

ROLE OF COPPER AND ZINC SUPPLEMENTATION ON IMMUNITY OF ANIMALS EXPOSED TO LEAD AND CADMIUM: A REVIEW

Kumar, M.¹, Kaur, H.¹, Sk. Asraf H.¹, Sontakke, U. B.¹, Tyagi, A.² and Chandra, G.²

¹Dairy Cattle Nutrition Division, National Dairy Research Institute, Karnal-132001 (India)

²Dairy Cattle Physiology Division, National Dairy Research Institute, Karnal-132001 (India)

Lead and cadmium are the common environmental heavy metal pollutants and have widespread distribution. Both natural and anthropogenic sources including mining, smelting and other industrial processes are responsible for human and animal exposure. These pollutants, many a times, are co-pollutants leading to concurrent exposure to living beings and resultant synergistic deleterious health effects. Several mechanisms have been explained for the damaging effects on the body immune system. Oxidative stress has been implicated in the pathogenesis of the lead and cadmium induced pathotoxicity. Several ameliorative measures to counteract the oxidative damage to the body system during exposure to these toxicants have been assessed with the use of antioxidants in the past one decade. The present review focuses on mechanism of lead and cadmium induced oxidative damages and the ameliorative effect of Cu and Zn supplementation on immune status of animals.

Key words: Copper, Zinc, Lead, Cadmium, Oxidative stress, Reactive oxygen species, Antioxidants, Immune status and Animal

Technological advancement in industry and agriculture has introduced many chemicals such as heavy metals, pesticides, insecticides and herbicides in the environment. Among all the chemical pollutants, toxic heavy metals are of great concern because of their low threshold for toxicity and long persistence in the ecosystem. The diverse deleterious health effect upon exposure to toxic heavy metals in the environment is a matter of serious concern and a global issue. Heavy metals are recognized as environmental pollutants and are released from both industrial and agricultural sources. The intensive use of high-phosphate fertilizers in agriculture leads to increased accumulation of heavy metal ions, especially cadmium, in the soil (Taylor, 1997). Lead and cadmium are the two most abundant toxic metals in the environment. The common sources of lead and cadmium are diverse in nature including natural and anthropogenic processes such as combustion of coal and mineral oil, smelters, mining and alloy processing units, paint industries, etc. The anthropogenic activities and vehicular emissions contribute to the entry of heavy metals to humans' and other animal's food chains (Okada et al., 1997). Heavy metal toxicity is common in farm animals as they get access to lead from soil, water, feed and fodder. Young animals are more susceptible to heavy metal toxicities because of higher rate of absorption from the intestinal tract. On the contrary, their detrimental effects on physiological, biochemical, and behavioral dysfunctions have been documented in animals and humans by several investigators (Goyer, et al., 1979). Presence of heavy metals in the diet of animals binds with the SH and CH₂ groups of essential enzymes, phospholipids and nucleic acids, and interferes with oxidative phosphorylation, which results in accumulation of reactive oxygen metabolites (ROM) such as superoxide (O₂⁻) leading to oxidative stress. Oxidative stress reduces the immunity status of the animals by affecting the cell-mediated immunity and immune cell function.

Supplementation of trace minerals such as copper and zinc can help overcome the adverse effects of heavy metals. In India, commonly fed feeds contain Zn and Cu content below the critical level of 40 ppm and 20 ppm respectively as recommended by NRC (2001) for dairy cattle. A survey conducted on mineral status of feeds and fodders in Haryana (Kaushlendra, 2006) revealed that the Zn and Cu contents in feeds are less

than the recommended level (NRC, 2001).

LEAD CONTAMINATION IN SOIL, FEEDS AND WATER

Lead is a bluish or silvery-grey metal with atomic number 82, atomic weight 207.19 and a specific gravity of 11.34. Earth's crust is the major source of lead in the environment. It enters the food and water supply (Williams et al., 1999) quite naturally and is absorbed by foodstuffs (particularly green leafy vegetables) growing on soil where lead is present. Lead intake occurs either by eating contaminated plants, eating the soil itself, or breathing soil dust. Singhal and Mudgal (1984) observed 14.0, 5.0, 22.0, 5.1 and 12.0 ppm lead in cotton seed cake (CSC), groundnut cake (GNC), mustard cake (MC), maize grains and wheat bran, respectively. Coppock et al. (1988) reported 25.89 ppm Pb in alfalfa and attributed this higher level to soil contamination. In industrial areas of Delhi, air-borne particulates discharged in the smoke from the burning of charcoal and battery scrap was suspected to have settled on animal fodders grown in nearby fields resulting in high lead content (319.0- 1017.0 ppm) and toxicity in livestock (Dey et al., 1996). Drinking water may also be contaminated with lead. Lead soldering in pipes and drinking fountains can leach into the water and contaminate the drinking water especially soft water. Soil water is reported to contain only about 0.05-0.13% of the total soil lead concentration (Davies, 1995). Arvind (2003) reported that lead content varied from 0.10 to 0.17 ppm in water samples collected from some cities of Haryana. The permissible level of lead in drinking water has been reduced from 50 Bg/l (WHO, 1984) to 10 Bg/l (WHO, 1993a).

CADMIUM CONTAMINATION IN SOIL, FEEDS AND WATER

Cadmium is an important environmental pollutant present in soil, water, air and food. Anthropogenic sources add 3-10 times more cadmium to the atmosphere than natural sources (Irwin et al., 2005). Major occupational exposure occurs from non-ferrous smelters during production and processing of cadmium, its alloys and compounds, and the exposure is increasingly become during recycling of electronic waste. Contaminants such as sewage sludge, polluted ground water and mining effluents are important sources of Cd. It may occur naturally or as a contaminant in pigments, batteries, metal coatings, plastics and fertilizers. The availability of Cd to plants from the soil is more than other heavy metals as it is more mobile in the system (Alloway, 1995). High concentration of Cd has been found in forages grown in fields near industrial zinc-plating sites. Setia et al. (1998) reported higher Cd concentration in various crops (greens, mustard leaves, fenugreek, garlic leaves, reddish, coriander leaves and wheat) grown in sewage water vs. tube well water irrigated soils. Nisha (2001) also reported higher level of cadmium in oats and berseem grown under sewage irrigation. Mineral supplements such as phosphate and zinc sources are the most likely source of cadmium exposure for animals (Linden et al., 2003). Cd concentration of five out of eight mineral mixture samples was found above the maximum acceptable limits (Kaur et al., 2007). The Cd concentration in natural surface and ground water is usually less than 1 Bg/l (ATSDR, 1999; Pinot et al., 2000). Datta et al. (1999) reported 1-202 Bg/l Cd content in the groundwater at some places of Delhi near industrial sites, Haryana and Uttar Pradesh. Pond water contained higher cadmium content than other sources of water; however, no sample exceeded the maximum permissible limits (Bharthidasan, 2008).

EFFECTS OF LEAD AND CADMIUM ON OXIDATIVE STRESS AND IMMUNITY

Evidence continues to accumulate on the modulating effects of environmental contaminants (such as organochlorines, oxymetholone, lead, cadmium, mercury and gallium arsenide) on immunity (Mishra, 2009; Ohsawa, 2009). The immune system appears especially sensitive to heavy metals such as lead, cadmium, arsenic and mercury (Fowler, 2009) due to accumulation of ROS such as superoxide (O_2^-) leading to oxidative stress.

Oxidative stress

Sies (1991) reported that oxidative stress in a living organism is a result of an imbalance between reactive oxygen metabolites (ROM) production and neutralizing capacity of antioxidant mechanisms. Oxidative stress is a medical term for damage to animal or plant cells caused by reactive oxygen species, which include superoxide, singlet oxygen, peroxy nitrite or hydrogen peroxide. Oxidative stress leads to peroxidative damage of lipids and other macromolecules (fig.1.), with consequent alteration of cell membranes and other cellular components (Toyokuni, 1999). Oxidative stress can lead to the modification of important physiological and metabolic functions. Oxidative stress can take place by three ways: 1) abstraction of a hydrogen atom, 2) abstraction of an electron or 3) addition of oxygen.

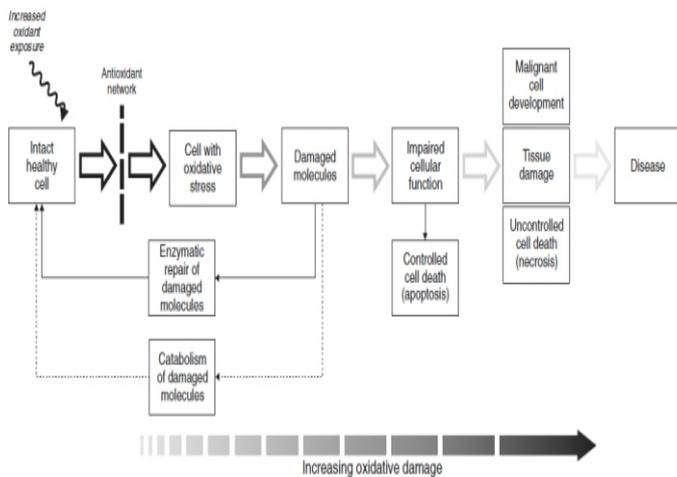


Fig.1. Schematic outline of cellular defences against oxidative stress-mediated cellular damage

Oxidative stress as a possible mode of action for lead and cadmium

Heavy metals result in accumulation of ROS such as superoxide (O_2^-) leading to oxidative stress. ROS damage the lipid bilayer of immune cells, because immune cells have very high level of unsaturated fatty acid such as arachidonic acid.

Mode of lead action

Pb causes oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense system of cells via depleting glutathione, inhibiting sulfhydryl-dependent enzymes, interfering with some essential metals needed for antioxidant enzyme activities, and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition.

Possible mechanisms for lead toxicity include competition with and substitution for calcium, disruption of calcium homeostasis, stimulation of release of calcium from mitochondria, and opening of mitochondrial transition pore. In addition, direct damage to mitochondria and mitochondrial membranes by generation of ROS is also seen. Disruption of tissue oxidant/ antioxidant balance, alteration of lipid metabolism, and substitution for zinc in various zinc-mediated processes are some of the metabolic repercussions of lead toxicity (Ahamed et al., 2007). Lead toxicity development due to calcium interaction has gained considerable

amount of attention by researchers. Calcium blocks the uptake of lead through the intestine because lead is a strong blocker of calcium channels. Lead and calcium compete for the same binding sites on a large family of ion-binding proteins composed of calmodulin and related proteins. Lead acts by displacing calcium ions bound to calmodulin. Lead impairs normal calcium homeostasis and uptake by calcium membrane channels and substitutes for calcium in calcium sodium ATP pumps. Lead also blocks heme synthesis, thereby increasing levels of the precursor aminolevulinic acid (ALA). ALA suppresses GABA mediated neurotransmission by inhibiting its release and also possibly by competing with GABA at receptors. By displacing zinc, lead can alter the regulation of genetic transcription through sequence-specific DNA-binding zinc finger protein or zinc-binding sites in receptor channels (Lidsky and Schneider, 2003).

The δ -aminolevulinic acid dehydrase (ALAD) is highly sensitive to the toxic effects of lead (Farant and Wigfield, 1982). The accumulation of δ -aminolevulinic acid (ALA) upon exposure to lead induces generation of ROS (Lima et al., 1991) and resultant oxidative stress (Bechara, 1996 and Douki et al., 1998a). Lead is shown to alter antioxidant activities by inhibiting functional SH groups in several enzymes such as ALAD, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glucose-6-phosphate dehydrogenase (G6PD) (Valle and Ulmer, 1972). GPx, CAT, and SOD are potential targets for lead toxicity because these antioxidant enzymes depend on various essential trace elements for proper molecular structure and activity (Gelman and Michaelson, 1978). Mercaptides are formed with the SH group of cysteine, that are less stable complexes.

Mode of action of cadmium

Cadmium is a well-recognized environmental pollutant with numerous adverse health effects. Cadmium stimulates the formation of metallothioneins and reactive oxygen species (ROS), thus causing oxidative damage to erythrocytes and various tissues resulting in loss of membrane functions (Sarker et al., 1998). The increase in lipid peroxidation due to Cd toxicity have been attributed to alterations in the antioxidant defense system which includes enzymes such as glutathione peroxidase (GPx), glutathione-S-transferase, superoxide diamutase (SOD) and catalase (CAT), and non-enzymatic molecule like glutathione, which normally protect against free radical toxicity. Cadmium is known to displace Cu, Zn and Fe ions from metalloproteins, resulting in their inactivation as well as the release of free Fe that can catalyze the generation of reactive oxygen species.

Cadmium has various effects on molecules, cells, and organelles. Oxidative stress has often been discussed as a primary effect of Cd exposure. Cell cycle progression, DNA replication and repair differentiation, proliferation, and apoptotic pathways are altered and affected by cadmium. Cd ion enters cells mediated by channels or transport proteins. After entering, Cd induces a decrease or increase (in a dose-dependent manner) in the activities of scavenging enzymes. Metallothionein (MT) and/or phytochelatins are activated as scavengers of the free radicals. Excess free radical production increases the levels of lipid peroxidation and disruption of membranes [cellular, mitochondrial, and chloroplast (plants)]. Furthermore, Cd causes a decrease in intracellular ATP levels, leading to mitochondrial membrane breaking. This causes Ca^{2+} and cytochrome C to escape into the cellular matrix and activate various caspases by binding to Apaf-1 (apoptosis protease activating factor 1) and induce apoptosis and/or necrosis. By activating cellular signals, it regulates cell cycle progression and inhibition of DNA methylation and/or interference with cell adhesion. The RAS signaling pathway involved in cell proliferation and differentiation is one of the primary sites of cadmium action as a consequence of which many genes involved in cell cycle regulation are overexpressed and many proteins are up-regulated. Cadmium also increases the level of several kinases of the RAS pathway, with MAPK being the one of the chief ones. Zn-dependent proteins and Zn-binding molecules are "candidate" targets of Cd toxicity. The chemical similarity of these two ions makes it a possibility that Cd ions can replace Zn and, in so doing, interfere with some of the many Zn reliant processes. Mammalian metallothioneins (MT) are cysteine-rich heavy-

metal-binding proteins that can protect against heavy metals toxicity and oxidative stress. They contain about 30% of cysteine residues, which are known for their ability to chelate free cadmium. Intoxication of cells or animals with cadmium results in an increase of the production of metallothioneins, which belong to the principal pathway of detoxification of this heavy metal.

The next important mode of action relates to the effects of Cd on heat shock proteins (HSPs). Cadmium intoxication alters the expression levels of HSPs, which are active stress responsive proteins. HSPs are cellular chaperone proteins, which can be induced by various environmental stresses including toxic exposure to cadmium. HSP induction is generally considered as an adaptive response of cells to stress, closely linked with cell survival. Denaturation and oxidation of proteins by cadmium are responsible for the overexpression of HSP chaperones (Gaubin et al., 2000). HSPs induction after Cd exposure is also linked to the increase of oxidative stress. Cadmium metallothionein (CdMT) may play a paradoxical role, providing protection against the cadmium ion in the intracellular milieu, but promoting cadmium toxicity when it is present in sufficient amounts in the parts of living systems where absorption is fast. Cadmium probably induces apoptosis through the mitochondrial pathway; Lopez et al. (2003) showed that the decreases in the ATP intracellular levels at the highest concentration of cadmium were accompanied by ATP release, indicating mitochondrial and cytosolic membrane breaking. This mechanism, produced by mitochondrial toxicity with fall in ATP, breakdown of mitochondrial membrane potential, and ROS formation, induces apoptosis with disruption of cellular membranes and necrosis. Cadmium inhibits all of the known pathways of cellular Ca²⁺ influx and acts as a competitive ion to Ca²⁺ at the voltage-dependent Ca²⁺ channels, and it is a potent blocker of the Ca²⁺-dependent neurotransmitter release. This effect on Ca²⁺ influx is due to the interaction of the heavy-metal ion with thiol groups of proteins involved in intracellular Ca²⁺ sequestration. On the other hand, Cd has been reported to elevate the intracellular Ca²⁺ concentrations. This sustained increase is believed to be the main cause for cellular death. In addition to causing cell death, neurotoxicity, and genotoxicity, cadmium targets the DNA repair mechanism itself, thus posing as a great threat among all the known toxic trace elements.

As Giaginis et al. (2006) have suggested, an ample amount of evidence shows that Cd might interfere with DNA repair procedure, leading to increased buildup of damaged DNA bases. The failure of DNA repair systems to correct affected bases can lead to harmful mutations, genomic instability, or cell death.

EFFECT OF LEAD AND CADMIUM ON IMMUNITY

General background of body immune system

The immune system is evolved in vertebrates to protect the body from invasion by ever-changing microbial pathogens. The immune system is divided into innate or non-adaptive or non-specific immune system and the acquired or specific or adaptive immune system (fig.2.).

Innate immunity is composed of physical barriers to infection (skin, mucous membranes in respiratory and other systems, saliva, and physical reflexes such as coughing, sneezing, tear production, inflammation, fever, etc.), phagocytic cells (neutrophils, macrophages, and natural killer cells), and their associated cytokines and acute-phase proteins (Devereux, 2002). Innate immunity has no “memory” effect (i.e., repeated stimulation of the innate immune system provokes very similar responses). Adaptive immunity can be further conceptually divided into two categories i.e. humoral and cell mediated immunity. The cells of the acquired immune system are responsible for synthesizing antibodies, providing memory, and killing invading micro-organisms.

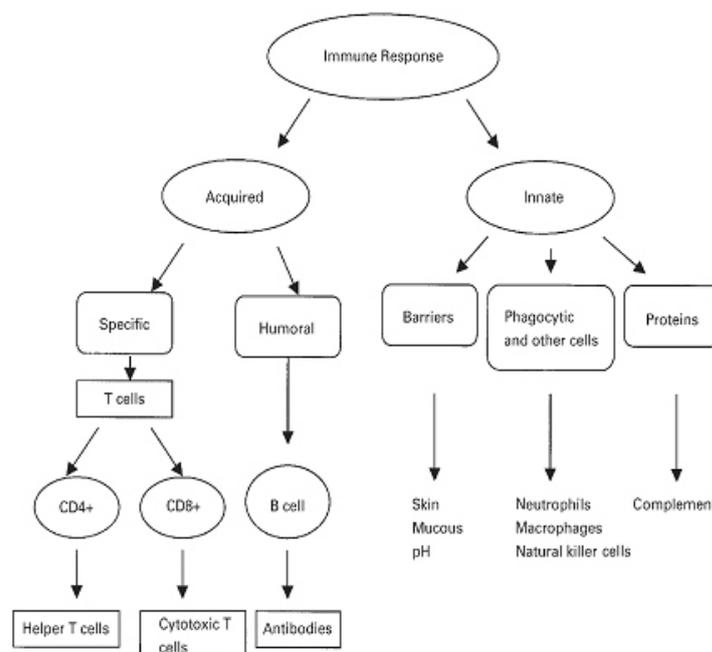


Fig.2 Over view of the immune system

Humoral immunity is that type of immune protection that can be transferred by blood serum. It encompasses antigen receptors called immunoglobulins, or antibodies that are attached to the surface of B cells (Devereux, 2002). Lymphocytes, macrophages and soluble components such as immunoglobulins compose the specific immune response (Sordillo et al., 1997; Tizard, 2000). B cell and T-cell lymphocytes each respond to different antigens and perform different roles. Cell mediated immunity is associated with T-cells. The T-cells differentiate into T-helper (types 1 [Th1] and 2 [Th2]) or T-cytotoxic subclasses, based primarily on the cytokine profile produced by the innate immune system. Th1 cells produce pro inflammatory cytokines and stimulate cell-mediated immunity, whereas Th2 cells produce anti inflammatory cytokines and stimulate humoral immunity. The T-cytotoxic cells attach to and destroy host cells that are infected by pathogens (Carroll and Forsberg,2007).

A series of in vitro studies have demonstrated that exposure of bovine peripheral blood mononuclear cells to Pb and Cd reduced their responsiveness to mitogens or decreased the number of viable cells (Jakway et al.,1971). Pb and Cd are responsible for reducing circulating antibody titers. Antibody synthesis has been decreased by Pb, Cd and As and the activity of B lymphocyte receptors was inhibited by Cd ((Jakway et al., 1971). Lead, and to a lesser extent cadmium, have been the most extensively studied in understanding how heavy metals impair immune function. While the overall effects of lead on antibody production appears to be minimal, if lead dosage and exposure are sufficient it can lead to depressed total antibody levels. In effect, lead results in switching of B lymphocytes from producing IgM and IgG antibody isotopes critical in conferring protection against infectious agents to IgE associated with allergic and hypersensitivity responses (Basaran and Undeger, 2000). However, it is the T lymphocyte subset that appears to be the most sensitive to the toxic effects of lead and cadmium. Lead inhibits antigen presentation through inhibiting specific T lymphocytes (Th1) stimulation while promoting presentation to Th2 lymphocytes (McCabe and Lawrence, 1991; Ohsawa 2009). By either mechanism, the overall effect of is to skew the immune response away from making protective antibody responses against specific pathogens. One of the leading effects of lead is the suppression of the ability to induce delayed type hypersensitivity (DTH) responses upon exposure to a new antigen. Lead, cadmium, arsenic and nickel like heavy metals are responsible for circulating antibody titers (Blakley et al., 1980).

Ameliorative effect of Cu/ Zn supplementation on lead and cadmium induced oxidative stress and immune suppression

Abatement of lead and cadmium toxicity with rebalancing the impaired prooxidant /antioxidant ratio through supplementation of antioxidant nutrients are still not completely clear. However, evidences suggest significant protective effects of antioxidant nutrients such as zinc and copper etc. The immune system appears especially sensitive to environmental contaminants such as lead and cadmium (Dieter and Piepenbrink 2006; Fowler 2009), and while lead exposure at low and moderate levels does not produce overt cellular cytotoxicity, the immune-associated health effects are a result of an impaired regulation of cell function.

Role of copper and zinc as antioxidant

First level of defense against oxidants is antioxidant network. Antioxidants can be broadly defined as any substance that delays, prevents or removes oxidative damage to a target molecule (Halliwell and Gutteridge, 2007). Under physiological conditions, the body usually has sufficient antioxidant reserves to cope with the production of free radicals (Miller et al., 1993; Castillo et al., 2005). Antioxidants function as inhibitors at both the initiation and promotion/progression/transformation stages and protect cells from oxidative damage (Sies, 1991). Mechanisms of antioxidant functions include, 1) preventive antioxidants 2) free radical scavengers 3) sequestration of elements by chelation and 4) quenching active oxygen species. Antioxidants are capable of donating electrons to oxidants and making them harmless.

Functional capabilities of leukocytes decrease due to inadequate levels of essential trace elements Cu and Zn that are needed for optimal antioxidant defenses during times of pro-oxidant challenge (Spears, 2000). Trace elements, such as copper (Cu) and zinc (Zn) are essential components of the endogenous enzymatic antioxidant defenses. Zn deficiency is reported to be associated with in vitro and in vivo oxidative stress (Zago et al., 2001). Chew (1995) reported that antioxidants are very important to immune defense and health of humans and animals. Antioxidants serve to stabilize these highly reactive free radicals, thereby maintaining the structural and functional integrity of cells. Antioxidant therapy provides a potentially important and cheap alternative treatment to diseases related to oxidative stress (Higdon and Frei, 2003; Keane et al., 2003). Zn can occupy iron- and copper-binding sites in lipids, proteins, and DNA and thus exert a direct antioxidant action (Tate et al., 1999). Kumar et al. (2010) also found the decreased value of plasma total antioxidant activity in lead supplemented group, which was however recovered due to addition of Zn in the diet of lead. Zn may play a key role in the suppression of free radicals and in the inhibition of NADPH-dependent lipid peroxidation, as well as in the prevention of lipid peroxidation via inhibiting glutathione depletion (Prasad et al., 1997). Zn is a cofactor of the main antioxidative enzyme Cu, Zn SOD. The zinc molecule in zinc-containing enzymes was found to act as an antioxidant and protect specific regions of the enzyme from free radical attack, thus preserving its stability and activity. The second mechanism by which Zn functions as an antioxidant is through the prevention of free radical formation by other metals, such as iron and copper. When Zn, instead of iron or copper, is incorporated into proteins, free radical generating reactions that may otherwise occur, are inhibited. As continued human population growth and industrialization encroach upon what were once pristine habitats, natural populations of organisms are increasingly exposed to environmental pollutants. Immunocompetence is vital in maintaining the overall health of an organism and is extremely sensitive to toxins, such as heavy metals (Dean and Murray, 1991; McMurphy et al., 1995; Institoris et al., 2001). The immune system is therefore a useful target for environmental risk assessment studies with wild animals.

Ameliorative effect of Cu supplementation

Cu is needed for proper development and maintenance of the immune system including the formation of antibodies and white blood cells. Copper deficiency results in decreased humoral and cell-mediated immunity, as

well as decreased nonspecific immunity (Jones and Suttle, 1981; Lukasewycz and Prohaska, 1983). Dietary Cu affects phagocytic as well as specific immune function regulated by phagocytic cells such as macrophages and neutrophils (Spears, 2000; Weiss and Spears, 2006). Mononuclear cells from heifers receiving the low Cu diet produced less interferon when stimulated with Con A than cells isolated from cows supplemented with Cu. Cu deficiency reduced lymphoid organ weights and lymphocyte proliferative responses (Mulhern and Koller, 1988; Bala et al., 1991) and IgM concentrations (Windhauser et al., 1991), and decreases in neutrophil function (Boyne and Arthur, 1986; Xin et al., 1991). Boyne and Arthur (1981) reported that the respiratory burst and microbicidal activity of bovine peripheral blood neutrophils were decreased by dietary Cu deficiency. Antibody production by spleen cells was significantly reduced in copper deficient animals (Lukasewycz et al., 1990). Copper deficiency resulted in decreases in both numbers and function of lymphocytes derived from the thymus (Lukasewycz, 1985). It has been suggested that the caeruloplasmin in plasma functions as an extracellular scavenger of free radicals and may protect immune cells against inappropriate lipid peroxidation by superoxide and other radicals released from neutrophils and macrophages (Saenko et al., 1994). The diminished activities of these phagocytic cells compromise the innate immune defense system and contribute to greater susceptibility to infections. Bala et al. (1991) showed suppressed proliferative response to T cell mitogens in Cu-deficient animals. Two copper-dependent enzymes, ceruloplasmin and superoxide dismutase, exhibit anti-inflammatory activity and may play critical roles in the prevention of oxidative tissue damage resulting from infection and inflammation (Suttle and Jones, 1986). Copper is involved in the antioxidant system via its involvement in the enzymes Cu/Zn superoxide dismutase (SOD). Kumar et al. (2010) found that mRNA SOD expression was higher in Pb and Cd treated group which was recovered to some extent in Cu and Zn supplemented group.

Ameliorative effect of Zn supplementation

Zinc is a trace element essential for living organisms. It plays an important role in the DNA replication, transcription, and protein synthesis, influencing cell division and differentiation (Frederickson, 1989). Zn has a relationship with more than 300 enzymes in the body and can prevent cell damage through activation of the antioxidant system (Powell, 2000). It is an essential component of the oxidant defense system and functions at many levels. Antibody synthesis is decreased by Pb by inhibiting the activity of B lymphocyte because B-cells are involved in the production of antibodies or immunoglobulins. As Pb is responsible for production of ROS and immune cells are particularly sensitive to oxidative stress. Kumar et al. (2010) found the decreased total immunoglobulin concentration in lead supplemented group which was recovered in some extent in addition of Zn in the diet.

Interactions between zinc and lead have been investigated at absorptive and enzymatic sites (Flora et al., 1982). Zinc and lead compete for similar binding sites on the metallothionein-like transport protein in the gastrointestinal tract. (Kagi and Vallee, 1961). The competition between zinc and lead might decrease the absorption of lead, thus reducing lead toxicity. Dietary supplementation with zinc and in combination with ascorbic acid (Papaioannou et al., 1978) and thiamine (Flora et al., 1989) reduces lead toxicity in humans and animals. In another study, zinc was administered to lead-exposed rats along with chelating agents CaNa₂EDTA, succimer, and D-penicillamine. Zinc enhanced the efficacy of lead chelation by reducing the blood, hepatic and renal lead level, and over turning the inhibited activity on blood ALAD (Flora and Tandon, 1990). A recent study has shown prevention of δ -ALAD inhibition and increased cellular SOD in the testis of lead-exposed rats following zinc supplementation (Batra et al., 1998). The competitive mechanism of interaction and Zn-induced metallothionein induction are the plausible mechanism behind protective effects of Zn against Cd toxicity. This is substantiated by the findings that Cd treatment decreases the testicular Zn concentration and elevates the levels of hepatic and renal metallothioneins (Bonda et al., 2004).

Zn may affect immunity via its important role in cell replication and

proliferation. Zn plays an important role in cell-mediated immunity (Meunier et al., 2005). Zn deficiency and heavy metal exposure reduces potency of both cellular and antibody response of the immune system to infections. Even minute alterations in the Zn level influence T cell development as well as T and B cell functions. Cellular functions, especially the cytokine production are also modulated by Zn. Zn is also known to be associated with enzymes involved in the phagocytic oxidative burst (Chandra and Au, 1980), in cellular maturation and functioning of B and T-lymphocytes. Zinc deficiency is associated with reduced phagocytosis and killing by macrophages and decreased blood lymphocyte population (Fraker and King, 1999) and resulted in atrophy of spleen and thymus. Spears (2000) also observed relationship between Zn status and immune function in cattle. Bartoskewitz et al. (2007) supplemented 1000 ppm Zn and 200 ppm Cu to deers maintained in captivity and observed improved lymphocyte proliferation. Kumar (2000) studied the effect of Pb and Cd on in vitro lymphocyte proliferation. They observed that the cell proliferation in the presence of mitogens was significantly reduced in higher concentration of Cd and Pb treatment as compare to lower concentration. They also found the protective effect of Cu and Zn supplementation over Pb and Cd exposed lymphocytes. Reduced activity of catalase and SOD enzymes in Pb supplemented group might be due to the susceptibility of these enzymes to the oxidative reactive molecules (Pigeolet et al., 1990). Nordenson and Beckman (1981) stated that SOD and catalase are more important radical scavenging enzymes against oxygen metabolites produced by transition heavy metals. Kumar et al. (2010) also found the higher level of antioxidant enzymes (SOD and catalase) in Zn supplemented group as compare to lead supplemented group.

CONCLUSION

Generation of highly reactive oxygen species after lead and cadmium exposure may result in systematic mobilization and depletion of the cell's intrinsic antioxidant defenses. Several mechanisms have been proposed to mediate the oxidative stress caused by lead and cadmium, including disrupted prooxidant/antioxidant balance. Although many investigators have shown lead and cadmium induced immune suppression by oxidative stress and some antioxidants were found to reduce lead and cadmium toxicity, the mechanisms of dietary supplementation of Cu and Zn remain to be further clarified in lead-exposed humans or animals. As less ROS are produced in the cytoplasm, the activities of antioxidant enzymes in the cytoplasm are not as high as the mitochondrial enzymes with lead and cadmium treatments. Thus, more oxidative stress is observed in the mitochondria than in the cytoplasm. Each antioxidant enzyme shows its own pattern of activation or inhibition upon exposure of cells to different concentrations of lead and cadmium. In summary, impaired antioxidant defenses can be a result of the inhibitory effects of lead and cadmium on various enzymes, which in turn causes the cells to be more susceptible to oxidative and immune suppression.

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