Role of Dietary Zinc as a Nutritional Immunomodulator

T. K. Goswami*, R. Bhar[1], S. E. Jadhav[1], S. N. Joardar[2] and G. C. Ram[3]
Section of Immunology, Indian Veterinary Research, Institute, Izatnagar-243122, India

ABSTRACT : Zinc is ubiquitous in all living cells. Structural and catalytic properties of cellular enzymes are zinc dependent. Zinc deficiency leads to a variety of pathological abnormalities with immune impairment. It is an established fact that nutritional status contributes to overall immune response of individuals. Outcome of zinc deficiency on immune system is so drastic that it is difficult to conceive at the first instance. Zinc supplementation has been advocated to prevent diarrheal disease in children with poor nutritional status. The bioavailability of zinc depends upon its sources. Moreover it varies between monogastrics and ruminants. Controversy still prevails between inorganic and organic sources of zinc with respect to their superiority in bioavailability. Zinc exerts immunostimulatory effects in various laboratory and farm animals. Animals having congenital zinc deficiency diseases like A46 lethal trait usually die due to impairment of the immune system unless treated with zinc. The immune mechanism of zinc and its effect on animals and man are discussed. Zinc has been considered as extremely safe at higher therapeutic doses, but does not provide any beneficial effect but rather may cause immunosuppression. More recently, zinc has been prescribed for immunodeficient hosts, to modulate the immune system so that to a certain extent it can combat against opportunistic pathogens. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 3 : 439-452)***

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INTRODUCTION

Historically the discovery of zinc dates back to 15th century. The importance of zinc in the life science was first came to notice in the year 1869, once it was realised that the growth of mold *Aspergillus niger* is very much zinc dependant (Raulin, 1869). Until 1934 the biological effect of zinc deficiency in animal physiology was not clearly reported (Todd et al., 1934). In 1939, recognition of zinc as an integral component of the enzyme carbonic anhydrase established the first biochemical role of zinc (Kelin and Mann, 1939). Subsequently zinc deficiency in pig was evidenced with clinical symptoms of parakeratotic lesions of the skin (Tucker and Salmon, 1955). Next to iron the zinc is the most abundant trace mineral of human body (Mc Cance and Widdowson, 1942). Although by late 1950 it was known that zinc was necessary for human health but not well acclaimed. Zinc deficiency was initially discovered in human with a syndrome of "adolescent nutritional dwarfism" identified principally in mid eastern countries (Prasad et al., 1961). The organs affected by severe zinc deficiency include epidermal, gastrointestinal, central nervous, immune, skeletal and reproductive systems (Hambidge and Walravener, 1982). Research findings indicate that the impact of zinc deficiency on immune system is too rapid and extensive than its impact on other tissues and organs. Reduced food intake has been observed in rats to maintain growth or cellular metabolism when maintained in zinc deficient diet.

The immune system is a physiological system, that can discriminate self from non-self, thereby protects the host against pathogens. The integral parts of immune system encompasses heterogeneous subpopulations of immunocompetent cells viz T-cells, B-cells, natural killer cells, monocytes and antigen presenting cells (dendritic cells) and their products like immunoglobulin, cytokines as well as complement proteins. The list of micronutrients, which have significant influence on immune function, is ever increasing and includes zinc, copper, iron, selenium magnesium and several vitamins. Practical consequence of micronutrient deficiency leads to increased susceptibility to infection. Zinc is a cofactor for more than 300 enzymes, which is involved in cell proliferation, DNA replication and signal transduction (Coleman, 1992). Zinc is an integral part for all of the six enzyme classes viz oxidoreductase, transferase, hydrolase, lyase, isomerase, and ligase. Immune system being a highly proliferating system, continuous cell (T and B-cells) proliferation takes place in order to produce immunoglobulin and cytokines. Zinc deficiency down regulates the system and can be restored to normalcy on supplementation. Deficiencies of zinc dependant metalloenzymes negatively influence the immune function. Cells with rapid turnover such as those involved in cell-mediated immune response, the intestinal mucosa, and the skin are particularly vulnerable to zinc deficiency. Most of the immune functions are down regulated by zinc deficiency (Table 1). Supplementation restores the immune function to its normal level. Many critical reviews on this

* Corresponding Author: T. K. Goswami. E-mail: tkgoswami101@rediffmail.com
[1] Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar-243122, India.
[2] West Bengal University of Animal and Fishery Sciences, Kolkata-700037, India.
[3] In-charge, Section of Immunology, Indian Veterinary Research Institute, Izatnagar-243122, India.
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Table 1. Effect of zinc deficiency on immune system

1	Serum thymulin	↓
2	Peripheral T cells count	↓
3	Delayed type of hypersensitivity	↓
4	Natural killer cell activity	↓
5	Macrophage function (phagocytosis and intracellular killing)	↓
6	Neutrophil function (oxidative burst)	↓
7	T-cell dependent antibody production	↓

topic dealing with molecular aspect of zinc and immune system are available, interested reader may go through a number of specific reviews (Sandstead, 1994; Aggett and Comerford, 1995; Prasad, 1995; Wellinghausen et al., 1997a; Rink and Gabriel, 2000). In the present brief review we have the goal of setting forth, to summaries the recent advancement on zinc from basic and applied fields in a simpler way that can be convincing to many, even to those who are not involved with molecular biology. We have tried to incorporate as many as relevant articles as possible with special reference to farm animals, however relevant citation dealing with mice and human experiments could not be excluded in order to give a comprehensive information. More over it was not within the scope of this review to cover many important points with the cited reference for which we apologize.

MOLECULAR *MECHANISM* OF ZINC ACTION

Zinc has catalytic, structural and regulatory role in many enzymes and presecretory polymers. It is an integral part of more than 300 metalloenzymes, which control general cellular functions, essential for cell survival and cell replication (Vallee, 1955; Vallee et al., 1948). Limited amount of zinc is absorbed from stomach and major quantity enters through brush border membrane of intestine by pericellular and carrier mediated process (Rucker et al., 1994). In ruminants endogenous excretion into the rumen and reticulum is higher than absorption from these stomachs. In monogastric animals dietary phytate makes zinc phytate complex that hinder zinc absorption, whereas low molecular weight binding ligands viz. aminoacids (histidine and glutamate), citrate, glucose, EDTA and picolinate, assists zinc uptake (Hambidge et al., 1986). Contrary to this, absorption of zinc from rat duodenal loop is reduced when fed with zinc-methionine or zinc chloride along with EDTA (Hempe and Cousins, 1989). Mounting evidence in avian species as well as ruminants suggest that zinc-methionine may either be absorbed differently or behave differently after absorption as compared to inorganic zinc (Greene et al., 1988; Spears, 1989). Exact mechanism of zinc entry inside the cell is yet to be known. Zinc ions being hydrophilic don't enter to the cell by passive diffusion. It is hypothesized that it may enter through cation channel, facilitated diffusion mediated by samino acid binding or by specific receptor (Simkin, 1997). It seems that the transferin receptor (CD71) has a definite roll on zinc uptake, as a sizable amount of zinc remains bound to transferin (Cunningham Rundless et al., 1980). Current literatures have shown that zinc uptake is mediated by zinc transporter proteins. Zinc transporters are membrane bound proteins having multiple membrane spanning region and most have intracellular histidine rich loop. Till date four zinc transporter proteins viz. Zn T-1, Zn T-2, Zn T-3 and ZnT-4, have been characterized. Some of these zinc transporters respond to immune challenge. Oxidative stress and other metabolic competitors in the diet regulate expressions of the transporter proteins (Cousins and Mc Mahon, 2000). Within minutes zinc enters inside the cells and exerts its function. Distribution of zinc in different cellular compartments are not uniform, about 30-40% of the total cellular zinc remains in the nucleus, about 50% remains in the cytoplasm and the remainder is associated with the cell membrane. Virtually cellular zinc does not remain as free ion rather it is in the bound form either as zinc protein/enzyme or nucleotides (Thiers and Vallee, 1957; Smeyers Verbeke et al., 1977). Zinc exerts different effects on monocytes and T-cells but zinc uptake does not differ between these two cell types (Wellinghausen et al., 1997b). Inspite of fragmentary information regarding the involvement of zinc in cell signaling, considerable researchers have favoured its role in protein tyrosine kinase enzyme, involved in cyclic AMP and cyclic GMP pathways (Wellinghausen et al., 1996a). In addition zinc alters the membrane fluidity and stability. Thereby contact with other cells and receptors are affected viz. altered assembly of cell surface receptor and ion channels (Simkin, 1997).

ROLE OF ZINC IN INNATE IMMUNNE FUNCTION

The effect of zinc on immune function is so acute that for some time it is difficult to accept. Most of the information on zinc deficiency is derived from mouse model. Innate immunity mediated by nonspecific immune effectors cell protects the host to a certain extent till the specific immunity develops. Chemotaxis, phagocytosis and generation of oxidative radicals are the major arms of neutrophil and monocyte mediated functions (innate immune system), which are impaired in zinc deficient human being (Keen and Gershwin, 1990). Influence of zinc on immunesystem has been described briefly in our earlier report (Goswami et al., 2002). Natural killer (NK) cell have receptor for MHC class-I molecules that inhibit cytotoxicity

against target cells. This killer cell inhibitory receptor (p58) on NK cell requires zinc for recognition of certain MHC molecules (HLA-C of human being) on target cells (Rajgopalon et al., 1995). Calprotectin, the cytosolic complex protein of neutrophil, which belongs to S-100 protein family, is known to have antifungal activity. It has been suggested that calprotectin may act as zinc chelator thereby zinc deprivation may lead to growth inhibition (Sohnle et al., 1991). Contrary to it, the finding of Murthy et al. (1993) suggested that zinc could block the anti-candidal activity of calprotectin. The possible explanation for the anti-candidal activity of zinc might be due to conformational alteration of calprotectin similar to S-100 protein family member (Klingman and Hilt, 1988). Earlier report have shown that zinc inhibit bacterial growth by altering the active transport system and blocking the initial step in bacterial mating (Sobocinski et al., 1977).

EFFECT OF ZINC ON MUCOSAL IMMUNE SYSTEM

Most of the pathogens get entry into the body through mucosal route like intestine and respiratory tract. Soluble proteins and microbes do cross the epithelial barrier. More detail of the trans-epithelial vesicular transport pathway by which microbes cross the barrier is reviewed elsewhere (Kraehenbuhl and Neutra, 2000). The lymphoid tissues in the mucosal tract specifically in the gut mucosa are organised in two compartments those are anatomically and functionally distinct. The organised lymphoid tissue with encapsulation are located in Peyer's patches, isolated lymphoid follicle and mesentric lymphnodes, the next one is diffuse lymphoid tissues without encapsulation are distributed in intra epithelial and lamina propria of intestine. Depending upon the antigenic stimulus the immunesystem is either activated to Th-1 or Th-2 type, as per their cytokine secretion pattern. Usually bacterial, viral and protozoan antigens favour Th-1 type response with IL-1, IL-12 and IFN-γ cytokine secretion, whereas nematodes infection favourably up regulate the gene for Th-2 type cytokines (IL-4, IL-5 and IL-13) in mesenteric lymphnode and Peyer's patches (Svetic et al., 1993). Information from experimental studies has indicated that rat fed with zinc deficient diets have higher burdens of *Trichinella spiralis* along with delayed expulsion as compare to control (Fenwick et al., 1990a,b). During a recent survey in northern India, decrease in the level of zinc in blood serum was recorded in cattle infected with microfilaria *Setaria cervi* (Sharma and Joshi, 2002). Similarly zinc deficiency promotes survival of *Heligmosomoides polygyrus* in mouse model (Shi et al., 1994;1998). Although parasites need higher concentration of zinc for their enzymatic function the zinc content of the worms from three different dietary groups did not differ significantly (Shi et al., 1995). It indicated that the survival of parasite is not due to zinc deficiency in the parasite itself rather deficiency of zinc, impairs the ability of mice T-cells to produce IL-4, the pivotal cytokine that influence the Th-2 type response (Urban et al., 1991). Insufficient IL-4 production impairs the Ig E and Ig G secretion as well mast cell proliferation, thereby mast cells degranulation are prevented. The other possible explanation is that zinc deprivation drastically reduces the feed intake resulting energy restriction, which contributes to the lower production of INFγ from T-cells (Scott and Koski, 2000). However force feeding of zinc depleted diet to rats failed to correct the situation (Park et al., 1986).

ROLE OF ZINC ON LYMPHOPOESIS

A limited period of zinc deficiency as little as 30 days has abrogated most of the immune function in the rat depending upon the severity of deficiency (Fraker et al., 1993; Keen and Gershwin, 1990). Zinc deficiency and protein calorie malnutrition (PCM) has a drastic effect on immune system exhibited by thymic atrophy and lymphopenia (Chandra and Newberne, 1977). PCM and deficiency of zinc are not identical but have many common features on immunological determinants with an increased occurrence of sepsis, pneumonia, flue and many other infections in human being. Moderate zinc deficiency due to suboptimal intake is more prevalent from low-income families manifested with low birth weight and chronic diarrhea (Sazwal et al., 1998). Acute zinc deficiency in human being is of rare occurrence except those suffering from genetic defect to assimilate zinc. The syndrome is known as "acrodermatitis enteropathica" characterized by thymic atrophy and lymphopenia, impaired cell mediated immune response (CMI) with high incidence of infection in those individuals (Endre et al., 1990). Critical study on mice model has shown that poor immune response in zinc deficiency, directly correlate with decrease in absolute population of peripheral lymphocytes and spleenocytes. It is obvious that the total out put of antibody production (from the reduced number of lymphocytes) is half of the antibody produced from the normal mice. But on per cell basis the IL-2 production from spleenocytes of zinc deficient mice was similar to control mice and respond equally well to known mitogen (Cook-Mills and Fraker, 1993; Dowd et al., 1986). Unlike IL-2 production, the IL-4 and IL-5 production was found to be down regulated by zinc deficiency in weanling mice (Shi et al., 1998). Thus the evidences support that diminished immune response is due to decrease in lymphopoesis. Glucocorticoides (GC) induced by suboptimal zinc might play role in down regulation of immune response (Alleyne and Young, 1967). Administration of dexamithasone (0.125 mg/calf/day) from

one week of age up to 8th week has shown decrease serum zinc level with concomitant rise in zinc content in liver of buffalo calf. Higher zinc content in liver has been suggested due to sequestration of zinc from circulation, which remains as bound form with metalothionin (Singh and Singha, 2002). The background information about the intrinsic mechanism of GC induced immuno-suppression came into light in 1984 by Cohen and Duke. They suggested that loss of thymocytes and precursor T-cells was due to apoptosis induced by GC. Flow cytometry study has shown that bone marrow B and T-cells are equally susceptible to GC (Garvy et al., 1993). As compare to precursor B-cells (bone marrow B cells) the mature B-cells are fairly resistant to zinc induced apoptosis. The bcl-2 gene a member of proto-oncogene family found on hematopoetic cells is one of the key factors for the survival of mature B-cells having high expression of bcl-2 gene, which prevent mitochondrial disintegration and rescued the death cycle (Mignotte and Vayssier, 1998). Low expression of bcl-2 may be the cause of immature B cell depletion; therefore it leads to insufficient replenishment to peripheral circulation. DNA synthesis and cell proliferation is zinc dependant phenomena (Chester, 1997; Chester et al., 1993). In apoptic death, a cascade of activation of endonuclease leads to fragmentation of cellular DNA (Cohen and Duke, 1984; Schwartzman and Cidlowski, 1993). Where the suboptimal zinc favours the apoptosis, the higher concentration of zinc supplementation inhibits the endonuclease activity, but it did not provide long-term protection to the cells (Fraker and Telford, 1997). High level of zinc beyond physiological limit has no beneficial effect rather found to be immunosuppressive (Wellinghausen et al., 1997b). Collective information suggests that suboptimal zinc along with GC have a synergistic role for removal of precursor T and B cells resulting depressed immune function.

EFFECT ON T-CELL

Zinc dependant hormone "thymulin" secreted from thymic stroma, governs T cell proliferation. Thymulin, a zinc containing hormone, is biologically inactive when stripped of zinc (Dardenne et al., 1982). Thus rapidly developing immature thymocytes in side the thymus are highly vulnerable to decrease in thymulin activity. Thymulin plays an important role in T-cell maturation, cytokine production and expression of IL-2 receptor on T cells (Tanaka et al., 1989; Coto et al., 1992). The effect of zinc is so specific that neither any biologically active divalent cation (calcium or magnesium) nor structurally similar ion like cobalt or iron can restore the effect. Zinc deficiency causes thymic atrophy, which is reversible with zinc supplementation (Moochegiani et al., 1995). T-cell proliferation induced by zinc is not due to direct action of zinc on T cells rather an indirect effect through (zinc induced) monocyte derived cytokines. Zinc fails to induce cytokine secretion in monocyte depleted T cells (Hadden, 1995; Wellinghausen et al., 1997b). The proliferating ability of spleenic T cell, levels of Ca^{+}, calmodulin, IL-2 and IL-2 receptors of thymocytes are reduced in zinc deficient rats (Feng and Chang, 1993; Wu et al., 1998). The number of CD4 and CD8 T cells and their ratio in the spleen of zinc deficient mice are declined (Zhou and Wu, 1995). Conversely high zinc concentration can inhibit T cell response by blocking the IL-1 type 1 receptor associated kinase (Wellinghausen et al., 1999). Deficiency or sufficiency of zinc in excess abrogates the T cell response towards T cell mitogen in chicks (Chandra, 1984; Zhang et al., 1999).

EFFECT ON MONOCYTE

Although zinc uptake does not differ in monocyte and T cells but it exerts different effect on these two cell types. Surprisingly monocytes have higher intracellular zinc concentration and high tolerance to extracellular zinc than T-cells. This attributes to their cytokine secreting ability at higher zinc concentration where the T-cell becomes suppressive (Wellinghausen et al., 1997b). Molecular mechanism by which higher zinc level induces T cell suppression is supposed to be due to zinc specific inhibition of IL-1 type-I receptor–associated kinase. Similar to *in vitro* study, the inhibitory effect after high dose zinc supplementation has been reported earlier (Chandra, 1984). It appears that physiological level of zinc delicately regulates T-cell function. Release of cytokines like IL-1, IL-6 and TNFα from monocyte is due to direct interaction of zinc, independent of the presence of lymphocytes (Driessen et al., 1994). Increased mean adherence of peritoneal macrophage to glass surface, improved clearance of *E. coli* from circulation and *in vivo* degradation of the pathogen are the attributes of enhanced activity of mononuclear phagocytic system of turkeys maintained with supplementation of zinc methionine above the basal level (Kidd et al., 1994a,b).

EFFECT ON MITOGEN

Since 1970, zinc ion has been described as a simplest mitogen and deficiency of zinc abrogate the mitogenic stimulation (Kirchner and Ruhl, 1970). Zinc has been shown to be having co-mitogenic property with phytohemaglutinin (Fraker et al., 1986). Similarly substimulatory concentration of zinc enhances the cytokine secretion from lipopolysaccharides induced leukocytes (Driessen et al., 1995a). Synergistic activity of zinc is due to direct interaction of zinc with LPS resulting structural

alteration of LPS making it less fluid thereby it become more biologically active (Wellinghausen et al., 1996a.b). Further more, the report of Driessen et al. (1995a) have suggested that the zinc enhances LPS activity so strongly that unstimulatory concentration of LPS combined with unstimulatory doses of zinc resulted in activation of monocytes. LPS interact with LPS binding protein (LPB) in the circulation that leads to polyclonal activation of the immune system (Bone, 1991). Two possible mechanism for zinc action has been proposed either (i) zinc up regulates expression of CD14 receptor on the cell surface, stabilize binding of LPS to LPB thus LPS-LPB complex can act as a ligand for CD14 receptor, or (ii) zinc can support the second messenger signals pathways for cell activation (Driessen et al., 1995b). LPS being a major pathogenic factor in Gram-negative bacterial infection, zinc therapy in those patients enhance the LPS activity with exaggerated acute phase response (Braunschweig et al., 1997). Supplementation of zinc may leads to devastating consequence in pig during sepsis (Klosterhalfen et al., 1996). Reduction in blood zinc level during inflammation of mammary gland due to clinical and sub clinical mastitis in lactating cow of an organized herd has been observed (Naresh et al., 2001). According to them the reduced blood zinc concentration can't be solely attributed to its increased excretion in milk. Rather the interaction between toxins and enzymes (released by the mastitis causing pathogen) with the host myeloepithelium may activate the utilization of zinc, resulting in less zinc in circulation. Lower level of detection in blood plasma might be due to enhanced utilization of zinc in inflamed cell has also been suggested by them. Zinc therapy has been recommended for prophylactic measure before any operation but not during sepsis. Zinc also influences the immunostimulative effects of some bacterial super antigens derived from Gram-positive bacteria, as well as tetanus toxin and diphtheria toxins. These super antigens act as non-specific mitogen through antigen presentation pathway bypassing the antigen processing mechanism. Super antigens bind to the β chain of MHC-II molecules. This binding process needs the requirement of zinc by forming a zinc clusters involving three amino acids of super antigens and histidine-81 of the β chain of MHC-II molecule (Fraser et al., 1992; Kim et al., 1994; Sundstrom et al., 1996). This zinc dependant interaction is diminished by zinc chelation (Bernatchez et al., 1997). Zinc mediated dimerization of superantigen derived from *Staphylococcus aureus*, facilitates T cell independent interaction resulting direct activation of monocytes (Sundstrom et al., 1996).

EFFECT OF ZINC ON FARM ANIMALS

Inherited deficiency

Similar to that of inherited severe zinc deficiency viz acrodermatitis enteropathica (AE) observed in human being spontaneously occurring inherited zinc deficiency condition is not uncommon in Friesian and Shorthorn cattle. This hereditary defect in farm animals is due to inheritance of an autosomal recessive lethal trait A46. It is characterized by the reduced capacity of dietary zinc absorption, resulting dermal and intestinal lesions along with impaired immunity that leads to death unless maintained with zinc therapy (Andressen et al., 1974; Brummerstedt et al., 1974; Kroneman et al., 1975; Flagstad, 1976; Price and Wood, 1982; Perryman et al., 1989). Experimental design consisting of four heifers heterozygous for lethal trait A46 when reared, only two of them developed clinical sign of zinc deficiency within 3 to 5 weeks of age. At birth the plasma zinc concentration was although normal, it gradually declined with onset of clinical symptoms, and it could be restored on supplementation of 200 mg of $ZnSO_4$ (7 H_2O) per day per calf (Perryman et al., 1989). Flow cytometry analysis of lymphocytes from these calves revealed fluctuation in T lymphocytes subpopulation. Antibody response towards a T-cell dependant antigen, ϕX 174 bacteriophage was of low magnitude during primary as well as secondary response, as compared to control calves. Whereas both the calves have shown immunoglobulin class switching (IgM and IgG isotypes). The antibody thus produced was equally capable to neutralise the bacteriophage like that of normal calves. The above study have indicated that the calves with A46 genetic disorders were immunologically normal (neither lymphopenic nor leukopenic) during birth but subsequently alteration in lymphocyte population (reduced B cell count) might have contributed for immunological impairment. In their findings authors have mentioned that the lymphocyte blastogenic responses of one of the animal towards the lectins (ConA, PHA and PWM) was subnormal up to 4 weeks of age, but following zinc supplementation beginning at 4 weeks of age, the response was increased. This has suggested that zinc therapy may ameliorate the disease condition to a certain extent. Simultaneously authors (Perryman et al., 1989) have judiciously described that the lymphocyte defects are not so prominent to explain the uniform mortality in calves having A46 genetic disorder.

Researchers (Arrayet et al., 2002) have also probed the role of zinc in the recovery of another inherited immunodeficiency disease of cattle known as bovine leukocyte adhesion deficiency disease (BLAD). In normal healthy cattle the leukocytes are capable to adhere to the cell surface due to the expression of a membrane protein known as integrin of β2 family, designated with a CD number (CD18). Due to lack of expression of CD18, the circulating leukocytes fail to adhere therefore subsequent down stream events and signal transduction pathways for cell activation is abrogated resulting severe immune

impairment. Molecular mechanism of BLAD is described in more detail by Kriegesmann et al. (1997). Calves homozygous for CD18 mutation have recurrent bacterial infection, poor growth and survive rarely beyond one year of age (Kehrli et al., 1992). In the report of Arrayet et al. (2002), a total of 421 Holestein calves including 33 female and 20 male heterozygous for BLAD character were maintained for 90 days from birth with supplementation of 100 mg of zinc/kg DM, which was much higher than the recommendation of NRC (1989b). The study period did not influence the growth performance in calves heterozygous for CD18 locus. Growth performance was also not affected in normal calves within 90 days of their study. Although animals were vaccinated with infectious bovine rhinotracheitis, parainfluenza type 3, bovine virus diarrhea, respiratory syncitial virus, leptospirosis and clostridia, nowhere the difference in magnitude of antibody response between normal and CD18 heterozygous calves has been mentioned. Possibly the animals were vaccinated to minimize the infection and to prevent mortality from these pathogens, as the BLAD carrier animals were more prone to recurrent infection. The above study has emphasized that heterozygous BLAD calves neither have an advantage nor disadvantage in growth over normal calves. Practical benefit of this study has shown that there is no need to incur added expenses to remove calves heterozygous for BLAD under the assumption of poor growth performance (Arrayet et al., 2002).

Effect on ruminants

Growth promoting effects of zinc on farm animals have witnessed variable results and at times the results are more conflicting than conclusive. Most frequently dietary zinc is supplemented either in the organic (zinc-methionine/zinc-lysine) or in the inorganic form like zinc oxide, zinc sulfate etc. Bioavailability of zinc among these two forms is still under debate.

About six week of experimental feeding of Holstein heifer calf with 300 ppm of dietary zinc either in the form of ZnO or organic form (Zinc-Met/Zn-Lys) resulted without any difference in growth performance and immune function in the recipient (Kincaid et al., 1997). Except higher bioavailability of zinc in organic form, there was no significant treatment effect recorded on mitogen induced blastogenesis, IL-2 production, cytotoxicity, and intra-cellular killing ability of neutrophil. Therefore it was concluded that supplementation of extra dietary zinc in either of the forms could not enhance the immune function of animals. Apparent absorption of zinc was higher in pregnant cattle and sheep as compare to non-pregnant one. Effect of gestational status has shown higher retention of zinc during last trimester of gestation (Vierboom et al., 2003). The results of zinc supplementation had become more puzzling due to nonuniformity in the results of their bioavailability between feed grade and analytical grade obtained from same zinc source viz ZnO (Edwards and Baker, 1999; Mavromichalis et al., 2000). Similar to that of cattle, the overall immune response of sheep could not be enhanced with higher supplementation of zinc (25 mg Zn-Met/kg) above the basal diet having 25 mg of ZnO/kg (Dorke et al., 1998). Unless there is severe deficiency of zinc, the immune-status is unaltered in lamb and even marginal zinc deficiency had shown similar immunological response and gain in body weight like that of zinc adequate lambs (Dorke et al., 1993). However, supplementation of zinc at very high level may have negative consequences. During 63 days of their study, Hatfield et al. (2002) observed reduced antibody titer against para influenza-3 vaccine in grazing sheep fed on supplemental zinc, seven times more than NRC (1985) recommendation. The exact mechanism for this negative impact although not been justified, possibly the high level of zinc in their study might have shown antagonistic effect on other minerals that positively modulate the immune mechanism of host. Severe zinc deficiency is known to cause immunosuppression (Chester, 1997), but additional zinc above the normal requirements not necessarily enhance over all immune status of animals (Spears et al., 2002). Grazing cattle usually suffer from zinc deficiency, for which supplementation is a common practice in farming. Report of Corah and his group has confirmed this view while surveying the forage samples from which only 2.5% samples were zinc adequate (Corah et al., 1996). In addition to lower feed intake zinc retention become negative during stress, which demands additional zinc supplementation along with the diets. Supplementation of zinc at the rate of 25 mg/kg above the basal diet (25 mg/kg) have shown enhance antibody response against bovine herpes virus-1 vaccine in steers, but no difference in antibody titer was observed against parainfluenza-3 viral vaccine (Spears et al., 1991).

Effect on monogastric animals

Importance of zinc in pig nutrition has been recognized since 1955 in relation to its role to treat skin disorder like parakeratosis (Tucker and Salmon, 1955). Its role in the farm animals was brought to light by subsequent studies. Like that of ruminants controversy still prevails regarding the superiority of organic versus inorganic form of zinc in pig nutrition. According to Lee et al. (2001a) in terms of growth performance, the organically chelated and complexed form of copper and zinc are equally effective as that of their inorganic salts. Subsequent reports form the same working groups have opined that zinc concentration in serum to be influenced by the dietary levels rather than by the source (Lee et al., 2001b). Contrary to the above

findings in a different experimental trial the same group has observed that weaned pigs rearing with organic form of zinc along with other trace minerals tended to grow faster rate, consumed less feed that was utilized more efficiently. Consequently organic form of zinc feeding resulted in greater accumulation of zinc in serum and bone (Acda and Choe, 2002).

More researchers have given justification in favour of zinc as an antimicrobial agent (Sorderberg et al., 1990; O' Quinn et al., 1997) as well as an immunomodulator that has influenced the physiology of recipient in a beneficial manner (Wirth et al., 1989; Chirase et al., 1994). It has been suggested that the action of zinc is not additive or synergistic with the antibiotics in the feed formulation, but authenticity of such hypothesis is yet to be verified in diverse environment and management conditions (O'Quinn et al., 1997). Role of zinc oxide as growth promoter and for the control of diarrhea has been described in detail in a recent review (Bosi et al., 2003). Interestingly in his previous experiment he could not observed any beneficial effect on growth and diarrhea intensity in enterotoxogenic *E. colli* challenged pigs, maintained at 3 g of dietary zinc (Bosi, 2000).

Closer to this observation, we failed to detect any major difference in antibody level against *Pasturella multocida* vaccine in pig with higher dietary zinc (Bhar et al., 2003). Possibly the zinc content in the basal diet was adequate for maintenance of normal health resulting moderate antibody response, without any detectable immune impairment. Our finding is in agreement with the recent findings of Roberts et al. (2002). In their report no gain in feed intake, feed efficiency and cellular immune response measurement was observed in pigs fed on additional supplementation of 150 ppm of zinc along with the 30 ppm already present in the basal diet. In our earlier finding additional zinc supplementation (30 mg $ZnSO_4$/kg) above the normal schedule was neither detrimental nor beneficial as far as immunological parameter was concerned, nevertheless the additional zinc enhanced the wound healing process (Bhar et al., 2001). Wound healing process is a complex phenomenon known to be controlled by various cytokines that regulate immune network of host. A local effect of zinc can be suspected considering its wound healing action in the topical use. In the pig during wound healing process an increased exogenous gene expression of insulin growth factor-I was observed. This growth stimulating factor could favour a better recovery of intestinal epithelium after the weaning stress or reduce its negative effect, has been described recently (Bosi et al., 2003). The immuno-potentiating effect of zinc to withstand the inflammatory response during sepsis in pig model has suggested its use as a prophylactic measure before any surgical intervention (Klosterhalfen et al., 1996). Zinc supplemented at 50 and 150 ppm to a corn-soyabean meal based diet containing 30 ppm of zinc enhanced the febrile response in pigs subjected to iatrogenic endotoxaemia, but did not affect growth performance. According to them supplementation above NRC recommendation for zinc may not be beneficial (Roberts et al., 2002).

Effect on poultry

Serum zinc concentration in birds has been found to be influenced by the source of dietary zinc. Organically bound minerals such as chelated and complexes have higher bioavailability in poultry birds (Lee at al., 2001b). Depletion of lymphocytes, degenerative changes in thymus, reduction of lymphoid follicle in bursa of Fabricious is characteristic features of zinc deficiency in broilers (Wight et al., 1980; Burns, 1983). Higher magnitude of antibody response was recorded by the fortification of zinc in poultry birds (Pimentel et al., 1991). Reduction in antibody titre against Newcastle disease and Marek's disease virus, substantially low B cell response against lipopolysaccharides and T cell response towards mitogen like Concanavalin-A have been reported in zinc deficient chicks (Zhang and Zhou, 1998; Zhang et al., 1999). Ducklings maintained with 22.9 mg of zinc/kg diet have shown much reduction in the weight and growth index of the bursa, spleen and thymus. Restrained proliferation and differentiation of lymphocytes within lymphoid organ might be the cause of reduced growth index of these lymphoid tissues (Cui et al., 2003). Enhanced antibody response in the progeny chicks from hen maintained with zinc fortified diet support the immunomodulatory property of this compound (Stahl et al., 1989). Dietary zinc supplementation in turkey enhanced mononuclear phagocytic system resulting an early clearance of *E. coli* from circulation (Kidd et al., 1994a,b). Recent information by Lim and his group has shown no significant increase in serum IgG level in birds fed with zinc-met alone or in combination with Mn-met. Similarly supplementation of zinc had no beneficial effects on laying performance and eggshell quality (Lim and Paik, 2003).

Human study has revealed an interesting application of zinc as an adjuvant in vaccination. The results of a number of vaccination trials, which were accompanied by zinc supplementation, are extremely contradictory (Rawer et al., 1987; Greskas et al., 1992; Turk et al., 1998). Comparison of above experimental results from various animals and human studies have shown either an enhanced immuneresponse or without any detectable beneficial effect of zinc. Such variable and contradictory results might be due to a plethora of factors like, different source of dietary zinc used in their respective studies, species of animals used, environmental or induced stress generated in their experiment proper, duration of the feeding trial, developmental stages of animal during trials, parameters

Table 2. Different terminology for dietary standards used by various countries

Terminology used		Name of the country
RDA	Recommended Dietary Allowances	USA
RDA	Recommended Daily Allowance	UK
RNI	Recommended Nutrient Intake	Canada
RDI	Recommended Daily Intake	Australia
LRNI	Low Reference Nutrient Intake	UK
EAR	Estimated average Requirement	UK
RNI	Reference Nutrient Intake	UK
LTI	Lowest threshold Intake	ECSCF
AR	Average Requirement	ECSCF
PRI	Population Reference Intake	ECSCF

used to detect the immuneresponse and many more like synergistic or antagonistic effect of other micronutrients present in the feed and so on.

ZINC SUPPLEMENTATION

Recommendation of zinc therapy for corrective measure is not an easy task. It should be considered along with bioavailability of dietary zinc. Dose recommendation based upon the plasma zinc concentration is never a realistic approach but there is no single assay system that could reflect the entire spectrum of zinc status to represent the deficiency to sufficiency. Dietary requirements of zinc in farm animals are immensely variable due to species difference. Moreover, within the same species requirements differ during various growth phases of its life cycle. When animals maintained on a well-formulated ration having adequate zinc, still few animals may show deficiency due to many reasons, like level of intake, previous nutritional status, interrelationship among nutrients, malabsorption, disease, environmental stress and many other known and unknown factors. Besides these a lot many midway correction is required for different stage of development, age, weaning stage, gestation, lactation, etc. For global uniformity the nutritional requirements for man and animals are followed by the guidelines designed by either National Research Council (NRC) of USA or by Agricultural Research Council (ARC) of UK. The recommended dietary allowance (RDA) for each nutrient has been fixed in NRC guidelines with the basic purpose of providing standard for good nutrition. For practitioner the guide lines of RDA are approximate, flexible and generous, and nutrition practitioner can use them to assess the adequacy of diets. In nutrition practice different terminology for dietary standards (Table 2) are used by various countries (Lachance, 1998).

Recommendations are usually made to meet the average demand of the population plus safety factor considering the individual variability and bioavailability of zinc. Most studies on zinc bioavailability determine the relative bioavailability and only a few reports address the absolute

Table 3. Minimum requirement of zinc for various farm animals as per recent guidelines

Category of species	Requirement/recommendation mg of zinc/kg DM	Reference
Calf	28-34	ARC (1980)
Milch cow	63	NRC (2001)
Beef cattle	40	GfE (2001)
Sheep	20-33	NRC (1985)
Goat	10	NRC (1981)
Pig	50-100	GfE (1987)
Poultry	44 mg	GfE (1999)

apparent bioavailability. Absolute bioavailability was found to be 19%, 22% and 23% for zinc acetate, zinc oxide and zinc sulfate, respectively (Poulsen and Carlson, 2001). The schedule of recommendation of zinc for various farm animals at different growth phase is so complex that it is beyond the scope of this review to accommodate. For recent information readers can refer to NRC (2001) and GfE (2003). Minimum requirement of zinc as per recent guidelines are given below (Table 3).

As per recent guidelines (http://europa.eu.int/comm/food/fs/sc/scan) a minimum of 60 mg/kg DM may be the requirement for dog provided calcium content in the diet is not excess. Exact requirement in gestation and during lactation may change in different breeds that may reach to 90 mg/kg. Considering the above requirement the recommendation given by AAFC (1998) is 120 mg/kg DM for dog and 75 mg/kg DM in cat. Fish are capable to absorb zinc from water to a certain extent, but predominantly absorption is through intestine for which a dietary requirement is about 15-30 mg/kg DM (Origino and Yang, 1978). Suggested minimum zinc requirement is 20 mg/kg DM for growth and normal maintenance of pregnancy in sheep, however during lactation requirements may increase to 33 mg/kg DM. It is recommended that 1 g/kg is definitely high and can cause growth reduction in sheep (NRC, 1985). The requirements for horses are like that of cattle, but for poultry and pigs it is 60 and 50 mg per kg diet, respectively (Tilden et al., 1990). Zinc supplementation to farm animals definitely relies upon the natural level of zinc present in the feeding stuff and all facts impacting on zinc availability. As per European Union up to 250 mg/kg DM are allowed as a feed supplement. A dose level of 2 to 6 g/kg is definitely being considered as prophylactic measure intended to correct the physiological disturbance and prevent diarrhea in young pigs (Poulsen, 1995; Carlson et al., 1999; Mavromichalis et al., 2000,2001). Zinc toxicity is not common, yet ruminants are more prone to its toxicity than pig and poultry. A dose level of 1 g $ZnSO_4$/litre was found to be toxic to broiler chicks (Donmezz et al., 2002).

For human nutrition different criteria like "reference nutrient intake", "estimated average requirement", "lower reference nutrient intake", "lowest thresh hold intake" have

been described (Aggett et al., 1995). An upper limit of 50 mg/day although recommended for an adult but for children it is less clear. Classical manifestation of zinc deficiency has been observed in children with acrodermititis enteropathica, whereas in animal model the deficiency is milder type with growth retardation as a crucial means of conserving zinc. Exact mechanism of zinc conservation is not known. Pathological expression of deficiency usually occurs, long before major depletion of total body zinc pool takes place.

Zinc supplementation is generally prescribed when suspected for retarded growth. However, chronic diarrhea, pneumonia also demand zinc therapy. It is also recommended when suspected for mild zinc deficiency status. Detection of zinc deficiency is usually hampered due to lack of adequate biomarker and the lack of pathognomonic clinical features. For many reasons mild zinc deficiency does not show typical clinical signs and remain undiagnosed till moderate deficiency arises. Serum zinc level although convenient but considered as a poor measure of marginal zinc deficiency (King, 1990). Other alternative approaches like hair zinc concentration, detection of zinc dependent enzymes etc are not so convincing. Presently zinc recommendation has become a practice in pregnancy. Additional zinc supplementation during pregnancy and lactation is a routine practice in animal feed formulation. The supplementation in pregnancy although have shown beneficial effect on the fetal brain development and high birth weight (Goldenberg et al., 1995), yet others have shown no real benefit out of it (Caulfield et al., 1999). Growing evidence of zinc deficiency in individuals where intake of zinc is adequate is more puzzling. In this situation poor bioavailability of dietary zinc may be one of the possible reasons. New approach for zinc status assessment has now looking for detection of serum ferritin, transferin and metallothionein. Detection of differential mRNA display in various tissues that are preferentially up-or-down regulated by zinc deficiency is under active investigation by the researchers for evaluating the zinc deficiency status. Technical advances in both "genomic" and "proteomic" investigation will hopefully add knowledge in zinc homeostasis (Humphery- Smith and Blackstock, 1997).

Prior detection of biomarkers of zinc deficiency before prescribing zinc therapy is neither always possible for professional nor always affordable for the patients and animal owner too. Symptomatic deficiency occurring either due to inadequate intake, malabsorption or increased loss may be suggestive for zinc recommendation. The safety margin of zinc is fairly high. Zinc is considered to be relatively non-toxic mineral at moderate dose level. Based on scientific knowledge the recommended dietary allowance (RDA) for human being is prescribed by NRC (1989a) which is far below the safe intake margin given by toxicologists in their prescribed oral reference dose (RfD). The RfD is 21 mg/day for men, whereas the RDA is 15 mg/day for adult men and pregnant women and 12 mg/day for non-pregnant women. Interestingly a lower level of recommendation has been practiced in UK. All these recommendations are definitely at a lower limit than the actual requirement in deficiencies. Immunosuppression at a higher dose of zinc supplementation may be a completely new therapeutic approach for the selective suppression of lymphocytes function required during transplantation. As compare to commonly prescribed immunosuppressive drugs, zinc is extremely nontoxic even at doses much higher than the RDA (Fosmire, 1990).

CONCLUSION

The importance of zinc has definitely proved to be true for proper functioning of immune system, which is delicately regulated by zinc level. For proper physiological function body requires a trace amount of zinc so it is referred as trace element. As there is no specialized zinc storage in the body daily intake is essentially required to achieve normal level. Deficiency of zinc not only hampers the overall immune function but also associated abnormalities are surfaced out. Unless there is any congenital defect regarding zinc assimilation the deficiency symptoms can be corrected by supplementation. Recommended intakes emphasize the values applicable only to the healthy population, based on average data, which is flexible and need correction depending upon the individual cases. There are no reliable and authentic parameters to detect zinc deficiency status correctly. Plasma zinc concentration although acceptable for many professionals, yet it has considerable limitations. For example during real zinc deficiency cases, plasma zinc level may be maintained within the reference range by the release of zinc from tissue catabolism. Leukocytes contain about 25 times as much zinc as the equivalent number of erythrocytes (Vallee and Gibson 1948; Vallee, 1955). Decrease in leukocyte zinc content together with reduction in alkaline phosphatase has been considered as diagnostic criteria, but its validity has been proposed as well questioned (Prasad, 1983; Milne et al., 1985). Until there is no suitable biomarker is available, to detect zinc deficiency status, therapeutic zinc administration must be adjusted according to the plasma zinc level, with consideration of associated clinical signs and previous managemental history. Routine dietary recommendation of zinc in farm animal is a quite common practice to sustain good health, as it can reduce the disease prevalence to a certain extent. Therapeutic use of zinc to induce immunosuppression is a novel approach practiced by the clinicians. Due to its low toxicity at higher dose level as compared to commercially

available immunosuppressive drugs it has become a choice for the treatment of rheumatoid arthritis, autoimmune diseases. and survival of organ transplantation in the recipient. Mice model has contributed much in this area yet farm animal studies are of paramount importance.

REFERENCES

AAFC. 1998. (Association of American Feed Control Officials). Official Publication, USA.

Acda, S. P. and B. J. Choe. 2002. Effects of organic trace minerals supplementation on sows reproductive and neonate's growth performance through 2nd week post weaning. Asian-Aust. J. Anim. Sci. 15:1312-1318.

Aggett, P. J. and J. G. Comerford. 1995. Zinc and human health. Nutr. Rev. 53:S16-S22.

Agricultural Research Council. 1980. The nutrient requirements of ruminant livestock. Commonwealth Agric. Bureaux..

Alleyne, G. A. and V. H. Young. 1967. Adrenocortical function in children with severe protein calorie malnutrition. Clin. Sci. 33:189-200.

Andresen, E., A. Basse, E. Brummmerstedt and T. Flagstad. 1974. Lethal trait A-46 in cattle. Additional genetic investigations. Nord. Veternaemed. 26:273-278.

Arrayet, J. L., A. M. Oberbauer, T. R. Famula, I. Garnett, J. W. Oltjen, J. Imhoof, M. E. Kehrli and W. T. Grahm. 2002. Growth of Holestein calves from birth to 90 days: the influence of dietary zinc and BLAD status. J. Anim. Sci. 80:545-552.

Bematchez, C., R. Al-Daccak, P. E. Mayer, K. Mehindate, L. Rink, S. Mecheri and W. Mourad. 1997. Functional analysis of micoplasma arthritidis-derived mitogen interaction with Class II molecules. Infect. Immu. 65:200-205.

Bhar, R., S. K. Maiti, T. K. Goswami, A. K. Garg, A. Chabra and Satyapal. 2001. Effect of Vitamin C and zinc as feed supplement on wound healing, antibody response and productivity in swine. In: 8th Annual Conference of Indian Association for Advancement of Veterinary Research, Ludhiana, India. p. 27.

Bhar, R., S. K. Maiti, T. K. Goswami, R. C. Patra, A. K. Garg and A. K. Chabra. 2003. Effect of dietary vitamin C and zinc supplementation on wound healing, immune-response in swine. Ind. J. Anim. Sci. 73:674-677.

Bone, R. C. 1991. The pathogenesis of sepsis. Ann. Intern. Med. 115:457-469.

Bosi, P. 2000. Modulation of immune response and barrier function in the piglets gut by dietary means. Asian-Aust. J. Anim. Sci. 13(Supp):278-293.

Bosi, P., C. Gremo Kolini and P. Trevisi. 2003. Dietary regulation of the intestinal barrier functions at weaning. Asian-Aust. J. Anim. Sci. 16:596-608.

Braunschweig, C. L., M. Sowers, D. S. Kovacevich, G. M. Hill and D. A. August. 1997. Parenteral zinc supplementation in adult human during the acute phase response increases the febrile response. J. Nut. 122:70-74.

Brummerstedt, E., E. Andresen, A..Basse and T. Flagstad. 1974. Lethal trait A-46 in cattle. Immunological investigations. Nord. Veternaemed. 26:279-293.

Burns, R. B. 1983. Antibody production suppressed in the domestic fowl by zinc deficiency. Avian. Pathol. 12:141-146.

Carlson, M. S., G. M. Hill and J. E. Link. 1999. Early-and traditionally weaned nursery pigs benefit from phase feeding pharmacological concentration of zinc oxide; effect on metallothionein and mineral concentrations. J. Anim. Sci. 77:1199-1207.

Caulfield, L. E., N. Zavateta and A. Figueroa. 1999. Adding zinc to prenatal iron and folate supplements improve maternal and neonatal zinc status in a peruvian population. Am. J. Clin. Nutr. 69:1257-1263.

Chandra, R. K. 1984. Excessive intake of zinc impairs immune responses. J. Am. Med. Assoc. 252:1443-1446.

Chandra, R. K and P. Newberne. 1977. Nutrition immunity and infection. Plenum Press. New. York.

Chesters, J. K., L. Patrie and K. Lipson. 1993. Two zinc dependant steps during G_1 to S phase transition. J. Cell. Physiol. 155:445-451.

Chesters, J. K. 1997. Zinc. In: Hand Book of Nutritionally Essential Mineral Elements (Ed. B. L. O'Dell and R. A. Sunde) Marcel Dekker Inc. New York. pp. 185-230.

Chirase, N. K., D. P. Hutcheson, G. B. Thompson and J. W. Spears. 1994. Recovery rate and plasma zinc and copper concentration of steer calves fed organic and inorganic zinc and manganese source with or without injectable copper and challenged with infectious bovine rhinotracheitis virus. J. Anim. Sci. 72:212-219.

Cohen, J. J. and R. C. Duke. 1984. Glucocorticoid activation of calcium dependant endonucleases in thymocyte nuclei leads to cell death. J. Immunol. 132:38-42.

Coleman, J. E. 1992. Zinc proteins: enzymes, storage proteins, transcription factors and replication proteins. Annu. Rev. Biochem. 16:897-946.

Cook-Mills, J. and P. J. Fraker. 1993. Functional capacity of residual lymphocytes from zinc-deficient adult mice. Br. J. Nutr. 69:835-848.

Corah, L. R., D. A. Dargatz and C. W. Peters. 1996. NAHMS forage Survey. Trace mineral analysis of 352 forage samples collected in 18 states. J. Anim. Sci. 74(Suppl 1):202 (Abstr.).

Coto, J. A., E. M. Hadden, M. Sauro, N. Zom and J. W. Hadden. 1992. Interleukin-1 regulate secretion of zinc-thymulin by human thymic epithelial cells and its action on T lymphocyte proliferation and nuclear protein kinase C. Proc. Natl. Acad. Sci. USA. 89:7752-7758.

Cousins, R. J. and R. J. McMahon. 2000. Integrative aspects of zinc transporters. J. Nutr. 130 (Suppl):1384-1387.

Cui, H., F. Jing and P. Xi. 2003: pathology of the thymus, spleen and bursa of Fabricius in zinc deficient ducklings. Avian Pathol. 32(3):259-264.

Cunningham-Rundles, S., R. S. Bockman, A. Lin, P. V. Giardina, M. W. Hilgartner, D. Caldwell-Brown and D. M. Carter. 1980. Physiological and pharmacological effects of zinc on immuneresponse., Ann. NY. Acad. Sci. 1980, 587, 113-122.

Dardenne, M., J. M. Pleau, B. Nabarra, P. Lefancier, M. Derrien, J. Choay and J. E. Bach. 1982. Contribution of zinc and other metals to the biological activity of serum thymic factor. Proc. Natl. Acad. Sci. USA. 79:5370-5373.

Donmezz, H. H., M. A. Karsli, I. Meral, N. Donmez and N. Simsek. 2002. Effects of increasing zinc supplementation in

drinking water on growth and thyroid gland function and histology in broiler chicks. Deutsche Tierarztliche Wochenschrift 109(10):438-442.

Dorke, E. A. and J. W. Spears. 1993: *In vitro* and *in vivo* immunological measurements in growing lambs fed diets deficient, marginal or adequate in zinc. J. Nutr. Immunol. 2:71.

Dorke, E. A., G. P. Gengelbach and J. W. Spears. 1998. Influence of level and source (inorganic versus organic) of zinc supplementation on immune function in growing lambs. Asian-Aust. J. Anim. Sci. 11:139-144.

Dowd, P. S., J. Kelher and P. J. Guillou. 1986. T-lymphocytes subsets and interleukin-2 production in zinc deficient rats. Br. J. Nutr. 55:59-69.

Driessen, C., K. Hirv, L. Rink and H. Kirchner. 1994. Induction of cytokines by zinc ions in human peripheral blood mononuclear cells and separated monocytes. Lymphokine Cytokine Res. 13:15-20.

Driessen, C., K. Hirv, H. Kirchner and L. Rink. 1995a. Zinc regulates cytokines induction by super antigens and lipopolysaccharides, Immunology. 84:272-277.

Driessen, C., K. Hirv, H. Kirchner and L. Rink. 1995b. Divergent effects of zinc on different bacterial pathogenic agents. J. Infect. Dis. 171:486-489.

Edwards, H. M. and D. H. Baker. 1999. Bioavailability of zinc in several sources of zinc oxide, zincs sulfate, and zinc metal. J. Anim. Sci. 77:2730-2735.

Endre, L., F. Beck and A. Prasad. 1990. The role of zinc in human health. J. Trace Elem. Exp. Med. 3:337.

Feng, J. and J. Cheng. 1993: Effect of zinc on the function of immune cells *in vitro*. Acta. Nutriment Sincia. 15:275-279.

Fenwick, P. K., P. J. Aggett, D. Macdonald C. Hubber and D. Wakelin 1990a. Zinc deficiency and zinc repletion: effect on the response of rat to infection with *Trichinella spiralis*. Am. J. Clin. Nutr. 52:166-172.

Fenwick, P.K., P. J. Aggett, D. Macdonald, C. Hubber and D. Wakelin. 1990b. Zinc deprivation and zinc repletion: effect on the response of rats to infection with *Strongyloides ratii*. Am. J. Clin. Nutr. 52:173-177.

Flagstad, T. 1976. Lethal trait A-46 in cattle. Intestinal zinc absorption. Nord. Veternaermed. 28:160-169.

Fosmire, G. J. 1990. Zinc toxicity. Am. J. Clin. Nutr. 51:225-227.

Fraker, P. J., M. E. Gershwin, R. A. Good and A. Prasad. 1986. Interrelationships between zinc and immune functions. Fed. Proc. 45:1474-1479.

Fraker, P. J., L. King, B. Garvy and C. Medina. 1993. Immunopathology of zinc deficiency: a role for apoptosis In: Human Nutrition: A comprehensive Treatise (Ed. D. Klurfeld). Plenum Press. New York. pp. 267-283.

Fraker, P. J. and W. Telford. 1997. A reappraisal of the role of zinc in the life and death decisions of life. Proc. Soc. Expt. Med. 215:229-236.

Fraser, J. D., R. G. Urban, J. L. Strominger and H. Robinson. 1992. Zinc regulates the functionof two super antigens. Proc. Natl. Acad. Sci. USA. 89:5507-5511.

Garvy, B., L. King, W. Telford, L. Morford and P. J. Fraker. 1993. Chronic level of corticosterone reduces the number of cycling cells of the B lineage in murine bone marrow and induces apoptsis. Immunology 80:587-592.

GfE (Gesellschaft fur Ernahrungsphysiologie) Ausschuβ fur Bedarfsnormen. 1987. Empfehlungen zur Energie-und Nahrstoffversorgung der Schweine DLG-Verlag, Frankfurt (Main).

GfE (Gesellschaft fur Ernahrungsphysiologie) Ausschuβ fur Bedarfsnormen. 1999. Empfehlungen zur Energie-und Nahrstoffversorgung der Legehennen und Masthuhnerr (broiler). DLG Verlag Frankfurt (Main).

GfE (Gesellschaft fur Ernahrungsphysiologie) Ausschuβ fur Bedarfsnormen. 2001. Empfehlungen zur Energie-und Nahrstoffversorgung der Mastrinder DLG-Verlag, Frankfurt am (Main).

GfE (Gesellschaft fur Ernahrungsphysiologie)Ausschuβ fur Bedarfsnormen. 2003. Empfehlungen zur Energie-und Nahrstoffversorgung der Mastputen DLG Verlag, Frankfurt (Main).

Goldenberg, R. L., T. Tamura, Y. Neggers, R. L. Copper, K. E. Johnson, M. B. Dubard and J. C. Hauth. 1995. The effect of zinc supplementation on pregnancy outcome, J. Am. Med. Assoc. 274:463-468.

Goswami, T. K., G. C. Ram and D. K. Singh. 2002. Influence of zinc on the immune system. Pashudhan, 17:4,8.

Greene, L. W., D. K. Lunt, F. M. Byers, N. K. Chirase, C. E. Richmond, R. E. Knutson and G. T. Schelling. 1988. Performance and carcass quality of steers supplemented with zinc oxide and zinc methionine. J. Anim. Sci. 66:1818-1823.

Greskas, D., P. Alivanis, N. Kotzadamis, M. Kiriazopoulos and A. Tourkantonis. 1992. Influenza vaccination in chronic haemodialysis patients. The effect of zinc supplementation. Renal Failuure. 14:575-578.

Hadden, J. W. 1995. The treatment of zinc as immunotherapy. Int. J. Immunopharmacol. 17:697-701.

Hambidge, K. M., C. E. Casey and N. F. Krebs. 1986. Zinc. In: Trace elements in human and animal nutrition, Vo.2 (Ed. W. Mertz) Academic Press, San Diego. pp. 1-37.

Hambidge, K. M. and P. A. Walravens. 1982. Disorders of mineral metabolism. Clin. Gastroenterol. 11:87-118.

Hatfield, P. G., B. L. Robinson, D. L. Minikheim, R. W. Kott, N. I. Roth, J. T. Daniels and C. K. Swenson. 2002. J. Anim. Sci. 80:1329-1334.

Hempe, J. M. and R. J. Cousins. 1989: Effects of EDTA and zinc methionin complex on zinc absorption by rat intestine. J. Nutr. 119:1179-1187.

Humphery-Smith, I. and W. Blackstock. 1997. Proteome analysis: genomic via the out put rather than input code. J. Prot. Chem. 16:537-544.

Keen, C. and M. Gershwin. 1990. Zinc deficiency and immune function. Ann. Rev. Nutr. 10:415-431.

Kehrli, M. E., Jr., M. R. Ackermann, D. E. Shuster, M. J. Van Der Maaten, F. C. Schmalstieg, D. C. Anderson and B. J. Hughes. 1992. Animal model of human disease: Bovine leucosis adhesion deficiency: B2 integrin deficiency in young Holstein cattle. Am. J. Pathol. 140:1489-1492.

Kelin, D. and T. Mann. 1939. Carbonic anhydrase. Nature 144:442-443.

Kidd, M. T., M. A. Qureshi, P. R. Ferkett and L. N. Thomas. 1994a. Blood clearance of *Escherichia coli* and evaluation of mononuclear phagocytic system as influenced by supplemental dietary zinc-methionine in young turkeys. Poult. Sci. 73:1381-1389.

Kidd, M. T., M. A. Qureshi, P. R. Ferkett and L. N. Thomas. 1994b Dietary zinc-methionine enhances mononuclear phagocytic function in young turkeys. Biol. Trace. Elmt. Res. 42:217-229.

Kim, J., R. G. Urban, J. L. Strominger and D. C. Wiley. 1994. Toxic shock syndrome toxin-1 complexed with a Class-II major histocompatibility molecule HLA-DR-I. Science 266:1870-1878.

Kincaid, R. L., B. P. Chew and J. D. Cronrath. 1997. Zinc oxide and amino acids as source of dietary zinc for calves: Effects on uptake and immunity. J. Dairy. Sci. 80:1381-1388.

King, J. C. 1990. Assessment of zinc status. J. Nutr. 120 (Suppl): 1474-1479.

Kirchner, H. and H. Ruhl. 1970. Stimulation of human peripheral lymphocytes by Zn^{2-} *in vitro*. Exp. Cell. Res. 61:229-230.

Klingman, D. and D. C. Hilt. 1988. The S-100 protein family. TIBS. 13:437.

Klosterhalfen, B., C. Tons, S. Hauptmann, L. Tietze, F. A. Offner, W. Kupper and C. J. Kirpatrick. 1996. Influence of heat shock protein 70 and metallothioneins induction by zinc-bis (DL-hydrogenaspartate) on the release of inflammatory mediators in a porcine model of recurrent endotoxemia. Biochem. Pharmacol. 52:1201-1210.

Kraehenbuhl, J. P. and M. R. Neutra. 2000. Epithelial M cells: differentiation and function. Ann. Rev. Cell. Devp. Biol. 16:301-332.

Kriegesmann, B., S. Jansen, B. Baumgartner and B. Brening. 1997. Partial genomic structure of bovine CD 18 gene and the refinement of test for bovine leukocyte adhesion deficiency. J. Dairy. Sci. 80(10):2547-2549.

Kroneman, J., G. J. W. Van de Mey and A. Helder. 1975. Hereditary zinc deficiency in Dutch Frisian cattle. Zentrabl. Veterinaermed. Reihe A. 22:201-208.

Lachance, P. A. 1998. International perspective: Basis need and application of RDA. Nutr. Rev. 56(4):S2-S4.

Lee, S. H., S. C. Choi, B. J. Chae, S. P. Acda and Y. K. Han. 2001a. Effects of feeding different chelated copper and zinc sources on growth performance and fecal excretion of weaning pigs. Asian-Aust. J. Anim. Sci. 14:1616-1620.

Lee, S. H., S. C. Choi, B. J. Chae, J. K. Lee and S. P. Acda. 2001b. Evaluation of metal amino acid chelates and complexes at various levels of copper and zinc in weanling pigs and broilers chicks. Asian-Aust. J. Anim. Sci. 14:1734-1740.

Lim, H. S. and I. K. Paik. 2003. Effects of supplementing minerals methionine chelates (Zn, Cu, Mn) on the performance and eggshell quality of laying hens. Asian-Aust. J. Anim. Sci. 16:1804-1808.

Mavromichalis, I., C. M. Peter, T. M. Parr, D. Ganessunker and D. H. Baker. 2000: Growth promoting efficacy in young pigs of two sources of zinc oxide having either a high or a low bioavailability of zinc. J. Anim. Sci. 78:2896-2902.

Mavromichalis, I., D. M. Webel, E. N. Parr and D. H. Baker. 2001. Growth promoting efficacy of pharmacological doses of tetrabasic zinc chloride in diets for nursery pigs. Can. J. Anim. Sci. 81:367-391.

Mc Cance, R. A. and E. M. Widdowson. 1942. The absorption and excretion of zinc. Biochem. J. 36:692-696.

Mignotte, B. and J. Vayssier. 1998. Mitochondria and apoptosis. Eur. J. Biochem. 252:1-15.

Milne, D. B., N. V. Ralston and J. C. Wallwork. 1985. Zinc content of blood cellular components and lymphnode and spleen lymphocytes in severely zinc deficient rats. J. Nutr. 115:1073-1078.

Moochegiani, E., L. Santareilli, M. Muzzioli and N. Fabris. 1995. Reversibility of the thymic involution and of age related peripheral immune dysfunction by zinc supplementation in old mice. Int. J. Immunopharmacol. 17:703-718.

Murthy, A. R. K., R. I. Lehrer, S. S. L. Harwig and K. T. Miyasaki. 1993. Invitro candidastatic properties of the human neutrophil calprotectin complex. J. Immunol. 151:6291-6301.

Naresh, R., S. K. Dwivedi, S. Dey and D. Swarup. 2001. Zinc, Copper and cobalt concentration in blood during inflammation of the mammary gland in dairy cow. Asian-Aust. J. Anim. Sci. 14:564-566.

National Research Council. 1981. Nutrient requirements of Goats; Angora, Dairy and Meat goats in temperate and tropical countries. 5th Ed. National Academy Press, Washington, DC.

National Research Council. 1985. Nutrient requirements of sheep. 6th Ed. National Academy Press. Washington, DC.

National research Council. 1989a. Recommended Dietary Allowances, 10th Ed. National Academy Press. Washington, DC.

National research Council. 1989b. Nutrient Requirements of dairy cattle. 6th Ed. National Academy Press. Washington, DC.

National Research Council. 2001. Nutrient Requirements of dairy cattle. 7th revised Ed. National Academy Press, Washington, DC.

O'Quinn, P. R., J. R. Bergstorm, J. L. Nelssen, M. D. Tokach, S. S. Dritz and R. D. Goodband. 1997. The interactive effects between diet complexity, zinc oxide and feed grade antibiotics on performance of segregated early weaned pigs. J. Anim. Sci. 75 (Suppl):192 (Abstr.).

Origino, C. and G. Yang. 1978. Requirement of rainbow trout for dietary zinc. Bull. Jpn. Soc. Sci. Fish 44:1015-1018.

Park, J. H., C. J. Grandjean, D. L. Antoson and J. A. Vander hoof. 1986. Effects of isolated zinc deficiency on the composition of skeletal muscles, liver and bone during growth in rats. J. Nutr. 116:610-617.

Perryman, L. E., D. R. Leach, W. C. Davis, W. D. Mikelsen, S. R. Heller, H. D. Ochs, J. A. Ellis and E. Brummerstedt. 1989. Lymphocyte alterations in zinc deficient calves with lethal trait A 46. Vet. Immuunol. Immunopathol. 21:239-248.

Pimentel, J. L., M. E. Cook and J. L. Gregor. 1991. Immuneresponse of chicks fed various level of zinc. Poult. Sc. 70:947.

Poulsen, H. D. 1995. Zinc oxide for weanling pigs. Acta. Agric. Scandinavica Sect. A 45:159-167.

Poulsen, R. D. and D. Carlson. 2001. Bioavailabality of zinc from different zinc sources. In: proc. 52nd Annual Meeting of European Association of Animal Production, Budapest, Hungary. EAAP publication. p. 123 (Abstr).

Prasad, A. S., J. A. Halsted and M. Nadimi. 1961. Syndrome of iron deficiency anemia, hepato spleenomegaly, hypogonadism, dwarfism and geophagia. Am. J. Med. 31:532.

Prasad, A. S. 1983. Clinical, biochemical and nutritional spectrum of zinc deficiency in human subjects an update. Nutr. Rev. 41:197-208.

Prasad, A. S. 1995. Zinc: An overview. Nutr. 11:93-99.

Price, J. and D. A. Wood. 1982. Zinc responsive parakeratosis and

ill thrift in Friesian cattle. Vet. Rec.110:478.

Rajagopalan, S., C. C. Winter, N. Waghman and E. O. Lung. 1995. The immunoglobulin related killer cell inhibitory receptor binds zinc and require zinc for recognition on HLA-C on target T cells. J. Immunol. 155:4143-4146.

Raulin, J. 1869. Etudes chimique sur Ia vegetation (Chemical studies on plants). Annales des Sciences Naturrelles Botanique at Biologie Vegetale 11:293-299.

Rawer, P., W. R. Willems, T. Breidenbach, W. Guttmann, W. Pabst and G. Schutterle. 1987. Seroconverson rate of hepatitis B vaccination, haemodialysis and zinc supplementation. Kidney. Intn. 22S:149-152.

Rink, L. and P. Gabriel. 2000. Zinc and immune system. Proc. Nutr. Soc. 59:541-552.

Roberts, E. S., E. VanHeugten, K. Lloyd, G. W. Almond and J. W. Spears. 2002. Dietary zinc effects on growth performance and immune response of endotoxemic growing pigs. Asian-Aust. J. Anim. Sci. 15:1496-1501.

Rucker, R. B., B. Lonnerdal and J. L. Keen. 1994. Intestinal absorption of nutritionally important trace minerals. In: Physiology of the intestinal tract, 3rd Ed. (Ed. L. R. Johnson) Raven Press, New York. pp. 2183-2202.

Sazawal, S., R. E. Black, S. Jalla, S. Mazumdar, A. Sinha and M. K. Bhann. 1998. Zinc supplementation reduces the incidence of acute lower respiratory infection in infants and preschool children: a double blind control trial. Pediatrics. 102:1-4.

Schwartzman, R. and J. Cidlowski. 1993. Apoptosis the biochemistry and molecular biology of programmed cell death. Endicr. Rev. 14:133-151.

Scott, M. E. and K. G. Koski. 2000. Zinc deficiency impairs immune response against parasitic nematode infections at intestinal and systemic sites. J. Nutr. 130 (Suppl):1412 S-1420 S.

Sharma, M. C. and C. Joshi. 2002. Serum mineral and hematological profile of microfilaria infected cattle in India. Its effects on production and therapy. Asian-Aust. J. Anim. Sci. 15:357-367.

Shi, H. N., M. E. Scott, M. M. Stevenson and K. G. Koski. 1994. Zinc deficiency impairs T cell function in mice with primary infection of *Heligmosomoides polygyrus*. Parasite Immunol. 16:339-350.

Shi, H. N., M. E. Scott, K. G. Koski and M. M. Stevenson. 1995. Energy restriction and severe zinc deficiency influence growth survival and reproduction of *Heligmosomoides polygyrus* Parasitology 100:599-609.

Shi, H. N., M. E. Scott, M. M. Stevenson and K. G. Koski. 1998. Energy restriction and zinc deficiency impair the function of murine T cells and antigen presenting cells during gastrointestinal nematode infection. J. Nutr. 128:20-27.

Simkin, P. A. J. 1997. Zinc, Again. Rheumatol. 24:626-628.

Singh, C. and S. P. S. Singha. 2002. Effect of dexamethasone stress on concentration of zinc in blood plasma and subcellular fraction of various tissues of neonatal buffalo calves. Asian-Aust. J. Anim. Sci. 15:1022-1025.

Smevers-Verbeke, J., C. May, P. Drochmans and D. L. Massart. 1977. The determination of Cu, Zn. and Mn in subcellular rat liver fractions. Anal. Biochem. 83:746-753.

Sobociniski, P. J., W. J. Canterbury Jr. and M. C. Powanda. 1977. Differential effect of parenteral zinc on the course of various bacterial infections. Proc. Soc. Exp. Biol. Med. 156:334-339.

Sohnle, P. G., C. Collins-Lech and J. H. Wiessner. 1991. The zinc reversible antimicrobial activity of neutrophil lysates and abscess fluid supernatants. J. Infct. Dis. 164:137-142.

Sorderberg, T. A., B. Sunzel, S. Holm, T. Elmros, G. Hallman and S. Sjoberg. 1990. Antibacterial effect of zinc oxide *in vitro*. Scand. J. Plast. Reconstr. Surg. Hand. Surg. 24:193-197.

Spears, J. W. 1989. Zinc methionine for ruminants: Relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. J. Anim. Sci. 67:835-843.

Spears, J. W. and E. B. Kegley. 2002. Effect of zinc source (zinc oxide vs. zinc proteinate) and level on performance carcass characteristics, and immune response of growing and finishing steers. J. Anim. Sci. 80:2747-2752.

Spears, J. W., R. W. Harvey and T. T. Brown. 1991. Effect of zinc methionine and zinc oxide on performance, blood characteristics, and antibody titre response to viral vaccination in stressed feeder calves. J. Am. Vet. Med. Assoc. 199:1731-1733.

Stahl, J. L., M. E. Cook, M. L. Sunde and J. L. Greeger. 1989. Enhanced humoral immunity in progeny chicks from hens fed practical diets supplemented with zinc. Appl. Agric. Res. 4(2):86-89.

Sandstead, H. H. 1994. Understanding zinc: recent observation and interpretation. J. Lab. Clin. Med. 124:322-327.

Sundstrom, M., L. Abrahamsen, P. Antonsson, K. Mehindate, W. Mourad and M. Dohlsten. 1996. The crystal structure of staphylococcal enterotoxin type D reveals $zinc^{2+}$ mediated homodimerization. EMBO. J. 15:6832-6840.

Svetic, A., K. B. Madden, Z. di zhou, P. Lu, I. M. Katona, F. D. Finkelman, J. F. Urban and W. C. Gause. 1993. A primary intestinal helminthic infection rapidly induces a gut-associated elevation of Th-2 associated cytokines and IL-3. J. Immunol. 150:3434-3441.

Tanaka, Y., S. Shiozawa, I. Morimoto and T. Fujita. 1989. Zinc inhibit pokeweed mitogen-induced development of immunoglobulin-secreting cells through augmentation of both CD4 and CD8 cells. Int. J. Immunopharmacol. 11:673-679.

Thiers, R. E. and B. L. Vallee. 1957. Distribution of metals in subcellular fractions of rat liver. J. Biol. Chem. 226:911-920.

Tilden, W. P., E. C. Arthur and S. L. Roberts. 1990. The macro minerals In: feeds and feeding. Prentice Hall, New Jersey. pp. 114-116.

Todd, W. R., C. A. Elvehejem and E. B. Harrt. 1934. Zinc in the nutrition of the rat. Am. J. Physiol. 107:146-156.

Tucker, H. F. and W. D. Salmon. 1955. Parakeratosis or zinc deficiency disease in the pig. Proc. Soc. Exp. Biol. Med. 88:613-616.

Turk, S., S. Bozfakioglu, S. T. Ecder, T. Kahraman, N. Gurel, N. Aysuna, A. Turkmen, N. Bekiroglu and E. Ark. 1998. Effects of zinc supplementation on the immune system and on antibody response to multivalent influenza vaccine in haemodialysis patients. Int. J. Artif. Org. 21:274-278.

Urban, J. F., I. M. Katona, W. E. Paul and F. D. Frinkelman. 1991. Interleukin-4 is important in protective immunity in gastrointestinal nematodes of mice. Proc. Natl. Acad. Sci. USA. 88:3513-3517.

Vallee, B. L. 1955. Zinc and metalloenzymes. Adv. Protein. Chem. 10:317-325.

Vallee, B. L. and J. G. Gibson. 1948. The content of normal human whole blood plasma leukocytes and erythrocytes. J. Biol. Chem. 176:445-457.

Vierboom, M. M., T. E. Engle and C. V. Kimberling. 2003. Effect of gestational status on apparent absorption and retention of copper and zinc in mature Angus cows and suffolk ewes. Asian-Aust. J. Anim. Sci. 16:515-518.

Wellinghausen, N., A. B. Schromm, U. Seydel, K. Brandenburg, J. Lulm, H. Kirchner and L. Rink. 1996a. Zinc enhances lipopolysaccharides induced monokines secretion by a fluidity change of lipopolysaccharides. J. Immunol. 157:3139-3145.

Wellinghausen, N., A. Fischer, H. Kirchner and L. Rink. 1996b. Interaction of zinc ions with human peripheral blood mononuclear cells. Cell. Immunol. 171:255-261.

Wellinghausen, N., H. Kirchner and L. Rink. 1997a. The immunobiology of zinc. Immunol. Today. 18:519-521.

Wellinghausen, N., M. Martin and L. Rink. 1997b. Zinc inhibit IL-1 dependant T cell stimulation. Eur. J. Immunol. 27:2529-2535.

Wellinghausen, N., M. Martin and L. Rink. 1999. Zinc status in patients with alveolar echinococcus is related to disease progression. Parasit. Imm. 21:237-241.

Wight, P. A. L., W. A. Dewar and G. M. Mackenzie. 1980. Monocytes in experimental zinc deficiency of domestic birds. Avian. Pathol. 9:61-66.

Wirth, J. J., P. Praker and F. Kierszenbaum. 1989. Zinc requirement for macrophage function: effect of zinc deficiency on uptake and killing of a protozoan parasite. Immunology. 68:114-119.

Wu, J., J. He and D. Xu. 1998. Effect of zinc deficiency on thymus development and its mechanism in rats. Acta. Nutriment Sincia. 20:303-306.

Zhang, R. and Y. Zhou. 1998. The effect of zinc on immune function in animals. Anim. Sci. Abroad. 25:3-8.

Zhang, R., Y. Zhou, Y. Huang and H. Yang. 1999. The modulation effects of zinc on immune organs development and function in broiler. Acta Veterinaria Zootechnia Sinica. 30:504-512.

Zhou, P. and J. Wu. 1995. The effect of zinc deficiency on T cellular immunity in mice. Acta Nutriment Sincia 17:78-81.