceware, Pequannock, NJ) immediately prior to PHA injection (0 h) and at 4, 8, 12, 24, and 48 h post injection. The two measurements collected on each animal were averaged and the change in skin thickness was used to evaluate the CMI response to PHA.

Two primary humoral immune responses [during the growing and(or) finishing phases] and one secondary humoral immune response (during the finishing phase only) were compared across treatments via administration of one or more foreign proteins into cattle. On d 28 (Year 1) and 31 (Year 2) of the growing phase, 5 ml of a 20% pig red blood cell (**PRBC**) solution (Sigma-Aldrich, St. Louis, MO) diluted in autoclaved PBS (pH 7.4) was injected intramuscularly into the neck of a subset of randomly selected

animals (three per pen) in order to elicit and evaluate a primary immune response during the growing phase. Blood was collected via jugular venipuncture into non-heparinized vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) immediately prior to PRBC injection (d 0), and on 7, 14, and 21 d post-injection from the same animals. Samples were stored on ice for approximately 2 h prior to being centrifuged at 2,000 × *g* for 15 min. Serum was harvested and stored in polyethylene tubes at –80°C until analyzed.

A secondary humoral immune response to PRBC was evaluated on d 84 (Year 1) and 80 (Year 2) of the finishing phase in 22 head (randomly selected, 1 to 2 head per pen) of the 45 animals previously used to evaluate a primary humoral immune response to PRBC. Five milliliters of a 20% PRBC solution, identical to that used to evaluate primary humoral immune response during the growing phase, was injected (i.m.) into the neck of each animal to elicit a secondary humoral immune response. Blood was collected via jugular venipuncture into non-heparinized vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) immediately prior to the second PRBC injection (d 0), and at 7, 14, and 21 d post-injection from the same animals. Samples were handled as previously described.

Serum samples collected from animals administered PRBCs were thawed and antibody titers to PRBC were measured using a microtiter hemagglutination assay (Ferket and Qureshi, 1992). Concentrations of total immunoglobulin (**Ig**), immunoglobulin G (**IgG**), and immunoglobulin M (**IgM**) specific for PRBC were determined.

A second evaluation of primary humoral immune response was performed during the finishing phase using ovalbumin (**OVA**) as the antigen (Ward et al., 1993). Of the original 45 head previously injected with PRBC to evaluate primary humoral immune

response during the growing phase, 23 of those animals (randomly selected, 1 to 2 head per pen) were injected with OVA to stimulate a primary immune response during the finishing phase. A solution containing OVA (106 mg; Sigma-Aldrich, St. Louis, MO), Freund's Incomplete Adjuvant (FIA; 60 ml; Sigma-Aldrich, St. Louis, MO), and PBS (60 ml) was injected into the neck of each animal both subcutaneously (2 ml) and intramuscularly (1 ml). A total of 4,000 μg of OVA was administered to each animal. Blood was collected via jugular venipuncture into non-heparinized vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) immediately prior to the injection (d 0), and at 7, 14, and 21 d post-injection from the same animals. Samples were handled as described for the PRBC sampling for primary humoral immune response. After storage, samples were thawed, heated for 30 min at 56°C, and analyzed for antibody titers specific to OVA using an ELISA procedure (Engvall and Perlmann, 1972). Data were expressed as a total concentration of antibody titers to OVA.

Interferon gamma concentrations were determined using blood collected on d 0 and 56 into heparinized vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ). After collection, samples were centrifuged at 2,000 × *g* for 15 min and plasma was harvested and stored at –80°C. After storage, interferon gamma concentrations were determined using a commercially available enzyme amplified sensitivity immunoassay (Bovine IFN-δ EASIA; Biosource Europe S.A., Nivelles, Belgium).

Red blood cell (**RBC**) superoxide dismutase (**SOD**) enzyme activity was determined from samples collected on d 0 and 28 of the growing phase. Heparinized vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) were used to collect blood, which was centrifuged at 2,000 × *g* for 15 min, and 1 ml of RBC was collected from each sample

and lysed in 4 ml of cold dH_2O. Lysed RBC samples were stored at –80°C and later analyzed for SOD activity using a spectrophotometric assay (Bioxytech SOD-525, Oxis Health Products, Inc., Portland, OR) read at 525 nm by a Spectronic Genesys 5 spectrophotometer (Thermo Electron Corp., Woburn, MA). Hemoglobin concentrations were determined using a hemoglobin reagent set (Pointe Scientific, Lincoln Park, MI) so that SOD activity data could be expressed per mg of hemoglobin.

Throughout the entire feeding period, cattle were visually monitored daily to detect signs of morbidity. Cattle observed displaying clinical symptoms of morbidity were transported to a working facility and a rectal temperature was collected. If an animal's rectal temperature was above 39.4°C, it was considered morbid and treated accordingly. Based on a visual diagnosis by trained personnel, morbid cattle were classified as morbid due to a respiratory disease or morbid due to other reasons. It was also noted if an animal was being treated for the first time, or re-treated for signs of morbidity.

Harvest and Carcass Data. At the conclusion of the feeding period, all cattle were transported 55 km and harvested at a commercial abattoir. In Year 1, all cattle were harvested after receiving the finishing diet for 140 d. In Year 2, equal numbers of steers and heifers per treatment (the heaviest from each treatment) were harvested after receiving the finishing diet for 145 d (70 animals) while the remaining 60 animals were harvested after receiving the finishing diet for 181 d. After harvest, carcasses were chilled for approximately 28 h (Year 1) and 36 h (Year 2) before complete carcass data were collected prior to carcass fabrication. Carcass data included: Hot carcass weight (**HCW**), dressing percent (**DP**), ribeye area (**REA**), twelfth rib backfat (**BFAT**), percent kidney, pelvic, and heart (**KPH**) fat, calculated yield grade (**YG**), bone maturity, lean

maturity, and marbling score (**MARB**). For analysis of percent lipid and fatty acid composition, in both years a longissimus dorsi muscle sample (approximately 100 g) encompassing the entire surface of the ribeye was collected from the face of the loin section from the same side of each chilled carcass (at the time of carcass data collection). Samples were placed into plastic bags, stored on ice, transported to the laboratory, and stored at –80°C. Immediately after thawing at room temperature, subcutaneous fat was trimmed from each sample prior to grinding the trimmed longissimus dorsi muscle sample in a small food processor (Cuisinart, Stamford, CT). After grinding, 1 g of the ground homogenate was weighed onto oven-dried filter paper, folded, oven dried for 24 h at 100°C, and re-weighed to calculate percent dry matter. Samples were then placed into a Soxhlet apparatus (Pyrex, Corning, Inc., Corning, NY), and lipid was extracted via ether distillation (AOAC, 1975). Samples were then removed from the apparatus, air dried under a fume hood for approximately 12 h, oven dried at 100°C for 24 h, and weighed to determine percent lipid content. To analyze for fatty acid composition, duplicate 1 g subsamples of longissimus muscle (ground homogenate) were used for lipid extraction (Engle et al., 2000a). Methyl ester derivatives of the fatty acids extracted from longissimus muscle were prepared in duplicate using a combination of $NaOCH_3$ followed by HCl/methanol (Kramer et al., 1997). After methylation, the methyl ester solution was dried under N_2 gas and reconstituted with 0.5 ml hexane. Fatty acid composition of longissimus muscle was determined via a gas chromatograph (**GC**; 6890 Series, Agilent Technologies, Wilmington, DE), as described by Engle et al. (2000a). The GC was equipped with a 6B90 series injector (Agilent Technologies, Wilmington, DE) and flame ionization detector and fitted with a fused silica capillary column (100 m × 0.25 mm i.d.;

SP-2560 Supelco Inc., Bellefonte, PA). Samples were injected using the split mode with helium as the carrier gas and a split ratio of 100:1 at 180°C. Data collected and reported were the normalized area percentages of fatty acids.

Statistical Analysis. Performance (BW, ADG, DMI, and GF), immune (interferon gamma, SOD, and humoral and cell-mediated immune responses), and mineral status data (liver and plasma mineral concentrations) were all assessed for the growing and finishing phases separately using a restricted maximum likelihood-based, mixed effects model repeat measures analysis (PROC MIXED, SAS Inst. Inc., Cary, NC). Pen within treatment × yr was included as a random variable and the autoregressive covariate design was used when variation was homogeneous and time intervals were equal. Autoregressive with heterogeneous variation was used when variation was heterogeneous, and a spatial power covariance structure was used when time intervals were not equal. Initial models for performance, health status, immune response, and mineral status included the fixed effects of treatment, sex, time, year, and all possible interactions. Observational health data including morbidity rate were assessed using chi square analysis (PROC FREQ, SAS Inst. Inc., Cary, NC). Data for carcass characteristics, percent lipid, and fatty acid content were analyzed using least squares analysis of variance models (PROC MIXED, SAS Inst. Inc. Cary, NC). Initial models for carcass characteristics, percent lipid, and fatty acid content included the fixed effects of treatment, sex, year, harvest date, and all possible interactions. For all analyses, pen (replicate) was used as the experimental unit. When an interaction was not significant, it was removed and the model was reduced. If the treatment × yr interaction was not significant, data were pooled across years. Differences among means were determined

using preplanned single degree of freedom contrasts; comparisons made were: 1) Control vs. trace mineral supplemented cattle, and 2) ORG vs. ING.

RESULTS AND DISCUSSION

In Year 2, one steer died from bloat at the beginning of the growing phase, shortly after arrival to the feedlot. All data collected from this steer were removed prior to any statistical analyses. Results of the cow/calf (pre-weaning) phase of this experiment have been reported elsewhere (Ahola et al., 2004).

Performance

Growing Phase. During the initial 56-d growing phase, no yr × treatment interactions were present ($P > 0.10$) for initial or final BW, ADG, or DMI (Table 4.4). Initial BW was similar ($P > 0.10$) across treatments. Calf weaning weights, collected at the ranch (ECRC), were impacted by both trace mineral supplementation and source (Ahola et al., 2004); however, at the time of weaning on the ranch (prior to shipment to the feedlot) equal numbers of calves across treatments were culled. Culling decisions were based predominantly on poor pre-weaning performance (i.e. light weaning weight, generally unthrifty, or poor performing calves). Culled calves were never incorporated into the feedlot phase of the experiment. At the end of the growing phase (d 56) no BW differences were observed between supplemented and control cattle ($P = 0.72$) or between ORG- and ING-supplemented cattle ($P = 0.79$). Similarly, neither ADG nor DMI were affected by either trace mineral supplementation ($P = 0.60$ and $P = 0.75$, respectively) or source ($P = 0.86$ and $P = 0.25$) throughout the growing phase. There was

a tendency ($P = 0.10$) for a yr × treatment interaction for G:F, therefore G:F ratios are reported separately for each year. In Year 1, no difference ($P = 0.92$) was observed for G:F between supplemented and non-supplemented control cattle, although ORG-supplemented cattle had a greater ($P < 0.03$) G:F than ING-supplemented cattle. In Year 2, no differences were observed for G:F between control and supplemented cattle ($P = 0.70$) or between ORG and ING cattle ($P = 0.47$).

The combined effects of lifetime Cu, Zn, and Mn supplementation and source on growing phase performance have not been well addressed in previous beef cattle trace mineral research. The majority of trace mineral experiments in feedlots have focused on the supplementation of a single trace mineral (i.e. Cu or Zn) only during the growing and(or) finishing phases. The current experiment evaluated the combined effects of Cu, Zn, and Mn supplementation and source during the pre-natal, neonatal (pre-weaning), and post-weaning (feedlot) periods on ultimate growing and finishing phase performance. Therefore, it is not possible to determine the individual effects of Cu, Zn, or Mn.

George et al. (1997) evaluated the combined effects of Cu, Zn, Mn, and cobalt (**Co**) supplementation and source on weaned heifer calf performance during a 42-d growing phase using trace mineral treatments of: 1) inorganic at NRC (1984) recommended concentrations, 2) organic at NRC (1984), and 3) organic at three times NRC (1984) for the first 14 d and then at NRC (1984) recommended concentrations for the remainder of the growing phase. The authors reported that DMI, ADG, and G:F were not affected by either supplementation or source; however, heifers receiving the organic form at three times the NRC (1984) had greater ADG and G:F compared to those receiving either inorganic or organic minerals at NRC (1984) recommended concentrations.

Although not directly comparable to our data, the effects of either Cu or Zn supplementation and(or) source on growing phase performance have been evaluated extensively. The majority of researchers have concluded that performance during the growing phase is not affected by Cu supplementation (Ward et al., 1993; Engle and Spears, 2000b; Engle and Spears, 2001; Engle et al., 2000c; Lee et al., 2002) or source (Engle and Spears, 2000b; Lee et al., 2002). Relative to the greater G:F observed in ORG- vs. ING-supplemented cattle in the current experiment, Ward and others (1993) reported that feed efficiency was greater during the first 21 d of a 98-d growing phase in steers receiving supplemental Cu as $CuSO_4$ than steers receiving Cu-lysine. Effects of Zn supplementation and source on growing phase performance have been variable. Results have included higher ADG in steers (Spears and Kegley, 2002), higher G:F and ADG in lambs and heifers (Spears, 1989), and higher ADG and DMI in steers (Rust and Schlegel, 1993) receiving supplemental Zn compared to non-supplemented controls and no effect of Zn source on growing phase performance (Spears and Kegley, 2002; Engle et al., 1997; Spears, 1989).

Based on our results and previous research, the supplementation of Cu, Zn, and Mn (alone or in combination) can have a limited effect on growing phase performance, but the reported effects have been variable. Also based on previous research, neither Cu nor Zn source appears to impact growing phase performance, although trace mineral source did impact G:F in Year 1 of the current experiment. No benefit of lifetime supplementation of Cu, Zn, and Mn was observed on growing phase performance, although this has not been well addressed in the literature. Since cattle receiving the control treatment in the current experiment were able to maintain liver mineral

concentrations above deficient levels (Tables 4.6 and 4.7), it is likely that any potential benefit of trace mineral supplementation was not detectable since adequate amounts of the trace minerals were most likely available from the basal diet throughout the growing period.

Finishing Phase. No yr × treatment interactions were detected ($P > 0.10$) for initial or final BW, ADG, or DMI, so data for both years were combined (Table 4.5). Neither initial nor final BW differed between control and supplemented cattle ($P = 0.86$) or ORG and ING cattle ($P = 0.78$). Similarly, no effect of trace mineral supplementation or source was present for ADG ($P = 0.75$ and $P = 0.84$, respectively). There was a tendency ($P < 0.10$) for greater DMI in supplemented vs. non-supplemented controls during the finishing phase, but no effect ($P = 0.28$) of trace mineral source on DMI. A yr × treatment interaction was present ($P < 0.04$) for finishing phase G:F. In Year 1, non-supplemented control cattle had a greater ($P < 0.01$) G:F than supplemented cattle, while ORG cattle had a greater ($P < 0.04$) G:F than ING cattle. However in Year 2, no differences were observed between control and supplemented cattle ($P = 0.87$) or between ORG and ING cattle ($P = 0.69$).

Reported results on the impact of simultaneous Cu, Zn, and Mn supplementation during the finishing phase on beef cattle performance are limited. However, the effect of concentration and source of four trace minerals (Cu, Zn, Mn, and Co) on finishing phase performance has been evaluated previously. Steers received Cu, Zn, Mn, and Co in an organic form at NRC (1996) recommended concentrations or at 1.5 times NRC (1996), or in an inorganic form at 1.5 or 3.0 times NRC (1996) throughout a 198- or 230-d finishing period (Rhoads et al., 2003). In contrast to the current experiment, the authors reported

that DMI was greater in steers receiving the organic form at NRC (1996) recommendations vs. the other three treatments.

Relative to the effect of Cu supplementation on finishing phase performance, Cu can have a positive (Ward and Spears, 1997; Engle et al., 2000c), negative (Engle and Spears, 2000b), or no effect (Engle and Spears, 2000a; Engle and Spears, 2001; Engle et al., 2000b) on performance compared to non-supplemented controls. It has also been reported that Cu source had no effect on finishing phase performance (Engle and Spears, 2000b), but led to greater final BW and a tendency for greater ADG in steers receiving organic Cu vs. inorganic Cu (Lee et al., 2002). Zinc supplementation in finishing cattle led to a tendency ($P < 0.10$) for greater ADG (Rust and Schlegel, 1993), and no effect on ADG, DMI, or G:F (Spears and Kegley, 2002) vs. non-supplemented controls, while DMI decreased as Zn concentration increased (Malcolm-Callis et al., 2000). Relative to Zn source, there was tendency for ADG ($P = 0.10$) and G:F ($P = 0.07$) to be greater in cattle supplemented with organic Zn compared to inorganic Zn (Spears and Kegley, 2002), while in contrast ADG was greater in cattle receiving $ZnSO_4$ compared to Zn-methionine (Nunnery et al., 1996). Several researchers have also reported that Zn source had no impact on performance (Rust and Schlegel, 1993; Greene et al., 1988; Malcolm-Callis et al., 2000).

Reaching an objective conclusion about the impact of lifetime Cu, Zn, and Mn supplementation (alone or in combination) on finishing phase performance is difficult. The effect of individual trace mineral supplementation on finishing phase performance has been extremely variable, but appears to be limited. In the current experiment, the tendency for trace mineral supplementation to improve DMI and the inconsistent effects

of trace mineral supplementation and source on G:F (present in Year 1, but not in Year 2) may be spurious, or may reflect subtle differences in feed intake and(or) daily gain.

Mineral Status

Plasma Mineral. Plasma Cu and Zn concentrations, determined from samples collected at four points during the growing and finishing phases of Year 1, are listed in Table 4.6. Neither trace mineral supplementation nor source affected ($P > 0.10$) plasma Cu concentrations at any point during the feeding period in Year 1. Plasma Zn concentration tended ($P < 0.08$) to be greater in supplemented cattle only at the end of the finishing phase, while trace mineral source did not impact ($P > 0.10$) plasma Zn concentrations at any point during the feeding period. Utilizing plasma Zn concentration as a method of classifying Zn status may be inaccurate if cattle are not truly Zn deficient (Underwood and Suttle, 1999; Hambidge et al., 1986).

All treatments (including the non-supplemented controls) were able to maintain plasma concentrations of both Cu and Zn above levels considered deficient (0.6 mg Cu/L and 0.4 mg Zn/L; Puls, 1994). Therefore, throughout the duration of this experiment (both cow/calf and feedlot phases), it appears that cattle receiving the control treatment were able to consume adequate amounts of Cu and Zn via the basal diet alone.

Liver Mineral. No yr × treatment interactions were present ($P > 0.10$) for liver concentrations of Cu, Zn, or Mn (Table 4.7). At every time point, supplemented cattle had greater ($P < 0.01$) liver Cu concentrations than non-supplemented controls. However, liver Cu concentrations were not different ($P > 0.10$) between ORG and ING cattle at any time during the experiment. Liver Zn concentration was not impacted by

either Zn supplementation ($P > 0.10$) or source ($P > 0.10$) during the growing or finishing phases. However, liver Zn concentrations did decline numerically during the feeding period from the initial liver biopsy collection (on d 0 of growing phase) to the final liver biopsy collection (on d 112 and 140 of the finishing phase, Year 1 and Year 2, respectively). Liver Mn concentration was affected ($P < 0.02$) by trace mineral supplementation, but only at the end of the feeding period when supplemented cattle had greater ($P < 0.02$) liver Mn concentration than non-supplemented controls. There was no effect ($P > 0.10$) of trace mineral source on liver Mn concentration at any point during the feeding period.

Neither liver Cu nor Mn concentration was affected by trace mineral source when Cu, Zn, Mn, and Co were supplemented to cattle during the receiving and finishing phases (Rhoads et al., 2003). However, the authors reported that liver Zn concentration was greater in steers receiving the organic form of Zn at 1.5 times NRC (1996) vs. steers receiving the inorganic form of Zn at the same concentration.

Although differences in average liver Cu and Mn concentrations were observed in the current experiment, all treatments (including the non-supplemented controls) were able to maintain liver concentrations of Cu, Zn, and Mn at levels considered to be adequate (25 mg Cu/kg DM, 25 mg Zn/kg DM, and 2.5 mg Mn/kg DM; Puls, 1994). Therefore, throughout the duration of this experiment (during both the cow/calf and feedlot phases), cattle receiving the non-supplemented control treatment were apparently able to consume adequate amounts of Cu, Zn, and Mn via the basal diet.

Immunity and Health

The effect of lifetime trace mineral supplementation and source on health status during the growing and finishing phases was evaluated using several methods, including SOD activity, interferon gamma concentration, immune response, and morbidity rate. There were no treatment × yr interactions ($P > 0.10$) for SOD activity or interferon gamma concentration. Activity of the SOD enzyme, a Cu/Zn-dependent metalloenzyme responsible for catalyzing the conversion of a superoxide anion into oxygen and hydrogen peroxide, did not differ between supplemented and control cattle ($P = 0.85$) or between ORG and ING cattle ($P = 0.84$; Appendix A). In addition, neither trace mineral supplementation nor source ($P > 0.10$) affected interferon gamma concentration (Appendix A).

Limited literature has evaluated the combined effects of Cu, Zn, and Mn on SOD activity, however experiments have evaluated the effect of Cu alone on the enzyme. Supplementation of Cu did not affect SOD activity in feedlot cattle (Dorton et al., 2003; Ward et al., 1993), however greater SOD activity has been reported in Cu-supplemented cattle on d 0, 28, and 56 of the growing phase but not after d 56 (Ward and Spears, 1997). Since a reduction in SOD activity typically only occurs during extended Cu deficiency (Paynter, 1987), the lack of an effect of trace mineral supplementation or source on SOD activity observed in the present experiment is not unusual since cattle were above deficiency levels of Cu throughout the experiment (Puls, 1994).

There was no treatment × yr interaction ($P > 0.10$) for CMI to PHA. An *in vivo* CMI response to PHA was observed in all treatments when evaluated at 4 h after injection with PHA, however during the 48-h period following stimulation of a CMI response, the skin-

fold thickness in non-supplemented control cattle did not differ ($P = 0.18$) from that of supplemented cattle, nor did ORG cattle differ from ING ($P = 0.35$; Appendix A).

When Cu, Zn, Mn, and Co were combined and supplemented to stressed weaned heifer calves, no effect of concentration or source of trace minerals on CMI response at 12 or 24 h after PHA injection on d 7 after arrival to the feedlot was reported (George et al., 1997). However the authors did report a lower CMI response at 48 h after PHA injection in calves supplemented with the organic form at three-times NRC (1984) recommended concentrations compared to calves receiving the inorganic form at NRC (1984) recommended concentrations. The authors also reported that 21 d after arrival at the feedlot, greater and longer CMI responses to PHA at 12, 24, and 48 h were observed in calves supplemented with organic trace minerals at three-times the NRC (1984) recommended concentrations versus calves receiving the trace minerals in an organic or inorganic form at NRC (1984) recommended concentrations. Of the calves receiving Cu, Zn, Mn, and Co at NRC (1984) recommended concentrations, calves receiving the organic form had a greater CMI response to PHA than the inorganic form, but only at 48 h post PHA injection (George et al., 1997).

There were no treatment × yr interactions ($P > 0.10$) for primary humoral immune response to PRBC or OVA. In response to the first PRBC injection during the growing phase, there was no effect of trace mineral supplementation on IgG ($P = 0.87$), IgM ($P = 0.25$), or total Ig ($P = 0.79$) concentrations, and no effect of trace mineral source on IgG ($P = 0.77$), IgM ($P = 0.42$), or total Ig ($P = 0.18$) concentrations (Appendix A). Likewise, concentrations of primary antibody titers raised to OVA during the finishing

phase were not impacted by either trace mineral supplementation ($P = 0.16$) or source ($P = 0.55$).

Although limited research has evaluated the combined effect of Cu, Zn, and Mn on humoral immune response, previous researchers have reported that primary immune response to PRBC was not affected by Cu supplementation in 70 d old calves (Ward et al., 1997), and was lower in non-stressed calves receiving supplemental Cu and higher in stressed calves receiving supplemental Cu vs. non-supplemented controls (Ward and Spears, 1999). Growing calves receiving inorganic Cu had greater IgG and total Ig concentrations than calves supplemented with organic Cu (Dorton et al., 2003). Copper-supplemented cattle did not respond differently than non-supplemented controls to an injection of OVA (Ward et al., 1993), however a greater immune response to OVA has been reported in Cu-supplemented cattle vs. non-supplemented controls (Dorton et al., 2003; Ward and Spears, 1999) and in calves receiving supplemental Cu as organic vs. inorganic on d 14 and 21 after injection with OVA (Dorton et al., 2003).

There was no treatment × yr interaction ($P > 0.10$) for secondary humoral immune response to PRBC. In the evaluation of secondary immune response to PRBC during the finishing phase, concentrations of IgG, IgM, and total Ig were not impacted by trace mineral supplementation ($P = 0.58$, $P = 0.42$, and $P = 0.14$, respectively; Appendix A). Likewise, concentrations of IgG and IgM were not affected by trace mineral source ($P = 0.62$ and $P= 0.22$, respectively); however, there was a tendency ($P < 0.07$) for greater total Ig concentration in ORG compared to ING cattle (Appendix A).

There were no treatment × yr interactions ($P > 0.10$) for any of the morbidity rate variables evaluated, and trace mineral supplementation ($P > 0.10$) did not impact the

number of cattle treated or re-treated for symptoms of a respiratory disease or treated for ailments other than respiratory disease during the feeding period. The combination of a low morbidity rate (only 14.8% of cattle were treated for symptoms of a respiratory disease), and the fact that morbidity data are binomial, may have prevented the observation of differences across treatments for morbidity rate.

Grotelueschen et al. (2001) evaluated lifetime supplementation of trace minerals (Cu, Zn, Mn, and Co) on calf morbidity with 641 cow/calf pairs. The authors reported that fewer organic and non-supplemented calves were treated during the first 28-d compared to the inorganic treatment, fewer organic calves were treated throughout the feeding period compared to inorganic calves, and organic calves were re-treated less than inorganic and non-supplemented control calves during the first 28-d period and throughout the entire feeding period. When Zn source was evaluated, twice as many steers were treated in the basal and low organic Zn treatments compared to the high organic and inorganic Zn treatments (Galyean et al., 1995).

Carcass Traits

Carcass Characteristics. A summarization of carcass characteristics for both years is presented in Table 4.8. Carcass data were not affected ($P > 0.10$) by slaughter time, and no treatment × yr interactions were detected for HCW ($P = 0.82$), DP ($P = 0.85$), REA ($P = 0.59$), BFAT ($P = 0.66$), KPH ($P = 0.67$), YG ($P = 0.16$), or MARB ($P = 0.65$). No differences were observed for HCW between control and supplemented cattle ($P = 0.76$) or ORG and ING cattle ($P = 0.61$). Similarly, DP did not differ between control and supplemented cattle ($P = 0.66$) or ORG and ING cattle ($P = 0.22$). There was no effect of

supplementation or source on REA ($P = 0.21$ and $P = 0.85$, respectively), BFAT ($P = 0.33$ and $P = 0.40$, respectively), or KPH ($P = 0.64$ and $P = 0.53$, respectively). Calculated YG was not different between control and supplemented cattle ($P = 0.73$) or between ORG and ING cattle ($P = 0.20$). Marbling score was not impacted by either trace mineral supplementation ($P = 0.23$) or source ($P = 0.38$). These data are in agreement with those reported by Rhoads et al. (2003) who detected no effect of trace mineral source on carcass traits (HCW, DP, REA, YG, BFAT, KPH or MARB) was detected when Cu, Zn, Mn, and Co were supplemented to finishing cattle.

No treatment × yr interaction was present ($P = 0.63$) longissimus muscle lipid content (Appendix A). Longissimus muscle lipid content was similar between control and supplemented cattle ($P = 0.69$) and similar between ORG and ING cattle ($P = 0.15$). This is consistent with the absence of a trace mineral supplementation or source effect on MARB (Table 4.8).

Fatty Acid Profile. The results of fatty acid analysis of longissimus dorsi samples are listed in Table 4.9. No treatment × yr interactions were observed for any of the fatty acids or combinations analyzed, so data were pooled across years. Neither supplementation nor source of trace minerals impacted ($P > 0.10$) fatty acid composition of longissimus dorsi samples. At concentrations 1.5 times NRC (1996) recommendations, cattle receiving inorganic trace minerals had greater weight percentages of C18:1 cis and C20:4 than cattle receiving organic trace minerals; however, the weight percentage of C18:2 cis-9 trans-11 was greater in cattle receiving the organic form than the inorganic form (Rhoads et al., 2003).

It is unclear why neither supplementation nor source affected fatty acid profile, unlike previous research. The absence of any fatty acid differences may have been due to breed differences between the current experiment and previous research, differences in trace mineral concentrations and(or) sources supplemented to cattle, and(or) differences in duration of supplementation.

IMPLICATIONS

The combined effects of lifetime (prenatal through harvest) copper, zinc, and manganese supplementation and source on feedlot performance, mineral status, health, immune response, and carcass traits have not been well addressed in the literature. Experiments that have evaluated individual trace minerals such as copper or zinc indicate that both supplementation and source can impact mineral status, performance, health, and carcass characteristics during the feeding phase. However, when trace minerals were supplemented to cattle from the late fetal stage through harvest in the current experiment, only liver copper and manganese concentrations and the gain-to-feed ratio (in the first year only) were affected. It appears that lifetime supplementation and source of copper, zinc, and manganese has limited impact on the performance, health, and carcass characteristics of feedlot cattle when trace mineral stores can be maintained above levels considered to be deficient.

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Table 4.1. Ingredient composition, mineral analysis, and proximate analysis of the basal diet fed during the growing and finishing phases[a]

Ingredient	Growing phase	Finishing phase
Steam flaked corn, %	25.0	81.5
Corn silage, %	55.3	8.0
Ground alfalfa hay, %	13.1	6.7
Protein supplement, %:	6.6	3.8
Cotton seed meal, %	41.0	41.3
Soybean meal (46.5%), %	20.0	20.0
Limestone, %	11.75	11.75
Sunflower meal (32%), %	11.2	10.7
Urea, %	7.0	7.1
KCl, %	3.9	3.9
$CaPO_4$, %	3.85	3.85
NaCl, %	0.90	0.88
Monensin, %	0.19	0.31
Se (0.16%), %	0.08	0.08
I (20g), %	0.063	0.060
Vitamin A, %	0.067	0.070
Vitamin E, %	0.048	0.050
Sulfur flowers, %	0.029	0.030
$CoCO_4$, %	0.0003	0.0003
Mineral analysis of total diet:		
Ca, %	0.65	0.63
P, %	0.39	0.35
Cu, mg/kg DM	6.90	6.42
Zn, mg/kg DM	15.7	15.0
Mn, mg/kg DM	16.4	15.7
Proximate analysis[b]:		
DM, %	72.1	76.9
CP, %	13.6	12.4
TDN, %	84.3	85.6
CF, %	10.47	9.29
ADF, %	11.24	9.64
NDF, %	17.95	16.59
CFAT, %	3.91	4.00
Ash, %	2.70	2.47
NEg, Mcal/kg	1.41	1.50

[a]Dry matter basis.

[b]DM = Dry matter, CP = Crude Protein, TDN = Total Digestible Nutrients, CF = Crude Fiber, ADF = Acid Detergent Fiber, NDF = Neutral Detergent Fiber, CFAT = Crude Fat.

Table 4.2. Mineral analysis of three trace mineral treatments supplemented to feedlot cattle during the growing phase[a]

	Dietary treatment		
Ingredient	Control	ORG[b]	ING[c]
Cu, mg/kg DM	5.4	143	143
Zn, mg/kg DM	10.4	548	546
Mn, mg/kg DM	21.9	326	312

[a]Dry matter basis.
[b]ORG = Organic treatment (33% organic, 67% inorganic Cu, Zn, and Mn).
[c]ING = Inorganic treatment (100% $CuSO_4$, $ZnSO_4$, and $MnSO_4$).

Table 4.3. Mineral analysis of three trace mineral treatments supplemented to feedlot cattle during the finishing phase[a]

	Dietary treatment		
Ingredient	Control	ORG[b]	ING[c]
Cu, mg/kg DM	5.1	263	265
Zn, mg/kg DM	10.0	842	838
Mn, mg/kg DM	20.1	552	555

[a]Dry matter basis.
[b]ORG = Organic treatment (33% organic, 67% inorganic Cu, Zn, and Mn).
[c]ING = Inorganic treatment (100% $CuSO_4$, $ZnSO_4$, and $MnSO_4$).

Table 4.4. Effect of trace mineral supplementation and source on feedlot performance during the 56-day growing phase[a]

	Dietary treatment				Contrasts ($P <$)	
Item	Control	ORG[b]	ING[c]	SEM[d]	Cont vs Suppl	ORG vs ING
Initial BW, kg	218.9	212.2	214.9	6.47	0.51	0.78
Final BW, kg	299.6	294.8	297.7	7.31	0.72	0.79
ADG, kg	1.44	1.46	1.47	0.038	0.60	0.86
DMI[e], kg/d	7.74	7.64	8.00	0.214	0.75	0.25
G:F[f,g]						
Year 1	0.167	0.177	0.158	0.0057	0.92	0.03
Year 2	0.205	0.204	0.210	0.0057	0.70	0.47

[a]Least squares means.
[b]ORG = Organic treatment (33% organic, 67% inorganic Cu, Zn, and Mn).
[c]ING = Inorganic treatment (100% $CuSO_4$, $ZnSO_4$, and $MnSO_4$).
[d]SEM = Standard error of the mean.
[e]DMI = Dry matter intake.
[f]G:F = Gain to feed ratio.
[g]There was a tendency ($P = 0.10$) for a treatment × yr interaction for G:F during the growing phase, therefore data are reported separately for each yr.

Table 4.5. Effect of trace mineral supplementation and source on feedlot performance during the finishing phase[a,b]

	Dietary treatment				Contrasts ($P <$)	
Item	Control	ORG[c]	ING[d]	SEM[e]	Cont vs Suppl	ORG vs ING
Initial BW, kg	299.6	294.8	297.7	7.31	0.72	0.79
Final BW[b], kg	554.4	550.1	554.2	9.96	0.86	0.78
ADG, kg	1.53	1.54	1.55	0.031	0.75	0.84
DMI[f], kg/d	8.41	8.65	8.95	0.185	0.10	0.28
G:F[g,h]						
Year 1	0.208	0.197	0.181	0.0052	0.01	0.04
Year 2	0.163	0.163	0.166	0.0052	0.87	0.69

[a]Least squares means.
[b]Length of the finishing phase was 140 d (Year 1) and 145 d or 181 d (Year 2).
[c]ORG = Organic treatment (33% organic, 67% inorganic Cu, Zn, and Mn).
[d]ING = Inorganic treatment (100% $CuSO_4$, $ZnSO_4$, and $MnSO_4$).
[e]SEM = Standard error of the mean.
[f]DMI = Dry matter intake.
[g]G:F = Gain to feed ratio.
[h]There was a treatment × yr interaction ($P < 0.04$) for G:F during the finishing phase, therefore data are reported separately for each yr.

Table 4.6. Effect of trace mineral supplementation and source on plasma mineral concentration in feedlot cattle[a,b]

Item	Dietary treatment				Contrasts ($P <$)	
	Control	ORG[c]	ING[d]	SEM[e]	Cont vs Suppl	ORG vs ING
Cu, mg/kg DM						
d 0 growing	1.16	1.24	1.10	0.087	0.99	0.28
d 56 growing	0.93	0.99	1.03	0.087	0.44	0.74
d 84 finishing	1.12	1.00	0.97	0.087	0.22	0.85
d 140 finishing	1.17	1.14	1.05	0.087	0.50	0.46
Zn, mg/kg DM						
d 0 growing	0.92	0.89	1.04	0.083	0.66	0.21
d 56 growing	1.09	0.99	1.14	0.083	0.81	0.24
d 84 finishing	1.15	1.07	1.19	0.083	0.86	0.32
d 140 finishing	1.04	1.15	1.30	0.083	0.08	0.21

[a]Least squares means.
[b]Data reported includes plasma that was analyzed for mineral concentration in Year 1 only.
[c]ORG = Organic treatment (33% organic, 67% inorganic Cu, Zn, and Mn).
[d]ING = Inorganic treatment (100% $CuSO_4$, $ZnSO_4$, and $MnSO_4$).
[e]SEM = Standard error of the mean.

Table 4.7. Effect of trace mineral supplementation and source on liver mineral concentration in feedlot cattle[a,b]

Item	Dietary treatment				Contrasts ($P <$)	
	Control	ORG[c]	ING[d]	SEM[e]	Cont vs Suppl	ORG vs ING
Cu, mg/kg DM						
d 0 growing	70.7	81.3	141.3	17.23	0.01	0.25
d 56 growing	211.4	321.2	323.5	17.06	0.01	0.24
d 112 or 140 finishing[f]	183.2	291.7	287.9	16.94	0.01	0.15
Zn, mg/kg DM						
d 0 growing	100.4	102.3	100.0	3.88	0.88	0.69
d 56 growing	88.2	90.2	93.4	3.96	0.46	0.58
d 112 or 140 finishing[f]	91.0	86.7	85.1	3.88	0.29	0.78
Mn, mg/kg DM						
d 0 growing	7.59	7.85	7.71	0.457	0.75	0.84
d 56 growing	7.96	7.61	7.50	0.466	0.48	0.88
d 112 or 140 finishing[f]	5.35	7.24	6.49	0.466	0.02	0.26

[a]Least squares means.
[b]There was no treatment × yr interaction ($P > 0.10$) for liver Cu, Zn, or Mn concentrations, therefore data are combined for both years.
[c]ORG = Organic treatment (33% organic, 67% inorganic Cu, Zn, and Mn).
[d]ING = Inorganic treatment (100% $CuSO_4$, $ZnSO_4$, and $MnSO_4$).
[e]SEM = Standard error of the mean.
[f]Liver biopsy samples were collected on d 112 (Year 1) and d 140 (Year 2) of the finishing phase.

Table 4.8. Effect of trace mineral supplementation and source on carcass characteristics of feedlot cattle[a]

	Dietary treatment				Contrasts ($P <$)	
Item[b]	Control	ORG[c]	ING[d]	SEM[e]	Cont vs Suppl	ORG vs ING
HCW, kg	330.0	325.4	330.0	6.17	0.76	0.61
DP, %	60.52	60.09	60.62	0.290	0.66	0.22
REA, cm^2	74.27	76.98	76.54	1.566	0.21	0.85
BFAT[f], cm	1.23	1.41	1.29	0.103	0.33	0.40
KPH fat, %	1.97	1.98	2.01	0.067	0.64	0.53
YG[g]	3.20	3.05	3.25	0.106	0.73	0.20
Marbling score[h]	398.5	406.9	418.7	9.39	0.23	0.38

[a]Least squares means.

[b]HCW = hot carcass weight; DP = dressing percent; REA = ribeye area; BFAT = backfat; KPH = kidney, pelvic, and heart; YG = calculated yield grade.

[c]ORG = Organic treatment (33% organic, 67% inorganic Cu, Zn, and Mn).

[d]ING = Inorganic treatment (100% $CuSO_4$, $ZnSO_4$, and $MnSO_4$).

[e]SEM = Standard error of the mean.

[f]Backfat measurement collected and adjusted at the 12^{th} rib.

[g]Yield grade was calculated using the formula: yield grade = 2.5 + (2.5 × adjusted backfat) + (0.2 × percent kidney, pelvic, and heart fat) – (0.32 × ribeye area) + (0.0038 × hot carcass weight).

[h]Marbling scores: 400 = slight; 500 = small; 600 = modest.

Table 4.9. Effect of trace mineral supplementation and source on fatty acid composition in longissimus dorsi samples[a]

	Dietary treatment				Contrasts ($P <$)	
Item[b]	Control	ORG[c]	ING[d]	SEM[e]	Cont vs Suppl	ORG vs ING
Fatty acid C14:0, %	2.73	2.68	2.74	0.152	0.91	0.77
Fatty acid C16:0, %	20.63	20.28	22.07	1.075	0.69	0.25
Fatty acid C18:0, %	12.38	11.93	12.03	0.372	0.39	0.86
Fatty acid C18:1, %	34.10	35.02	35.10	1.167	0.51	0.97
Fatty acid C18:2, %	2.66	2.68	2.61	0.142	0.92	0.71
Fatty acid C18:3, %	0.73	0.87	0.76	0.099	0.51	0.44
Fatty acid C20:4, %	0.42	0.36	0.42	0.032	0.52	0.21
Unsaturated fatty acids, %	37.89	38.91	38.86	1.17	0.49	0.98
Saturated fatty acids, %	35.75	34.89	36.85	1.17	0.94	0.25
Ratio of unsaturated to saturated fatty acids	1.07	1.12	1.07	0.032	0.63	0.31
Monounsaturated fatty acids, %	34.10	35.02	35.10	1.17	0.51	0.97
Polyunsaturated fatty acids, %	3.78	3.88	3.75	0.210	0.89	0.67

[a]Least squares means.
[b]Reported as a percent, except for the ratio of unsaturated to saturated fatty acids.
[c]ORG = Organic treatment (33% organic, 67% inorganic Cu, Zn, and Mn).
[d]ING = Inorganic treatment (100% $CuSO_4$, $ZnSO_4$, and $MnSO_4$).
[e]SEM = Standard error of the mean.

CHAPTER V

EFFECT OF COPPER STATUS, SUPPLEMENTATION, AND SOURCE ON PITUITARY RESPONSIVENESS TO EXOGENOUS GONADOTROPIN RELEASING HORMONE IN OVARIECTOMIZED BEEF COWS

ABSTRACT

The effect of copper (Cu) status/supplementation and source on pituitary responsiveness to gonadotropin releasing hormone (GnRH) was evaluated using 12 multiparous, non-pregnant, non-suckling, ovariectomized Angus cows (7.1 ± 3.3 yr, 622.9 ± 49.8 kg, BCS 6.0 ± 0.5). Nine of the cows were considered Cu deficient (based on liver Cu concentrations < 30 mg Cu/kg) after receiving a low Cu forage-based ration supplemented with known Cu antagonists (5 mg molybdenum/kg diet and 0.3% sulfur) for 216 d. Once Cu deficient, cows were stratified based on age, BW, BCS, and liver Cu concentration and randomly assigned to repletion phase treatments. Treatments included: 1) control (deficient Cu status; no supplemental Cu), 2) organic (ORG; adequate Cu status; 100% organic Cu), and 3) inorganic (ING; adequate Cu status; 100% inorganic $CuSO_4$). Treatments were formulated to meet all NRC (1996) requirements except for Cu, which was supplemented to ORG and ING cows at 10 mg Cu/kg diet. Throughout the 159-d repletion phase, liver biopsy samples were collected to classify Cu status, and

every 14 d cows received a new source of exogenous progesterone intra-vaginally (via CIDRs) to mimic luteal-phase progesterone concentrations and negatively feedback on the hypothalamic-pituitary axis. During the repletion phase liver Cu concentrations were not different ($P > 0.10$) between ORG and ING cows at any time. By d 77 of the repletion phase all supplemented cows were considered Cu adequate, and liver Cu concentrations were greater ($P < 0.05$) in supplemented cows vs. non-supplemented controls on d 77, and were greater ($P < 0.01$) throughout the repletion phase. On d 99, the first of two experiments was initiated, which involved the administration of GnRH to all cows at low (0, 3, and 9 μg; Experiment 1) and high amounts (0, 27, and 81 μg; Experiment 2). Cows were catheterized every fifth day, and blood samples were collected at 15 min intervals for 1 h prior to GnRH administration (to establish basal LH concentration) and for 4 h after GnRH administration and analyzed for luteinizing hormone (LH) concentration. In Experiment 1, Cu status/supplementation did not affect ($P > 0.10$) basal or peak LH release, but total LH released tended ($P < 0.07$) to be greater in supplemented cows vs. non-supplemented controls at 3 μg GnRH, but was not different ($P > 0.10$) at either 0 or 9 μg GnRH. Similarly, in Experiment 2 there was no effect ($P > 0.10$) of Cu supplementation or source on basal, peak, or total LH released. Concentrations of LH in pituitary tissue collected immediately after humane euthanasia were not different ($P > 0.10$) across treatments. Based on these data, neither Cu status/supplementation nor source affected pituitary sensitivity to GnRH or pituitary stores of LH in beef cows.

INTRODUCTION

Copper (**Cu**) is an essential element required for normal reproductive function in beef cattle and other mammalian species (Underwood and Suttle, 1999). However, the mechanism(s) of action has not been clearly identified. Improved reproductive performance due to Cu supplementation (Muehlenbein et al., 2001; Kropp, 1990) and source (Kropp, 1993) have been reported, although due to binomial response variables and limited numbers, interpretation of data is difficult.

Phillippo et al. (1987a) proposed that depressed reproductive function associated with Cu deficiency may actually be due to a direct effect of supplementation of high concentrations of the known Cu antagonist molybdenum (**Mo**), based on hypothalamic-pituitary axis differences among Cu-deficient heifer calves receiving Mo and iron (**Fe**). Subsequent researchers were unable to remove the confounding effect of Mo on luteinizing hormone (**LH**) and pituitary sensitivity in Cu-deficient dairy steers (Xin et al., 1993); however, early researchers observed that Cu administration (i.v. injection of cupric acetate) caused ovulation in rabbits (Fevold et al., 1936; Suzuki and Bialy, 1964). More recently, researchers reported that gonadotropin releasing hormone (**GnRH**) increased LH and follicle stimulating hormone (**FSH**) release from rat pituitaries when Cu was present in the portal blood (Kochman et al., 1992), possibly by influencing GnRH receptor binding (Kochman et al., 1997) and(or) intracellular calcium activity (Hazum, 1983; Schvartz and Hazum, 1986) in anterior pituitary cells.

Differences in Cu availability in cattle have been reported, including greater availability of organic Cu in calves (Nockels et al., 1993) compared to inorganic Cu in the absence of antagonists. However, no differences in Cu availability between organic

and inorganic sources were observed in steers fed a high Mo diet (Wittenberg et al., 1990).

Since Cu can affect reproduction in beef cows and the hypothalamic-pituitary axis in other species, availability may differ among Cu sources, and Cu-adequate and Cu-deficient mature beef cows have not been compared in the absence of antagonist(s), we hypothesized that pituitary sensitivity to GnRH (as determined by LH release from the anterior pituitary after exogenous GnRH administration) will vary with Cu status, supplementation, and source. Therefore, the objectives of this experiment were to examine the effects of Cu in ovariectomized beef cows on the amount of LH: 1) released due to exogenous GnRH at low concentrations, 2) released due to exogenous GnRH at high concentrations, and 3) stored in the anterior pituitary.

MATERIALS AND METHODS

Experimental Animals. Prior to the initiation of this experiment, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee. Twelve mature, cycling, multiparous, non-pregnant, and non-suckling purebred Angus cows (7.1 ± 3.3 yr, 622.9 ± 49.8 kg, BCS 6.0 ± 0.5) from the Colorado State University (**CSU**) Beef Improvement Center (Saratoga, WY) were used for this experiment. Cows were transported to the CSU Agricultural Research, Development, and Education Center (**ARDEC**) and placed into one 7 m × 40 m feedlot pen equipped with an automatic waterer and concrete bunk. Immediately after arrival an initial BW (average BW collected over 2 d) and BCS (scale of 1 to 9, 1 = emaciated, 9 = obese; Richards et al., 1986) were collected, and the Cu status of each cow

was determined via the collection of a liver biopsy and blood sample. The liver biopsy sample was collected using the true-cut technique described by Pearson and Craig (1980), as modified by Engle and Spears (2000). Immediately post-collection, samples were rinsed with 0.01 M PBS solution, placed into acid-washed polypropylene tubes, capped, placed on ice for approximately 1 h, transported to the laboratory, and stored at –20°C. Liver samples were analyzed for Cu concentration as described by Engle et al. (1997). At the same time that liver biopsy samples were collected, blood samples were collected via jugular venipuncture into heparinized, trace mineral-free vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ). Once collected, blood samples were placed on ice for approximately 1 h, transported to the laboratory, centrifuged at 2000 × *g* for 15 min at room temperature, and plasma was transferred to acid-washed polypropylene storage vials and stored at –20°C. Plasma Cu concentrations were determined as described by Ahola et al. (2004).

All cows were ovariectomized via a standing flank procedure (Youngquist et al., 1995) 13 d after arrival to the feedlot to eliminate the effects of ovarian hormones on hypothalamic and pituitary sensitivity to GnRH (Martin et al., 1988). Briefly, the ovariectomy site was clipped of hair, scrubbed three times with povidone iodine solution (Agri Laboratories, Ltd., St. Joseph, MO) and 70% ethyl alcohol (Agri Laboratories, Ltd., St. Joseph, MO), and locally anesthetized with 50 ml of 2% lidocaine (Pro Labs Ltd., St. Joseph, MO) injected subcutaneously in an inverted-L pattern. After the onset of local anesthesia, a 20 cm incision was made (centered in the left paralumbar fossa area) that penetrated the abdominal cavity in order to remove both ovaries. An umbilical cord clamp (Double-grip umbilical cord-clamp, Hollister Inc., Libertyville, IL) was placed

around the mesovarium proximal to the ovary, so that only the ovarian pedicle was included, and then clamped shut. The ovary was then severed with an ecraseur and removed. The procedure was repeated for each ovary. Muscle incisions were then closed with absorbable sutures and skin incisions were closed with polyamide thread, non-absorbable sutures (Braunamid; Tuttlingen, Germany). Banamine (Flunixin Meglumine; 1.1 mg/kg BW, i.m.) was administered once immediately post ovariectomy and all cows were administered penicillin for three consecutive days.

Throughout the entire experiment, cow BW and BCS data were collected. Body condition scores were assigned by the same technician throughout the experiment.

Depletion Phase. In an attempt to attain Cu deficiency (liver < 30 mg of Cu/kg DM, Mills, 1987) in all cows, a ration was developed to decrease Cu availability from forages. To accomplish this, a forage-based basal ration (50% ground alfalfa, 50% ground corn stalks, as-fed basis; average daily dry matter intake = 8.0 kg/hd; Table 5.1) with low Cu and a corn-based supplement (0.54 $kg \cdot hd^{-1} \cdot d^{-1}$; DM basis) with 5 mg Mo/kg diet (DM basis) and 0.3 % sulfur (S; DM basis) were used to decrease Cu status. Due to the presence of elevated concentrations of Mo and S in the rumen, thiomolybdates will form and dramatically reduce the availability of Cu in the gastrointestinal tract (Suttle, 1991). On a daily basis, supplement was fed in the concrete feed bunk approximately 1 h prior to delivery of the forage-based ration to ensure individual animal intake of the supplement. Basal forage and water trace mineral concentrations were determined using samples collected from ground alfalfa hay, ground corn stalks, and water sources. Mean trace mineral concentrations (± SD) in the basal ration were: alfalfa hay = 6.5 ± 4.1 mg Cu/kg of DM, 0.30 ± 0.13 % S, 3.4 ± 3.1 mg Mo/kg of DM; corn stalks = 2. 6 ± 0.2 mg Cu/kg

of DM, 0.067 ± 0.01 % S, < 1.0 mg Mo/kg of DM; and water = 0.01 μg Cu/L, 0.02 % S, < 0.02 μg Mo/L. Mean trace mineral concentrations (± SD) in the depletion-phase corn-based supplement were: 19.05 ± 18.2 mg Cu/kg of DM, 5.87 ± 0.51 % S, 105.8 ± 52.91 mg Mo/kg of DM, and 243.3 ± 31.24 mg Fe/kg of DM.

To ensure daily individual intake of the supplement, 76 d after arrival at the feedlot, all cows were moved from the group pen to individual pens (2 m × 15 m) where they continued to receive the basal ration and supplement. On a daily basis, each cow received 0.54 $kg \cdot hd^{-1} \cdot d^{-1}$ of supplement approximately 1 to 2 h prior to delivery of the forage-based ration. To monitor the Cu status of each animal during the depletion phase, liver biopsy and blood samples were collected on d 55, 125, and 216 post-initiation of the depletion phase and analyzed for Cu concentration, as previously described. After cows received the depletion diet for 216 d, nine cows were classified as Cu deficient based on liver Cu concentration (liver < 30 mg of Cu/kg DM, Mills, 1987). Immediately after liver biopsy samples were analyzed, and nine of the original 12 cows were deemed Cu deficient, the depletion phase was terminated and the repletion phase was initiated. The three cows that failed to reach a Cu deficient status were removed from the experiment and their depletion phase data were not included in the analysis or results of this experiment.

Repletion Phase. The nine Cu-deficient cows were stratified based on age, BW, BCS, and liver Cu concentration and randomly assigned to one of three treatments: 1) control (deficient Cu status; no supplemental Cu; n = 3), 2) organic (**ORG**; adequate Cu status; 100% organic Cu; n = 3), and 3) inorganic (**ING**; adequate Cu status; 100% inorganic $CuSO_4$; n = 3). All cows were fed to meet NRC (1996) requirements for all trace

minerals (fed as inorganic) with the exception of Cu. Animals receiving the control treatment did not receive any supplemental Cu, while inorganic Cu was supplemented as $CuSO_4$ and organic Cu was provided from a commercially available mineral proteinate source (Bioplex^{TM} trace mineral, Alltech Inc., Nicholasville, KY). Cows receiving either the ING or ORG treatments received Cu at the NRC (1996) recommended concentration of 10 mg Cu/kg of diet DM in the form of a corn-based supplement. Trace mineral concentrations of the three treatments were: Control = 29.1 mg Cu/kg of DM, 0.287 % S, 620.0 mg Zn/kg of DM, <1.0 mg Mo/kg of DM, 146.0 mg Fe/kg of DM; ORG = 209.0 mg Cu/kg of DM, 0.271 % S, 649.0 mg Zn/kg of DM, <1.0 mg Mo/kg of DM, 147.0 mg Fe/kg of DM; and ING = 184.0 mg Cu/kg of DM, 0.256 % S, 676.0 mg Zn/kg of DM, <1.0 mg Mo/kg of DM, 111.0 mg Fe/kg of DM.

The basal forage-based ration of 50% ground alfalfa and 50% ground corn stalks (as-fed basis) was fed for the first 66 d of the repletion phase. However, to increase the rate of Cu repletion in the ORG and ING cows (by decreasing overall amount of fiber in the ration, a known antagonist of Cu), on d 66 of the repletion phase the basal ration was changed to 100% ground alfalfa (average daily dry matter intake = 8.0 kg/hd). The corn-based supplement that carried each of the three repletion treatments was maintained at a daily consumption of 0.54 kg/d (DM basis).

For the first 42 d of the repletion phase, all treatments included supplemental S (from $CaSO_4$) at NRC (1996) concentrations. After collection and analysis of the first liver biopsy sample for Cu concentration on d 42, and realization that liver Cu concentrations in cows receiving the ORG and ING treatments were not responding to the supplemental Cu (most likely due to the presence of elevated S concentrations in the total diet), all

supplemental S was removed from the three treatments and new batches were reformulated to meet NRC (1996) recommended concentrations for all minerals with the exception of S. Treatments were analyzed for trace mineral concentrations as described previously.

Copper status of each animal was monitored during the repletion phase via the collection and analysis of liver biopsies and blood samples. After receiving the repletion diet for 77 d, all cows receiving the ORG and ING treatments were considered adequate in liver Cu concentrations (Table 5.2; liver > 30 mg Cu/kg DM, Mills, 1987) while cows receiving the Control treatment were still classified as deficient in Cu, based on low liver Cu concentrations (liver < 30 mg Cu/kg DM, Mills, 1987). Immediately after liver biopsy samples collected on d 77 of the repletion phase were analyzed, and cows were classified as adequate or deficient in Cu, the responsiveness of the anterior pituitary to exogenous GnRH was evaluated in two experiments on d 99.

Pituitary Responsiveness. After receiving treatments for 99 d, cows were subjected to two dose-response experiments involving the administration of exogenous GnRH at low (Experiment 1; 0, 3, 9 μg GnRH) and high (Experiment 2; 0, 27, and 81 μg GnRH) concentrations. Circulating concentrations of LH released by the anterior pituitary in response to GnRH were determined from blood samples collected immediately before and after GnRH administration, since secretion of LH is completely synchronized with GnRH presence in the portal blood (Clarke and Cummins, 1982). This analysis of LH concentration allowed direct evaluation of sensitivity of the anterior pituitary to GnRH. To ensure that all cows remained within their classification as Cu adequate (ORG and ING) or Cu deficient (Control), blood and liver biopsy samples were collected

immediately prior to Experiment 1, in between Experiments 1 and 2, and after Experiment 2. Experiments 1 and 2 were completed over a 26-d period (d 99 through 126 of the repletion phase).

Since cows had been ovariectomized, no endogenous ovarian hormones were present to negatively feedback on the hypothalamic-pituitary axis and affect the release of GnRH or LH. However, in the absence of negative feedback from ovarian hormones, circulating concentrations of LH increase dramatically (Reeves et al., 1972). Thus, utilizing an ovariectomized cow as a model for an intact cow to evaluate a treatment difference in the sensitivity of the anterior pituitary to GnRH (as measured by circulating LH concentrations) would be difficult, possibly even impractical. Therefore, 19 d prior to the initiation of repletion phase, exogenous progesterone cattle inserts (1.38 g progesterone/insert; EAZI BREED CIDR, Pharmacia-UpJohn, Kalamazoo, MI) were inserted vaginally into all cows in order to provide an exogenous source of progesterone to negatively feedback on the hypothalamic/pituitary axis that would be similar in all cows. Inserts were replaced every 14 d with new inserts throughout the repletion phase, and were removed from all cows approximately 2 h prior to euthanasia at the end of the experiment.

Experiment 1. In a Latin square design, three low concentrations of GnRH (Cystorelin, Merial, Iselin, NJ) were randomly administered (i.v.) to all cows [0 (sterile water), 3, and 9 μg] once every 5 d throughout an 11 d period beginning 99 d after the repletion phase began. Concentrations of GnRH were randomly assigned to cows so that all concentrations were administered to all treatments on each day that cows were challenged. Gonadotropin releasing hormone from the stock solution (50 μg/ml

gonadorelin diacetate tetrahydrate; Cystorelin, Merial, Iselin, NJ) was diluted with sterile water (Pro Labs Ltd., St. Joseph, MO) to create the appropriate concentrations of 3 and 9 μg of GnRH. The 0 μg GnRH concentration contained 100% sterile water (Pro Labs Ltd., St. Joseph, MO. The total volume administered, regardless of GnRH concentration, was 5 ml. The GnRH used in these experiments has been approved for the treatment of cystic ovaries in cattle (i.m. or i.v.) at a dose of 100 μg (2 ml total volume; NADA 098-379, List No. 8283-03, Merial, Iselin, NJ).

Approximately 24 h prior to each GnRH administration, all cows were nonsurgically fitted with indwelling jugular catheters. Jugular catheter insertion sites were clipped of hair, scrubbed three times with povidone iodine solution (Agri Laboratories, Ltd., St. Joseph, MO) and 70% ethyl alcohol (Agri Laboratories, Ltd., St. Joseph, MO) and the area was locally anesthetized with 3 ml of lidocaine (Pro Labs Ltd., St. Joseph, MO). Catheter tubing (1.016 mm i.d., 1.778 mm o.d., 270 cm long; Tygon tubing, Saint-Gobain Performance Plastics, Akron, OH) was inserted through a 12-guage needle inserted into jugular vein, and the tubing was threaded 25 cm into the jugular vein. The 12-gauge needle was removed and patency was maintained with sterile 3.5% sodium citrate saline solution. The catheter was plugged using a blunted (2 cm long; 18 gauge) disposable hypodermic needle inserted into the free end of the catheter with the tip of a tuberculin syringe (cut and heat-sealed) used to prevent leaking. The catheter was kept in place at the site of entrance into the jugular with a foam patch (6 cm × 6 cm) that was glued (Kamar Adhesive, Kamar, Inc., Steamboat Springs, CO) flat against the neck and with a small slit in the center for the catheter. A second foam patch was glued against the first to keep the catheter in place. The free end of the catheter was extended toward the top of

the animal's neck (approximately 6 cm anterior to the poll), and the neck was wrapped with bandaging tape (3M Vetrap, 3M Animal Care Products, St. Paul, Mn) and elastic bandage with adhesive (Elastikon, Ethicon Inc., Somerville, NJ) to protect the catheter, which was coiled and stored in a denim pouch secured to the top of the animal's neck. After catheter insertion, cows were housed overnight in a group pen (50 m × 30 m) and were given ad libitum access to water only. Early the next morning cows were restrained in a squeeze chute and catheters were removed from the denim pouches, tested for patency, and extended down the topline of each cow. Cows were then placed into covered AI palpation chutes (1 m × 3 m) and blood samples were obtained (5 ml/sample; via the catheter) at 15 min intervals for a period of 5 h. Patency was maintained throughout the 5 h blood collection period with sterile 3.5% sodium citrate in saline. Five blood samples were collected from each cow at 15 min intervals for 1 h before GnRH administration (–60, –45, –30, –15, and 0 min) in order to determine baseline basal LH concentrations. Immediately after the 0 min sample was collected, one of the three randomly assigned concentrations of GnRH (0, 3, or 9 μg) was administered slowly (approximately 20 to 30 s) through the catheter (i.v.) prior to re-filling the catheter with sodium citrate to maintain patency. Beginning 15 min after GnRH infusion, sixteen blood samples were collected at 15 min intervals for 4 h (+15, +30, +45, +60, +75, +90, +105, +120, +135, +150, +165, +180, +195, +210, +225, and +240 min; Reeves et al., 1970). Blood was collected into 12 cc syringes and immediately transferred to pre-labeled disposable borosilicate glass culture tubes (13 mm × 100 mm; Fisher Scientific, Pittsburgh, PA). Samples were placed on ice for approximately 1 h, allowed to clot, centrifuged on-site at 3,0000 × *g* for 20 min at 4°C, and serum was transferred into

polystyrene tubes (12 mm × 75 mm, Fisher Scientific, Pittsburgh, PA). Harvested serum was then placed on ice for approximately 4 h, transferred to the laboratory, and stored at –20°C. After the sixteenth blood sample was collected on each day, cows were released from the AI palpation chutes and restrained in a squeeze chute for removal of catheters prior to returning cows to their individual pens for access to the daily ration and supplement. On the fourth and ninth days after the first catheters were removed (d 103 and 108 of the repletion phase) catheters were re-inserted into cows on alternating sides of the neck using the procedure described previously.

Experiment 2. In a Latin square design as described in Experiment 1, in Experiment 2 three high concentrations of GnRH were administered (i.v.) to all cows [0 (sterile water), 27, and 81 μg] once every 5 d throughout an 11 d period beginning 6 d after the completion of Experiment 1 (d 115, 120, and 125 of the repletion phase). Concentrations of GnRH were randomly assigned to cows so that all concentrations were administered to all treatments at each GnRH administration. All catherization, blood collection, and blood analysis procedures performed in Experiment 2 were identical to those described for Experiment 1, except that greater concentrations of GnRH were administered; however, the same total volume (5 ml) administered in Experiment 1 was also administered in Experiment 2.

Pituitary Tissue Collection. Thirty four days after the completion of Experiment 2 (d 159 of the repletion phase) all cows were transported to the CSU commercial packing facility (10 km) and humanely euthanized. Immediately following exsanguinations (approximately 15 min) the anterior pituitary gland tissue was harvested from each cow. Briefly, the pituitary gland from each cow was collected, trimmed, hemisected

midsagitally, and each half was wrapped in aluminum foil and immediately frozen in liquid nitrogen. After approximately 6 h in liquid nitrogen, frozen pituitary samples were transported back to the laboratory and stored at –80°C. Prior to determination of pituitary LH concentrations, samples were thawed at room temperature, and 250 mg of chilled wet tissue was weighed and homogenized in 750 µl of chilled PBS (0.15 M) with a hand-held homogenizer (PowerGen 125, Fisher Scientific, Pittsburgh, PA) in a 1.5 mL micro-centrifuge tube (Eppendorf, Brinkman Instruments Inc., Westbury, NY). Homogenized samples were then centrifuged at 12,000 × *g* for 15 min at 4°C. The supernatant was removed from each sample and stored at –80°C. Supernatant was then thawed at room temperature prior to determination of LH concentration.

Assays for LH Concentrations. Concentrations of LH in serum and pituitary samples were determined via a double antibody RIA (Niswender et al., 1969). Serum LH concentration was determined from 100 µl diluted in 400 µl of 1% PBS gel in polypropylene tubes (12 mm × 75 mm; Becton Dickinson, Franklin Lakes, NJ). Pituitary LH concentrations were determined from 100 µl diluted supernatant (diluted 1:100,000 in 1% PBS gel) in 400 µl of 1% PBS gel. Serum and pituitary samples were prepared in duplicate with an automated pipetting system. At the initiation of each assay, 200 µl of primary antibody was added, and tubes were incubated at 4°C for 24 h before 100 µl of radiolabelled (^{125}I) ovine LH was added. After another 24-h period of incubation at 4°C, 200 µl of a second antibody (anti-rabbit gamma globulin) was added, and samples were incubated for 72 h at 4°C. After incubation, 3 ml of cold PBS was added to all tubes (except for total count tubes) prior to centrifugation at 2,500 × *g* for 30 min. Supernatant

was removed from all tubes via pouring and blotting, and tubes were analyzed for LH concentration.

Analysis of serum samples was completed in four assays from Experiment 1 and five assays for Experiment 2. All pituitary samples were analyzed in one assay. A different set of bovine LH standards was used for each experiment, however the pituitary samples were assayed with the same standards used for Experiment 2 samples. The sensitivities of the assays were 0.15 and 0.28 ng/tube for Experiments 1 and 2, respectively. The intra- and interassay CV were 5.87 and 9.72% for Experiment 1, and 4.41 and 14.38 % for Experiment 2, respectively. Analysis of all count data was performed using RIANAL software.

Once LH concentrations were determined for each serum sample, the maximum concentration of LH at peak surge during the 4 h post-GnRH administration was determined. In addition, the total amount of LH released during the 4 h period following GnRH administration (area under curve) was calculated using the trapezoid rule in SigmaPlot (SPSS Inc., Chicago, IL).

Statistical Analysis. Copper status (liver and plasma Cu concentrations) and cow performance data (BW and BCS change) were assessed using a restricted maximum likelihood-based, mixed-effects model, repeated measures analysis (PROC MIXED, SAS Inst. Inc., Cary, NC). Initial models for Cu status and performance contained the fixed effects of treatment, time, and a treatment × time interaction, and animal was included as a random effect. A spatial power covariance structure was used in the analysis and the containment approximation was used to calculate denominator degrees of freedom. All LH concentation data (compiled via analysis of serum and pituitary tissue) were log

transformed prior to statistical analysis and determination of P-values; however, calculated raw means for LH concentrations (with standard errors calculated using non-transformed data) are reported in the tables and figures. Basal LH concentration data and data characterizing response to GnRH (peak LH release and area under the curve) were analyzed using a mixed-effects model (PROC MIXED, SAS Inst. Inc., Cary, NC). Initial models for response to GnRH included the fixed effects of treatment, dose sequence, and the interaction, and animal was included as a random effect. Animal was used as the experimental unit for all analyses. When an interaction was not significant, it was removed from the model and the reduced model was run. Differences among means were determined using preplanned single degree of freedom contrasts; comparisons made were: 1) Control vs. supplemented cattle, and 2) ORG vs. ING.

RESULTS AND DISCUSSION

Depletion Phase

Liver Mineral Status. Initial liver Cu concentrations were higher than anticipated (Figure 5.1), but not different ($P > 0.10$) across treatments. Since initial liver Cu concentrations were greater than 100 mg Cu/kg, cows were fed the depletion ration for a period of 216-d, until liver Cu concentrations were considered deficient (< 30 mg Cu/kg liver Cu; Mills, 1987). Throughout this 216-d period, mean loss of Cu from the liver averaged 0.49 ± 0.12 mg $Cu \cdot kg^{-1} \cdot d^{-1}$.

Previously reported rates of liver Cu loss during a depletion phase have been variable. Similar to the current experiment, liver Cu concentrations decreased approximately 0.27 mg $Cu \cdot kg^{-1} \cdot d^{-1}$ (from approximately 55 to 38.9 mg Cu/kg) during a 60-d period when Mo

(from Na_2Mo_4) was supplemented at 1.5 times the concentration of Cu in the forage (forage Cu = 1.54 mg Cu/kg) and S supplemented at 0.3% of the diet DM in pregnant Hereford × Angus heifers (Arthington et al., 1995). However, in young Hereford × Holstein heifer calves, supplementation of 5 mg Mo/kg diet (from Na_2Mo_4) alone decreased liver Cu approximately 1.88 mg $Cu \cdot kg^{-1} \cdot d^{-1}$ (from approximately 124.7 to 19.5 mg Cu/kg) over a 56-d period, and approximately 0.26 mg $Cu \cdot kg^{-1} \cdot d^{-1}$ throughout the next 56 d, resulting in liver Cu concentrations of 4 mg Cu/kg after 224 d of depletion via Mo supplementation (Phillippo et al., 1987b). The authors reported that these calves were fed a low Cu diet prior to initiation of the experiment, that started when calves were 91 to 133 d of age, which may have impacted the rate of liver Cu loss, and calves also had a small body mass compared to cattle in the current experiment. A substantial rate of liver Cu loss was also reported in young Hereford × Holstein heifer calves (122 to 175 d old), where liver Cu decreased approximately 1.37 mg $Cu \cdot kg^{-1} \cdot d^{-1}$ (from approximately 104.8 to 28.3 mg Cu/kg) during 56 d followed by a drop of 0.14 mg $Cu \cdot kg^{-1} \cdot d^{-1}$ during the next 168 d (resulting in liver Cu concentration of less than 4 mg Cu/kg) due to supplementation of 5 mg Mo/kg diet only (Mo source not reported; Humphries et al., 1983). In older dairy steers, supplementation of 10 mg Mo/kg diet [from ammonium Mo = $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$] led to a decrease of approximately 1.34 mg $Cu \cdot kg^{-1} \cdot d^{-1}$ (from over 100 to less than 25 mg Cu/kg) throughout a 56-d period, followed by a drop in liver Cu of 0.05 mg $Cu \cdot kg^{-1} \cdot d^{-1}$ over the subsequent 112 d to less than 20 mg $Cu \cdot kg^{-1}$ (Xin et al., 1993).

The rates of liver Cu loss during a depletion phase discussed above that were substantial (in excess of 1.5 mg $Cu \cdot kg^{-1} \cdot d^{-1}$) during the first 56-d and limited (less than

0.5 mg $Cu \cdot kg^{-1} \cdot d^{-1}$) during the subsequent period, were not observed in the current experiment. These rate differences in liver Cu loss that exist between our data and previous research may be due to one or more factors, which include composition of the basal ration, initial animal liver Cu concentrations, source of Mo, presence of other antagonists (e.g. S) supplemented at elevated concentrations, physiological status of the animal (i.e. growing, gestating, lactating, etc.), breed (dairy vs. beef), age, and(or) body size.

With the exception of depigmentation and defective keratinization, other commonly reported clinical (bone disorders, connective tissue disorders, and diarrhea) and subclinical (growth retardation) symptoms of Cu deficiency (Underwood and Suttle, 1999) were not readily observed, even though all cows in the present study reached Cu deficiency status (based on liver Cu concentrations). Prior to the end of the depletion phase, extremely wavy, harsh, and slightly de-pigmented hair coats were observed in all cows. Humphries et al. (1983) reported limited clinical symptoms of Cu deficiency, including loss of hair pigment and texture, skeletal changes, and a 'stilted' gait, in young heifer calves that received 5 mg Mo/kg diet for 20 weeks. Since cows in the current experiment were mature, bone and growth symptoms commonly observed in growing cattle were not detected. Interestingly, once the repletion phase was initiated and supplemental Mo/S was removed from the diet the hair coat symptoms described above dissipated, including cows receiving the control treatment that remained deficient in Cu for the remainder of the experiment, indicating a possible effect of Mo and S on hair coat apperance.

Cow BW and BCS. No treatment × time interaction was present for BW ($P = 0.75$); however, there was a time effect of BW ($P < 0.01$). During the depletion phase, initial BW was not different ($P > 0.10$) from final BW (Figure 5.2). Relative to BCS, there was no treatment × time interaction ($P = 0.63$), and there was no time effect ($P = 0.21$). Additionally, initial BCS was not different from final BCS ($P > 0.10$) during the depletion phase (data not shown). Since cows were mature, and daily forage intake varied due to changing Colorado weather conditions from late summer through spring, the time effect and variation in BW that occurred over the course of the depletion phase appears normal.

Limited research has addressed changes in BW and BCS to mature beef cows fed a Cu depletion diet. In non-mature cattle, a depletion phase diet had no affect on ADG or final BW in pregnant beef heifers (Arthington et al., 1995) or dairy steers (Xin et al., 1993); however, cattle in both experiments were much younger than the current experiment. In very young calves, ADG decreased substantially (to levels 72% of controls) after 16 and 20 weeks of Mo supplementation in two experiments with non-pregnant heifer calves, possibly due to decreased feed intake (Phillippo et al., 1987a).

Repletion Phase

Liver mineral status. At the initiation of the repletion phase, liver Cu concentrations were not different ($P > 0.10$) across treatments (Table 5.2), however all treatments were at liver Cu concentrations considered deficient (liver < 30 mg of Cu/kg DM, Mills, 1987). As expected, during the 159-d repletion phase, there was a treatment × time interaction ($P < 0.01$) for liver Cu concentration. By d 77 of the repletion phase, all supplemented cows

reached liver Cu concentrations considered adequate (liver > 30 mg of Cu/kg, Mills, 1987) and Cu concentrations were greater ($P < 0.05$) in supplemented than non-supplemented control cows. Supplemented cows also had greater liver Cu concentrations at the initiation of Experiment 1 (d 99; $P < 0.01$), at the initiation of Experiment 2 (d 155; $P < 0.01$), and at the end of the repletion phase (d 159; $P < 0.01$). Liver Cu concentrations were not different ($P > 0.10$) between ORG and ING treatments at any point during the repletion phase. When liver Cu concentrations determined at the initiation of Experiment 1 were compared with Experiment 2 within each treatment, liver Cu concentrations in Experiment 1 were not different ($P > 0.10$) from Experiment 2; however, in ORG cows liver Cu concentrations in Experiment 1 were lower ($P < 0.01$) than Experiment 2 and liver Cu concentrations in Experiment 1 tended ($P = 0.08$) to be lower than Experiment 2 in ING cows. These differences were expected since Cu was being repleted at an increasing rate; however, results of Experiments 1 and 2 cannot be combined since liver Cu concentrations were not the same throughout both experiments.

The magnitudes of response to Cu supplementation by treatments (± SEM) were: control = –0.3 ± 19.65 mg Cu/kg, ORG = 69.2 ± 13.16 mg Cu/kg, and ING = 75.6 ± 6.18 mg Cu/kg. Among these concentrations, supplemented cows had greater ($P < 0.01$) magnitudes of response to Cu supplementation than non-supplemented controls while ORG and ING cows did not differ ($P > 0.10$). During the 159-d repletion phase, the rate of Cu repletion was: Control = –0.002 ± 0.124 mg $Cu \cdot kg^{-1} \cdot d^{-1}$, ORG = 0.44 ± 0.083 mg $Cu \cdot kg^{-1} \cdot d^{-1}$, and ING = 0.48 ± 0.039 mg $Cu \cdot kg^{-1} \cdot d^{-1}$.

In pregnant beef heifers Arthington et al. (1995) reported that by d 45 after initiation of the Cu repletion phase (10 mg Cu/kg diet supplemented via either an organic or

inorganic form), Cu-supplemented heifers had greater liver Cu concentrations than controls not supplemented with Cu but still receiving supplemental Mo at 1.5 times that of the forage Cu concentration. However, unlike the current experiment, no treatment was included which provided no supplemental Cu and Mo/S. The authors reported that the overall magnitude of response to Cu supplementation throughout the 45-d repletion phase did not differ by Cu source (organic vs. inorganic). Although liver Cu concentrations were adequate, maximum concentrations of liver Cu were numerically lower (48.9 and 67.7 mg Cu/kg diet in the inorganic and organic Cu treatments, respectively) than those observed in the current experiment.

Relative to the availability of Cu, several experiments have been reported; however, results have varied. In young Cu-deficient calves (induced by Mo from ammonium Mo), organic Cu had greater availability than inorganic in the presence of Mo (Kincaid et al., 1986). However, in older steers depleted of Cu (via the consumption of hay with elevated Mo concentrations), no difference in Cu availability between sources (organic vs. inorganic) in the presence of Mo was reported (Wittenberg et al., 1990). In pregnant beef heifers, when no supplemental Mo or S were provided, no differences in Cu availability by source was observed (Arthington et al., 1995).

The rate of liver Cu repletion was slower than has previously been reported in beef cattle, after a repletion phase. In pregnant heifers over a 45-d period, Arthington et al. (1995) reported repletion rates of 0.95 (first 14 d) to 1.45 (next 31 d) mg $Cu \cdot kg^{-1} \cdot d^{-1}$ in heifers receiving 10 mg Cu/kg diet (in either organic or inorganic forms) after becoming Cu deficient (average liver Cu concentration = 38.9 mg Cu/kg) due to Mo/S supplementation. However, the authors also reported liver Cu gains of 0.1 (first 14-d

period) to 2.1 (next 31-d period) mg $Cu \cdot kg^{-1} \cdot d^{-1}$ in heifers supplemented with 8 mg Cu/kg inorganic Cu after beginning the supplementation period with an adequate liver Cu status (128 mg Cu/kg). It is unknown why rates of liver Cu repletion between the current experiment and previously reported research are inconsistent; however, variables including animal size, age, stage (pregnant, growing, lactating), diet consumed, and previous nutritional history may affect the rate of Cu repletion into the liver.

Cow BW and BCS. Body weight and BCS data during the repletion phase are presented in Table 5.3. During the repletion phase, there were no treatment × time interactions ($P > 0.10$) for cow BW or BCS; however, a time effect was present ($P < 0.01$) for BW but not ($P > 0.10$) for BCS. Initial repletion phase BW and BCS were not different ($P > 0.10$) across treatments. Repletion phase initial BW and BCS were not different ($P > 0.10$) from final BW and BCS within the Control or ING treatments; however, BW increased ($P < 0.05$) from initial to final in the ORG cows, while BCS was not different ($P > 0.10$). At the start of Experiment 1, neither BW nor BCS was different between supplemented and non-supplemented controls ($P > 0.10$), or between ORG and ING cows ($P > 0.10$). Similarly, at the start of Experiment 2 neither BW nor BCS was affected by Cu status or source ($P > 0.10$), and final BW and BCS (collected on d 159 of the repletion phase) were not different between supplemented and non-supplemented control cows ($P > 0.10$) or between ORG and ING cows. There is little research by others on BW and BCS changes due to Cu repletion after cattle became deficient in Cu due to the feeding of known Cu antagonists.

Pituitary Sensitivity to GnRH.

Experiment 1. Sensitivity of the anterior pituitary gland to low concentrations of exogenous GnRH (0, 3, and 9 μg) was evaluated via the collection and analysis of LH data, including basal LH concentration, LH peak amplitude, and total LH released (area under curve) during the 4 h period (Table 5.4). Basal LH concentrations present prior to infusion of GnRH were not affected by either Cu status ($P > 0.10$) or source ($P > 0.10$) in Experiment 1, and were not affected ($P > 0.10$) by the concentration of GnRH administered. Amplitude of the LH peak surge in response to GnRH was affected ($P < 0.01$) by GnRH concentration but there was no ($P = 0.67$) treatment × GnRH concentration interaction. Mean peak LH released across all treatments (± SEM) was 3.0 ± 0.38, 12.3 ± 2.14, and 39.0 ± 9.20 ng/ml in response to 0, 3, and 9 μg GnRH, respectively. Amplitude of the LH peak surge was not affected ($P > 0.10$) by either Cu status/supplementation or source at GnRH concentrations of 3 and 9 μg; however, at 0 μg GnRH control cows tended ($P < 0.06$) to have greater peak LH concentrations than supplemented cows. However, since no GnRH was administered (sterile water was used), this elevated LH concentration was not in response to the effects of GnRH on the anterior pituitary. Data for time to peak LH (after GnRH administration) were not reported since blood was sampled only once every 15 min, which is too infrequent to characterize time to peak LH accurately. No treatment × GnRH concentration interaction was found ($P > 0.10$) for total LH released; however, GnRH concentration did affect ($P < 0.01$) total LH released in response to GnRH. Mean total LH released across all treatments (± SEM) was 192.0 ± 61.0, 584.0 ± 125.7, and 2,190.5 ± 373.0 $ng \cdot ml^{-1} \cdot min^{-1}$ in response to 0, 3, and 9 μg GnRH, respectively. Total LH released tended ($P < 0.07$) to

be greater in supplemented vs. control cows when 3 μg GnRH was administered; however, Cu supplementation did not affect ($P > 0.10$) total LH release when 9 μg GnRH was administered. Total LH released was also not affected by Cu source at any concentration of GnRH.

Experiment 2. Higher concentrations of exogenous GnRH (0, 27, and 81 μg) were used to evaluate pituitary sensitivity (Table 5.5) at doses similar to those used by cow/calf producers for synchronization of breeding cattle or treatment of a persistent follicle. A dose concentration of 100 μg (in 2 ml total volume) is commonly used for estrous synchronization of beef cows at the time of AI and(or) when initiating a progestin treatment (e.g. MGA) to force ovulation and(or) initiate a new follicular wave. Similarly to Experiment 1, basal LH concentrations before GnRH infusion were similar ($P > 0.10$) across treatments in Experiment 2, and were not affected ($P > 0.10$) by the concentration of GnRH administered. There was no ($P = 0.88$) treatment × GnRH concentration interaction for amplitude of the LH peak surge in response to GnRH, although peak LH was affected ($P < 0.01$) by GnRH concentration. Across all treatments, mean peak LH released (± SEM) was 3.7 ± 1.05, 64.3 ± 8.37, and 65.2 ± 12.41 ng/ml in response to 0, 27, and 81 μg GnRH, respectively. Peak LH released due to 27 μg GnRH was greater ($P < 0.01$) than 0 μg, however peak LH released due to 81 μg GnRH was not different ($P = 0.99$) than 27 μg, indicating that pituitary sensitivity did not increase when the amount of GnRH infused increased three-fold, possibly because pituitary response to GnRH reached saturation. Amplitude of the LH peak surge was not affected ($P > 0.10$) by either Cu status/supplementation or source at GnRH concentrations of 0, 27 or 81ug GnRH. No treatment × GnRH concentration interaction was present ($P > 0.10$) for total LH released

in Experiment 2, however GnRH concentration did affect ($P < 0.01$) total LH released in response to GnRH. Mean total LH released across all treatments (± SEM) was 200.2 ± 99.0, 3,948.0 ± 519.2, and 5,378.2 ± 916.9 $ng \cdot ml^{-1} \cdot min^{-1}$ in response to 0, 27, and 81 μg GnRH, respectively. Total LH released due to 27 μg GnRH was greater ($P < 0.01$) than 0 μg, however total LH released due to 81 μg GnRH was not different ($P = 0.37$) than 27 μg, indicating that total LH released throughout the 4 h period did not increase when the amount of GnRH infused increased three-fold, likely because the majority of LH stores were released when either 27 or 81 μg GnRH was administered. Total LH released was not affected ($P > 0.10$) by status/supplementation or source at 27 or 81 μg GnRH; however, when 0 μg GnRH was administered, ORG cows tended ($P < 0.08$) to have greater total LH release than ING cows, although this was not due to effects of exogenous GnRH on the pituitary, since no GnRH was administered. Total LH released due to 0 μg GnRH was not affected ($P > 0.10$) by Cu supplementation.

The effect of Cu status on LH secretion in dairy steers has been reported (Xin et al., 1993). When serum LH concentrations were evaluated (in the absence of stimulation via exogenous GnRH), calves supplemented with 5 mg Mo/kg diet had lower ($P < 0.09$) serum mean LH concentrations compared to non-supplemented controls or calves receiving 20 mg Cu/kg (0.35, 0.40, and 0.39 ng/ml in Mo-supplemented, control, and Cu-supplemented calves, respectively; Xin et al., 1993). However, the authors reported no difference in LH pulse frequency (pulse/h) after 16 weeks of Cu depletion with Mo supplementation, and no difference in response to GnRH (concentration not reported) based on total LH released and peak LH height. However, the authors acknowledged that the LH concentrations reported for peak LH height and total LH released were

approximately 20-fold lower than previous research (range of 2.5 to 3.0 ng/ml and 165.5 to 195.6 ng/min/ml, for peak LH height and total LH released, respectively), likely due to differences in the purity of the LH standard and the antiserum utilized in the assay. Additionally, since Mo-supplemented calves were Cu deficient while the non-supplemented control and Cu-supplemented calves were not, Cu status was confounded with Mo supplementation, making interpretation of data challenging.

In a second experiment, when Cu-deficient steer calves that received 10 mg Mo/kg diet for 8 months were compared to Cu-adequate calves receiving 20 mg Cu/kg diet, Xin et al. (1993) reported that serum mean LH and LH pulse frequency were not different across treatments. The authors also reported that average LH concentrations declined over the duration of the 112-d experiment due to absence of negative feedback in castrated steers and a possible "adjustment" of the hypothalamic-pituitary axis. In these steers, no effect of exogenous GnRH (concentration not reported) was observed on peak LH height or total LH released. Reported LH concentrations were low (ranges of 3.0 to 3.1 ng/ml and 176.1 to 189.2 ng/min/ml for peak LH height and total LH released, respectively).

Dose response curves were created for peak LH and total LH released for Experiments 1 and 2 separately (Figures 5.3 through 5.6), since data from Experiments 1 and 2 cannot be combined. Maximal LH release in response to GnRH apparently occurred when either 27 or 81 μg GnRH was administered.

Pituitary LH Concentration. Least squares means for LH concentrations in anterior pituitaries collected at the end of the repletion phase were: 1.66, 1.31, and 1.41 mg LH/g wet tissue for Control, ORG, and ING treatments, respectively (standard error of the

mean = 0.17). Based on these data, Cu status/supplementation did not affect ($P = 0.21$) pituitary LH concentration, and pituitary LH concentrations were not different ($P = 0.70$) between ORG and ING cows.

Lower ($P < 0.09$) pituitary LH concentrations were reported in dairy steers receiving 5 mg Mo/kg diet compared to control calves receiving 20 mg Cu/kg diet (154.6, 213.7, and 213.5 μg LH/g wet tissue in Mo-supplemented, non-supplemented control, and Cu-supplemented calves; Xin et al., 1993). However, these concentrations of LH are substantially lower than those reported in the current experiment. The authors explained that their low LH concentrations, in comparison to previous research, may have been due to the fact that their LH concentrations were consistently 20-fold lower due to the level of purification of LH standards and the antiserum used (Xin et al., 1993). Despite these results, the effect of Cu on pituitary LH concentration cannot be assessed since Cu status was confounded by Mo supplemenation. Luteinizing hormone concentrations could have been affected by Cu deficiency and(or) Mo supplementation, since Mo-supplemented cattle were also Cu deficient, while both Cu-supplemented and control cattle had adequate liver Cu concentrations (> 50 mg Cu/kg in non-supplemented control and > 250 mg CU/kg in Cu-supplemented cattle).

Based on our data, Cu status, supplementation and source did not impact pituitary sensitivity to GnRH in the current experiment. However, enhanced reproductive performance in grazing beef cows has been shown due to supplementation (Ahola et al., 2004) and source (Stanton et al., 2000), as seen by increased pregnancy rates early in the breeding season. Based on this evidence, and laboratory animal research where Cu was shown to impact LH release and ovulation, we hypothesized that Cu may affect the

endocrine regulation of estrous cycle by impacting pituitary responsiveness to GnRH, and thus reproductive performance. However, since neither basal LH, peak LH, nor total LH released was affected by Cu supplementation/status or source in a dose-response experiment, and pituitary LH concentrations did not differ, it appears that Cu may be impacting reproduction in areas other than at the level of the hypothalamic-pituitary axis. Other areas may include hypothalamic sensitivity to ovarian hormones, ovarian response to circulating LH or FSH, ovarian activity including follicular and luteal function, uterine repair and functioning, and(or) biological activity of circulating reproductive hormones.

IMPLICATIONS

In multiparous, ovariectomized beef cows, liver copper depletion due to elevated dietary concentrations of molybdenum and sulfur (copper antagonists) was slow. In addition, repletion of liver copper via supplementation was slow, possibly due to carryover effects of molybdenum and(or) sulfur on copper metabolism; however, the availability of organic and inorganic sources of copper did not differ. To avoid a copper deficiency in situations where cows are consuming forages high in copper antagonists, copper status and visual appearance of animals should be monitored more closely than changes in body weight or body condition score. Although benefits of copper supplementation and source on beef cattle reproductive performance have been documented in applied research settings, neither pituitary responsiveness to exogenous gonadotropin releasing hormone nor the amount of luteinizing hormone in the pituitary was affected by copper status/supplementation or source. Since the mechanism(s) of action by which copper affects reproduction do not appear to be at the level of the

hypothalamic-pituitary axis, further research is needed to examine other areas where copper may be affecting reproduction.

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Table 5.1. Ingredient composition, mineral analysis, and proximate analysis of the basal diet[a]

Ingredient	Depletion phase	Repletion phase
Ground alfalfa hay, %	47.56	93.70
Ground corn stalks, %	46.04	--
Corn-based supplement, %:	6.40	6.30
Fine ground corn, %	43.82	54.96
Cracked corn, %	21.58	27.07
CaSO4, %	16.50	--
Na2Mo4, %	0.0201	--
MnSO4, %	0.112	0.111
ZnSO4, %	0.151	0.151
Se (0.16%), %	0.112	0.111
EDDI, %	0.0011	0.0011
$CoCO_4$, %	0.0004	0.0004
Dried molasses, %	17.70	17.60
Mineral analysis of basal diet:		
Ca, %	1.05	1.31
P, %	0.15	0.24
S, %	0.42	0.30
Cu, mg/kg of DM	5.03	7.90
Zn, mg/kg of DM	52.60	58.38
Mo, mg/kg of DM	7.58	3.20
Fe, mg/kg of DM	207.3	127.26
Proximate analysis[b]:		
DM, %	88.01	89.28
CP, %	10.97	18.20
TDN, %	55.61	62.01
CF, %	29.90	27.12
CFAT, %	1.49	2.28
Ash, %	13.99	8.30
NEg, Mcal/kg	0.608	0.797

[a]Dry matter basis.
[c]EDDI = Ethylenediamine dihydroiodide.
[b]DM = Dry matter, CP = Crude Protein, TDN = Total Digestible Nutrients, CF = Crude Fiber, CFAT = Crude Fat.

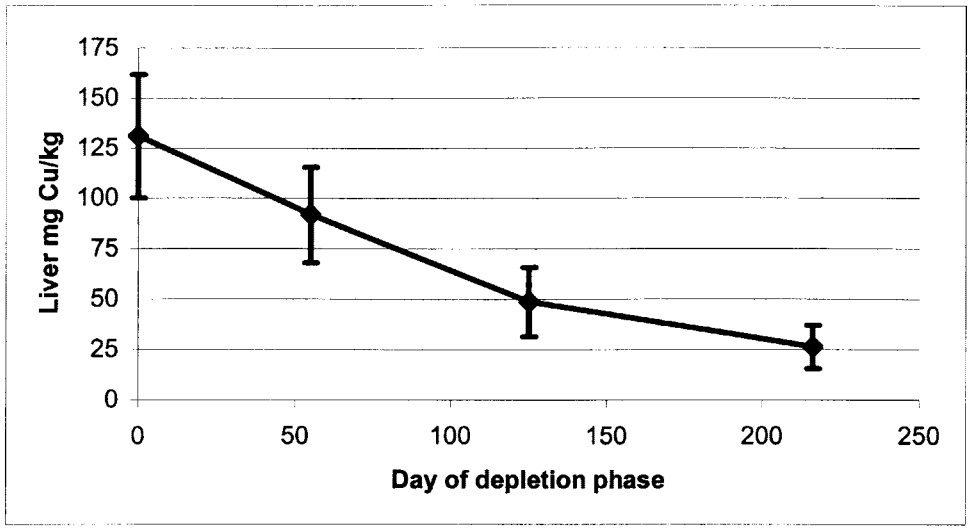


Figure 5.1. Least squares means (± SD) for liver copper concentration change during the 216-day depletion phase

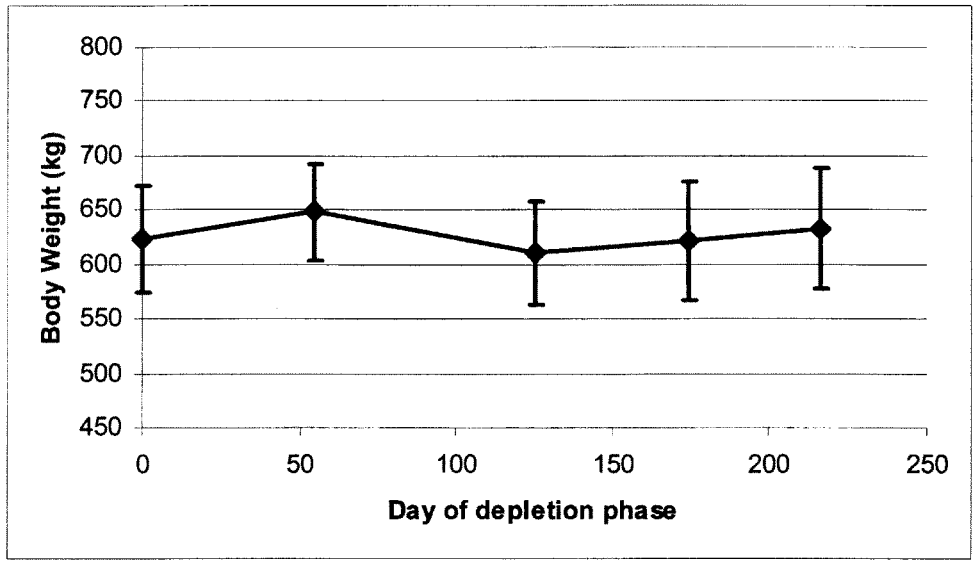


Figure 5.2. Least squares means (± SD) for cow body weight change during the 216-day depletion phase.

Table 5.2. Effect of Cu supplementation and source on liver Cu concentration during the repletion phase[a]

Item	Dietary treatment			SEM[d]	Contrasts ($P <$)	
	Control	ORG[b]	ING[c]		Cont vs Suppl	ORG vs ING
Cu, mg/kg of DM						
Repletion phase start	27.15	26.65	27.03	4.56	0.90	0.81
Experiment 1	11.44	44.74	41.72	5.22	0.01	0.76
Experiment 2	14.20	63.46	57.36	5.36	0.01	0.32
Repletion phase end	24.19	92.94	102.29	4.81	0.01	0.38

[a]Least squares means.
[b]ORG = Organic treatment (100% organic Cu).
[c]ING = Inorganic treatment (100% $CuSO_4$).
[d]SEM = Standard error of the mean.

Table 5.3. Effect of Cu supplementation and source on cow body weight and body condition score[a]

	Dietary treatment				Contrasts ($P <$)	
Item	Control	ORG[b]	ING[c]	SEM[d]	Cont vs Suppl	ORG vs ING
Repletion phase, start						
BW, kg	640.2	610.6	647.7	33.02	0.81	0.49
BCS, kg	6.02	5.99	6.65	0.27	0.51	0.27
Experiment 1						
BW, kg	628.8	611.37	645.4	33.02	1.00	0.46
BCS, kg	6.02	5.99	6.65	0.27	0.51	0.27
Experiment 2						
BW, kg	642.4	620.4	651.5	33.02	0.89	0.55
BCS, kg	6.02	5.99	6.65	0.27	0.51	0.27
Repletion phase, end						
BW, kg	647.7	626.5	675.0	33.02	0.95	0.33
BCS, kg	6.02	6.32	6.65	0.27	0.19	0.42

[a]Least squares means.
[b]ORG = Organic treatment (100% organic Cu).
[c]ING = Inorganic treatment (100% $CuSO_4$).
[d]SEM = Standard error of the mean.

Table 5.4. Effect of Cu supplementation and source on luteinizing hormone (LH) released in response to low concentrations of exogenous gonadotropin releasing hormone (GnRH; Experiment 1)[a]

Item	Dietary treatment				Contrasts ($P <$)	
	Control	ORG[b]	ING[c]	SEM[d]	Cont vs Suppl	ORG vs ING
Basal LH release, ng/ml						
0 μg GnRH	2.67	1.81	1.48	0.48	0.30	0.60
3 μg GnRH	3.14	1.93	1.59	0.57	0.27	0.55
9 μg GnRH	2.35	1.87	1.56	0.43	0.36	0.65
Amplitude of peak LH surge, ng/ml						
0 μg GnRH	4.14	2.98	1.73	0.38	0.06	0.17
3 μg GnRH	13.13	12.21	11.52	2.14	0.60	0.55
9 μg GnRH	47.12	38.29	31.64	9.20	0.33	0.67
Area under curve, $ng \cdot ml^{-1} \cdot min^{-1}$						
0 μg GnRH	210.2	231.2	134.6	61.0	0.89	0.57
3 μg GnRH	438.7	673.7	639.7	125.7	0.07	0.71
9 μg GnRH	2,005.6	2,517.5	2,048.3	373.0	0.66	0.84

[a]Raw averages are reported, however log transformed data were used to determine P values.
[b]ORG = Organic treatment (100% organic Cu).
[c]ING = Inorganic treatment (100% $CuSO_4$).
[d]SEM = Standard error of the mean.

Table 5.5. Effect of Cu supplementation and source on luteinizing hormone (LH) released in response to high concentrations of exogenous gonadotropin releasing hormone (GnRH; Experiment 2)[a]

	Dietary treatment				Contrasts ($P <$)	
Item	Control	ORG[b]	ING[c]	SEM[d]	Cont vs Suppl	ORG vs ING
Basal LH release, ng/ml						
0 μg GnRH	4.85	4.05	3.52	0.86	0.18	0.37
27 μg GnRH	4.72	4.40	3.81	0.76	0.71	0.36
81 μg GnRH	3.90	3.97	3.64	0.63	0.66	0.64
Amplitude of peak LH surge, ng/ml						
0 μg GnRH	3.86	4.03	3.23	1.05	0.68	0.82
27 μg GnRH	54.51	75.60	62.79	8.37	0.32	0.58
81 μg GnRH	65.23	68.79	61.63	12.41	0.74	0.71
Area under curve, $ng \cdot ml^{-1} \cdot min^{-1}$						
0 μg GnRH	159.4	290.8	150.4	99.0	0.71	0.08
27 μg GnRH	3,367.4	4,700.3	3,776.3	519.2	0.62	0.74
81 μg GnRH	5,438.8	6,085.0	4,610.8	916.9	0.99	0.60

[a]Raw averages are reported, however log transformed data were used to determine P values.
[b]ORG = Organic treatment (100% organic Cu).
[c]ING = Inorganic treatment (100% $CuSO_4$).
[d]SEM = Standard error of the mean.

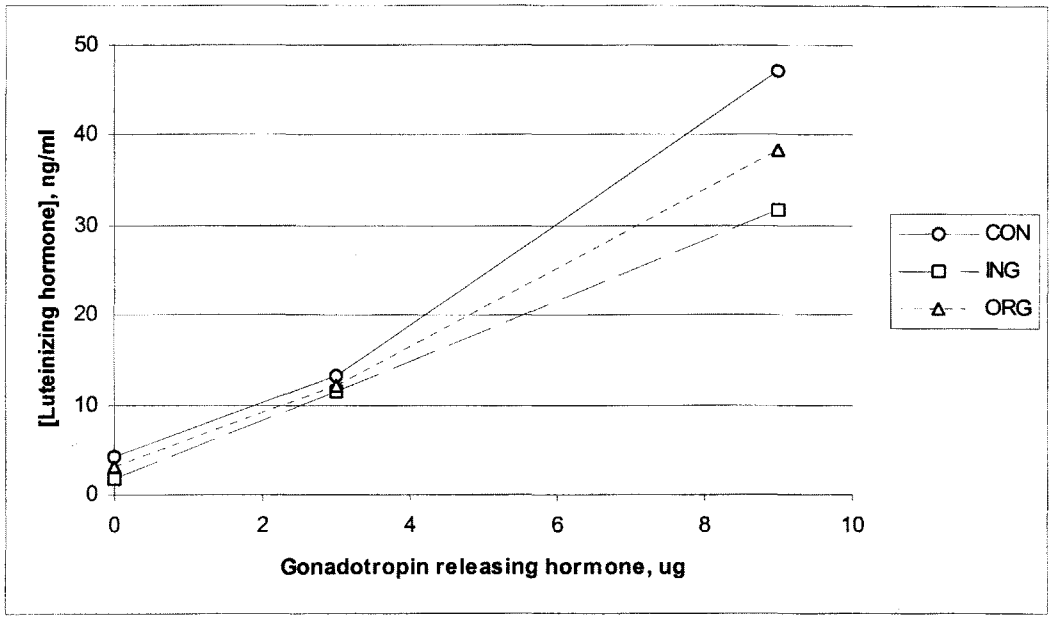


Figure 5.3. Dose response curve for peak luteinizing hormone concentration in experiment one when 0, 3, and 9 ug of gonadotropin releasing hormone was administered.

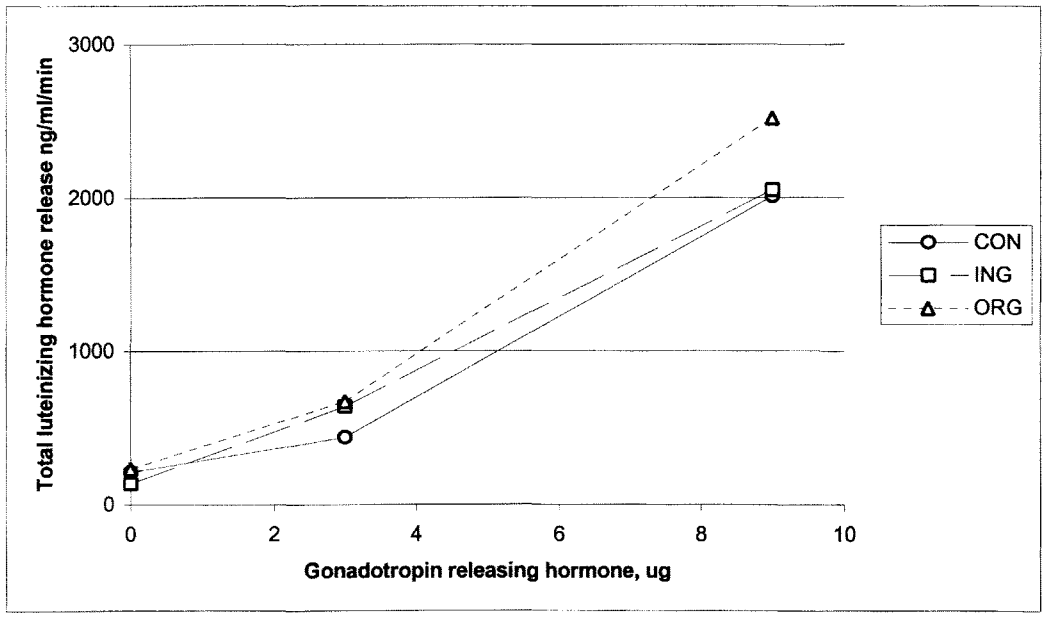


Figure 5.4. Dose response curve for total luteinizing hormone released in experiment one when 0, 3, and 9 ug of gonadotropin releasing hormone was administered.

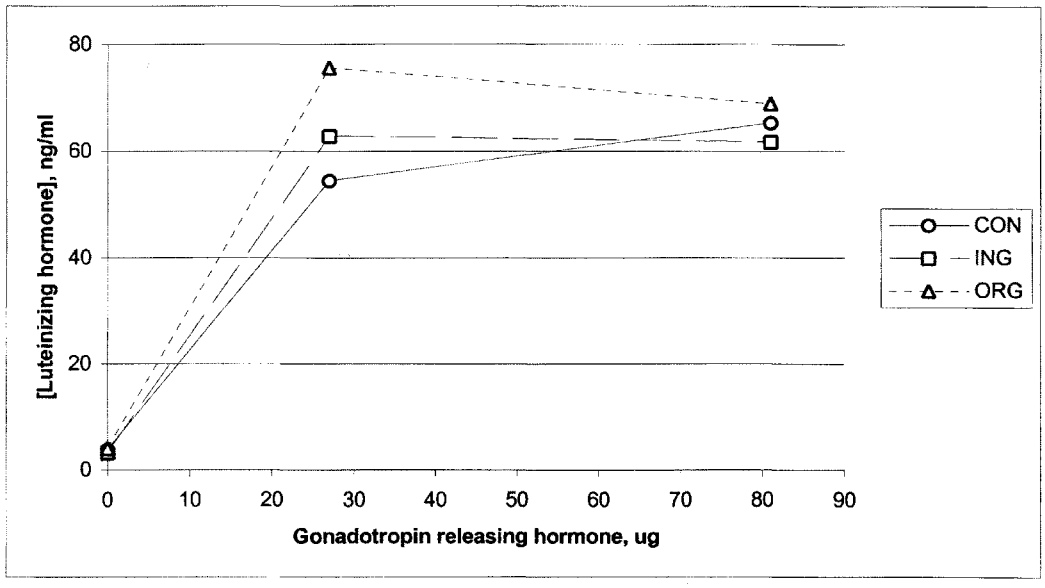


Figure 5.5. Dose response curve for peak luteinizing hormone concentration in experiment two when 0, 27, and 81 ug of gonadotropin releasing hormone was administered.

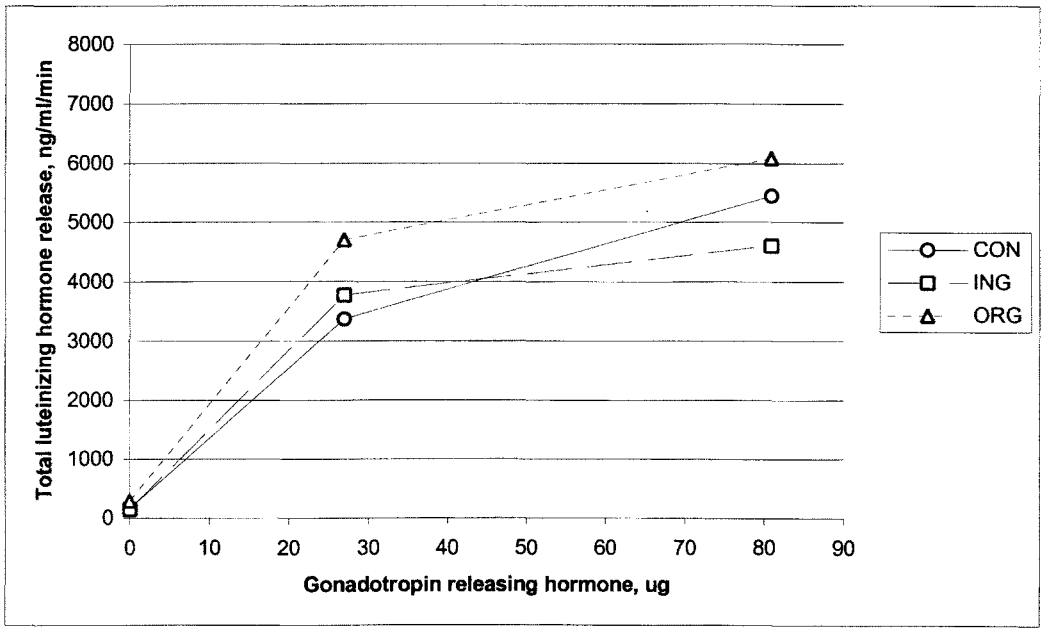
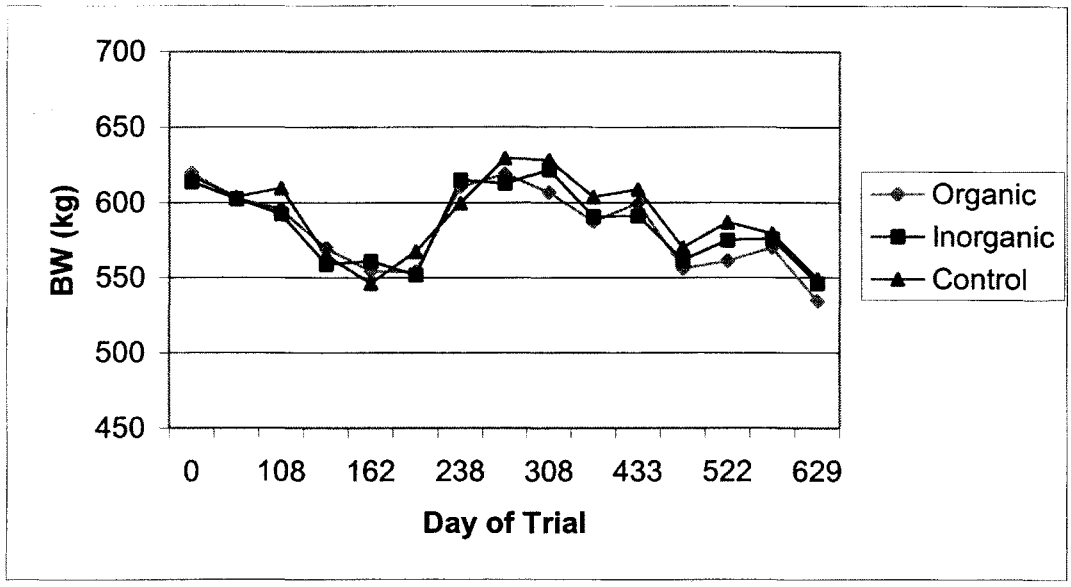
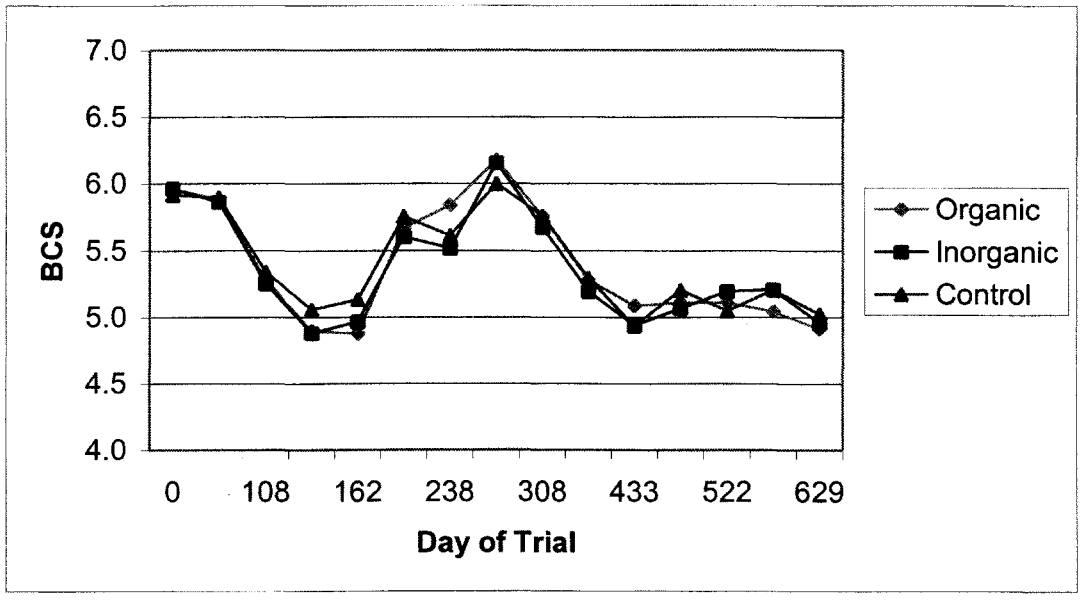


Figure 5.6. Dose response curve for total luteinizing hormone released in experiment two when 0, 27, and 81 ug of gonadotropin releasing hormone was administered.

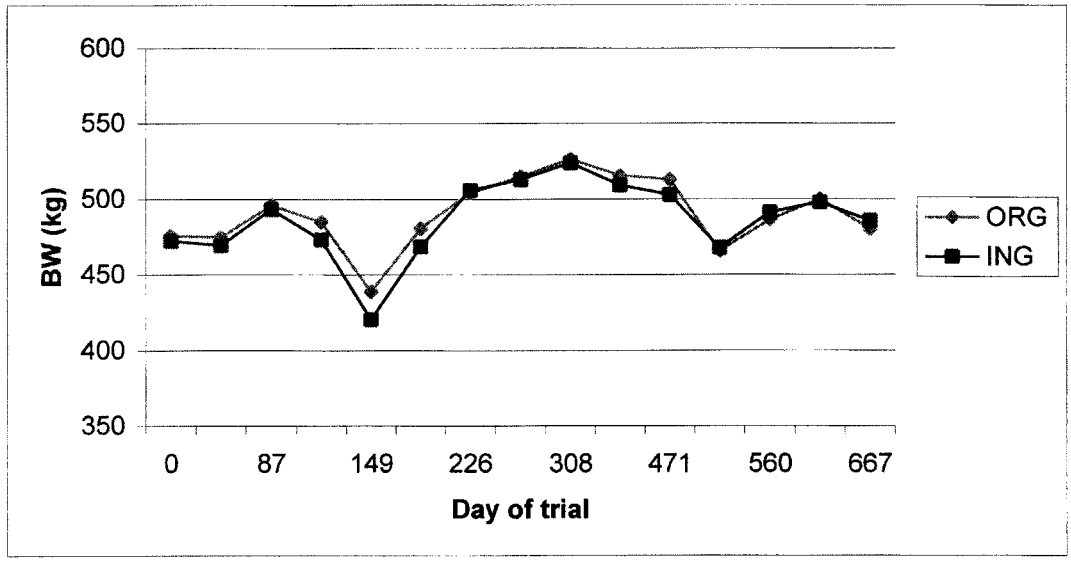
APPENDIX A



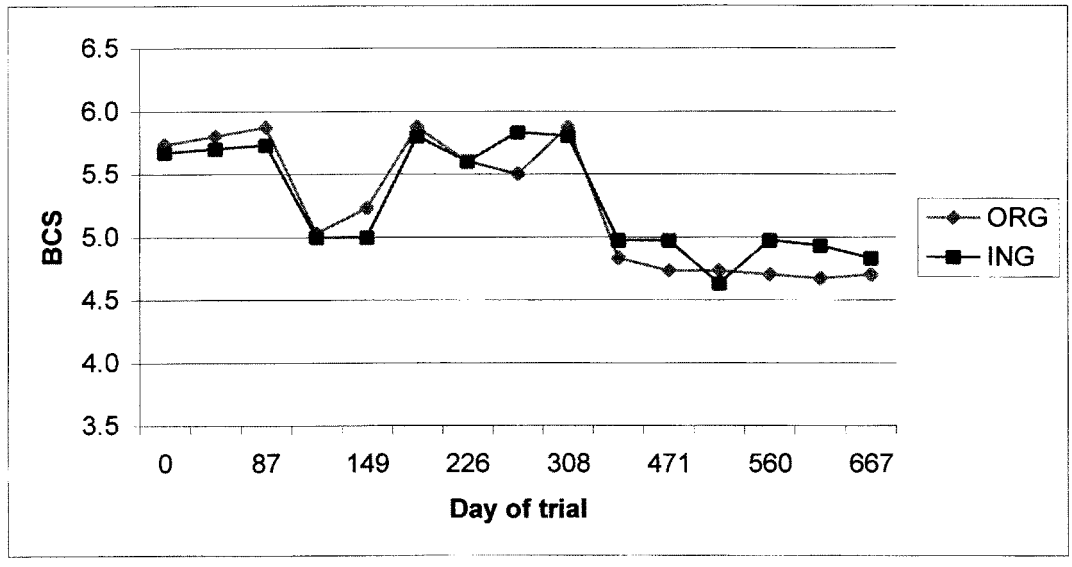
Least squares means for body weight (BW) change in multiparous cows throughout the 2-yr experiment. Treatment × time ($P > 0.10$); time ($P < 0.01$); treatments did not differ ($P > 0.10$).



Least squares means for body condition score (BCS) change in multiparous cows throughout the 2-yr experiment. Treatment × time ($P > 0.10$); time ($P < 0.01$); treatments did not differ ($P > 0.10$).



Least squares means for body weight (BW) change in young beef females throughout the 2-yr experiment. Treatment × time ($P > 0.10$); time ($P < 0.01$); treatments did not differ ($P > 0.10$).



Least squares means for body condition score (BCS) change in young beef females throughout the 2-yr experiment. Treatment × time ($P > 0.10$); time ($P < 0.01$); treatments did not differ ($P > 0.10$).

Least squares means for plasma mineral concentrations of multiparous cows and primiparous heifers supplemented with different trace mineral treatments[a]

Item	Treatments			SEM	Contrasts ($P <$)	
	Control	ORG[b]	ING[c]		Cont. vs. Suppl.	ORG vs. ING
Multiparous cows:						
Cu, mg/L						
d –82 yr 1[d]	0.71	0.70	0.73	0.04	0.78	0.35
d + 30 yr 1	0.71	0.80	0.77	0.04	0.19	0.57
d +110 yr 1	0.71	0.80	0.82	0.04	0.09	0.63
Zn, mg/L						
d –82 yr 1[d]	0.13	0.13	0.13	0.005	0.59	0.45
d + 30 yr 1	0.12	0.12	0.13	0.005	0.16	0.24
d +110 yr 1	0.11	0.13	0.13	0.005	0.02	0.95
Primiparous heifers:						
Cu, mg/L						
d –54 yr 1[d]	--	0.75	0.79	0.035	--	0.47
d + 30 yr 1	--	0.88	0.87	0.035	--	0.81
d +110 yr 1	--	0.87	0.91	0.035	--	0.47
Zn, mg/L						
d –54 yr 1[d]	--	0.13	0.13	0.008	--	0.92
d + 30 yr 1	--	0.11	0.11	0.008	--	0.74
d +110 yr 1	--	0.12	0.13	0.008	--	0.36

[a]Day is relative to average calving date (d 0) of the cow herd.
[b]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[c]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[d]Initial (d –82 and –54) plasma values indicate concentrations measured before supplementation, which began on January 17, 2001.

Health status, immune response, and morbidity data during the feedlot phase[a]

Item	Treatments			SEM[d]	Contrasts ($P <$)	
	Control	ORG[b]	ING[c]		Cont. vs. Suppl.	ORG vs. ING
SOD, U/mg Hb[e]	0.061	0.061	0.158	0.009	0.89	0.81
Interferon gamma, log_{10}:						
d 0	0.00	0.13	0.00	0.054	0.38	0.08
d 56 (yr 2 only)	0.00	0.07	0.00	0.054	0.66	0.29
CMI to PHA, mm[f]	1.82	1.38	1.63	0.189	0.18	0.35
Primary humoral immune response:						
To PRBC antigen, log_2 (growing):[h]						
IgG[i]	2.86	2.86	2.78	0.198	0.87	0.77
IgM[i]	0.64	0.52	0.34	0.151	0.25	0.42
Total Ig[i]	3.37	3.51	3.10	0.200	0.79	0.18
To OVA antigen, log_{10} (finishing)[g]	0.0033	0.0017	0.0023	0.0007	0.16	0.55
Secondary humoral immune response:						
To PRBC antigen, log_2 (growing phase):[h]						
IgG[i]	2.22	2.44	2.29	0.205	0.58	0.62
IgM[i]	0.29	0.72	0.31	0.225	0.42	0.22
Total Ig[i]	2.51	3.18	2.61	0.204	0.14	0.07
Respiratory symptoms:[j]						
No. head treated	13 (n=87)	15 (n=93)	12 (n=90)		0.33	0.62
No. head re-treated	2 (n=13)	2 (n=15)	0 (n=12)		0.45	0.14
Non-respiratory symptoms:[k]						
No. head treated	4 (n=87)	5 (n=93)	0 (n=90)		0.81	0.09

[a]Least squares means.
[b]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[c]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[d]SEM = Standard error of the mean.
[e]SOD = Superoxide dismutase enzyme activity, reported in units/mg hemoglobin (Hb).
[f]CMI = Cell-mediated immune response; PHA = Phytohemagglutinin.
[g]PRBC = Pig red blood cells; a foreign protein injected intramuscularly and subcutaneously to stimulate a primary humoral immune response (growing) and a secondary humoral immune response (finishing).
[h]IgG = Immunoglobulin G; IgM = Immunoglobulin M; and Total Ig = total immunoglobulins.
[i]OVA = Ovalbumin; a foreign protein injected intramuscularly and subcutaneously to stimulate a primary humoral immune response during the finishing phase.
[j]Respiratory includes animals treated for clinical symptoms of a respiratory disease, including the presence of an elevated rectal temperature.
[k]Non-respiratory includes animals treated for clinical symptoms of an ailment other than a respiratory disease (e.g. diptheria, bloat, foot rot, etc.).

Lipid content of longissimus dorsi samples[a]

Item	Treatments			SEM[d]	Contrasts ($P <$)	
	Control	ORG[b]	ING[c]		Cont. vs. Suppl.	ORG vs. ING
Total lipid, % of wet weight[e]	5.63	5.00	5.75	0.400	0.61	0.20
Percent DM of muscle sample, %[f]	28.62	28.10	28.01	0.468	0.34	0.90

[a]Least squares means.
[b]ORG = 50% organic and 50% inorganic Cu, Zn, and Mn.
[c]ING = 100% inorganic $CuSO_4$, $ZnSO_4$, and $MnSO_4$.
[d]SEM = Standard error of the mean.
[e]Total percent lipid in longissimus dorsi samples, on a wet weight basis.
[f]Percent dry matter of longissimus dorsi samples.

APPENDIX B

Liver Trace Mineral Concentrations (Chapter 2):

```
proc mixed;
*model livcu = trt time trt*time year trt*year time*year trt*time*year;
*initial model;

class trt rep time year;
model livcu = trt time trt*time year trt*year/ddfm=satterth;
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time year trt*year/pdiff;

proc mixed;
*model livzn = trt time trt*time year trt*year time*year trt*time*year;
*initial model;

class trt rep time year;
model livzn = trt time trt*time year trt*year time*year/ddfm=satterth;
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time year trt*year time*year/pdiff;

proc mixed;
*model livmn = trt time trt*time year trt*year time*year trt*time*year;
*initial model;

class trt rep time year;
model livmn = trt time trt*time year trt*year time*year/ddfm=satterth;
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time year trt*year time*year/pdiff;
```

Plasma Trace Mineral Concentrations (Chapter 2):

```
proc mixed;
*model livcu = trt time trt*time; *initial model;

class trt rep time;
model bldcu = trt time trt*time/ddfm=satterth;
random rep(trt);
repeated time/type=sp(pow)(day) subject=rep(trt) rcorr;
lsmeans trt time trt*time/pdiff;

proc mixed;
*model livzn = trt time trt*time; *initial model;

class trt rep time;
model bldzn = trt time trt*time/ddfm=satterth;
random rep(trt);
repeated time/type=sp(pow)(day) subject=rep(trt) rcorr;
lsmeans trt time trt*time/pdiff;
```

Reproductive Performance (Chapter 2):

```
proc genmod descending;
class trt rep bcs;
model cycling = trt/dist=b link=logit type3;
lsmeans trt/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model estrus = trt year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model preg_ai = trt bcs year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model preg_60d = trt year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model preg_eai = trt year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model preg_tai = trt year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;
```

Calf Performance (Chapter 2):

```
proc mixed;
*model RAWww = trt aoc year trt*aoc trt*year aoc*year; *initial model;

class trt rep year;
model RAWww = trt aoc year trt*year; *final model;
lsmeans trt year trt*year/pdiff;

proc mixed;
*model BIFww = trt year trt*year; *initial model;

class trt rep year;
model BIFww = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;

proc mixed;
*model ADJww = trt aoc aod year trt*aoc trt*aod trt*year aoc*aod
aoc*year; *initial model;

class trt rep aod year;
model ADJww = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;
```

```
proc mixed;
*model adj = trt aoc aod year trt*aoc trt*aod trt*year aoc*aod
aoc*year; *initial model;

class trt rep aod year;
model adj = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;
```

Body Weight and Body Condition Score Changes (Chapter 2):

```
proc mixed;
*model wt = trt time trt*time;

class rep trt time year; *final model;
model wt = trt time trt*time/ddfm=satterth;
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time/pdiff;

proc mixed;
*model bcs = trt time trt*time;

class rep trt time year; *final model;
model bcs = trt time trt*time/ddfm=satterth;
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time/pdiff;
```

==========

==========

Liver Trace Mineral Concentrations (Chapter 3):

```
proc mixed;
*model livcu = trt time trt*time year trt*year time*year trt*time*year;

class trt rep time year;
model livcu = trt time trt*time year trt*year/ddfm=satterth;
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time year trt*year/pdiff;

proc mixed;
*model livzn = trt time trt*time year trt*year time*year trt*time*year;

class trt rep time year;
model livzn = trt time trt*time year trt*year time*year/ddfm=satterth;
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time year trt*year time*year/pdiff;

proc mixed;
*model livmn = trt time trt*time year trt*year time*year trt*time*year;

class trt rep time year;
model livmn = trt time trt*time year trt*year time*year/ddfm=satterth;
```

```
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time year trt*year time*year/pdiff;
```

Plasma Trace Mineral Concentrations (Chapter 3):

```
proc mixed;
*model livcu = trt time trt*time;

class trt rep time;
model bldcu = trt time trt*time/ddfm=satterth;
random rep(trt);
repeated time/type=sp(pow)(day) subject=rep(trt) rcorr;
lsmeans trt time trt*time/pdiff;

proc mixed;
*model livzn = trt time trt*time;

class trt rep time;
model bldzn = trt time trt*time/ddfm=satterth;
random rep(trt);
repeated time/type=sp(pow)(day) subject=rep(trt) rcorr;
lsmeans trt time trt*time/pdiff;
```

Primary Immune Response to Pig Red Blood Cells (Chapter 3):

```
proc mixed;
*model igm = trt time trt*time; *inital model;

class rep trt time;
model igg = trt time trt*time/ddfm=satterth; *final model;
random rep(trt);
repeated time/type=ar(1) subject=rep(trt) rcorr;
lsmeans trt time trt*time/pdiff;

proc mixed;
*model igg = trt time trt*time; *inital model;

class rep trt time;
model igm = trt time trt*time/ddfm=satterth; *final model;
random rep(trt);
repeated time/type=ar(1) subject=rep(trt) rcorr;
lsmeans trt time trt*time/pdiff;

proc mixed;
*model total = trt time trt*time; *inital model;

class rep trt time;
model total = trt time trt*time/ddfm=satterth; *final model;
random rep(trt);
repeated time/type=ar(1) subject=rep(trt) rcorr;
lsmeans trt time trt*time/pdiff;
```

Reproductive Performance (Chapter 3):

```
proc genmod descending;
class trt rep bcs;
model cycling = trt/dist=b link=logit type3;
lsmeans trt/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model estrus = trt year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model preg_ai = trt bcs year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model preg_60d = trt year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model preg_eai = trt year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;

proc genmod descending;
class trt rep aod bcs year;
model preg_tai = trt year trt*year/dist=b link=logit type3;
lsmeans trt year trt*year/pdiff;
```

Calf Performance (Chapter 3):

```
proc mixed;
*model RAWww = trt aoc year trt*aoc trt*year aoc*year; *initial model;

class trt rep year;
model RAWww = trt aoc year trt*year; *final model;
lsmeans trt year trt*year/pdiff;

proc mixed;
*model BIFww = trt year trt*year; *initial model;

class trt rep year;
model BIFww = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;

proc mixed;
*model ADJww = trt aoc aod year trt*aoc trt*aod trt*year aoc*aod
aoc*year; *initial model;

class trt rep aod year;
model ADJww = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;
```

```
proc mixed;
*model adj = trt aoc aod year trt*aoc trt*aod trt*year aoc*aod
aoc*year; *initial model;

class trt rep aod year;
model adj = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;
```

Body Weight and Body Condition Score Changes (Chapter 3):

```
proc mixed;
*model wt = trt time trt*time;

class rep trt time year; *final model;
model wt = trt time trt*time/ddfm=satterth;
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time/pdiff;

proc mixed;
*model bcs = trt time trt*time;

class rep trt time year; *final model;
model bcs = trt time trt*time/ddfm=satterth;
random rep(trt*year);
repeated time/type=sp(pow)(day) subject=rep(trt*year) rcorr;
lsmeans trt time trt*time/pdiff;
```

==========

Initial Body Weight (Chapter 4):

```
proc mixed;
*model wt = sex trt year sex*trt sex*year trt*year sex*trt*year;
*inital model;

class pen trt sex time year;
model wt = trt year trt*year/ddfm=satterth; *final model;
random pen(trt*year);
lsmeans trt year trt*year/pdiff;
```

Growing Phase Body Weight (Chapter 4):

```
proc mixed;
*model wt = sex time trt year sex*time sex*trt sex*year time*trt
time*year trt*year sex*trt*time sex*time*year sex*trt*year
time*trt*year sex*time*trt*year; *initial model;

class pen trt sex time year;
model wt = time trt year time*trt time*year trt*year
       time*trt*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated time/subject=pen(trt*year) type=ar(1) r rcorr;
lsmeans time trt year time*trt time*year trt*year
       time*trt*year/pdiff ;
```

Growing Phase Average Daily Gain, by period (Chapter 4):

```
proc mixed;
*model adg = sex period trt year sex*period sex*trt sex*year period*trt
period*year trt*year sex*trt*period sex*period*year sex*trt*year
period*trt*year sex*period*trt*year; *initial model;

class pen trt sex period year;
model adg = sex period trt year period*trt period*year trt*year
      period*trt*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated period/subject=pen(trt*year) type=ar(1) r rcorr;
lsmeans sex period trt year period*trt period*year trt*year
      period*trt*year/pdiff ;
```

Growing Phase Average Daily Gain (entire phase; Chapter 4):

```
proc mixed;
*model adg = sex trt year sex*trt sex*year trt*year sex*trt*year;
*initial model;

class pen trt sex period year;
model adg = sex trt year trt*year/ddfm=satterth; *final model;
random pen(trt*year);
lsmeans sex trt year trt*year/pdiff ;
```

Growing Phase Dry Matter Intake (by period; Chapter 4):

```
proc mixed;
*model dmi = trt sex trt*sex period trt*period sex*period
trt*sex*period year trt*year sex*year period*year trt*sex*year
trt*period*year sex*period*year trt*sex*period*year; *initial model;

class pen trt sex period year;
model dmi = trt sex period trt*period year trt*year
     period*year trt*period*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated period/subject=pen(trt*year) type=ar(1) r rcorr;
lsmeans trt sex period trt*period year trt*year
     period*year trt*period*year/pdiff;
```

Growing Phase Dry Matter Intake (entire phase; Chapter 4):

```
proc mixed;
*model dmi = sex trt sex*trt year sex*year trt*year sex*trt*year;
*initial model;

class pen trt sex period year;
model dmi = sex trt year trt*year; *final model;
random pen(trt*year);
lsmeans sex trt year trt*year/pdiff;
```

Growing Phase Gain-to-Feed (by period; Chapter 4):

```
proc mixed;
```

```
*model gf = sex period trt sex*period sex*trt period*trt sex*period*trt
year sex*year period*year trt*year sex*period*year sex*trt*year
period*trt*year sex*period*trt*year; *initial model;

class pen trt sex period year;
model gf = period trt period*trt year period*year
      trt*year period*trt*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated period/subject=pen(trt*year) type=ar(1) r rcorr;
lsmeans period trt period*trt year period*year
      trt*year period*trt*year/pdiff;
```

Growing Phase Gain-to-feed (entire phase; Chapter 4):

```
proc mixed;
*model gf = sex trt sex*trt year sex*year trt*year sex*trt*year;
*initial model;

class pen trt sex period year;
model gf = trt year trt*year/ddfm=satterth; *final model;
random pen(trt*year);
lsmeans trt year trt*year/pdiff;
```

Finishing Phase Body Weight (Chapter 4):

```
proc mixed;
*model wt = sex time trt year sex*time sex*trt sex*year time*trt
time*year trt*year sex*trt*time sex*time*year sex*trt*year
time*trt*year sex*time*trt*year; *initial model;

class pen sex trt time year;
model wt = sex time trt year sex*time sex*trt sex*year time*trt
time*year trt*year
      sex*trt*time sex*time*year time*trt*year; *final model;
random pen(trt*year);
repeated time/subject=pen(trt*year) type=arh(1) r rcorr;
lsmeans sex time trt year sex*time sex*trt sex*year time*trt time*year
trt*year
      sex*trt*time sex*time*year time*trt*year/pdiff ;
```

Finishing Phase Average Daily Gain (by period; Chapter 4):

```
proc mixed;
*model adg = sex period trt year sex*period sex*trt sex*year period*trt
period*year trt*year sex*trt*period sex*period*year sex*trt*year
period*trt*year sex*period*trt*year; *initial model;

class pen trt sex period year;
model adg = sex period trt year period*trt period*year trt*year
       period*trt*year; *final model;
random pen(trt*year);
repeated period/subject=pen(trt*year) type=ar(1) r rcorr;
lsmeans sex period trt year period*trt period*year trt*year
       period*trt*year/pdiff;
```

Finishing Phase Average Daily Gain (entire phase; Chapter 4):

```
proc mixed;
*model adg = sex trt year sex*trt sex*year trt*year sex*trt*year;
*initial model;

class pen trt sex period year;
model adg = sex trt year trt*year/ddfm=satterth; *final model;
random pen(trt*year);
lsmeans sex trt year trt*year/pdiff ;
```

Finishing Phase Dry Matter Intake (by period; Chapter 4):

```
proc mixed;
*model dmi = trt sex trt*sex period trt*period sex*period
trt*sex*period year trt*year sex*year period*year trt*sex*year
trt*period*year sex*period*year trt*sex*period*year; *inital model;

class pen trt sex period year;
model dmi = trt sex period trt*period year trt*year period*year
trt*period*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated period/subject=pen(trt*year) type=ar(1) r rcorr;
lsmeans trt sex period trt*period year trt*year period*year
trt*period*year/pdiff;
```

Finishing Phase Dry Matter Intake (entire phase; Chapter 4):

```
proc mixed;
*model dmi = sex trt sex*trt year sex*year trt*year sex*trt*year;
*initial model;

class pen trt sex period year;
model dmi = sex trt year trt*year; *final model;
random pen(trt*year);
lsmeans sex trt year trt*year/pdiff;
```

Finishing Phase Gain-to-Feed (by period; Chapter 4):

```
proc mixed;
*model gf = sex period trt sex*period sex*trt period*trt sex*period*trt
year sex*year period*year trt*year sex*period*year sex*trt*year
period*trt*year sex*period*trt*year; *initial model;

class pen trt sex period year;
model gf = period trt period*trt year
      period*year trt*year period*trt*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated period/subject=pen(trt*year) type=ar(1) r rcorr;
lsmeans period trt period*trt year
      period*year trt*year period*trt*year/pdiff;
```

Finishing Phase Gain-to-Feed (entire phase; Chapter 4):

```
proc mixed;
*model gf = sex trt sex*trt year sex*year trt*year sex*trt*year;
*initial model;

class pen trt sex period year;
```

```
model gf = trt year trt*year; *final model;
random pen(trt*year);
lsmeans trt year trt*year/pdiff;
```

Cell-mediated immune response (Chapter 4):

```
proc mixed;
*model change = trt sex trt*sex time trt*time sex*time trt*sex*time
year trt*year sex*year time*year trt*sex*year trt*time*year
sex*time*year trt*sex*time*year; *inital model;

class pen trt sex time year;
model change = trt sex time trt*time sex*time year trt*year
      time*year trt*time*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated time/type=sp(pow)(hours) subject=pen(trt*year) rcorr;
lsmeans trt sex time trt*time sex*time year trt*year
      time*year trt*time*year/pdiff;
```

Interferon Gamma (Chapter 4):

```
proc mixed;
*model conc = trt sex trt*sex time trt*time sex*time trt*sex*time year
trt*year sex*year trt*sex*year; *inital model;

class pen sex trt year time;
model conc = trt sex time trt*time year trt*year
sex*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated time/type=arh(1) subject=pen(trt*year) rcorr;
lsmeans trt sex time trt*time year trt*year sex*year/pdiff;
```

Primary Immune Response to Pig Red Blood Cells (Chapter 4):

```
proc mixed;
*model igg = trt sex trt*sex time trt*time sex*time trt*sex*time year
trt*year sex*year time*year trt*sex*year trt*time*year sex*time*year
trt*sex*time*year; *inital model;

class pen trt sex time year;
model igg = trt time trt*time year trt*year
      time*year trt*time*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated time/type=ar(1) subject=pen(trt*year) rcorr;
lsmeans trt time trt*time year trt*year
      time*year trt*time*year/pdiff;
```

Secondary Immune Response to Pig Red Blood Cells (Chapter 4):

```
proc mixed;
*model igg = trt sex trt*sex time trt*time sex*time trt*sex*time year
trt*year sex*year time*year trt*sex*year trt*time*year sex*time*year
trt*sex*time*year; *inital model;

class pen trt sex time year;
model igg = trt time trt*time year trt*year time*year
trt*time*year/ddfm=satterth; *final model;
```

```
random pen(trt*year);
repeated time/type=arh(1) subject=pen(trt*year) rcorr;
lsmeans trt time trt*time year trt*year time*year trt*time*year/pdiff;
```

Primary Immune Response to Ovalbumin (Chapter 4):

```
proc mixed;
*model ova = trt sex trt*sex time trt*time sex*time trt*sex*time year
trt*year sex*year time*year trt*sex*year trt*time*year sex*time*year
trt*sex*time*year; *inital model;

class pen trt sex time year;
model ova = trt time trt*time/ddfm=satterth; *final model;
random pen(trt*year);
repeated time/type=ar(1) subject=pen(trt) rcorr;
lsmeans trt time trt*time/pdiff;
```

Superoxide Dismutase Activity (Chapter 4):

```
proc mixed;
*model sod = trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen trt sex year;
model sod = trt sex year sex*year trt*year; *final model;
random pen(trt*year);
lsmeans trt sex year sex*year trt*year/pdiff;
```

Carcass Characteristics (Chapter 4):

```
proc mixed;
*model HCW = trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen trt sex year;
model HCW = trt sex year trt*year; *final model;
lsmeans  trt year trt*year/pdiff;

proc mixed;
*model DP = trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen trt sex year;
model DP =  trt year trt*year; *final model;
lsmeans  trt year trt*year/pdiff;

proc mixed;
*model REA =  trt sex trt*sex  year sex*year trt*year trt*sex*year;
*inital model;

class pen trt sex year;
model REA =  trt sex year sex*year trt*year; *final model;
lsmeans  trt sex year sex*year trt*year/pdiff;

proc mixed;
*model BFAT =  trt sex trt*sex  year sex*year trt*year trt*sex*year;
*inital model;
```

```
class pen trt sex year;
model BFAT = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;

proc mixed;
*model KPH =  trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen trt sex year;
model KPH = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;

proc mixed;
*model FYG =  trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen trt sex year;
model FYG = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;

proc mixed;
*model USYG =  trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen trt sex year;
model USYG = trt sex year sex*year trt*year; *final model;
lsmeans trt sex year sex*year trt*year/pdiff;

proc mixed;
*model MARB =  trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen trt sex year;
model MARB = trt sex year trt*year; *final model;
lsmeans trt sex year trt*year/pdiff;

proc mixed;
*model USQG =  trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen trt sex year;
model USQG = trt year trt*year; *final model;
lsmeans trt year trt*year/pdiff;
```

Fatty Acid Profile (Chapter 4):

```
proc mixed;
*model A = trt sex trt*sex year sex*year trt*year trt*sex*year; *inital
model;

class pen sex trt year; *Fatty Acid 14:0;
model A = trt sex trt*sex year sex*year trt*year trt*sex*year; *final
model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
```

```
*model B = trt sex trt*sex year sex*year trt*year trt*sex*year; *inital
model;

class pen sex trt year; *Fatty Acid 16:0;
model B = trt sex trt*sex year sex*year trt*year trt*sex*year; *final
model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model C = trt sex trt*sex year sex*year trt*year trt*sex*year; *inital
model;

class pen sex trt year; *Fatty Acid 18:0;
model C = trt sex trt*sex year sex*year trt*year trt*sex*year; *final
model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model D = trt sex trt*sex year sex*year trt*year trt*sex*year; *inital
model;

class pen sex trt year; *Fatty Acid 18:1;
model D = trt sex trt*sex year sex*year trt*year trt*sex*year; *final
model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model E = trt sex trt*sex year sex*year trt*year trt*sex*year; *inital
model;

class pen sex trt year; *Fatty Acid 18:2;
model E = trt sex trt*sex year sex*year trt*year trt*sex*year; *final
model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model F = trt sex trt*sex year sex*year trt*year trt*sex*year; *inital
model;

class pen sex trt year; *Fatty Acid 18:3;
model F = trt sex trt*sex year sex*year trt*year trt*sex*year; *final
model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model G = trt sex trt*sex year sex*year trt*year trt*sex*year; *inital
model;

class pen sex trt year; *Fatty Acid 20:4;
model G = trt sex trt*sex year sex*year trt*year trt*sex*year; *final
model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model UNSAT = trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;
```

```
class pen sex trt year; *Fatty Acid UNSAT;
model UNSAT = trt sex trt*sex year sex*year trt*year trt*sex*year;
*final model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model SAT = trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen sex trt year; *Fatty Acid SAT;
model SAT = trt sex trt*sex year sex*year trt*year trt*sex*year; *final
model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model UNvsSAT = trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen sex trt year; *Fatty Acid UNSATvsSAT;
model UNvsSAT = trt sex trt*sex year sex*year trt*year trt*sex*year;
*final model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model MONO = trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen sex trt year; *Fatty Acid MONO;
model MONO = trt sex trt*sex year sex*year trt*year trt*sex*year;
*final model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;

proc mixed;
*model POLY = trt sex trt*sex year sex*year trt*year trt*sex*year;
*inital model;

class pen sex trt year; *Fatty Acid POLY;
model POLY = trt sex trt*sex year sex*year trt*year trt*sex*year;
*final model;
lsmeans trt sex trt*sex year sex*year trt*year trt*sex*year/pdiff;
```

Liver Trace Mineral Concentrations (Chapter 4):

```
proc mixed;
*model cu = trt sex trt*sex time trt*time sex*time trt*sex*time year
trt*year sex*year time*year trt*sex*year trt*time*year sex*time*year
trt*sex*time*year; *initial model;

class pen trt sex time year;
model cu = trt time trt*time year trt*year time*year
trt*time*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated time/subject=pen(year*trt) type=sp(pow)(day) r rcorr;
lsmeans trt time trt*time year trt*year time*year trt*time*year/pdiff;

proc mixed;
```

```
*model zn = trt sex trt*sex time trt*time sex*time trt*sex*time year
trt*year sex*year time*year trt*sex*year trt*time*year sex*time*year
trt*sex*time*year; *initial model;

class pen trt sex time year;
model zn = trt time trt*time year trt*year time*year
trt*time*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated time/subject=pen(year*trt) type=sp(pow)(day) r rcorr;
lsmeans trt time trt*time year trt*year time*year trt*time*year/pdiff;
```

proc mixed;

```
*model mn = trt sex trt*sex time trt*time sex*time trt*sex*time year
trt*year sex*year time*year trt*sex*year trt*time*year sex*time*year
trt*sex*time*year; *initial model;

class pen trt sex time year;
model mn = trt time trt*time year trt*year time*year
trt*time*year/ddfm=satterth; *final model;
random pen(trt*year);
repeated time/subject=pen(year*trt) type=sp(pow)(day) r rcorr;
lsmeans trt time trt*time year trt*year time*year trt*time*year/pdiff;
```

Plasma Trace Mineral Concentrations (Chapter 4):

proc mixed;

```
*model cu = trt sex trt*sex time trt*time sex*time trt*sex*time;
*initial model;

class pen trt sex time;
model cu = trt time trt*time/ddfm=satterth; *final model;
random pen(trt);
repeated time/subject=pen(trt) type=sp(pow)(day) r rcorr;
lsmeans trt time trt*time/pdiff;
```

proc mixed;

```
*model zn = trt sex trt*sex time trt*time sex*time trt*sex*time;
*initial model;

class pen trt sex time;
model zn = trt sex time trt*time sex*time/ddfm=satterth; *final model;
random pen(trt);
repeated time/subject=pen(trt) type=sp(pow)(day) r rcorr;
lsmeans trt sex time trt*time sex*time/pdiff;
```

==========

Dry Matter Intake - Repletion phase (Chapter 5):

proc mixed;

```
*model DMI = trt time trt*time BW Age/ddfm=satterth; *initial model;

class id trt time;
model DMI = trt time trt*time/ddfm=satterth; *final model;
random id(trt);
repeated time/type=sp(pow)(day) subject=id(trt) rcorr;
```

```
lsmeans trt time trt*time/pdiff;
```

Pituitary LH Concentration (Chapter 5):

```
proc mixed;
*model first_actual = trt BW BCS Age Cu; *initial model;

class id trt;
model pituitary_LH = trt age; *final model;
lsmeans trt/pdiff;
```

LH Response to GnRH (Chapter 5):

```
proc mixed;
*model log_basal = trt|dose period BW Age Cu; *inital model;

class id trt period dose;
model log_basal = trt|dose BW; *final model;
random id(trt);
lsmeans trt dose trt*dose/pdiff cl;

proc mixed;
*model log_peak_min_basal = trt|dose period BW Age Cu; *inital model;

class id trt period dose;
model log_peak_min_basal = trt|dose; *final model;
random id(trt);
lsmeans trt dose trt*dose/pdiff cl;

proc mixed;
*model log_auc_all = trt|dose period BW Age Cu; *inital model;

class id trt period dose;
model log_auc_all = trt|dose; *final model;
random id(trt);
lsmeans trt dose trt*dose/pdiff cl;
```

Liver Concentrations of other minerals during repletion (e.g. Fe; Chapter 5):

```
proc mixed;
*model Fe = trt time trt*time/ddfm=satterth; *initial model;

class id trt time;
model Fe = trt time trt*time/ddfm=satterth; *final model;
random id(trt);
repeated time/type=sp(pow)(day) subject=id(trt) rcorr;
lsmeans trt time trt*time/pdiff;
```

Repletion Phase BCS, BW, and Liver Cu changes (Chapter 5):

```
proc mixed;
*model BCS = trt time trt*time bw age trt*bw trt*age bw*age
trt*time*bw/ddfm=satterth; *initial model;

class id trt time;
model BCS = trt time trt*time age/ddfm=satterth; *final model;
```

```
random id(trt);
repeated time/type=sp(pow)(day) subject=id(trt) rcorr;
lsmeans trt time trt*time/pdiff;

proc mixed;
*model BW = trt time trt*time age trt*age trt*time*age/ddfm=satterth;
*initial model;

class id trt time;
model BW = trt time trt*time/ddfm=satterth; *final model;
random id(trt);
repeated time/type=sp(pow)(day) subject=id(trt) rcorr;
lsmeans trt time trt*time/pdiff;

proc mixed;
*model Cu = trt time trt*time age trt*age time*age trt*time*age bw
trt*bw time*bw age*bw initial_Cu/ddfm=satterth; *initial model;

class id trt time;
model Cu = trt time trt*time age time*age bw time*bw
initial_Cu/ddfm=satterth; *final model;
random id(trt);
repeated time/type=sp(pow)(day) subject=id(trt) rcorr;
lsmeans trt time trt*time/pdiff;
```

Initial BW, BCS, and Liver Cu (Start of repletion phase; Chapter 5):

```
proc mixed;
*model BW = trt age trt*age; *initial model;

class id trt;
model BW = trt/ddfm=satterth; *final model;
random id(trt);
lsmeans trt/pdiff;

proc mixed;
*model BCS = trt age trt*age bw; *initial model;

class id trt; *final model;
model BCS = trt/ddfm=satterth; *final model;
random id(trt);
lsmeans trt/pdiff;

proc mixed;
*model Cu = trt age bw trt*age age*bw; *initial model;

class id trt; *final model;
model Cu = trt/ddfm=satterth; *final model;
random id(trt);
lsmeans trt/pdiff;
```