RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES
PRINT ISSN 2221-1896, ONLINE ISSN 2223-0343
www.roavs.com

# Zinc deficiency (hypozincemia) in local Iraqi cattle

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## Abstract

Clinical, hematological, pathological and some biochemical parameters have been studied in local cattle and calves affected naturally with hypozincemia in Mosul, Iraq. The study was conducted on 78 local Iraqi cattle and calves, among these animals, 30 calves were less than six months of age and 38 animals were more than three years old. Ten clinical healthy cattle of different ages were used as control. Affected cattle showed signs of alopecia in different body regions (73.6%), abnormal skin (rough, thickened, wrinkled, cracked and with dandruff ) (73.6%), paleness of mucous membranes (47.3%), intermittent diarrhoea (39.4%), decreased milk production (31.5%) and loss of appetite (26.3%), whereas affected calves showed alopecia in various body regions (90%), abnormal skin (83.3%), decreased growth rate (53.3%), swelling of joints and stiff gait (43.3%) and pica (36.6%). No significant difference has been detected in body temperature, whereas respiratory and heart rates were significantly increased in affected animals in comparison with control. Statistical analysis showed significant decrease in the total erythrocytes (TRBCs), hemoglobin (HB) and packed cell volume (PCV) in diseased cattle and calves and macrocytic normochromic type of anemia was found. The results also indicated significant decrease in lymphocytes and platelets counts**,** however significant increase was encountered in platelets volume, platelets distribution width, prothrombine time and activated partial thromboplastine time in diseased animals. The biochemical results revealed significant decrease in serum zinc and fibrinogen and haptoglobin level was higher in diseased cattle and calves. Microscopic lesions of the skin of zinc deficient cattle and calves were in the form of epidermal hyperplasia, parakeratosis, hyperkeratosis, acanthosis and the formation of thickened adherent scale.

**Keywords:** Zinc Deficiency, Cattle, Hematology, Pathology, Biochemistry

## Introduction

Zinc has a wide spectrum of biological activities and its deficiency has been related to various dysfunctions and alterations of normal cell metabolism **(**Chirase et al., 1991). It is an integral component of a wide range of metalloenzymes and acts as a cofactor for RNA and DNA polymerases (Mozaffari and Derakhshanfar, 2007). Its presence is of particular importance in rapidly-dividing cells, including those of the epidermis (Nishi, 1996). Zinc is also essential for the biosynthesis of fatty acids and participates in both the inflammatory and immune responses and also involved in the metabolism of vitamin A (Watson, 1998).

The risk of zinc deficiency increases when soil pH rises above 6.5 and use of fertilizers like nitrogen and phosphorus increases (Miller et al., 1991). Some legumes contain less zinc than grasses grown on the same soil and zinc concentration decreases with aging of the plant (Arrayet et al., 2002). Several factors may affect the availability of zinc to ruminants and cause secondary zinc deficiency. These include the consumption of immature grass, feeding of late-cut hay, and the presence of excessive dietary sulphur. Moreover, the contamination of silage with soil at harvesting time can also affect the digestibility of zinc (Radostits et al., 2007).

Cattle have a small, zinc storage unit, therefore clinical signs and laboratory abnormalities associated with zinc deficiency occur rapidly after removal of zinc from diets and return to normal after supplementation, therefore, optimal zinc in nutrition is required (Campbell and Miller, 1998).

Zinc deficiency results in failure of keratinization, which leads to parakeratosis, loss and failure of growth of hair, lesions of coronary bands, retarded testicular development and cessation of spermatogenesis (Oberleas and Harland, 2008). This probably reflects the importance of zinc in protein synthesis (Meglia et al., 2008). The lesions of the arteriolar walls of the dermis have also been reported (Engle et al., 1997).

Natural cases of zinc deficiency occur in cattle and characterized by parakeratosis and alopecia which may affect about 40-50% of the skin area, the lesions are most marked on the muzzle, vulva, anus, tail head, ears, backs of the hind legs, knee folds, flank and neck. Most animals are below average body condition and are stunted in growth.

The purpose of the current work is to present the clinical, hematological, biochemical and pathological features of clinical hypozincemia in local breed of cattle and calves in Mosul, Iraq.

## Materials and Methods

Seventy eight cattle and calves of local breeds, of different ages and of both sexes were brought to the Consultant Veterinary Hospital, College of Veterinary Medicine, and University of Mosul. Diseased animals exhibited signs of loss of appetite, alopecia in various parts of the skin, thickening and scaling of the skin and signs of anemia. Among these animals 30 calves were less than six months old and 38 animals were more than three years old .All of the examined cattle was negative for gastrointestinal, blood and external parasites. Ten clinical healthy cattle of different ages were served as control. Hematological examinations were performed using automatic full digital cell counter (Beckman coulter, USA). Blood was drained from each animal by jugular vein- puncture and from it 2.5 milliliters of blood were mixed with EDTA and used to determine total erythrocyte count (TRBCs), hemoglobin concentration (Hb), packed cell volume (PCV), platelets count (PLT), mean platelets volume (MPV), platelets distribution width (PDW) and total leucocytes count. Differential leucocytes count was done using giemsa stained blood smears (Coles, 1986). The other 2.5 milliliters of blood were mixed with trisodium citrate (used plasma) to determine prothrombine time (Prt), activated partial thromboplastine time (APTT) and fibrinogen using commercial kits (Biolabo/France). Clotting time (CT) was also estimated according to (Bush, 1975). Skin specimens were collected from alopecic patches fixed in 10% formalin solution for 48 hours, trimmed to suitable sizes, washed, dehydrated, cleared in xylol, embedded in paraffin wax, sectioned at 5-6µm thickness, and stained with hematoxlyin and eosin and examined with light microscope (Kiernan, 1999). Blood serum samples were tested spectrophotometrically for zinc values using atomic absorption spectrophotometer (PYE Unicam spg atomic absorption spectrophotometer). Bovine-Haptoglobin-ELISA was estimated according to (Hiss et al., 2004). Microtiter plates were coated with purified bHp (5 ng in 100 *µ*L of 50 m*M* NaHCO3, pH 9.6) at 4°C for 20 h. After blocking with 300 µL of 2.5% casein in 0.05 M NaCl, pH 7.4, at room temperature for 1.5 h, the plates were stored at −20°C. Prior to use, the plates were washed 5 times. To each well, 50 µL of test sera (dilution 1/100 in healthy cows or 1/1000 in diseased cows) was added in duplicate. Calibration curves were created using 50 µL of purified bHp at dilutions from 0.0 to 10 µg/mL in duplicate. An amount of 50 µL of the antiserum (dilution 1/50,000) was then added and incubated for 2 h at room temperature. After 3 washes, 100 µL of the second antibody conjugated to peroxidase (1/20,000 dilution) was added and incubated for 30 min. After 5 washes, the wells were filled with 150 µL of a freshly prepared substrate solution containing 0.05 M citric acid, 0.055 M Na2HPO4, 0.05% urea hydrogen peroxide, 2% ProClin 150, and 2% of a tetramethylbenzidine solution (12.5 mg/mL dimethylsulfoxide). The reaction was stopped after 30 min with 50 µL of 1M oxalic acid, and the optical density (OD) was determined at 450 nm with a microtiter plate reader. The Hp concentrations in unknown samples were then calculated from the calibration curve.

The significance of variations in the various values of cattle and calves with zinc deficiency and those of normal control animals were analyzed statistically using one way analysis of variance (SPSS program) ( Leech et al., 2007).

## Results

Diseased cattle with hypozincemia showed sings of alopecia in different body regions (73.6%) (Fig. 1), abnormal skin (rough skin, thickened, wrinkled, cracked and with dandruff (73.6%) (Fig. 2), paleness of mucus membranes (47.3%), intermittent diarrhea (39.4%) decreased milk production (31.5%) and loss of appetite (26.3%). Hypozincemic calves showed alopecia in various body regions (90%), abnormal skin (83.3%), decreased growth rate (53.3%), swelling of joints and stiff gait (43.3%) and pica (36.6%) (Table1).

No significant difference was detected in body temperature, whereas respiratory and heart rates were significantly increased ($P<0.05$) in diseased animals in comparison with control (Table 2).

There was significant reduction ($P<0.05$) in the mean values of TRBc, HB and PCV in diseased cattle and calves affected with hypozincemia, and anemia was of macrocytic normochromic type. Results also indicated significant decrease in lymphocytes in diseased cattle and calves affected with zinc deficiency (Tables 3 and 4).

Changes of blood clotting indices were also noticed in diseased cattle and calves affected with hypozincemia compared with control animals. The results showed significant decrease ($P<0.05$) in the mean values of total platelets count, whereas significant increase ($P<0.05$) was encountered in platelets volume,

**Table 1: Clinical signs of cattle and calves affected with zinc deficiency (n=68)**

| Clinical sings /Cattle(n=38) | No. of cases | Percentage of cases |
|---|---|---|
| Alopecia in various body regions | 28 | 73.6 |
| Abnormal skin | 28 | 73.6 |
| Pale mucous membranes | 18 | 47.3 |
| Intermittent diarrhea | 15 | 39.4 |
| Decreased milk production | 12 | 31.5 |
| loss of appetite | 10 | 26.3 |
| Clinical sings /Calves (n=30) | | |
| Alopecia in various body regions | 27 | 90 |
| Abnormal skin | 25 | 83.3 |
| Decreased growth rate | 16 | 53.3 |
| Swelling of joints and Stiff gait | 13 | 43.3 |
| Pica | 11 | 36.6 |

**Table 2: Clinical parameters of cattle and calves affected with zinc deficiency**

| Parameters | Body temperature (°C) | Respiratory rate/min | Heart rate/ min |
|---|---|---|---|
| Control | 38.6±0.5 7[a] | 25±7.37[a] | 80±3.62[a] |
| Cattle | 39.0±0.55[a] | 38.0±10.62[b] | 97.0±13.44[b] |
| Calves | 38.8±0.63[a] | 44.0±5.73[b] | 130±10.53[b] |

[a,b] Values with different superscript within a column differ significantly (P<0.05)

**Table 3: Blood parameters of diseased cattle and calves affected with zinc deficiency and control group**

| Parameters | RBC ($\times 10^6$/ml) | Hb (g/dl) | PCV (%) | MCV (fl) | MCHC (gm/100ml) |
|---|---|---|---|---|---|
| Control | 6.92 ± 1.34[a] | 12.37± 0.54[a] | 36.32± 2.11[a] | 52.39±3.77[a] | 34.05±2.86[a] |
| Cattle | 4.8±1.23[b] | 9.76±1.43[b] | 28±2.72[b] | 58.33±4.45[b] | 34.85±3.54[a] |
| Calves | 5.1±1.76[b] | 10.2±1.22[b] | 29±2.65[b] | 56.86 ±3.52[b] | 35.17±5.78[a] |

[a,b] Values with different superscript within a column differ significantly (P<0.05)

**Table 4: Total and differential leukocytes count of cattle and calves affected with zinc deficiency and control group**

| Parameters | TLC($\times 10^3$/ml) | N% | L% | M% | E% | B% |
|---|---|---|---|---|---|---|
| Control | 10.58±2.46[a] | 45.16±3.33[a] | 48.67±4.21[a] | 3.51±1.21[a] | 3.29±1.36[a] | 1.2± 0.25[a] |
| Cattle | 9.78±1.93[a] | 50.33±7.63[a] | 40.12±2.53[b] | 4.33±2.11[a] | 4.58±1.22[a] | 1.3±0.33[a] |
| Calves | 9.11±1.23[a] | 49.22±4.32[a] | 40.34±2.54[b] | 4.53±2.23[a] | 4.71±1.34[a] | 1.4±0.12[a] |

[a,b] Values with different superscript within a column differ significantly (P<0.05)

**Table 5: Indices of clotting factors in diseased cattle and calves affected with zinc deficiency and control group**

| Parameters | PLT ($\times 10^3$/ml) | MPV(fl) | PDW (%) | CT (min.) | PRT (sec) | APPT(sec) |
|---|---|---|---|---|---|---|
| Control | 423.72±34.41[a] | 8.41±2.52[a] | 18.62±4.52[a] | 3.2 5±1.12[a] | 13.74±1.85[a] | 56.24±3.42[a] |
| Cattle | 333.64±55.46[b] | 15.13±2.71[b] | 22.45±3.25[b] | 4.83±1.42[a] | 18.58±3.87[b] | 62.82±6.44[b] |
| Calves | 350.14±75.56[b] | 13.23±3.61[b] | 21.65±4.28[b] | 4.53±1.72[a] | 17.28±4.57[b] | 61.73±5.34[b] |

[a,b] Values with different superscript within a column differ significantly (P<0.05)

**Table 6: Biochemical changes in cattle and calves affected with zinc deficiency**

| Parameters | Serum zinc (µg/ml) | Fibrinogen (mg/100ml) | Haptoglobin (mg/100ml) |
|---|---|---|---|
| Control | 85.61±1.23[a] | 410.30±28.55[a] | 0.22±0.31[a] |
| Cattle | 33.26±5.71[b] | 320.24±22.67[b] | 0.61±0.32[b] |
| Calves | 42.16±3.51[b] | 351.72±15.75[b] | 0.66±0.24[b] |

[a,b] Values with different superscript within a column differ significantly (P<0.05)

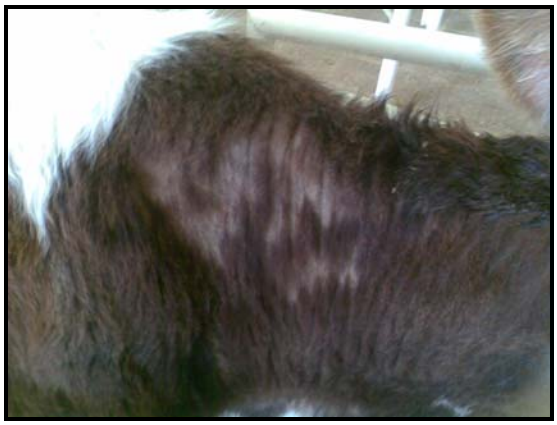

**Fig. 1: Alopecia in different body regions**

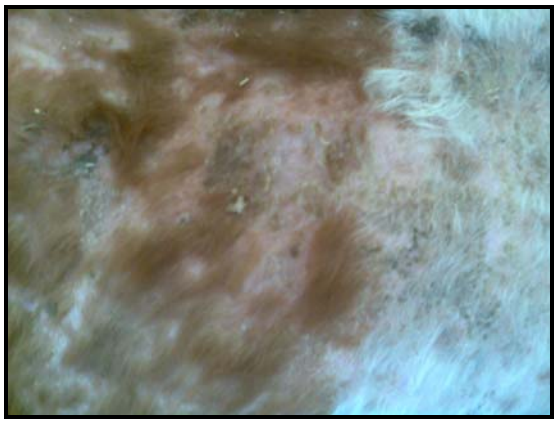

**Fig. 2: Dandruff with thickening of the skin**

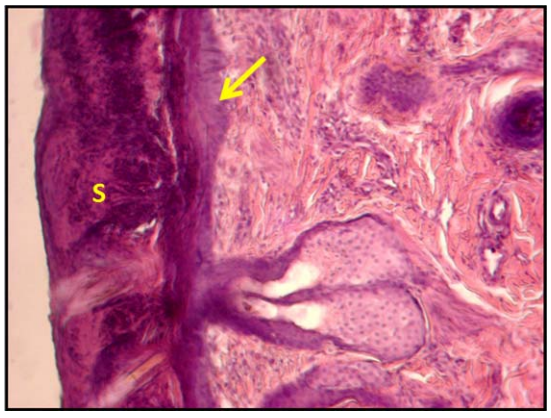


**Fig. 3: Cross section of the skin lesion of a calf affected with zinc deficiency. Note the increased thickness of the epidermis (arrow), the para-keratotic hyperkeratosis, and the moderate acanthosis leading to the formation of thickened adherent scale (S). H&E. 165.**

platelets distribution width, prothrombin time and activated partial thromboplastin time (Table 5).

Histopathological examination of the skin lesions revealed thickening of the epidermis with parakeratosis, hyperkeratosis and moderate acanthosis. These changes lead to the formation of an adherent scale (Figs 3 and 4).

Results of biochemical changes indicated significant decrease ($P<0.05$) in zinc values and fibrinogen in diseased cattle and calves compared with control animals. However, hapotoglobin level was increased in cattle and calves affected with hypozincemia (Table 6).

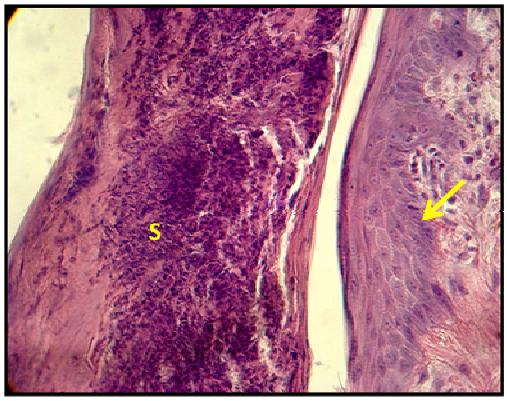


**Fig. 4: Cross section of cutaneous lesion in a calf affected with zinc deficiency. Note the thickened epidermis (arrow) and the thick adherent scale (S). H&E. 370x.**

## Discussion

Deficiencies in oligo-elements always negatively affect health, production and reproduction in cattle (Engle et al., 1997). Mineral supplementation in rations for cattle is important not only for animals but also for farmer who could benefit a greater productivity of his cattle and a better financial gain if the trace element status in animals would be appropriate (Underwood, 1977). Once the animals are deficient, their products (meat, milk) are also deficient. Meat and milk are consumed by people who need a sufficient intake of oligo-elements to their health (Spears, 1995).

Diseased cattle and calves showed different clinical signs which were also mentioned by others (Spears, 1994, Radostits et al., 2007) that zinc deficiency in cattle results in reduced growth and feed intake, loss of hair and skin lesions which are most severe on legs, neck, head, around the nostrils, excessive salivation, swollen feet with open, scaly lesions and impaired reproduction. Arrayet et al. (2002) added that deficiency of zinc in males reduces testicular

development and sperm production while oestrus and conception rate are decreased in females. The extent that marginal or subclinical zinc deficiency exists is unknown, but is likely more widespread and based on zinc supplementation studies, subclinical zinc deficiency can result in impaired reproduction and decreased weight gains (Berleas, 2008).

Zinc deficiency has been known to cause hypogonadism and several mechanisms of hypogonadism due to zinc deficiency have been suggested. Moreover, zinc affects growth hormone (GH) metabolism and conversely, GH affects zinc metabolism, as zinc deficiency may result in reduced GH production and/or insulin-like growth factor-I (IGF-I). Zinc deficiency may also affect bone metabolism and gonadal function and the interrelationships among zinc, growth, gonadal function and GH-IGF-I axis appear to be complex (Nishi, 1996). Increasing calf age was associated with lower serum zinc concentration which could reflect an actual age effect, or alternatively, an effect caused by the shift from a dam's milk-based diet to pasture or supplemental forage and concentrates (Campbell and Miller, 1998).

Studies in various species, including rodents, domestic fowl, calves and lambs have found that dietary zinc deficiency significantly reduces red blood cell carbonic anhydrase activity which may impair respiratory functions (Lukaski, 2005). This might be the cause of the increased respiratory rate which was detected in diseased cattle and calves in our study. Rapid respiration may occur due to hypoxia (anemic hypoxia), caused by decreased Hb concentration, affecting oxygen transportation to body tissues. Therefore, the body may receive inadequate supply of oxygen which result in panting in animals (Radostits et al., 2007). Results of hemogram revealed a significant decrease in TRBCs, HB, PCV, reflecting macrocytic normochromic type of anemia. Similar results were recorded by Al-Saad et al. (2006, 2010) in buffalo calves and sheep. The cause of anemia in zinc deficient animals might be due to impairment of cell replication and protein synthesis and thus the generation of blood cells (O´Dell et al., 1987; Payne, 1989).

It has been suggested that dietary zinc deficiency stimulates the hypothalamus–pituitary–adrenal stress axis, leading to increased plasma corticosterone levels. This may explain the lymphopenia and thymic atrophy associated with dietary deficiencies (Fraker et al., 1995). Moreover Fraker et al. (2000) added that zinc deficiency and energy malnutrition in mice are characterized by reduced growth, atrophy of lymphoid tissue, reduced lymphocyte numbers and increased susceptibility to infection.

Platelet count was significantly lower in diseased cattle and calves than normal control animals. These findings are in accordance with those of Gordon et al. (1982) who found that low zinc diet causes poor platelet aggregation and increased bleeding tendency in adult males. However, it has been shown that hypozincemia predisposes to increased coagulability, poor platelet aggregation and increased bleeding time. The blood clotting disturbances can be regressed by appropriate zinc intake management. Considering the importance of zinc as an essential element, its participation in regulation of the equilibrium between pro- and anti-thrombotic factors originating in platelets and endothelium has been reported (Tubek et al., 2007). Moreover, it has been found that zinc deficiency impairs thrombin-stimulated platelet aggregation; thereby increasing bleeding tendency due to impair and abnormal platelet function (Xia and O´Dell, 1995).

The indices of other clotting factors (platelet volume, platelet distribution width, prothrombin time and the activated partial thromboplastin time) were significantly higher in zinc deficient cattle and calves than normal control animals. Similar results were recorded by Marx and Eldor (1985) who mentioned that procoagulant effect of $Zn^{+2}$ occurs in the presence of $Ca^{+2}$ but was inhibited by metal chelating agents. Higher levels of $Zn^{+2}$ (greater than 0.2 mM final concentration) were required to accelerate thrombin-induced clot formation in the presence of citrate or oxalate. Moreover, 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. Van Nostrand (1995) added that $Zn^{+2}$ at concentrations >1 microM increased the inhibition of coagulation factors X1a (FX1a) by protease nexin-2 (PN-2)/amyloid beta-protein precursor.

Results of biochemical analysis of serum samples indicated a significant decrease in the level of serum zinc in both diseased cattle and calves as compared to that of healthy control animals. In cattle and other ruminants, haptoglobin (α-2 globulin produced in the liver) has been one of the acute phase proteins most commonly monitored as a marker of inflammation and has been found to be effective in detecting serious inflammatory conditions in cows (Ganheim et al., 2007, Nazifi et al., 2008). Haptoglobin, binds free hemoglobin released from erythrocytes with high affinity, thereby inhibits its oxidative activity (Wassell, 2000). In current study, results indicated increased level of haptoglobin in diseased cattle and calves affected with zinc deficiency than in normal animals. It has been shown that zinc-deficiency induces stress responses (Someya et al., 2009) and higher levels of haptoglobin related to stress were mentioned by Steel and Whitehead, (1994) and Gymnich et al. (2003). Stress stimulates the hypothalamus–pituitary–adrenal axis through several intermediates, including adrenocorticotropic hormone (ACTH), which increases the

release of corticosterone from the adrenal glands and corticosterone act on the liver to increase the induction of acute-phase proteins such as haptoglobin. Thus, the stress axis involves interactions among many components, including ACTH, corticosterone, and haptoglobin. Moreover, Godson et al. (1995) added that the acute phase response is characterized by localized changes such as the aggregation of platelets and clot formation, dilatation and leakage of blood vessels, accumulation of leukocytes and activation of stromal cells to release biological respons. The release of mediators by resident and invading cells then results in the initiation of systemic responses, including activation of complement and clotting systems, alterations in the plasma concentration of trace minerals and changes in liver metabolism, including the production of a set of proteins called acute phase proteins and haptoglobin is one of them.

The histopathological changes reported in cattle and calves were similar to those reported in zinc deficiency in human (Samady et al., 2002; Wilson et al., 2006) and animals (Singer et al., 2000; Tyler et al., 2003). These changes are commonly reported in zinc deficiency but are not entirely diagnostic for this type of deficiency. They have been attributed to the involvement of zinc, copper, selenium and molybdenum in many biochemical processing supporting life such as utilization of oxygen, DNA and RNA reproduction, maintenance of cell membrane integrity and sequestration of free radicals (Wright and Spears, 2004).

**Acknowledgement**

This research work was financially supported by the College of Veterinary Medicine, University of Mosul, Iraq.

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