A PRELIMINARY STUDY ON COPPER AND ZINC LEVELS AND ASSOCIATED HAEMATOLOGICAL CHANGES IN THE BLOOD OF GOATS SLAUGHTERED AT BODIJA ABATTOIR, NIGERIA

JUBRIL, Afusat Jagun, KADRI, Zaharat Olamide and ADEKUNLE, Latifat Ajoke
Department of Veterinary Pathology, University of Ibadan, Ibadan, Oyo State, Nigeria.

Corresponding Author: Jagun, A. J. Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria. **Email:** afusatjagun@yahoo.com **Phone:** +234 8034701005

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ABSTRACT

Copper and zinc are two micro-minerals important for maintenance of health and optimal production. This research sought to establish the levels of copper and zinc in goats to provide data for further studies on micronutrients, their interaction and deficiency states. Health status of sampled goats were clinically evaluated. Blood samples were collected through jugular venu-puncture. One (1) ml of each blood sample was acid digested with concentrated nitric acid, assayed for zinc and copper using atomic absorption spectrophotometer. Haematology was also done using whole blood as a health index. Most of the goats looked apparently healthy, while few goats showed emaciation, crusty skin lesions, varying degrees of achromotrichia (change in coat colour) and rough hair coats. 89.9 % had hypocupraemia, while none of the sampled goats showed zinc deficiency. However, zinc toxicity occurred in 84.4 % of the sampled goats when compared to reference values. Haematology showed varying result, ranging from monocytic hypochromic anaemia to polycytaemia. All anaemic animals were found to be deficient in copper. The study has highlighted the presence of copper deficiency and or zinc toxicity in goats and forms a preliminary data to the study of the fate of small ruminant health in the face of micronutrient imbalance.

Keywords: Copper, Zinc, Micronutrient, Haematology, Hypocupraemia*,* Achromotrichia*,* Goats

INTRODUCTION

Micro minerals are mineral elements required in minute quantities for the maintenance of health and immunity of man and animals (Yatoo *et al.,* 2013). There are seven important micro minerals required in the diet of animal and these include; iron, copper, zinc, cobalt, iodine, manganese and selenium (NRC, 2003). Iron, copper and zinc have been reported to be important micro minerals, required in adequate amounts in the diet of grazing animals to make a balanced diet and for maintenance of health and optimal production. Micro minerals perform several important functions in the body system. They act as cofactors of enzymes such as superoxide dismutase, glutathione peroxidise, glutathione reductase etc. (Antonyuk *et al*., 2009) which are important enzymes in maintenance of immunity of an animal (Tomlinson *et al*., 2004). They act as antioxidants and thus prevent oxidative stress. They also help to maintain proper homeostatic mechanisms and are essential for a large number of digestive, physiological and biosynthetic processes within the body (Close, 1998) thus contributing to proper growth, production and reproduction of animals Boland and Lonergan, 2003). Blood parameters are often used in assessment because they are significantly correlated to nutritional status and some trace minerals (Claypool *et al*., 1975; Levander, 1986; Mills, 1987) and blood is less invasive for sampling than liver (Ayman, 2007).

Many studies in mineral nutrition and metabolism have resulted in establishing requirement guidelines for different animal species (Haenlein, 1980; Puls, 1988; Corah, 1996; Fernandes *et al.*, 2012). Copper and zinc levels in small ruminant have been determined in health and diseases. The deficiency states of copper and zinc could be further studied and associated with disease conditions in goats.

Copper performs essential roles in an animal's health largely because it allows many critical enzymes to function properly (Harris, 2001). It is a component of cytochrome oxidase which is important in oxidative phosphorylation and electron transfer (Fisher, 2008). It also functions in copper-zinc superoxide dismutase which is an important antioxidant defense (Suttle, 2010). Its physiological functions include; cellular respiration, bone formation, connective tissue development (McDowell, 1992; Underwood and Suttle, 1999). Given these numerous roles, problems associated with copper deficiency include; anaemia as a result of reduced iron mobilization, spontaneous bone fracture, poor hair pigmentation, reduced growth and reduced fertility (Legleiter and Spears, 2007), while copper toxicity presents symptoms such as; weight loss, depression, weakness, lethargy, jaundice and dehydration (Johnston *et al.*, 2014).

Zinc on the other hand act as a component of numerous metalloenzymes, influences transcription and cell replication (Prasad, 1996; Prasad, 2004; Vázquez-Armijo *et al.*, 2011). It is important for catalytic activity of more than 200 enzymes (Sandstead, 1994). It also plays a role in immune function, wound healing, protein synthesis, DNA synthesis and cell division (Prasad, 1996). Zinc ions are effective antimicrobials even at low concentrations (Osredkar and Sustar, 2011). It is important for immunity, male reproduction, skin and hoof health (Classen *et al.*, 2011). Deficiency symptoms includes; impaired spermatogenesis and development of secondary sex organs in males, and reduced fertility, while toxicity symptoms will include; reduced weight gain and feed efficiency, depressed feed intake, and eventually pica (Vázquez-Armijo *et al.*, 2011).

Extensive research work on micronutrients and especially copper and zinc has been carried out on different species of animals including man. In cattle, copper and zinc has been reported below recommended levels in diets led to decreased productivity, delayed cow-calf production cycle and warranting supplementation (Ansotegui *et al.*, 1994). Larson (2005) had earlier reported that cattle with subclinical deficiency status can continue to reproduce or grow but at a reduced rate, with decreased feed efficiency and a depressed immune system. Stabel *et al.* (1993) also reported that copper deficiencies affect various physiological characteristics that may be important in immunological defence to pathogenic challenge. During stressful periods in cattle, provision of adequate dietary zinc supplementation is critical because stress has been shown to impact negatively on zinc retention (Nockels *et al.*, 1993). Feed intake was shown to be increased after supplying zinc to steer calves that have undergone stress (Spears and Kegley, 1991).

However, there are relatively few original scientific research reported on mineral nutrition metabolism and levels in goats that can be used in monitoring studies and evaluation. Given the importance of micronutrient to the physiology of animals, this project work seeks to establish the levels of copper and zinc in goats for further studies as copper and zinc are one of the most important and essential micronutrient in animals and considering that the deficiency of one element can potentiate the toxicity of the other. The symptoms of deficiency or toxicity of these micronutrients are similar to those caused by infectious disease agents therefore it is important to observe the changes associated with normal health.

Finally, this work aims to serve as a preliminary study for further research on micronutrient and how they interact with other elements and or influence heavy metal toxicity.

MATERIALS AND METHODS

Study Area and Animal: The blood samples from goats used for this study were obtained from the Bodija Abattoir, Ibadan. Ibadan (7°23′47″N 3°55′0″E) is the capital city of Oyo State and the third largest metropolitan city by population in Nigeria, after Lagos and Kano (FRN, 2007). The location of Bodija Market makes it a popular hub for the sales and distribution of livestock and foodstuff from different parts of Nigeria especially Northern Nigeria (Filani, 2005).

A total of 99 adult male goats were selected for this study over a period of seven days from the goats slaughtered at the Bodija Municipal Abattoir, Ibadan. The selected goats were all Red Sokoto breed and the weight ranges from to 12 – 18 kg. The goats were examined antemortem for obvious clinical signs to ascertain their health status.

Sample Collection: Blood samples were obtained from the goats through jugular veni-puncture and stored in lithium heparinised bottles. The samples were preserved in iced pack containers immediately after collection for transportation to the laboratory for analysis.

Sample Analysis: The collected blood was used for micronutrient assay (copper and zinc) and complete haematology.

Micronutrient Assay: 1 ml of the blood was digested in 1 ml of buffered nitric acid. The mixture was boiled at 100^0 C for two hours, filtered and the filtrate collected into universal sample bottles and made up to 25 mls. Copper and zinc concentrations from the digested blood were measured using Atomic Absorption Spectrophotometer (AAS) with graphite furnace and background correction (SR-BDG). All blanks, standards and samples were analysed in triplicates and levels of copper and zinc were compared to the standard. The calibration curve gave a linear response across this range with a correlation coefficient of 0.999. The averages of the triplicate values were calculated from the data.

Haematology: Complete blood cell count was carried out on the collected blood sample for the red cell, leukocyte and platelet using routine haematological methods as described by Duncan *et al.* (1994). Other haematological parameters measured include; packed cell volume using the micro haematocrit. Haemoglobin concentration was estimated using the spectrophotometer. Haematometric indices of mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were also calculated.

Statistical Analysis: All data were analysed for their central tendencies and expressed as means ± standard errors of means (SEM) and percentages. Analysis of variance was used to compare means. P-value of <0.05 was accepted as significant.

RESULTS

Clinical evaluation of the animals showed mostly apparently healthy animals with few showing emaciation, crusty skin lesions, achromotrichia and rough hair coats.

Hypocupremia was recorded in 89.9 % of the sampled goats with only 2 % showing copper toxicity and a mean value of 0.4 ± 0.2 mg/l. Zinc on the other hand had a mean value of 3.7 ± 1.0 mg/l and was found to be toxic in 84.4 % of the sampled goats and no deficiency was recorded. These values are represented in Tables 1 and 2.

Table 1: The levels and distribution of copper and zinc in sampled Sokoto red goats slaughtered in Bodija Abattoir

Levels	Copper	
	Number of goats	Values (ppm)
All	99	0.4 ± 0.2
High	2	1.7 ± 0.1
Marginal	8	0.7 ± 0.2
Low	89	0.3 ± 0.1*
Reference	-	0.6 – 1.2[1]
	Zinc	
All	99	3.7 ± 1.0
High	84	4.0 ± 0.9
Marginal	15	2.1 ± 0.3
Low	0	-
Reference	-	0.8 – 2.7[2]

[1]*Suttle (2010)*, [2]*Plumb (1999)*

Table 2: Percentage deficiency and toxicity of copper and zinc in Sokoto red goats slaughtered in Bodija Abattoir

Micronutrients	Range	% Deficient	% Toxic
Copper	1.7- 0.3	89.9	2.0
Zinc	4.0-2.1	-	84.4

The haematological findings of the micro nutrient status of the goats are presented in Table 3 for copper and includes; polycythaemia in 21.2 %, macrocytic hypochromic anaemia in 8.1 % and 70.7 % were normal. Zinc levels are presented in Table 4 with polycythaemia observed in 95.2 % of the sampled goats. The haematological profile of Sokoto red goats slaughtered in Bodija Abattoir with varying levels of copper indicated that goats with high levels of copper had higher RBC, WBC, PCV MCV and MCHC among other parameters than goats with minimal and low levels of copper (Table 5). Similarly, the haematological profile of Sokoto red goats slaughtered in Bodija Abattoir with varying levels of zinc indicated that goats with high levels of zinc had higher RBC, WBC, PCV, MCV and MCHC among other parameters than goats with minimal and low levels of zinc (Table 6).

DISCUSSION

A high percentage of sampled goats (89.9%) exhibited copper deficiency, a condition that has been reported as one of the major micro minerals limiting production of grazing animals in Africa (McDowell and Arthington, 2005) and also in humans (McDowell, 2003). Lazzaro (2001) also reported copper deficiency as a serious problem, that can be as important as infectious or parasitic disease, among grazing livestock in tropical countries including Nigeria. Copper deficiencies among ruminants in the tropics has been associated with common clinical signs of disease such as depressed growth, bone disorders, infertility, diarrhoea, hair depigmentation, susceptibility to bacterial infections and anaemia (Mohammed *et al.*, 2014).

Various researchers have reported a mutual antagonism existing between copper and zinc where excess zinc is known to interfere with copper absorption and is regarded as a prime example of competitive biological interactions between trace elements with similar chemical and physical properties. Thus, excessive zinc has been shown to inhibit intestinal absorption, hepatic accumulation and placental transfer of dietary copper as well as to induce clinical and biochemical signs of copper deficiency (Bremner *et al.*, 1976; Hall *et al.*, 1979; L'Abbé and Fischer, 1984; Yadrick *et al.*, 1989). Zinc toxicity induces the secretion of intestinal protein, metallothionein which has more affinity for copper and certain other elements. This results in copper displacing zinc from the protein in vivo (Bremner *et al.*, 1987). This could result in increased renal uptake and urinary excretion of copper as Cu-metallothionein (Bremner and Beattie, 1995). This may be the case in this study as there was a high percentage of zinc toxicity (84.4 %) as against a high percentage of copper deficiency (89.9 %).

Anaemia recorded for all copper deficient goats in this study was in agreement with Suttle *et al.* (1987) who reported different classes of anaemia in copper deprived animals and also in the report of Mohammed *et al.* (2014) where they stated that the disorders related to copper deficiency are due to the functional roles of copper containing enzymes in cellular respiration, membrane stability, immune function and erythropoiesis. The high prevalence of polycythaemia recorded in this study in both copper deficient and zinc toxic animals is however inconclusive. This unusual blood picture may be related to the long distance of transportation and associated transport stress and accompanying dehydration, the animals understudied were subjected to prior to blood sample collection (Minka and Ayo, 2010). But there was an increase in MCV and a decrease in MCHC in both copper deficient and zinc toxic samples indicating a macrocytic hypochromic type of anaemia in agreement with the work of Suttle in 1987 on cows and ewes. This find Suggest that though the blood picture could have been masked by dehydration, some clinical deviations were still apparent.

Table 3: The distribution of copper levels and effects on packed cell volume of Sokoto red goats slaughtered in Bodija Abattoir

Packed cell volume status	Number of goats	Copper Deficiency (%)	Marginal (%)	Copper Toxicity (%)
Polycythaemia	21	95.2	-	4.8
Anaemia	8	100	-	-
Normal	70	88.6	10	1.4

Table 4: The distribution of zinc levels and effects on packed cell volume of Sokoto red goats slaughtered in Bodija Abattoir

Packed cell volume status	Number of goats	Zinc Deficiency (%)	Marginal (%)	Zinc Toxicity (%)
Polycythaemia	21	-	4.8	95.2
Anaemia	8	-	25	75
Normal	70	-	17.1	82.9

Table 5: The haematological profile of Sokoto red goats slaughtered in Bodija Abattoir with varying levels of copper

Haematological Parameters	Copper Levels		
	High	Marginal	Low
Number of goats	2	8	89
PCV (%)	45.5 ±1.5	30.4 ±1.0	33.5 ±1.0
HB (g/dL)	14.6 ±0.4	10.1 ±0.3	12.5 ±1.5
RBC (µL)	12.9 ±0.1	11.2 ±0.1	11.7 ±0.2
MCV (fL)	35.1 ±1.0	26.8 ±1.0	28.2 ±0.5
MCHC (g/dL)	11.2 ±0.3	8.9 ±0.2	10.4 ±1.1
Platelets x10^3 /µL	103 ±1.3	92 ±1.3	122 ±5.0
WBC X10^3/µL	6.0 ±0.5	5.0 ±0.2	7.0 ±0.3
Lymphocytes x10^3/µL	4.0 ±0.4	3.0 ±0.1	4.0 ±0.2
Neutrophiles x10^3/µL	1.0 ±0.1	1.0 ±0.1	0.3 ±0.1
Monocytes x 10^3/µL	0.1 ±0.0007	0.1 ±0.01	0.2 ±0.01
Eosinophils x10^3 /µL	0.3 ±0.03	0.2 ±0.007	0.2 ±0.01

PCV=packed cell volume, HB=haemoglobin concentration, RBC=red blood cell count, MCV=mean corpuscular volume, MCHC=mean corpuscular haemoglobin concentration, WBC=white blood cell count

Table 6: The haematological profile of Sokoto red goats slaughtered in Bodija abattoir with varying levels of zinc

Haematological Parameters	Zinc Levels		
	High	Marginal	Low
Number of goats	84	14	0
PCV (%)	34.3 ±1.1	29.1 ±1.0	-
HB (g/dL)	12.9 ±1.5	9.5 ±0.3	-
RBC (µL)	11.8 ±0.2	11.1 ±0.2	-
MCV (fL)	28.7 ±0.5	25.9 ±0.5	-
MCHC (g/dL)	10.6 ±1.1	8.4 ±0.1	-
Platelets X10^3 /µL	121 ±5.0	111 ±4.2	-
WBC X10^3/µL	7.0 ±0.3	5.5 ±0.2	-
Lymphocytes x10^3 /µL	4 ±0.2	3 ±0.2	-
Neutrophils x10^3 /µL	2 ±0.1	1 ±0.1	-
Monocytes x10^3 /µL	0.1 ±0.01	0.1 ±0.01	-
Eosinophils x10^3 /µL	0.1 ±0.01	0.1 ±0.01	-

PCV=packed cell volume, HB=haemoglobin concentration, RBC=red blood cell count, MCV=mean corpuscular volume, MCHC=mean corpuscular haemoglobin concentration, WBC=white blood cell count

Conclusion: the present study has highlighted the prevalence of copper deficiency and or zinc toxicity in goats slaughtered for consumption in Bodija, Nigeria. It has also shown the relationship of these states and their haematological picture. It is a preliminary study that will provide the basis and knowledge for further studies of micronutrient imbalance in small ruminants, its role in heavy metal toxicity, the impact of which has been reported in goats in certain areas of heavy mining activity in Nigeria. It is recommended that this project work be carried out under a controlled condition to determine at which level they transform to become deficient and or toxic and the relationship of these imbalances with haematological parameters of the animals.

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REFERENCES

ANSOTEGUI, R. P., SWENSEN, C. K., MILNER T. T., BRYAN, K. S. and PATERSON, J. A. (1994). Effects of chemical form and intake of mineral supplementation on blood profiles and inflammatory reaction to phytohemagglutinin in pregnant heifers. *Proceedings*, *Western Section*, *American Society of Animal Science,* 45: 222 – 225.

ANTONYUK, S. V., STRANGE, R. W., MARKLUND, S. L. and HASNAIN, S. S. (2009). The structure of human extracellular copper–zinc superoxide dismutase at 1.7 Å resolutions: insights into heparin and collagen binding. *Journal of Molecular Biology*, 388(2): 310 – 326.

AYMAN B. M. Y. (2007). *Microminerals level in Grasses, Some Organs and Serum of Camel in Butana Region, Sudan.* Master's Thesis, Department of Animal Nutrition, Faculty of Animal Production, University of Khartoum, Sudan.

BOLAND, M. P. and LONERGAN, P. (2003). Trace minerals in production and reproduction in dairy cows. *Advances in Dairy Technology*, 15: 319 – 330.

BREMNER, I. and BEATTIE, J. H. (1995). Copper and zinc metabolism in health and disease: speciation and interactions. *Proceedings of the Nutrition Society*, 54(2): 489 – 499.

BREMNER, I., HUMPHRIES, W. R., PHILLIPPO, M., WALKER, M. J. and MORRICE, P. C. (1987). Iron-induced copper deficiency in calves: dose-response relationships and interactions with molybdenum and sulphur. *Animal Science*, 45(3): 403 – 414.

BREMNER, I., YOUNG, B. W. and MILLS, C. F. (1976). Protective effect of zinc supplementation against copper toxicosis in sheep. *British Journal of Nutrition*, 36(3): 551 – 561.

CLASSEN, H. G., GRÖBER, U., LÖW, D., SCHMIDT, J. and STRACKE, H. (2011). Zinc deficiency. Symptoms, causes, diagnosis and therapy. *Medizinische Monatsschrift fur Pharmazeuten*, 34(3): 87 – 95.

CLAYPOOL, D. W., ADAMS, F. W., PENDELL, H. W., HARTMANN JR, N. A. and BONE, J. F. (1975). Relationship between the level of copper in the blood plasma and liver of cattle. *Journal of Animal Science*, 41(3): 911 – 914.

CLOSE, W. H. (1998). The role of trace mineral proteinates in pig nutrition. Pages 469 – 483. *In:* LYONS, T. P, and JACQUES, K. A. (Eds.). *Biotechnology in the Feed Industry.* Nottingham University Press, Nottingham, United Kingdom.

CORAH, L. (1996). Trace mineral requirements of grazing cattle. *Animal Feed Science and Technology*, 59(1-3): 61 – 70.

DUNCAN, R. J., PRASSE, K. W. and MAHAFFEY, E. A. (1994). *Veterinary Laboratory Medicine: Clinical Pathology*. 3rd Edition, Iowa State University Press, Ames, Iowa, USA.

FERNANDES, M. H. M. R., RESENDE, K. T. D., TEDESCHI, L. O., TEIXEIRA, I. A. M. A. and FERNANDES, J. S. (2012). Macromineral requirements for the maintenance and growth of Boer crossbred kids. *Journal of Animal Science*, 90(12): 4458 – 4466.

FILANI, M. O. (2005). *Transport Market Study – The Bodija Cattle Market in Ibadan*. Department for International Development, United Kingdom.

FISHER, G. E. J. (2008). Micronutrients and animal nutrition and the link between the application of micronutrients to crops and animal health. *Turkish Journal of Agriculture and Forestry*, 32(3): 221 – 233.

FRN (2007). *Official Gazette, Legal Notice on Publication of the Details of the Breakdown of the National and State Provisional Totals 2006 Census*. Federal Republic of Nigeria (FRN), Abuja.

HAENLEIN, G. F. W. (1980). Mineral nutrition of goats. *Journal of Dairy Science*, 63(10): 1729 – 1748

HALL, A. C., YOUNG, B. W. and BREMNER, I. (1979). Intestinal metallothionein and the mutual antagonism between copper and zinc in the rat. *Journal of Inorganic Biochemistry*, 11(1): 57 – 66.

HARRIS, E. D. (2001). Copper homeostasis: the role of cellular transporters. *Nutrition Reviews*, 59(9): 281 – 285.

JOHNSTON, H., BEASLEY, L. and MACPHERSON, N. (2014). Copper toxicity in a New Zealand dairy herd. *Irish Veterinary Journal*, 67(1): 20. https://doi.org/10.1186/2046-0481-67-20

L'ABBÉ, M. R. and FISCHER, P. W. (1984). The effects of dietary zinc on the activity of copper-requiring metalloenzymes in the rat. *Journal of Nutrition*, 114(5): 823 – 828.

LARSON, C. K. (2005). Role of trace minerals in animal production. *In: Nutrition Conference,* Department of Animal Science, University of Tennessee, Tennessee, USA.

LAZZARO, J. (2001). Basic Information on copper deficiency in dairy goats In Southern California. *California Animal Health and Food* Safety *Laboratory System (CAHFS)*, 14: 345 – 351.

LEGLEITER L. R. and SPEARS, J. W. (2007). Plasma diamine oxidase: a biomarker of copper deficiency in the bovine. *Journal of Animal Science*, 85(9): 2198 – 2204.

LEVANDER, O. A. (1986). Selenium. Pages 209 – 275. *In:* MERTZ, W. (Ed.). *Trace Elements in Human and Animal Nutrition*. 5th Edition, Volume 2, Orlando Academic Press, USA.

MCDOWELL, L. R. (1992). *Minerals in Animal and Human Nutrition*. Academic Press, San Diego, California, USA.

MCDOWELL, L. R. (2003). *Minerals in Animals and Human Nutrition*. 2nd Edition, Elsevier Science, Amsterdam, Netherlands.

MCDOWELL, L. R. and ARTHINGTON, J. D. (2005). *Minerals for Grazing Ruminants in Tropical Regions*. 5th Edition, University of Florida, Gainesville, Florida, USA.

MILLS, C. F. (1987). Biochemical and physiological indicators of mineral status in animals: copper, cobalt and zinc. *Journal of Animal Science*, 65(6): 1702 – 1711.

MINKA, N. S. and AYO, J. O. (2010). Physiological responses of food animals to road transportation stress. *African Journal of Biotechnology*, 9(40): 6601 – 6613.

MOHAMMED, A., CAMPBELL, M. and YOUSSEF, F. G. (2014). Serum copper and haematological values of sheep of different physiological stages in the dry and wet seasons of Central Trinidad. *Veterinary Medicine International*, 2014: Article ID 972074, http://dx.doi.org/10.1155/2014/972074

NOCKELS, C. F., DEBONIS, J. and TORRENT, J. (1993). Stress induction affects copper and zinc balance in calves fed organic and inorganic copper and zinc sources. *Journal of Animal Science*, 71(9): 2539 – 2545.

NRC (2003). *Nutrient Requirements of Nonhuman Primates*. National Research Council (NRC), National Academic Press, Washington DC, USA.

OSREDKAR, J. and SUSTAR, N. (2011). Copper and zinc, biological role and significance of copper/zinc imbalance. *Journal of Clinical Toxicology*, 2011: http://dx.doi.org/10.4172/2161-0495.S3-001

PLUMB, D. C. (1999). *Veterinary Drug Handbook*. Iowa State University Press, Iowa, USA.

PRASAD, A. S. (1996). Zinc deficiency in women, infants and children. *Journal of the American College of Nutrition*, 15(2): 113 – 120.

PRASAD, A. S. (2004). Zinc deficiency: its characterization and treatment. *Metal Ions in Biological Systems*, 41: 103 – 138.

PULS, R. (1988). *Mineral Levels in Animal Health: Diagnostic Data*. Sherpa International, Toronto, Canada.

SANDSTEAD, H. H. (1994). Understanding zinc: recent observations and interpretations. *Journal of Laboratory and Clinical Medicine*, 124(3): 322 – 327.

SPEARS, J. W. and KEGLEY, E. B. (1991). Effect of zinc and manganese methionine on performance of beef cows and calves. *Journal of Animal Science*, 69(1): 59.

STABEL, J. R., SPEARS, J. W. and BROWN JR, T. T. (1993). Effect of copper deficiency on tissue, blood characteristics, and immune function of calves challenged with infectious bovine rhinotracheitis virus and *Pasteurella hemolytica*. *Journal of Animal Science*, 71(5): 1247 – 1255.

SUTTLE, N. F. (2010). *Mineral Nutrition of Livestock.* 4^{th} Edition, CABI, Cambridge.

SUTTLE, N. F., JONES, D. G., WOOLLIAMS, C. and WOOLLIAMS, J. A. (1987). Heinz body anaemia in lambs with deficiencies of copper or selenium. *British Journal of Nutrition*, 58(3): 539 – 548.

TOMLINSON, D. J., MÜLLING, C. H. and FAKLER, T. M. (2004). Invited review: formation of keratins in the bovine claw: roles of hormones, minerals, and vitamins in functional claw integrity. *Journal of Dairy Science*, 87(4): 797 – 809.

UNDERWOOD, E. J. and SUTTLE, N. F. (1999). *The Mineral Nutrition of Livestock.* 3^{rd} Edition, CABI Publishing, Wallingford, United Kingdom.

VÁZQUEZ-ARMIJO, J. F., ROJO, R., LÓPEZ, D., TINOCO, J. L., GONZÁLEZ, A., PESCADOR, N. and DOMÍNGUEZ-VARA, I. A. (2011). Trace elements in sheep and goats reproduction: a review. *Tropical and Subtropical Agroecosystems*, 14: 1 – 13.

YADRICK, M. K., KENNEY, M. A. and WINTERFELDT, E. A. (1989). Iron, copper, and zinc status: response to supplementation with zinc or zinc and iron in adult females. *American Journal of Clinical Nutrition*, 49(1): 145 – 150.

YATOO, M. I., SAXENA, A., DEEPA, P. M., HABEAB, B. P., DEVI, S., JATAV, R. S. and DIMRI, U. (2013). Role of trace elements in animals: a review. *Veterinary World*, 6(12): 963 – 967.