Trace Mineral Nutrition and Immune Competence in Cattle

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Introduction

The importance of essential trace minerals on livestock productivity has been realized for several decades. In improved agricultural systems, the supplementation of essential trace minerals has lead to increased livestock production as measured by body weight gain and reproductive performance. Recently, the importance of trace mineral nutrition on immune competence in livestock has gained attention. Research supporting these efforts has focused on multiple areas of immunity, including both specific and non-specific branches of the immune system. The three trace elements, which have received the most attention include copper, selenium, and zinc. Chromium, which is not yet approved for use for cattle in the US, has also been investigated. This paper will focus on these elements.

Trace Mineral Deficiency

Trace mineral deficiencies in livestock are often divided into two distinct categories:

1. Primary: A deficiency resulting from the consumption of an essential trace mineral at levels inadequate to support the physiological functions associated with that element.

2. Secondary: A deficiency resulting from the consumption of an element which antagonizes the pre- or post-absorption of an essential trace mineral rendering the element incapable of supporting the physiological functions associated with that element.

Research investigating the influence of trace minerals on health and performance of cattle has often depended upon the experimental feeding of one or more antagonists to induce a deficiency of the essential trace element under investigation. This methodology creates confounding in the experimental design causing the investigator difficulty in separating outcomes between the trace mineral deficiency and the potential

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direct effect of the antagonist. An example of this procedure relates to a common technique used in our laboratory as well as others, whereas, molybdenum and sulfur are supplemented to cattle diets to induce a secondary copper deficiency. It is difficult to separate the outcomes of this design between complications associated with the induced copper deficiency or the direct effect of feeding high levels of molybdenum and sulfur. Nevertheless, the presence of antagonists in common production systems is the most likely culprit leading to a deficiency (secondary deficiency). In improved agricultural production systems a primary deficiency, resulting from the reduced intake of trace mineral, is usually uncommon. The remedy for either deficiency (primary or secondary) is often achieved by increasing the amount of the supplemental trace mineral offered to the animal. Complete removal of the antagonist is difficult and sometimes impossible depending on its source. The data summarized in this paper may entail experiments that utilize trace mineral antagonists. Outcomes, therefore, may be dependent on the lack of trace mineral being studied, or directly related to the presence of the antagonist, or both.

Complications associated with dietary sulfur antagonism are a major consideration for beef cattle. Both copper and selenium may be strongly antagonized by high dietary sulfur concentrations. We have found complications with this antagonist in many regions of the US and the world. Contributions of sulfur to the total diet can be achieved from the forage via rainfall (acid rain) or the application of sulfur-containing fertilizers (i.e. ammonium sulfate). Dietary sulfur can also be realized through the supplementation of byproduct feeds that contain high concentrations of sulfur (i.e. molasses, brewer's grains, and feather meal). One of the most troublesome sources of dietary sulfur is high-sulfate water, which is very difficult to remove from the diets of cattle reared in these environments.

Categories of the Immune System

The data presented in this paper will cover multiple branches of the immune system; therefore, it is necessary to briefly outline two of the major categories of immunity and the tissues, cells, and cell products associated with these categories.

Nonspecific Immunity

Components of the nonspecific immune system include physical barriers (e.g. skin), phagocytic cells (e.g. macrophages & neutrophils), and soluble proteins derived from phagocytic cells (e.g. cytokines). Components of nonspecific immunity are present both prior to and following antigen exposure and they typically are indiscriminant against most foreign substances, in other words, not "specific" for any given antigen.

Specific Immunity

Components of the specific immune system include the category of leukocytes called lymphocytes. Lymphocytes orchestrate specific immune responses within two broad categories:

1. *Humoral immunity* – mediated by circulating antibodies produced from B-lymphocytes and in response to specific antigen recognition.

2. *Cell-mediated immunity* – mediated by T lymphocytes which both recruit and stimulate phagocytic (nonspecific) activity as well as participate in direct lysis of infected cells (e.g. viral infected cells).

Several nutrients have been shown to impact immune competence, including protein, energy, vitamins, and minerals. Each of these nutrients may impact one or both of the major arms of the immune system, specific and nonspecific immunity. It is important to consider the intimate interrelationship between many nutrients. Deficiencies or toxicity in any single nutrient will often impact the functionality of another nutrient. In regards to the brevity of this review, only those nutrients that have accumulated substantial research interest will be reviewed, including 1) selenium and vitamin E, 2) zinc, 3) copper, and 4) chromium.

Selenium and Vitamin E

The antioxidant roles of vitamin E and selenium, via the metalloenzyme glutathione peroxidase, share common biological activities. The sparing of one will impact the functionality of the other. Therefore, researchers studying vitamin E and selenium take into account the dietary concentrations of each nutrient and to what degree they may impact the results of the study. Results from studies investigating either nutrient without direct consideration to the other should be considered questionable.

Weiss et al. (1990) reported on a survey taken on commercial dairy herds. These results indicate that cattle with high serum selenium and alpha-tocopherol concentrations also had fewer incidences of clinical mastitis. Indeed, as the mean herd plasma selenium concentration increased the bulk tank somatic cell count (SCC) decreased (Figure 1). This effect may be due in part to the well-observed link between vitamin E and selenium nutrition and neutrophil function. Neutrophils, nonspecific phagocytic cells, are important in the control of intramammary infections. Neutrophils isolated from the milk of selenium supplemented cows were shown to have improved killing capacity when compared to cows fed a selenium deficient diet (Grasso et al., 1990). Similarly, blood neutrophils from vitamin E and Selenium supplemented cows also were shown to have improved killing ability (Figure 2; Hogan et al., 1990).

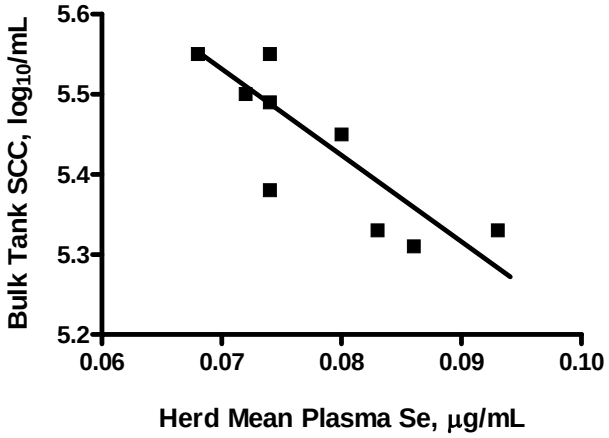

Figure 1. Relationship between herd Se status and milk SCC (adapted from Weiss et al. (1990).

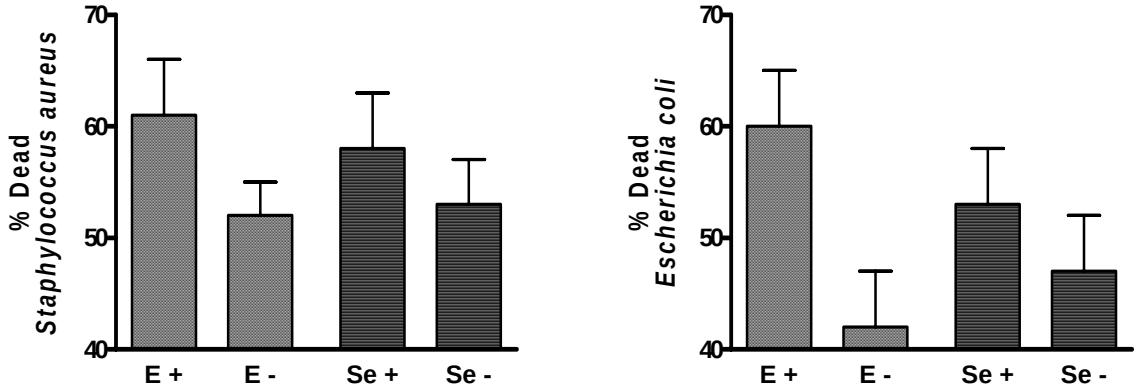

Figure 2. Percent intracellular killing by bovine neutrophils. Means represented (± SE); E = vitamin E and Se = selenium supplemented cows. There were no significant vitamin E x selenium interactions. Intracellular *S. aureus* killing was greater for vitamin E ($P < 0.01$) and selenium ($P < 0.05$) supplemented cows. Intracellular *E. coli* killing was greater for vitamin E ($P < 0.01$), but not selenium ($P > 0.05$) supplemented cows. Figure adapted from Hogan et al. (1990).

The majority of vitamin E and selenium research supports this important role on nonspecific neutrophil function. Although vitamin E and selenium may also play a part in specific immune functions, specific examples through published literature have been lacking.

Vitamin E, as alpha-tocopherol, does not readily cross the placenta. Newborn calves are generally born with low concentrations of vitamin E, dependent on colostrum and milk for their vitamin E intake. Maternal concentrations of alpha-tocopherol typically decline just prior to and following parturition. Injections as well as high-level supplementation of vitamin E prior to calving have been shown to maintain alpha-tocopherol concentrations during this critical period (Smith et al., 1997). Taking these points into consideration, a focus on cow vitamin E nutrition is most important during the last few months of gestation and during early lactation. Optimized vitamin E nutrition during this period will both bolster the immune system of the cows by aiding neutrophil function and decreasing mammary infections while ensuring delivery of vitamin E nutrition to the offspring.

Selenium has been shown to affect multiple measures of immune competence in cattle. Of course, as discussed previously, the interconnection of vitamin E within these individual factors is not well understood, but should be considered. One of the most recognized roles of selenium in normal cattle production systems is its apparent direct link to postpartum uterine involution. Multiple studies have shown that selenium deficient cattle have a higher incidence of retained placentas compared to cattle with adequate selenium nutrition. Retained placentas are closely related to the animal's immune system through the resulting inflammatory responses associated with expulsion or retention of the placental membranes. Some of the frequently cited early data reporting this association came from The Ohio State University. In one study (Julien et al., 1976a); the authors reported a 38% reduction in retained placentas in selenium treated versus control cows. In a second field study (Julien et al., 1976b), the authors observed a 42% reduction in the incidence of retained placentas in cows receiving a prepartum injection of a blend of selenium and vitamin E compared to control cows. Finally, selenium supplementation also appears to be effective in lessening the recovery time of cows inflicted with metritis. Harrison et al. (1986) reported on a study indicating that selenium may be important to cows diagnosed with metritis. In their study, metritis-inflicted cows receiving supplemental selenium had fewer uterine recovery days compared to metritis-inflicted cows not supplemented with selenium.

Zinc

Zinc has enjoyed the distinction of being one of the most studied trace elements in animal nutrition. Zinc is essential for the function of numerous enzymes. In particular, zinc has been shown to be important in biochemical processes involved in nucleic acid metabolism and cell division (Chesters, 1974). For this reason, zinc has long been recognized as an important nutrient for spermatogenesis and male fertility in farm animals. Recently however, the role of zinc in immune competence has received some attention. Many investigators have suggested that the manifestation of zinc deficiency in cattle will be realized by impaired apatite and growth before reduced immune competence is observed. Significant data related to this concept is currently lacking for cattle, but studies in sheep (Droke and Spears, 1993) and mice (Beach et al., 1981) support this supposition.

Chirase et al. (1991) investigated the effect of an organic form of dietary zinc (zinc methionine) on feedlot steers challenged with a respiratory virus. In this study, zinc methionine supplemented steers experienced an improved rate of disease recovery. Considering the link between zinc and cell divisional processes it would appear probable that zinc may play an important role in lymphocyte functions which are dependent on a rapid cell division process called "clonal expansion" following antigen stimulation. Prasad and Kundu (1995) investigated the effect of zinc fortified milk fed to newborn calves. In this study, zinc supplementation resulted in greater immunoglobulin (antibody) responses to antigen challenge. In another study (Spears et al., 1991; Table 1), stressed steers supplemented with zinc methionine experienced an increase in antibody titers to BHV-1 vaccination compared to steers receiving no supplemental zinc. As well, steers provided supplemental zinc (inorganic or organic) experienced a greater dry matter intake compared to control steers receiving no supplemental zinc.

Table 1. Effect of supplemental zinc on antibody titer to BHV-1 and dry matter intake of stressed feedlot calves.[1]

	Dietary treatment[2]			
Item	Control	ZnMet	ZnO	SE
BHV-1 titer[3]	0.49	0.72	0.55	0.09
Dry matter intake, kg[4]	6.32	6.65	6.60	0.14

[1]Adapted from Spears et al. 1991. JAVMA. 199:1731.
[2]Control = no zinc, ZnMet = 25 mg supplemental zinc methionine / kg of diet, and Zinc oxice = 25 mg of supplemental zinc oxide / kg of diet.
[3]Serum neutralizing titer expressed as negative $\log_{10}$ of highest dilution of serum causing neutralization of virus. Steers provided supplemental zinc tended to have higher ($P < 0.16$) BHV-1 antibody titers than Control steers.
[4]Control vs zinc ($P < 0.11$).

Copper

The influence of copper on immune function has been widely investigated but with quite variable results. Copper is the essential element in at least two enzymes important in immune competence, copper/zinc-superoxide dismutase and ceruloplasmin (Prohaska, 1990). It is likely that that copper may exert its effect on immunity through the attenuation of one or both of these enzyme complexes.

Several groups have investigated the effect of copper on neutrophil function. Although results have been variable, some studies suggest that low copper status results in reduced neutrophil phagocytic capacity (Boyne and Arthur, 1981; Xin et al., 1991). However, our research has not supported a link between copper status and neutrophil killing capacity or chemotactic ability (Arthington et al., 1995; Arthington et al., 1996a). Copper status does appear to result in an increase in blood neutrophil number (neutrophilia) in cattle (Arthington et al., 1996a, Arthington et al., 1996b) and mice (Karimbakas et al., 1998). This phenomena is in contrast to that which is seen in humans, where copper deficiency results in marked neutropenia (Percival, 1995). Although neutrophil number is elevated in cattle, there is currently no data suggesting that immune competence is changed as a result of this effect.

Research investigating the effect of copper on specific immune responses in cattle have also been variable and tend to suggest little or no effect of dietary copper on lymphocyte function (Stable et al., 1993; Arthington et al., 1995; Ward et al., 1997; Ward and Spears, 1999). In some cases lymphocyte proliferative responses to mitogen stimulation appear to be increased in response to copper deficiency (Torre et al., 1994; Arthington et al., 1996b). It is impossible to suggest that this response infers an improvement in immune competence without measuring disease resistance. It may be just as likely that this response reflects an over-active immune system, draining energy away from growth and reproduction to fuel the production of immune cells and cell products.

Copper is transported throughout peripheral circulation complexed with its transport protein, ceruloplasmin. During instances of copper deficiency blood ceruloplasmin concentrations are decreased (Ward and Spears, 1999; Arthington 1996b). Ceruloplasmin is a sensitive acute phase protein, which increases in blood concentration following stress stimuli. Copper deficient calves are not able to mount a normal acute phase protein response following inflammatory challenge. In contrast, fibrinogen, another sensitive acute phase protein, is dramatically increased following inflammatory challenge in copper deficient calves (Figure 4; Arthington et al., 1996b).

Figure 4

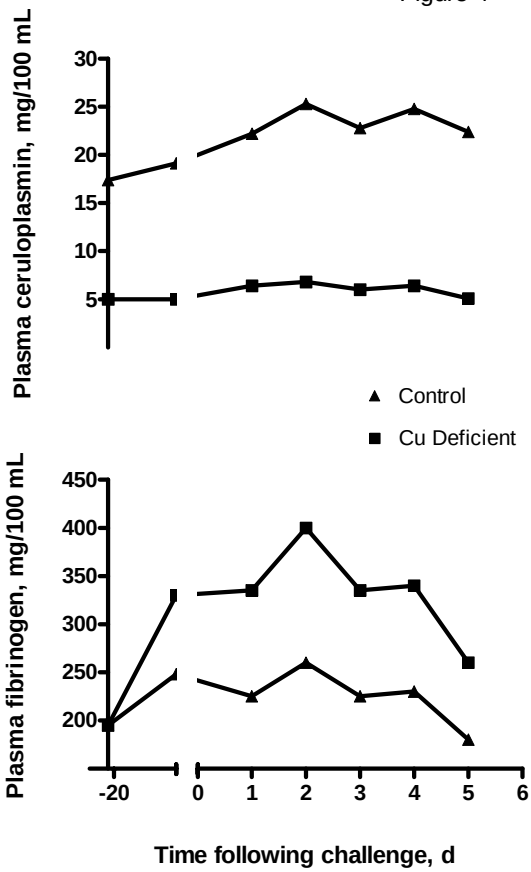

Figure 4. Effect of copper status on the acute phase protein response following IBR challenge (d=0) in growing heifers (Arthington et al. JAS. 1996. 74:2759).

A normal acute-phase reaction lasts only 24 to 48 h. Critical negative feedback mechanisms exist, including the important anti-inflammatory action of glucocorticoids on the activation of inflammatory cells such as macrophages and neutrophils. These inflammatory cells are the primary producers of the proinflammatory cytokines responsible for stimulating acute phase protein production. Any alteration in this negative feedback control mechanism or in the products of continued stimulation may result in a chronic inflammatory condition (Baumann and Gauldie, 1994). Chronic inflammation in livestock, resulting from an altered inflammatory response, will have direct implications upon growth and performance. These effects are likely a result of the highly pleiotropic actions of the pro-inflammatory cytokines (Johnson, 1997; Klasing and Korver, 1997). It is likely that

that the pathogenesis of copper deficiency in cattle manifests itself through this altered proinflammatory response. The marked neutrophilia and altered acute phase protein response in stressed, copper deficient cattle support this supposition.

Chromium

Recently dietary chromium has received attention as a potential nutrient involved in immune competence. Several studies have indicated that chromium can be an important modulator of insulin-like activities (Schwarz and Mertz, 1959). More recently, studies from Canada have shown marked improvements in measures of health and stress when feeder steers where supplemented with chromium. Chang and Mowat (1992) reported a significant decrease in serum cortisol concentrations of chromium supplemented, stressed feeder calves (75.0 vs 55.6 nmol/L for control and chromium supplemented calves, respectively). Further, chromium supplemented calves also had increased serum immunoglobulin concentrations. Currently, this response has not been widely supported, as other laboratories have reported no effect of chromium supplementation on blood cortisol concentrations in stressed feeder steers (Kegley and Spears, 1995) or steers challenged with a respiratory virus (Arthington et al., 1997). When evaluating these results it is important to investigate the type of stress under consideration, as each of these studies utilized a differing type and intensity of stressor.

In another study, rectal temperatures of chromium supplemented stressed feeder calves, were reduced by 0.5^{o} C (Moonsie-Shageer and Mowat, 1993). This decreased temperature response was also associated with an increase in feed intake. Other researchers have noted increases in *in vitro* lymphocyte proliferative responses to concanavalin-A (ConA) stimulation (Burton et al., 1995). In this study, lymphocytes, cultured with serum from chromium supplemented cows, experienced increased proliferation following ConA stimulation. Other researchers have reported no effect of chromium supplementation on lymphocyte responsiveness to mitogen stimulation in cattle (Arthington et al., 1997; Kegley and Spears, 1995; Kegley et al., 1996).

Currently, supplemental chromium sources are available for use in swine, but not ruminant diets. The acceptance of this technology in ruminant nutrition will surely increase the amount of research and subsequent knowledge available to the consumer.

Summary

Focusing improvements in nutrition on immune competence holds great promise in future livestock management strategies. Other nutrients, not described in this document, also have been shown to be potentially important in maintaining immune competence, these include, but are not limited to, B-vitamins, beta-carotene, vitamins A & D, manganese, cobalt, and potassium.

Many of the research results, presented in this document and in this area of science in general, are highly variable. Efforts to control the sources of variation within experimental models will result in improved results and ultimately a better

understanding of the application of these nutritional technologies. This variation may be best explained from at least four sources:

1) The level of deficiency achieved in the experiment
2) The source, concentration, and integrity of the supplemental nutrient provided
3) Influence of antagonistic factors found in the basal diet
4) Physiological state of the experimental animal (stage of pregnancy, growth, stress, etc.)

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