EFFECTS OF A SUSTAINED-RELEASE COPPER BOLUS ON GROWTH AND REPRODUCTIVE TRAITS OF BEEF BULLS

by

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Thesis submitted to the faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Animal Science

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August 1995

Blacksburg, Virginia

Keywords: Copper, Bulls, Bolus, Growth, Reproduction

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(ABSTRACT)

Two experiments were conducted to determine the effects of sustained -release copper (Cu) boluses on growth and reproductive traits of beef bulls. Two groups of bulls were used in consecutive years (n=26 in experiment 1; n=20 in experiment 2). Bulls in each experiment were allotted by weight and breed into two equally numbered treatment groups (control (C); treated (T)). Bulls were housed together and offered a balanced diet containing 9 ppm Cu for an ADG of 1.4 kg. Treated bulls in each experiment were given 1 Cu bolus (Cuprax® Pitman-Moore, Mundelein, IL in experiment 1; Copasture™ Copasture-25 bolus Schering-Plough, Kenilworth, NJ in experiment 2). All bulls were bled, had blood samples taken and had scrotal circumferences (SC) measured on d 0, 28, 56, 84, 112, and 140. Blood was collected via jugular venipuncture to determine serum Cu and plasma ceruloplasmin (Cp) concentrations. Breeding soundness examinations (BSE) were conducted on all bulls on d 140 and 185 in experiment 1 and d 168 in experiment 2. Body weight and SC were not affected by treatment in either experiment ($P > .05$). Serum Cu and plasma Cp concentrations were not affected by treatment in experiment 2 ($P > .05$). Serum Cu was higher for the T bulls on d 56, 84, and 112 in experiment 1 ($P < .05$). All serum Cu concentrations for both experiments were within the normal range for cattle. Plasma Cp concentrations were recorded only for d 1, 112, and 140 in experiment 1 and were significantly higher for the C group on d 112 ($P < .05$). There were no significant effects of treatment on the results of the BSE (SC, percentage of normal sperm, progressive motility, and primary or secondary abnormalities) in either experiment. These data suggest that the Cu bolus in experiment 2 had no effect on body weight gains, SC, serum Cu and plasma Cp concentrations, sperm normality, progressive motility, and primary and secondary abnormalities. These data also suggest that the Cu bolus in experiment 1 had no effect on body weight gains, SC, and sperm normality, progressive motility, and primary and secondary abnormalities; however, treatment in experiment 1 did increase serum Cu concentrations on some but not all days and did not increase concentrations above the normal range for beef cattle. The conclusion made from these experiments is that the sustained Cu boluses used in these studies do not significantly affect growth and reproductive traits in beef bulls with adequate Cu status.

Keywords: Copper, Bulls, Bolus

ACKNOWLEDGMENTS

I would like to express my gratitude to Dr. D. E. Eversole for his educational and financial support and his patience in providing me an opportunity to earn a Master of Science degree.

I would also like to thank the other members of my committee, Dr. J. P. Fontenot, Dr. W. S. Swecker, and Dr. M. L. Wahlberg for their guidance, advice, and technical expertise.

My sincere appreciation is extended to Christi McAvoy and Chad Joines of the Virginia Tech Beef Center for their generous support and help with the cattle used in these and other experiments.

To the staff of the Virginia-Maryland Regional College of Veterinary Medicine, Megan Irby, Rachel Bethard, Jeanette Baker, Linda Correll and Kam Ko I extend appreciation for all of their technical assistance. Much of my research would not have been completed if not for their generous help.

There are many more individuals who offered assistance throughout my stay here at Virginia Tech. I wish to thank Dr. Notter, Wanda, Jean, Ellie, Gwen, Danny, and all of the graduate students within the department for their help.

To my parents Peter and Betsy, a truly heartfelt thanks, for without their support, this degree would have never been.

Finally, my never-ending thanks to my wife Heather and son Caleb, for loving and supporting me and keeping me sane throughout this endeavor. Thank you for bringing meaning into all I do.

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CHAPTER I.

Introduction

The trace element copper (Cu) is essential for growth and for the prevention of a wide range of clinical and pathological disorders in all types of farm animals. Under normal feeding conditions, grazing ruminants are most susceptible to Cu deficiencies with the incidence of Cu-deficiency generally highest in young, growing animals due to their higher Cu requirements (McDowell, 1992). Copper is essential for growth as it is required for cellular respiration, bone formation, connective tissue development, proper cardiac function, and is essential to several important metallo- enzymes (Underwood, 1977).

Growth rates and reproductive performance of Cu-deficient animals have been shown to be reduced in some studies (Whitelaw et al., 1984; Richards et al., 1985); however, no abnormalities were observed in other studies (van Ryssen et al., 1990; Ward et al., 1993). Most studies on the effects of Cu on reproductive traits in cattle have been conducted in the female. The objectives of these experiments were to determine the effects of sustained-release Cu boluses on growth and reproductive traits in growing beef bulls.

CHAPTER II.

Review of Literature

The Copper Requirement of Beef Cattle

Requirement and availability. Copper is required by the ruminant for a number of physiological functions including reproduction, maintenance of the central nervous system, normal growth, immune function and nutrient metabolism (McDowell, 1992). These and other Cu-dependent, physiological functions will be discusssed later in this paper. In beef cattle, the suggested level of dietary Cu is 8 ppm with a range of 4 to 10 ppm (NRC, 1984).

Forages are generally higher in Cu than concentrates. Most forages will provide 3 to 4 times the Cu requirement of beef cattle; however, plants that contain high levels of molybdenum (Mo), sulfur (S), phytate, or lignin may reduce Cu absorption (Underwood, 1977). Fresh forages are less effective at improving Cu status in an animal than dried forages of equivalent Cu content (Hartmans and Bosman, 1970). Chapman and Bell (1963) investigated the absorption of ^{64}Cu from several different inorganic compounds by beef cattle. The relative absorption and subsequent appearance of ^{64}Cu in the blood was in the following order: $CuCO_3 > Cu(NO_3) > CuSO_4 > CuCl_2 > Cu_2O >$ CuO (powder) > CuO (needles) > Cu (wire).

Toxicity. The continued ingestion of Cu in excess of requirement will lead to the accumulation of Cu in body tissues, especially the liver.

Abnormally high tissue levels may lead to Cu toxicosis. The most sensitive to Cu toxicosis are the ruminants, while the nonruminants have a relatively high tolerance for Cu. The apparent differences between ruminants and nonruminants seem to be determined by their differences in S metabolism (McDowell, 1992). The maximum tolerable level of dietary Cu in beef cattle is 115 ppm (NRC, 1984). Sheep are much more susceptible to Cu toxicosis than mature cattle, yet calves are as susceptible as sheep (Hill, 1975).

Animals suffering from Cu poisoning may experience nausea, vomiting, salivation, abdominal pain, convulsions, paralysis, collapse, and death. Hypercupremic animals may also be predisposed to anemia, muscular dystrophy, decreased growth, and impaired reproduction. Hypercupremia in sheep promotes renal tubular necrosis; spongy lesions in the white matter of the brain; dark, stained kidneys; and swollen livers (NRC, 1980).

Copper Metabolism

Absorption. Copper is poorly absorbed in most animal species (Underwood, 1977). Only 5 to 10% of the Cu in the diet is absorbed by adult non-ruminant animals and only 1 to 3% of Cu is absorbed by ruminants (McDowell, 1992). The intestinal absorption of Cu is regulated by the nutritional status of the individual animal (absorption increases with deficiency), the chemical form in which the element is present, and by a number of interactions with other dietary factors that affect Cu bioavailability (Mertz, 1987). Age also affects Cu absorption; young animals may absorb 10 to 20 % more Cu than adult animals (McDowell, 1992). The preruminant animal absorbs Cu at a much higher rate than mature ruminants (Suttle, 1975).

The small intestine appears to play the major role of Cu absorption; however, depending on the species studied, Cu can be absorbed in all segments of the gastrointestinal tract (Mertz, 1987). The primary site of Cu absorption is the upper part of the duodenum. There is evidence that Cu is absorbed in the intestine by two mechanisms: one saturable active transport mechanism and the other an unsaturable simple diffusion mechanism. Similar to other transport mechanisms, low Cu concentrations are predominantly transported via the active pathway, whereas the higher Cu concentrations are transported via simple diffusion (Bronner and Yost, 1985). Cousins (1985) reviewed the potential role of the protein, metallothionein, as a regulator of Cu absorption. Metallothionein may bind intracellular Cu ions and remove them from transport into the portal circulation until the intestinal cell is sloughed off allowing the Cu to become part of the intestinal contents again. This method of binding ionic Cu may protect the cell from possible damaging effects of high levels of this metal. Copper-metallothioneins have also been isolated from the liver and kidneys of several species (Bremner, 1980).

Copper transport across the brush border membrane of the small intestine may be influenced by a variety of dietary components, including amino acids, phytate, fiber, ascorbic acid, and thiomolybdate (Cousins, 1985). High protein diets have generally been shown to positively affect Cu absorption (Davis et al., 1962; Greger and Snedeker, 1980). Dietary phytate, fiber, ascorbic acid, and thimolybdate appear to complex with Cu and limit its absorption (Cousins, 1985).

Transport. Albumin and(or) amino acids are believed to be the ligands responsible for the transport of Cu from the intestine to the portal circulation (Frieden, 1980). These are then taken up by the liver and other tissues. The hepatic Cu is then incorporated into ceruloplasmin (Cp) and (a) released into the plasma (b) released into the bile or (c) stored temporarily in the liver (Bremner, 1980). Plasma Cu is distributed among three major constituents comprising two pools. Ceruloplasmin represents a tightly bound pool that accounts for at least 90 % of total plasma Cu in most species. Amino acid- and albumin-bound Cu constitute the second, less tightly bound pool (Cousins, 1985). Marceau and Aspin (1972) found that Cu derived from albumin was readily removed from the plasma and, presumably, readily exchangeable with tissue Cu, whereas Cu from Cp was not. Ceruloplasmin appears to act as a donor of Cu for certain enzymes, including cytochrome *c* oxidase and lysyl oxidase (Bremner, 1980; Cousins, 1985).

Storage. The main storage site for Cu is the liver, although it can also accumulate in other tissues. Three proteins found in the liver may be Cu storage specific proteins. These proteins are mitochondrial cuprein, hepatocuprein (superoxide dismutase) and metallothionein which is present in the cytosol (Bremner, 1980; McDowell, 1992). In the ruminant, as little as 4 % of the Cu may occur as superoxide dismutase (Bremner, 1980).

Excretion. In most species a high proportion of ingested Cu appears in the feces. Most of this is unabsorbed Cu but active excretion occurs via the bile. Urinary output is negligible (Underwood, 1977).

Growth and Development

Copper is required by most species for normal growth and development. The roles of Cu become most evident in the case of Cu-deficient animals. Reported values for serum Cu are .7 to 1.5 mg/mL with less than .5 mg/mL, considered deficient (Corah and Ives, 1991).

Pigmentation and keratinization of hair and wool. Often the first sign of a hypocupremic condition is achromatrichia (lack of pigmentation). This condition is commonly observed in the wool and hair of mammals and is attributed to the reduction in synthesis and activity of the Cu-containing enzyme tyrosinase (polyphenol oxidase). Tyrosinase is required for the conversion of tyrosine to melanin and for pigmentation of hair, wool and feathers (Underwood, 1977).

Reduced keratinization, signified by the reduction of crimp in fibers, is another effect of Cu deficiency. Keratinization requires Cu for the incorporation of disulfide groups which provide the cross-linkages of keratin and the alignment of the long chain keratin fibrillae in the fiber (Underwood, 1977; McDowell, 1992).

Iron metabolism and cellular respiration. Copper plays an important role in iron (Fe) absorption and mobilization. Serum Fe levels tend to be reduced in Cu deficiency, and hypochromic anemia can develop while intestinal mucosa and liver Fe levels are higher than normal (Mills, 1980). Freiden and Hsieh (1976) proposed an explanation based on the fact that plasma Fe is bound to the Fe-transport protein transferrin only as Fe^{3+}. Iron is absorbed from the intestinal lumen most effectively as Fe^{2+} and is released from Fe stores (ferritin) in the liver as Fe^{2+}. The oxidation rate of

Fe^{2+} by oxygen limits the rate of Fe mobilization, and this reaction is catalyzed by a ferroxidase. Ceruloplasmin (ferroxidase I) is one of at least two plasma ferroxidases involved in the mobilization of Fe and(or) its incorporation into hemoglobin or myoglobin.

Cytochrome *c* oxidase, a Cu-containing terminal respiratory enzyme, catalyzes the reduction of O_2 to water, an essential step in cellular respiration (McDowell, 1992). Loss of cytochrome *c* oxidase activity, due to Cu deficiency, results in the failure of synthesis of heme, an important component of hemoglobin (Underwood, 1977).

Cross-linking of connective tissue. Copper plays an important role in the cross-linking of the two important connective tissue proteins, elastin and collagen. Its role in connective tissue is linked to the enzyme lysyl oxidase and deficiency leads to a failure of collagen to undergo cross-linking and maturation (Harris and O'Dell, 1974; Harris et al., 1980). The role of Cu in connective tissue lies with the understanding of the function of lysyl oxidase. Copper is a cofactor for this enzyme and a determinant of its activity in connective tissues. Lysyl oxidase catalyzes the oxidative deamination of certain lysine and hydroxylysine (collagen only) residues in tropoelastin and tropocollagen before the formation of crosslinks (Harris et al., 1980).

Neurological function. Neurological dysfunction is apparent in Cu-deficient mammals. Enzootic ataxia in lambs is caused by Cu deficiency and is associated with incoordination of the hind quarters (Hunt, 1980). Ataxia, tremor and swaying gait have been reported in Cu-deficient guinea pigs (Everson et al., 1968). Brain growth is reduced in dietary Cu-deficient animals and extensive tissue necrosis may occur (Hunt, 1980). There is also demyelination in ataxic lambs and reduced myelination in Cu-deficient rats (Prohaska and Wells, 1974). Myelin is composed largely of phospholipid, and the loss of cytochrome *c* oxidase in Cu deficiency leads to depressed phospholipid synthesis in the liver mitochondria (Howell and Davidson, 1959). Other central nervous system effects of Cu deficiency are the reduction of two neurotransmitters, dopamine and norepinephrine (O'Dell, 1984).

Lipid metabolism. Petering et al. (1977) reported that Cu deficiency in the rat resulted in elevated levels of serum triglycerides, phospholipids, and cholesterol. The reduction of the Cu-dependant enzyme, superoxide dismutase, may be responsible for alterations in lipid and long-chain fatty acid metabolism (McDowell, 1992).

Immune function. Copper has been shown to influence the resistance of sheep to bacterial infections (Woolliams et al., 1986). Copper deficiency results in decreased humoral and cell-mediated immunity regulated by phagocytic cells such as macrophages and neutrophils. The relationship of Cu to the immune system may be through superoxide dismutase and its role in the microbial systems of phagocytes. Reductions in superoxide dismutase activity in leukocytes from Cu-deficient sheep were accompanied by an increased release of superoxide anion. This accumulation of superoxide may serve to weaken the phagocyte (Jones and Suttle, 1981). However, in cells from the Cu-deficient bovine, candidacidal activity was reduced before leukocyte superoxide dismutase activity declined and intracellular concentration of superoxide anion was lowered (Boyne and Arthur, 1986). The role of superoxide may vary among species and with the severity of Cu deficiency. There is no evidence that Cu deficiency influences the resistance of ruminants to viral infection (Suttle and Jones, 1989).

Mineral Interactions With Copper

The nutritional significance of trace elements is determined not only by their physiological and biochemical roles in metabolism, but also by their capacity to interact with other elements. Many interactions among trace elements are of potential importance because they can be beneficial or harmful in terms of animal health.

Copper, molybdenum and sulfur. The interrelationship among Cu, Mo, and S provides a good example of the complex interrelationships which can exist among trace elements. In ruminants, investigations of the dose:response relationships have established the practical significance of the interaction (Suttle, 1975). Dick (1956) determined that Mo could impair the Cu metabolism of sheep under experimental conditions, provided that the diet contained adequate sulfate.

Both inorganic and organic S can reduce plasma Cu concentrations (Suttle, 1975). The involvement of Mo and the entire S component of the diet in an antagonism has been investigated. Suttle and Field (1974) and Suttle (1974) found that the administration of Mo in the diet and Cu by subcutaneous injection or continuous intravenous infusion circumvented the antagonism. This indicated that the important site of antagonism was located in the gut. When the rumen was bypassed by administering Mo by continuous abomasal infusion while Cu was given in the diet, Mo no longer inhibited Cu repletion in hypocupremic ewes. Therefore, it appeared that the Cu x Mo x S antagonism involved the lowering of the availability of both Cu and Mo in the rumen (Suttle, 1975). A hypothesis for the mechanism of this Cu x Mo x S antagonism is based on two *in vitro* reactions. Dowdy and Matrone (1968a,b) determined that Cu^{2+} and MoO_4^{2-} readily combine to form $CuMoO_4$. Tridot and Bernhard (1962) found that MoO_4^{2-} is readily converted to thiomolybdate (MoS_4^{2-}) in a sulfide-rich alkaline medium. Since both organic and inorganic forms of S are readily degraded to S^{2-} in the rumen, it appeared conceivable that a triple complex, cupric thiomolybdate, is formed, in which the Cu is of low biological activity.

When considering the amount of dietary Cu, Mo and S in the bovine diet, the ratio can be important. For optimal production, ratios of 6:1 to 10:1 Cu:Mo are suggested. Forages containing 7 to 10 μg Cu per g should meet bovine requirements unless Mo or S intake is high (Corah and Ives, 1991).

Iron. High Fe content of feeds and water supplies have been shown to cause hypocuprosis in cattle (Humphries et al., 1983). Reduced liver Cu concentrations in cattle and sheep given diets with 1500-2300 mg Fe/kg have been observed (Standish et al., 1969, 1971; Campbell 1974). Little is known about the mechanisms whereby Fe affects Cu metabolism and it is not clear whether the effect of Fe is on the absorption or the hepatic retention of Cu. Humphries et al. (1983) suggested that the inhibitory effect of Fe on Cu utilization in cattle is, like Mo, dependent on interaction with S^{2-} in the rumen. The authors supported their view through the observation that liver Cu concentrations in preruminant calves are not affected by additional Fe (500 mg/kg DM) in their milk substitute ration and that the biliary excretion of Cu by weaned calves is not affected by dietary Fe supplementation.

Other elements. Elements that interact with Cu metabolism include calcium (Ca), cadmium (Cd), zinc (Zn) and others. Less Cu was absorbed by mice from a high Ca than a low Ca diet due to an increase in intestinal pH (Thompsett, 1940). High Zn intakes can depress Cu absorption and Cu absorption is greatly increased in Zn deficiency. High dietary levels of Cd depress Cu uptake (Underwood, 1977).

Copper Supplementation and Growth in Ruminants

Supplementation with Cu in ruminant diets has been shown to successfully alleviate Cu deficiency (Steacy et al., 1983; Whitelaw et al., 1984; Richards et al., 1985). However, Cu supplementation in ruminants has yielded conflicting results in terms of animal performance, in particular, body weight gains (Whitelaw et al., 1984; Sankoh and Boila, 1987; Ward et al., 1993). The conflicts among these reports appear to be based primarily on the Cu status of the animals at the beginning of each study. Weight gains are generally improved with Cu supplementation if the animals begin with a Cu-deficient status or if there are Mo or S antagonisms. Both Whitelaw et al. (1984) and Richards et al. (1985) observed significant body weight gains in cattle supplemented orally with copper oxide (CuO) needles versus unsupplemented cattle. Suttle (1981) observed similar responses when CuO needles were given to hypocupremic sheep and cattle. Boila (1987) recorded no increase in weight gains in grazing cattle supplemented orally with CuO when compared to the unsupplemented group. Steacy et al. (1983) observed a decrease in ADG when 312 hypocupremic bulls were supplemented with a mineral mix containing .2% Cu. In this study there was a positive correlation ($P < .05$) between plasma Cu levels and ADG in the presupplementation period of the trial. This correlation became negative, however, during the Cu supplementation period. Engel et al. (1964) and Sankoh and Boila (1987) also observed lower weight gains in Cu-supplemented beef cattle; however, these results were not statistically significant. Ward et al. (1993) found that Cu supplementation of steers with adequate Cu status did not improve animal performance.

Copper and Reproduction

Bovine testicular measurements can be used as indicators of sire reproductive performance and productive traits in their progeny. Techniques used to measure testicular characteristics that are correlated with sperm output, seminal quality, and fertility are the measurement of SC and testicular consistency.

Testicular weight is an important trait and can provide an accurate estimate of sperm-producing parenchma (Almquist and Amann, 1961). Because testicular weight can not be measured directly in live bulls, indirect measurements were developed. These measurements include testes diameter and length, paired-testes width and SC (Boyd and VanDemark, 1957; Almquist et al., 1976). The measurement of SC is an accurate predictor of both testicular weight and sperm output in growing bulls. Correlations between SC and testes weight in dairy bulls range from .89 to .95 (Coulter, 1979) and was .95 in Hereford and Angus bulls (Coulter and Foote, 1979). The correlation between SC and sperm output in young dairy bulls was .81 (Hahn et al., 1969).

Testicular consistency, which measures the firmness of the testes, is related to seminal quality. Poor seminal quality and reduced fertility or sterility is related to a soft consistency of the testes (Haq, 1949).

Investigations into the effects of Cu or Cu deficiency on male ruminant reproductive traits are limited. Copper deficiency can negatively affect female ruminant reproductive performance. Disorders linked to Cu deficiency include decreased conception rates, infertility, anestrus and fetal resorption (Corah and Ives, 1991). In many cases, decreased reproductive performance due to a Cu deficiency may actually be due to secondary deficiencies complicated by Mo (Baldwin et al., 1981; Phillippo et al., 1987; Van Niekerk and Van Niekerk, 1989a,b)

In the bull calf, increased Mo in the diet decreased libido and induced sterility. These conditions were linked to a marked damage in interstitial and germinal epithelium and lowered spermatogenesis (Thomas and Moss, 1951). In rams, semen volume was suppressed by a severe Cu deficiency induced by feeding diets high in Mo alone or Mo and sulfate (SO_4). This was attributed to a lower pH of the semen when compared to semen pH levels of Cu-supplemented rams. In addition, rams supplemented with Mo and SO_4 had lower sperm concentration, poorer sperm motility and morphology, lower fructose concentrations in the ejaculates and lower plasma testosterone concentrations than rams supplemented with Cu or Mo alone. After this induced Cu deficiency was reversed, the above parameters reverted to normal (Van Niekerk and Van Niekerk, 1989a). In a second study by Van Niekerk and Van Niekerk (1989b), macro- and microscopic changes of the testes caused by Mo alone or Mo and SO_4 induced Cu deficiency were investigated. The diameter and length of testes from rams supplemented with high levels of Mo and SO_4 were smaller than rams supplemented with Mo or Cu. This decrease in testes size was accompanied by a marked decrease in testes mass. Histological examinations revealed that the seminiferous tubules were less developed and less active in the severely Cu-deficient (Mo and SO_4 group) rams. This was attributed to an inactivity of the Sertoli cells which also accounted for the total lack of spermiogenesis. Van Ryssen et al. (1990) observed no significant effects of high Mo intakes on ram fertility; however, they did not establish a Cu-deficient state in any of the rams in the study. The effects of Cu on spermatozoa in the human have been investigated. The role of Cu in sperm is unclear, but it appears to be involved in spermatozoan mobility and it may also act at the pituitary receptors which control the release of interstitial cell stimulating hormone (LH). In the seminal fluid, Cu levels may be decreased in cases of azoospermia and may be increased in cases of oligo- and asthenozoospermia. Copper concentrations in the ejaculate, however, do vary considerably from one day to the next and can also vary in different fractions from a single ejaculate (Skandhan, 1992).

CHAPTER III.

Effects of a Sustained-Release Copper Bolus on Growth and Reproductive Traits of Beef Bulls

Abstract

Two experiments were conducted to determine the effects of sustained -release copper (Cu) boluses on growth and reproductive traits of beef bulls. Two groups of bulls were used in consecutive years (n=26 in experiment 1; n=20 in experiment 2). Bulls in each experiment were allotted by weight and breed into two equally numbered treatment groups (control (C); treated (T)). Bulls were housed together and offered a balanced diet containing 9 ppm Cu for an ADG of 1.4 kg. Treated bulls in each experiment were given 1 Cu bolus (Cuprax® Pitman-Moore, Mundelein, IL in experiment 1; Copasture™ Copasture-25 bolus Schering-Plough, Kenilworth, NJ in experiment 2). All bulls were weighed, had blood samples taken, and had scrotal circumferences (SC) measured on d 0, 28, 56, 84, 112, and 140. Blood was collected via jugular venipuncture to determine serum Cu and plasma ceruloplasmin (Cp) concentrations. Breeding soundness examinations (BSE) were conducted on all bulls on d 140 and 185 in experiment 1 and d 168 in experiment 2. Body weight and SC were not affected by treatment in either experiment ($P > .05$). Serum Cu and plasma Cp concentrations were not affected by treatment in experiment 2 ($P > .05$). Serum Cu was higher for the T bulls on d 56, 84, and 112 in experiment 1 ($P < .05$). All serum Cu concentrations for both experiments were within the normal range for cattle. Plasma Cp concentrations were recorded only for d 1, 112, and 140 in experiment 1 and were significantly higher for the C group on d 112 ($P < .05$). There were no significant effects of

treatment on the results of the BSE (SC, sperm normality, progressive motility, and primary or secondary abnormalities) in either experiment. These data suggest that the Cu bolus in experiment 2 had no effect on body weight gains, SC, serum Cu and plasma Cp concentrations, sperm normality, progressive motility, and primary and secondary abnormalities. These data also suggest that the Cu bolus in experiment 1 had no effect on body weight gains, SC, and percentage of normal sperm, progressive motility, and primary and secondary abnormalities; however, treatment in experiment 1 did increase serum Cu concentrations on some but not all sampling days and did not increase concentrations above the normal range for beef cattle. The conclusion made from these experiments is that the sustained Cu boluses used in these studies did not significantly affect growth and reproductive traits in beef bulls with adequate Cu status.

Introduction

Copper is required by the ruminant for a number of physiological functions including growth and reproduction (McDowell, 1992). In beef cattle, Cu-deficiency is often a problem and can be affected by a number of factors including age and stage of production and interactions with other elements in the diets (Underwood, 1977). Supplementation with Cu in ruminant diets has been shown to alleviate Cu-deficiency (Steacy et al., 1983; Whitelaw et al., 1984; Richards et al., 1986) but has yielded conflicting results in terms of animal performance, particularly, body weight gains (Whitelaw et al., 1984; Sankoh and Boila, 1987; Ward et al., 1993). Smith et al. (1989) established a relationship between sire SC and reproductive and growth traits of his progeny. Thomas and Moss (1951) and Van Niekerk and Van Niekerk (1989ab) determined that Cu-deficiency in ruminants can reduce spermatogenesis, sperm motility, libido and testes development. The objectives of these experiments were to determine the effects of sustained-release Cu boluses on growth and reproductive traits in growing beef bulls.

Materials and Methods

Experimental Design

Experiment 1. Beef bulls (7 to 9 months old; n=26) were blocked by initial mean body weight (296.4 kg) and breed (16 Angus (A); 8 Polled Hereford (H); 2 Gelbvieh (G)) into equally numbered treatment groups. Within each breed, the bulls were allotted in pairs. The heaviest bulls in each breed were allotted into different groups and this process was continued with the next heaviest pairs always allotting the heavier bull of a pair to the group that had been assigned the lowest body weight of the two bulls in the previous pair. Initial mean weights were determined by recording the body weights prior to feeding on two consecutive days. On the second day of weighing and subsequent allotment into one of two treatment groups (d 1), bulls in the assigned treated (T) group were given a Cu bolus and the remaining group served as the control (C) group. Treated bulls (n=13) were given 1 Cuprax® (Pitman-Moore, Mundelein, IL) bolus containing Cu oxide (CuO) needles. Bulls were housed together and were weighed, had blood samples taken, and had scrotal circumferences (SC) recorded on d 1, 28, 56, 84, 112, and 140. Breeding soundness examinations (BSE) were conducted using the procedures and guidelines outlined in the

Theriogenology Handbook (Chenoweth et al., 1993) on all bulls on d 140 and 185.

Experiment 2. Beef bulls (7 to 9 months old; n=20) were blocked by initial mean body weight (272.3 kg) and breed (14 A and 6 H) into equally numbered treatment groups. Within each breed, the bulls were allotted in pairs. The heaviest bulls in each breed were allotted into different groups and this process was continued with the next heaviest pairs always allotting the heavier bull of a pair to the group that had been assigned the lowest body weight of the two bulls in the previous pair. Initial mean weights were determined by recording the body weights prior to feeding on two consecutive days. On the second day of weighing and subsequent allotment into one of two treatment groups (d 1), bulls in the assigned treated (T) group were given a Cu bolus and the remaining group served as the control (C) group. Treated bulls (n=10) were given 1 Copasture™ Copasture-25 bolus (Schering-Plough, Kenilworth, NJ) containing CuO needles. Bulls were housed together and weighed, had blood samples taken, and had SC recorded on d 1, 28, 56, 84, 112, and 140. Breeding soundness examinations were conducted as stated in experiment 1 on all bulls on d 140 and 168. Two bulls from T had to be removed from the experiment due to injury after d 140 and prior to d 168.

Diets and Sample Collection

Diets. Bulls in experiment 1 were fed a total mixed diet consisting of 909 g of SBM per head per day, 1 % of expected body weight in whole shelled corn (DM basis) per head per day, and ad libitum access to corn silage. The diet contained 8 ppm Cu. The bulls were also offered ad libitum access to limestone and salt in a mixture of 2.3 kg per 36.4 kg, respectively. No other source of supplemental minerals were offered. The chemical composition of the diet in experiment 1 is shown in Table 1. Feeding and the diet in experiment 2 was designed identically to that of experiment 1 with the exception that whole shelled corn was offered at 1.5 % of expected body weight (DM basis) per head per day and the level of Cu in the ration was 11 ppm. The chemical composition of the diet in experiment 2 is shown in Table 2. In both experiments acid deterdent fiber was analyzed according to Goering and Van Soest (1970) and nitrogen was determined by the Kjeldahl method. Mineral analyses were conducted using the Inductively Coupled Plasma method (ICP 9000, Fisher Scientific, Pittsburgh, PA).

Sample collection. Two 10 mL blood samples were collected via jugular venipuncture from each bull in each experiment on d 1, 28, 56, 84, 112 and 140. One of the blood samples was collected into a heparinized tube for plasma collection and the other for serum collection (Vacutainer™ , Becton Dickinson, Rutherford, NJ). Blood samples were centrifuged for 15 min at 1800 x *g*. Both serum and plasma samples from experiment 1 were stored in polypropylene tubes at -20°C until analyzed. In experiment 2, serum samples were stored in polypropylene tubes at -20°C while plasma samples were stored in polypropylene tubes at -70°C until their analyses.

Scrotal circumferences were recorded in cm using a metal scrotal measuring tape.

Semen was collected via electro-ejaculation (Electrojac III™, Ideal® Instruments, Chicago, IL) into sterile sample collection bags that had been hand-warmed. Semen analysis was conducted immediately following ejaculation of each bull.

Serum Copper Analysis

Table 1. Chemical composition (DM basis) of total mixed diet on d 38 of experiment 1

	----- % -----		----- ppm -----
DM	39.39	Na	22
CP	13.89	S	1255
ADF	21.79	Zn	25
TDN	73.06	Cu	8
Ca	.22	Mn	40
P	.24	Fe	215
Mg	.17		
K	.46		

Table 2. Chemical composition (DM basis) of total mixed diet on d 75 of experiment 2

	%		ppm
DM	47.29	Na	39
CP	13.09	S	2482
ADF	15.39	Zn	48
TDN	70.00	Cu	11
Ca	.33	Mn	44
P	.52	Fe	312
Mg	.30		
K	1.47		

Sample preparation. Serum samples were thawed at room temperature and diluted three fold (1 part sample, 2 parts water) with de-ionized and distilled water in polypropylene tubes.

Standards preparation. A five-point calibration curve (.125, .250, .500, .750 and 1.00 ppm) was constructed using Cu reference standard solution (Fisher # SC194-100, Fisher Scientific, Pittsburgh, PA). Standards were prepared with de-ionized, distilled water.

Sample analysis. Samples were analyzed through flame atomic absorption using the Varian SpectrAA 20 (Varian, Sunnyvale, CA). Instrument operating conditions are shown in Table 3. De-ionized, distilled water (blank) was used to zero the instrument. Standards and samples were introduced manually. Three readings were taken on each standard and sample. Readings were accepted within 3% relative SD. Sample concentrations were multiplied by three to obtain ppm.

Plasma Ceruloplasmin Assay

Procedures for performing the plasma Cp assay were adapted from a serum ceruloplasmin determination kit (Sigma Chemical, St. Louis, MO).

Buffer solution. An .8 M sodium acetate buffer solution was prepared using sodium acetate and de-ionized water. Five-hundred mL of de-ionized water was mixed with 54.43 g of sodium acetate. Acetic acid was used to adjust to pH 8.7.

Substrate solution. A para-phenylenediamine substrate solution was prepared just prior to performing the assay. Para-phenylenediamine dihydrochloride was diluted in the sodium acetate buffer in a ratio of 34 mg per 20 mL. This substrate was refrigerated and protected from light.

Table 3. Varian SpectrAA 20 instrument operating conditions for serum Cu analysis

Instrument mode	Absorbance
Calibration mode	Concentration
Measurement mode	Integration
Lamp position	1
Lamp current (mA)	4
Slit width (nm)	0.5
Wavelength (nm)	324.8
Flame	Air-Acetylene
Sample introduction	Manual
Delay time	0
Time constant	0.05
Measurement time (sec)	1.0
Replicates	2
Background correction	Off
Oxidant flow	3.5
Acetylene flow	1.5

Reaction inhibiting agent. A sodium azide (NaN_3) inhibiting agent was prepared by mixing 45 mg NaN_3 per mL sodium acetate buffer.

Blank solution. A blank solution was prepared using .27 mL of substrate per .01 mL of reaction inhibiting agent. This solution was refrigerated and protected from light.

Standards. Standards were prepared and used to calibrate the computer program for each sample set (Appendix A; Figure 1). Bovine Cp (Oxidase activity = 3100 units/mL and 38 units/mg ceruloplasmin protein) (Sigma Chemical, St. Louis, MO) was used to prepare the standards. A 2 mL solution of 2 mg of Cp protein per mL saline was prepared using .05 mL ceruloplasmin and 1.95 mL saline. Standards were then prepared by diluting the 2 mg/mL solution with saline to 1.5, 1, .5, .25, .125, .0625 mg/mL solutions. Standards were refrigerated and protected from light.

Assay Procedures. Plasma samples were thawed on ice and protected from light. Computer template 1 was set up with unknowns, unknown blanks, standards, and standard blanks (Appendix A; Figure 2) using Softmax computer software (Molecular Devices, Menlo Park, CA). Subsequent templates were set up with unknowns and unknown blanks only (Appendix A; Figure 3). Each template represented one sample plate. Flat bottom, non-sterile, 96 well plates were used. Plate 1 was loaded first with 20 μL of the six standards in triplicate and the remaining wells were filled with 20 μL of plasma in triplicate on ice. Subsequent plates were loaded without standards. The plate was removed from the ice and .28 mL of blank solution was added to all blank wells using a repeating pipettor. Sample wells were then loaded with .28 mL of substrate solution. The loaded plate was then agitated twice to mix and placed in a 37°C water bath for exactly 20 min. At that time, the plate was removed, the bottom wiped to remove excess water and placed in a microplate reader (Molecular Devices UV*max*, Menlo Park, CA) to measure ceruloplasmin activity in mg/mL at a wavelength of 550 nm.

Semen Analysis

Semen evaluation was conducted through procedures outlined in the Theriogenology Handbook (Chenoweth et al., 1993). Results of the d 140 BSEs were not recorded or analyzed in either experiment because of their ages at that time (12-14 months) and to give the younger bulls in each group a greater chance to sexually mature.

Motility. Percent progressive motility of spermatozoa was assessed under a microscope. Specimens were placed on a warmed slide and covered with a coverslip. The percentage of active, progressively motile cells was estimated and rated (Table 4). The minimum threshold for individual motility was set at 30 %.

Normality. A drop of semen was mixed with a drop of Hancock stain and spread over the surface of a slide and allowed to air dry. Normality (percentage of sperm free from abnormalities) was assessed microscopically. The minimum threshold for sperm normality was set at 70 % normal. Abnormalities were determined to be either primary or secondary (Table 5).

Statistical Analysis

Data were analyzed as a two-way analysis with two treatment groups. Analysis of variance was conducted using the GLM and Repeated Time Polynomial procedures of SAS (1985).

Table 4. Guidelines for estimating and rating sperm motility (Chenoweth et al., 1993)

Mass activity (Gross)	Rating	Individual (%)
Rapid swirling	Very Good (VG)	$\geq$ 70
Slower swirling	Good (G)	50 - 69
Generalized oscillation	Fair (F)	30 - 49
Sporadic oscillation	Poor (P)	< 30

Table 5. Categories of sperm abnormalities (Chenoweth et al., 1993)

Primary abnormalities	Secondary abnormalities
Underdeveloped	Small normal heads
Double forms	Giant & short broad heads
Acrosome defects	Free normal heads
Crater-Diadem defect	Detached, folded, loose acrosome membranes
Pear-shaped head	Abaxial midpiece
Abnormal head contour	Distal droplet
Small and free abnormal heads	Simple bent tail
Proximal droplet	Terminal coiled tail
Double bent and coiled tail	
Accessory tail	

Results

Experiment 1. Body weight gains of bulls were not affected by treatment (Table 6; P > .05); however, body weight did increase in both treatment groups significantly over the length of the experiment (Figure 1; P < .05). Least squares mean body weights were 312 and 290 kg on d 1 and 477 and 448 kg on d 140 for C and T bulls, respectively.

Scrotal circumferences were not affected by treatment (Table 7; P > .05) but increased in both groups over time from 25 .6 and 24.9 cm on d 1 to 35.2 and 34 cm on day 140 for C and T bulls, respectively (Figure 2; P < .05).

Least squares mean serum Cu concentrations were not significantly different (P > .05) between C and T bulls on d 1 (.89 and .85 ppm, respectively) and 28 (.87 and .85 ppm, respectively; Table 8). Serum Cu concentrations were higher (P < .05) for T than for C bulls on d 56 (.98 and .90 ppm, respectively), 84 (.86 and .75 ppm, respectively) and 112 (.99 and .86 ppm, respectively). By the end of the feeding trial (d 140), differences between Cu concentrations were no longer significant (1.15 and 1.22 ppm for C and T bulls, respectively; P > .05). There were significant increases in serum Cu concentrations over time for both treatment groups (Figure 3; P < .05). On d 56, 84, and 112 serum Cu concentrations were significantly different among breeds (Table 9; P < .05). These differences became nonsignificant when the two Gelbvieh bulls with markedly low serum Cu concentrations were removed from the statistical analysis. A significant interaction between treatment and breed on d 84 and 112 was observed (P < .05).

Table 6. Least squares means for body weight of bulls in experiment 1

	Treatment[a]			
Day	Control (kg)[b]	SE	CuO (kg)[c]	SE
1	312.9	13.8	290.9	13.8
28	356.9	15.3	327.2	15.3
56	396.1	16.8	365.6	16.8
84	411.7	17.3	383.7	17.3
112	448.4	17.6	419.1	17.6
140	477.5	19.6	448.4	19.6

[a]Differences on each day are not significant ($P > .05$).
[b]No Cu: $n = 13$.
[c]Cu bolus: $n = 13$.

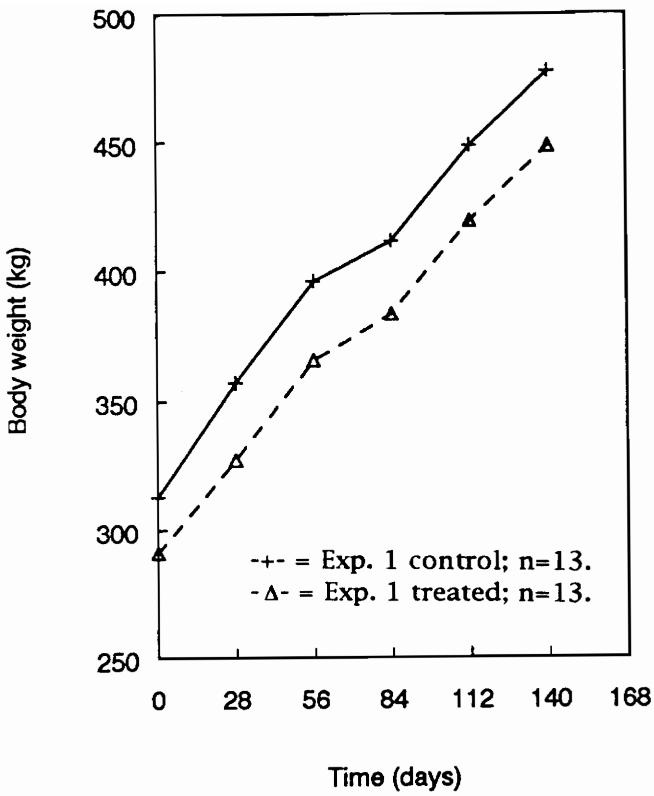

Figure 1. Least squares mean body weight gains for bulls in experiment . Differences within days are not significant (P > .05). Differences over time are significant (P < .05).

Table 7. Least squares means for scrotal circumference of bulls in experiment 1

	Treatment[a]			
Day	Control (cm)[b]	SE	CuO (cm)[c]	SE
1	25.7	.8	25.0	.8
28	28.7	.9	27.8	.9
56	31.2	.9	30.4	.9
84	32.6	.9	32.0	.9
112	34.5	1.0	33.6	1.0
140	35.2	1.2	34.0	1.2

[a] Differences on each day are not significant ($P > .05$).
[b]No Cu: n = 13.
[c]Cu bolus: n = 13.

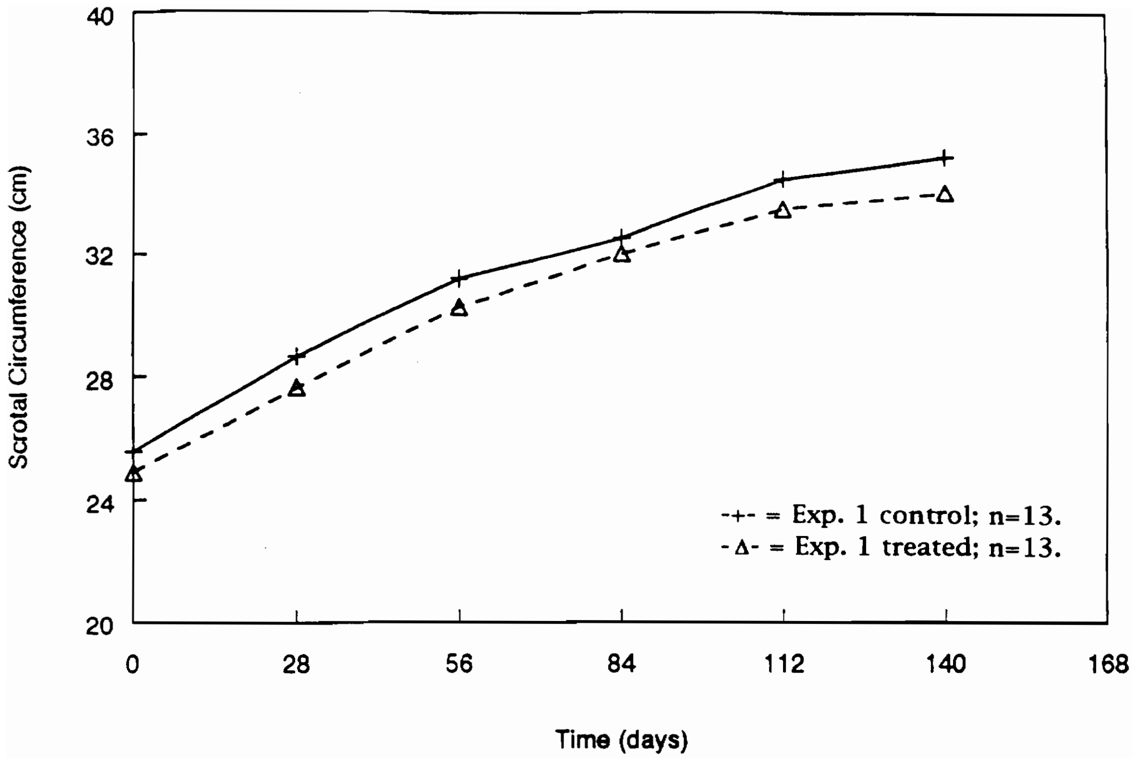

Figure 2. Least squares mean scrotal circumferences for bulls in experiment 1. Differences within days are not significant (P > .05). Differences over time are significant (P < .05).

Table 8. Least squares means for serum Cu concentrations from bulls in experiment 1

	Treatment			
Day	Control (ppm)[a]	SE	CuO (ppm)[b]	SE
1	.89[c]	.07	.85[c]	.07
28	.87[c]	.06	.85[c]	.06
56	.90[c]	.06	.98[d]	.06
84	.75[c]	.05	.86[d]	.05
112	.85[c]	.05	.99[d]	.05
140	1.15[c]	.06	1.22[c]	.06

[a]No Cu: n = 13.
[b]Cu bolus: n = 13.
[c,d]Values within a row with different superscript differ ($P < .05$).

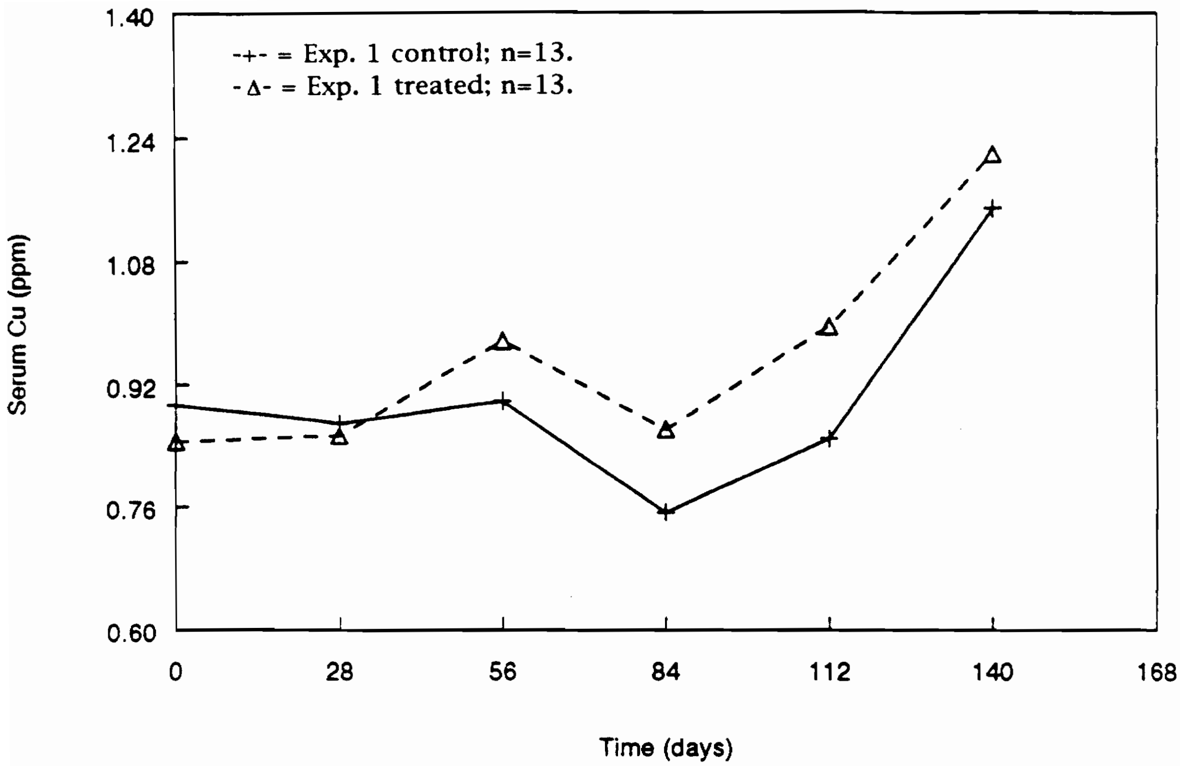

Figure 3. Least squares mean serum Cu concentrations for bulls in experiment 1. Differences within days 56, 84, and 112 are significant ($P < .05$). Differences over time are significant ($P < .05$).

Table 9. Least squares mean serum Cu concentrations by breed of bulls in experiments 1 and 2

	Serum Cu concentration (ppm)					
	Day					
Exp. 1 Breed	1	28	56	84	112	140
Angus ± SE (n = 16)	.93[a] ± .04	.92[a] ± .04	1.11[a] ± .04	.93[a] ± .03	1.00[a] ± .03	1.29[a] ± .04
Gelbvieh ± SE (n = 2)	.78[a] ± .13	.77[a] ± .11	.74[b] ± .11	.63[b] ± .09	.73[b] ± .09	1.06[a] ± .10
Polled Hereford ± SE (n = 8)	.90[a] ± .06	.90[a] ± .06	.97[a] ± .06	.86[a] ± .04	1.04[a] ± .05	1.21[a] ± .05
Exp. 2 Breed						
Angus ± SE (n = 14)	.99[c] ± .04	.97[c] ± .04	.93[c] ± .07	.81[c] ± .05	.98[c] ± .04	.92[c] ± .04
Polled Hereford ± SE (n = 6)	1.02[c] ± .06	.89[c] ± .07	.96[c] ± .10	.85[c] ± .07	.92[c] ± .06	.90[c] ± .06

[a,b]Values within a column with different superscript differ ($P < .05$).

[c]Differences within columns are not significant ($P > .05$).

Least squares mean plasma Cp concentrations were not different between treatment groups on d 1 and 140 (.39 and .39 mg/mL for C and T bulls, respectively; $P > .05$); however, Cp concentrations of bulls from C were higher (.88 versus .63 mg/mL) on day 112 (Table 10; $P < .05$). Due to lab errors, plasma samples from days 28, 56, and 84 were not included in the analysis. Plasma Cp concentrations increased over time in both treatment groups (Figure 4; $P < .05$).

There were no differences ($P > .05$) in SC between treatment groups on d 185 when the BSE was conducted. In addition, percentage of normal sperm (76 and 72 % for C and T bulls, respectively), motility (78 and 73 % for C and T bulls, respectively), and primary and secondary abnormalities (19.7 and 22.5 % and 5.8 and 5.5 % for C and T bulls, respectively) were not affected by Cu treatment (Table 11; $P > .05$). Two bulls failed the BSE; one from each treatment group.

Experiment 2. Body weight gains of bulls were not affected by treatment (Table 12; $P > .05$). Weight gains significantly increased over time in each treatment group (Figure 5; $P < .05$). Least squares mean body weights were 269 and 268 kg on d 1 and 437 and 430 kg on d 140 for C and T bulls, respectively.

Treatment did not affect SC of bulls on any d (Table 13; $P > .05$); however, SC did increase significantly ($P < .05$) from d 1 (25.7 and 26.3 cm for C and T bulls, respectively) to d 140 (34.3 and 33.7 cm for C and T bulls, respectively)for both treatment groups (Figure 6; $P < .05$).

Serum Cu concentrations were not affected by treatment throughout the study (Table 14; $P > .05$). Least squares mean serum Cu concentrations were 1.03 and .97 ppm on d 1 and .91 and .91 ppm on d 140 for C and T bulls,

Table 10. Least squares means for plasma Cp concentrations from bulls in experiment 1

Day	Treatment: Control (mg/ml)[a]	SE	CuO (mg/ml)[b]	SE
1	.39[c]	.12	.39[c]	.12
112	.88[c]	.12	.63[d]	.12
140	.68[c]	.15	.72[c]	.15

[a]No Cu: n = 13.
[b]Cu bolus: n = 13.
[c,d]Values within a row with different superscript differ ($P < .05$).

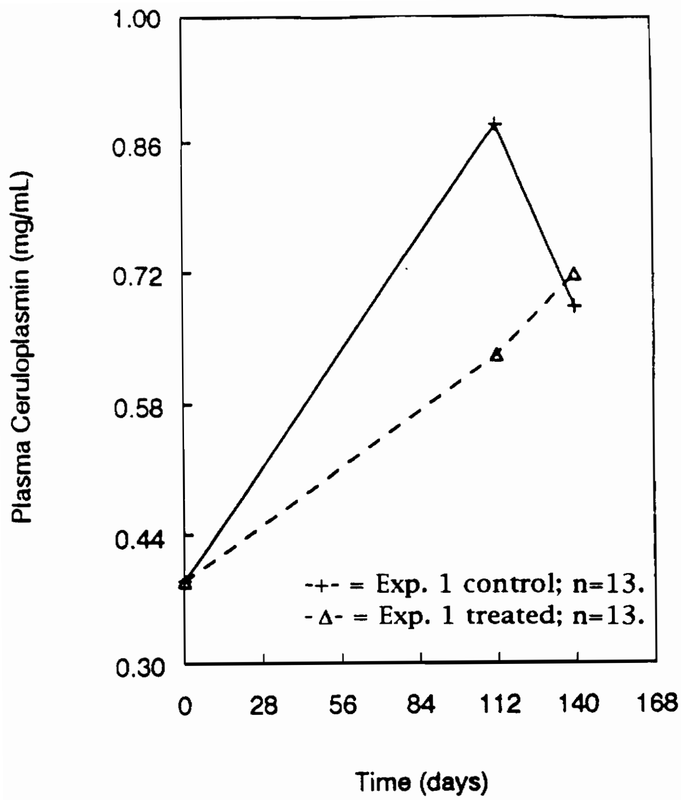

Figure 4. Least squares mean plasma ceruloplasmin concentrations for bulls in experiment 1. Differences within d 112 are significant ($P < .05$). Differences over time are significant ($P < .05$).

Table 11. Results of breeding soundness examination conducted on d 185 of experiment 1

	Treatment[a]			
	Control[b]	SE	CuO[c]	SE
	cm			
Scrotal circumference	34.04	1.15	33.68	1.15
	%			
Sperm Normality	76.00	8.95	72.41	8.95
Sperm Motility	78.12	9.05	73.33	9.05
Sperm 1° Abnormalities	19.70	8.94	22.54	8.94
Sperm 2° Abnormalities	5.79	·3.58	5.45	3.58

[a]Values are least squares means.
[b]No Cu: n = 13.
[c]Cu bolus: n = 13.

Table 12. Least squares means for body weight of bulls in experiment 2

	Treamtent[a]			
Day	Control (kg)	SE	CuO (kg)	SE
1	269.0	11.1	268.4	11.1
28	318.7	12.9	314.2	12.9
56	348.8	14.5	344.5	14.5
84	380.7	15.5	376.8	15.5
112	397.9	16.5	391.9	16.5
140	437.1	17.6	430.4	17.6

[a]Differences on each day are not significant ($P > .05$).
[b]No Cu: n = 10.
[c]Cu bolus: n = 10.

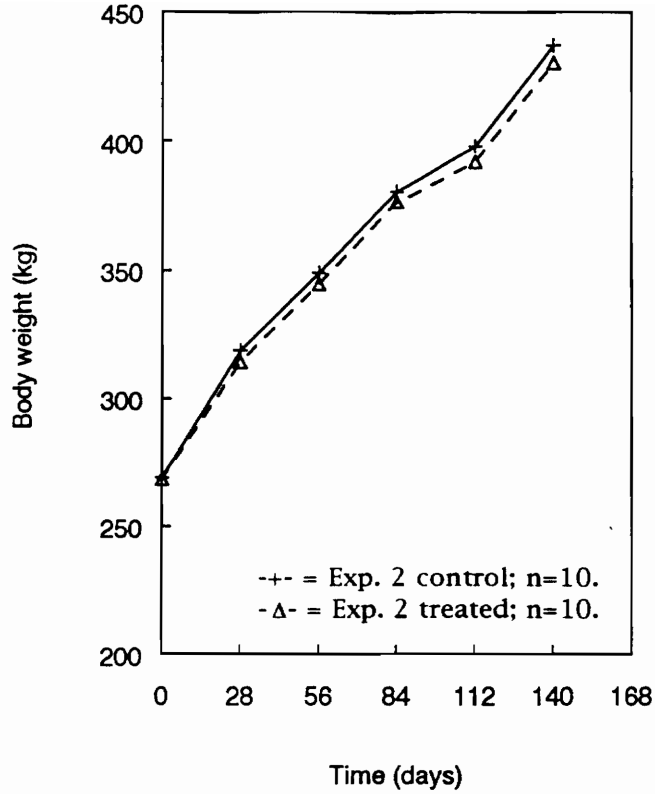

Figure 5. Least squares mean body weight gains for bulls in experiment 2. Differences within days are not significant (P > .05). Differences over time are significant (P < .05).

Table 13. Least squares means for scrotal circumference of bulls in experiment 2

	Treatment[a]			
Day	Control (cm)[b]	SE	CuO (cm)[c]	SE
1	25.7	.9	26.3	.9
28	26.9	1.0	27.6	1.0
56	28.8	1.0	29.6	1.0
84	30.8	1.0	31.0	1.0
112	33.8	.8	33.5	.8
140	34.3	1.2	33.7	1.2

[a]Differences on each day are not significant ($P > .05$).
[b]No Cu: n = 10.
[c]Cu bolus: n = 10.

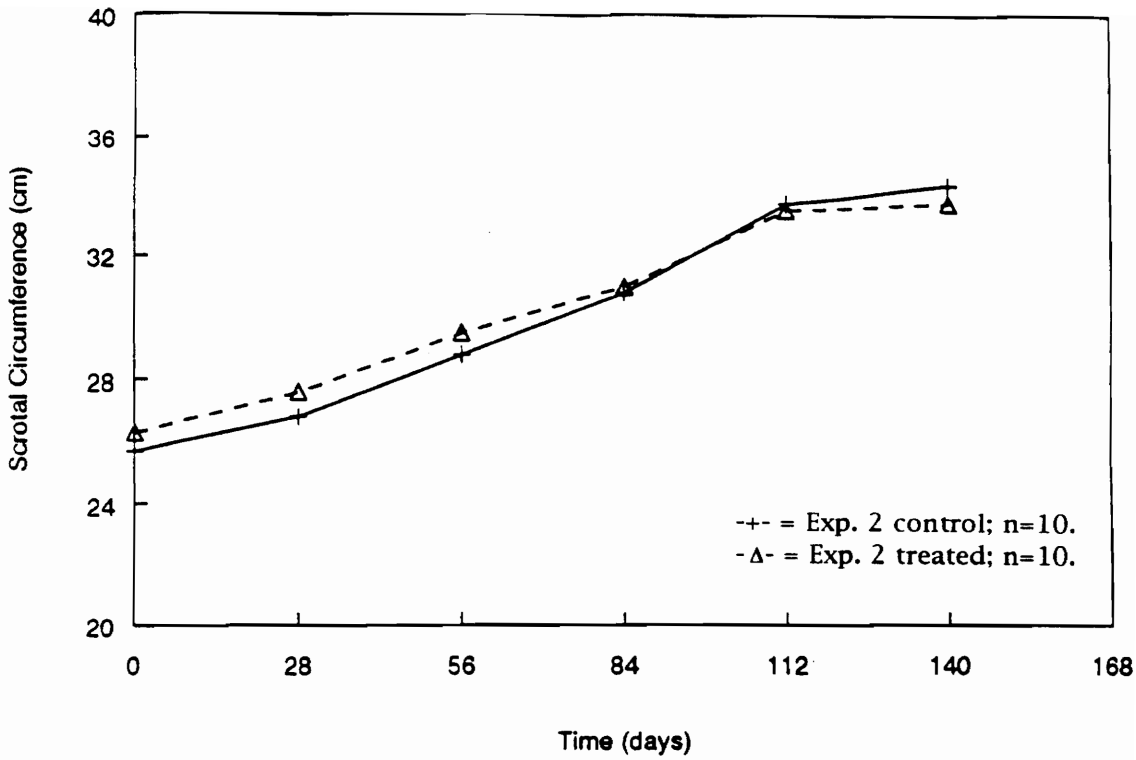

Figure 6. Least squares mean scrotal circumferences for bulls in experiment 2. Differences within days are not significant (P > .05). Differences over time are significant (P < .05).

Table 14. Least squares means for serum Cu concentrations from bulls in experiment 2

	Treatment[a]			
Day	Control (ppm)[b]	SE	CuO (ppm)[c]	SE
1	1.03	.06	.97	.06
28	.98	.06	.88	.06
56	.99	.09	.91	.09
84	.83	.06	.83	.06
112	.92	.05	.99	.05
140	.91	.0	.91	.05

[a]Differences on each day are not significant ($P > .05$).
[b]No Cu: n = 10.
[c]Cu bolus: n = 10.

respectively (Table 9). Serum Cu was, however, significantly different for each group over time (Figure 7; $P < .05$).

Plasma Cp concentrations were not different between treatments on any day (Table 15; $P > .05$). Least squares mean plasma Cp concentrations were 1.64 and 1.42 mg/mL on d 1 and .95 and .93 mg/mL on d 140 for C and T bulls, respectively. Plasma Cp concentrations were significantly different over time for both treatment groups (Fiqure 8; $P < .05$).

On the day of the BSE (d 168), SC did not differ between treatments ($P > .05$). Percentage of normal sperm (70 and 61 % for C and T bulls, respectively), motility (62.5 and 57 % for C and T bulls, respectively), and primary and secondary abnormalities (11 and 6.7 % and 11 and 21 % for C and T bulls, respectively) were not affected by Cu treatment (Table 16; $P > .05$). Two bulls were removed from the treated group prior to d 168 due to injuries. Two bulls failed the BSE; one from each treatment group.

The breed of bull had no significant effect on any of the parameters investigated in experiment 2 ($P > .05$).

Discussion

Body weight gains. Bulls in experiment 1 and 2 showed no response in terms of body weight gains to Cu supplementation (Tables 6 and 12, respectively). Copper status for bulls in each treatment group within each experiment were within the normal ranges throughout the studies and dietary Cu levels met the NRC (1984) minimum recommended levels of 8 mg/kg (Tables 1 and 2). Because blood Cu levels were well within the normal range and not indicative of a metabolic deficiency of Cu, a growth

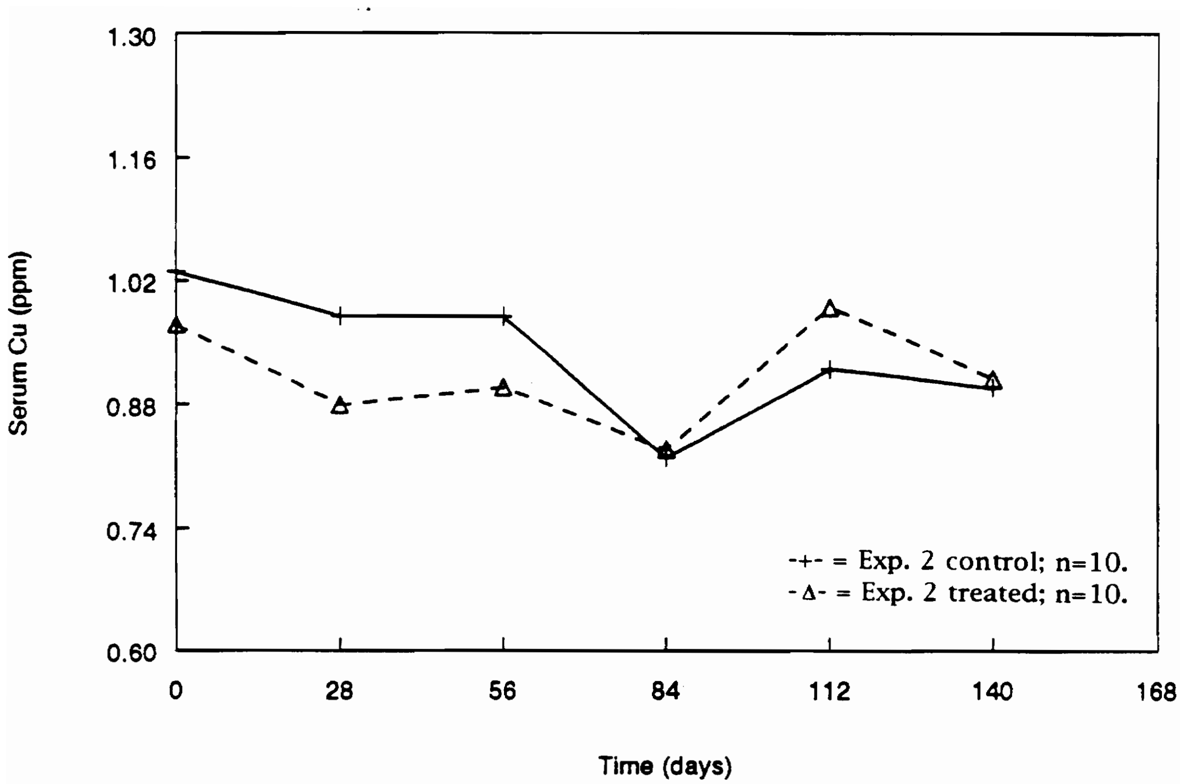

Figure 7. Least squares mean serum Cu concentrations for bulls in experiment 2. Differences within days are not significant ($P > .05$). Differences over time are significant ($P < .05$).

Table 15. Least squares means for plasma Cp concentrations from bulls in experiment 2

	Treatment[a]			
Day	Control (mg/ml)[b]	SE	CuO (mg/ml)[c]	SE
1	1.64	.12	1.42	.12
28	.95	.12	.96	.12
56	.89	.15	.77	.15
84	1.04	.18	1.05	.18
112	1.40	.25	1.46	.25
140	.95	.13	.93	.13

[a]Differences on each day are not significant ($P > .05$).
[b]No Cu: n = 10.
[c]Cu bolus: n = 10.

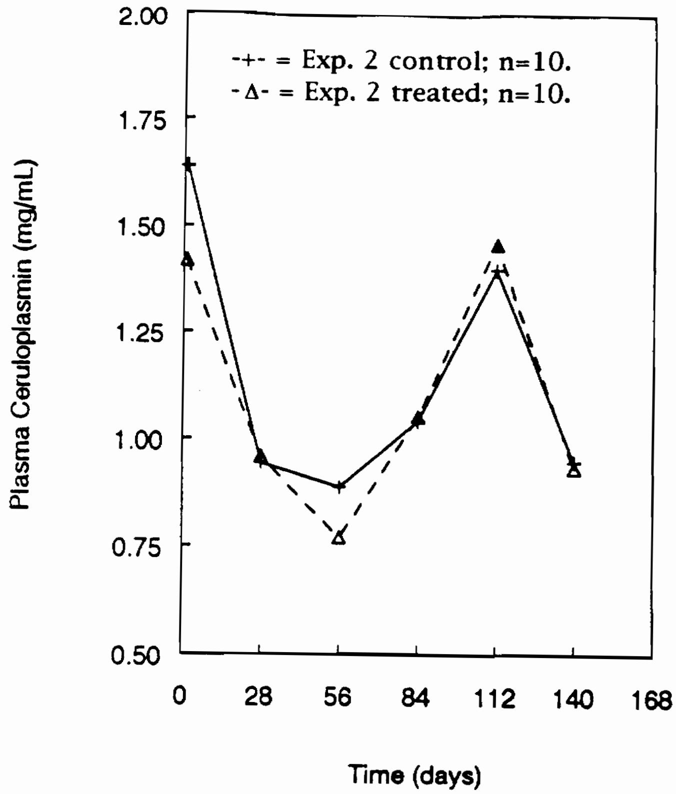

Figure 8. Least squares mean plasma ceruloplasmin concentrations for bulls in experiment 2. Differences within days are not significant ($P > .05$). Differences over time are significant ($P < .05$).

Table 16. Results of breeding soundness examination conducted on d 168 in experiment 2

	Treatment[a]			
	Control[b]	SE	CuO[c]	SE
	cm			
Scrotal circumference	35.39	.99	34.33	1.05
	%			
Sperm Normality	70.30	11.2	61.30	11.80
Sperm Motility	62.52	8.90	57.33	9.42
Sperm 1° Abnormalities	11.30	4.53	6.70	4.79
Sperm 2° Abnormalities	11.21	7.20	21.16	7.62

[a]Values are least squares means.
[b]No Cu: n = 10.
[c]Cu bolus: n = 8.

response to the CuO boluses should not have been expected. Boila (1987) and Ward et al. (1993) recorded no weight gain responses in cattle supplemented with dietary Cu with adequate Cu status. The results were similar when Sankoh and Boila (1987) used injectable Cu. Results of experiments 1 and 2 provide further evidence that supplementation of Cu to cattle with adequate Cu status does not improve animal growth.

Bulls from each treatment group in both studies gained weight at similar rates as would be expected when their dietary requirements have been met (Figures 1 and 5).

Scrotal circumference. Treated bulls in experiments 1 and 2 showed no significant increase in SC over the control group. Studies of Cu-deficient rams have shown increases in SC when the rams were supplemented with Cu (Van Niekerk and Van Niekerk, 1989a,b). These same studies and a study by van Ryssen et al. (1990) also reported, however, that Cu supplementation did not increase SC in rams with adequate Cu status. Because all bulls in both experiments were of adequate Cu status, no response to Cu supplementation should have been expected. Scotal circumferences did increase significantly over time (Figures 2 and 6), a result of normal growth patterns of animals with adequate nutrition.

Serum copper. Serum Cu concentrations were within normal ranges for all bulls in both experiments. In experiment 1, serum Cu did not differ between treatment groups on d 1 and 28. By d 56, serum Cu concentrations from the treated group were significantly higher ($P < .05$) and remained higher through d 112 (Table 8). Specifications of the CuO bolus used in experiment 1 stated that the expected rate of Cu absorption was over a period of approximately 6 wk (Cuprax®, Pitman-Moore, Mundelein, IL). If an increase in serum Cu was to be expected, it would most likely not be expected until after d 28. Differences in serum Cu were no longer significant by d 140 ($P > .05$). It is important to note that statistically different blood Cu concentrations that are each within the normal range for a species may not be biologically significant. It appears the CuO bolus played a role in the treated group: however, it is not certain that the Cu status of these bulls was significantly improved. At a low Cu status, liver and blood samples both give information on the status of the animal. A normal blood Cu concentration does not indicate the size of the reserve, particularly in the liver. A normal serum Cu concentration outside of a significant stressor does not give any indication of what may be expected in the critical period of stress (Hartmans, 1974). The interaction that occurred on d 84 and 112 between treatment and breed is unexplained, appears to have occurred at random, and is not considered to be a significant event in this study.

In experiment 2 there were no significant differences between serum Cu concentrations between treatments on any d (Table 14). Serum Cu concentrations were within the normal range for cattle. Similar to experiment 1, these results say nothing about the Cu reserves, but it appears that the CuO bolus had little effect on blood Cu status in the treated group. Serum Cu was different within treatments over time (Figure 7); however, the differences were not a significant increase or decrease from the start of the study. These differences most probably result from the normal variation in serum Cu levels over time.

Plasma ceruloplasmin. Due to lab errors, experiment 1 plasma samples from d 28, 56, and 84 were removed from the analysis. Plasma Cp concentrations were not significantly affected by treatment on d 1 and 140.

On d 112, Cp concentrations were significantly higher for the control group (Table 10; Figure 4). This conflicts with serum Cu of the treated bulls which had significantly higher concentrations in experiment 1 on d 112. Blakely and Hamilton (1985) reported that serum and plasma Cu levels were positively correlated, regardless of the bound form of Cu. The same study also reported a poor correlation between Cp and liver Cu concentrations. The stark contrast in serum Cu and plasma Cp concentrations on d 112 is most likely attributed to a lab error while conducting the highly sensitve Cp assay.

Experiment 1 plasma Cp concentrations did increase significantly above d 1 concentrations over time (Figure 4). This more accurately follows the pattern of serum Cu concentrations over time.

In experiment 2, plasma Cp concentrations were not different on any d between treatments (Table 15). This supports the results of the serum Cu concentrations and provides further evidence that the CuO bolus did not affect blood Cu concentrations. Over time, the Cp concentrations differed significantly (Figure 8). Ceruloplasmin concentrations were lowest on d 56 and highest on d 1.

Breeding soundness exams. The results of the BSEs in experiment 1 are shown in Table 11. Breeding soundness examinations were conducted using the procedures and guidelines outlined in the Theriogenology Handbook (Chenoweth et al., 1993). Results from the BSE on d 140 were not included in the statistical analysis in either experiment. It was estimated at that time that some of the younger bulls may have needed additional time to sexually mature. There were no differences between treatments in SC or percentage of normal sperm, motility, and primary and secondary abnormalities. Eight bulls from the control group and nine from the treated group passed the examination. A total of seven bulls received a deferred status, four from the control group and three from the treated. A deferred status was given to bulls which failed to meet one of the two minimum requirements in the sperm motility (> 30 % progressive motility) or normality (> 70 % normal) categories. Two bulls failed the BSE based on semen characteristics, one from each treatment group. Both of these bulls failed to meet the minimum threshold for motility and normality.

The results of BSEs in experiment 2 were not affected by treatment. There were no differences between treatments in SC or percentage of normal sperm, motility, and primary and secondary abnormalities. Two bulls from the treated group were removed from the experiment prior to the BSE on d 168. Seven bulls from the control group and four from the treated group passed the examination. Two bulls from the control group and three from the treated group received a deferred status and two bulls failed the examination by failing to ejaculate, one from each treatment group.

Results from the blood Cu concentrations provide evidence as to why there was no response in terms of reproductive traits in the treated group in either experiment. Since blood levels did not differ significantly or were not considered to be biologically significant, no response should have been expected. In addition, Van Niekerk and Van Niekerk (1989a,b) and van Ryssen et al. (1990) reported no improvement in reproductive traits in rams with an adequate Cu status. Van Niekerk and Van Niekerk (1989a,b) did, however, report that reproductive traits in Cu-deficient rams improved significantly upon Cu supplementation.

Conclusions

The results of these experiments reinforce the findings of Whitelaw et al. (1984), Sankoh and Boila (1987) and Ward et al. (1993) that Cu supplementation to cattle with an adequate Cu status does not improve body weight gains. The studies also support the findings of Hartmans (1974) who determined that blood Cu concentrations are not necessarily indicative of the Cu status of an animal. Liver samples from bulls in each of the groups would have allowed for the determination of the effects that Cu supplementation via a bolus would have on Cu status. Caution should be taken when evaluating blood Cu concentrations, with emphasis being given only to those results that show a deficiency. From the results of other experiments (Van Niekerk and Van Niekerk 1989a,b; Skandhan, 1992), Cu clearly plays a role in maintaining normal reproductive capabilities in ruminants; however, its effects have only been appreciated in cases of severe deficiency. Further experiments examining the effects of high levels of dietary Cu may provide evidence that Cu may improve reproductive traits, particularly semen characteristics.

CHAPTER IV.

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APPENDIX A

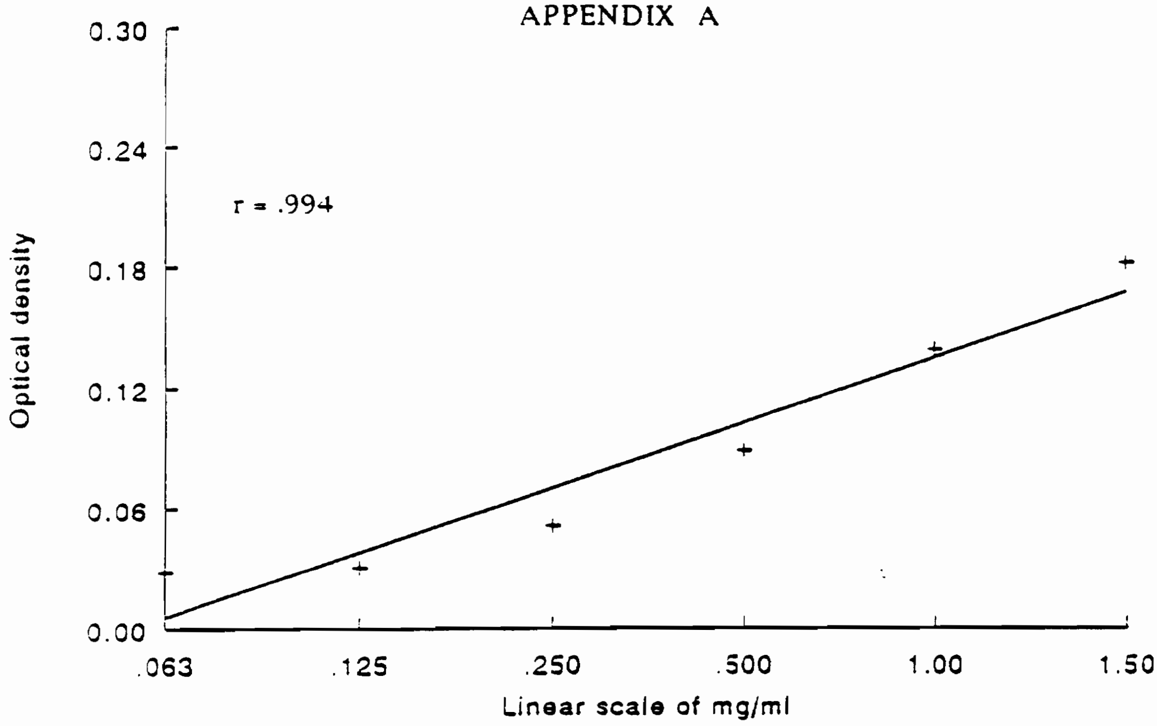

Figure 1. Example of the standard linear curve used in calibration for the bovine ceruloplasmin assay.

APPENDIX A

	1	2	3	4	5	6	7	8	9	10	11	12
A	S01	S01	S01	S02	S02	S02	S03	S03	S03	S04	S04	S04
B	S01	S01	S01	S02	S02	S02	S03	S03	S03	S04	S04	S04
C	S05	S05	S05	S06	S06	S06	U09	U09	U09	U10	U10	U10
D	S05	S05	S05	S06	S06	S06	U09	U09	U09	U10	U10	U10
E	U01	U01	U01	U02	U02	U02	U03	U03	U03	U04	U04	U04
F	U01	U01	U01	U02	U02	U02	U03	U03	U03	U04	U04	U04
G	U05	U05	U05	U06	U06	U06	U07	U07	U07	U08	U08	U08
H	U05	U05	U05	U06	U06	U06	U07	U07	U07	U08	U08	U08

Figure 2. Computer template 1 with standards (S) and unknowns (U).

APPENDIX A

	1	2	3	4	5	6	7	8	9	10	11	12
A	U11	U11	U11	U12	U12	U12	U13	U13	U13	U14	U14	U14
B	U11	U11	U11	U12	U12	U12	U13	U13	U13	U14	U14	U14
C	U15	U15	U15	U16	U16	U16	U17	U17	U17	U18	U18	U18
D	U15	U15	U15	U16	U16	U16	U17	U17	U17	U18	U18	U18
E	U19	U19	U19	U20	U20	U20	U21	U21	U21	U22	U22	U22
F	U19	U19	U19	U20	U20	U20	U21	U21	U21	U22	U22	U22
G	U23	U23	U23	U24	U24	U24	U25	U25	U25	U26	U26	U26
H	U23	U23	U23	U24	U24	U24	U25	U25	U25	U26	U26	U26

Figure 3. Computer template with unknowns (U) and with out standards.

Vita

Clinton Van de Mark Giustra was born February 17, 1968 in Brooklyn, New York to Peter and Betsy Giustra. He graduated from Medomak Valley High School in Waldoboro, Maine in June of 1986. He received an Associate of Applied Science degree in Building Construction and Design at Southern Maine Vocational Technical Institute in May of 1988.

He spent one year at Chamberlayne Junior College in Newton, Massachusetts and received his Bachelor of Science degree in Animal Science from the University of Maine at Orono in May of 1993. In August 1993, he began study towards a Master of Science degree in Animal Science at Virginia Polytechnic Institute and State University.

On November 12, 1993, he married the former Heather L. Umphrey and adopted his son Caleb Michael in August of 1994.

Clinton V. Giustra