The Importance of Copper and the Effects of Its Deficiency and Toxicity in Animal Health

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Abstract

Copper is an important trace element that plays a very important role in the biochemistry of all living organisms and affects enzymes activity as a cofactor or as a fundamental structure of many metalloenzymes as superoxide dismutase, ceruloplasmin, lysyl oxidase, cytochrome oxidase and tyrosinase. Therefore copper is essential for cellular respiration, free radical defence, neurotransmitter function and tissue biosynthesis. Excessive copper accumulation is toxic in all species as it leads to hepatic cirrhosis, hemolytic anemia and degeneration of basal ganglia. The aim of this review is to give a view on the health issue surrounding copper and animal health including many interested points about copper and fetal maternal relationship, the role of copper in the different body system and the effect of its deficiency and toxicity, as well as focusing on the relationship of copper with metalloenzymes, immunity and DNA.

Key words: Copper, Metalloenzymes, Deficiency, Toxicity, Immunity, DNA

Introduction

Copper (Cu) is an essential trace element for all biological organisms, from bacterial cells to human. Depeneding on the source of the biological material, Cu content ranges from parts per billion to parts per million. Cu deficiceicny has been linked to a variety to clinical signs, including pale coat, poor sheep fleece quality, anemia, spontaneous fractures, poor capillary integrity, myocardial degeneration, hypomyelinization of the spinal cord, impaired reproductive performance, decreased resistance to infectious disease, diarrhoea and generalized ill-health (Tessman *et al.*, 2001), causing sever economic losses. Hypocuprosis is the second most widspread mineral deiciciency affects on grazzing animals, many investigations concerning the mechanisms of Cu activity in the body have dealt primarily with the distribution of Cu in various tissues, tha changes which occur in the blood after different conditions and the interrelationships between Cu and variuos enzymes systems, vitamins and minerals .

Cerone *et al.* (2000) explained that Cu is an essential trace element that has an important role in many physiological functions in nervous, hematological, cardiovascular, reproduction and immune systems. Moreover, Cu plays a significant role, being associated with specific proteins. The majority of the biological functions of Cu are believed to be associated with copper's role as a ligand in the active site of metalloenzymes. Among the principal enzymes, ceruloplasmin (a plasma glyco-protein, may function as a Cu transport and as an antioxidant), Dopamine-β-monooxygenase (located in noradrenergic neurons and involved in conversion of dopamine to norepinephrine), Cytochrome-c-oxidase (the terminal mitochondrial electron carrier), lysyl oxidase (responsible for oxidative deamination of peptidyl lysine), Cu- Zn-Superoxide dismutase (a cytosolic protein that speeds up the dismutation of superoxide) and Tyrosinase (located in melanocytes and involved in the conversion of tyrosine into melanin) and Cu is needed for proper development of antibodies and white blood cells, in addition to antioxidant enzyme production (Sharma *et al.*, 2005). Cu deficient goats are more susceptible to be infected by infectious diseases and do not respond as well to the vaccinations, in addition, they tend to be less resistant to parasitic challenge. Goats receiving proper Cu nutrition tend to be less susceptible to infections and have less severe infections when disease does occur.

The focus of this review article is the health issue surrounding Cu and animal health including many intereseted points about Cu and fetal maternal relationship, the role of Cu in the different body systems and the effect of its defeciency and toxicity, as well as focusing on the relationship of Cu with metalloenzymes, immunity and DNA.

Cu and Metalloenzymes

Cu is essential both for its role in antioxidant enzymes, like Cu/zinc superoxide dismutase and ceruloplasmin, as well as its role in lysyl oxidase, essential for the strength and integrity of the heart and blood vessels. With such a central role in cardiovascular health, Cu has been generally overlooked in the debate over improving the cardiovascular health. Cu deficiency has produced many of the same abnormalities present in cardiovascular disease. It seems almost certain that Cu plays a large role in the development of this killer disease, not because of its excess in the diet, but rather its deficiency (Al-Bayati *et al.*, 2015).

The biochemical role for Cu is primarily catalytic, with many Cu metalloenzymes acting as oxidases to achieve the reduction of molecular oxygen. Many Cu metalloenzymes have been identified (Harris and Gitlin, 1996). Diamine oxidase inactivates histamine release during allergic reaction, monoamine oxidase is important in serotenin degradation to metabolites and in the metabolism of catecholamines (epinephrine, norepinephrine and dopamin), lysyle oxidase uses lysine and hydroxylysine found in the collagen and elastin as substrates for posttransational process to produce cross-linkage needed for the

development of conective tissues including those of bone, lung and the circulatroy system, as wll as lysyle oxidase activity in the skin, which declined with low dietary Cu and increased with repletion, is potentaiily a useful indicator of Cu status. .

Cu is an essentail part of ferroxidases enzymes found in plasma (Gambling *et al.*, 2011a), with a function in ferrus ion oxidation that is needed to achieve iron binding to transferrin. Ferroxidase I, also called ceruloplasmin, is the predominant Cu protein in plasma and may also have antioxidant functions. Defects in ceruloplasmin function produce cellular iron accummulation, a result that support its ferroxidase role (Harris and Gitlin, 1996). Ceruloplasmin concentration is also a reliable indicator of Cu deficiciency as it carries between 60-95% of serum Cu, and changes in serum Cu concentration usually parallel the ceruloplasmin concentration in the blood (Prohaska, 2011). Each molecule of ceruloplasmin contains six to eight atoms of Cu which infleunce its biological activity and it have been isolated from several animal species including man and have a simillar chemical structure (Crisponi *et al.*, 2010), biochemical properties of ceruloplasmin such as optimal pH are often similar in various animal species.

The impact of Cu deficiency on the antioxidant system and oxidative damage of cellular components has been reported in several species and tissues, as well as in cultured cells. Ceruloplasmin is the main cupremic determinant and appears to be one of the enzymes most sensitive to Cu deficiency (Hussein and Staufenbiel, 2012). In this sense, it is well known that low ceruloplasmin levels are related to an increased susceptibility to infections and tissue injuries. It has been suggested that Ceruloplasmin in plasma acts as an extracellular scavenger of free radicals and may thus protect the cells against reactive oxygen species released from neutrophils and macrophages (Picco *et al.*, 2004).

In domestic animals, ceruloplasmin has been used diagnostically to investigate Cu deficiency (El-khaiat. *et al.*, 2012). Animals treated with Cu preparations show increased ceruloplasmin activity (Dezfoulian *et al.*, 2012) and blood Cu concentrations, however a number of variables must be considered to fully evaluate the Cu status. Similar correlations between ceruloplasmin and blood Cu concentrations have been reported by other investigators as well as ceruloplasmin appears to be a useful indicator of nutritional Cu status in cattle and sheep. Ceruloplasmin activity and the serum or plasma Cu concentration decrease with nutritional Cu depletion of cattle (Hepburn *et al.*, 2009) .

In cattle, correlation coefficients as high as 0.93 have been observed using plasma samples while in sheep, comparisons between serum ceruloplasmin and whole blood Cu concentrations have also produced good correlations (r = 0.75) and since ceruloplasmin was reported to contain greater than 95% of the circulating Cu in normal animal (Nakazato *et al.*, 2014), ceruloplasmin synthesis occurs in the liver, a major site of Cu storage, it could be expected that ceruloplasmin activity would be a useful indicator of hepatic Cu concentrations. Plasma has more ceruloplasmin activity than serum, suggesting a relatively greater sequestration of ceruloplasmin than Cu during the clotting process.   Under routine

diagnostic laboratory conditions, ceruloplasmin appears to be a useful diagnostic aid to evaluate Cu status in cattle and sheep . Ceruloplasmin may function as an antioxidant in two different ways: by binding to Cu, ceruloplasmin prevents free Cu ions from catalyzing oxidative damage. The other way is through the oxidation of ferrous iron by ceruplasmin, facilitating iron load into its transport protein, transferrin, and preventing free ferrous ions from participating in harmful free radical generating reactions (Al-Bayati et al., 2015).

Two forms of superoxide dismutase are expressed in mammalian cells, a mangano and Cu /zinc form (Al-Bayati et al., 2015, Nakazato et al., 2014). There is substantial documentation from animal studies that diets low in Cu reduce the activities of many of these Cu metalloenzymes. Activities of some Cu metalloenzymes have been shown to decrease in Cu depletion. Erythrocyte superoxide dismutase activity, though not as specific as serum Cu while, ceruloplasmin concentration, may be a reliable indicator of Cu status, and some suggest it is more sensitive .

The consequences of hypocuprosis include a failure of Cu metaloenzymes many of which form part of the antioxidant defence system. Cu is associated with several enzymes, either as a cofactor or as an allosteric component. Cu acts as an electron transfer intermediate in redox reactions, being an essential cofactor for oxidative and reductase enzymes (Uauy *et al.*, 1998). Cu, as well as other essential trace elements, is an atypical antioxidant because it works indirectly. Cu /Zinc superoxide dismutase catalyzes dismutation of the superoxide anion, producing molecular oxygen and hydrogen peroxide, with the latter product usually metabolized by glutathione peroxidase and catalase. The ferroxidase activity of caeruloplasmin mediates the oxidation of ferrous ions to the ferric state, thereby preventing ferrous ion-dependent formation of hydroxyl radicals via the Fenton reaction. Thus, in enabling Cu /Zinc superoxide dismutase and caeruloplasmin to function, Cu can be classified as part of the antioxidant defence system of cells (Pan and Loo, 2000). The impact of Cu deficiency on the antioxidant defence system and oxidative damage to cellular components. The activity of Cooper/Zinc superoxide dismutase, catalase and glutathione peroxidase is decreased in animals with Cu deficiency and increased in animal with Cu supplementation (Genther and Hansen, 2014). Machado *et al.* (2014$_b$) found that supplementaion with trace mineral containing Cu increased serum superoxide dismutase activities in lactating Holstein cows.

Cattle hypocuprosis was associated with a decrease in Cu /Zinc Superoxide dismutase,, ceruloplasmin and cytochrom oxidase activity and with an increase in lipid peroxidation (Picco et al., 2004). Collectively, these studies indicate that Cu deficiency weakens the antioxidant defense mechanism. Several enzymes with antioxidant activity which do not require Cu as a cofactor, such as catalase and glutathione peroxidase, are known to be negatively influenced by Cu deficiency, increasing free radicals generated in the cells . Cu deficiency impairs secretion of tyrosine hydroxylase and dopamine beta enzyme systems,

which are both Cu-containing in hypothalamic neurons. This causes inhibition of synthesis of thyroid hormone-releasing factor (Sharma *et al.*, 2008). Soetan *et al.* (2010) demonstrated that Cu is a constituent of enzymes like cytochrome-c-oxidase, amineoxidase, catalase, peroxidase, ascorbic acid oxidase, plasma monoamine oxidase, erythrocuprin (ceruloplasmin), lactase, uricase, tyrosinase and cytosolic superoxide dismutase. Moreover, Cu is necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems, helps in the incorporation of iron in hemoglobin, assists in the absorption of iron from the gastrointestinal tract and in the transfer of iron from tissues to the plasma. Fry (2011) demonstrated that Cu is required for the activity of superoxide dismutase and that two Cu ions are bound to the enzyme. This enzyme is responsible for the reduction of the superoxide to hydrogen peroxide and oxygen in the cell.

Cu and Immunity

Cu is known to play an important role in development and maintenance of the immune system which requires Cu to perform several functions, of which little is known about the direct mechanism of action. There are several reports of dysfunction in vitro in immune cells from Cu-deficient ruminants. The ability of peripheral blood granulocytes from Cu-deficient sheep and cattle to ingest Candida albicans in vitro may (Boyne and Arthur, 1986) or may not be impaired, but their capacity to kill the engulfed organism is invariably reduced (Jones and Suttle, 1981). Both deficiency and excessive intake of Cu have been reported to reduce several aspects of immune response in animal models, including neutrophil numbers and its phagocytic activity (Yatoo MI *et al.*, 2013), lymphocyte proliferation, and antigen-specific antibody production (Pocino *et al.*, 1991). Treated calves with Cu had increased neutrophil activity compared with non-treated calves (Teixeira *et al.*, 2014) while (Machado *et al.*, 2014a) found that, Cu supplemented lactating Holstein cow had significant increased serum superoxide dismutase activity although leukocyte function was not affected by supplementation.

Cu deficiency, in general, reduces the effectiveness of the acquired response (Prohaska *et al.*, 1983). Some studies of Cu deficiency, in which ceruloplasmin was almost non-detectable, determined that the Cu-deficient animals were anemic, their thymus weights were significantly lower, and their spleen weights were significantly higher than in normal animals as well as antibody production was significantly reduced in Cu-deficient animals (Paolicchi *et al.*, 2013). Splenocytes in Cu-deficient animals had a reduction in the incorporation of tritiated thymidine into cellular DNA in a standard mitogenic proliferation assay and the mitogen-induced synthesis of DNA was impaired by Cu deficiecny (Percival, 1998).

Cu also is utilized in host immune systems to prevent infection, not only is Cu required for proper development of the immune system (Rowland and Niederweis, 2012), but also, new evidence shows that

Cu is employed at a cellular level to kill invading bacteria (Rowland and Niederweis, 2013). Cu was accumulated in the granulomas of guinea pigs infected with Mycobacterium tuberculosis and that Cu resistance was required for full virulence in Mycobacterium tuberculosis (Wolschendorf *et al.*, 2011). Multicopper oxidases play a crucial role in Cu detoxification in many bacteria, including *Escherichia coli* (Grass and Rensing, 2001), Pseudomonas syringae and Salmonella enterica (Achard *et al.*, 2010).

Reductions in Superoxide dismutase activity in leucocytes from Cu-deficient sheep accompanied by increased release of Superoxide anion, suggesting that superoxide might accumulate and weaken the phagocyte after the pathogen triggers the respiratory burst (Jones and Suttle, 1981). However, in cells from the Cu-deficient bovine, candidacidal activity is reduced before leukocyte superoxide dismutase activity declined, while decreased nitro blue tetrazolium reduction indicated lowered intracellular concentration of superoxide anion (Boyne and Arthur, 1986).

The physiological role of Superoxide may vary between species and with the severity of Cu deficiency, while Cu is an appropriate element with which to begin, since it has been clearly shown to influence resistance of sheep to bacterial infections (Boyne and Arthur, 1986).The Scottish Blackface hill breed, which was naturally susceptible to copper deficiency, and a line selected for low plasma Cu both produced lambs that were highly vulnerable to microbial infections when pasture improvement by liming and reseeding lowered their Cu status.

Schuschke *et al.* (1994) found an increase in the mast cell population in the cremaster muscle of Cu-deficient animals, suggesting that Cu deficiency might alter the distribution of blood cells into tissues or the maturation patterns of the leukocyte population. Cu supplemetation influences the resistance of ruminants to viral infection as bovine herps virus-1(Arthington and Havenga, 2012) rift vally virus (El-khaiat *et al.*, 2013) and *Haemonchus contortus* infection in lambs as it reduced egg per gram feces in Cu supplemented lambs (Leal *et al.*, 2014) and goat (Vatta *et al.*, 2012) while Schafer *et al.* (2015) found that, Cu supplementation enhancing the immune response in lambs experimentally infected with *Haemonchus contortus*. However, it did not reduce egg counts in the feces or the number of adult parasites in the abomasum. Cu supplemntation decreased antibiotic resistence of E-coli in pigs (Agga *et al.*, 2014) and in dairy cows (Scaletti and Harmon, 2012), increased colostrum immunoglobulins and decreased calve mortility during calving specially when it was supplemented in the form of organic trace minerals (Formigoni *et al.*, 2011). Sharma et al. (2008) stated that the antimicrobial activity of the neutrophils from Cu-deficient calves decreased compared with neutrophils from Cu supplemented calves. Zhou *et al.* (2009) mentioned that ruminants with Cu deficiency have lower lymphocytes percentages than normal and tend to have decreased cytokine responses to disease challenge.

Cu and Maternal Fetal Relationship

Pregnancy is a period of rapid growth and cell differentiation for both the mother and fetus. Consequently, it is a period when both are vulnerable to changes in dietary supply, especially of those nutrients that are marginal under normal circumstances (Gambling and Mcardle, 2004). Each fetus is completely dependent on its dam via the placenta for its supply of essential trace elements (Abdelrahman and Kincaid, 1993) , but embyro quality not affected by Cu supplementation during the gestitional period in cattle (Hackbart *et al.*, 2010). Cu is often one of the most limiting trace elements for the fetus and neonate for normal development and it has a major etiologic role in decrease of fetal growth and development (Ergaz *et al.*, 2012). Deficiency of Cu impairs fetal growth and causes serious consequences (Gambling *et al.*, 2011b) and can cause death. Calves normally are born with liver Cu concentrations of approximately 400 ppm, compared with adult concentrations of 200 ppm (Underwood, 1977). When intakes of Cu are deficient, maternal transfer of Cu to the fetus is insufficient for normal development, and abnormalities to the central nervous system (Bastian *et al.*, 2010), skeleton, and metabolism result .

It was reported that there was extraordinary metabolic demands on both the mother and developing fetus associated with gestation because adequate maternal Cu nutritive was essential for normal embryogenesis (Keen *et al.*, 1998). Cu is an essential trace element that plays an important role in the biochemical reactions of the body; however, its requirement and interaction with other minerals is not clearly understood (Solaiman *et al.*, 2001). Hepatic concentrations of trace elements were commonly used to estimate trace element storage pools because dietary intake was rarely available and nutrient interactions affected on the availability or retention trace elements (García-Vaquero *et al.*, 2011).

Maternal liver Cu was negatively correlated with fetal age, (Abd Elghany *et al.*, 2011) while, Graham *et al.* (1994) found that maternal liver Cu was not correlated with fetal size. Fetal liver Cu increased as fetal age increased and was less than to maternal Cu in early gestation and there was no differences between maternal and fetal liver Cu in late gestation, while Gonneratne and Christensen (1989$_b$) found that, fetal liver Cu was significantly higher than that of the maternal liver through gestation, as well as Cu concentration was significantly increased in early gestation than that of late gestation in the fetal liver and kidney (Abdelrahman and Kincaid, 1993) while Richards (1999) found that in the fetal kidney, Cu concentration did not change significantly with gestation.

Ovine maternal and fetal liver Cu were negatively correlated in this and previous reports (Gonneratne and Christensen, 1989b). Presence of significant negative relationship between age of the fetus and maternal liver Cu concentration as well as the relationships between maternal liver and amniotic and allantoic fluid Cu concentrations were significantly negative, while the relationship between age of the fetus and maternal plasma, fetal liver, amniotic fluid, allantoic fluid and fetal kidney Cu concentrations were significantly positive might indicate that, the dam and fetus depended on the maternal liver Cu contents during gestation (Abd Elghany et al., 2011) and liver Cu can be used as an indicator of the Cu status

(Johnston *et al.*, 2014) through gestation and fetuses had a capacity to sequester maternal Cu, even when the dam was Cu deficient (Graham et al., 1994). Parkinson (1981) found that amniotic fluid Cu concentration gradually increased during pregnancy.

Because Cu is essential for development of the central nervous system of the embryonic lamb, an acute maternal hypocuprosis can cause gross brain lesions in the fetal or neonate lamb (Hidiroglou and Knipfel, 1981). Adequate maternal intake of Cu is essential for development of the central nervous system of the embryonic lamb. Consequences of Cu deficiency during intrauterine life may include gross brain lesions, with affected lambs born dead or dying shortly after birth.

Enzootic ataxia of the unborn or the unweaned lamb is primarily from Cu deficiency (Hidiroglou and Knipfel, 1981) a degeneration of myelin in the spinal cord being responsible for the ataxia that frequently affects the hind limbs. The pathological process of enzootic ataxia of lambs suggested a disorder of nervous parenchyma in myelination areas in the form of a spongy inhibition associated with functional vascular disorders. Ewes deficient in Cu give birth to lambs characterized by a partial herniation of the cerebellum such that anteriorly the fissura prima lay beneath the tentorium cerebelli and posteriorly formed a "tail" in the foramen magnum (Suttle and Field, 1969). Lesions in the brain and spinal cord characteristic of enzootic ataxia could be detected as early as 99 days postconception in fetal lambs whose dams were grazing on land where enzootic ataxia (swayback) occurred. However, the characteristic lesion of delayed swayback was not present at birth but developed in the postnatal period (Hidiroglou and Knipfel, 1981).

Administration of therapeutically effective amounts of Cu to the ewe could be delayed until the last month of pregnancy and still be effective in preventing swayback in the offspring. It appeared that an inadequate supply of Cu to the fetus during the last 3 or 4 weeks of gestation can cause swayback. In guinea pigs deficient of Cu that showed gross brain changes at birth, it was postulated that the supply of Cu was inadequate during fetal development to maintain the necessary oxidase activity. Moreover, the deficiency of Cu also might limit synthesis of phospholipid (Hidiroglou and Knipfel, 1981).

Because of high demand for Cu by the developing embryo, the Cu-deficient ewe is unable, apparently, to maintain a Cu reserve adequate for normal functional purposes during late gestation. However, in some cases, Cu-deficient ewes give birth to an unaffected lamb; in these ewes more Cu crossed the placenta than in the ewe giving birth to an affected lamb. There was constant increase in Cu deposition throughout the fetal period and, therefore, an increasing demand for Cu by the fetus. The pregnant ewe appeared to be equipped poorly to protect her lamb against effects of a dietary deficiency of Cu and plasma Cu decreased during pregnancy and again after parturition, perhaps from the physiological disturbances that accompany pregnancy, such as an increase in blood volume and demands of the developing fetus (Abd Elghany et al., 2011).

When Cu is absorbed by the ewe, it is transferred to the liver and converted into hepatocuprein and then into haemocuprein for liberation into the blood stream. It may be that the hepatocuprein or other Cu complexes in the liver govern transfer of Cu from dam to fetus. The concentration of Cu in the mammalian liver is higher at birth than at any other time during life. More than 50% of the total Cu in the body of most newborn lambs is in the liver (Hoffmann, 2009). Much of the Cu in fetal bovine liver is in the mitochondria as a Cu-protein complex called neonatal hepatic mitochondrocuprein, which contains about 40 mg Cu/kg (Graham et al., 1994, Hidiroglou and Knipfel, 1981). Between 15 and 35% of the total hepatic Cu of the sheep fetus, corresponding to most of the cytosol Cu, is in the metallothionein-containing fraction. Cu concentrations in liver increased towards the end of gestation in ewes. (Abd Elghany et al., 2011). Liver Cu begins to decrease soon after birth, presumably from mobilization to meet the needs of other tissues of the growing animal. The substantial store of Cu in the liver of newborn animals would be of advantage when the sole source of exogenous Cu is milk, which is usually low in Cu (Hidiroglou and Knipfel, 1981).

Cu deficiency during embryonic and fetal development can result in numerous gross structural and biochemical abnormalities. Such a deficiency can arise through a variety of mechanisms, including low maternal dietary Cu intake, disease-induced or drug-induced changes in maternal and conceptus Cu metabolism, or both (Keen et al., 1998). High Cu content in most newborn animals has suggested placental transfer and storage before birth.

Cu and DNA

Pan and Loo (2000) showed an increase in DNA damage measured by the comet assay in Jurkat T-lymphocytes cultured with the Cu chelator 2,3,2-tetraamine after exposure to hydrogen peroxide. Cu levels are necessary to maintain the structural integrity of DNA during oxidative stress as Cu has essential role of inhibition of oxidative damage of DNA (Al-Qudah *et al.*, 2010, Picco *et al.*, 2012). However, Cu deficiency in Jurkat T-lymphocytes itself did not have genotoxic effects and the increase of DNA damage and an increase in the frequency of chromosomal aberrations found in hypocupremic animals could be explained by higher oxidative stress suffered by these animals (Picco et al., 2004). In Cu-deficient rat embryos a tendency to higher 8-hydroxy-2-deoxyguanosine concentrations was observed by (Hawk *et al.*, 2003). As well as a clear relationship between Cu deficiency and the yield of DNA damage was observed by (Picco et al., 2004).

Excess Cu can be lethal which acts predominantly through formation of highly reactive hydroxyl radicals by Fenton type reaction which damages DNA and other macromolecules (Banu *et al.*, 2004), further studies also found that in chromatin isolated from frozen calf thymus has been reported to contain 25ng of tightly boound Cu while, (Sagripanti *et al.*, 1991) reported that there was an average of one Cu atom bound for every two nucleotides equivalent to 1.2μmol/mg of double DNA as well as (Prasad *et al.*, 2006)

concluded that direct interaction of transitional metal (Cu) to DNA in the presence of hydrogen peroxide caused destabilization and fragmentation of chromatin structure i.e. when DNA was not protected within a nuclear cellular milieu, nucleotides bases were more prone to interact with transitional metal (Cu) and caused DNA fragmentation. (Linder (2012) cited that studies of the structural integrity of the nuclear matrix associated with chromosomal DNA indicated that Cu ions were important for maintaining at least one level of folding of the DNA strands. Moreover, it was clear that nuclei contain a significant proportion of cellular Cu and that much of that was actually bound to DNA bases. Goats with experimentally-induced Cu deficiency had DNA fragmentation as detected by gel electrophoresis and the DNA ladder represented a series of fragments that is multiples of 180–200 bp. which suggested a significant role of Cu deficiency in induction of DNA damage and cell apoptosis in goats (El-khaiat et al., 2013).

Cu Deficiency

Physiologic consequences resulting from Cu deficiency include defects in connective tissue that lead to vascular and skeletal problems, anemia associated with defective iron utilization, and possibly specific aspects of central nervous system dysfunction. Some evidence suggested that immune and cardiac dysfunction which may occure in experimental Cu deficiency and the development of such signs of deficiency had been demonstrated in infants. Cu deficiency causes a disease in lambs called enzootic ataxia (also known as swayback). This disorder, is characterized by spastic paralysis (especially of the hind limbs), severe uncoordination, and anemia. The brains of affected animals are typically smaller than normal, have collapsed cerebral hemispheres and shallow convolutions, and are hypomyelinated (Keen et al., 1998).

Clinical symptoms of Cu deficiency vary and include poor appetite in congenital forms, weakness of limbs, twisted joints, edema, head tremors, incoordination, ataxia, paresis, and paralysis (Ozkul *et al.*, 2012), osteochondrosis (Handeland and Bernhoft, 2004), poor body condition, growth rate and coats . Degeneration and necrosis of the motor neurons in the medulla spinalis and cerebellum as well as demyelinization were also reported in cases of Cu deficiency (Dinev I *et al.*, 2005). Cu deficiency is clearly teratogenic and also induces adverse developmental and neurobehavioral effects.

The nature and magnitude of these effects depend on (a) timing of Cu deficiency during reproduction and development, (b) extent of Cu deficiency, and (c) animal species (Mason *et al.*, 1989). The major target organs for Cu deficiency are the blood and hematopoietic system, the cardiovascular system, connective tissue and bone, the nervous system, and the immune system (Ralph and McArdle, 2001). Reported adverse effects of Cu deficiency include anemia, decreased erythropoiesis and altered hematology,

impaired immune function and neurological development, altered cardiac function and lipid metabolism were recorded by further researches.

Cu is important for thyroid hormones due to its role in synthesis or conversion of thyroid hormones (Abdollahi E. *et al.*, 2013, Bastian et al., 2010). Cu deficiency impairs secretion of tyrosine hydroxylase and dopamine beta enzymes which are both containing, in the hypothalamic neurons, this causes inhibition of synthesis of thyroid hormone eleasing factor (Yatoo MI et al., 2013), so Cu deficiency may have effect on the sexual development and speramatogenesis. In female rats, severe Cu deficiency during gestation induces fetal resorptions or stillbirths . The offspring of pregnant rats given a Cu-deficient diet during gestation have increased postnatal mortality and a high incidence of structural and behavioral abnormalities, including brain lesions, skeletal malformations, cardiovascular lesions, severe growth retardation, convulsions, and hyperirritability to noise. Low Cu levels in cattle can result in many problems from poor hair coat to reduced weight gains, impaired immune system, broken bones , or lower reproduction rates (Murawski *et al.*, 2006) and when deficiency is corrected, they do better.

One of the most visible signs of Cu deficiency is change in hair color. Black animals develop a red tint and red animals become bleached and light colored. The coat becomes dull and animals may be slow to shed in the spring and in young animals, Cu deficiency can result in diarrhea (Shalaby *et al.*, 2010a) and higher incidence of diseases, lameness and poor response to vaccination. Affected animals may have a stiff gait; the ends of the cannon bones may be enlarged and painful, with sore fetlock joints. Pasterns may be upright, with the calf walking on its toes. Bones may be weak and brittle. Heifers may be late reaching puberty and fertility may be impaired. Cows may be slow to cycle after calving. Cattle may develop severe Cu deficiency due to excess of other trace minerals such as molybdenum or sulfur. Deficiency may be primary when there's not enough Cu in the soil or plants grown on those soils, or secondary when other factors prevent utilization of Cu. Elements that bind with Cu to prevent absorption by the body include molybdenum, iron, zinc, sulfur, lead and calcium carbonate . Cu deficiency has been linked to a variety of clinical signs, including pale coat, poor sheep fleece quality, anaemia (Aref *et al.*, 2009), spontaneous fractures, poor capillary integrity, myocardial degeneration, hypomyelinization of the spinal cord, impaired reproductive performance, decreased resistance to infectious disease, diarrhea and generalized ill-health causing severe economic losses. Cu-deficiency in cattle include poor weight gain/weight loss, poor hair coat, pale mucous membranes, anemia, and neonatal ataxia (Tiffany *et al.*, 2002) and impaired reproductive functions ,alterations in cardiac function, anaemia and fragile bones. Ozkul et al. (2012) observed that clinical symptoms of congenital Cu deficiency include poor appetite, weakness of limbs, twisted joints, edema, head tremors, incoordination, ataxia, paresis, and paralysis.

Cu deficiency during pregnancy can result in early embryonic death and gross structural abnormalities in the embryo and fetus, including skeletal, neuronal, pulmonary, and cardiovascular defects. Morphologically, the Cu-deficient embryos were characterized by blisters, blood pooling, heart anomalies, and swollen hindbrain (Hawk et al., 2003). Swayback disease is caused by Cu deficiency and it affects several species of domestic and wild animals. Clinical signs in animals include decreased growth rate, anemia , ataxia, bone disorders, diarrhea, abnormal pigmentation (Hasan *et al.*, 2009, Sharma et al., 2005), and poor reproductive performance. The clinical disease is affected by several factors, including the species, age, and sex of the affected animals, and the duration and severity of the Cu deficiency; ruminants are the species most highly susceptible to Cu deficiency (Adogwa *et al.*, 2005).

Legleiter and Spears (2007) reported that Cu deficiency in the bovine, a wide spread problem in many areas, may result in decreased growth, anemia, weak bones, cardiac failure, depigmentation of hair, and reduced reproductive efficiency. Handeland *et al.* (2008) stated that Cu deficiency causes various disease syndromes in ruminants as general unthrift, poor body condition, growth rates and coats, as well as enzootic ataxia and osteochondrosis. Sharma et al. (2008) founded that the Cu-deficient animals were listless, showed depigmentation of the skin and stiff gait and were anemic and diarrheic. Moreover, the serum haemoglobin values in Cu-deficient animals were significantly lower than in the animals on Cu-rich diet.

Shalaby *et al.* (2010b) mentioned that the wool of Cu deficiency sheep losses its crimp and become steely, the fact that steely wool has more sulphahydryl groups (SH) and fewer disulphide group (S-S) suggests that Cu required for the oxidation of SH to S-S groups in keratin synthesis. Soetan et al. (2010) stated that clinical disorders associated with Cu deficiencies include anemia, bone disorders, neonatal ataxia, depigmentation and abnormal growth of hair or wool, impaired growth and reproductive performance, heart failure and gastrointestinal disturbances.

Cu Toxicity

The importance of Cu in animal health and disease is well documented. Both Cu deficiency and Cu toxicity can occur in natural conditions and may lead to diminished animal production, reproduction (Murawski et al., 2006), various organs dysfunctions (Kumar *et al.*, 2015), development of pathological lesions and, ultimately, to death of the animal. Cu toxicity is caused by an unbalance between the influx of Cu into the body and the excretion of Cu from the body, leading to accumulation of Cu in the liver with consequent liver cell damage. This imbalance may be due to an increased dietary supply of Cu, to an increased availability of the ingested Cu, or to a decreased biliary excretion of Cu. The diagnosis of Cu toxicity was based on toxicological analysis of biological, plant, and soil samples as well as the

supportive medical history, clinical signs, necropsy lesions, liver (Minervino *et al.*, 2009) and kidney concentrations of Cu (Oruc *et al.*, 2009) and microscopic findings in sections of liver.

There are two forms of Cu poisoning - acute and chronic. Acute Cu poisoning can result from the accidental administration of large quantities of Cu (often via oral Cu salts, parenteral Cu administration, or grazing pasture recently fertilised with Cu (Parkinson *et al.*, 2010). Chronic Cu poisoning is associated with the slow accumulation in the liver of smaller amounts of Cu ingested over a long period of time, but with no change in blood Cu levels. When the liver's capacity to accumulate Cu is overloaded, usually after a stressful event, there is a release of Cu into the bloodstream that leads to intravascular haemolysis. Combined with liver damage this causes acute toxicosis and recumbency with affected animals often dying within 24-48 hours; these animals show symptoms of profound depression, thirst, anorexia, pale or icteric mucous membranes and haemoglobinuria (Johnston et al., 2014)

Cu is a well-documented cause of liver toxicity in many domestic species, including sheep, dogs, cats, horses, cattle, goats, pigs, and camelids (Carmalt *et al.*, 2001, Morgan *et al.*, 2014). Sheep is the most sensitive domestic animal to Cu toxicity because their Cu excretory mechanism is less efficient. Acute Cu toxicity is usually seen after accidental administration of excessive amounts of soluble Cu salts, which may be present in anthelmintic drenches, mineral mixes, or improperly formulated rations . In most cases, sheep undergo chronic exposure to copper causing liver necrosis and resulting in massive haemolysis, haemoglobinuria and eventually in renal failure (Mendel *et al.*, 2007).

There are 3 main causes of hepatic Cu accumulation: excessive dietary Cu, inherent defects in Cu metabolism, or impaired Cu excretion in bile. Excessive gastrointestinal Cu absorption may exceed the metabolic capacity for storage in the liver (Oruc et al., 2009), this is the chief mode of Cu toxicity in sheep on pastures rich in Cu -containing plants or deficient in molybdenum (Lorgue G *et al.*, 1996). In domestic animals, most cases of acute Cu toxicity result from the parenteral administration of Cu-containing compounds (Cu glycinate) or the consumption of Cu sulfate–containing footbaths, licks, or salt-mineral mixes (Hoenerhoff and Williams, 2004).

Liver damage is an important feature of all Cu storage diseases. The toxicity of Cu has been demonstrated in in vitro studies and is mainly derived from its ability to bind to sulphydryl groups, nucleic acids, and tubulin, thus impairing such cellular functions as enzyme activity, protein synthesis, and intracellular transport. The elevated blood Cu leads to erythrocyte damage, methaemoglobin production, and Heinz body formation with subsequent haemolysis. According to (Soli, 1980) the possible causes of haemolysis of erythrocytes are three alternative mechanisms: decreased red cell deformability due to Heinzbody formation, chemical and/or mechanical changes in red cell membranes due to Heinz body attachment, and direct oxidative injury to the red cell membrane. Although in his view Heinz body formation may be the principal cause, evidence is available suggesting that Cu may cause lipid peroxidation in the erythrocyte

membrane, leading to its disruption (Hochstein *et al.*, 1980), this process may somehow be related to an inhibition of glycolytic enzymes and a concomitant decrease of the glutathione concentration in the erythrocyte and decrease the activity of glucose-6-phosphate dehydrogenase (Sansinanea *et al.*, 1996).

When Cu is ingested in large amounts in the diet, it may accumulate within the liver over a period of a few weeks to more than a year without clinical signs followed by a sudden release of liver Cu stores with resultant toxicity. Many factors that alter Cu metabolism can influence chronic Cu toxicity by enhancing the absorption or retention of Cu. Chronic Cu toxicity may result from excessive intake of Cu; low intake of molybdenum, sulphur, zinc, or calcium; or liver damage (Hasan et al., 2009). In Cu toxicity, blood Cu concentrations may increase suddenly, causing lipid peroxidation and intravascular hemolysis.

Acute toxicity may follow ingestion of 20 to 100 mg of Cu/kg body weight in sheep, while chronic toxicity of sheep may occur with daily intake of 3.5 mg of Cu/kg body weight when grazing pastures that contain 15–20 mg of Cu/kg on a dry weight basis with concurrent low concentrations of molybdenum and sulfur (Lorgue G et al., 1996) and chronic Cu toxicity was appeared when Cu supplementation was 2mg Cu /kg body weigh daily for 105 days in cattle and buffalos (Minervino et al., 2009). Acute Cu toxicity causes severe gastroenteritis with abomasal erosions and ulcerations, abdominal pain, diarrhea, anorexia, dehydration, and shock.

Hemolysis and hemoglobinuria may develop after 3 days if the animal survives these gastrointestinal disturbances. Icterus usually develops in animals that survive more than 24 hr. The sudden onset of clinical signs in chronic Cu toxicity is associated with the development of a hemolytic crisis. Affected animals exhibit depression, weakness, rumen stasis, anorexia, hematuria, hemoglobinuria, icterus, incoordination, and ptyalism(Oruc et al., 2009). Methemoglobinemia, hemoglobinuria, anemia, and decreased blood glutathione concentrations are usually observed during hemolytic crisis (Mendel et al., 2007) as well as a severe decrease in haemoglobin concentration and haematocrit was recorded (Van Niekerk *et al.*, 1994). Animals with these clinical signs and laboratory abnormalities often die within 1–2 days. Although herd morbidity is often, 5%, usually 75% of affected animals die. Furthermore, losses from Cu toxicity may continue for up to 2 months after the dietary problem has been rectified (Hasan et al., 2009).

Cu storage begins in the centrilobular hepatocytes, where most of the Cu is sequestered in hepatic lysosomes (Rolfe and Twedt, 1995). Lysosomal membranes lose integrity as Cu accumulates, and Cu lysosomal hydrolases are released, irreversibly injuring the cell. Hepatocellular necrosis and apoptosis occur, the accelerated loss of hepatocytes leads to acute massive Cu release causing hemolysis and accumulation of hemoglobin casts in the renal tubules (Lorgue G et al., 1996). Renal tubular hemoglobinuric casts cause ischemia and direct damage to the epithelium, resulting in tubular necrosis. In acute hepatic toxicity, the metabolic capacity of the liver to store Cu is rapidly compromised, and necrosis

occurs rapidly with subsequent renal tubular nephrosis due to hemolytic crisis (Mendel et al., 2007). Bile ductular hyperplasia, bridging portal fibrosis, hepatocellular regeneration are (Bosje *et al.*, 2003, Carmalt et al., 2001) and damage of the morphometrical structure of testes (Babaei *et al.*, 2012) were recorded in Cu toxicity.

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Page 20