

## Correlation Between Zinc Deficiency and Immune Status of Sheep

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**Abstract:** From birth, the human being and animals are exposed to a continuous stream of microorganisms that have adverse effects on their body conditions and health status. In a global scale, there are many factors affecting the immune status such as systemic disorders, malnutrition, infectious diseases, deficiencies of minerals and trace element including copper, zinc, selenium and iron, protein-energy malnutrition, vitamins deficiency and fetal malnutrition. A total number of 20 diseased sheep, 1-2 years old from different localities had been subjected to this study. The selected animals were thoroughly examined and the results of physical and laboratory investigations were reported. The diseased animals showed the clinical signs of zinc deficiency mainly emaciation, alopecia and parakeratosis of the skin covering the vulva, anus, tail, head and ears. In addition ten clinically healthy sheep were served as control group. Lymphopenia with decreased activity of superoxide dismutase enzyme are characteristic for zinc deficiency. Monocytosis also recorded in such clinical cases, followed by significant decrease in monocytes % after zinc supplementation. In addition, decreased levels of serum total proteins, albumin, globulins and immunoglobulins in such cases were observed. The treatment with zinc improves cellular and humeral immunity. In addition, there was a significant ( $p<0.05$ ) reduction in the levels of superoxide dismutase enzyme ( $0.4\pm0.02$ ) in zinc-deficient sheep. The obtained results of laboratory investigations of zinc deficient sheep revealed that there was a significant reduction in the levels of zinc associated with non significant variations in the levels of copper. Concerning the leucocytic picture the results revealed that there was a significant ( $p<0.05$ ) reduction in the total leucocytic count and a significant ( $p<0.05$ ) decrease in lymphocyte % with a significant increase in monocytes % in cases of zinc deficiency when compared with their values in healthy animals. It could be concluded from this study that immune system is extremely affected by several factors. It is observed that micronutrient such as zinc is the major element affecting the immune status in sheep. This elemental deficiency adversely affects the cellular and humeral immune status in sheep.

**Key words:** Sheep, effect, zinc deficiency, immune status, blood chemistry, Egypt

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### INTRODUCTION

Copper, zinc, selenium and iron are essential trace minerals associated with specific and non specific immune mechanisms (Caroline *et al.*, 1994; Chandra, 1997). It was declared by Erickson *et al.* (2000) 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 micronutrients are required to prevent damage of cells participating in innate immunity. Deficiency of one ore more of the essential micronutrients has an adverse

effect on animals' health status. 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 (Shenkin, 2000).

Bhaskaram (2002) found that micronutrient deficiencies and infectious diseases often coexist and exhibit complex interactions. Several micronutrients such as copper, zinc and iron have immunomodulating functions and thus influence the susceptibility of the host to infectious diseases and the course and outcome of such diseases. Certain micronutrients also possess antioxidant functions that not only regulate immune

homeostasis of the host but also alter the genome of the microbes, particularly viruses. Cunningham-Rundles *et al.* (2002) stated that normal maturation of immune response at birth is both supported and stimulated by the gastrointestinal microenvironment, which provides both nutrients and antigenic microbial exposure to the developing animals. Micronutrients and vitamins are present in the local environment and have important regularity effects on the adaptive immune cell function through effects on type of cytokine response.

Copper/Zinc Superoxide Dismutase (Cu/Zn SOD) is a key antioxidant enzyme involved in superoxide detoxification in normal cellular metabolism and after cell injury, component of the antioxidant defense system and thymulin which is essential for the formation of T-lymphocytes (Mocchegiani *et al.*, 2004; Peluffo *et al.*, 2005) and it undergoes a significant reduction in blood samples from animals deficient in copper and/or zinc (Cerone *et al.*, 2000).

Moreover, zinc is known to play a central role in the immune system and in case of zinc deficiency there was an increased susceptibility to a variety of pathogens. Zinc is crucial for normal development and function of cells mediating non specific immunity such as neutrophils and natural killer cells. Moreover, zinc is important for gene regulation within the lymphocytes, T-cell activation, T-helper 1 (Th1) cytokine production and B lymphocyte help (Shankar and Prasad, 1998).

High (1999) stated that the immunologic function, particularly cell-mediated immunity, declines with age, contributing to the increased incidence of infectious diseases and supplementation of zinc can modulate the immune response in elderly. The main effect of zinc deficiency appears to be a reduction in cell mass that may indirectly affect immune cell function, particularly, whenever T-helper cell numbers are reduced (Thurnham, 1997).

Moreover, Underwood and Suttle (1999) reported that zinc deficiency is further characterized by impairment of the immune system and atrophy of the thymus with subsequent reduction in the humoral immune capacity. Splenic macrophages from severely zinc deficient animals were less able to facilitate T-cell mitogenesis. Zinc deficiency in addition causes a reduction in cytokine production from T and B cells in animals with gut nematodes and given a diet devoid of zinc. In animals and humans, zinc deficiency results in rapid and marked atrophy of the thymus, impaired cell-mediated cutaneous sensitivity and lymphopenia. Primary and secondary antibody responses are reduced following zinc deficiency. In addition, antibody response and the generation of

splenic cytotoxic T-cells after immunization are reduced. Moreover, zinc also inhibits the production of tumor necrosis factors which are implicated in the pathophysiology of cachexia and wasting in acquired immune deficiency syndrome (Spears, 2000).

Strand *et al.* (2001) concluded that mice fed a diet low in zinc for a relatively short period were more prone to a severe streptococcus pneumonia infection than mice fed a normal diet. In addition it was reported by Mocchegiani *et al.* (2004) that infection may cause mortality in old age due to damaged immune response. As zinc is required as a catalyst, structural (zinc fingers) and regulatory ion, it is involved in many biological functions, including immune responses. Low zinc ion bioavailability and impaired cell mediated immunity are common in ageing and may be restored by physiological supplementation with zinc for 1-2 month. Consequently, the main objective of this study was to investigate the correlation of zinc deficiency and the immune status of sheep.

## MATERIALS AND METHODS

**Animals:** A total number of 20 diseased sheep, 1-2 years old from different localities had been subjected to this study. The selected animals were thoroughly examined and the results of physical and laboratory investigations were reported. The diseased animals showed the clinical signs of zinc deficiency.

### 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 -20°C for further biochemical analysis (Coles, 1986) (Fig. 1).

### Adopted methods

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

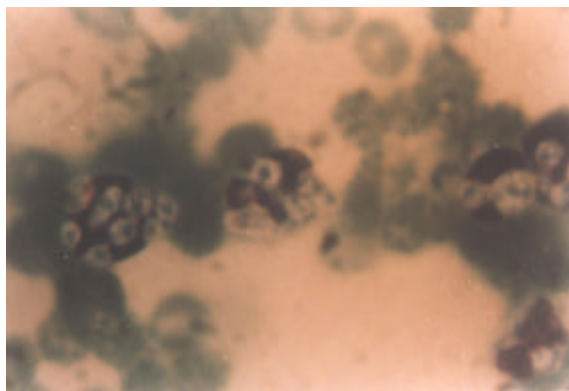


Fig. 1: Phagocytic activity of neutrophils engulfed heat killed candida in control group

**Superoxide Dismutase enzyme (SOD):** SOD was estimated according to the method described by Mirsa and Fridovich (1985).

**Phagocytic activity of neutrophils:** The phagocytic activity of neutrophils was measured according to the method described by Lehrer and Clins (1969).

**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), Wootton (1965) and Drupt, respectively.

**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 protocols:** The diseased cases were treated with zinc oxide powder in a dose rate of 4 mg kg<sup>-1</sup> body weight administered orally for 4 successive weeks.

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

## RESULTS AND DISCUSSION

The most observed signs among the diseased sheep were emaciation, alopecia and parakeratosis of the skin

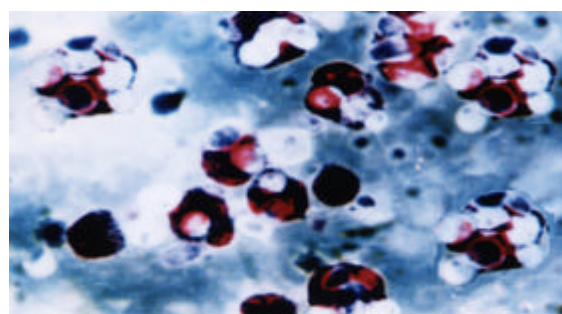


Fig. 2: Phagocytic activity of neutrophils engulfed heat killed candida in zinc deficient cases

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

Animal groups	Variables	
	Copper µg/100 mL	Zinc µg/100 mL
Control group	98.6±3.3 <sup>a</sup>	75.30±1.30 <sup>a</sup>
Zinc deficient sheep before treatment	95.4±4.1 <sup>a</sup>	48.80±1.01 <sup>b</sup>
Zinc deficient sheep after treatment	96.4±2.7 <sup>a</sup>	62.45±1.91 <sup>a</sup>

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

covering the areas over vulva, anus, tail, head and ears and around the nostrils. The obtained results of laboratory investigations of zinc deficient sheep revealed that there was a significant reduction in the levels of zinc associated with non significant variations in the levels of copper (Table 1, Fig. 2). Zinc is crucial for normal development and function of cells mediating non specific immunity such as neutrophils and natural killer cells (decreased phagocytic % and phagocytic index) (Table 2). Lymphopenia with decreased activity of superoxide dismutase enzyme are characteristics for zinc deficiency (Table 3). Monocytosis also recorded in such clinical cases, followed by significant decrease in monocyte % after treatment (Table 3). In addition, significant decrease in the levels of serum total proteins, albumin, globulins and immunoglobulins in such cases were recorded (Table 4). The treatment with zinc oxide powder improves the cellular and humoral immunity (Fig. 3).

There is no doubt that trace element levels affect the immune status of animal. It was documented by many authors that trace elements particularly copper, zinc, selenium and iron are of paramount importance in controlling cellular and/or humeral immunity through specific and non specific immune mechanisms (Caroline *et al.*, 1994; Ashworth and Antipatis, 2001; Chandra, 1997). The diseased sheep showed the clinical signs of alopecia and parakeratosis of the skin covering

Table 2: Mean values  $\pm$ SE of phagocytic % and phagocytic index in clinically healthy sheep and diseased sheep suffered zinc deficiency before and after treatment

The phagocytic % (Zinc-deficient sheep)			The phagocytic index (Zinc-deficient sheep)		
Control	Before treatment	After treatment	Control	Before treatment	After treatment
83.2 $\pm$ 2.3 <sup>a</sup>	47.4 $\pm$ 1.09 <sup>b</sup>	69.6 $\pm$ 0.42 <sup>c</sup>	3.7 $\pm$ 0.4 <sup>a</sup>	1.24 $\pm$ 0.3 <sup>b</sup>	2.86 $\pm$ 0.5 <sup>c</sup>

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

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

Leucogram	Apparently Healthy sheep	Zinc deficient sheep before treatment	Zinc deficient sheep after treatment
Total leucocytic count ( $\times 10^9$ )	9.8 $\pm$ 0.79 <sup>a</sup>	6.60 $\pm$ 0.52 <sup>b</sup>	7.59 $\pm$ 0.46 <sup>c</sup>
Neutrophils (%)	50.1 $\pm$ 2.30 <sup>a</sup>	51.40 $\pm$ 1.70 <sup>a</sup>	50.90 $\pm$ 1.89 <sup>a</sup>
Lymphocytes (%)	48.5 $\pm$ 2.50 <sup>a</sup>	37.25 $\pm$ 1.96 <sup>b</sup>	46.15 $\pm$ 1.93 <sup>a</sup>
Eosinophils (%)	0.6 $\pm$ 0.26 <sup>a</sup>	0.50 $\pm$ 0.42 <sup>a</sup>	0.55 $\pm$ 0.41 <sup>a</sup>
Basophils (%)	0.5 $\pm$ 0.16 <sup>a</sup>	0.45 $\pm$ 0.02 <sup>a</sup>	0.50 $\pm$ 0.11 <sup>a</sup>
Monocytes (%)	0.3 $\pm$ 0.15 <sup>a</sup>	7.20 $\pm$ 0.95 <sup>b</sup>	0.75 $\pm$ 0.17 <sup>a</sup>
Blood Superoxide Dismutase (SOD): $\mu\text{mol L}^{-1}$	1.5 $\pm$ 0.02 <sup>a</sup>	0.40 $\pm$ 0.02 <sup>b</sup>	1.30 $\pm$ 0.02 <sup>a</sup>

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

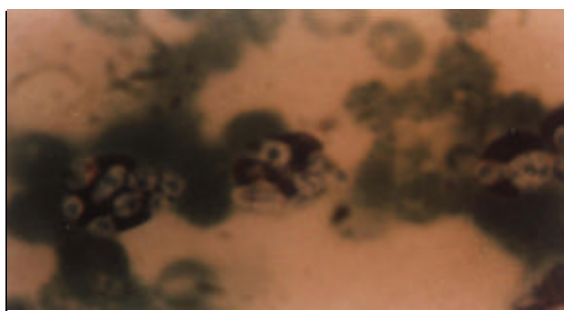


Fig. 3: Phagocytic activity of neutrophils engulfed heat killed candida after treatment with zinc

the vulva, anus, tail, head and ears. There was clinical improvement of the general health condition of the affected animals after treatment. These clinical findings were in concurrence with those stated by Underwood and Suttle (1999). Alopecia and parakeratosis may be attributed to the role of zinc in several enzymes as carboxyl peptidase, alcoholic dehydrogenase, super oxide dismutase and other enzymes necessary for carbohydrate, lipids, protein and nucleic acid metabolism and for many other biochemical reactions in the body metabolism. Furthermore, zinc metalloenzymes linked with vitamin A metabolism (reductase and alcohol dehydrogenase) which is necessary for healthy skin (Underwood and Suttle, 1999; Chandra, 1997).

The obtained results of laboratory investigations of zinc deficient sheep revealed that there was a significant ( $p < 0.05$ ) reduction in the levels of zinc

(48.8 $\pm$ 1.01  $\mu\text{g}/100 \text{ mL}$ ) in group two associated with non significant variations in the levels of copper, selenium and iron. There was a significant ( $p < 0.05$ ) increase in the zinc levels (62.45 $\pm$ 1.91  $\mu\text{g}/100 \text{ mL}$ ) after treatment. These results were in agreement with those obtained by Lamand (1978) and Suliman *et al.* (1988).

The decreased levels of zinc could be attributed to the low availability of zinc in the diet by presence of certain substances competing with zinc absorption as phytic acid, excess dietary calcium and increasing copper levels in the diet (Suliman *et al.*, 1988; Gehan, 2000).

There were significant ( $p < 0.05$ ) reductions in the levels of total proteins (5.29 $\pm$ 0.55  $\text{g dL}^{-1}$ ), albumin (1.66 $\pm$ 0.1  $\text{g dL}^{-1}$ ) and globulins (3.08 $\pm$ 0.12  $\text{g dL}^{-1}$ ) in cases of zinc deficiency (Table 4). Moreover, there were significant ( $p < 0.05$ ) reductions in the levels of IgG (7.6 $\pm$ 0.42  $\text{mg mL}^{-1}$ ), IgM (1.54 $\pm$ 0.01  $\text{mg mL}^{-1}$ ) and IgA (0.11 $\pm$ 0.02  $\text{mg mL}^{-1}$ ) in cases of zinc deficiency. There were significant increases in total proteins (5.93 $\pm$ 0.75  $\text{g dL}^{-1}$ ), albumin (2.16 $\pm$ 0.33  $\text{g dL}^{-1}$ ), globulins (3.7 $\pm$ 0.45  $\text{g dL}^{-1}$ ), IgG (14.7 $\pm$ 0.44  $\text{mg mL}^{-1}$ ), IgM (2.79 $\pm$ 0.12  $\text{mg mL}^{-1}$ ) and IgA (0.24 $\pm$ 0.03  $\text{mg mL}^{-1}$ ) toward the normal values after treatment. These results were in concurrence with those obtained by El-Sangary (1999), Gehan (2000), Chandra (1997) and Baltaci *et al.* (2005).

The decreased levels of total proteins, albumin and globulins of such clinical conditions could be attributed to faulty nutrition and loss of appetite which considered as stress factors adversely affect the hepatic parenchyma resulting in failure of protein synthesis (McDonald, 1980). Moreover, the decreased levels may be attributed to the 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 (McDonald, 1980; Dovarax *et al.*, 1980).

The decreased levels of immunoglobulins may be due to decreased number of immunoglobulins forming cells especially T and B lymphocytes and faulty nutrition (Chandra, 1997; Baltaci *et al.*, 2005). Concerning the leucocytic picture the results revealed that there was a significant ( $p < 0.05$ ) reduction in the total

Table 4: Mean values±SE of serum total protein, albumin, globulin and immunoglobulin in clinically healthy sheep and diseased sheep suffered zinc deficiency before and after treatment

Animal groups	Variables					
	Total proteing dL <sup>-1</sup>	Albumin g dL <sup>-1</sup>	Globulin g dL <sup>-1</sup>	IgG mg mL <sup>-1</sup>	IgM mg mL <sup>-1</sup>	IgA mg mL <sup>-1</sup>
Control group	6.09±0.45 <sup>a</sup>	3.85±0.4 <sup>a</sup>	3.85±0.64 <sup>a</sup>	19.0±1.01 <sup>a</sup>	3.08±0.32 <sup>a</sup>	0.35±0.03 <sup>a</sup>
Zinc deficient sheep before treatment	5.29± 0.55 <sup>b</sup>	1.66±0.1 <sup>b</sup>	3.08±0.12 <sup>b</sup>	7.6±0.42 <sup>b</sup>	1.54±0.01 <sup>b</sup>	1.11±0.02 <sup>b</sup>
Zinc deficient sheep after treatment	5.93±0.75 <sup>a</sup>	2.16±0.33 <sup>c</sup>	3.7±0.45 <sup>a</sup>	14.7±0.44 <sup>c</sup>	2.79±0.12 <sup>a</sup>	0.24±0.03 <sup>a</sup>

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

leucocytic count ( $6.6 \pm 0.52 \times 10^3$ ) and a significant ( $p < 0.05$ ) decrease in lymphocyte % ( $37.25 \pm 1.96$ ) with a significant increase in monocytes % ( $7.2 \pm 0.95$ ) in cases of zinc deficiency when compared with their values in healthy animals. The decreased values of total leucocytic count may be attributed to the stress of malnutrition which leads to secretion of adrenocorticotrophic hormone from adenohypophysis resulting in increase of blood cortisol concentration. The increased levels of monocytes were attributed to the need for increased phagocytic activity (Henely *et al.*, 1985; Mgongo *et al.*, 1984). Furthermore, decreased lymphocyte % may be attributed to the reduction of 3H-thymidine (thymulin) which determines the splenic lympho-proliferation. In addition, zinc is essential for T-cell division, maturation and differentiation; lymphocyte response to mitogens, programmed cell death of lymphoid and myeloid origins; gene transcription and biomembrane function.

In addition, there was a significant ( $p < 0.05$ ) reduction in the levels of superoxide dismutase enzyme ( $0.4 \pm 0.02$ ) in zinc-deficient sheep. There were significant ( $p < 0.05$ ) increases in the levels of total leucocytic count ( $7.59 \pm 0.46 \times 10^3$ ) and lymphocytes % ( $46.15 \pm 1.93$ ) with a significant ( $p < 0.05$ ) reduction in monocytes % ( $0.75 \pm 0.17$ ) after treatment. Furthermore, there was a significant ( $p < 0.05$ ) increase in the levels of superoxide dismutase enzyme ( $1.3 \pm 0.02$ ) towards the normal values after treatment. These results were in harmony with those obtained by Chandra (1997) and Taylor and Giessbrech (2000). The decreased levels of super oxide dismutase enzyme could be attributed to the entrance of zinc in the formation of super oxide dismutase through the regulation of reactive oxygen species. These results were in agreement with those obtained by Lastra *et al.* (1997), Thurnham (1997), Cerone *et al.* (2000) and Mocchegiani *et al.* (2004).

Regarding the phagocytic function there were significant ( $p < 0.05$ ) reductions in the levels of phagocytic percentage ( $47.4 \pm 1.09$ ) and phagocytic index ( $1.24 \pm 0.3$ ) of phagocytic cell in zinc-deficient sheep. Meanwhile, there was a significant ( $p < 0.05$ ) increase in the levels of phagocytic percentage ( $69.6 \pm 0.42$ ) and phagocytic index ( $2.86 \pm 0.5$ ) toward the normal values following zinc

supplementation. The results of decreased phagocytic function were in concurrence with those obtained by Thurnham (1997) and Cerone *et al.* (2000). These results could be attributed to the critical role of zinc in formation of superoxide dismutase enzyme and its responsibility in regulation of cytokine production. These results were in harmony with those obtained by Thurnham (1997), Cerone *et al.* (2000) and Mocchegiani *et al.* (2004). The obtained results of laboratory investigations revealed that there were significant reductions in both cellular and humeral immunity in cases of zinc deficiency.

## CONCLUSION

It could be concluded from this study that immune system is extremely important affected by several factors. As the immune system can protect the body against several diseases and infection, it is well observed that micronutrients such as zinc are the major elements affecting the immune status in sheep. This elemental deficiency adversely affects the cellular and humeral immune status in sheep.

## REFERENCES

- Ashworth, C.J. and C. Antipatis, 2001. Micronutrient programming of development throughout gestation. *Reproduction*, 122: 527-535.
- Baltaci, A.K., R. Mogulkoc, C.S. Bediz and A. Pekel, 2005. Effects of zinc deficiency and pinealectomy on cellular immunity in rats infected with *Toxoplasma gondii*. *Biol. Trace. Elem. Res.*, 104: 47-56.
- Bhaskaram, P., 2002. Micronutrient malnutrition, infection and immunity. *Nutr. Rev.*, 60: 40-45.
- 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.
- Cerone, S.I., A.S. Sansinanea, S.A. Streitenberger, M.C. Garcia and N.J. Auza, 2000. Cytochrome c oxidase, Copper, Zinc-superoxide dismutase and ceruloplasmin activities in copper deficient bovines. *Biol. Trace Elem. Res.*, 73: 269-278.

- Chandra, R.K., 1997. Nutrition and the immune system: An introduction. *Am. J. Clin. Nutr.*, 66: 460S-463S.
- Coles, E.H., 1986. *Veterinary Clinical Pathology*. 4th Edn., W.B. Saunders Co., Philadelphia, London, ISBN-13: 978-0721618289.
- Cunningham-Rundles, S., S. Abrn, R. Abav-Nussbaum and A. Dnistrian, 2002. Development of immunocompetence: Role of microutrients and microorganisms. *Nutr. Rev.*, 60: 68-72.
- Dovarax, M., D. Raszk, M. Toulouva and Q. Mastowska, 1980. Effect of oestradiol progesteron or estestrone on adrenocortical activity and on various metabolic parameters in overiectomized gilts. *Vet. Medizin*, 27: 765-766.
- 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. MVSc Thesis, Zagazig University, Egypt.
- Henely, M.K., M.D. Vaitukaitis and L.V. Judith, 1985. Hormonal changes associated with changes in body weight. *Clin. Obeste. Gynecol.*, 28: 615-631.
- High, K.P., 1999. Micronutrient supplementation and immune function in the elderly. *Clin. Infect. Dis.*, 28: 717-722.
- 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.
- Lamand, M., 1978. Copper and zinc deficiency treatment by intramuscular injections in sheep. *Ann. Rech. Vet.*, 3: 495-500.
- Lastra, M.D., R. Pastelin, M.A. Herrera, V.D. Orihuela and A.E. Aguilar, 1997. Increment of immune responses in mice perinatal stages after zinc supplementation. *Arch. Med. Res.*, 28: 67-72.
- Lehrer, R.I. and M.J. Clins, 1969. Interaction of *Candida albicans* with human leucocytes 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.
- McDonald, L.E., 1980. *Veterinary Endocrinology and Reproduction*. 1st Edn., Lea and Febiger, Philadelphia, pp: 274-398.
- McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan, 1995. *Animal Nutrition*. 5th Edn., Addison Wesley Longman, Edinburgh, 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. Ogaa, 1984. The influence of cobalt/vitamin B<sub>12</sub> deficiency as a «stressor» affecting adrenal cortex and ovarian activities in goats. *Repord. Nutr. Dev.*, 24: 845-854.
- Mirsa, H.P. and I. Fridovich, 1985. *Bioactivation of foreign compounds*. Academic Press, Orlando.
- Mocchegiani, E., R. Giacconi, C. Cipriano, E. Muti, N. Gasparini and M. Malovolta, 2004. Are zinc-bound metallothionein isoforms (I+II and III) involved in impaired thymulin production and thymic involution during ageing. *Immun. Ageing.*, 1: 5-10.
- 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.
- Shankar, A.H. and A.S. Prasad, 1998. Zinc and immune function: The biological basis of altered resistance to infection. *Am. J. Clin. Nutr.*, 68: 447S-463S.
- Shenkin, A., 2000. Micronutrients in the severely-injured patient. *Proc. Nutr. Soc.*, 59: 451-456.
- Snedcor, G.W. and W.G. Cochran, 1982. *Statistical Methods*. 7th Edn., The Iowa State University Press, Ames, Iowa, USA., pp: 507.
- Spears, J.W., 2000. Micronutrient and immune function in cattle. *Proc. Nutr. Soc.*, 59: 587-594.
- Strand, T.A., D.E. Briles, H.K. Gjessing, A. Maage, M.K. Bhan and H. Sommerfe, 2001. Pneumococcal pulmonary infection, septicemia and survival in young zinc-depleted mice. *Br. J. Nutr.*, 86: 301-306.
- Suliman, H.B., A.I. Abdel Rahim, A.M. Zakia and A.M. Shommein, 1988. Zinc deficiency in sheep: Field cases. *Trop. Anim. Health Prod.*, 20: 47-51.
- Taylor, C.G. and J.A. Giessbrech, 2000. Dietary zinc deficiency and expression of T Lymphocyte sign transduction proteins. *Conj. physiol. Pharmacol.*, 78: 823-828.
- Thurnham, D.I., 1997. Micronutrient and immune function: Some recent developments. *J. Clin. Pathol.*, 50: 887-891.
- Underwood, E.J. and N.F. Suttle, 1999. *The Mineral Nutrition of Livestock*. 3rd Edn., CAB International, Wallingford, Oxon, UK.
- Wootton, I.D.P., 1965. *Micro-Analysis in Medical Biochemistry*. 4th Edn., Grune and Stratton Inc., New York, pp: 863-864.