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Title: Effect of Zinc, Copper and Selenium Supplementation on the Humoral Immune Response of Weaned Beef Steers.

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Two studies were conducted to determine the effects of zinc (Zn), copper (Cu) and/or selenium (Se) on the humoral immune response of weaned steers fed diets deficient in these minerals. Steers were bled weekly for eight weeks to measure plasma mineral levels. Humoral immune response was measured as antibody response to Keyhole limpet hemocyanin (KLH) over seven weeks of the trials. Steers were injected with 0.5 ml KLH vaccine on weeks 2 and 5 of the Zn study. Ten steers (5 per treatment) were randomly assigned to either a Zn supplemented (z), 3.25 ml injectable zinc-oxide suspension (100 mg/ml oil) or a control (c) treatment. Plasma Zn was maintained at

higher (P<.05) levels in supplemented steers from week 3 through week 8. Humoral immune response was not different (P>.10) between treatments. In 1988, 20 steers were stratified by weaning weight and randomly assigned to one of four treatments: 1) Injectable Se 1 ml Mu-Se/ 90.9 kg body weight, 2) Injectable Molycu 2 ml, 3) control, or 4) Se + Cu. Plasma Cu was higher (P<.05) in treatments 2 and 4 from week 2 through 8. Steers were injected with 0.5 ml of KLH vaccine on weeks 2 and 6. Immune response was not different (P>.10) between Cu supplemented steers and other treatments. Plasma Se was higher (P<.05) in treatment 1 as compared to 2 and 3, but not different (P>.10) from 4. Immune response was highest (P<.05) in treatment 1 with others not different(P>.10). In 1990, a second trial was conducted with 48 steers stratified by weight and randomly assigned to one of the same four treatments. Plasma Cu was higher (P<.05) on week 3 for both groups receiving Cu. Plasma Se was higher (P<.05) on weeks 2 through 5 for both groups receiving Se. Humoral immune response was higher (P<.05) on weeks 7 and 8 for both groups receiving Se. Humoral immune response was uneffected by Zn status of weaned beef steers. Selenium had a positive effect and Cu had no effect on humoral immune response of weaned beef steers. Copper also tended (P=.07) to reduce the

positive response from Se.

Effect of Zinc, Copper and Selenium Supplementation on the Humoral Immune Response of Weaned Beef Steers

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INTRODUCTION

Weaning is a time of high stress on calves. Many producers wean and vaccinate at the same time. It has been observed that many calves exhibit morbidity shortly after weaning. This may be due to stress, vaccinations, improper nutrition or a combination of these factors. Plasma zinc levels have been found to be deficient in weaned calves at the Eastern Oregon Agricultural Experiment Station near Burns (Whanger et al., 1987). Plasma levels of selenium and copper have been found to be deficient in weaned calves at the Station near Union (Whanger et al., 1987). These trace minerals have been demonstrated to be involved with the health and immune system functions of animals (National Research Council, 1984).

The cost of vaccines and treatment of sick animals is one of the major factors in reduced profits for beef cattle operations. A secondary cost is the poor performance of these animals. Producers are constantly examining management options to maximize profits. If trace mineral

deficiencies have an adverse affect on the immune systems response, these animals would be more susceptible to disease and vaccination of these animals when deficient may reduce the efficacy of vaccines and therefore increase the cost of vaccination and subsequent treatment. The purposes of this research project were to: 1) evaluate the efficacy of zinc oxide injections to maintain adequate zinc plasma levels in weaned steers, 2) to determine the length of time sodium selenite and cupric glycinate would elevate selenium and copper plasma levels, and 3) to evaluate the humoral immune response of weaned steers under adequate and deficient zinc, copper and selenium nutrition. Some understanding of the immune system function and response is necessary to understand information presented in this thesis. For those not familiar with the functioning of the immune system, a brief overview is presented in Appendix I.

LITERATURE REVIEW

ZINC

Nutritional factors are important in maintaining efficient growth, reproductive performance and in maintaining the adequate health of all animals. When animals were allowed to roam freely, nutritional deficiencies were less of a factor due to the variety of herbaceous material available and the need for survival as opposed to domestic needs for optimum production. The diets may have been inadequate and therefore, did not allow for maximum growth rate and productivity, but generally the variety in the diet helped prevent some of the vitamin and mineral deficiencies observed today. Our domestic animals are dependent on the feed we offer them and therefore, the science of nutrition has become very important. To maintain optimum production the entire nutritional requirements of animals must be known and met with proper feeds, including supplements when needed. This must also include vitamins and trace minerals.

The need for adequate trace minerals to allow for efficient growth and reproduction of animals has been documented by many researchers (Mayland et al., 1980; Underwood, 1980). Ammerman and Goodrich (1983) also have

stressed the need for adequate trace mineral nutrition for maintenance of normal health in animals. Other researchers have studied aspects of animal health and the response of the immune system when trace mineral nutrition is adequate or deficient for approximately 50 years. However, this continues to be an area of concern and intensive study in recent years. Three trace minerals that have received the most attention are zinc (Zn), copper (Cu) and selenium (Se).

Zinc is known to be essential in the nutrition of numerous species. The need for supplemental Zn has been observed to be due to low total amounts of Zn in the diet and poor bioavailability in that which is in the diet (Underwood, 1980). The level of Zn in the diet can influence net absorption, metabolism and the function of other elements. With low levels of Zn in the diet, symptoms include growth retardation, alopecia, delayed sexual maturity, abnormal feathering, skin lesions, hyperkeratinization of the esophagus, abnormal skeletal development, fetal abnormalities and reduced numbers of circulating lymphocytes (Underwood, 1980).

Zinc is absorbed from the intestinal tract in proportion to the need of the animal (Underwood, 1980). The movement of Zn into the body is regulated at the point of

intake and varies with the requirement and the level available in the diet. Binding sites for Zn are found in sulfhydryl, amino, imidazole and phosphate groups. Therefore, amino acids, proteins, nucleic acids and other organic molecules bind Zn under physiological conditions. Limited storage of Zn is readily available to the body from tissues in times of deficiencies. This tissue reserve is quite small and is reflected in the drop of plasma Zn levels within 24 hours on a Zn deficient diet.

Zinc not only activates some enzymes, but is also a component in several important metalloenzyme. Riordan and Vallee (1976) reported Zn was a constituent in many important metalloenzymes including carbonic anhydrase, D-glyceraldehyde-3-phosphate dehydrogenase, lactic and malic dehydrogenase, alkaline phosphatase, aldolase, superoxide dismutase, ribonuclease, DNA polymerase and others. The active site of these enzymes contains Zn, and it is involved in the catalytic process. Zinc may also function in the maintenance of the structure of some enzymes and does play a role in the configuration of DNA and RNA (Underwood, 1980).

Tucker and Salmon (1955) reported the importance of Zn supplementation for a food-producing animal when they demonstrated that swine parakeratosis could be prevented

with adequate dietary Zn. In their study it was determined that the deficiency was created by high dietary calcium in the presence of vegetable proteins containing phytates. This reduced the bioavailability of Zn and resulted in skin lesions. When the animals were supplemented with Zn in their diet, this disorder was eliminated.

Zinc deficiency symptoms that have been observed in sheep include skin thickening, wool loss, moderate anemia, poor growth of lambs and mortality in lambs and ewes. In a study conducted by Mahmoud et al. (1985) it was demonstrated that subcutaneous injections of zinc chloride suspended in water (100 mg Zn per ml) and zinc oxide suspended in olive oil (100 mg Zn per ml) were effective in reducing and eventually eliminating the clinical signs of Zn deficiencies. When the animals were injected with zinc chloride the response was gradual and a few of the sheep developed skin abscesses and ulcers at the injection site. However, when the zinc oxide suspension was used, the response was more rapid than with zinc chloride and no abscesses or ulcers were observed. One month after administration of the injections plasma Zn levels were within the normal range and were maintained for an additional two months.

Lamand (1985) has also reported efficient use of

intramuscular injections of Zn metal suspended in oil to restore plasma Zn levels to normal within 10 days of the administration. These animals had been fed a diet deficient (13 mg Zn/kg dry matter (DM)) in Zn for 69 days and then supplemented with 50 mg Zn/kg DM for 29 days. This failed to increase the plasma Zn to normal levels. The supplement was then increased to 100 mg Zn/kg DM. The protein content of the diet was 70 g crude protein throughout the study. The plasma Zn levels did not increase until the injections of Zn were administered. In a companion study, sheep were fed two levels of crude protein (94 and 148 g/kg DM) and supplemented with 50 mg Zn/kg DM. The low protein group continued to exhibit deficient plasma Zn levels while the high protein group exhibited a return to normal plasma Zn levels. From these experiments the author reported that intestinal Zn absorption in ruminants was apparently restrained by an inadequate protein diet. This observation has also been reported in mice (Filteau and Woodward, 1982) and rats (Pedersen and Eggum, 1983).

Zinc deficiencies in cattle have also been reported (Price and Wood, 1982; Legg and Sears, 1960; Mayland et al., 1980). Zinc responsive parakeratosis was successfully treated in a Friesian calf submitted to the North of Scotland College of Agriculture in 1982 (Price and Wood, 1982). The calf was delivered with the symptoms of low

growth rate and skin lesions of parakeratosis, which are general symptoms of severe Zn deficiency. A genetic disorder, lethal trait A 46, has been described as an inability to absorb intestinal Zn, and was thought to be the cause of morbidity in this calf. The calf was fed a diet of 500 g DM milk replacer and drenched daily with a zinc sulfate solution containing 75 mg Zn per dose for four days. Plasma Zn levels failed to increase and the symptoms continued to get worse until the Zn supplement was increased to 1000 mg Zn per day. When this level of Zn was used, the calf progressively improved and 11 weeks after Zn therapy was initiated the calf had completely recovered with normal skin and regrowth of hair. Legg and Sears (1960) have also reported successful treatment of parakeratosis in cattle. Zinc supplementation has been shown to increase the performance of calves and cows grazing range (Mayland et al., 1980).

The effects of Zn deficiency on the immune system of animals has become an area of interest in recent years. In a study conducted by Miller et al. (1968), young Zn deficient pigs had reduced thymus weight, increased leukocyte counts but lower percentages of lymphocytes and an increased percentage of immature neutrophils. When these animals were challenged with Salmonella pullorum, they died.

No mortality was observed in pigs with adequate levels of Zn in the diet. Fewer immunoglobulin mu (IgM) and gamma (IgG) forming cells per spleen were observed in young Zn deficient mice when challenged with sheep red blood cells (Fraker et al., 1977). These authors concluded that Zn deficiency caused a rapid atrophy of the thymus and interfaced with the T-cell helper function. Fraker (1983) reported substantial losses in the humoral immune capacity occurred with Zn deficiency while no morbidity or loss of weight was observed in adequate mice.

Zinc has been found to be essential for support of T helper cell function which is required for the stimulation of B-cell action in the humoral immune system response (Hambidge et al., 1987). The thymus is also important in T-cell formation and therefore, has an effect on the cell-mediated immune system response (Tizard, 1987). Based on these findings a deficiency in Zn may severely impair the immune system by causing the thymus to atrophy. Research in the area of Zn deficiency and immune system response in cattle has been conducted. Spears (1988) reported increased antibody titers in cattle challenged with Parainfluenza-3 (PI_3) when zinc methionine was included in their diet.

COPPER

Copper (Cu) was first recognized as an essential mineral by Hart et al. (1928) when their study demonstrated the need for Cu in the formation of hemoglobin in rats. Deficiency of Cu can be observed in almost any species but, the symptoms can vary to a great extent depending on the species and age of the animal. Some of the deficiency symptoms that have been observed in ruminants include bone disorders, scouring, neonatal ataxia, achromotrichia, impaired keratinization, infertility and cardiovascular disorders (Davis and Mertz, 1987). Many of the disorders can be relieved with 4 to 10 parts per million (ppm) Cu in the diet.

Absorption of Cu occurs mainly in the intestine but, may occur throughout the gastrointestinal tract (Davis and Mertz, 1987). Copper absorption appears to be regulated through the nutritional status of the individual but, is also influenced by the chemical form of Cu and several interactions with other dietary factors affecting Cu bioavailability. The absorbed Cu first appears in plasma as cupric ion bound loosely to albumin and then is deposited in the liver for synthesis of ceruloplasmin (Scheinberg and Sternlieb, 1960). The ceruloplasmin is then released to the

general circulatory system and is found as cuproprotein in many parts of the body with excretion occurring through the biliary system, during perspiration and lactation, as well as in the feces (Underwood, 1980). Two of the main factors that affect Cu absorption in ruminants are the amounts of molybdenum (Mo) and sulfur (S) contained in the diet.

The first indication of a Cu interaction with Mo was from work by Ferguson et al. (1943). These authors observed scouring in cattle grazing forage with high amounts of Mo and adequate levels of Cu. They found that the scouring could be stopped by drenching the cattle with copper sulfate. Dick (1952) demonstrated that the Mo effect on Cu occurred only when adequate amounts of inorganic sulfate was present, thus suggesting a three-way interaction. In 1975, Dick et al. proposed the three-step sequence of reduction of sulfate to sulfide in the rumen; reaction of the sulfide with molybdate to form thiomolybdate; and the reaction of thiomolybdate with Cu to form an insoluble copper tetra-thiomolybdate.

Copper is essential for the proper functioning of several metalloenzymes. These include amine oxidases, cytochrome C oxidase, dopamine ß-hydroxylase, ferroxidase, tyrosinase and superoxide dismutase as well as others

(Underwood, 1980). Copper is essential for proper bone metabolism and skeletal abnormalities have been observed in Cu deficient swine (Lorenzen and Smith, 1947). It is postulated that a reduction in lysyl oxidase activity leads to diminished stability and strength of bone collagen due to impaired cross-linkage of polypeptide chains (Davis and Mertz, 1987).

Scouring in cattle has been determined to be caused by Cu deficiencies in some cases. Fell et al. (1975) when working with young Friesian steers, found a depletion of cytochrome oxidase in the epithelium of the duodenum, jejunum and ileum under Cu deficiency. The authors also observed mitochondrial abnormalities in these same tissues. These findings may represent pathological changes of significance in the incidence of scouring in Cu deficient cattle.

Neonatal ataxia which is a nervous disorder in lambs, has been linked to Cu deficiency. Bennetts and Chapman (1937) demonstrated that this abnormality could be prevented by supplementing Cu to ewes during pregnancy. Neonatal ataxia was initially characterized by a demyelinating encephalopathy with cavitation of the white matter of the cerebral hemispheres (Innes and Shearer, 1940). However, in

1960, Barlow et al., found cell necrosis and nerve fiber degeneration in the brainstem and spinal chord of Cu deficient lambs with symptoms of the disease. A report by Gallagher and Reeve (1971) suggested a causal relationship between the loss of cytochrome oxidase and the impaired phospholipid synthesis. Therefore, it is postulated that at the time when myelin is being laid down most rapidly in the fetal lamb, Cu deficiency causes a depression in cytochrome oxidase activity which leads to inhibition of aerobic metabolism and phospholipid synthesis needed for myelin synthesis (Davis and Mertz, 1987).

Depigmentation of hair and wool color can be controlled by changes in the Cu, Mo or S content of the diet (Davis and Mertz, 1987). Along with pigment changes, the growth and appearance of hair can be altered when Cu is deficient. A deterioration in keratinization of wool has led to "stringy" or "steely" wool in sheep. This decreased crimping of the wool fibers can be dramatically reversed if Cu supplementation is initiated (Davis and Mertz, 1987).

Infertility in cattle grazing Cu deficient pastures has been observed. The infertility has been associated with delayed or depressed estrus in several areas of the world (Allcroft and Parker, 1949; Bennetts et al., 1948).

The role of Cu in the immune system has also been documented by researchers. Lamand and Levieux (1981) observed an increase in plasma Cu, as ceruloplasmin, in sheep diagnosed with subclinical chronic metritis or mastitis. The ewes were also found to have low plasma Zn levels. The decrease in Zn and increase in Cu levels was thought to be due to the infection. The infected ewes had lower plasma albumin but higher fibrinogen and IgG levels. Beisel (1976) also reported increased plasma Cu levels for an extended period of time in convalescent animals. Stress, trauma and infections tend to increase plasma glucagon, glucocorticoids and interleukin 1 (Cousins, 1985). These increases in turn elevate the metallothionein levels in liver cells and promote Cu-ceruloplasmin secretion. The elevated ceruloplasmin relates to increases in ferroxidase activity, Cu transport to tissues and serum antioxidant properties, such as superoxide dismutase increases. These factors in turn may preclude the deleterious oxidative damage to tissues caused by the respiratory burst from macrophages and neutrophils.

A decrease in antibody forming cells has been reported by Chandra and Daton (1982) in cases of Cu deficient animals. An increase in the ability of polymorphonuclear leukocytes (PMN) to phagocytize infectious agents has been

reported in humans after Cu supplementation (Heresi et al., 1985). Mice born to Cu deficient dams had decreased activity of plasma ceruloplasmin and splenocyte superoxide dismutase when Cu was deficient in the diet during lactation and post lactation periods (Prohaska and Lukasewycz, 1989). The Cu deficient mice also demonstrated a decreased antibody response to sheep red blood cells, this response was proportional to the degree of the deficiency. This response was reversed when Cu supplementation began. Microbicidal activity of neutrophils was found to be lower in Cu deficient cattle in comparison to those that were adequate (Boyne and Arthur, 1981). The authors first thought that the decline was due to reduced activity of superoxide dismutase. However, in a more recent study the decline in microbicidal activity was observed before the decline in superoxide dismutase (Arthur and Boyne, 1985). Superoxide dismutase is an enzyme that contains Zn and Cu and acts as a scavenger of free oxygen radicles and hydrogen peroxides (Underwood, 1980).

In a review of Cu in the immune system, Bull (1988) reported that Cu affects the antibody-forming cells of the immune system, thus affecting the susceptibility of infection by organisms controlled by the humoral immune system. Results from sheep with marginal Cu deficiency

showed an increase in lamb mortality associated with infectious diseases (Bull, 1988). Chicks infected with Salmonella gallinarum showed a 6-fold increase in ceruloplasmin activity. The author in the review, reported the function of ceruloplasmin may have been to transport Cu to the infected target tissue where it is used as components of intermediary metabolism. Through these activities, Cu has been hypothesized as playing an important role in the immune response of animals.

SELENIUM

Selenium (Se) toxicity has been a concern in animal production for over 55 years (Franke, 1934). The symptoms of toxicity reported by Franke (1934) were also recorded in a journal kept by Marco Polo on his journey through China (Underwood, 1980). Twenty-three years after Franke's report, the requirement of Se as a nutrient was demonstrated by Schwarz and Folts (1957). They discovered that Se would prevent liver necrosis in rats. Nesheim and Scott (1958) reported Se would also prevent exudative diathesis in chicks. The need for Se was confirmed by it's presence in the metalloenzyme, glutathione oeroxidase in 1973(Rotruck et al., 1973). Symptoms of Se deficiency include nutritional

myopathy (white muscle disease), diarrhea, stiff muscles, rough hair coat, reduced gain in young animals, poor fertility, higher incidence of retained placentas, a low viability of offspring and reduced immune response (Reynolds, 1978).

Selenium absorption, retention and distribution in the body varies with the chemical form and amount ingested (Underwood, 1980). When Se is ingested at or near toxic levels, absorption is rapid and efficient. In toxicity studies using rats, higher absorption occurred when Se was fed in seleniferous grains than from selenites and selenates, with even lower rates from selenides and elemental Se (Franke and Painter, 1938; Smith et al., 1988). Some organic compounds, such as selenodiacetic and selenopropionic acids, are less toxic to rats (Moxon et al., 1938). These authors felt this may be a result of lower absorption rates. Wright and Bell (1966) determined that absorption occurred through the duodenum, with no absorption occurring in the rumen or abomasum of sheep or through the stomach of pigs.

Transport of Se occurs mostly in connection with plasma proteins and deposition is in tissues throughout the body (Buescher et al., 1960; McConnell and Levy, 1962). Tissue Se is readily available for transport to needed sites.

Initially losses are rapid and will then slow down when animals are transferred from an adequate to a Se deficient diet (Underwood, 1980). The major pathway of excretion is through the urine (Lopez et al., 1969). Selenium is incorporated as a component of glutathione peroxidase, a metalloenzyme that acts as a scavenger of peroxides and converts them to less harmful compounds such as alcohols in the body (Rotruck et al., 1973).

There is a metabolic interaction between Vitamin E and Se. Scott (1979) reported studies that showed Se lessened the Vitamin E requirement in three ways: 1) it is required to maintain proper function of the pancreas which provides for normal fat digestion and Vitamin E absorption, 2) the proper amounts of glutathione peroxidase is produced, which protects the polyunsaturated fatty acids of lipid membranes from peroxides thereby reducing the Vitamin E required to maintain the membrane integrity, and 3) Se aids in the retention of Vitamin E in blood plasma. They also reported that Vitamin E reduces Se requirements by: 1) maintaining body Se in an active form, and 2) preventing membrane lipid breakdown thereby reducing the amount of peroxides produced. Vitamin E and Se can be supplemented alone or in combination to prevent many of the same symptoms observed in deficiency of one or both (Cheeke, 1971).

In 1958, Muth et al., reported success in preventing white muscle disease in lambs from ewes fed legume hay deficient in Se. Ewes were supplemented with 0.1 ppm Se and their lambs showed no symptoms of the disease and only one of the 16 had heart lesions when necropsied at 6 weeks of age. Since that time Se has been found to be a necessity for normal growth (Doyle and Spaulding, 1978). Supplemental Se has also been demonstrated to increase lambing percentages from 25% to 90% in New Zealand (Scott and Schurman, 1980). This was accomplished by administration of Se to the ewes just prior to mating.

Increased reproductive performance has been observed in swine when Se was supplemented (Wilkinson et al., 1976). These authors reported increased number of pigs weaned and increased weaning weights from Duroc sows supplemented with Se. Selenium has also been reported to prevent hepatosis dietetica in pigs (Eggert et al., 1957). Vitamin E and Se each enhanced the immune response of pigs and when used in combination, there appeared to be an additive effect on increasing the hemagglutination titers (Peplowski et al., 1980). The same results were observed by Larsen and Tollersrud (1981) when pigs were fed alpha-tocopherol or sodium selenite. Whitehair et al. (1983) conducted a study which suggested that the mastitis-metritis-agalctia syndrome

may be improved when the gestation-lactation diet of swine is supplemented with Vitamin E and Se.

Several studies have been conducted to evaluate the effects of Vitamin E and Se on the incidence and duration of environmental mastitis in dairy herds (Smith et al., 1985 a, b; Smith et al., 1984). In one such study Smith et al. (1984) found a reduced incidence and shorter duration of clinical symptoms when Vitamin E and Se were supplemented to dairy cows during the nonlactating period. Conrad (1985), in a review paper, examined reports of decreased incidence of retained placentas, metritis, cystic ovaries and less time required for uterine involution all ocurred with adequate Vitamin E and Se fed to dairy cows during the dry period. The immuno-competence of dairy animals has also been examined in relation to Vitamin E and/or Se status (Gyang et al., 1984; Aziz et al., 1984; Aziz and Klesius, 1986a and b). Gyang et al. (1984) reported that PMN intracellular killing of Staphylococcus aureus was reduced in dairy cows deficient in Se when compared to those injected with Vitamin E and Se. Aziz et al. (1984) demonstrated reduced chemiluminescence from Se deficient PMN's incubated with opsonized zymosan. These studies also showed reduced migration and chemotaxis of PMN's from Se deficient goats. Depressed production of chemotactic

factors has been observed in PMN's from Se deficient goats (Aziz and Klesius, 1986 a,b).

The effects of Se and Vitamin E have also been studied in beef cattle (Byers and Moxon, 1979; Swecker et al.,1989; Perry et al., 1976). Perry et al. (1976) reported a positive gain response to Se supplementation in growing calves when the total protein consumption was low. The same trend was observed by Byers and Moxon (1979) when growing calves were fed inorganic Se with three levels of protein. However, in a second experiment, no differences were found when 0.1 ppm Se was added to diets that nearly met the protein requirement of growing animals. The authors reported that the difference in response to Se supplementation may have been due to the amount of protein in the diet of the animals. Spears et al. (1986) reported an increase in adjusted calf weaning weight and a decrease in death loss with injections of Se plus vitamin E.

Summer gains of spring-born calves and their dams were higher when the animals were supplemented on range with Se (Lanka and Vavra, 1982). Steer and Heifer calves had improvements of 12.2 and 14%, respectively when supplemental Se was available. The dams gained 41 kg more than the controls when supplemented. The same calves were maintained

in the study during the fall and winter with increased variability in performance within treatment groups observed. There were no differences observed between treatments and the authors hypothesized this may have been due to a decrease in the Se content of the salt from 50 to 30 ppm. The gain at the lower level was .05 kg per day less in supplemented calves than in controls. In the same study 15 calves were also injected with Se at weaning and an additional 15 were injected at weaning and again 90 days later. Calves receiving one injection gained 9.4% more than controls and those receiving two injections gained 8.2% more than controls. This study shows the need for supplemental Se to obtain optimum growth of calves, whether supplementation occurs through feed or injection, it is beneficial in Se deficient areas.

A topic of much recent research has been the effects of Se on the immune system of cattle. The humoral immune system response to hen egg lysosome antigen was increased in weaned beef calves fed 80, 120, 160 or 200 mg Se/kg of trace mineralized salt (Swecker et al., 1989). In the same study calves fed 20 mg Se/kg and injected with 0.1 mg Se plus 0.22 IU Vitamin E/kg body weight also had increased titers. Sheffy and Schultz (1979) observed a decreased stimulation of IgG forming cells in animals deficient in Se. The T-cell

dependent antibody response has been demonstrated to be decreased when Se is deficient, The same response was noted and further decreases were seen when Vitamin E was also deficient (Chandra and Daton, 1982). Droke and Loerch (1989) conducted two trials to examine the effects of Se and/or Vitamin E on the performance, health status and humoral immune response to Pasteurella haemolytica vaccination of calves received at a feedlot. In the first trial calves given injections of Vitamin E and sodium selenite responded with trends toward higher ADG and feed efficiency. There was also an increased enzyme linked immunosorbent assay (ELISA) response in supplemented calves. However, no differences were observed in feed intake or days sick due to treatment. In the second study, four treatments were used: 1) control, 2) 25 mg Se, 3) 340 IU Vitamin E, and 4) both Se and Vitamin E. The authors reported no effect due to treatment in ADG, feed intake or feed efficiency. A higher 7 day ELISA titer was observed in calves treated with both Se and Vitamin E and a trend toward higher titers was seen on day 13 for the same treatment. However, again there was no difference in morbidity, number of head treated or percent treated for sickness among any of the treatments.

The immune response of calves challenged with Infectious Bovine Rhinotracheitis (IBR) virus was higher for

calves fed diets containing 0.2 mg Se/kg than for those fed a deficient diet (0.03 mg Se/kg) (Reffett et al., 1988). The primary and secondary immune responses were measured in this study as well as the glutathione peroxidase activity. At the end of a 42 day adaptation period, whole blood glutathione peroxidase activity was lower for calves fed the deficient diet. The glutathione peroxidase activity increased in Se sufficient calves but not in Se deficient calves after challenge with IBR. Serum IgM increased in animals from both treatments after primary challenge and was not affected by a secondary challenge. However, antibody titers were higher by day 49 of the challenge period in calves fed a diet adequate in Se. Calves weaned, transported and challenged with Pasteurella hemolytica had higher IgM levels if their dams had received sodium selenite injections every 60 days prior to weaning (Stabel et al., 1989). Selenium supplemented calves had lower antibody titers but equal body temperatures, plasma creatine phosphokinase, serum IgG and albumin concentrations to nonsupplemented calves in the same study.

MATERIALS AND METHODS

Trial I

A preliminary study was conducted in the fall of 1988 to determine if detectable differences due to trace mineral nutrition could be observed in the humoral immune response of weaned steers. This was a two phase study with Zn being studied at the Eastern Oregon Agricultural Experiment Station near Burns. Copper and Se were studied at the Eastern Oregon Agricultural Experiment Station near Union. The two phases will be described separately.

Zinc Phase

Ten Hereford X Angus steers, approximately 7 months of age, from first calf heifers were weighed and randomly assigned to either a Zn or a control treatment and returned to their dams for one week. One day after weighing, the steers were bled via jugular venipuncture into tubes containing sodium heparin to obtain a baseline plasma Zn level. Injections of 3.25 ml zinc oxide suspension or 3.25 corn oil were administered intramuscular in the middle rear third of the neck. The zinc oxide suspension contained 100 mg zinc oxide per ml of corn oil and was administered at a rate of 0.022 mg zinc oxide per kg body weight. Control

steers were injected with corn oil to eliminate the effect of injection from the study.

The steers remained with their dams on range for six additional days and were then weaned and transported 20.5 km to the winter headquarters. After being allowed to settle for one day, the steers were bled for plasma Zn level and for a baseline humoral immune response. After bleeding the steers were vaccinated with 5 ml of Vira Shield-4, 5 ml of Fermicon-7 clostridium and 0.5 ml of an experimental Key hole limpet hemocyanin vaccine (KLH). The Vira Shield-4, produced by Grand Laboratories (Larchwood, IA), contained killed virus from IBR, cytopathic and noncytopathic bovine viral diarrhea (BVD) and PI_3. The Fermicon-7, produced by Bio-centric (St. Joseph, MO), contained bacterin from Clostridium chauvoei-septicum-novyi-sordellii-perfringens types C and D. The KLH vaccine was prepared by Dr. Lynn Woodard (Wyoming State Veterinary Laboratory) and contained:

50 mg Key hole limpet hemocyanin
2.5 ml hexadecane
1.75 ml Tween 80
0.75 ml Span 80
100 µg T1501 adjuvant
45 ml normal saline.

All vaccines were administered intramuscular in the hindquarter of the steers.

Steers were then placed in a pen and received 1.36 kg of ground corn-biuret (.05 kg biuret) and fed 6.8 kg native meadow hay per head per day. Feed bunks were covered and feed was measured in daily. The hay was analyzed and found to contain 3.9 ppm Zn (Appendix II).

Three weeks after the initial zinc oxide or corn oil injections, the steers were given a second injection of either zinc oxide suspension or corn oil at a dose of 3.5 ml in the opposite side of the neck from the initial injection. One week later the steers were vaccinated again with 5 ml of Vira Shield-4, Fermicon-7 and 0.5 ml of KLH.

The steers were bled via jugular venipuncture each week for the 8 weeks of the study period. Blood for plasma Zn level was collected in a tube containing sodium heparin, centrifuged at 2000 rpm's for 12 minutes with plasma removed and stored in 4 ml glass vials in a freezer until analyzed. The collection tubes had a royal blue stopper that contained no Zn to avoid contamination of the plasma. Blood for the immune response was collected in normal red topped clot tubes, centrifuged at 2100 rpm's for 15 minutes to obtain serum which was then removed and stored in 4 ml glass vials in a freezer until analyzed. The initial or baseline serum was used as a negative control in analysis. The immune

response was followed through weekly collections from week 2 through week 8 of the trial.

Plasma Zn levels were measured by atomic absorption spectrophotometry. Plasma samples were thawed, mixed with a vortex mixer and diluted at a 1 to 5 ratio with 0.1 normal nitric acid in 5 ml glass tubes. Sample mixtures were then mixed and analyzed by atomic absorption spectrophotometer.

Humoral immune response for KLH was measured using an indirect ELISA (Voller et al., 1979) (Appendix III). In this procedure, KLH is diluted to 5 µg/ml of a PBS buffer solution (Appendix IV), and 0.1 ml deposited in each well of a 96 well Linbro/Titertek EIL plate, sealed with parafilm and refrigerated at 4° C for at least overnight. Plates were then washed 3 times with PBS-Tween 20 buffer solution (Appendix IV). Serum was thawed, mixed with a vortex mixer and diluted 1 to 1000 in PBS-Tween 20 buffer solution and mixed. The diluted serum was then placed in the wells of the plate with each serum being placed in 4 wells at a rate of 0.05 ml/well. A positive control was obtained by hyperimmunizing 5 fall calves from another experimental herd. These calves were vaccinated four times at 2 week intervals with 0.5 ml KLH in each injection. One week after

the final vaccination, blood was collected, centrifuged to obtain serum, pipetted and the serum was pooled and frozen. The positive control serum was thawed, diluted to 1 to 250 in the PBS-Tween 20 solution and 0.05 ml was placed in each of 4 wells in the plate. Four wells of the plate also contained 0.05 ml of the PBS-Tween 20 buffer only. This allowed for the removal of background interference when reading the plate. The plate, having been coated with the diluted serums, was then incubated for 1 hour at 37° C while rotating on a platform at 120 rpm's. Plates were then removed and washed 3 times with the PBS-Tween 20 buffer. A solution of alkaline phosphatase enzyme-conjugated affinipure F(ab') 2 fragment goat anti-bovine IgG antibody diluted to 1 to 2000 in PBS-Tween buffer was placed in each well at a rate of 0.1 ml. The plates were then again incubated for 1 hour at 37° C while rotating on a platform at 120 rpm's. After incubation, the plates were washed 3 times with the PBS-Tween 20 buffer and 150 µl of p-nitrophenyl phosphate disodium·6 H_2O dissolved in a substrate buffer (Appendix IV) to 1 mg/ml was placed in each well and allowed to incubate at room temperature. The plates were then read using a Dynatech MR 700 set on dual wavelength with filters 3 (reference, 490 nm) and 1 (test, 410 nm) when the average of the four positive control serums reached an optical density (OD) of 1.000. (Dynatech MR 700

settings are listed in Appendix V).

Copper and Selenium Phase

The Cu and Se phase of the preliminary trial was conducted at the Union Station of the Eastern Oregon Agricultural Experiment Station. Twenty Hereford X Angus steers,approximately 10 months of age, were stratified by weight and randomly assigned to one of four treatments. Treatments included: 1) control, 2) Se supplemented, 3) Cu supplemented, and 4) Cu and Se supplemented. Control steers received 2 injections of 2 ml each saline solution (1 subcutaneous and 1 intramuscular) to simulate stress equal to those steers receiving supplement injections. Selenium steers received an injection of 1 ml Mu-Se/90.9 kg body weight(Schering-Plough), intramuscular and 2 ml saline solution subcutaneously in the dewlap. Copper steers received 2 ml Moly-Cu (Schering-Plough) subcutaneously in the dewlap and 2 ml saline solution intramuscular in the rear quarter. Copper plus Se steers received 2 ml Moly-Cu subcutaneously in the dewlap and 1 ml Mu-Se/90.9 kg body weight intramuscular in the rear quarter. The mineral or saline injections were administered at the time of weighing 1 week prior to weaning. The steers were also bled via jugular venipuncture as described in the Zn phase and placed

back with their dams on pasture for one week. Blood samples were then collected weekly for the 8 weeks of the trial to determine Cu and Se plasma levels.

At weaning (week 2) the steers were also bled for a baseline immune response as described in the Zn phase. Vaccinations were administered at this time and included 5 ml Vira Sheild-4, 5 ml Fermicon-7 and 0.5 ml KLH. Vaccinations were repeated 4 weeks later at the same levels. Weekly blood samples were collected to track the humoral immune response through the remaining 7 weeks of the trial.

Following weaning the steers were placed in pens with covered feed bunks and fed 8.2 kg per head per day native hay that was deficient in Se (0.02 ppm) and marginal in Cu (5.3 ppm) (Appendix VI). Steers were allowed access to a mineral free salt and water at all times.

Plasma Cu levels were determined in the same manner as that described for plasma Zn. Plasma Se levels were determined using an automated fluorimetric method (Brown and Watkinson, 1977) after acid digestion. Samples were prepared by weighing 1 g plasma into a 50 ml Erlenmeyer flask, adding 10 ml concentrated nitric acid and 3 ml concentrated perchloric acid, placed on a hot plate and

digested until thick white fumes were observed for 15 minutes. The flasks were then cooled and 1 ml of concentrated hydrochloric acid was added, then reheated until white fumes were observed for 15 minutes. Flasks were then allowed to cool before adding 15 ml of 0.027 EDTA solution and 3 drops of an indicator solution (0.1 g Bromocresol green plus 0.1 g Cresol red dissolved in 13 ml 0.02 normal sodium hydroxide brought to 250 ml with double distilled water). The flasks were then titrated to a straw yellow color with 5 normal ammonium hydroxide. This color indicates a pH in the range of 2 to 3. Flasks were then weighed and flask weights subtracted to determine the volume. Approximately 3 ml of the sample was then placed in a plastic vial and placed in the automated analyzer. Known standards of 0, 10, 25, 50, 75, 100 and 200 ppm Se were also run through the analyzer to obtain a standard curve. The heights of the peaks were measured and the solution concentrations

were calculated by:

$$\frac{\text{peak height}}{\text{volume}} .$$

The concentration of Se was then calculated by the equation:

$$\frac{\text{solution concentration X volume}}{\text{initial weight of the sample}} .$$

This equation calculates the ng of Se per g of serum or parts per billion (ppb). By dividing the ppb by 1000 the Se concentration in ppm is obtained.

TRIAL II

A second trial was conducted based on the results of Trial I. The Zn phase was eliminated from the second trial due to no observed differences in treatments. The number of steers in the Cu and Se phase of Trial II was increased in an attempt to detect differences in trends of the effect of Cu on the immune response in Trial I. Based on the equation:

$$n=2(Z_{\alpha/2}+Z_{\beta})^2(\sigma)^2/\delta^2$$

(Steel and Torrie, 1980), 48 Hereford X Angus steers, approximately 10 months of age, were stratified by weight and randomly assigned to pens. There were four treatments within each of two blocks. Treatments were the same as those described in the Cu and Se phase of Trial I. A map of the treatment pens and blocks is shown in Appendix VII. Plasma Cu and Se levels were determined using the same protocol as described in Trial I. One exception to this protocol was made in the plasma Se determination and that a pooling of the serum samples based on the treatment groups

due to the number of samples. This reduced the number of samples from 384 to 128 however, this left adequate samples per treatment per period for analysis, this was enough to determine if a difference existed.

Vaccinations and humoral immune response were determined using the same protocol as described in Trial I. The steers were fed native hay that was marginal in Se (0.05 ppm) and adequate in Cu (10.9 ppm) on a dry matter basis (Appendix VIII). Steers were maintained in pens with 6 steers per pen and fed in covered bunks for the 8 week trial.

A final weight was taken on the last day of the trial in both Trial I and Trial II after restriction from feed and water over night. Average daily gain was then calculated for the 8 week period.

STATISTICAL ANALYSIS

Trial I

The experimental design for Trial I was a completely random design (CRD). No differences ($P>.10$) existed in the birth date or weaning weight of the steers used in the Zn phase and all animals were housed in the same pen.

Therefore, the CRD was appropriate for this phase of the trial. Each steer was used as a replication. Analysis was completed on treatment means, using contrasts in the General Linear Models (GLM) procedures of the Statistical Analysis System (SAS). Contrasts were determined to be significant if p values were less than .05, and a trend was determined if the p value was between .1 and .05, The null hypothesis was H_0: difference = 0, and the alternative hypothesis was H_a: difference = 0. The model used to test the hypothesis was difference = treatment + error. In this phase of Trial I the Type 4 sums of squares was used due to unequal replications within treatments. This was caused by the death of one calf in the Zn treatment. The treatment by time interaction was tested and found to be not significant (P>.10), therefore, the contrasts tested were between treatment means within each sampling period.

There was a greater variation in weaning weights of steers used in the Cu plus Se phase. Therefore, steers were stratified by weight to remove the effect of weight from treatment differences. The same model and tests described for the Zn phase were used for this phase. There was again no significant treatment by time interaction.

Trial II

With more animals available in Trial II, the experimental design was changed to a randomized complete block. The blocking criterion was based on location of pen. This allowed for each of four treatments to be represented in each of two blocks. There was no treatment by block interaction and block was not significant when tested. Therefore block was dropped from the model which allowed for 12 replications per treatment. There was also no significant interaction between treatment and time.

RESULTS AND DISCUSSION

TRIAL I

Zinc Phase

The plasma Zn level of all steers was adequate at the first sampling date. Mean plasma Zn levels for all sampling dates are reported in Table I. This indicates that a sufficient level of Zn had been consumed by the calves either through consumption of range plants or through milk provided by the dam. There was a slight drop in the plasma Zn levels on the second week; however, both treatment groups remained adequate. On week 3, the control group was deficient (<0.8 ppm) in Zn while the Zn treatment group remained adequate. Week 2 of the trial was when the steers were weaned, transported the 45 miles to the Winter Headquarters and vaccinated. The stress occurring over this time could have caused the depression in plasma Zn. However, those steers that received a zinc oxide injection maintained adequate plasma Zn levels. Week 4 was the time of the second injection of either zinc oxide in oil or oil alone. At this time both treatment groups were deficient. Throughout the remainder of the trial, the control steers remained at deficient levels of plasma Zn. Those steers given Zn injections had adequate plasma Zn levels for 3

TABLE I. MEAN PLASMA ZINC LEVELS (ppm)

OF STEERS (TRIAL I)

	WEEK							
TREATMENT	1[a]	2	3	4[a]	5	6	7	8
ZINC	.97	.84	.87	.73	.81	.94	1.00[b]	.75
CONTROL	1.07	1.02	.75	.62	.73	.80	.73[c]	.69
SE	.07	.08	.09	.09	.04	.08	.09	.05

a- Week of mineral injection.
b,c- Means within columns with different superscripts differ (P<.05).

weeks but, were again deficient on week 8. The efficacy of supplementing Zn through injections of zinc oxide suspended in corn oil was found to be adequate if they were reinjected every 3 to 4 weeks. This creates a problem due to the need to work the animals so often and is not feasible for many operations.

The performance of the steers was measured as ADG for the 8 week trial and means are reported in Table II. This time frame was too short and the number of steers used limited the inference of the data collected. Supplemented steers had ADG's approximately one half of the nonsupplemented group (.16 kg and .31 kg, respectively). This trend of higher ADG of nonsupplemented steers may be due to several factors. The length of time of the study (8 weeks) and the number of animals per treatment (5 steers) are only two of the factors. The additional stress of dehorning three of the animals, one control and two of the supplemented, may have also effected the performance. In addition, one of the supplemented steers was disabled unable to stand one day after the second injection was given. The calf was given antibiotics and showed no improvement over a two week period. During this time his appetite remained high, but the steer was unable to stand and feed and water were carried to him. The steer was killed by lethal

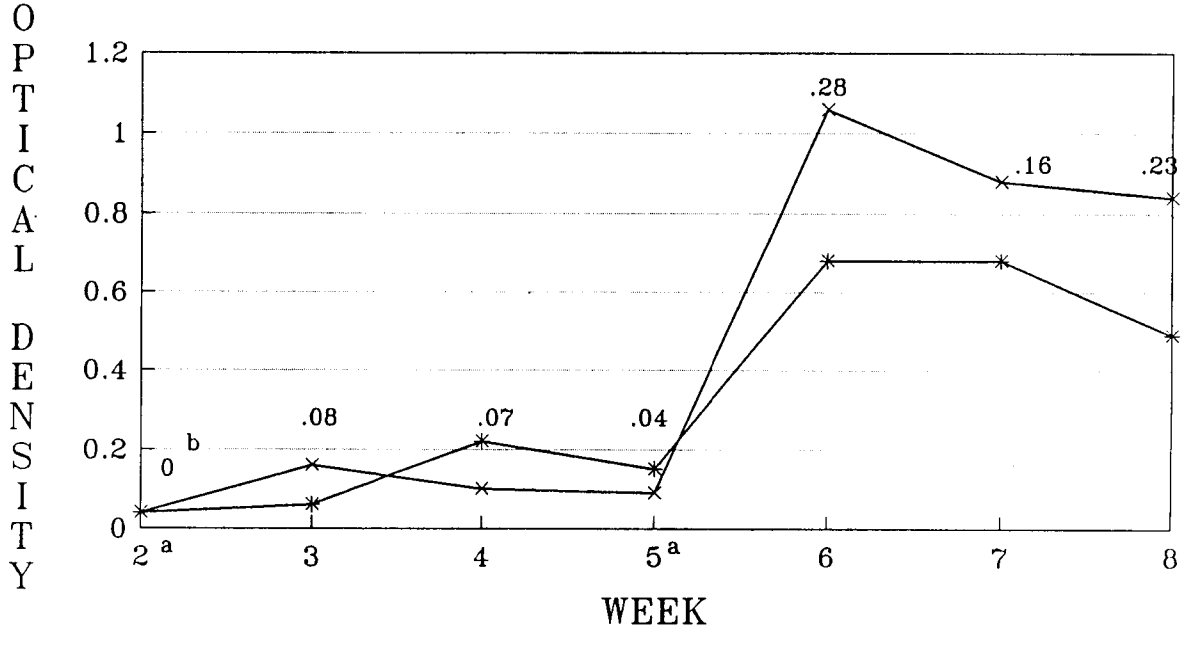
TABLE II. MEAN AVERAGE DAILY GAINS OF STEERS IN THE ZINC PHASE (TRIAL I).

TREATMENT	ADG (kg)
ZINC	.16
CONTROL	.31
SE	.21

injection on week 6 and posted. Upon gross examination, the calf appeared normal and when liver, kidney, brain and blood samples were analyzed, all samples were normal.
We were unable to determine the cause of the problem. This animal's data was therefore dropped from the study.

The OD readings of the serum from steers in the Zn phase are presented in Figure I. Initial OD readings for both treatment groups was .05. This sample was collected at the time of vaccination and therefore was used as the negative control for the remaining samples of this phase. The low initial levels also confirmed that the steers had no previous contact with KLH. Throughout the study there were no differences ($P>.10$) in the OD readings between treatment groups, although numerical differences did occur. The control steers had numerically higher initial and secondary immune responses to KLH which is in contrast to the findings of Spears (1988). This may have occurred because both treatment groups had adequate plasma Zn levels when initially vaccinated. In the study by Spears, (1988) the animals were deficient at the time of initial vaccination. Since the secondary immune response is affected by the primary response through the number of IgG producing daughter cells (Tizard, 1987) it would be expected that the secondary response would not be different. Two of the five

FIGURE I. MEAN OPTICAL DENSITY FOR STEERS IN ZINC PHASE (TRIAL I)



a-Week of vaccination b-std. errors

Zn supplemented calves were dehorned and one of the control group was also dehorned on week 4 of the study. Individual OD readings on week 3 were nearly equal but, for the following week were depressed in all three of the steers that were dehorned. Therefore, more stress may have been added to the animals, and since more of the supplemented steers were dehorned, the mean immune response for the treatment group could have been lowered. With dehorning, the steers could be more susceptible to other infectious agents and their immune system mechanisms may have been needed to fight infection and protect the new wounds.

Copper and Selenium Phase

Mean plasma Cu levels are reported in Table III. Adequate (>0.6 ppm) levels of plasma Cu were observed in all treatment groups on week 1 of the trial. With the forage of the area traditionally marginal in Cu, the steers were able to maintain adequate Cu intake through grazing selectivity or through the milk provided by their dams. These animals had perviously grazed a forested range and the forage in this area may have contained adequate Cu levels. One week later, at weaning, the nonsupplemented steers had deficient(<0.6 ppm) plasma Cu levels while those animals supplemented with either Cu or Cu plus Se had adequate

TABLE III. MEAN PLASMA COPPER LEVELS (ppm) OF STEERS (TRIAL I)

	WEEK							
TREATMENT	1[a]	2	3	4	5	6	7	8
COPPER	.71	.89[b]	1.05[b]	.87[b]	.84[b]	.97	.94	.96[b]
CONTROL	.65	.53[c]	.57[c]	.57[c]	.62[c]	.81	.76	.76[c]
COPPER PLUS SELENIUM	.63	.79[b]	.98[b]	.81[b]	.82[b]	.96	.90	.95[b]
SE	.17	.22	.03	.13	.14	.11	.27	.20

a-Week of mineral injection.
b,c-Means within columns with different superscripts differ (P<.05).

levels. Nonsupplemented steers remained deficient from week 2 through week 4 and were adequate for the remaining. 4 weeks of the trial. From the initiation of the trial the supplemented steers maintained adequate plasma Cu levels that were higher ($P<.05$) than nonsupplemented steers except on weeks 1, 5 and 6. The Cu level in the hay fed post weaning was marginal (5.3 ppm). The main storage tissue for Cu in the body is the liver, where Cu is then made available for transport to other tissues (Underwood, 1980). The steers were apparently able to build Cu stores in the liver when dietary Cu was adequate, and remobilize it for use when they became deficient. However, in an earlier study conducted by Whanger et al. (1987), hepatic and plasma Cu levels were low throughout December and April, respectively. Based on this study, a single injection of cupric glycinate (2 ml) was adequate to maintain plasma Cu levels in weaned steers.

All the steers had deficient (<0.03 ppm) plasma Se levels at the initiation of the trial. Mean plasma Se levels are reported in Table IV. Forages have been reported to be deficient in Se throughout the area and animals have shown deficient plasma and hepatic Se levels unless supplemented (Whanger et al., 1987). One week following Se injections (1 ml/90.9 kg body weight), the

TABLE IV. MEAN PLASMA SELENIUM LEVELS (ppm) OF STEERS (TRIAL I)

	WEEK							
TREATMENT	1[a]	2	3	4	5	6	7	8
SE	.008	.038[b]	.039[b]	.026[b]	.032[b]	.022	.019[b]	.016[b]
CONTROL	.010	.010[c]	.011c	.010[c]	.009[c]	.017	.011[c]	.010[c]
CU PLUS SE	.010	.038[b]	.034[b]	.026[b]	.021[b]	.023	.021[b]	.016[b]
STD ERROR	.007	.031	.010	.008	.022	.024	.004	.002

a-Week of mineral injection.
b,c-Means within columns with different superscripts differ (P<.05).

supplemented steers, had adequate (>0.03 ppm) Se plasma levels. These levels were maintained through week 3 in the Cu plus Se and the Se treatment groups. Steers in the Se treatment group also had adequate levels of plasma Se on week 5 of the trial but, were deficient on week 4. For the remaining sampling dates all treatment groups were deficient. Even though the levels were deficient on most sampling dates, the supplemented groups maintained higher ($P<.05$) plasma Se levels except on weeks 1 and 6 of the trial. Based on this portion of the trial, injections of sodium selenite were sufficient to maintain adequate plasma Se for up to 21 days.

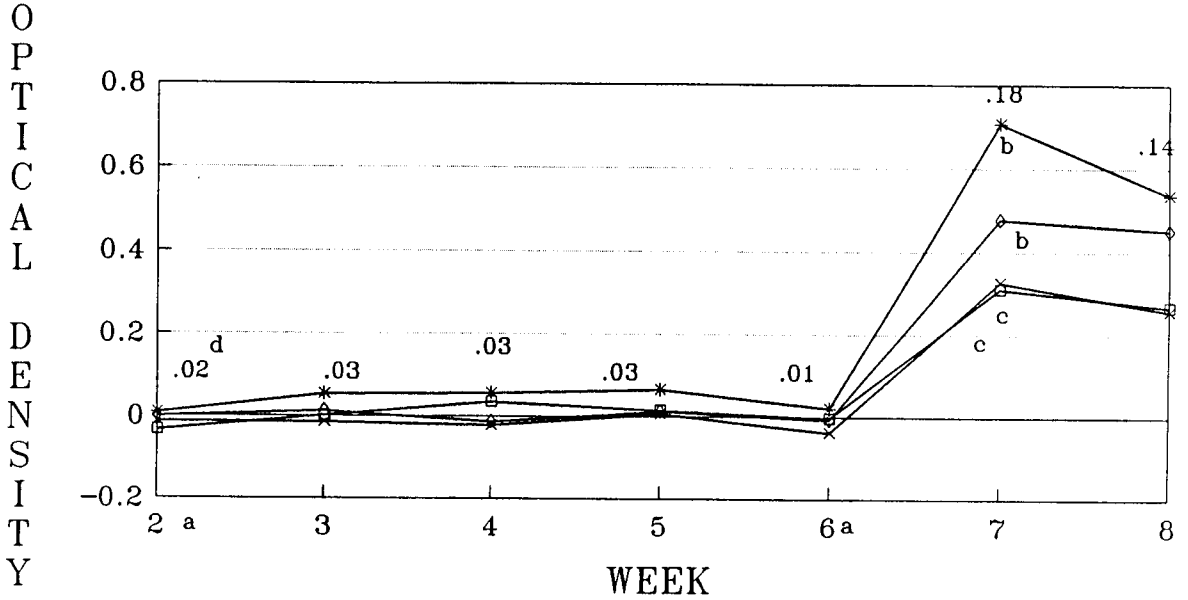
There was no difference ($P>.10$) in the ADG of the steers with respect to treatment. Mean ADG's are reported in Table V. The time period for this trial was 8 weeks, which is too short to obtain accurate performance response to the treatments. The low number of animals also contributes to the weakness of analyses of performance data reported here.

The initial KLH vaccination was administered on week 2 of the trial. The OD readings from that sample were 0 ± .03 for all treatment groups. Mean OD readings are presented in Figure III. These first samples were used as a negative

TABLE V. MEAN AVERAGE DAILY GAIN OF STEERS IN THE COPPER AND SELENIUM PHASE (TRIAL I)

TREATMENT	ADG (kg)
SELENIUM	.39
COPPER	.41
COPPER PLUS SELENIUM	.46
CONTROL	.41
SE	.21

FIGURE II. MEAN OPTICLE DENSITY FOR STEERS CU AND SE (TRIAL I).



a-Week of vaccination
b,c-Means with different letters differ (P<.05) d-std. errors

control and demonstrated that these steers had no previous contact with KLH. The OD readings for control steers remained at or near 0 through week 6 of the trial, while Cu steers maintained readings at .02 from week 2 through week 6. Selenium treated steers maintained an OD of .06 from week 2 through week 5 while Cu plus Se steers had an OD of .06 on weeks 2 and 3 but fell to 0 from week 4 through wee 6. The low OD readings for this primary immune response may have been due to the sampling time or the analysis procedure. The ELISA analysis is designed to test the level of IgG. In the classic primary immune response, the first immunoglobulin produced is IgM and IgG levels are very low. As time progresses IgG is produced. Immunoglobulin gamma is produced at peak levels 7 to 10 days after infection therefore, the bulk of it's production may have occurred after the 7 day post vaccination sample was taken. If the primary response was sufficient, the levels of IgG should have been detected 14 days after the initial vaccination and they were not. It is this time period when IgG synthesis should be nearly equivalent to the catabolism rate, therefore maintaining a steady level of IgG in the lymphatic system (Tizard, 1987). However, if a primary immune response is inadequate, the secondary response will also be low. The primary immune response has an effect on the secondary response through the number of IgG producing

daughter cells that have been exposed to the infectious agent. If a high primary response is produced, more daughter B-cells would be produced. Therefore, a high and rapid secondary immune response will be obtained (Tizard, 1987).

The second vaccination with KLH was administered on week 6. On week 7 the OD readings were higher ($P<.10$) for the Se treatment group compared to the control and Cu treatment groups. The Cu plus Se treatment group was not different ($P>.10$) from any of the other groups. There were no other sampling dates when OD readings were different ($P<.10$), however, there were some interesting numerical tendencies. The steers treated with Se only had numerically higher OD readings throughout the trial. This also occurred in studies conducted by Swecker et al. (1989) and Reffett et al. (1988). Steers receiving Cu alone had OD readings essentially equal to those of the control group. The Cu plus Se treated steers had OD readings below the Se group but higher than the control and Cu treatment groups. Further examination of this phenomenon should be undertaken and was attempted in Trial II of this research. From Trial I it can be stated that Se supplementation had a positive effect on the secondary humoral immune response of weaned steers. There appears to be no benefit to the humoral

immune response of weaned steers with Cu supplementation. Copper, when used with Se supplementation, may partially reduce the positive effects of the Se.

Trial II

The Zn phase of Trial I showed no differences ($P>.10$) in any of the parameters measured. Therefore, this phase of the trial was eliminated from Trial II. Based on the results of Trial I, the number of animals per treatment on the copper and selenium phase was increased from 5 to 12 steers according to the formula from Steel and Torrie (1980). This procedure was intended to allow differences in the humoral immune response due to Cu and Se to be detected and allow better comparisons when used in conjunction with each other.

Mean plasma Cu levels are presented in Table VI. All treatment groups had adequate (>0.6 ppm) plasma Cu levels throughout the trial. On week 2, one week after mineral injections were administered, those that received a cupric glycinate (2 ml) injection tended to have higher ($P<.10$) plasma levels of Cu, even though none of the groups were deficient. The following week, the Cu and Cu plus Se treatment groups had higher ($P<.05$) plasma Cu levels than

TABLE VI. MEAN PLASMA COPPER LEVELS (ppm) OF STEERS (TRIAL II).

	WEEK							
TREATMENT	1[a]	2	3	4	5	6	7	8
COPPER	.8	1.1	1.3[b]	1.1	1.1	1.1	1.2	1.1
SELENIUM	1.0	.9	1.0[c]	1.0	1.1	1.2	1.3	1.2
CONTROL	.9	.8	.9[c]	1.0	1.0	1.1	1.1	1.1
COPPER PLUS SELENIUM	.9	1.0	1.2[b]	1.1	1.1	1.1	1.2	1.1
SE	.08	.13	.18	.06	.05	.05	.08	.08

a-Week of mineral injection.
b,c- Means within columns with different superscripts differ (P<.05).

control and Se treatment groups. The reason for the steers not becoming deficient (<0.6 ppm) in plasma Cu was due to the adequate level of Cu contained in the hay. In Trial I the Cu level in hay was 5.3 ppm on a dry matter basis, while in Trial II Cu was 10.9 ppm (Appendices VI and VII, respectively). The requirement for Cu in growing cattle is 8 ppm (NRC, 1984) and therefore, the steers were consuming adequate Cu to meet their needs.

Mean plasma Se levels are reported in Table VII. All treatment groups had plasma Se levels at or slightly above the deficient (0.03 ppm) level on week 1. One week following the administration of treatments, both groups receiving sodium selenite (1 ml/90.9 kg body weight) had higher ($P<.05$) plasma Se levels than control or Cu treatment groups. When Se was supplemented alone, Se plasma levels were maintained at or above deficient levels for the entire trial period. However, when Cu plus Se were supplemented together, plasma Se was deficient on weeks 7 and 8. Steers with no Se supplementation were deficient from week 4 (control) and week 5 (Cu) through the end of the trial. This is in contrast to the results in Trial I where all treatment groups were deficient from week 6 through week 8. Both Se supplemented groups had higher ($P<.05$) plasma Se levels on weeks 2 and 3 of Trial II. On week 4 the groups

TABLE VII. MEAN PLASMA SELENIUM LEVELS (ppm) OF STEERS (TRIAL II).

	WEEK							
TREATMENT	1[a]	2	3	4	5	6	7	8
SELENIUM	.03	.06[b]	.05[b]	.04[b]	.03	.03	.03	.03
COPPER	.04	.04[c]	.03[c]	.03[bc]	.02	.02	.02	.02
CONTROL	.03	.04[c]	.03[c]	.02[c]	.02	.02	.02	.02
COPPER PLUS SELENIUM	.03	.06[b]	.04[b]	.04[b]	.03	.03	.02	.02
SE	.005	.01	.009	.009	.005	.005	.005	.005

a-Week of mineral injection.
b,c-Means within columns with different superscripts differ (P<.05)

supplemented with Se had higher (P<.05) plasma Se levels than the control group, but only tended to be higher (P<.10) than the Cu treatment group. The hay fed in Trial II had 0.05 ppm Se which was higher than that fed in Trial I (0.02 ppm). The suggested range of Se for growing cattle is 0.05 to 0.30 ppm (NRC, 1984). The 0.05 ppm in the hay for Trial II is at the very bottom of this range and it may have been enough to maintain the higher levels of plasma Se when compared to Trial I.

Performance of the steers was again measured as ADG over the 8 week trial. This time period is too brief to obtain an accurate measure of performance. With 12 steers per treatment, the number of animals may have been inadequate to give a strong estimate of performance. However, steers supplemented with Se alone had a higher (P<.05) ADG than the Cu supplemented steers and tended to gain more (P<.10) than the Cu plus Se and control treatment groups (Table VIII). The Cu plus Se and control treatment groups also had higher (P<.05) ADG's than the Cu treatment group. This trend is interesting in the aspect that the Cu treatment group had the lowest ADG and the Cu plus Se treatment group gained equivalent to the control group. This trend was also observed in Trial I when the immune response was examined. However, as stated earlier , the

TABLE VIII. MEAN AVERAGE DAILY GAIN OF STEERS IN THE COPPER AND SELENIUM PHASE (TRIAL II)

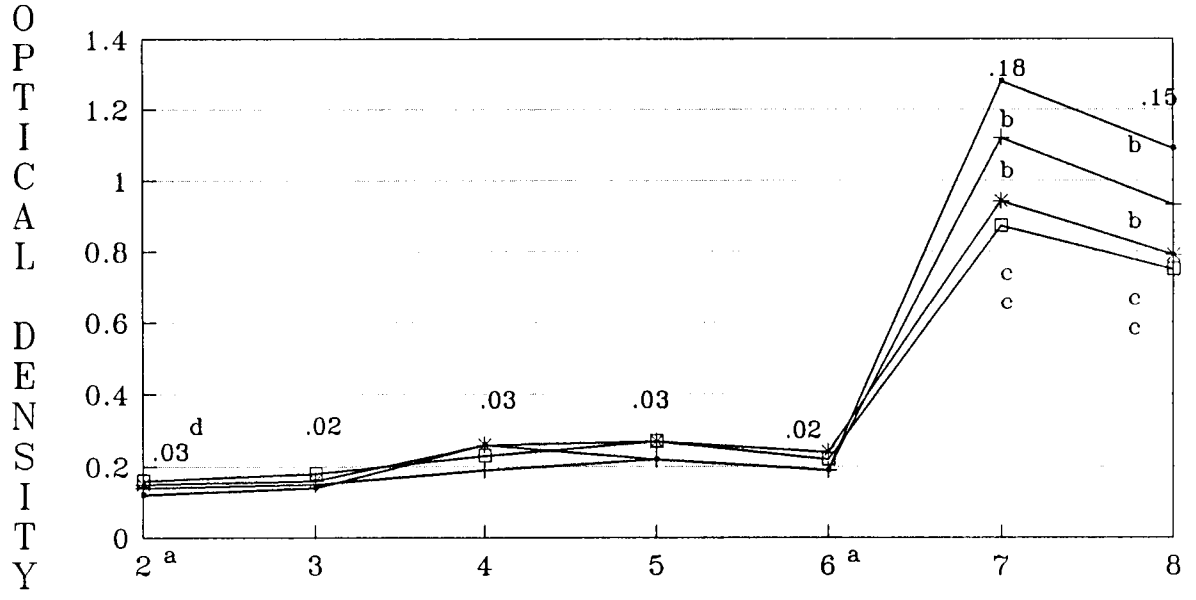
TREATMENT	ADG (kg)
SELENIUM	.33[a]
COPPER	.25[c]
CONTROL	.31[b]
COPPER PLUS SELENIUM	.31[b]
SE	.03

a,b,c-Means within columns with different superscripts differ (P<.05)

length of time was fairly short, so concrete statements about performance of the animals cannot be made. Additional research with more animals is needed in this area.

The initial KLH vaccination was administered on week 2 of the trial. Initial OD readings from that sample date were .12, .15, .16 and .14 for Se, Cu, control and Cu plus Se, respectively. The means for all sample dates are presented in Figure III. Samples from week 2 were taken prior to vaccination and served as negative controls for this phase. Optical densities were not different ($P>.10$) between treatment groups until after the second KLH vaccination. On week 7, one week after the second vaccination, the Se and the Cu plus Se treatment groups had higher ($P<.05$) OD readings compared to the Cu or control groups. The following week (week 8) Se treated steers had the highest ($P<.05$) OD readings. Steers supplemented with Cu plus Se had higher ($P<.05$) OD readings than Cu or control groups. There was a trend for lower ($P<.10$) OD readings in the Cu plus Se treatment group compared to the Se group, this was a significant difference at $P=.07$. This trend was also observed in Trial I but, was not significant ($P>.05$). In Trial II this occurred from week 4 through the end of the trial with the exception of week 6. The higher level of Cu

FIGURE III. MEAN OPTICAL DENSITY FOR STEERS. CU AND SE PHASE (TRIAL II).



a-Week of vaccination d-std. errors

b,c-Means with different letters differ (P<.05)

in the hay may have masked this response since all steers had adequate plasma Cu levels throughout the trial. The response is interesting and should receive additional attention.

Two steers were afflicted with pneumonia during Trial II. They were both mild cases and recovered after administration of Tetracycline. One was in the Cu treatment group and the other was in the Cu plus Se treatment group. There was no difference ($P>.10$) in the total number of animals sick, number treated or in number of sick days between the treatment groups. However, numbers were too low to draw conclusions from this data. Droke and Loerch (1989) observed the same trend of higher antibody titers but no difference in morbidity among animals.

SUMMARY

Zinc oxide suspended in corn oil was an effective means of supplementing Zn to maintain plasma Zn levels of weaned steers. The major problem with such Zn supplementation is the amount of handling required. Since the injections were effective for only 21 days, the animals would need to be reinjected each month. This would cause increased expense due to labor and supplement costs as well as an increase in stress on the animals which may be reflected in lower performance. The Zn supplementation had was ineffective with regard to modifying the humoral immune system response. This may have been due to the adequate level of plasma Zn on the date of vaccination. If initial levels had been deficient, a treatment response may have been detected.

Copper supplementation was effective in maintaining adequate plasma Cu levels in two trials. Even though in Trial II, adequate Cu was provided in the feed, supplemented steers tended to have higher plasma Cu levels. Copper had a positive effect on the secondary humoral immune response of steers in Trial II but not in Trial I. There was a tendency for Cu to be antagonistic to Se in the immune response. This phenomenon needs further research to determine the

cause and establish the validity of these results. Copper also appeared to depress the performance of steers in Trial II. However, larger numbers of animals are needed in order to reach conclusive results.

Selenium supplementation was effective in maintaining higher plasma Se levels whether used alone or in combination with Cu. Supplemental Se tended to increase the performance of steers in Trial II when compared to control or Cu plus Se treated steers. The humoral immune response was improved with Se supplementation in both trials which has also been reported by other researchers. However, when used in conjunction with Cu the improvement in response was not as high when compared with Se alone. The increased immune response should be conducive to fewer animals needing treatment for sicknesses. However, this was not observed in these trials. If the additional cost of supplementation is not compensated for through increased performance or reduced morbidity, it would be a questionable practice.

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APPENDIX

APPENDIX I

Immune System Overview

Immunity, a relative term, is used to describe the resistance to infection by a microorganism that an animal has developed due to previous exposure. The immune system is composed of several components that account for an animal's immunity. These components are the phagocytic system, lymphocyte system, humoral immune system, cell-mediated system and the complement system.

The first line of defense against invasion of microorganisms is the phagocytic system (Dr Donald Mattson, Personal Communication). This system consists of several cell types that phagocytize or ingest invading microorganisms. Included are the polymorphonuclear leukocytes (PMN) and the monocytes in blood and macrophages in tissue. These cells may be circulating free within or may be fixed in a specific area of the body. A subset of

monocytes, macrophages and fixed macrophage-like cells are also responsible for initiation of the humoral immune or cell-mediated immune response. This occurs through phagocytosis and partial digestion of foreign microorganisms followed by presentation of the remnants to lymphocytes.

The lymphocytes are cells of the immune system that are responsible for initiation of the humoral and cell-mediated immune response (Tizard, 1987). Lymphocytes are produced in the yolk sac of the fetus at first and then, during the latter period of development in the fetal liver. In adults, lymphocytes are produced in the bone marrow and then differentiate in two locations. The location of differentiation determines the type of lymphocyte. Lymphocytes involved in the humoral immune response are differentiated in the Bursa of Fabrcius in chickens and are therefore called B-cells. In mammals, the Bursa equivalent or bone marrow is the site of differentiation for B-cells. From there, these cells migrate to seed other organs of the lymphatic system in the body. In the cell-mediated immune response, the thymus is the site of differentiation of lymphocytes referred to as T-cells. There are several classes of T-cells, some of which regulate the humoral immune response and some that regulate the cell-mediated immune response. These cells then migrate to other organs

of the lymphatic system. Some cells involved in the cell-mediated immune response have an unknown lineage. These cells, known as killer cells (K-cells) and natural killer cells (NK-cells), are responsible for destroying host cells that have been altered by virus infection or cancer production. Some of these cells are derivatives of T-cells, while some have no identifiable markers.

The humoral immune response is mediated by antibodies or immunoglobulins (Halliwell and Gorman, 1989). These are large molecular weight proteins found in the gamma globulin fraction of blood serum. Antibodies are produced by B-cells in response to invasion and macrophage processing of microorganisms. The remnant of the microorganism is then presented to the B-cells with assistance of T-helper cells. As antibodies are produced they are released in the lymphatic system and can neutralize the specific antigen (microorganism) that initiated their production. This may be a virus, bacterium, parasite or a toxin produced by the microorganism. The antibodies can also coat host cells that have been altered. This allows for more efficient attack by PMN's, macrophages and K-cells. A special adaptation of the humoral immune system is the secretory immune system. This system is composed of B-cells placed under mucous membrane cells and is responsible for producing antibodies against

foreign material invading the mucous membranes of the body. Anyibody (IgA) in this case is liberated on mucous membrane surfaces.

The cell-mediated immune response is initiated in the same manner as the humoral immune response (Dr. Donald Mattson, personal communication). With macrophage processed antigen being presented to T-helper cells and through their assistance, T-effector cells are stimulated into three types of activity. These include: a) inhibition of growth or destruction of altered host cells, b) production of lymphokines which initiate action by other cells, or c) recruitment of additional T-cells from the infected area into action. In this case, the cell mediated immunity may be referred to as the branch of the immune response that destroys altered host cells (usually due to infection by a microorganism).

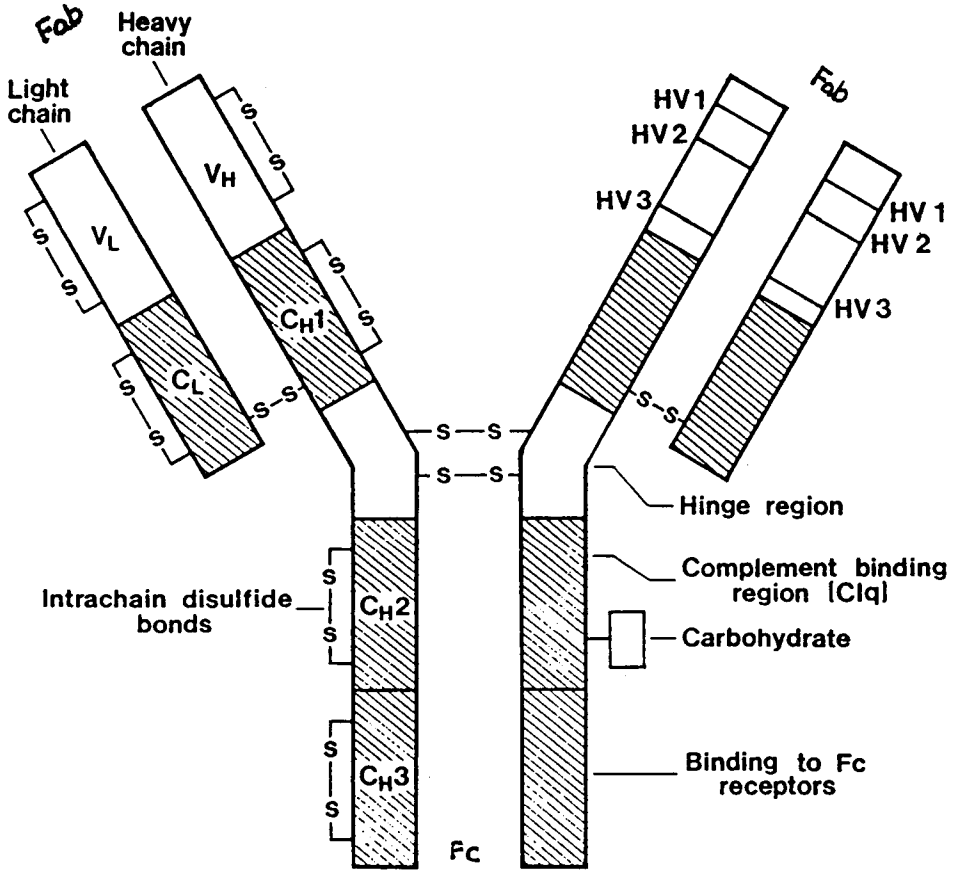
The complement system of the immune response is composed of a number of serum proteins which enhance the effect of antibodies reacting with an invading microorganism (Tizard, 1987). This enhancement can occur through complement production that enzymatically digests holes in the cell membrane of some microorganisms, production of chemotactic substances to cause migration of PMN's and macrophages into the area, release of by-products which

cause inflammation and in turn brings in more disease fighting cells of all types and an increased affinity for attachment of PMN's and macrophages which enhances phagocytosis.

The nature of the humoral immune response is pertinent in this research and therefore, needs some further explanation. There are several classes of antibodies produced by B-cells (Halliwell and Gorman, 1989). Each has a specific structure and is directed against the specific antigen presented to the B-cell. Within the lymph nodes, spleen and bone marrow the antibodies produced include immunoglobulin mu (IgM) and immunoglobulin gamma (IgG). These two antibodies are generally responsible for the majority of the humoral immune response. Each immunoglobulin contains at least one unit or monomer (Figure A1). This unit is comprised of four polypeptide chains held together by non-covalent and covalent interchain cysteine bridges and is bilaterally symmetrical. Each of these antibodies has an Fab portion and an Fc portion. The Fc portion is the base of the Y structure and is the portion that binds to specific cells, attaches to phagocytes, activates complement, controls catabolic rate, controls solubility and is the area where the J chain joins to form the pentamere of IgM and the secretory component of

FIGURE A1. IgG STRUCTURE

Schematic diagram of an IgG pentamere showing the two types of fragment and the heavy and light chains. The chains are held together by disulfide bonds. The interchain disulfide bonds maintain the three-dimensional structure. (Figure from Halliwell and Gorman,1989).



secretory immunoglobulin alpha. The Fab areas are the arms of the Y structure and are responsible for binding the antigen.

Upon invasion by a microorganism, phagocytosis, processing and presentation of the remnant to B-cells, the production of IgM begins. The IgM is a pentamere of immunoglobulins and is released to fight the infection. Initial production of IgM generally occurs during the primary immune response and can be detected in the serum in three to five days. This is the second highest immunoglobulin found in the serum, based on concentration. Once IgM has been produced for a time period the B-cell goes through a gene switch reaction which causes the production of IgG or other class of antibody. The cause of this reaction is not known.

The highest concentration of immunoglobulin in serum is IgG (Halliwell and Gorman, 1989). This is a monomer and easily disperses through tissues. Detectable concentrations can be found in seven to ten days after primary infection. During a secondary infection IgM can be detected within two days and IgG within five days. In the secondary infection, the IgG is produced mostly from B-cell daughters, called memory cells, that are circulating and have been previously exposed to the antigen. The IgM production is from B-cells

that have not had previous contact with the antigen.

APPENDIX II

Feed Analysis for Zinc Phase (Trial I).

	REPORTED AS % DRY MATTER
Dry Matter	93.5%
Crude Protein	6.82
Acid Detergent Fiber	42.93
Neutral Detergent Fiber	66.74
Magnesium	0.09
Phosphorus	0.15
Copper	2.05 ppm
Zinc	3.90 ppm

APPENDIX III

Enzyme-linked Immunosorbent Assay Protocol

Collect blood from each animal, allow to clot, and centrifuge. Remove serum and freeze undiluted.

Coating wells with antigen:

1. Dilute antigen to 5 µg/ml in PBS coating buffer.
2. Add 0.1 ml of diluted antigen to each well of a 96-well microtiter plate. (Linbro\Titertek EIA Plate, Flow Laboratories, Cat. # 76-381-04).
3. Seal wells with parafilm and store at 4° C until needed. (At least overnight, but good up to 10 days).

ELISA Procedure

1. Remove antigen coated plate from refrigerator and empty by use of a plate washer or by inverting and shaking plate over a sink.
2. Wash plate with washer using PBS-Tween 20 three times.
3. Dispense diluted serum samples into wells, at a rate of 0.05 ml per well. Place PBS-Tween 20 into 4 wells for a background reading. Samples should be diluted to 1:1000 with PBS-Tween 20. The positive control serum was diluted to 1:250 with PBS-Tween 20. Incubate the plate for 1 hour at 37° C while spinning on a platform at 120 rpm's.
4. Wash plate as in step 2.

5. Dilute enzyme-conjugated antibody to 1:750 with PBS-Tween 20, and add 0.10 ml to each well. Incubate for 1 hour at 37° C while spinning on a platform at 120 rpm's.

6. Wash plate as in step 2.

7. Dissolve substrate (p-nitrophenyl phosphate disodium·$6H_2O$) in substrate buffer to a concentration of 1 mg/ml.

8. Add 150 µl substrate solution to each well.

9. Incubate at room temperature until the positive serum reaches an Optical Density of 1.0.

APPENDIX IV

Buffer Solutions Used In ELISA Procedure

PBS-Tween 20 (For 1 liter of solution)

NaCl	8.0 g
KH_2PO_4 (anhydrous)	0.2 g
Na_2HPO_4 (anhydrous)	1.15 g
KCl	0.2 g

pH to 7.4 and q.s. to 1 liter.
Add 0.5 ml Tween 20.

SUBSTRATE BUFFER (For 1 liter of solution)

Diethanolamine	97 ml
H_2O	800 ml

pH to 9.8 with HCl and q. s. to 1 liter.

PBS-Coating buffer (For 1 liter of solution)

NaCl	8.5 g
KH_2PO_4	0.227 g
K_2HPO_4	1.91 g

pH to 7.4 and q. s. to 1 liter.

APPENDIX V

Dynatech MR700 Settings for ELISA Procedure

Option	1.2
Dual Mode	
Reference Filter	3 (Wavelength 490 nm)
Test Filter	1 (Wavelength 410 nm)
Threshold	1.99
Calibration	0.99

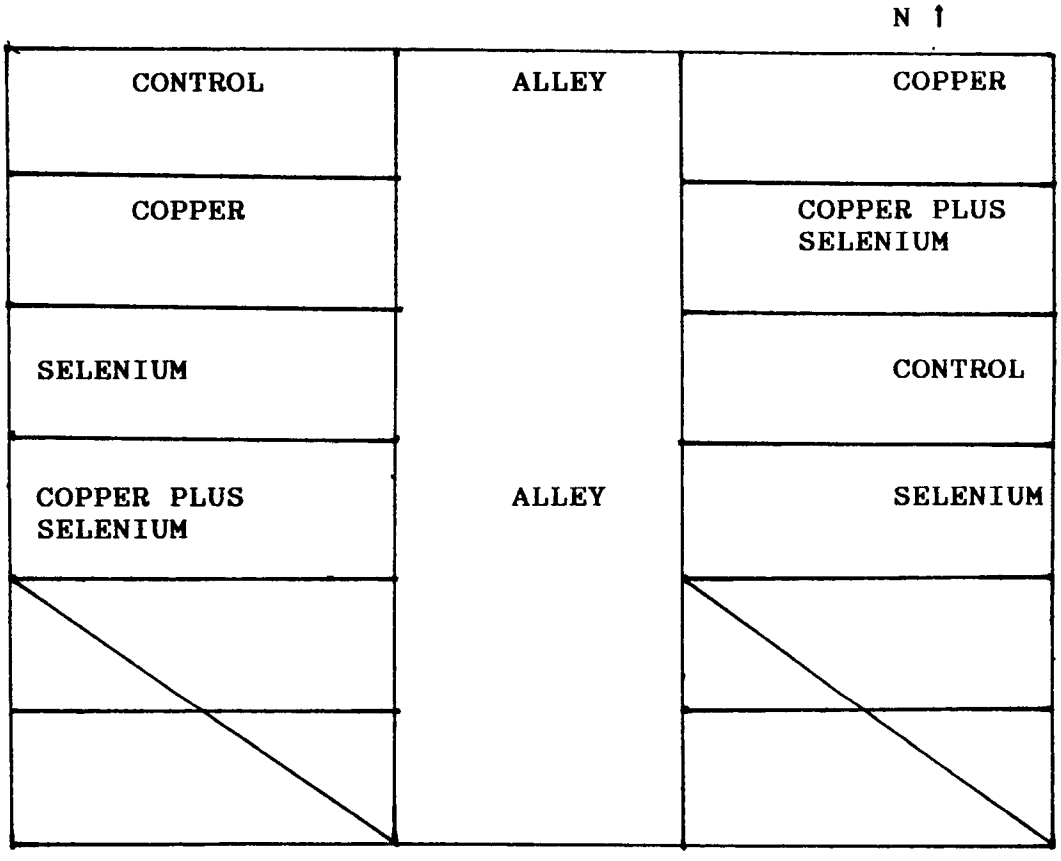
APPENDIX VI

Feed Analysis For Copper Plus Selenium Phase (Trial I).

	REPORTED AS DRY MATTER %
Dry Matter	92.2
Crude Protein	11.40
Acid Detergent Fiber	46.24
Neutral Detergent Fiber	67.62
Phosphorus	0.33
Copper	5.30 ppm
Molybdenum	3.60 ppm
Selenium	0.022 ppm
Manganese	58.6 ppm

APPENDIX VII

Pen Map For Trial II.



APPENDIX VIII

Feed Analysis Trial II.

	REPORTED AS % DRY MATTER
Dry Matter	94.8
Crude Protein	8.75
Acid Detergent Fiber	45.55
Neutral Detergent Fiber	66.63
Phosphorus	0.43
Copper	10.97 ppm
Molybdenum	3.60 ppm
Selenium	0.054 ppm