

Serum Copper Concentration and Immune Status of Sheep: Clinical and Laboratory Study

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Abstract: This study was designed to investigate the effect of serum copper concentration on the immune status of sheep. Therefore, this study was carried on 40 sheep (20 diseased sheep and 20 clinically healthy) 1-2 years old from different localities. The selected animals were thoroughly examine and the clinical findings were reported. Copper deficient-sheep showed the clinical signs of ill thrift, limp and glossy wool with depigmentation and retardation of growth in addition to depression of animal viability. Two blood samples were obtained from each sheep and were used for determination of total and differential leucocytic counts, determination of superoxide dismutase enzyme, phagocytic percentage, phagocytic index, copper, zinc, selenium, iron, total proteins, albumin, globulins and immunoglobulins. The obtained results revealed that the phagocytic ability and killing capacity of neutrophils (phagocytic % and phagocytic index) were reduced. Moreover, the activity of the antioxidant enzyme copper, zinc-superoxide dismutase in ovine neutrophils is decreased. Furthermore, there was decreased levels of blood total proteins, albumin, globulins and immunoglobulins. The treatment with copper improves cellular (phagocytic % and phagocytic index) and humoral immunity (immunoglobulins levels). It could be concluded that copper as a micronutrient is of paramount importance for the immune status and its deficiency adversely affects the cellular and humeral immune status in sheep.

Key words: Sheep, serum, copper, deficiency, immune status, Egypt

INTRODUCTION

Copper, zinc, selenium and iron are essential trace minerals associated with specific and non specific immune mechanisms (Caroline *et al.*, 1994; Chandra, 2003). Erickson *et al.* (2000) declared that micronutrient such as zinc, copper and selenium can influence several components of innate immunity. Micronutrients play an important role in alteration of oxidant-mediated tissue injury and phagocytic cells produce reactive oxidants as part of the defense against infectious agents. Thus, adequate micronutrient are required to prevent damage of cells participating in innate immunity.

Micronutrients deficiency have a great effect on animals. The main effects of subclinical deficiency are an altered balance of reactive oxygen species and antioxidants, leading to oxidative damage of polyunsaturated fatty acids and nucleic acid and impaired immune function with increased likelihood of infectious complication. Copper appears to be essential

for the normal functioning of the immune system in ruminants just as it does in small laboratory animals. In cases of copper deficiency the effect was in numbers of cell mediating immunity, increasing mast cells (non specific immune cells) in muscle and decreasing some populations of T-cells (specific immune cells). Moreover, it affects the activities of copper-zinc SOD which has a protective functions (Underwood and Suttle, 1999).

Spears (2000) reported that copper deficiency reduces the ability of isolated neutrophils to kill yeast and/or bacteria. Moreover, copper deficiency reduces antibody production, interferon and tumor necrosis factor by mononuclear cell. From other point of view micronutrient imbalance or deficiency specially zinc and copper will affect pregnancy outcome through alterations in maternal and conceptus metabolism. Moreover, their deficiency can reduce defense against free radicals damage (Ashworth and Antipatis, 2001). Copper/zinc superoxide dismutase (Cu/Zn SOD) is a key antioxidant enzyme involved in superoxide detoxification in normal cellular

metabolism and after cell injury (Peluffo *et al.*, 2005). Consequently, the main objective of this study was to investigate the effect of copper deficiency on the immune status of sheep.

MATERIALS AND METHODS

Animals: A total number of 40 sheep (20 diseased and 20 clinically healthy) 1-2 years old from different localities had been subjected to this study. The selected animals were thoroughly examined and the clinical findings were reported. Copper deficient-sheep showed the clinical signs of ill thrift, limp and glossy wool with depigmentation and retardation of growth in addition to depression of animal viability.

Samples and sampling protocol

Blood samples: Blood samples were collected by jugular veinpuncture after clipping and disinfecting the area with disinfectant solution. The blood samples were obtained in two occasions, one before treatment and second 30 days post treatment. Two blood samples were obtained from each sheep. The first sample was collected in a clean, dry vacutainer tube with anticoagulant. These blood samples were used for total and differential leucocytic counts, determination of superoxide dismutase enzyme, phagocytic percentage and phagocytic index. The second blood samples were obtained by jugular veinpuncture in vacutainer tubes without anticoagulant for obtaining blood sera. Only clear non hemolysed serum samples were obtained and kept frozen at -200°C for further biochemical analysis (Coles, 1986).

Adopted methods

Total and differential leucocytic count: Total leucocytic count ($\times 10^3 \text{ cumm}^{-1}$) was carried out using hemocytometer while differential leucocytic count was carried out using stained blood film with Wright's stain according to Coles (1986).

Superoxide Dismutase enzyme (SOD): SOD was estimated according to the method described by Anders (1985).

Phagocytic activity of neutrophils: The Phagocytic activity of neutrophils was measured according to the method described by Lehrer and Clins (1989) (Fig. 1-3).

Biochemical analysis of blood sera samples: The biochemical analysis of blood sera for the selected parameters particularly copper, zinc, total proteins and albumin were measured spectrophotometrically using the commercial test kits according to the methods described by Meret and Henkin (1971), Johnsen and Eliasson (1987) and King and Wootton (1959), respectively.

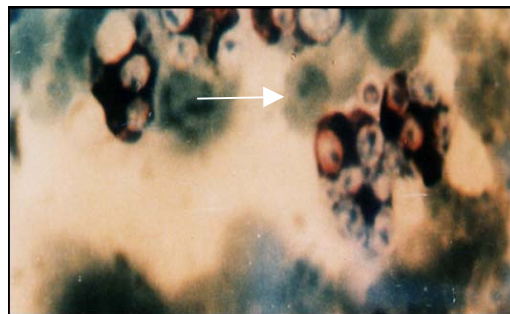


Fig. 1: Phagocytic activity of neutrophils engulfed heat killed candida in clinically healthy sheep

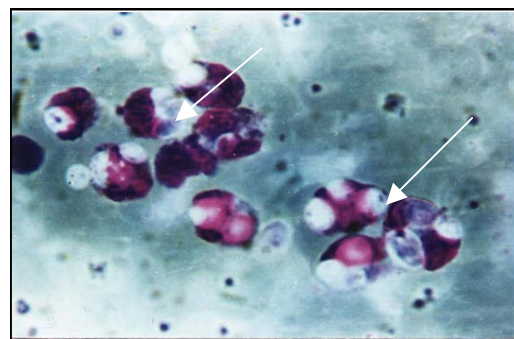


Fig. 2: Phagocytic activity of neutrophils engulfed heat killed candida in copper deficient sheep

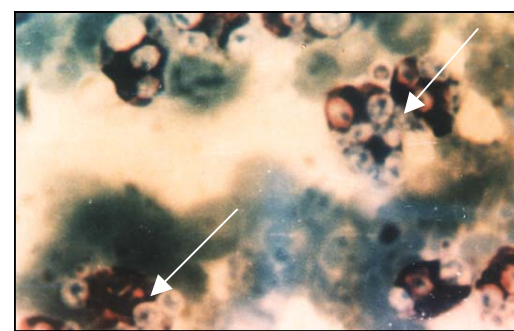


Fig. 3: Phagocytic activity of neutrophils engulfed heat killed candida after treatment with copper

Serum globulins: Serum globulins were calculated by subtraction of the amount of serum albumin from the amount of total serum protein.

Serum IgG, IgM and IgA: Serum IgG, IgM and IgA were determined by using available test kits by using single radial immunodiffusion technique according to the method described by Mancini *et al.* (1965).

Therapeutic trial: The diseased conditions suffered copper deficiency were treated by oral administration of copper sulfate powder at dose rate 1 g/head/week for 4 successive weeks.

Statistical analysis: The obtained data were statistically analyzed for means and significance between the groups using ANOVA according to Snedecor and Cochran (1982) by using SPSS computerized system.

RESULTS AND DISCUSSION

In this study, copper deficient sheep showed the clinical signs of ill thrift, limp and glossy wool with depigmentation and retardation of growth. Regarding laboratory findings the results showed significant reduction in serum copper levels in deficient sheep with non significant changes in serum zinc concentrations when compared to their herd mates (Table 1). Also, there was significant decrease in the levels of total proteins, albumin, globulins and immunoglobulins IgG, IgM and IgA in deficient sheep (Table 2). Moreover, there was significant decrease in the activity of the antioxidant enzyme copper, zinc-superoxide dismutase in ovine neutrophils (Table 3). There was significant decrease in phagocytic ability and killing capacity of neutrophils expressed as phagocytic % and phagocytic index (Table 4). The treatment with copper sulphate improves both cellular (phagocytic % and phagocytic index) and humoral immunity (immunoglobulins levels) in treated sheep.

Table 1: Mean values±SE of serum levels of zinc and copper in clinically healthy sheep and diseased sheep suffered copper deficiency before and after treatment

Animal groups	Variables	
	Copper µg/100 mL	Zinc µg/100 mL
Control group	97.6±2.26 ^a	74.2±1.70 ^a
Copper-deficient sheep before treatment	74.38±1.76 ^b	73.8±1.30 ^b
Copper-deficient sheep after treatment	94.4±1.80 ^a	73.7±1.80 ^b

The different superscripts within the column are significantly differ while the same superscript within the column are non significantly differ; p<0.05

Table 2: Mean values±SE of serum total protein, albumin, globulin and immunoglobulin in clinically healthy sheep and diseased sheep suffered copper deficiency before and after treatment

Animal groups	Variables					
	Total protein (g dL ⁻¹)	Albumin (g dL ⁻¹)	Globulin (g dL ⁻¹)	IgG (mg mL ⁻¹)	IgM (mg mL ⁻¹)	IgA (mg mL ⁻¹)
Control group	6.08±0.65 ^a	2.23±0.40 ^a	3.85±0.45 ^a	19.0±1.01 ^a	3.08±0.32 ^a	0.35±0.03 ^a
Copper-deficient sheep before treatment	5.01±0.40 ^b	1.75±0.50 ^b	3.28±0.36 ^b	9.65±0.51 ^b	1.65±0.81 ^b	0.11±0.02 ^b
Copper-deficient sheep after treatment	5.9±0.50 ^a	2.16±0.33 ^a	3.79±0.50 ^a	17.75±0.74 ^a	2.79±0.12 ^a	0.28±0.02 ^a

The different superscripts within the column are significantly differ while the same superscript within the column are non significantly differ, p<0.05

In this study, copper-deficient sheep showed the clinical signs of retarded growth, limp and glossy wool, depigmentation of wool and anemia. There was an obvious clinical improvement of the general health condition of the affected animals after treatment. The clinical findings observed in copper deficient sheep were in concern with those achieved by El-Sangary (1999), Underwood and Suttle (1999), Gehan (2000) and Radostits *et al.* (2000). Retardation of growth in such clinical conditions could be attributed to interference of intermediary metabolism due to failure of tissue oxidation. Limp and glossy wool may be attributed to the requirement for copper to oxidized the SH into S-S (disulphide bridge in the two adjacent cystine molecules) groups in keratin synthesis. Also depigmentation of wool may be due to deficiency of copper containing enzyme tyrosine (polyphenyloxidase) which catalyses the conversion of L-tyrosine into melanin. Moreover, anemia could be attributed to the importance of copper in the formation of hemoglobin as copper is necessary for the reutilization of iron liberated from the normal breakdown of hemoglobin (McDonald *et al.*, 1979; Radostits *et al.*, 2000).

The obtained results of laboratory investigations of copper deficient sheep (Table 1) revealed that there were significant reductions in the levels of copper (74.38±1.7 µg/100 mL) in diseased sheep associated with non significant variation in the levels of zinc. There was a significant improvement of copper levels (94.4±1.8 µg/100 mL) toward the normal values 30 days after treatment. These results were in harmony with those obtained by Whitelaw *et al.* (1982), Caroline *et al.* (1994), Underwood and Suttle (1999) and Radostits *et al.* (2000). The decreased copper levels in such clinical conditions could be attributed to low copper levels in the diet or to the presence of excess amounts of molybdenum, zinc, iron, lead and calcium carbonate in the diet of affected animals (Whitelaw *et al.*, 1982; Underwood and Suttle, 1999).

There were significant (p<0.05) reductions in the levels of total proteins (5.01±0.4 g dL⁻¹), albumin (1.75±0.5 g dL⁻¹) and globulins (3.28±0.36 g dL⁻¹) in blood serum of copper deficient sheep if compared to the levels

in their herd mates (Table 2). In addition, there was significant ($p < 0.05$) reduction in the levels of immunoglobulin G ($9.65 \pm 0.51 \text{ mg mL}^{-1}$), immunoglobulin M ($1.85 \pm 0.65 \text{ mg mL}^{-1}$) and immunoglobulin A ($0.11 \pm 0.02 \text{ mg mL}^{-1}$) in those sheep. There was significant ($p < 0.05$) increase in the levels of total proteins ($5.9 \pm 0.5 \text{ g dL}^{-1}$) albumin ($2.1 \pm 0.4 \text{ g dL}^{-1}$), globulins ($3.79 \pm 0.5 \text{ g dL}^{-1}$) immunoglobulin G ($17.75 \pm 0.74 \text{ mg mL}^{-1}$), immunoglobulin M ($3.14 \pm 0.1 \text{ mg mL}^{-1}$) and immunoglobulin A ($0.28 \pm 0.02 \text{ mg mL}^{-1}$) towards the normal values after treatment. The obtained results were in agreement with those obtained by Scrimshaw and SanGiovanni (1997), Ashworth and Antipatis (2001) and Chandra (2003).

The decreased levels of total proteins, albumin and globulins could be attributed to faulty nutrition and loss of appetite which is considered as stress factors that adversely affect the hepatic parenchyma resulting in failure of protein synthesis (Mc-Donald, 1975). Moreover, the decreased levels may be attributed to increased levels of blood cortisol in diseased animals. Since, there are some evidences that the increased concentration of cortisol causes catabolism of protein leading to negative nitrogen balance and increased urinary elimination of nitrogen (Mc-Donald, 1975; Dovarax *et al.*, 1980). The decreased levels of immunoglobulin might be a result of the decreased number of immunoglobulin forming cells and faulty nutrition (Ashworth and Antipatis, 2001; Chandra, 2003). There was a significant ($p < 0.05$) decrease in the total leukocytic count (8.5 ± 0.467) with a significant

($p < 0.05$) increase in monocytes % (3.80 ± 1.01) in copper deficient sheep. Moreover, there was a significant ($p < 0.05$) decrease in the superoxide dismutase enzyme (0.398 ± 0.02) in the same animals (Table 3). Meanwhile, there was a significant ($p < 0.05$) increase in the levels of total leucocytic count (10.2 ± 0.467) and the levels of superoxide dismutase enzyme (1.16 ± 0.02) with a significant ($p < 0.05$) decrease of monocyte levels (0.75 ± 0.175) towards the normal levels after treatment. These results were in harmony with those obtained by Jones and Suttle (1981), Gehan (2000) and Chandra (2003). The decreased total leukocytic count could be attributed to the stress of malnutrition which cause secretion of adrenocorticotrophic hormone from adenohypophysis and a resultant increase in blood cortisol concentration while the increased levels of monocytes may be attributed to the need for increased phagocytic activity. This interpretation was in concern with Henley and Vaitukaitis (1985) and Mgongo *et al.* (1985).

From another point of view Schonland *et al.* (1972) attributed the decreased total leucocytic count to the decreased levels of circulating lymphocytes in response to stress of malnutrition. While the decreased levels of super oxide dismutase enzyme may be attributed to entering of copper in the synthesis of super oxide dismutase enzyme (Ashworth and Antipatis, 2001; Chandra, 2003).

There was a significant ($p < 0.05$) reduction in the levels of phagocytic percentage (43.8 ± 0.96) and phagocytic index (1.28 ± 0.3) of phagocytic cell in cases of copper deficiency (Table 4). Meanwhile, there was a significant ($p < 0.05$) increase in the levels of phagocytic percentage (78.0 ± 1.36) and phagocytic index (3.5 ± 0.4) of phagocytic cell resulting in improvement of phagocytic cell function after treatment.

These results were in agreement with those obtained by Boyne and Arthur (1986), Gengelbach (1994) and Chandra (2003). The decreased levels of phagocytic percentage and phagocytic index may be attributed to the role of copper in tissue oxidation by supplementing cytochrome oxidase system or entering into their formation in addition to entering in the formation of super oxide dismutase enzyme (Boyne and Arthur, 1986; Gengelbach, 1994; Chandra, 2003). The obtained results of

Table 3: Mean values±SE of total and differential leucocytic count and blood Superoxide Dismutase (SOD) in clinically healthy sheep and diseased sheep suffered copper deficiency before and after treatment

Leucogram	Apparently healthy sheep	Copper-deficient	
		sheep before treatment	sheep after treatment
Total leucocytic×10 ³	10.45±0.79 ^a	8.5±0.460 ^b	10.2±0.460 ^a
Neutrophils (%)	50.1±7.400 ^a	45.65±1.89 ^b	50.95±1.89 ^a
Lymphocytes (%)	48.5±7.930 ^a	40.15±1.86 ^b	45.60±1.84 ^a
Eosinophils (%)	0.60±0.84 ^a	0.50±0.38 ^a	0.60±0.41 ^a
Basophils (%)	0.50±0.53 ^a	0.50±0.11 ^a	0.50±0.11 ^a
Monocytes (%)	0.30±0.15 ^a	3.80±1.01 ^b	0.75±0.17 ^a
Blood Superoxide Dismutase (SOD) (μmol L ⁻¹)	1.55±0.02 ^a	0.39±0.02 ^b	1.16±0.02 ^a

The different superscripts within the column are significantly differ while the same superscript within the column are non significantly differ; $p < 0.05$

Table 4: Mean values±SE of phagocytic % and phagocytic index in clinically healthy sheep and diseased sheep suffered copper deficiency before and after treatment

The phagocytic (%)			The phagocytic index		
Control	Copper-deficient sheep before treatment	Copper-deficient sheep after treatment	Control	Copper-deficient sheep before treatment	Copper-deficient sheep after treatment
83.2±2.3 ^a	43.8±0.96 ^b	78.0±1.36 ^a	3.7±0.55 ^a	1.28±0.3 ^b	3.5±0.4 ^a

The different superscripts within the column are significantly differ while the same superscript within the column are non significantly differ; $p < 0.05$

laboratory investigations revealed a significant reduction in both cellular and humoral immunity in cases of copper deficiency.

CONCLUSION

From this study it could be concluded that micronutrient (copper) is a major element affecting the immune status in sheep. Such elemental deficiency can adversely affect the cellular and humoral immune status in sheep.

REFERENCES

- Anders, M.W., 1985. Bioactivation of Foreign Compounds. Academic Press, Orlando, USA., ISBN-13: 9780120594801, Pages: 555.
- Ashworth, C.J. and C. Antipatis, 2001. Micronutrient programming of development throughout gestation. *Reproduction*, 122: 527-535.
- Boyne, R. and J.R. Arthur, 1986. Effects of molybdenum or iron induced copper deficiency on the viability and function of neutrophils from cattle. *Res. Vet. Sci.*, 41: 417-419.
- Caroline, N.N., D. Blodgett, D. Eversol, G. Gerhardt, G.G. Schurig and C.D. Thatcher, 1994. Effect of copper and Iron on neutrophil function and humoral immunity of gestating beef cattle. *JAVMA.*, 204: 1796-1800.
- Chandra, R.K., 2003. Nutrition and immune system: An introduction. *Am. J. Clin. Nutr.*, 66: 460S-463S.
- Coles, E.H., 1986. *Veterinary Clinical Pathology*. 4th Edn., W.B. Saunders Company, Philadelphia, London, Toronto.
- Dovarax, M., D. Raszyk, M. Toulouva and Q. Mastowskova, 1980. Effect of oestradiol, progesteron or testosterone on adrenocortical activity and on various metabolic parameters in ovariectomized gilts. *Zentralblatt Vetrinar Medizin*, 27: 765-765.
- El-Sangary, F.M., 1999. Studies on causes, diagnosis, biochemical changes and treatment of unthriftiness in sheep. Ph.D. Thesis, Vet Medical Science, Zagazig University, Egypt.
- Erickson, K.L., E.A. Medina and N.E. Hubbard, 2000. Micronutrient and innate immunity. Department of cell biology and human anatomy, University of California, School of Medicine. *J. Infect. Dis.*, 182: 5-10.
- Gehan, K.A., 2000. Relationship of certain blood constituents with ill thriftiness in weaner sheep. M.VSc Thesis, Zagazig University, Egypt.
- Gengelbach, G.P., 1994. Effect of copper deficiency on cellular immunity in cattle. Ph.D. Thesis, North Carolina State University, Raleigh, USA.
- Henley, K.M. and J.L. Vaitukaitis, 1985. Hormonal changes associated with changes in body weight. *Clin. Obstet. Gynecol.*, 28: 615-631.
- Johnsen, O. and R. Eliasson, 1987. Evaluation of a commercially available kit for the colorimetric determination of zinc in human seminal plasma. *Int. J. Androl.*, 10: 435-440.
- Jones, D.G. and N.F. Suttle, 1981. Some effects of copper deficiency on leukocyte function in sheep and cattle. *Res. Vet. Sci.*, 31: 151-156.
- King, E.J. and I.D.P. Wootton, 1959. *Micro-Analysis in Medical Biochemistry*. 3rd Edn., Grune and Stratton, London, UK., Pages: 306.
- Lehrer, R.I. and M.J. Clins, 1989. Interaction of *Candida albicans* with human leukocytes and serum. *J. Bacteriol.*, 98: 996-1004.
- Mancini, G., A.O. Tarbonara and J.F. Heremans, 1965. Immuno-chemical quantitation of antigen by single radial immunodiffusion technique. *Immunochemistry*, 2: 235-254.
- Mc-Donald, L.E., 1975. *Veterinary Endocrinology and Reproduction*. 2nd Edn., Lea and Febiger, Philadelphia, pp: 157-161.
- McDonald, P., R.A. Edwards and J.F.D. Greenhalgh, 1979. *Animal Nutrition*. 3rd Edn., Longman Group Ltd., London, UK.
- Meret, S. and R.I. Henkin, 1971. Simultaneous direct estimation by atomic absorption spectrophotometry of copper and zinc in serum, urine and cerebrospinal fluid. *Clin. Chem.*, 17: 369-373.
- Mgongo, F.O.K., S. Gombe and J.S. Oгаа, 1985. The influence of cobalt/vitamin B12 deficiency as a stressor affecting adrenal cortex and ovarian activities in goats. *Nutr. Dev.*, 24: 845-854.
- Peluffo, H., L. Acarin, M. Faiz, B. Castellano and B. Gonzalez, 2005. Cu/Zn superoxide dismutase expression in the postnatal rat brain following an excitotoxic injury. *J. Neuroinflammation*, 2: 1-12.
- Radostits, O.M., D.C. Blood and C.C. Gay, 2000. *Veterinary Medicine*. 8th Edn., Bailliere, London, Philadelphia.

- Schonland, M.M., B.C. Shanley, W.E. Loening, M.A. Parent and H.M. Coovadia, 1972. Plasma-cortisol and immunosuppression in protein-calorie malnutrition. *Lancet*, 2: 435-436.
- Scrimshaw, N.S. and J.P. SanGiovanni, 1997. Synergism of nutrition, infection and immunity: An overview. *Am. J. Clin. Nutr.*, 66: 464-477.
- Snedecor, G.W. and W.G. Cochran, 1982. *Statistical Methods*. 7th Edn., Iowa State University Press, Ames, Iowa, USA.
- Spears, J.W., 2000. Micronutrient and immune function in cattle. *Proc. Nutr. Soc.*, 59: 587-594.
- Underwood, E.J. and N.F. Suttle, 1999. *The Mineral Nutrition of Livestock*. 3rd Edn., CAB International, Wallingford, Oxon, UK.
- Whitelaw, A., A.R. Fawcett and A.J. MacDonald, 1982. Cupric oxide needles in the prevention of sway back. *Vet. Rec.*, 110: 522-532.