ZIBELINE INTERNATIONAL PUBLISHING

ISSN: 2521-0807 (Print)
ISSN: 2521-0424 (Online)

CODEN : MSMAGK

Matrix Science Medica (MSM)

DOI : http://doi.org/10.26480/msm.01.2018.18.21

CrossMark

COPPER DEFICIENCY IN RUMINANTS IN PAKISTAN

Maryam Riaz, Ghulam Muhammad

Department of Clinical Medicine and Surgery, University of Agriculture Faislabad, Pakistan.
****Corresponding Author Email: drmaryamriazvet@gmail.com, profdrgm54@gmail.com***

ARTICLE DETAILS

Article History:

Received 26 June 2018
Accepted 2 July 2018
Available online 1 August 2018

ABSTRACT

Copper (Cu) is a vital micronutrient in all living organisms. It is essential for the proper functioning of the different enzymes like ceruloplasmin, dopamine β, superoxide dismutase, cytochrome c oxidase, lysyl oxidase, monoamine oxidase, tyrosinase, peptidylglycine α, glactose oxidate, ascorbate oxidase and monooxigenase. These enzymes play a vital role in a variety of physiological functions of hematological, nervous, cardiovascular, reproductive, antioxident and immune systems. Hypocuprosis may be due to primary or secondary copper deficiency. The main reason of copper deficiency in Pakistani livestock is most probably due to the presence of dietary Cu antagonist, e.g sulphur and in particular molybdenum. Clinical symptoms of copper deficiency include ataxia, paralysis, paresis, incoordination, head tremors, edema, twisted joints, weakness of limbs, poor quality fleece, bespectacled eyes, fractures, anemia, depigmentation of hair and skin, rough body coats, thin bellies, degeneration of myocardial muscles, ataxia, head tremors and falling disease. In Pakistan copper has been used for the treatment of different health disorders of livestock e.g nutritional hemoglobinuria, parturient hemoglobinuria, mastitis, anestus, leukoderma, chronic diarrhea.

KEYWORDS

Copper, deficiency, enzymes, micronutrient, ruminants.

1. INTRODUCTION

Primary and secondary deficiencies of macro and trace elements are significant impediments to livestock production because of their associations with low production, reproduction and a variety of health disorders. Copper is a vital micronutrient in all living organisms ranging from plants to mammals. It is required in extremely miniscule amounts. This element plays an essential role in a variety of physiological functions of hematological, nervous, cardiovascular, reproductive and immune systems [1]. Cu plays a key role in antioxidant system of the body by virtue of its association in the enzymes Cu-Zn superoxide dismutase (SOD) and ceruloplasmin (ferroxidase). In the cytosol, Cu-Zn SOD is capable of dismutation of superoxide radicals to hydrogen peroxide [2].

Deficiency of Cu in dairy cattle is mostly due to the presence of dietary antagonist, e.g sulphur and in particular molybdenum [3]. As a consequence of antagonism between copper and molybdenum, copper-molybdenum complex formation occurs with poor intestinal absorption of copper [4]. Some researchers reported that production and reproduction in livestock is reduced due to the deficiency of trace minerals in soil and forages [5]. Biological activity of sulphur, iron and molybdenum is increased due to the alkalinity of soils that reduces the availability of Cu [6].

There are many clinical symptoms of copper deficiency including poor quality fleece, poor reproductive performance, fractures, anemia, myocardial degeneration, decreased immunity to infectious diseases and diarrhea [7]. On a global level, copper deficiency is one of the most prevalent mineral deficiencies in ruminants second only to phosphorus [8]. Animals deficient in copper are very prone to infections [9]. The focus of this review article is the health problems in livestock surrounding role of copper in animal health and disease, primary and secondary copper deficiency, normal serum copper levels in dairy cows and buffaloes, copper related studies in Pakistan.

2. ROLE OF COPPER IN ANIMAL HEALTH AND DISEASE

A study stated that in the biochemistry of the living organisms, copper is a very important element [10]. It plays a vital role as a cofactor in the activity of enzymes. It work as a basic structure of metalloenzymes like tyrosinase, superoxide dismutase (SOD), cytochrome oxidase, ceruloplasmin and lysyl oxidase. So, Cu is important for cellular respiration, in function of neurotransmitter, in tissue biosynthesis and defence against free radical.

Copper is important for its role in lysyl oxidase, for the integrity of blood vessels and heart, as well as for its role in antioxidant enzymes like ceruloplasmin and Cu-Zn superoxide dismutase. Copper deficiency (hypocuprosis) plays a big role in the development of cardiovascular disease [11]. Copper deficiency has been investigated by using ceruloplasmin diagnostically in domestic animals [12].

Superoxide dismutase has two forms *viz.,* copper-zinc form and mangano form in mammalian cells [11]. With supplementation of copper, the activity of catalase and Cu-Zn superoxide dismutase increased and with its deficiency, the activity of these enzymes decreased [13]. Cu deficiency in cattle was associated with a decrease in ceruloplasmin, Cu-Zn SOD and cytochrome oxidase activity [14].

Copper is essential for the proper growth of immune system. At cellular level, Cu performs bacteriocidal activity to prevent infections [15]. Ruminants with hypocuprosis have a lower lymphocyte count than those with normal Cu levels and have decreased inflammatory responses to disease [16].

Copper is important in the development of connective tissue in cattle through lysl oxidase enzyme. This enzyme is involved in the construction of inter-moleculer cross linkage in elastin and collagen and thus plays important role in strengthing the collagen and elastin. Heart problems and bone problems have been found in copper deficient animals due to less development of connective tissue. This enzyme also influences bone development in animals. It is also important in cartilage mineralization and formation of collagen in bone. So, it has a direct effect on bone development and growth. Basically, bone strength is dependent upon the cross links of collagen. Due to copper deficiency, there is reduction in cross links of collagen. If proper Cu supplementation is performed then bone problems in livestock can be reversed [10,17].

Copper has effect on the CNS through the enzyme cytochrome-c-oxidase because it is important in the growth of the myelin sheath of nerves. So, Cu is vital in the development of CNS. Cytochrome c activity decreased in the brain of lambs affected with hypocuprosis [17,18]. Appetite regulation in mammals is a complex biological phenomenon. Cu dependent enzymes are involved in the formation of appetite regulating hormones *viz.,* cholecystokinin and gastrin. These enzymes are very important to maintain the balance of appetite regulating hormones which are essential to drive hunger and so important for production and performance of animals [19].

Hypocuprosis causes anemia due to less release of iron leading to less availability of iron for erythropoiesis and many other functions. Cu is necessary for the proper distribution, absorption and release of Fe throughout the body. So, less Fe is available for erythropoiesis [20]. Suttle and Jones stated that copper status also affects the hemoglobin concentration and Heinz body count [21]. In hypocuprosis, Heinz body counts were high and hemoglobin level was significantly low in animals. After copper supplementation, haemoglobin concentration increased and Heinz body counts decreased in sheep.

Table 1: summarizes the major copper dependent enzymes, their functions and clinical significance in the mammalian body.

Enzymes	Biological Functions	Clinical Significance
Ceruloplasmin	Fe^{2+}- Fe^{2+}, copper and iron transport	Anemia
Lysyl oxidase	X-linkages in connective tissue (collagen and elastin)	Aortic rupture, joint problems, in strength of bones
Copper-zinc superoxide dismutase (SOD)	Dismutation of O_2^- to H_2O_2	Antioxidation
Cytochrome c oxidase	Terminal electron transfer and function of central nervous system	Anorexia
Peptidylglycine α-amidating monooxygenase	Elaboration of numerous biogenic molecules	Appetite
Dopamine β monoxygenase	Catecholamine metabolism	Depigmentation
Tyrosines	Tyrosinase to Melanin	Behaviour

Adopted from Underwood and Suttle [17].

Copper has effect on the normal function of immune system in livestock. There are two types of immune cells which are used for defense against pathogens. When copper deficiency is present in animals then these immune cells are reduced, so responses to pathogens are reduced. The superoxide dismutase enzyme has effect on phagocytes function in ruminants. Oxidative damage to phagocytes increases when there is reduction in superoxide dismutase. The engulfing capability of phagocytes can be reduced due to hypocuprosis. When copper deficiency remains for 8 month period in cattle, superoxide dismutase activity is significantly reduced. This correlation between immune function, neutrophils function and copper superoxide dismutase shows the importance of copper in immune system in livestock [17]. Cu associated superoxide dismutase enzyme (SOD) has some functions in the reproductive system of dairy cattle and giving security to the developing and growth of oocyte during early pregnancy against oxidative demage [10,17].

Copper is vital micro element in the pigmentation of skin and hair in mammals. A Cu containing enzyme tyrosinase (enzyme required for the conversion of tyrosine to melanin) is important for the pathway by which pigmentation occurs. So, in copper deficiency, the concentration of tyrosinase is reduced and depigmentation in ruminants can be seen as an early sign of hypocuprosis in ruminants [17, 18]. A study stated that if maternal transfer of copper to the fetus is less for growth, then abnormalities occur in skeleton, metabolism and in central nervous system [22]. In neonate lambs, Cu deficiency can cause gross brain lesions. Unborn and unweand calves can develop enzootic ataxia due to hypocuprosis [23]. Copper is the vital microelement for the fetus for its normal growth [24].

A study shows the functional parts of copper and related enzymes focusing on clinical signs of hypocuprosis. These include degenerative arthiritis, weakness, aortic break in cows, anemia, enzootic ataxia and copper's immune function in battling disease. Variable factors influencing copper intakes, absorption and metabolism influencing copper storage have been described. Copper dietary mineral associations are highlighted with interactions including, iron-sulphur-copper, iron-copper, copper-molybdenum-sulphur, sulphur-copper and zinc-copper. The role of high iron intakes repressing copper storage is of specific significance to tropical environment and is discussed. Next, tissue Cu levels enzootic ataxia, goats and sheep blood copper levels and requirements for copper are put forward [25].

Clinical symptoms of copper deficiency include ataxia, paralysis, paresis, incoordination, head tremors, edema, twisted joints and weakness of limbs [26]. Copper deficiency mainly targets the immune system, CNS and hematopoietic system, connective tissue, bones and the cardiovasculer system [27]. Hypocuprosis can be linked with a variety of clinical signs like low graded sheep wool, pale coat color, anemia, myocardial degeneration, poor reproduction rate, less resistance to infectious diseases and diarrhea [28]. A group researchers reported that weight loss, anemia, pale mucous membranes, enzootic ataxia, poor reproductive performance and soft bones can be linked with copper deficiency in cattle [29].

In domesticated animals, un-even bone development and feeble bones are due to copper deficiency which mostly happens during growth of animals. Seriously copper insufficient calves and sheep will show swelling or broadening of the end of leg bones, and bones will break (fragile bone) easily because of soft bone tissues. Beading at the centre point of the rib cage may likewise be found in Cu lacking sheep and calves because of the over development of the costrochondral junctions [17]. In Cu inadequate red deer, skeletal abnormalities have been reported. Clinical signs and symptoms show a skeletal variations e.g, carpal joints, the outward pivot of the rear legs, swollen hocks, and lameness [30].

3. PRIMARY AND SECONDARY COPPER DEFICIENCY

If copper deficiency is due to complete insufficiency in copper intake, then it is called primary copper deficiency. And if the less supply of copper to pastures is due to some interfering factors then it is called secondary copper deficiency. When copper to molybdenum ratio is 5 to 1 to 10 to 1, cattle perform normally. So, secondary Cu deficiency results due to the excess amount of molybdenum in the presence of sulphur. As thiomolybdate complex is formed it is poorly absorbed from intestine [31]. In cows, scouring or diarrhea is more observable than in sheep as the excreta is for the most part more liquid [17]. Looseness of the bowels in cows is caused by high molybdenum (molybdenosis) levels in the blood. The Mo over-abundance causes a Cu insufficiency [32]. Within 12 hour, dairy cows with extreme diarrhea will react to copper treatment [8,17].
Animals may intake high level of Fe due to soil ingestion or high Fe level in drinking water and high level of Fe in pasture. Fe has been found as antagonist to lower the Cu status of both cow and sheep [33]. Ruminants

may develop signs of copper deficiency (hypocuprosis) in cases where the Cu level >10ppm. This is occurs when copper antagonists are high in ingested diet. The minerals which antagonize Cu are molybdenum (Mo), sulphur (S) and iron (Fe) [34].

Copper supplementation is used in crop and fruits (especially in citrus fruits) production to control different bacterial and fungal disease in Pakistan. In Pakistani soil, availability of micronutrients is very poor. Soil in Pakistan are predominantly calcareous and alkaline. High pH and calcareous soil are responsible for the low availability of the micronutrient. Copper status is 20 to 100 mg/kg (average is 30 to 40 mg/kg), molybdenum 0.2 to 5 mg/kg and sulphur status is 60 to 75 mg/kg in Pakistani soil (www.nfdc.gov.pk).

In northern irrigated zone of Punjab, out of 15 districts, Cu deficiency in blood was reported only in district Sialkot. In southern irrigated zone, no data were available on Cu deficiency. In arid zone, Cu deficiency in animal blood was reported only in district Jehlum [35-39]. Estimated dietary copper requirement is 72.6 mg/day and dietary copper concentration is 12 mg/kg diet in 300kg heifer [40].

4. NORMAL SERUM COPPER LEVEL IN DAIRY COWS AND BUFFALOES

Some researchers reviewed 9 previous studies reporting serum Cu and Zn levels in dairy cows [41]. The reference Cu values ranged from 5.2 to 39 μmol/L. They further contented that the recommended reference values in bovine serum that reflect at least a sufficient dietary supply of Cu and Zn are 8 to 19 μmol/L for both of these trace minerals. The serum Cu level of healthy buffaloes 118.4 ± 5.2 μg/dl [42].

5. COPPER RELATED STUDIES IN PAKISTANI LIVESTOCK

A scanning of available literature indicated that a very little research work has been done thus far on copper status and deficiency in Pakistani livestock. A study focusing on infertility and disease surveillance in district Sahiwal revealed that deficiency of phosphorus and Cu is widespread in this district and that the low reproductive efficiency of cattle and buffaloes is mainly due to deficiencies of these two minerals along with low level of feeding and poor management [43]. Forages accessible at livestock farm Khizerabad, Sargodha, Pakistan were found to be deficient in copper, cobalt and zinc [39]. Deficiencies of Cu, Co, Zn and Se either singly or in combination were responsible for anestrous in dairy buffaloes managed at Buffalo Research Institute, Pattoki, district Kasur, Pakistan [44].

A group researchers reported a marginal deficiency of copper during summer in the sera of lactating goats sampled from district Layyah of Southern Punjab, Pakistan [45]. A research study conducted in urban and peri urban areas of Faislabad, Pakistan, reported that the combined therapy of $ZnSO_4$, $CuSO_4$ and enrofloxain was better compared to as alone treatment with $CuSO_4$ and $ZnSO_4$ for sub- clinically mastitic buffaloes [46]. Abbas surveyed copper deficiency related chronic diarrhea in cattle and buffaloes in urban and peri urban areas of Faislabad, Pakistan [47]. He selected 384 buffaloes and cattle with unknown prevalence. In the study areas, he collected blood samples from animals which were suffering from chronic diarrhea. Serum copper level was determined by atomic absorption spectrometry. It was concluded 89% of cattle and buffaloes in this area were deficient in Cu.

A Pakistani study on parturient hemoglobinuria of dairy buffaloes reported that diseased buffaloes had significantly ($P<0.001$) lower levels (65.4 ± 6.0 ug/dl) of serum copper as compared to serum copper levels (118.4± 5.2 ug/dl) of healthy buffaloes [42]. Hussain reported low serum copper level (0.584±0.114 mg/dl) in 25 dairy buffaloes affected with nutritional hemoglobinuria [48]. The serum copper level is unaffected dairy buffaloes was 0.650± 0.071 mg/dl. Other researchers successfully treated leukoderma affected buffalo with copper sulphate [49].

A study was conducted to determine the micro mineral status of pastures having high population of ruminants in Punjab, Pakistan. Soil and forages samples were collected for two seasons [50]. It was discovered that sampling period influenced soil Cu, Zn and Se while all forage minerals except Se were influenced by sampling times. Seasonal impacts were seen in soil Fe, Mn and Se, and forages Cu, Fe, Zn, Mn and Se. All soil mineral levels with the exception of Co and Se were observed to be over the critical levels and liable to be satisfactory for normal development of plants developing in that, though soil Co and Se were in extreme insufficient levels during the two seasons for the normal plant growth. The levels of Fe, Zn, Co, and Se in soil were higher, while those of Cu and Mn were bring down during winter season than those during summer season. Forages contained marginal inadequate level of Co during winter season, those of Cu and Se during the summer season. Moderate deficient levels of Fe and extreme lacking level of Zn, Mn and Co were found during the summer season. Forages Co, Fe, Zn, Mn and Se during winter season were observed to be satisfactory for the needs of ruminants. Thus, grazing animals at this area require continuous mineral supplementation of these elements to prevent infections caused by supplement inadequacy, and to help ideal animal productivity.

Some researchers has been reported the concentrations of some vital micro elements (Mg, Cu and Zn) in soil, forage and blood plasma of grazing sheep and goats in two fields (managed pastures and unmanaged pastures) in the South Western Punjab, Pakistan during two consective seasons of the year [51]. In light of the data acquired from soil, field forages and plasma it is conceivable that low levels of copper and zinc in soil and forage could potentially restrict ruminant production. It is concluded that mineral supplement may constantly be given to the grazing animals to upgrade the mineral status for boosting the production potential of animals at this region.

A study was carried out to determine the concentration of certain vital elements like Zn, Mg and Cu in soil and forages from two different areas in the field in Sargodha, Pakistan during two consecutive periods of the year [52]. The objective of this investigation was to assess the concentration of vital micro elements like Zn, Mg and Cu in soil and forages in connection to the requirements of animals grazing in that area. In light of these discoveries, it was concluded that field soil should be altered with copper and zinc containing fertilizers and the ruminants feeding on these forages should be consistently supplemented with particularly custom-made mineral mixture containing these elements to stay away from diseases caused by the deficiency of these mineral elements.

REFERENCES

[1] Cerone, S.I., Sansinanea, A.S., Streitenberger, S.A., Garcia, M.C., Auza, N.J. 2000. Cytochrome c oxidase, Cu, Zn-superoxide dismutase, and ceruloplasmin activities in copper-deficient bovines. Biological Trace Element Research, 73, 269-278.

[2] Halliwell, B., Gutteridge, J.M.C. 1999. In: Free Radicals in Biology and Medicine, third ed. Oxford University Press, New York, USA.

[3] Spears, J.W. 2003. Trace mineral bioavailability in ruminants. The Journal of Nutrition, 133, 1506–1509.

[4] Smart, M.E., Cymbaluk, N.F., Christensen, D.A. 1981. A review of copper status of cattle in Canada and recommendation for supplementation. The Canadian Veterinary Journal, 33, 163-170.

[5] Underwood, E.J., Suttle, N.F. 1999. The Mineral Nutrition of Livestock (3rd sd.): CABI Publishing.

[6] Dua, K. 2009. Importance of micronutrient and relevance of their supplementation in buffaloes. Zoological Society of Pakistan, (9), 541-549.

[7] Tessman, R.K., Lakritz, J., Tyler, J.W., Casteel, S.W., Williams, J.E., Dew, R.K. 2001. Sensitivity and specificity of serum copper determination for detection of copper deficiency in feeder calves. Journal of the American Veterinary Medical Association, 218 (5), 756-60.

[8] McDowell, L.R. 2003. Minerals in Animal and Human Nutrition, seconded. Elsevier, Amsterdam, 235–276.

[9] Chew, B.P. 2000. Micro nutrients play role in stress, production in dairy cattle. Feedstuffs, 72, 11.

[10] Hefnawy, A., Elkhaiat, H. 2015. The importance of copper and the effects of its deficiency and toxicity in animal health. International Journal of Livestock Research, 5 (12), 1-20.

[11] Al-Bayati, M.A., Jamil, D.A., Al-Aubaidy, H.A. 2015. Cardiovascular effects of copper deficiency on activity of superoxide dismutase in diabetic nephropathy. North American Journal of Medical Sciences, 7, 41-46.

[12] El-khaiat, H.M., Abd El-Raof, Y.M., Ghanem, M.M. 2012. Clinical, haemato-biochemical changes in goats with experimentally- induced copper deficiency with trials of treatment. Benha Veterinary Medical Journal, 23, 137-147.

[13] Genther, O.N., Hansen, S.L. 2014. A multielement trace mineral injection improves liver copper and selenium concentrations and manganese superoxide dismutase activity in beef steers. Journal of Animal Science, 92, 695-704.

[14] Picco, S.J., Abba, M.C., Mattioli, G.A., Fazzio, L.E., Rosa, D., De Luca, J.C., Dulout, F.N. 2004. Association between copper deficiency and DNA damage in cattle. Mutagenesis, 19, 453-456.

[15] Rowland, J.L., Niederweis, M. 2012. Resistance mechanisms of Mycobacterium tuberculosis against phagosomal copper overload. Tuberculosis (Edinburgh, Scotland), 92, 202-210.

[16] Zhou, L., Long, R., Pu, X., Qi, J., Zhang, W. 2009. Studies of a naturally occurring sulfur induced copper deficiency in Przewalski's gazelles. The Canadian Veterinary Journal, 50, 1269-1272.

[17] Underwood, E.J., Suttle, N.F. 2000. The Mineral Nutrition of Livestock (3rd sd.): CABI Publishing.

[18] Howell, J.C., Gawthorne, J.M. 1987. Copper in Animals and Man. Vol 1: CRC Press Inc.

[19] Underwood, E.J. 1999. Trace Elements in Human and Animal Nutrition. 3rd Ed. Academic Press, New York, USA, pp: 255-305.

[20] Williams, D.M., Scott, W.F., Green, G.B. 1983. Hepatic iron accumulation in copper-deficient rats. British Journal of Nutrition, 50, 653- 660.

[21] Suttle, N.F., Jones, D.G. 1987. Heinz body anemia in lambs with deficiencies of copper or selenium. British Journal of Nutrition, 58, 539-548.

[22] Bastian, T.W., Prohaska, J.R., Georgieff, M.K. 2010. Perinatal iron and copper deficiencies alter neonatal rat circulating and brain thyroid hormone concentrations. Endocrinology*,*151**,** 4055-4065.

[23] Hidiroglou, M., Knipfel, J.E. 1981. Maternal-fetal relationships of copper, manganese, and sulfur in ruminants. Journal of Dairy Science, 64, 1637-1647.

[24] Ergaz, Z., Shoshani-Dror, D., Guillemin, C. 2012. The effect of copper deficiency on fetal growth and liver anti-oxidant capacity in the Cohen diabetic rat model. Toxicology and Applied Pharmacology, 265**,** 209-220.

[25] Mohammad, A., Osman, N.I., Youssef, F.G. 2016. Review on Copper's Functional Roles, Copper X Mineral Interactions Affecting Absorption, Tissue Storage, and Cu Deficiency Swayback of Small Ruminants. AJAVS, 2 (2), 1-14.

[26] Ozkul, I.A., Alcigir, G., Sepici-Dincel, A. 2012. Histopathological and biochemical findings of congenital copper deficiency: are these similar to those of caprine arthritis-encephalitis? Journal of Veterinary Science, 13, 107-109.

[27] Ralph, A., McArdle, H.J. 2001. Copper metabolism and requirements in the pregnant mother, her fetus, and children. International Copper Association, New York. Journal of Pharmacy and Pharmacology, 6 (1), 30-35.

[28] Aref, N.M., Abd Ellah, M.R., Khamis, G.F. 2009. Some trace elements and antioxidants profile in ill-thrift Friesian calves. Assiut Veterinary Medical Journal, 55, 144-152.

[29] Tiffany, M.E., McDowell, L.R., O'Connor, G.A. 2002. Effects of residual and reapplied biosolids on performance and mineral status of grazing beef steers. Journal of Animal Science, 80, 260-269.

[30] Audige, L., Wilson, P.R., Morris, R. 1995. Osteochondrosis, skeletal abnormalities and enzootic ataxia associated with copper deficiency in a farmed red deer (Cervuselaphus) herd. New Zealand Veterinary Journal, 43, 70-76.

[31] Ahmad, M.W., El Khadraway, H.H., Emtenan, M.H. 2009. Effect of copper deficiency on ovarian activity in Egyptian buffalo- cows. World Journal of Zoology, 4 (1), 41-46.

[32] Mills, C.F., Dalgarno, A.C., Wenham, G. 1976. Biochemical and pathological changes in tissues of Friesian cattle during the experimental induction of copper deficiency. British Journal of Nutrition, 35, 309-331.

[33] Lee, J., Grace, N.D. 1997. A New Zealand perspective on copper, molybdenum and suphur interactions in ruminats. Proc. Of the society of the sheep and beef cattle veterinarians of the NZ Veterinary association, 27 (1), 25-38.

[34] Suttle, N.F. 1974. Recent studies of the copper-molybdenum antagonism. Proceedings of the Nutrition Society, 33, 299-305.

[35] Ali, M.A. 2008. Studies on calving related disorders (dystocia, uterine proplapse and retention of fetal membrane) of the river buffalo (*Bubalus bubalis*) in different agro-ecological zones of Punjab province, Pakistan. Ph.D. Thesis. Department of Theriogrnology, University of Agriculture Faisalabad.

[36] Pasha, T.N., Khan, M.Z., Farooq, U. 2009. Studies on mineral imbalances in the livestock of canal irrigated districts of the Punjab (report). University of Veterinary and Animal Sciences, Lahore, Pakistan.

[37] Akhtar, M.S., Bachaya, A., Lodhi, L.A. 2013. Mineral status of soil, fodder, and in lactating Nili-Ravi buffaloes in irrigated agro-ecological zone of Punjab, Pakistan. International Buffalo Information Centre (IBIC) Buffalo Bulletin, 32 (2), 824-827.

[38] Khan, M.Z., Pasha, T.N., Farooq, U. 2012. Mapping of calcium and phosphorous status of buffaloes in different cropping zone of Punjab Province. Journal of Animal and Plant Sciences, 22 (3), 315-318.

[39] Khan, Z.I., Ashraf, M., Ahmad, N. 2009. Availability of nutritional minerals (cobalt, copper, iron and zinc) in pastures of central Punjab for farm Livestock. Pakistan Journal of Botany, 41, 1603-1609.

[40] NRC. 2001. National Research council, Nutrients requirements of dairy cattle 7th edition 133-136.

[41] Spolders, M., Holtershinken, M., Meyer, U. 2010. Assessment of reference values for copper and zinc in blood serum of first and second lactating dairy cows. Veterinary Medicine International, 1-8.

[42] Akthar, M.Z., Khan, A., Sarwar, M., Javaid, A. 2007. Influence of soil and forage minerals on buffalo (*Bubalusbubalis*) parturient haemoglobinuria. Asian-Australasian Journal of Animal Sciences, 20, 393-398.

[43] Pederson, C.H. 1989. Infertility and disease surveillance using a milk recording scheme in The Sahiwal district of Pakistan. Tropical Animal Health and Production, 21, 263-272.

[44] Akhtar, S.M., Farook, A.A., Mushtaq, M. 2009. Serum concentrations of copper, iron, zinc and selenium in cyclic and anoestrous Nili-Ravi buffaloes kept under farm condition. Pakistan Veterinary Journal, 29, 47-48.

[45] Khan, Z.I., Hussain, A., Ashraf, M. 2007. Micromineral status of grazing sheep in a semi region of Pakistan. Small Ruminant Research, 68, 279-284.

[46] Latif, M.A. 2012. Therapeutic efficacy of zinc and copper alone and in combination with enrofloxacin for the treatment of sub-clinical mastitis in dairy buffaloes. M.Phil. thesis Dept. CMS Faislabad, Pakistan.

[47] Abbas, Q. 2016. A survey of copper deficiency associated with chronic diarrhea in cattle and buffaloes in urban and periurban areas of Faisalabad. M.Phil. thesis Dept. CMS Faislabad, Pakistan.

[48] Hussain, S. 2016. Investigation on serum copper levels in nutritional haemoglobinuria in buffaloes (Bubalus Bubalis) in Urban and Peri- urban areas of Faislabad.

[49] Muhammad, G., Zia, T., Razzaq, A. 1998. Successful treatment of leukoderma in a buffalo heifer with prolonged administration of copper sulphate. Pakistan Veterinary Journal, 18 (4), 227-228.

[50] Khan, Z.I., Hussain, A., Ashraf, M. 2005. Evaluation of variation in soil and forage micro-mineral concentrations in a semiarid region of Pakistan. Pakistan Journal of Botany, 37 (4), 921-931.

[51] Khan, Z.I., Ashraf, M., Ahmad, K. 2008. A Comparative study on mineral status of blood Plasma of small ruminants and pastures in Punjab, Pakistan. Pakistan Journal of Botany, 40 (3), 1143-1151.

[52] Ahmad, K., Khan, Z.I., Shaheen, M. 2012. Dynamics of magnesium, copper and zinc from soil to forages grown in semiarid area in Sarghodha, Pakistan. Legume Research Journal, 35 (4), 294-302.