

## Micronutrients and immune function in cattle

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Complex inter-relationships exist between certain micronutrients, immune function and disease resistance in cattle. Several micronutrients have been shown to influence immune responses. The relationship between deficiencies of some micronutrients and disease resistance is less clear. A number of studies have indicated that Cr supplementation may improve cell-mediated and humoral immune response as well as resistance to respiratory infections in stressed cattle. With respiratory-disease challenge models Cr generally does not affect disease resistance. Deficiencies of Cu, Se, vitamin E and Co in cattle reduce the ability of isolated neutrophils to kill yeast and/or bacteria. Cu deficiency reduces antibody production, but cell-mediated immunity is generally not altered. However, Cu deficiency appears to reduce production of interferon and tumour necrosis factor by mononuclear cells. Numerous studies have linked low vitamin E and/or Se status to increased susceptibility of dairy cows to intramammary infections. In contrast to findings in laboratory animals, marginal Zn deficiency does not appear to impair antibody production or lymphocyte responsiveness to mitogen stimulation in ruminants. Co deficiency has been associated with reduced resistance to parasitic infections. It is well documented that vitamin A-deficient animals are more susceptible to various types of infections.  $\beta$ -Carotene, possibly via its antioxidant properties, may affect immune function and disease resistance independent of its role as a precursor of vitamin A.

### Immune responses: Trace elements: Vitamins

A number of micronutrients have been shown to affect various aspects of immunity in cattle. The interactions between nutritional status, immunology and disease resistance are extremely complex. Despite the apparent involvement of certain micronutrients in the immune system, deficiencies of micronutrients have not always increased the susceptibility of animals to natural or experimentally induced infections. Suttle & Jones (1986, 1989) cautioned against assuming that changes, especially in *in vitro* measures of immune function, are related to decreased disease resistance in the whole animal. They suggested that when micronutrient supplies are limited disease resistance may have priority for nutrients over processes less critical for survival. The purpose of the present paper is to review the effects of trace minerals and vitamins on immune responses and disease resistance in cattle.

### Chromium

Considerable research has indicated that Cr can affect immune response and disease resistance in cattle. However,

responses to supplemental Cr have been highly variable. Factors that may contribute to the inconsistent findings include differences between studies in: (1) the initial Cr status of the animals; (2) the amount of available Cr in the control diet; (3) the form of Cr supplemented; (4) the type or degree of stress imposed on the animals. Cr is present in the body at very low concentrations, and unfortunately no reliable measure of Cr status is currently available. Concentrations of Cr in control diets have not been reported in a number of studies and, furthermore, little is known regarding the bioavailability of Cr from various feeds.

### Cellular immunity

A number of studies have investigated the effect of dietary Cr on the ability of isolated peripheral lymphocytes to proliferate in response to mitogen stimulation. Lymphocytes from dairy cows supplemented with 0.5 mg Cr as a Cr–amino acid chelate/kg diet from 6 weeks prepartum to 16 weeks post-partum had increased blastogenic responses to concanavalin A (Con A) stimulation (Burton *et al.* 1993).

**Abbreviations:** Con A, concanavalin A; IBRV, infectious bovine rhinotracheitis virus; IL, interleukin; PHA, phytohaemagglutinin; PWM, pokeweed (*Phytolacca americana*) mitogen; TNF, tumour necrosis factor.

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Addition of Cr to the diet prevented the decrease in blastogenic response that was observed in control cows 2 weeks prepartum. Addition of blood serum from Cr-supplemented cows to cultures of lymphocytes isolated from control cows also increased Con A-induced lymphocyte blastogenesis (Burton *et al.* 1995). The enhanced blastogenesis observed did not appear to be related to concentrations of insulin or other hormones present in serum from Cr-supplemented cows. Direct addition of Cr as either a Cr–amino acid chelate or CrCl<sub>3</sub> to lymphocytes obtained from non-Cr-supplemented cattle also increased blastogenic response to Con A stimulation (Chang *et al.* 1994, 1996).

In stressed calves Cr supplementation (0.14 mg/kg; as an amino acid chelate) increased Con A-induced lymphocyte blastogenesis in calves showing signs of morbidity, but not in calves with normal body temperatures and no visual signs of sickness (Chang *et al.* 1994). In contrast, Cr supplementation (3 mg/d; as a high-Cr yeast) did not affect Con A, phytohaemagglutinin (PHA), or pokeweed (*Phytolacca americana*) mitogen (PWM)-induced blastogenesis of lymphocytes obtained from calves following inoculation with bovine herpesvirus-1 (Arthington *et al.* 1997). Other studies in cattle have reported no effect of Cr supplementation (0.4 mg/kg; as either a Cr–nicotinic acid complex, high-Cr yeast or CrCl<sub>3</sub>) on PHA or PWM-stimulated lymphocyte blastogenesis (Kegley & Spears, 1995; Kegley *et al.* 1996).

Effects of Cr on cell-mediated immunity have also been evaluated *in vivo* by measuring induration responses following percutaneous application of dinitrochlorobenzene or intradermal injection of PHA. In stressed calves that had been sensitized to dinitrochlorobenzene, Cr supplementation did not affect response to dinitrochlorobenzene application (Moonsie-Shageer & Mowat, 1993; Kegley *et al.* 1997). Addition of 0.4 mg Cr/kg diet (as either CrCl<sub>3</sub> or a Cr–nicotinic acid complex) increased inflammatory responses to an intradermal injection of PHA in young calves receiving milk (Kegley *et al.* 1996).

Mononuclear cells from dairy cows supplemented (0.5 mg Cr/kg) with a Cr–amino acid complex produced lower concentrations of interleukin (IL) 2, interferon- $\gamma$  and tumour necrosis factor (TNF)- $\alpha$  following stimulation with Con A than cells from control cows (Burton *et al.* 1996). However, plasma TNF- $\alpha$  concentrations were not affected by Cr supplementation (high-Cr yeast) in calves before or following inoculation with bovine herpesvirus-1 (Arthington *et al.* 1997).

There is no evidence to suggest that Cr alters the ability of phagocytic cells to ingest or kill micro-organisms. Neutrophils isolated from dairy cows supplemented with 0.5 mg Cr/kg diet and control cows had a similar ability to phagocytize fluorescent beads (Chang *et al.* 1996). Similarly, the addition of high-Cr yeast to calf diets did not affect the ability of neutrophils to kill *Staphylococcus aureus* (Arthington *et al.* 1997).

#### Humoral immunity

The effects of dietary Cr on humoral immunity have been evaluated by measuring specific antibody production following administration of either a foreign protein or a

vaccine. Dairy cows supplemented with 0.5 mg Cr/kg diet (as an amino acid chelate) had greater primary and secondary antibody responses to ovalbumin than control cows (Burton *et al.* 1993). The primary injection of ovalbumin was given 2 weeks before parturition and the secondary injection was administered 2 weeks post-partum. Cows were also injected with human erythrocytes in this study, and Cr did not affect antibody response to this antigen. In calves that had been stressed due to transportation and feed restriction Cr supplementation (0.2–1.0 mg Cr/kg) from high-Cr yeast increased primary, but not secondary, antibody responses to human erythrocytes (Moonsie-Shageer & Mowat, 1993). In contrast, the addition of 0.4 mg Cr/kg diet (as a Cr–nicotinic acid complex) did not affect antibody responses to porcine erythrocyte immunization in stressed cattle (Kegley *et al.* 1997). Similarly, in young calves receiving milk diets, neither CrCl<sub>3</sub> nor the Cr–nicotinic acid complex enhanced specific antibody responses to porcine erythrocytes (Kegley *et al.* 1996). Supplementing Cr as an amino acid chelate for 6 d before and 28 d following vaccination increased antibody titres to infectious bovine rhinotracheitis virus (IBRV), but not to parainfluenza virus type 3 (Burton *et al.* 1994).

#### Disease resistance

Studies with cattle that had been stressed due to weaning, transportation and feed restriction indicate that Cr supplementation may alleviate effects of stress on animal performance and health. The incidence of respiratory disease is frequently high in stressed calves. Cr supplementation of stressed calves has reduced morbidity following transportation in some studies (Moonsie-Shageer & Mowat, 1993; Mowat *et al.* 1993; Lindell *et al.* 1994), but not in other studies (Chang & Mowat, 1992; Chang *et al.* 1995; Mathison & Engstrom, 1995). Supplementing dairy cows with a Cr–amino acid chelate did not affect mammary gland health status (Chang *et al.* 1996).

The effects of dietary Cr on physiological responses of calves to an experimental disease challenge have also been evaluated. Cr supplementation was provided for 49–75 d before disease challenge in these studies. Calves supplemented with 0.4 mg Cr/kg diet (as either a Cr–nicotinic acid complex or CrCl<sub>3</sub>) tended to have lower body temperatures at certain time points after intranasal inoculation with IBRV followed by *Pasteurella haemolytica* intratracheally 5 d later (Kegley *et al.* 1996). Supplementing calves with 0.4 mg Cr/kg diet (as a Cr–nicotinic acid complex) for 56 d before transportation did not affect body temperature or feed intake responses to an IBRV challenge (Kegley *et al.* 1997). Rectal temperature responses, also, were not affected by Cr (high-Cr yeast) in calves inoculated with bovine herpesvirus-1 (Arthington *et al.* 1997).

Stress results in elevated blood concentrations of cortisol which is known to depress immune functions. Cr supplementation in cattle decreased serum cortisol concentrations in some studies (Chang & Mowat, 1992; Moonsie-Shageer & Mowat, 1993; Kegley *et al.* 1996), but not in others (Lindell *et al.* 1994; Kegley & Spears, 1995; Kegley *et al.* 1997). When serial blood samples were obtained from jugular-cannulated calves at 4 h intervals for

6 d Cr supplementation (high-Cr yeast) did not affect serum cortisol concentrations following inoculation with bovine herpesvirus-1 (Arthington *et al.* 1997).

### Copper

Cu deficiency in ruminants is generally caused by antagonists present in forage that reduce Cu absorption. It is well documented that high dietary concentrations of Mo, S and Fe reduce Cu status in ruminants (Suttle, 1991). Studying the role of Cu in immune responses and disease resistance is made even more complex because of the numerous interactions that occur between Cu and other minerals. In several immune studies Cu deficiency was induced by feeding high concentrations of Mo or Fe. In these studies it is impossible to separate the effects of Cu deficiency *per se* from possible direct effects of high dietary Mo or Fe on immune variables.

#### Phagocytic function

Administration of Cu to Cu-depleted calves increased the ability of isolated peripheral blood granulocytes (primarily neutrophils) to kill ingested *Candida albicans* by over 2-fold (Jones & Suttle, 1981). Granulocyte-killing ability remained low in Cu-depleted calves that were not given Cu. This finding indicates that impaired phagocytic killing activity due to Cu deficiency can be reversed by Cu supplementation. Neutrophils isolated from calves fed on diets severely deficient in Cu also had impaired ability to kill ingested *C. albicans* compared with calves receiving adequate Cu (Boyne & Arthur, 1981). Cu deficiency did not alter the ability of phagocytic cells to phagocytize yeast (Boyne & Arthur, 1981; Jones & Suttle, 1981).

Neutrophils obtained from calves born to cows fed on a control diet marginally deficient in Cu or the control diet supplemented with 2.5 mg Mo/kg diet tended to have depressed ability to kill *S. aureus* (Gengelbach *et al.* 1997). Marginal Cu deficiency in dairy heifers also reduced neutrophil killing of *S. aureus* (Torre *et al.* 1996).

Cu deficiency induced by feeding cattle either 5 mg Mo or 500 mg Fe/kg diet depressed the ability of neutrophils to ingest and kill *C. albicans* (Boyne & Arthur, 1986). Compared with Cu-supplemented animals, neutrophils isolated from steers supplemented with 10 mg Mo/kg diet for 8 months had reduced ability to kill *S. aureus* (Xin *et al.* 1991). However, marginal Cu deficiency produced by feeding heifers a low Cu:Mo value of 1:1.5 for 60 d did not affect neutrophil bactericidal capacity relative to Cu-adequate controls (Arthington *et al.* 1995).

In rats Cu deficiency also impairs the ability of macrophages to kill yeast cells (Babu & Failla, 1990). The effect of Cu deficiency on macrophage function in cattle has not been studied extensively. Cu deficiency in calves did not affect the ability of macrophages to phagocytize porcine erythrocytes (Gengelbach *et al.* 1997).

#### Specific immune function

A number of studies in rats and mice have indicated that both cell-mediated and humoral immunity are greatly

depressed by Cu deficiency (Prohaska & Failla, 1993). However, studies in cattle have failed to show consistent effects of Cu deficiency on either cell-mediated or humoral immune response.

Cu deficiency in calves produced by feeding a semi-purified diet containing approximately 1 mg Cu/kg diet did not affect *in vitro* mitogen-induced lymphocyte blastogenesis (Stabel *et al.* 1993; Ward *et al.* 1997). In both studies calves were severely deficient in Cu, based on plasma (<0.3 mg/l) and liver Cu (<10 mg/kg DM) concentrations, when blastogenic assays were performed. Furthermore, the addition of 5 mg Mo/kg to the semi-purified diet to produce a more severe Cu deficiency did not reduce the lymphocyte blastogenic response to PHA or PWM (Ward *et al.* 1997). Other studies have also found no effect of marginal Cu deficiency or Mo-induced Cu deficiency on the response of lymphocytes to mitogen stimulation (Ward *et al.* 1993; Arthington *et al.* 1995, 1996; Torre *et al.* 1995). However, in a recent study low Cu status was associated with a reduced response of peripheral-blood lymphocytes to stimulation with T-cell mitogens (PHA and Con A) following weaning and an IBRV challenge (Wright *et al.* 2000). In this study calves were born to dams that received no supplemental Cu or 25 g CuO particles 60–90 d before calving. Calves born to dams that received Cu also received 12.5 g CuO particles 90 d before weaning.

*In vivo* cell-mediated immunity, assessed by measuring the response to an intradermal injection of PHA, was higher in calves supplemented with 10 mg Cu/kg compared with calves fed on a low-Cu diet (1.1 mg Cu/kg) for 126 d (Ward *et al.* 1997). However, the addition of 5 mg Mo/kg to the low-Cu diet also increased the swelling response to PHA (Ward *et al.* 1997). In contrast, cellular immune response to PHA was reduced in growing cattle supplemented with 5–10 mg Mo/kg diet for 77 d (Ward *et al.* 1993). The depressing effect of Mo on cellular immune response in this study was observed regardless of whether dietary Cu was present at 6.2 or 11.2 mg/kg diet. Neither dietary Cu or Mo affected the delayed-type hypersensitivity response to dinitrochlorobenzene in steers (Ward & Spears, 1999).

Limited research suggests that dietary Cu may affect cytokine production in cattle. Mononuclear cells from lactating dairy cows receiving a marginal level of Cu (6–7 mg/kg diet) produced less IFN- $\gamma$  when stimulated with Con A than cells isolated from cows fed on adequate levels of Cu (Torre *et al.* 1995). IL-2 production by mononuclear cells was not affected by Cu in this study. Production of TNF- $\alpha$  and IL-1 by isolated peripheral-blood monocytes following stimulation with *Escherichia coli* lipopolysaccharide was not significantly affected by dietary Cu or Mo (Gengelbach & Spears, 1998). However, TNF- $\alpha$  and IL-1 production tended to be lower from monocytes obtained from calves fed on a low-Cu diet supplemented with 5 mg Mo/kg compared with calves receiving adequate Cu. Cu-adequate calves had higher plasma TNF- $\alpha$  concentrations than Cu-deficient calves (Gengelbach *et al.* 1997).

Humoral immune response, also, has not been consistently affected by dietary Cu or Cu antagonists. Antibody production following primary injection of porcine erythrocytes tended to be lower in calves fed on a Cu-deficient diet

(1.1 mg Cu/kg) compared with those supplemented with 10 mg Cu/kg diet (Gengelbach & Spears, 1998). Calves fed on the Cu-deficient diet supplemented with 5 mg Mo/kg had a lower primary immune response than Cu-adequate calves. The secondary immune response to porcine erythrocytes also tended to be lower in calves fed on the Cu-deficient diet supplemented with Mo (Gengelbach & Spears, 1998). The addition of 5 mg Cu/kg to a diet marginally deficient in Cu (5 mg/kg) increased antibody responses to ovalbumin administration in growing steers (Ward & Spears, 1999). The addition of 5 mg Mo/kg to the marginal- or adequate-Cu diet did not affect antibody responses to ovalbumin injection on day 133 of the study. The humoral response to porcine erythrocytes was measured later in this study, and was affected by a Cu  $\times$  stress interaction. Half the steers in each treatment group were stressed on day 168 by transportation and feed deprivation for 12 h. Steers were inoculated with porcine erythrocytes at the end of the 12 h stress period. Cu supplementation increased antibody production in stressed steers, but decreased response to erythrocytes in steers that were not stressed (Ward & Spears, 1999).

In other studies, Cu deficiency induced by high dietary Fe or Mo did not consistently affect primary or secondary antibody production in cattle injected with porcine erythrocytes (Gengelbach, 1994; Ward *et al.* 1997). Cu deficiency also did not alter antigen-specific antibody responses in calves inoculated with IBRV and *P. haemolytica* (Stabel *et al.* 1993).

#### *Disease resistance*

Naturally occurring Cu deficiency in lambs grazing improved pastures resulted in increased susceptibility to bacterial infections and greater mortality (Woolliams *et al.* 1986). Cu deficiency observed in this study was at least partly due to Mo, as pastures contained 1.2–3.1 mg Mo/kg.

Disease resistance has been studied experimentally in Cu-deficient cattle by inoculating animals with pathogenic organisms. Clinical signs of respiratory disease following inoculation with IBRV and *P. haemolytica* were similar in Cu-deficient and Cu-adequate calves (Stabel *et al.* 1993). Before the disease challenge calves in the deficient group had been fed on a diet containing 1.5 mg Cu/kg for 150 d and their plasma Cu concentrations had declined to approximately 0.2 mg/l. The rectal temperature response to intranasal inoculation with bovine herpesvirus-1 was similar in heifers fed on a diet supplemented with adequate Cu and those fed on the basal diet supplemented with Mo to achieve a dietary Mo:Cu of 2.5:1 (Arthington *et al.* 1996). However, following the viral challenge plasma fibrinogen (an acute-phase protein) increased in heifers fed on the high-Mo diet, but not in those fed on the Cu-adequate diet.

Gengelbach *et al.* (1997) evaluated the effects of dietary Cu, Fe and Mo on the response of newly weaned calves to an IBRV challenge followed by a *P. haemolytica* challenge. Calves in this study were born to heifers fed on a control diet containing 4.5 mg Cu/kg or the control diet supplemented with either 5 mg Mo, 600 mg Fe or 10 mg Cu/kg. Calves in the control, Fe and Mo treatments were severely deficient in Cu before weaning, based on plasma Cu concentrations (<0.20 mg/l) and caeruloplasmin activities. Cu-adequate

calves and those fed on high levels of Fe had higher rectal temperatures than control and Mo-supplemented calves following the disease challenge. Consistent with the higher body temperatures, feed intake after the disease challenge was lower in the Fe- and Cu-supplemented calves. Plasma TNF- $\alpha$  concentrations were higher in Cu-adequate calves, and may explain the higher body temperature and lower feed intakes in the Cu-supplemented animals. It is unclear why body temperature and feed intake of Fe-supplemented calves responded differently from that of control and Mo-supplemented calves.

#### **Selenium and vitamin E**

Se and vitamin E will be discussed together because both nutrients are involved in the cellular antioxidant defence system. Vitamin E functions as an antioxidant that scavenges free radicals and protects against lipid peroxidation. The role of vitamin E as an antioxidant may explain its importance in immune responses (Bendich, 1993). Se functions as an essential component of the enzyme GSH peroxidase, which destroys H<sub>2</sub>O<sub>2</sub> and lipid hydroperoxides (Rotruck *et al.* 1973). Although Se and vitamin E function independently, studies have shown that administration of both Se and vitamin E may result in synergistic enhancement of the immune response (Stabel & Spears, 1993). The effects of Se and vitamin E on immune responsiveness and disease resistance have been reviewed (Stabel & Spears, 1993; Finch & Turner, 1996). Thus, this section will only briefly highlight previous findings and focus primarily on papers published since these reviews were written.

#### *Phagocytic function*

Research has clearly indicated that Se deficiency in cattle reduces the ability of blood and milk neutrophils to kill yeast and bacteria (Boyne & Arthur, 1979; Grasso *et al.* 1990; Hogan *et al.* 1990). Reduced neutrophil killing activity in Se-deficient steers was associated with non-detectable GSH peroxidase activity in neutrophils (Boyne & Arthur, 1979). Se deficiency does not affect the ability of isolated neutrophils to ingest either yeast or bacteria. In goats both random and chemotactic migration of neutrophils *in vitro* were reduced by Se deficiency (Aziz *et al.* 1984). Recently, bovine mammary endothelial cells grown in Se-deficient cell culture media were found to exhibit enhanced neutrophil adherence when stimulated with TNF- $\alpha$  and IL-2 (Maddox *et al.* 1999). This factor could affect neutrophil migration into tissues and subsequent inflammation.

Vitamin E supplementation of lactating dairy cows (Hogan *et al.* 1990) and young calves fed on a milk substitute (Eicher *et al.* 1994) also increased blood neutrophil bactericidal activity. Compared with non-vitamin E-supplemented cows, supplementing dairy cows with 3000 mg vitamin E/d for 4 weeks prepartum and 8 weeks post-partum prevented a decline after parturition in neutrophil superoxide anion production and IL-1 production, and major histocompatibility class II antigen expression by blood monocytes (Politis *et al.* 1995).



### Specific immune function

Vitamin E supplementation increased the blood lymphocyte blastogenic response to PHA in young calves (Reddy *et al.* 1986). High supplemental concentrations (1000 or 2000 mg/d) of vitamin E also increased lymphocyte response to Con A stimulation in steers fed on a high-vitamin E diet (Garber *et al.* 1996).

Low dietary Se does not consistently affect cell-mediated immune response in ruminants (Stabel & Spears, 1993). Cao *et al.* (1992) reported that Se deficiency in dairy cows resulted in a reduced response of isolated peripheral-blood lymphocytes to mitogen stimulation with Con A. They suggested that the impaired response of lymphocytes from Se-deficient cows may be related to altered arachidonic acid oxidation by lymphocytes via the 5-lipoxygenase pathway. Lymphocytes from Se-deficient cows produced less products of arachidonic acid oxidation, specifically 5-hydroxyeicosatetraenoic and leukotriene B<sub>4</sub> when stimulated.

Humoral immune response has been enhanced by increasing dietary Se and/or vitamin E in several studies (Stabel & Spears, 1993; Finch & Turner, 1996). Antibody responses have been most consistent when both Se and vitamin E have been supplemented.

### Disease resistance

The vitamin E and Se status of dairy cows has been shown to affect their susceptibility to intramammary infections. Supplementing a diet low in Se and vitamin E with 740 mg vitamin E/d throughout the dry period reduced the incidence of clinical mastitis at calving by 37 % (Smith *et al.* 1984). In this study injecting 1 mg Se/kg body weight at 21 d before calving did not affect the incidence of clinical mastitis, but the duration of clinical symptoms was reduced by 46 %. Cows supplemented with both vitamin E and Se had a shorter duration of clinical signs of mastitis than cows supplemented with either nutrient alone. Vitamin E and Se status was also related to the rate of clinical mastitis and the bulk-tank milk somatic cell count in a survey study of dairy herds (Weiss *et al.* 1990). High serum Se concentrations were associated with reduced rates of mastitis and lower bulk-tank milk somatic cell concentrations. High blood concentrations of vitamin E were associated with a decreased rate of clinical mastitis. Weiss *et al.* (1997) recently reported that cows supplemented with high levels of vitamin E had a considerably lower incidence of clinical mastitis during the first 7 d of lactation. Cows in the high-vitamin E treatment group received 1000 mg/d during the first 46 d of the dry period, 4000 mg/d during the last 14 d of the dry period and 2000 mg/d during lactation. Cows in the low-vitamin E treatment were given 100 mg/d during the dry period and lactation, while those in the intermediate vitamin E treatment received 1000 mg/d during the dry period and 500 mg/d during lactation. All the experimental diets were marginal in Se (0.1 mg/kg diet).

Experimental mastitis, induced by intramammary challenge with *E. coli*, was more severe and of longer duration in cows receiving 0.04 mg Se/kg compared with those receiving 0.14 mg Se/kg diet (Erskine *et al.* 1989).

However, the severity and duration of infection was not affected by Se deficiency when mastitis was induced by intramammary challenge with *S. aureus* (Erskine *et al.* 1990).

A study with beef cows and calves fed on feeds marginally deficient in Se indicated that bimonthly Se-vitamin E injections reduced calf death losses (4.2 % *v.* 15.3 %) from birth to weaning (Spears *et al.* 1986). Most of the deaths in the study were attributed to diarrhoea and subsequent lack of thrift. Incidence of diarrhoea was lower in calves born to cows receiving 1000 mg vitamin E/d during the third trimester of pregnancy than in calves from cows receiving 80 mg vitamin E/d (Zobell *et al.* 1995).

Clinical signs and duration of infection were not affected by Se deficiency in calves inoculated with IBRV (Reffett *et al.* 1988). Se deficiency, also, did not affect susceptibility of stressed steers to *P. haemolytica* challenge (Stabel *et al.* 1989). Injecting stressed steers with Se and/or vitamin E did not affect health status during the receiving period (Droke & Loerch, 1989).

### Zinc

An array of research in human subjects and laboratory animals has indicated that Zn deficiency reduces immune responses and disease resistance (Chesters, 1997). In cattle surprisingly little research has been carried out to examine the relationship between dietary Zn and immune function. However, controlled studies in cattle (JW Spears and EB Kegley, unpublished results) and lambs (Droke & Spears, 1993; Droke *et al.* 1993) suggest that marginal Zn deficiency does not impair cell-mediated or humoral immune responses. This finding is in contrast with research in rats and human subjects in which even marginal Zn deficiency reduces immune responses (Fraker *et al.* 1984).

A genetic disorder (lethal trait A46) of Zn metabolism has been reported in Holstein and Shorthorn calves that results in severe Zn deficiency due to impaired Zn absorption. Calves with lethal trait A46 exhibit thymus atrophy and reduced lymphocyte response to mitogen stimulation (Perryman *et al.* 1989). The addition of 25 mg Zn/kg to a control diet that contained 33 mg Zn/kg increased body-weight gain by 10 % in growing steers (JW Spears and EB Kegley, unpublished results). However, Zn supplementation did not increase *in vitro* lymphocyte responses to PHA or PWM stimulation, *in vivo* cellular response to intradermal PHA administration or antibody response following vaccination for IBRV. In young calves the addition of 150 or 300 mg Zn/kg to a control diet containing 65 mg Zn/kg did not affect mitogen-induced blastogenesis, cytotoxicity and IL-2 production by lymphocytes, or phagocytic and bactericidal activity of isolated neutrophils (Kincaid *et al.* 1997). Calves receiving 17 mg Zn/kg diet had a reduced induration response to intradermal injection of PHA compared with Zn-supplemented animals (Engle *et al.* 1997).

Lambs fed on a semi-purified diet severely deficient in Zn (3.7 mg Zn/kg) showed a reduced blastogenic response to PHA (a T-cell mitogen), but an increased response to PWM (a T-dependent  $\beta$ -cell mitogen; Droke & Spears, 1993). Zn-deficient lambs also had a lower percentage of

lymphocytes and a higher percentage of neutrophils in their peripheral blood. The *in vivo* inflammatory response to intradermal PHA administration was similar in Zn-deficient and Zn-adequate lambs. Immune responses in lambs fed on diets marginally deficient in Zn (8.7 mg/kg) did not differ from those observed in lambs fed on adequate Zn (44 mg/kg; Droke & Spears, 1993). Following administration of dexamethasone to depress the immune response, susceptibility to intratracheal inoculation with *P. haemolytica* and measures of cell-mediated and humoral immune response did not differ among lambs fed on diets deficient (5.5 mg/kg), marginal (10.5 mg/kg) or adequate (45 mg/kg) in Zn (Droke *et al.* 1993). Marginal Zn deficiency in pregnant ewes did not affect cellular immunity in their lambs (White *et al.* 1994). Also, the addition of 25 mg Zn/kg to a control diet containing 28 mg Zn/kg did not affect cellular or humoral immune responses in lambs following adrenocorticotrophin administration (Droke *et al.* 1998).

Despite the lack of effects of marginal Zn deficiency on immune function in ruminants, the limited research carried out suggests that the addition of Zn to practical diets may affect disease resistance. Increasing the level of supplemental Zn from 30 to 100 mg/kg diet slightly reduced morbidity from respiratory diseases in newly weaned calves that had been transported (Galyean *et al.* 1995). The addition of Zn as Zn-methionine to a control diet containing approximately 30 mg Zn/kg reduced rectal temperature and increased feed intake in steers after inoculation with IBRV (Chirase *et al.* 1991).

### Cobalt

Limited research indicates that Co deficiency affects neutrophil function and resistance to parasitic infection. Neutrophils isolated from calves deficient in Co had reduced ability to kill *C. albicans* (MacPherson *et al.* 1987; Paterson & MacPherson, 1990). Co-deficient calves had a decreased prepatent period and increased faecal egg output following experimental infection with *Ostertagia ostertagi* (MacPherson *et al.* 1987). Higher faecal egg counts were also observed in Co-deficient lambs after natural infection with gastrointestinal nematodes (Vellema *et al.* 1996).

### Vitamin A and $\beta$ -carotene

$\beta$ -Carotene is the major precursor of vitamin A that occurs naturally in feedstuffs. Research suggests that  $\beta$ -carotene may affect immune function, independent of its role as a source of vitamin A.  $\beta$ -Carotene, as such, can serve as an antioxidant, while vitamin A is not an important antioxidant.

It is well documented that vitamin A-deficient animals are more susceptible to bacterial, viral and parasitic infections (Chew, 1987). Cows supplemented with  $\beta$ -carotene around dry-off had lower rates of new mammary gland infections during early dry-off (Chew, 1993). However, in dairy cows supplemented with 17.2 mg retinyl acetate/d neither increasing supplemental vitamin A nor the addition of 300 mg  $\beta$ -carotene/d affected the incidence of clinical mastitis or new intramammary infections (Oldham *et al.* 1991).

Michal *et al.* (1994) evaluated the effects of  $\beta$ -carotene and vitamin A on immune function and the incidence of retained placenta and metritis in dairy cows. Control cows in this study received no supplemental vitamin A. Cows supplemented with 300 or 600 mg  $\beta$ -carotene for 4 weeks before calving had a lower incidence of retained placenta and metritis than control cows. The incidence of retained placenta was similar in cows receiving  $\beta$ -carotene and those receiving 66 mg retinyl palmitate/d; however, the incidence of metritis was lower in cows receiving  $\beta$ -carotene.  $\beta$ -Carotene also enhanced lymphocyte proliferation induced by Con A, PHA and PWM immediately before calving and after calving (Michal *et al.* 1994). The addition of low concentrations of  $\beta$ -carotene to bovine lymphocyte cultures also stimulated mitogen-induced blastogenesis (Daniel *et al.* 1991). Supplementation of  $\beta$ -carotene did not consistently affect neutrophil bactericidal activity (Michal *et al.* 1994).

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