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IMMUNE RESPONSES AFFECTED BY MICRONUTRIENTS

Dr. Cheryl F. Nockels
Colorado State University, Fort Collins 80523

Introduction

Antioxidant nutrients such as vitamin E, ß-carotene and the trace elements selenium, copper, zinc and manganese in enzymes are very important in protecting an animal's tissues from oxidative destruction. Evidence is presented that this protective benefit also results in an improved immune response which decreases infectious disease incidences arising in stressed cattle following shipping. The amounts of the nutrients needed for immunoenhancement is higher than the suggested required amounts by NRC.

Reactive Oxygen Molecules -- Production and Control

There needs to be a balance between oxidants and antioxidants within any animal cell. If the cell becomes more oxidative, then oxidative stress results which can result in cell damage such as lipoperoxidation, DNA damage and protein destruction which may lead to cell death. During metabolism several powerful oxidants are produced such as the free radicals, superoxide ($O_2^{\cdot}$), peroxyl radical (ROO·), and hydroxyl radical (OH·) and other nonfree radical oxidants hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). Free radical and peroxide oxidants have been termed reactive oxygen molecules or metabolites (ROM) by Powell (1991). For a thorough review of ROM production and their control see Miller and Madsen (1994). To control oxidants produced in cells antioxidants, electron donors, such as lipid soluble vitamin E, ß-carotene (at low oxygen pressure) and perhaps vitamin A and water soluble ones such as vitamin C, glutathione and urates act as chain breaking antioxidants. Another means of controlling oxidants is by enzymes. Superoxide dismutases containing either copper and zinc or manganese convert superoxide to hydrogen peroxide. Peroxides are degraded to water by peroxidases such as selenium containing glutathione peroxidase. This enzyme may also convert lipid peroxides as well to alcohols. A similar enzyme, manganese superoxide dismutase, is present in mitochondria. The amount and function of these enzymes may be limited not only by the quantity of the mineral but by protein availability. Both zinc and copper are bound to other proteins metallothionein and ceruloplasmin, respectively, which have antioxidant capabilities.

Of critical importance in determining when antioxidants may become deficient are those physiological events which increase metabolic pathways producing ROM. An increase in ROM arises when oxidative metabolic reactions are increased as in aerobic exercise, pregnancy, stress, tissue injury, infection and the detoxification of numerous compounds (Nockels, 1991a). Animals, in response to infectious disease and stress, elicit increased ROM production (Nockels, 1991b). An active immune response invokes the production of millions of new cells, proteins and hormones which requires an extremely large production of energy through pathways producing ROM. Also phagocytic cells such as neutrophils and macrophages produce free radicals in the respiratory burst reactions which are used in killing phagocytized organisms

(Tizard, 1988). In stress, many of the same hormones, glucocorticoids and epinephrine, are produced as in infection. Concomitant ROM production occurs with corticoid synthesis and epinephrine enhanced eicosanoid metabolism (Nockels, 1991b).

Stress often precedes an infectious episode in animals, which decreases antioxidants later needed by an active immune system. For instance, if a neutrophil is producing ROM for killing a bacterium, the released ROM may also destroy the macrophage as inadequate antioxidant is present in its cells to protect it from its own oxidants. Also, surrounding cells may be destroyed as hydroxyl radicals may pass through membranes to other tissues. Corticoids produced in stress also are antagonistic to immunity (Breazile, 1988), but can be decreased by vitamin E (Reddy et al., 1987b). This vitamin also aids the immune response by reducing the production of the immunosuppressive prostaglandin, PGE_2 (Lawrence et al., 1985).

When cells are unable to control ROM production or removal, then tissue membrane destruction is one consequence. Membrane destruction results in enzymes leaking from cells and entering the circulation. Frequently creatine kinase or lactate dehydrogenase are enzymes in blood used to predict tissue damage.

Overview of Immunity

A brief overview of the immune system follows. Antigens, such as bacteria, viruses, and parasites are substances foreign to the body that cause the immune system to respond. This response is acquired, which means that specific proteins and cells are being produced to neutralize or destroy that specific antigen. In order to respond immunologically, whether it be to an invading foreign antigen or one that has been given in a vaccine, the animal needs to have a responsive immune system. Regardless of the antigen source, the nutrient requirements for this immune response are going to be similar.

The immune system based on its acquired response may be divided into the cellular and humoral portions. Bone marrow produces lymphocytes and primary lymphoid organs regulate the production and differentiation of these lymphocytes. The cell mediated immune response involves T-lymphocytes which go through a period of maturation within the thymus, accumulate in lymph nodes and other lymphoid areas, and then are able to respond to a particular antigen as T-cells. One of the means of measuring cellular immunity is by quantifying lymphocyte replication in response to a mitogen in an *in vitro* system. Another means of measuring cellular immunity is by giving a mitogen intradermally and measuring a skin swelling response. In some of the research I will be reporting skin swelling to the mitogen, phytohemagglutinin (PHA) which causes infiltration of lymphocytes into the area.

B-lymphocytes will be processed in intestinal lymphoid tissues before migrating to thymus independent areas and are then B-cells. From these B-cells is derived the humoral immune response which involves the production of antibodies or immunoglobulins. The time course of antibody development following the administration of a particular antigen that the animal has never previously received will take about 10 days to become maximal. The amount of antibody response is measured as a titer, will not become very high, and persist but two to three weeks.

This first response to the antigen is the primary immune response. If a second administration of the same antigen was given, the secondary immune response will be much more rapid, give a higher antibody titer, and persist for months. Since the T- and B-cell response may require days to respond, then the immediate immune defense requires the phagocytic system which utilizes neutrophils and macrophages.

The remainder of the paper will document the importance of ß-carotene, vitamin E, copper, zinc and selenium in aiding immunity, particularly following stress in cattle.

Nutrients Increasing Immunity

Carotenoids

In addition to ß-carotene other carotenoids (having no pro vitamin A activity) have been found to be beneficial as antioxidants to the immune system. Excerpts from a current review of ß-carotene in the immune response of cattle (Chew, 1994) will be presented. In two studies, one of the following supplements was fed daily to Holstein cows in three treatments: 1) 53,000 IU vitamin A, 2) 173,000 IU vitamin A, 3) 53,000 IU vitamin A + 300 mg ß-carotene (equivalent to 173,000 IU vitamin A). In the first study the supplements were fed from three weeks prior to dry off through three weeks after dry off. The incidence of new intramammary quarter infections was 27% in cows fed ß-carotene, 49% fed 53,000 IU vitamin A and 50% fed 173,000 IU vitamin A. In the second study, cows were fed the supplements from four weeks prepartum through 8 weeks postpartum. Milk from cows fed ß-carotene had 38% lower somatic cell content compared to the two vitamin A only fed groups. ß-carotene has been shown to increase lymphocyte blastogenesis and to stimulate neutrophil phagocytosis and killing ability during prepartum and dry periods.

Vitamin E

A study determined the amount of vitamin E and the route of administration which would correct vitamin E deficiency and improve immunity. Holstein heifer calves were allotted at birth to one of four vitamin E treatments: 1) 0 mg, 2) 1400 mg 3) 2,800 mg dl-α-tocopheryl acetate fed orally weekly for 12 weeks, or 4) 1400 IU dl-α-tocopherol was injected intramuscularly weekly (Reddy et al., 1985, 1986). The calf starter contained .44 ppm of selenium. Lymphocyte stimulation to PHA was increased ($P < .05$) at 4 and 8 weeks in the calves injected with vitamin E (Reddy et al., 1986). Serum from both the calves fed 2800 mg of vitamin E and those injected with the vitamin had lower ($P < .05$) viral replication titers. Serum IgM was increased ($P < .05$) at week 6 by feeding the highest vitamin E amount. When averaged over the 12 week period, serum creatine kinase was reduced ($P < .05$) by oral and injected vitamin E supplementation compared to controls. The authors pointed out that the higher creatine kinase content in the unsupplemented calves was a preclinical indication of muscular dystrophy which was not prevented by selenium alone, and that both vitamin E and selenium are necessary for prevention. In an additional experiment, 2000 IU of dl-α-tocopherol was injected into yearling

heifers and the serum α-tocopherol and lymphocyte stimulation indices were increased ($P < .01$) seven days later (Reddy et al., 1986).

Reddy et al. (1987a,b) in subsequent experiments, fed one of four dl-α-tocopherol quantities of 0, 125, 250 or 500 IU daily to Holstein heifer calves in their milk for the first 8 weeks. Thereafter dl-α-tocopheryl acetate was fed in .45 kg of dry feed daily until the calves were 24 weeks old. Vitamin E improved several immune responses in the calves. Overall weight gains were improved ($P < .05$) by vitamin E (125 and 250 IU/d) supplementation. Serum glutamic oxalacetic transaminase and lactic dehydrogenase were decreased by 125 IU of vitamin E/day. Serum tocopherol concentrations less than .2 mg/dl, as well as enzyme amounts indicated the controls were deficient throughout the entire study (Reddy et al., 1987a). The secondary immune response antibody titers developed to antibovine herpesvirus type I were inceased ($P < .05$) in calves fed 125 IU vitamin E daily (Reddy et al., 1987b). Lymphocyte stimulation indices to PHA, Con A, PWM, and LPS were effectively enhanced ($P < .05$) by the vitamin at different supplemental levels. The overall mean serum cortisol concentrations were reduced ($P < .05$) by all vitamin E supplemental amounts.

Following shipping, calves weighing 532 lb were each supplemented with 800 IU vitamin E daily for the first 21 days and 400 IU for the next 7 days (Hicks, 1985). During this 28-day period, average daily gain ($P < .01$) and feed conversion were increased and number of days sick, percent morbidity and mortality were reduced by vitamin E fortification. In another study newly arrived stressed feeder cattle were fed either 0 or 1600 IU vitamin E per kg diet (Gill et al., 1986). The cattle fed vitamin E gained 22.2% faster ($P < .05$), had 11.7% less morbidity and 12.5% fewer sick days compared to controls.

Following the arrival of stressed calves, ten were injected with 1500 IU d-α-tocopherol and ten received the carrier of the vitamin (Nockels et al., 1993a). All the calves were injected with keyhole limpet hemocyanin and hen egg ovalbumin. Antibody development to the two antigens was increased ($P < .05$) in those given the vitamin E.

Selenium

Steers made selenium (Se) deficient did have reduced neutrophil candidacidal and myeloperoxidase activities (Boyne and Arthur, 1979, 1981; Arthur and Boyne, 1985).

Calves made selenium deficient and given a primary and secondary inoculation with infectious bovine rhinotracheitis virus (IBRV) had reduced ($P < .05$) glutathione peroxidase activity, increased plasma creatine kinase activity, decreased ($P < .05$) IgM after both inoculations, but no change in IgG, and a decrease ($P < .05$) in antibody titer after the secondary challenge (Reffett et al., 1988). In another study, calves were offered selenium in a mineral supplement at 20, 80, 120, 160 and 200 ppm (Swecker et al., 1989). The gains were not affected by the selenium (suggesting that if body weight gain is the criteria of adequacy then the 20 ppm Se was adequate), but IgG titers produced in response to hen egg lysozyme inoculation increased ($P < .05$) in calves ingesting all selenium concentrations above 20 ppm. This data

compliments immune research conducted with other nutrients, which demonstrates that the immune needs are higher than that for growth.

In dairy cows it was found that plasma selenium increases with dietary selenium and a high correlation ($r^2 = .70$) exists between high blood selenium and reduced mastitis incidence (Weiss et al., 1990).

Selenium and Vitamin E Interact

When stressed cattle arrived at the feedlot and were given by injection either 25 mg Se or 340 IU of vitamin E or both together and immunized with *Pasteurella hemolytica*, IgG titers developed only when the two nutrients were given together (Droke and Loerch, 1989). The same result occurred when the amount of each nutrient was doubled which also further increased the IgG titers. Compared to controls dairy cows either injected with .1 mg Se/kg body weight 21 days prior to parturition or fed .74 g vitamin E/head/day during the dry period had a decrease in the incidence and duration of mastitis and when given together, a further reduction in the duration of the disease was noted (Smith et al., 1984).

Mastitis in dairy cows has been effectively controlled by supplementing vitamin E and selenium (Smith and Conrad, 1987). In the first trial cows during the dry period were fed 1000 IU vitamin E/cow/day and given a single selenium IM injection of .1 mg sodium selenite/kg body weight 21 days before anticipated calving. The 4 groups of cows were controls (no supplementation), Se injected, vitamin E fed, and vitamin E fed-Se injected. New clinical cases of mastitis were reduced 37% in both groups getting vitamin E, and 12% in those getting just Se compared to controls. All three supplemental groups had a reduction ($P < .05$) in duration of clinical symptoms. Vitamin E supplemented-Se injected cows had a shorter duration of clinical signs than those receiving either vitamin E or Se alone.

In a second trial beginning 60 days prior to calving, control heifers received no supplemental vitamin E or Se (Smith and Conrad, 1987). The experimental heifers received 2 IU dl-α-tocopheryl acetate/kg body weight/day and 2 μg of selenium from sodium selenite/kg body weight/day, and at 21 days prepartum were subcutaneously injected with 100 μg of selenium/kg body weight. During lactation the supplemented group received 88 IU vitamin E/kg and .3 mg selenium/kg feed. Supplementation with vitamin E and selenium increased blood levels of vitamin E, Se, and glutathione peroxidase at parturition. Improved udder health in the supplemented cows compared to controls was evidenced by: 1) a 42.4% reduction in prevalence of infected quarters at calving; 2) a 59% reduction in quarter lactation days infected; 3) a 32.1% reduction in clinical mastitis over the entire lactation and a 57.2% reduction during the first four days of lactation; and 4) significantly lower milk somatic cell numbers at 14 days of lactation and for the entire lactation.

However, positive additive effects of administering the two nutrients together are not always found as Hogan et al. (1990) reported that each nutrient independently increased neutrophil killing. An extensive review of the role of vitamin E and selenium in immune

resistance to mastitis and their affects on neutrophil function has been presented (Hogan et al., 1993).

Zinc

Supplementing 350 mg of zinc daily per steer for the first 28 days after arrival at the feedlot was beneficial (Hutcheson, 1985). The diet contained 43 ppm zinc which met the recommended amount of 20-40 ppm according to the NRC (1984). The added zinc increased ($P < .05$) feed intake and daily gain of the morbid steers and reduced the number repulled for treatment.

The influence of zinc amount and source on the immune response in calves was investigated. Cattle were divided into three groups (Spears et al., 1991). The basal control diet contained 26.4 mg zinc/kg, which was within the NRC suggested requirement range of 20-40 ppm. To this diet was added 25 mg zinc/kg diet coming either from zinc methionine or from zinc oxide. The newly arrived feeder cattle were immunized with bovine herpes virus. Relative to the controls, the zinc methionine-fed cattle had a significant improvement in antibody titer. The titer was intermediate for those given the zinc oxide. The negative seroconversion percents or the percent of cattle from each treatment that did not form antibody to the antigen by day 14 were 33% of the controls, 10% of those given zinc methionine, and 23% of those fed zinc oxide. Feeding zinc methionine to cattle on pasture was found to decrease the incidence of footrot relative to those fed a zinc-deficient diet (Brazle, 1993).

In a study conducted by Chirase et al. (1991), 60 ppm zinc was added from zinc methionine to the control diet that contained 31 ppm zinc. After injecting the cattle with IBRV, the rectal temperature response was higher in the controls, and less in those receiving the 90 ppm zinc. The cattle fed the higher zinc amount stayed on feed better and gained better than those fed less dietary zinc.

Copper

Neutrophils from copper (Cu) deficient cattle did not have a depression in phagocytosis, but killing of ingested *C. albicans* was compromised relative to controls (Boyne and Arthur, 1981). In another study, neutrophils from Cu deficient cattle were again found to have reduced candidacidal activity (Arthur and Boyne, 1985). Since the previous studies utilized tetrathiomolybdate to enhance the effects of low dietary Cu, the intent of the next research was to determine if Cu deficiency produced by feeding iron (Fe), would cause similar changes in cattle neutrophil activity as that produced when using molybdenum (Mo). In 1986 Boyne and Arthur produced copper deficiency in cattle by feeding groups either high iron or high molybdenum and a group was fed the control diet limited to 80% of the control intake to take into account the effect of reduced feed intake in the copper deficient cattle. Neutrophil killing of *Candida albicans*, phagocytic activity, nitroblue tetrazolium (NBT) reduction (an estimate of free radical production), superoxide dismutase activity, and neutrophil viability were all reduced ($P < .05$) by copper deficiency produced by feeding either antagonist. The influence of feed

restriction on neutrophil function also reduced ($P < .05$) killing of *Candida albicans*, NBT reduction and neutrophil viability. This later information is particularly important for cattle newly arrived at a feedlot that may not have eaten for a few days or will have a depressed feed intake for the next two to three weeks.

Xin et al. (1991) reported the Holstein steers made copper deficient by feeding molybdenum had decreased ($P < .05$) liver copper concentration, no change in serum copper, increased ($P < .05$) liver molybdenum content, and decreased polymorphonuclear neutrophil copper quantity and killing ability.

Copper deficiency effects on immune responsiveness and disease resistance were studied in calves (Spears, 1988). When calves became Cu deficient based on serum Cu and serum ceruloplasmin activity, they and controls were inoculated with IBRV followed 7 days later with *Pasteurella haemolytica*. Serum IgM concentrations were lower in Cu deficient calves following disease exposure. Ceruloplasmin activity rose following inoculations in controls but remained low in Cu deficient calves. No change in rectal temperature or feed intake occurred in either group.

Recently, Ward et al (1993) reported reduced cell mediated immune response, as indicated by skin swelling response to PHA when cattle were made copper deficient by feeding molybdenum and sulfur. Stabel et al. (1993) fed copper deficient or adequate diets to Holstein calves and found that serum IgM and antigen specific antibodies to *P. hemolytica* tended to be higher in copper adequate calves. Copper deficiency was found to reduce ($P < .05$) antibody titer development to IBRV in calves (Nockels et al., 1993b).

Mineral Combinations

Previous research has investigated the impact of supplementing single trace elements to cattle on their immune response. In a recent study, zinc and manganese from methionine complexes were added to the cattle's control diet which was low in zinc (27.8 ppm) and manganese (14.1 ppm) (Chirase et al., 1994). The cattle were infected with IBRV and relative to controls those receiving the supplement had greater ($P < .05$) dry matter intake, a reduced fever period, and lost less body weight. In recent studies (unpublished) conducted at our Colorado State University research feedlot, supplementing equal amounts of copper, zinc, manganese and cobalt from either inorganic compounds or organic complexes both before shipping or after arrival has resulted in increased ($P < .05$) antibody titer formation to vaccines, skin swelling response to PHA and feed efficiency.

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