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Nutrition in health and immune function of ruminants

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ABSTRACT

Nutrition, an important modulator of immune function, can often influence the balance between health and disease. Diets influence immunity through meeting substrate needs of immune system cells, deprivation of nutrients from pathogen, direct regulatory effects on cells of immune system, changing the balance of hormones that regulate immunity, reduction of collateral damage induced by an immune response, and physical and chemical immunomodulation action of non-nutrient components of feeds. Poor immune-competency can result in greater incidence and duration of infections causing decrease in food intake, nutrient losses, and impaired animal health and well-being. Substrates (energy, amino acids and all nutrients) are necessary for the anabolic activity of immune system's cells (leukocytes), such as proliferation and antibody production as well as the secretion by the liver of large quantities of immunologically active molecules, the acute phase proteins. The small size of the immune system, its capacity to appropriate nutrients from other tissues, and endowment with high priority nutrient transporters generally indicate that the immune system can usually obtain many of the nutrients that it needs to do its functions over a wide range of dietary levels. However, requirements of some nutrients may not be met by nutrient appropriation mechanism under low dietary intake because of their low concentration in muscle and their relatively high need within the immune system. Role of ß-carotene, vitamin E, copper, zinc and selenium in aiding immunity are mediated not only through maintenance of appropriate amounts of antioxidants, but also by the involvement of enzymes in aiding the production of energy, proteins, cells and hormones needed in defense mechanisms. This review discusses the complex relationship between nutritional status and immune function with special focus on mechanism of action of individual key nutrient.

Key words: Disease, Health, Immunity, Nutrition, Ruminants

Immunity refers to reactions by an animal's body to foreign substances such as microbes and various macromolecules, independent of a physiological or pathological result of the reaction (Abbas *et al*. 1991). In response to invasion of pathogens, the immune system first executes innate and then acquire host defense systems of high diversity. Nutritional status of host critically determines outcome of the war against invading pathogens. Nutrition also plays crucial role in preventing collateral damage to host tissue during an immune response.

Generally, immunity and disease resistance are correlated, which is true for most infectious diseases, but sometimes membrane integrity and types of receptors on epithelial cells, are the major deciding factors. Optimizing the immune system is important because responses with the wrong leukocyte populations or under-responsiveness can increase the incidence of infectious diseases, whereas over-exuberant responses result in a variety of problems in an animal. Poor immunocompetence can result in greater incidence and duration of infections, which cause decreased food intake, nutrient losses, and impaired animal health and well-being. Interaction of about 45 nutrients with different diseases is a complex subject but will be presented on the basis of general mechanism as well as on the role of individual important nutrient.

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Mechanisms by which diet influences immunity

Diets influence immunity through several mechanisms. These include meeting the substrate needs of immune system cells, deprivation of nutrients from pathogen, through direct regulatory effects on cells of immune system, through changing the balance of hormones that regulate immunity, through reduction of collateral damage induced by an immune response, and physical and chemical immunomodulatory action of non-nutrient components of feeds.

Meeting substrate needs of immune system cells: Although immune system is relatively small (weight of all leukocytes and their products involved in immune function is probably lesser than 2 % of body weight) yet it is a vital system and its requirements must be met. Substrates (energy, amino acids and all nutrients) are necessary for the anabolic activity of immune system's cells (leukocytes), such as proliferation and antibody production as well as the

secretion by the liver of large quantities of immunologically active molecules, the acute phase proteins. In young animals, a severe deficiency of any nutrient impairs immunocompetence (Cook 1991). When leukocytes become activated, they express high levels of nutrient transporters, which allow them to easily obtain necessary nutrients even when they are at low concentrations. The immune system can also mobilize nutrients from muscle and other tissues. When leukocytes become stimulated by pathogens, they release a series of pro-inflammatory cytokines like interleukin 1, tumor necrosis factor and interleukin 6 that go throughout the body and redistribute nutrients, especially those from skeletal muscle. The small size of immune system, its capacity to appropriate nutrients from other tissues, and its endowment with high priority nutrient transporters indicate that immune system can obtain many of the nutrients that it needs to do its functions over a wide range of dietary levels. However, requirements of trace nutrients such as iron, copper, and zinc may not be met by nutrient appropriation mechanism under condition of low dietary intake because of their low concentration in muscle and their relatively high need within the immune system. Evidence is accumulating that the dietary requirement for some trace nutrients may be higher for optimal immune function than it is for maximal growth or reproductive performance (Spears *et al*. 1991).

Deprivation of nutrients from pathogens: Immune cells sequester trace minerals, such as manganese and iron, when they engulf pathogens, and this action serves to starve pathogens and prevent their replication (NRC 2001). It is well documented that injecting or orally feeding of iron to baby pigs provides additional amounts of this limiting nutrient that enhance the growth of pathogens resulting in increased severity and duration of diarrhoea. In birds, a similar situation exists within the egg to deprive bacteria of nutrients (biotin) so that they are unable to colonize the albumen and infect the developing embryo. In situations where high levels of specific dietary nutrients compromise immune function, dietary restriction of that nutrient may be beneficial.

Direct regulatory effects on cells of the immune system: Nutrients in the diet can directly affect the regulatory functions (communicating apparatus) of leukocytes altering the type, duration, and vigor of the immune response. For example, type of dietary fat can change the proportion of prostaglandins and other eicosanoids that are released by leukocytes to coordinate their responses to disease challenges because the type of dietary fat changes the composition of the membrane fatty acids which are the precursors for the synthesis of eicosanoids. Thus, changing the fatty acid composition of immune cells through diet affect phagocytosis, T cell signaling and antigen presentation capability (Calder 2008). Fish oil is high in eicosapentaenoic acid, which causes macrophages to be predisposed to release interleukins that drive T helper cells toward a Th2 type of response and less predisposed to a Th1 type of response, especially the inflammatory response (Fritsche *et al.* 1999, Korver and Klasing 1997). These divergent responses are important in defense against different pathogens. It is important to note that nutrients, which affect communication within the immune system "modulate" or "change" the response, accentuating some components of the response while decreasing others; the dietary manipulations do not "boost" the entire immune system. Thus, host resistance to specific pathogens shifts - with better resistance to some pathogens, but greater susceptibility to others. In dietary fish oil, prevalence of those diseases in which protection is mediated by a Th2 response is diminished, whereas the incidence of those where protection is afforded by the inflammatory response is increased. Similarly, vitamins A, D, E, xanthophylls, as well as some amino acids and bioactive minerals also exert regulatory actions on communication within the immune system.

Changing balance of hormones that regulate immunity: Feeding regimes markedly affect insulin, glucagon, glucocorticoid and IGF levels, which can change type and duration of immune response. For example, chronic severe protein calorie malnutrition and zinc deficiency elevated levels of glucocorticoids, which impinge on T-cell function and decrease immune-competence (Prasad 2008). Other dietary factors that impact immunity through their effects on hormone levels include protein to calorie ratios and presenting food *ad lib.* versus a few large daily meals.

Reduction of collateral damage induced by an immune response: The immune system releases a variety of harmful substances at the site of infection to kill invading pathogens, which sometimes cause collateral damage to healthy cells in the area surrounding the site of infection. Nutritional factors that minimize the extent of collateral damage induced by immune responses mitigate the nutritional costs for repair and convalescence. For example, reactive oxygen intermediates released at the site of infection can cause damage to the cell membranes of healthy host cells and adequate levels of dietary antioxidants minimize this collateral damage (Chew 1995).

Physical and chemical actions of non-nutrient components of feeds in intestines: Certain non-nutrient components of feeds e.g. sugars, lectins, and mitogens, lignin and silica have specific effects on immuno-competence like influence on the function of leukocytes, integrity of intestinal epithelia or population of commensal microflora found in the intestines. Feeding of *Moringa* leaves @ 0.25% of diet to broiler chicks acted as functional feed to decrease mortality, total cholesterol and increased body weight gain and feed efficiency. Phyto-constituents present in *Moringa* leaves could have improved the performance (Dey and De 2013). Numerous dietary polysaccharides particularly glucans elicit diverse immunomodulatory effects in numerous animal tissues (Ramberg *et al.* 2010).

Role of different nutrients in immunity and health status of livestock

Role of important nutrients on immune response is

presented in Table 1. The cell mediated immune response is most consistently and most severely depressed in malnutrition. Total lymphocyte counts are depressed and most particularly, the proportion and absolute number of T cells are decreased. Consistently in acute or chronic malnutrition, the complement system, phagocytosis, and opsonic function are also reduced. Contrary to the effects of protein-calorie deprivation, effects of specific essential amino acid deficiencies appeared to affect primarily humoral responses, the exception being methionine, which markedly influences cellular immunity (Sheffy and Williams 1982). With the exception of vitamins E and B_6, vitamin deficiencies more consistently influence humoral immunity than cell mediated immunity. Detail role of different nutrients in immune function has been presented in following section.

Dietary energy concentration: Very little organized research has been conducted concerning the effects of energy intake on immune responsiveness. Fiske and Adams (1985) fed 3 groups of 13-month old steers at 3 levels of energy intake and reported that underfed steers had lowered plasma protein and circulating antibody values and reduced antibody response to the injection of fowl RBC, and thymus atrophy whereas the overfed steers had depressed lymphocyte response to pokeweed mitogen. However, antibody response to *Brucella abortus* strain 19 was similar in all the 3 groups. Jacobi *et al.* (1997) while reviewing the links between somatic cell counts (SCC) of milk and nutritional imbalances observed that insufficient energy content and/or excess protein led to increased SCC. It was suggested that ammonia and ketone bodies in milk inhibited T lymphocyte activity and protein synthesis affected by energy deficits reduced humoral immunity. In a study involving pregnant heifers fed either *ad lib.* (energy surplus) or restricted diet, Wentink *et al*. (1997) observed that hepatic lipidosis caused by energy surplus diet caused significant reduction in humoral and cellular immune responses after vaccination compared to the restricted fed groups.

Effect of protein insufficiency on immune responsiveness: Deficiency of dietary protein during growth and development interferes with the maturation of all organs and tissues. In a series of studies with mice Cooper *et al.*

Table 1. Role of important nutrients in immune function

Nutrient	Immune function/ inference	Animal/ reference
Energy /fat	Calorie malnutrition reduces cell mediated immunity and antibody response	Cattle (Fiske and Adams 1985, Jacobi *et al*. 1997)
	Changing the fatty acid composition of immune cells through diet affects phagocytosis, T cell signaling and antigen presentation capability	Mice (Fritsche *et al.* 1999)
Protein / amino acids	Protein is required for maturity of organs of immune system and protein deficiency/excess reduces immune response	Mice (Cooper *et al.* 1974), Cattle (Galyean *et al.* 1999)
	Specific amino acids are required for optimum immunity function of gut associated lymphoid tissue	Ruminants (Ruth and Field 2010)
Minerals		
Zinc	Zinc is crucial for normal development and function of cell mediating innate immunity, neutrophills and NK cells, phagocytosis and cytokine production	Cattle (Spears *et al.* 1991)
Copper	Cu deficiency affects innate immune response	Cattle (Boyne and Arthur 1986)
Chromium	Reduces serum cortisol and increases serum IgM and total immunoglobulins	Cattle (Chang and Mowat 1992)
Iron	Deficiency impairs the peripheral lymphoid compartment	Pig (Svoboda *et al.* 2004)
Selenium	Seleno-proteins catalyse oxido-reduction reactions and protect the host from oxidative stress; negative correlation between serum Se level and incidence of mastitis and other diseases were reported	Cattle (Sanders1984, Weiss *et al.* 1990)
Vitamins and carotenoids		
Vitamin E	Influences primary immune response neutrophil mediated antibody–dependent cellular cytotoxicity and phagocytosis as well as lymphocyte stimulation; affects virulence of viral infection	Cattle (Hogan *et al.* 1990), Mice (Beck *et al*. 1994)
Vitamin A	Influences cellularity of lymphoid organs; plays role in glycosulation in membrane of lymphocytes; Retinoic acid plays crucial role in migration of T and B cell to gut	Mora *et al*. 2008, Iwata *et al*. (2004)
Vitamin D	Has inhibitory effects on the adaptive immune response and stimulatory effect on monocyte proliferation	Holick (2007)
Vitamin C	Protects bio-membranes against lipid peroxidation damage; alleviates suppressive action of corticoids on neutrophils.	Cattle (Roth and Kaeberle 1985)
B vitamins	Riboflavin deprivation has negative effect on activity of macrophages	Mazur-Bialy *et al.* (2013)
Carotenoids	Prevents oxidative damage to cells of immune system and play important role in cell mediated immunity and cytotoxicity	Rats (Butera and Krakowa 1986)
Interaction of nutrients	Beta carotene is more active as antioxidant at low oxygen tension but alpha tocopherol is more active at ambient oxygen tension. Vitamin C acts synergistically with vitamin E.	Palozza and Krinsky (1992)

(1974) attempted to obtain basic information on effect of protein deficiency in mammals on various aspects of immune responsiveness. They observed that chronic protein insufficiency: (i) did not affect the primary humoral antibody response to *Brucella abortus* (humoral); (ii) depressed the primary humoral antibody response to sheep erythrocytes (humoral); (iii) enhanced graft-versus-host reaction as measured by spleen indices (cellular); (iv) accelerated rejection of skin allografts in nonneonatally thymectomized mice versus both normally fed, non-thymectomized, protein deficient animals (cellular); (v) enhanced the phytohaemagglutinin-induced blastogenic effect on spleen cells (cellular); (vi) enhanced phagocytic activity of peritoneal macrophages (cellular); (vii) enhanced resistance to viral infection while depressing resistance to bacterial infection (cellular). It was suggested that chronic protein insufficiency may lead to increased output of thymic hormones (thymin) which in turn causes an absolute increase in the T cell population and 20 increased immune-competence of each effector T cell. However, reports on effects of low protein diet on immune responsiveness in ruminants are scanty and measurements of humoral or cellular immunity is very much limited. In a study (Woodard *et al.* 1980) pregnant beef heifers were fed on diets deficient in protein, energy or both for about the last 5 months of pregnancy. After being fed on the diets for about 2 months, heifers given low-energy diets had significantly less amounts of total complement (C) hemolytic activity than did those fed on diets adequate in energy. The decrease in C was observed regardless of the amount of dietary protein, although low-protein intake seemed to exaggerate the effect of low-energy intake. Reduced protein in the diets had no effect on C titres when energy intake was adequate. Based on regression analysis of pooled data from feeding trials, Galyean *et al.* (1999) reported that bovine respiratory disease morbidity rates in cattle tended to increase with increasing CP level. Feeding of naturally protected protein through condensed tannins of *Ficus infectoria* and *Ficus bengalensis* leaves to lambs and crossbred cows, respectively, increased antioxidant status and cell mediated immunity (Dey *et al.* 2007, Dey and De 2014) due to increased non-ammonia nitrogen (NAN) flux to small intestine and increased absorption of essential amino acids (Dey *et al.* 2008). These researchers also reported that an increase in total thiol groups and antioxidant enzymes (CAT, SOD and GSH) and decrease in lipid peroxidation might have improved cellular integrity so that CMI response was increased. Both oral and parenteral feeding studies established convincing evidence that not only the total protein intake, but the availability of specific dietary amino acids (in particular glutamine, glutamate, and arginine, and perhaps methionine, cysteine and threonine) are essential to optimizing the immune functions of intestine and the proximal resident immune cells. These amino acids each has unique properties that include, maintaining the integrity, growth and function of the intestine, as well as normalizing inflammatory cytokine secretion and improving T-lymphocyte numbers, specific T cell functions, and the secretion of IgA by lamina propria cells (Ruth and Field 2013).

Interaction of viral and parasitic infection, nutrition and immunity: Infections cause direct impairment of immune function. The viruses of measles, distemper and bovine viral diarrhoea are highly immunosuppressive. Influenza, mumps, and chickenpox cause a transient depression of delayed type hypersensitivity. Lymphopenic viruses like parvoviridae and retroviridae are also recognized immune response suppressors.

Similarly parasitic infections were also found to be immune-suppressive. The damaging effects are systemic as well as complex functional aberrations relating to intestinal function. The resulting increased probability of malnutrition of parasitized hosts is obvious. Sometimes prophylactic nutritional regimes cause impairment of the immune system. For example, chronic iodism caused by iodine compounds used to prevent certain cattle diseases causes suppression of both the humoral and cell mediated immune response (Hillman and Curtis 1980).

Role of micronutrients in immune respons

The group of antioxidant vitamins (carotenoids, vitamin E and vitamin C) has recently received a great deal of attention because of their action on immunity and disease etiology. It is well established now that antioxidants improve immunity following stress. Free radicals are generated during metabolism, immune response, synthesis of corticosteroid, auto-oxidation of unsaturated organic molecules (e.g. polyunsaturated fatty acid esters), radiation, or from activities of some oxidases, dehydrogenases, and peroxidases. Also, phagocytic granulocytes undergo respiratory burst to produce oxygen radicals to destroy intracellular pathogens (Tizard 1988). The free radicals cause lipid peroxidation, destruction of protein and DNA, which in turn causes cancer, inflammatory diseases, aging, artherosclerosis, etc. Tissue defense mechanisms against free-radical damage generally includes vitamin C, vitamin E, and ß-carotene as the major antioxidant sources. In addition, several metallo-enzymes which include glutathione peroxidase (selenium), catalase (iron), and superoxide dismutase (copper, zinc, and manganese) are also critical in protecting the internal cellular constituents from oxidative damage. Mazur-Bialy *et al.* (2013) showed that riboflavin deprivation has negative impact on viability of macrophages and on their ability to generate an immune response. The details of role of carotenoids, vitamin E, copper, zinc and selenium in immunity are being presented in following section.

Carotenoids: Carotenoids consist of a group of over 600 coloured pigments found in nature. Only 10% of the carotenoids possess provitamin activities in mammals. Nowadays evidences are accumulating to indicate that carotenoids also have an important independent effect on immune responses, separate from its provitamin activity. Carotenoids (which may or may not have pro-vitamin

activity) with 9 or more conjugated double bonds may enhance immune function by quenching singlet oxygen and other reactive oxygen molecules, including free radicals. Vitamin A in contrast cannot quench singlet oxygen and is a relatively poor antioxidant (Burton and Ingold 1984).

Role of carotenoids in nonspecific host defense: Carotenoids play important role in nonspecific immune functions by protecting neutrophils (polymorphonuclear nutrophils, PMN) and macrophages from damage by oxidants produced at the time of phagocytosis. Dairy cows fed 300 mg of b-carotene /d during the dry period had lower incidence of intramammary infection than cows fed preformed vitamin A alone. The PMN generated oxygen radicals can also cause chromosomal damage to co-cultured cells, b-carotene, added to the culture media, reduced the level of sister chromatid exchange seen following exposure to activated PMN (Weitberg *et al*. 1985). Nutrophils possess a myelo-peroxidase:hydrogen peroxide:halide system, which is an important part in their antimicrobial action. But the oxidative products of this system may inhibit cellular host defense, like PMN motility and mitogen–induced lymphocyte transformation. The exact mechanisms by which carotenoids regulate immunity largely are unclear. The most recognized mechanism of carotenoids action is its antioxidant function. b-carotene can scavenge singlet oxygen and quench peroxyl radicals, especially under low oxygen tension.

Role of carotenoids in specific cellular host defense: Both b-carotene and vitamin A influence cellularity and function of the lymphoid organs and prevent and reverse stress-induced involution of thymus gland and spleen (Mora *et al.* 2008). Deficiency of vitamin A decreases localization of lymphocytes in the lymph nodes resulting in defective immune response. It has been suggested that defective lymphocyte trapping is the result of impaired lymphocyte surface membrane glycoproteins (vitamin A plays an important role in glycosulation), resulting in decreased antigen and lymphocyte recognition and contact.

Role of carotenoids and vitamin A in cell mediated immune response: Cell-mediated cytotoxicity– A form of cell-mediated immunity, cytotoxicity is mediated by cytotoxic T cells, a subset of T cells. Vitamin A and ß-carotene may enhance cell-mediated cytotoxicity by influencing the production of lymphokines. Probably vitamin A accomplishes this by enhancing the production of IL-2. Similarly vitamin A may enhance helper T cell and natural killer cell activities, because both of these cell types require IL-2 for proliferation and action. The immune system has 3 cell types capable of killing of tumor cells. Macrophages, natural killer cells and cytotoxic T-cells can recognize and kill tumor cells. Abril *et al.* (1989) reported that ß-carotene *in vitro* stimulated human peripheral blood mononuclear cells to secrete a novel cytokine that possess cytotoxic activity against human cancer cell lines. The cytotoxic activity induced by b-carotene was different from that of IFN, interleukins, lymphotoxin, or tumor necrosis factor. Shklar and Schwartz (1988) demonstrated that both ß-carotene and canthaxanthine, but not retinoic acid, could induce macrophages to produce tumor necrosis factor-a which can kill tumor cells directly. Canthaxanthin and astaxanthin, which are not precursors of vitamin A can also reduce the tumor burden by enhancement of cytotoxic T-cell activity.

Natural killer cells lyse tumor cells and their killing ability is greatly enhanced by IFN. Beta-carotene augments interferon (IFN) activity and thus stimulates natural killer (NK) cell activity. However, vitamin A augments NK cell activity significantly probably by mimicking the biological action of IFN although vitamin A decreases IFN action. Retinoic acid (a metabolite of vitamin A) plays crucial role in migration of T and B cell to gut (Iwata *et al.* 2004, Mora *et al*. 2008).

Humoral immunity – Humoral immunity involves interaction of various cell types and culminates in antibody formation. Antigen-presenting cells or macrophages first process the antigen and present it to helper T cells, which stimulate B cells, and also present it directly to B cells which produce antibodies. Several studies showed increased serum antibody titers in retinal-supplemented or retinyl palmitate-supplemented mice immunized with antigen. The mechanism by which vitamin A influences the local immune system is presently unclear. Vitamin A modulates glycoprotein synthesis. Secretory component of secretory antibodies is a glycoprotein. Further vitamin may also influence modulate transport glycoprotein and alter the glycoprotein surface properties of lymphocytes, and consequently may lead to depressed differentiation and proliferation of lymphocytes or to aberrant migration of lymphocytes.

Role of vitamin E in immune response and health status: Vitamin E acts as antioxidant by quenching free radicals generated during metabolism. α-Tocopherol was proposed to be the most important lipid-soluble radical scavenging chain breaking antioxidant in membranes and plasma. It functions by trapping peroxyl free radicals, especially at ambient oxygen tension (Burton and Ingold 1984). Vitamin E may guard against peroxidation of arachidonic acid (Lawrence *et al.* 1985). Studies showed that supplementation of vitamin E effectively increased antigen-specific antibody responses in various species of animals. Primary immune responses are associated with high concentrations of IgM and low concentration of IgG secretion, but the converse is true during secondary immune responses. Supplementation of chick diets with vitamin E resulted in higher concentration of serum interferon (a and b). In a time course experiment Stabel *et al.* (1992) were able to demonstrate that α-tocopherol added to PBMC (peripheral blood mononuclear cell) during the first 48 h of culture enhanced IgM production, suggesting that its presence is required during the initial stages of cellular activation. Vitamin E administration to calves enhanced immune response and weight gain, while enzymes of muscle origin (creatinine kinase and SGOT) and plasma cortisol concentration were decreased (Reddy *et al*. 1987b). Hogan

et al. (1990) reported that intake of 500 IU/d of supplemental vitamin E during the first 30 days of lactation increased the ability of blood neutrophols to kill bacterial pathogens compared with that of neutrophils collected from cows fed no supplemental vitamin E. Steers supplemented with α-tocopherol had higher antibody response to *Pasteurella haemolytica* vaccination (Droke and Loerch 1989). Smith *et al*. (1984) reported that intra-mammary infection was reduced 42.2% in vitamin E-selenium supplemented versus unsupplemented controls. The duration of all intra-mammary infections in lactation was reduced to 50 % in supplemented heifers. A known consequence of vitamin E and selenium deficiency is impaired PMN activity which acts as primary defense apparatus in mammary gland against bacterial infection. Vitamin E plays a role in resistance to viral infection. Vitamin E deficiency allows a normally benign virus to cause disease (Beck *et al.* 1994). A selenium or vitamin E deficiency leads to a change in viral phenotype, such that an avirulent strain of a virus becomes virulent and a virulent strain becomes more virulent (Beck 1997). Beneficial effects of supplementation of vitamin E in stressed cattle on morbidity and performance were noted in many experiments (Tengerdy *et al*. 1983, Hicks 1985, Lee *et al*. 1985, Reddy *et al.* 1987a, 1987b).

Vitamin C in immunity and health: Vitamin C or L-ascorbic acid is the most important antioxidant in extracellular fluids (Stocker and Frei 1991) and can protect bio-membranes against lipid peroxidation damage by eliminating peroxyl radicals in the acquous phase before the later can initiate peroxidation (Frei *et al*. 1989). One of the protective effects of vitamin C may partly be mediated through its ability to reduce circulating glucocorticoids. The suppressive action of glucocorticoids on neutrophil function in cattle was alleviated with vitamin C supplementation (Roth and Kaeberle 1985). In addition, ascorbate can generate the reduced form of α- tocopherol, perhaps accounting for observed sparing effect of these vitamins (Jacob 1995). In the process of sparing fatty acid oxidation, tocopherol is oxidized to the tocopheryl free radical. Ascorbic acid can donate an electron to the tocopheryl free radical, regenerating the reduced antioxidant form of tocopherol. The ascorbate radical (semi-dehydro-ascorbate) is again reduced to ascorbate by NADH-dependent semi-dehydro-ascorbate reductase. Ascorbic acid is reported to have a stimulating effect on phagocytic activity of leukocytes on function of the reticulo-endothelial system, and on formation of antibodies. Vitamin C can stimulate the production of interferons, the proteins that protect cells against viral attack (Siegel 1974).

Interaction of antioxidant vitamins: Considerable interactions exist among carotenoids, tocopherol and ascorbic acid in their antioxidant activities. Individually, β-carotene is a more active chain breaking antioxidant nutrient at low oxygen partial pressures, whereas α-tocopherol is more active at ambient oxygen tensions (Burton and Ingold 1984) thus β-carotene and α-tocopherol have complementary roles relative to the varying oxygen tensions in biological membranes. Vitamin C interacts synergistically with α-tocopherol at the membrane-cytosol interface to regenerate membrane-bound oxidized vitamin E. A combination of β-carotene and α-tocopherol results in an inhibition of lipid peroxidation significantly greater than the sum of the individual inhibitions (Palozza and Krinsky 1992). Ubiquinones and ubiquinols (fat soluble antioxidant compounds) also protect biological systems from oxidative damage. The antioxidant activity of ubiquinones-ubiquinols can be due either to a direct scavenging mechanism or to an indirect mechanism involving recycling of α-tocopherol. The interactions among antioxidant nutrients are likely very important in protecting cells because the concentrations of each antioxidants alone may not be adequate to effectively protect these cells against lipid peroxidation.

Vitamin D in immunity and health: Rickets from vitamin D deficiency is often accompanied by increased rates and severity of infections. Monocytes from ricketic children have reduced ability (30–40%) to phagocytize *E.coli* when compared to age matched controls. Neutrophils from ricketic children had defective motility. Administration of vitamin D leads to rapid improvement of these defects. Studies with rats and mice showed that vitamin D deficiency resulted in impaired macrophage function, which can be reversed by 1, 25-$(OH)_2$ D3 treatment. The 1, 25-$(OH)_2$ D_3 enhances the expression of class II major histocompatibility antigens (la antigens) which mediated antigen presentation to lymphocytes. Thus, vitamin D promotes monocyte to function more efficiently as antigen presenting cells. Rigby *et al.* (1985) hypothesized that 1, 25-$(OH)_2$ D_3 may act *in-vivo* in an inhibitory feedback loop to suppress T-cell proliferation once adequate g-interferon is produced by activated lymphocytes in the presence of activated macrophages. Excessive release of systemic 1, 25-$(OH)_2$ D_3 during hypocalcaemia can reduce proliferation of lymphocytes, which might explain the finding that cows with clinical milk fever are more susceptible to infectious diseases such as mastitis. Holick (2007) showed that 1, 25-$(OH)_2$ D_3 is synthesized by cells of immune system from its precursor in much higher concentration than circulatory levels and has inhibitory effects on the adaptive immune response and stimulatory effect on monocyte proliferation.

Selenium in immunity and health: Selenium is an essential element for normal immune function and health of animal and human being. Seleno-proteins are present in every cell type. At least 20–30 seleno-proteins exist, but only about 12 have been characterized. Seleno-proteins catalyse oxido-reduction reactions and protect the host from oxidative stress in various ways. The extracellular seleno-enzyme eGPX is less important in preventing oxidative damage in the absence of stress and may simply provide a buffer to ensure a continued Se supply when dietary supply is limited. Under the condition of stress, elimination of peroxides in the extracellular fluid is dealt with by extracellular (eGPX) or plasma form of GPX. Other GPXs and seleno-enzymes appear to be essential in preventing oxidative damage to the cell.

Although excellent results of Se supplementation were reported from mice and human being, in a review on effect of Se in ruminants, Suttle and Jones (1989) concluded that there is little convincing evidence that Se deficiency affects resistance to infection in ruminants. However, Sanders (1984) reported that in a 150 cow beef herd all newborn calves had diarrhoea, with 50% mortality. Examination post mortem revealed changes consistent with colibacillosis. Vaccination of cows in late pregnancy with *E.coli* and bovine viral diarrhoea (BVD) vaccines reduced morbidity but did not eliminate the problem. Serum selenium was low and supplementing the premix with Se at 90 mg/lb, with premix at 17 lb/ton of feed, resolved the problem.

Calves made Se deficient and given a primary and secondary inoculation with infectious bovine rhinotracheitis virus (IBRV) had reduced glutathione peroxidase activity, increased plasma creatinine kinase activity, decreased Ig M after both inoculations, but no change in IgG, and a decrease in antibody titer after secondary challenge (Reffett *et al.* 1988). Steers fed Se deficient did have reduced neutrophil, candidacidal and myeloperoxidase activities (Arthur and Boyne 1985). Weiss *et al.* (1990) observed negative correlation between serum Se level and incidence of mastitis.

Knight and Tyzink (1990) fed Se depleted ponnies either 0.02 or 0.22 ppm Se containing diet and challenged antigenically with sheep packed red blood cells and observed higher primary immune response in high Se supplemented ponnies compared to the low Se group.

Maternal nutrition and immunoglobulin status of colostrums

Nutritional deprivation during pregnancy has dramatic and lasting damaging effect on both humoral and cellular responses of offsprings. Of particular note was the observation that re-feeding did not fully restore optimal immune response, with some impairment being carried over from severely restricted F0 to F1 and F2 generation offspring (Suskind 1977). These data indicated the high vulnerability of the reticulo-endothelial system to nutritional and metabolic derangement during its period of formation and development. Halliday *et al.* (1978) found that feeding 25% higher energy (ME) than requirement during last 12 weeks of gestation increased colostral concentration of Ig G1, Ig G2 and IgM as compared to those fed as per requirement. Maternal protein restriction during prepartum period affected ability of the calf to absorb the Ig from colostrums (Blecha *et al.* 1981). Maternal nutritional restriction affects passive transfer of Ig through its effect on cortisol and tri-iodothyronine concentration, which was demonstrated to be necessary for maturation of the intestinal epithelium and which ultimately determined the rate of absorption of colostral antibodies from intestine (Hough *et al.* 1990). Klimes *et al.* (1986) suggested that the quality of colostrums could be used as an index for assessing the feeding status and general health of dairy cow during late pregnancy.

Colostrum from cows that are not supplemented with vitamin E during the dry period may provide inadequate vitamin E to calves after birth. Quigley and Drewry (1998) also suggested that these diets might increase the incidence of calves born in respiratory acidosis, which may impair the acquisition of passive immunity.

Iron and immunity

On the one hand, metabolic events associated with the mounting of a specific immune response may require Fe, on the other hand invading microorganisms may utilize Fe within the host's body for their own multiplication. Both Fe deficiency (Svoboda *et al.* 2004) and Fe overload (Cunningham-Rundles *et al.* 2000) may be associated with increased susceptibility to infectious disease and impaired immune mechanisms. However, while Fe deficiency is more frequently encountered, Fe overload is relatively rare and is more commonly associated with inherited disorders of Fe metabolism or on excessive blood transfusions (Brock and Mainou-Fowler 1986). The proteins of transferring class (serum transferring, lactoferrin and ovotransferrin) inhibit microbial growth by sequestering Fe. Studies with animal models showed that cell mediated immunity is impaired in Fe deficiency. Iron deficient animals had decreased skin responses and lymphocyte responses to mitogens which returned to normal after Fe repletion. Neutrophil function (defective intracellular killing) may be impaired in Fe deficiency due to reduced production of reactive oxygen compounds such as superoxide. The Fe is present in cytochrome b 559 which is required for the production of superoxide. On the other contrary certain forms of Fe can catalyse the reaction of superoxide with H_2O_2 to form the short lived but highly reactive hydroxyl radical (Baldwin *et al.* 1984), which is believed to be the most toxic of the reactive O_2 species.

Zinc and immune function

Zinc is crucial for normal development and function of cell mediating innate immunity, neutrophills and NK cells, phagocytosis and cytokine production (Prasad 2008). Animals with Zn deficiencies have lower concentrations of thymic hormone, resulting in the loss of lymphocytes positive for Thy-1. Zinc also is important in activation of B cells. Supplementing 350 mg extra zinc daily per steer fed with diet containing adequate (43 ppm) zinc resulted in increase in feed intake and daily gain of morbid steers and reduced the number requiring further treatment. Spears *et al.* (1991) observed that relative to the control (26.4 ppm Zn in diet), additional supplementation of 25 ppm Zn either as Zn methionine or Zn oxide improved antibody titre on immunization indicating higher requirement of Zn for immune function than NRC (1984) recommended level. However, addition of excess Zn (150–300 ppm) to diets containing 60 ppm Zn had no effect on immune responsiveness in calves (Kincaid *et al.* 1997). Droke *et al.* (1998) reported that there was no enhancement of immune response by supplementation of either organic or inorganic

Zn under the condition where basal diet was nearly adequate in Zn (27.6 ppm).

Copper deficiency and immune response: Copper deficiency alters immune response in animals. Minatel and Carfagini (2000) while reviewing the subject observed that innate immune response is impaired in ruminants with Cu deficiency. Microbiocidal activity, O_2 production, and superoxide dismutase activity of neutrophils from Cu deficient cattle and sheep are decreased. Phagocytic function might be affected in ruminants with severe induced-Cu deficiency. However, macrophage functions appear to be less impaired. Boyne and Arthur (1986) observed that copper deficiency produced either by high Mo or by high Fe in diet or reduction in feed intake result in significant reduction in neutrophil killing of *C. albicans*, phagocytic activity, nitroblue tetrazolium (NBT) reduction (an estimate of free radical production), superoxide dismutase activity, and neutrophil viability. Copper deficiency resulted in reduction in serum Ig M concentration following disease exposure (Spears 1988). Ward *et al.* (1993) reported reduced cell mediated immune response, as measured by skin swelling response to PHA when cattle were made Cu-deficient by feeding Mo and S. Gengelbach *et al.* (1997) in a study on cattle observed that copper supplemented calves had greater plasma tumor necrosis factor than Mo-induced Cu deficient animals. However, Gengelbach *et al.* (1997) in another study on cattle observed that Cu deficiency coupled with high Mo and Fe had inconsistent effects on immune function and suggested that Cu deficiency may not affect specific immune function in calves.

Chromium and immune function: Though the amount of published data about the beneficial roles of dietary Cr for laboratory animals is substantial, similar information for domestic animals is scanty. Experiments indicated that supplementation of Cr may improve health and immunity of animals under certain situations. Chang and Mowat (1992) examined the effect of supplementation of Cr to calves that had experienced the stressors associated with feedlot placement (travel, crowding, antigen exposure, limitation of feed and water supply). Supplementation of organic Cr from a high Cr yeast was @400 ppb until day 28 whereupon the Cr level was reduced to 200 ppb for the following 70 days. The supplementation of Cr reduced serum cortisol significantly and significantly increase serum Ig M and total immunoglobulins in calves fed a certain diet. A similar result was reported by Moonsie-Shageer and Mowat (1993). Chang *et al.* (1994) observed that supplemental Cr increased blastogenic responses of peripheral blood lymphocytes in morbid calves but no such effect on healthy calves. Kheiri and Toglyani (2009) observed that Cr supplementation improved antibody titre against Newcastle virus in chicks. However, some workers reported no positive effect of Cr supplementation on immunity parameters (Arthington *et al.* 1997).

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