

Clinical, Hematological, Biochemical and Pathological Studies on Zinc Deficiency (Hypozincemia) in Sheep

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Abstract: The clinical, hematological, biochemical and pathological changes in naturally occurring zinc deficiency in sheep were described. Loss of appetite, alopecia, abnormal skin and decreased body weight constituted the main signs and symptoms. The respiratory and heart rates were significantly higher ($p < 0.05$) in zinc deficient sheep than in normal control sheep. Values of total erythrocyte count, hemoglobin concentration and packed cell volume were significantly lower ($p < 0.05$) in zinc deficient sheep than in normal control sheep. Significant differences were not found in the mean corpuscular volume, mean corpuscular hemoglobin concentration, total leukocyte count and differential leukocyte count values in sick and normal control sheep. The platelet count was significantly lower ($p < 0.05$) in zinc deficient sheep than in normal control sheep. Values of other clotting indices (platelet volume, platelet distribution width, clotting time, prothrombin time and activated partial thrombo-plastin time) were significantly higher ($p < 0.05$) in zinc deficient than in normal control sheep. Zinc, total protein, calcium, alkaline phosphatase and fibrinogen values in the serum were significantly lower ($p < 0.05$) in zinc deficient than in normal control sheep. The levels of aspartate aminotransferase and alanine aminotransferase were significantly higher ($p < 0.05$) in zinc deficient than in normal control sheep. Microscopic lesions of the skin of zinc deficient sheep were in the form of parakeratosis and in some cases hyperkeratosis.

Key words: Zinc deficiency, hypozincemia, sheep, hemoglobin, serum, Iraq

INTRODUCTION

Only few reports are available on natural zinc deficiency in sheep in the literature (ARC, 1980; Mahmoud *et al.*, 1983). This could be due to the facts that pastures rarely contain < 20 mg zinc kg^{-1} DM and that sheep are able to absorb zinc very efficiently at low intakes (Suttle and Jones, 2007). The young rapidly growing lamb achieves its high requirement for zinc from the ewe's milk which is rich in zinc (around 7 mg L^{-1}) (Cao *et al.*, 2000). Among factors that predisposes sheep to zinc deficiency are increased calcium and phosphorus intake (decreases zinc absorption), diets rich in legumes (high calcium) or homemade high-phosphorus grain supplements (corn-soybean, corn-oats-barley) with no added minerals, elevation of soil pH above 6.5 and increased soil fertilization with nitrogen and phosphorus (Linklater and Smith, 1993; Anderson *et al.*, 2002). Experimentally induced zinc deficiency is associated with parakeratosis, growth retardation, wrinkled skin, wool loss, swollen hocks, excessive salivation and impaired

spermatogenesis (Kendall *et al.*, 2000). Pathologically, the cutaneous lesions include alopecia, scaling and crusting of the skin of the face, neck, distal extremities and muco-cutaneous junctions. The microscopic lesions consist of parakeratosis and sometimes hyperkeratosis (McGavin and Zachary, 2007).

Zinc is a component of almost 300 enzymes (Berger, 2002). It is vital to the activity of a variety of hormones such as glucagons, insulin, growth hormone and sex hormones (Al-Saad *et al.*, 2006). It also plays a key role in the immunological responses (Prasad and Kundu, 1995). Zinc also plays a role in maintaining hoof tissues through stimulation of growth of epidermal cells, production of keratin, improved wound healing and improved cellular integrity (Ginn *et al.*, 2007).

In a previous study, the clinical and pathological features of naturally occurring zinc deficiency in buffalo calves were described (Al-Saad *et al.*, 2006). The purpose of this study was to present the clinical, hematological, biochemical and pathological features of hypozincemia in sheep.

MATERIALS AND METHODS

Six herds of sheep consisting of 681 sheep were examined in Nineveh Province (Nineveh-Iraq). Among these sheep, a total of 125 sheep were found to be affected with hypozincemia and constituted the basis of the present study. Hypozincemic sheep were 32 rams (1-2 years old), 50 ewes (>2 years old) and 43 lambs (4-6 months old). Additionally, 25 normal sheep (4 months to >2 years old) were used as control. Both the clinical cases and the control sheep were of a local breed (Awassi). All of the used sheep were negative for gastrointestinal, blood and external parasites.

Blood samples were taken with and without anticoagulant from the jugular vein for hematological, biochemical and trace element analysis. Values of erythrocyte, hemoglobin, packed cell volume, Mean corpuscular volume, Mean Corpuscular Hemoglobin Concentration (MCHC), total Platelets count (Plt), Mean Platelets Volume (MPV), Platelet Distribution Width (PDW), Total Leukocyte Count (TLC) and Differential Leukocyte Count (DLC) were measured using automatic full digital cell counter (Bechman, USA). About 2.5 mL of blood were mixed with trisodium citrate (used plasma) to determine Prothrombine time (Prt), Activated partial thromboplastine time (Aptt) (Coles, 1986) and clotting time according to Bush (1975).

Blood samples were kept for 15 min at room temperature and then centrifuged to obtain serum for biochemical analysis. Total protein, AST, ALT, ALP, fibrinogen and calcium levels were determined using commercial kits (Biomere, France). The zinc values were determined using atomic absorption spectrophotometer (PYE Unicam spg atomic absorption spectrophotometer). The significance of variations in the various values of sheep with zinc deficiency and those of normal control sheep were analyzed statistically using SPSS version 11.5 (Leech *et al.*, 2007).

Skin specimens were collected from the alopecic patches and fixed in 10% formalin solution for 48 h, trimmed to suitable size, washed, dehydrated, cleared in xylol, embedded in paraffin wax, sectioned at 4-5 μ thickness, stained with hematoxylin and eosin and examined with a light microscope (Kiernan, 1999).

RESULTS AND DISCUSSION

The signs and symptoms of the 125 sheep are summarized (Table 1 and 2). These signs and symptoms arranged in order of decreasing frequency included anorexia (66.4%), alopecia (62.4%) (Fig. 1), abnormal skin (54.4%) (Fig. 2), decreased body weight (36.0%), pale mucous membranes (29.6%), watery saliva flow (26.4%), wool eating (20.0%), intermittent diarrhea (16.8%), decreased growth rate (12.8%) and swelling of joints (10.4%). Body temperature was similar in normal control and hypozincemic sheep (Table 2). The respiratory rate was significantly higher ($p<0.05$) in sick sheep than in normal control sheep. Within the groups of diseased sheep, the respiratory rate was significantly higher ($p<0.05$) in lambs than in rams and ewes (Table 2). The heart rate was significantly higher ($p<0.05$) in sick sheep than in control sheep. Among the groups of diseased sheep, the heart rate was significantly higher ($p<0.05$) in lambs than in rams and ewes (Table 2).

Results of hematological examination of samples from control and diseased sheep are presented in Table 3. Values of total erythrocyte count, hemoglobin concentration and packed cell volume were significantly lower in hypozincemic sheep than in normal control sheep. No significant differences were detected in these values for hypozincemic rams, ewes and lambs. Similarly, no significant differences were encountered in the MCV, MCHC, TLC and DLC values of normal control and diseased sheep. Also, significant differences were not found between these values in sick rams, ewes and lambs. Data concerning the indices of clotting factors of normal control and hypozincemic sheep are shown in Table 4. The platelet count was lower in diseased sheep than in

Table 1: Signs and symptoms in sheep affected with hypozincemia

| Signs and symptoms | No. of cases | Cases (%) |
|---------------------------|--------------|-----------|
| Loss of appetite | 83 | 66.4 |
| Alopecia in various parts | 78 | 62.4 |
| Abnormal skin | 68 | 54.4 |
| Decreased body weight | 45 | 36.0 |
| Pale mucous membranes | 37 | 29.6 |
| Watery saliva flow | 33 | 26.4 |
| Wool eating | 25 | 20.0 |
| Intermittent diarrhea | 21 | 16.8 |
| Decreased growth rate | 16 | 12.8 |
| Swelling of joints | 13 | 10.4 |

Table 2: Clinical parameters of sheep affected with hypozincemia

| Parameters | Control | Diseased | | |
|----------------------------------|-------------------------------|-------------------------------|-------------------------------|---------------------------------|
| | | Rams | Ewes | Lambs |
| Body temperature ($^{\circ}$ C) | 39.67 \pm 0.48 ^a | 39.72 \pm 0.76 ^a | 39.51 \pm 0.88 ^a | 39.8 \pm 0.83 ^a |
| Respiratory rate (min) | 24.0 \pm 1.8 ^a | 33.6 \pm 4.52 ^b | 35.82 \pm 6.71 ^b | 63.73 \pm 10.25 ^c |
| Heart rate (min) | 76.89 \pm 6.4 ^a | 98.71 \pm 4.62 ^b | 102.24 \pm 8.4 ^b | 130.21 \pm 10.51 ^c |

Values are mean \pm standard error of mean; values with different letters mean the presence of significant differences ($p<0.05$)

Table 3: Hematological values of normal control and hypozincemic sheep

| Parameters | Control | Diseased | | |
|----------------------------|-------------------------------|-------------------------------|-------------------------------|------------------------------|
| | | Rams | Ewes | Lambs |
| RBC count $\times 10^6$ | 7.45 \pm 0.94 ^a | 5.21 \pm 1.19 ^b | 5.02 \pm 1.12 ^b | 4.82 \pm 1.11 ^b |
| Hb (g dL ⁻¹) | 10.72 \pm 1.41 ^a | 8.31 \pm 1.07 ^b | 8.42 \pm 1.21 ^b | 7.92 \pm 0.4 ^b |
| PCV (%) | 35.24 \pm 4.8 ^a | 27.3 \pm 1.24 ^b | 28.41 \pm 1.6 ^b | 26.4 \pm 1.62 ^b |
| MCV (fl) | 53.77 \pm 7.2 ^a | 52.8 \pm 4.21 ^a | 54.11 \pm 3.61 ^a | 52.7 \pm 7.8 ^a |
| MCHC (g dL ⁻¹) | 30.75 \pm 4.5 ^a | 30.27 \pm 3.66 ^a | 31.24 \pm 4.81 ^a | 30.2 \pm 4.8 ^a |
| TLC $\times 10^9$ | 7.12 \pm 1.21 ^a | 7.24 \pm 1.12 ^a | 6.9 \pm 1.82 ^a | 7.4 \pm 1.33 ^a |
| Lymphocytes | 47.4 \pm 2.11 ^a | 46.2 \pm 1.33 ^a | 48.2 \pm 2.11 ^a | 47.3 \pm 1.81 ^a |
| Neutrophils | 49.12 \pm 6.11 ^a | 48.4 \pm 2.4 ^a | 46.3 \pm 3.2 ^a | 48.8 \pm 2.7 ^a |
| Monocytes | 3.12 \pm 1.09 ^a | 3.03 \pm 1.4 ^a | 3.21 \pm 0.91 ^a | 3.2 \pm 1.28 ^a |
| Eosinophils | 1.64 \pm 1.2 ^a | 1.52 \pm 1.4 ^a | 1.62 \pm 1.3 ^a | 1.59 \pm 1.0 ^a |
| Basophils | 0.48 \pm 0.49 ^a | 0.47 \pm 0.48 ^a | 0.46 \pm 0.48 ^a | 0.47 \pm 0.46 ^a |

Values are mean \pm standard error of mean; values with different letters mean the presence of significant differences ($p < 0.05$)

Table 4: Indices of clotting factors in control and hypozincemic sheep

| Parameters | Control | Diseased | | |
|-------------------|-----------------------------|------------------------------|------------------------------|------------------------------|
| | | Rams | Ewes | Lambs |
| Plt $\times 10^9$ | 477 \pm 33.8 ^a | 351 \pm 40.71 ^b | 360 \pm 22.8 ^b | 310 \pm 70.2 ^c |
| MPV (fl) | 8.26 \pm 0.6 ^a | 11.41 \pm 2.4 ^b | 10.61 \pm 1.9 ^b | 11.62 \pm 3.2 ^b |
| PDW (%) | 15.6 \pm 1.8 ^a | 119.8 \pm 7.3 ^b | 20.4 \pm 3.2 ^b | 18.7 \pm 9.4 ^b |
| Ct (min) | 3.5 \pm 0.51 ^a | 5.8 \pm 1.7 ^b | 5.2 \pm 1.2 ^b | 5.4 \pm 1.22 ^b |
| Ptt (sec) | 10.2 \pm 1.3 ^a | 17.6 \pm 2.3 ^b | 15.9 \pm 1.4 ^b | 17.8 \pm 2.4 ^b |
| appt (sec) | 32.0 \pm 2.4 ^a | 40.8 \pm 2.61 ^b | 42.5 \pm 2.9 ^b | 42.5 \pm 4.6 ^b |

Values are mean \pm standard error of mean; values with different letters mean the presence of significant differences ($p < 0.05$)

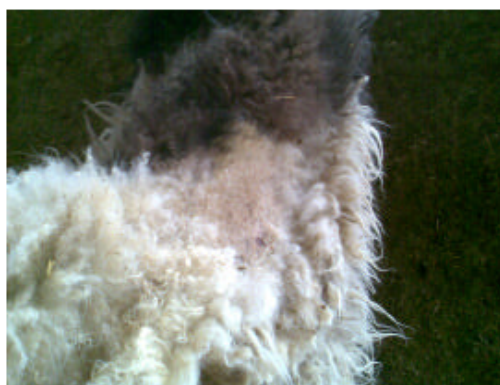


Fig. 1: A sheep affected with zinc deficiency, alopecia in different parts of the skin



Fig. 2: The neck of a sheep affected with zinc deficiency. Note the alopecia and abnormality of skin

control normal sheep. This difference was statistically significant ($p < 0.05$). This count was significantly lower in lambs than in rams and ewes. The platelet volume, platelet distribution width clotting time, prothrombin time and activated partial thromboplastin time values were significantly higher ($p < 0.05$) in diseased sheep than in normal control sheep. No difference was encountered between these values in various groups of diseased sheep.

Serum values of minerals, proteins and enzymes in control and diseased sheep are presented in Table 5. Zinc, total protein, calcium, alkaline phosphatase and fibrinogen values were significantly lower ($p < 0.05$) in diseased sheep than in control sheep. Among the various groups of diseased sheep, zinc level was the lowest in lambs than in rams and ewes. The AST and ALT levels were significantly greater ($p < 0.05$) in sick sheep than in normal control sheep.

Histopathology of the skin biopsies revealed parakeratosis, moderate acanthosis and sometimes hyperkeratosis. Furthermore, there were poor wool growth, intracellular edema, pseudoepitheliomatous hyperplasia and heavy infiltration of mononuclear cells (plasma cells, lymphocytes and mophages) in the dermis (Fig. 3 and 4).

In the present study, loss of appetite was the main clinical sign exhibited by zinc deficient sheep. Reduced appetite has been also reported in buffalo calves affected with zinc deficiency (Al-Saad *et al.*, 2006). In humans with zinc deficiency the reduced appetite has been attributed to reduced ability to taste (hypogeusia) and smell foods

Table 5: Serum values of some minerals, proteins and enzymes in normal control and hypozincemic sheep

| Parameters | Control | Diseased | | |
|---|------------------------------|------------------------------|------------------------------|------------------------------|
| | | Rams | Ewes | Lambs |
| Zn($\mu\text{g mL}^{-1}$) | 82.11 \pm 2.7 ^a | 33.61 \pm 5.4 ^b | 35.92 \pm 5.4 ^b | 20.61 \pm 3.4 ^c |
| Total protein(g dL ⁻¹) | 7.08 \pm 1.21 ^a | 6.21 \pm 1.27 ^b | 5.98 \pm 1.32 ^b | 5.5 \pm 1.08 ^b |
| AST(U L ⁻¹) | 39.37 \pm 9.9 ^a | 56.41 \pm 4.6 ^b | 62 \pm 8.91 ^b | 64.51 \pm 6.2 ^b |
| ALT(U L ⁻¹) | 19.67 \pm 4.7 ^a | 28.25 \pm 3.1 ^b | 27.4 \pm 4.21 ^b | 30.4 \pm 6.2 ^b |
| Calcium(mg/100 mL) | 11.3 \pm 0.86 ^a | 9.4 \pm 0.52 ^b | 8.9 \pm 1.21 ^b | 8.4 \pm 0.68 ^b |
| Alkaline phosphatase (U L ⁻¹) | 250 \pm 10.88 ^a | 201 \pm 6.68 ^b | 210 \pm 4.62 ^b | 200 \pm 7.86 ^b |
| Fibrinogen(mg dL ⁻¹) | 346 \pm 10.2 ^a | 310 \pm 8.71 ^b | 298 \pm 11.78 ^b | 305 \pm 7.43 ^b |

Values are mean \pm standard error of mean; values with different letters mean the presence of significant differences ($p < 0.05$)

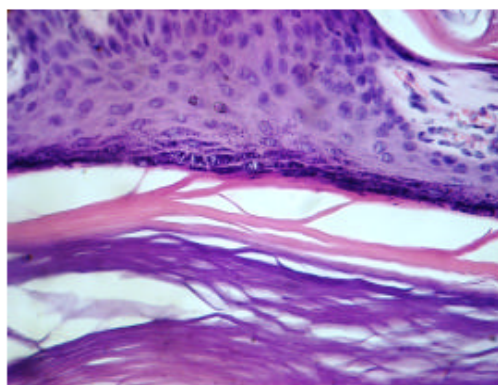


Fig. 3: Cross section of the skin lesion of a sheep affected with zinc deficiency. Note the presence of parakeratosis, hyperkeratosis and acanthosis. H and E stain

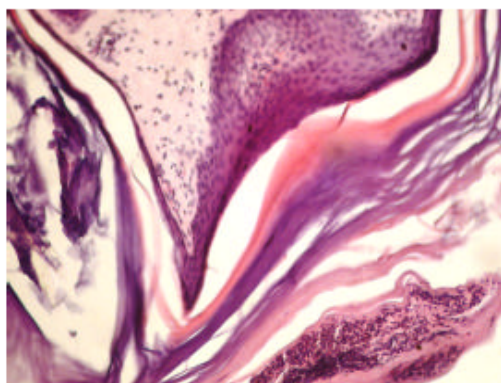


Fig. 4: Cross section of the skin lesion of a sheep affected with zinc deficiency. Note epidermal hyperplasia with marked hyper-keratosis. The parakeratotic hyperkeratosis and acanthosis form the thickened adherent scale. (arrow). H and E stain

(hyposmia) (Berger, 2002; Grodner *et al.*, 2004). It has been shown that changes in appetite are associated with changes in the concentration of amino acid derived neurotransmitters in the brain.

Zinc deficient rats have been noted to change their dietary preferences, avoiding carbohydrates and seeking protein and fat (Kennedy *et al.*, 1998). This might be explained by the lack of key enzymes required for carbohydrate metabolism because of reduced expression of zinc dependent messenger RNA needed to synthesize these enzymes (Kennedy *et al.*, 1998). As in humans, zinc deficiency in animals may reduce appetite by impairing the taste. A well known fact is that the sense of taste is mediated through the salivary zinc dependent polypeptide, gustin. Low salivary zinc concentration leads to a reduction of taste and reduced appetite (Droke *et al.*, 1993). The reduced appetite in zinc deficient animals could be the reason for paleness of mucous membranes, depression of growth rate and the lowered body weight. This suggestion is supported by the finding that feeding 3000 ppm zinc, added as zinc oxide, enhances growth and health of nursery pigs (Hahn and Baker, 1993; Hill *et al.*, 2000; Carlson *et al.*, 1999).

In this study, the finding of abnormal skin was common and this result is expectable since parakeratosis, thickening, hardening and cracking of skin is a common sign of zinc deprivation in human and in all animal species (Radostits *et al.*, 2000; Berger, 2002). Alopecia was the second most frequent sign in sheep with zinc deficiency. This finding is in accordance with those of others in calves (Radostits *et al.*, 2000; Machen *et al.*, 1996; Sharma and Joshi, 2005; Can *et al.*, 1999), buffalo calves (Al-Saad *et al.*, 2006) and in humans (Prasad, 1985, 1983; Gaveau *et al.*, 1987). The finding of wool eating in zinc deficient sheep is in accordance with the finding of others that alopecia and the wool eating habit in sheep were associated with reduction in serum copper and zinc levels (Akgul *et al.*, 2000). Zinc has been found to be crucial to proper skeletal growth during embryonic development (Berger, 2002). This could explain the occurrence of the skeletal disturbances that have been seen in zinc deficient sheep in this study. The respiratory and heart rates were significantly higher ($p < 0.05$) in zinc deficient sheep than in normal control sheep. These rates were significantly higher ($p < 0.05$) in lambs than in rams and ewes. These differences could be due to the fact that zinc is a

component of the enzyme carbonic anhydrase, which is located in the red blood cells and parietal cells of the stomach and is related to the transport of respiratory carbon dioxide and the secretion of hydrochloric acid by the gastric mucosa (Radostits *et al.*, 2000).

Results of the hematological examination indicated that the values of total RBC count, Hb concentration and PCV in zinc deficient sheep were significantly lower ($p < 0.05$) than those of normal control sheep. Significant differences were not found in these values in zinc deficient rams, ewes and lambs. The anemia of the zinc deficient sheep was of the normocytic normochromic type. These results were similar to those reported in hypozincemic buffalo calves (Al-Saad *et al.*, 2006) and could be explained on the basis that zinc deficiency leads to impairment of cell replication and protein synthesis and thus the generation of blood cells (Payne, 1989). Significant differences were not encountered in the rest of the hematological parameters in normal control and hypozincemic sheep.

In this study, the platelet count was significantly lower ($p < 0.05$) in diseased sheep than in normal control sheep. Additionally, this count was significantly lower in lambs than in rams and ewes. These findings are in accordance with those of (Gordon *et al.*, 1982; Tubek *et al.*, 2007) who found that a low zinc diet caused poor platelet aggregation and increased bleeding tendency in adult males. The indices of other clotting factors (platelet volume, platelet distribution width, clotting time, prothrombin time and the activated partial thromboplastin time) were significantly higher ($p < 0.05$) in zinc deficient sheep than in normal control sheep. These results are in accordance with the findings of others with either human or bovine thrombin that 0.01-0.1 mM Zn^{+2} induced significant reductions of clotting times in a concentration-dependent manner (Marx and Eldor, 1985). The procoagulant effect of Zn^{+2} occurred in the presence of Ca^{+2} but was inhibited by metal chelating agents. Higher levels of Zn^{+2} (> 0.2 mM final concentration) were required to accelerate thrombin-induced clot formation in the presence of citrate or oxalate. The presence of as little as 0.006 mM Zn^{+2} in an incubating mixture of thrombin and antithrombin-III has been found to severely reduce the inhibitory activity of antithrombin-III towards thrombin (Marx and Eldor, 1985). In another study, it was found that Zn^{+2} at concentrations > 1 microM increase the inhibition of coagulation factors XIa (FXIa) by protease nexin-2 (PN-2)/amyloid beta-protein precursor (Van Nostrand, 1995).

In this study, the serum values of zinc, total protein, calcium, alkaline phosphatase and fibrinogen were

significantly lower ($p < 0.05$) in zinc deficient sheep than in normal control sheep. This finding indicated clearly that the condition in sick sheep is hypozincemia. Zinc level was significantly lower ($p < 0.05$) in lambs than in rams and ewes. These results were similar to those reported in humans, cattle and buffalo calves (Machen *et al.*, 1996; Can *et al.*, 1999; Parade, 1981; Kirchessner *et al.*, 1975; Stockham and Scott, 2002). The level of aspartate aminotransferase was higher significantly ($p < 0.05$) in sick sheep as compared to normal control sheep. A similar finding has been reported in buffalo calves (Al-Saad *et al.*, 2006). Aspartate amino-transferase is known to be a cytoplasmic and mitochondrial enzyme that catalyzes a reversible reaction involved in the deamination of aspartate to form oxaloacetate, which can enter the Krebs cycle (Stockham and Scott, 2002). A high serum level of the enzyme occur in disorders or conditions that involve hepatocyte or muscle damage (Stockham and Scott, 2002).

Thus, it could be assumed that the increased level of the enzyme in this study could be due to hypoxia due to anemia. The serum level of alanine aminotransferase was significantly higher ($p < 0.05$) in zinc deficient sheep than in normal control sheep. This result differs from that reported in buffalo calves (Al-Saad *et al.*, 2006) in which no significant difference in the level of the enzyme was encountered in sick and normal buffalo calves. However, the result is similar to that reported by others (Akgul *et al.*, 2000).

Microscopic lesions of the skin of zinc deficient sheep described in this study were typical of zinc deficiency (Samady *et al.*, 2000; Walling *et al.*, 1989; Nakano *et al.*, 2003; Wilson *et al.*, 2006; Al-Saad *et al.*, 2006). Parakeratosis and in some cases hyperkeratosis are the main lesions. These pathological changes have been attributed to the involvement of zinc with cell replication in the skin (Wrightt and Spears, 2004). Furthermore, copper, zinc, selenium and molybdenum are involved in many biochemical processes supporting life. The most important of these processes are cellular respiratory, cellular utilization of oxygen, DNA and RNA reproduction, maintenance of cell membrane integrity and sequestration of free radicals (Wrightt and Spears, 2004).

CONCLUSION

Hypozincemia were affected sheep and exhibited different clinical signs, a significant changes were noticed between diseased and control sheep in hematological and biochemical values with differences indicated in blood clotting indices.

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