Study of the Experimentally Induced Zinc Deficiency in Iraqi Local Breed Goats

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ABSTRACT

The current study was conducted to study the experimentaly induced zinc deficiency and its hematological consequences in Iraqi goats. Twenty five goats, 5-6 months old were involved in this study. The study extended from November 2018 and continued for 10 weeks in the farm of College of Veterinary Medicine, University of Diyala. The animals were fed a high calcium content ration (400-500 gm/head/day), in addition to hay and water with ad libitum during the study. The main clinical signs were retardation in hair growth, especially of legs and head, swollen joint, poor growth, rough hair coat, loss of hair on head, limbs, and scrotum, cracking of the hooves. The skin was rough, thickened, and cracked, alopecia, crustng, and hyperkeratosis also occurred. Paleness of the mucous membranes of the eye, loss of appetite, itching, and emaciation were observed. Body weight, heart rates, and body temperature were not significantly changed, while respiratory rates significantly decreased in the 3rd and 6th weeks in comparison with the 1st week. The results of total red blood cells counts revealed significantly decrease in the 2nd week compared with the 1st week, then increased in the 4th, 6th and 8th weeks in comparison with the 1st week. Hemoglobin concentration significantly increased at the 2nd, 4th and 6th weeks in comparison with the 1st week. Packed cell volume increased at the 2nd, 4th, 6th and 8th weeks compared with 1st week. Serum zinc was significantly decreased from the 2nd week till the end of the experiment. Significant decrease in lymphocytes was detected at the 4th week compared with the 1, 2, 6 and 8 weeks. Eosinophil was also significantly decreased in the 2nd and 6th weeks compared with the 1st week, and increased during the 4th week and 8th week compared with the 2nd and 6th respectively. It is concluded from this study that zinc deficiency clearly affects the overall health status and may eventually lead to economic losses in local goats.

Keywords: Zinc deficiency, Iraqi local goats, Hematological alterations, Clinical sings

Introduction

Zinc (Zn) is an element of numerous metal-enzymes and transcription factors (1), which shows significant roles in the metabolism of necessary nutrients in ruminants (2). The zinc is the second metal greatest abundant trace element in the body, and as it is not stored in the body a continuous dietary intake is vital for the body’s appropriate physiological functions (3).

The two major sources of Zinc used by the animal feed industry are Zinc oxide (ZnO) and Zinc Sulfate Monohydrate (ZnSO4·H2O) (4). Zinc deficiency can be both primary, when milk and feed do not supply animals with sufficient quantities of the element, and secondary, when feed compositions adequate levels of zinc, but is unavailible from diet because is reduced by Zn antagonists (copper, magnesium, calcium, phosphates, divalent iron compounds) and amino acid deficiency (5). Zinc is one of the vital elements that are needed for body growth and important physiological processes. Zinc works as an activator of more than 200 enzymes and that is why its deficiency harms the organisms as a whole (6). Its presence is of particular importance in rapidly-dividing cells, including those of the epidermis (7).
Zinc is an important element necessary to ruminants for several biochemical functions. The first work suggested that zinc deficiency effects on growth, reproduction and gene expression in ruminants and is also necessary for normal activity of the immune system (8, 10).

As well as it is essential to nucleic acid and protein synthesis, carbohydrate metabolism, oxygen transport, and stabilizing cellular membranes (1,11).

Zinc is a part of proteins which are a family of beyond 2,000 transcription factors that bind DNA and trigger transcription of growth factors (12, 15), Cytoprotective proteins (16), and hematopoietic stem cells regulation in adults (17).

According to the reviews, there are few studies about zinc deficiency on the Iraqi local breeds of goats, so that this study aimed to evaluate the clinical and hematological alterations in experimentally induced Zn deficiency in Iraqi local breed goats.

Materials and Methods

Twenty-five healthy local breed goats, 5-6 months old and body weight of 15.52±1.05 kg were used. The current study was lasted from November 2018 and continued for 10 weeks in the farm of College of Veterinary Medicine, University of Diyala. The feces were examined to ensure that the animals were free from internal parasites, and the liver and urinary functions were checked before starting the study.

During two weeks of adaptation, the clinical examination of mucous membranes, appetite, behaviors, body condition, were carried according to (18). The animals were kept on a diet of high calcium and composition of the basal diet of the experimental ration (Table 1).

The diet was supplied at 400-500 gm/Head/day and hay with ad libitum to roughage and water supplies during all times of the study. Clinical and physical examinations included pulse, respiration rate, body temperature, and body weight, in addition to monitoring any abnormal changes in behavior, appetite, characters of feces, urine, and the appearance of hairs and skin.

While the hematological parameters were total and differential Leukocyte counts (WBC), (TLC and DLC), total erythrocytes count (TEC) and Erythrocytes indices. Blood samples were collected from jugular vein according to (19), and two blood samples were obtained from each animal, the first one was 2.5 ml blood collected into labeled test tube containing EDTA for Hemoglobin (Hb) and Packed cell volume (PCV) and estimated by the Hemoglobin (Hb) test strips method. Total red blood cells and differential leukocytes count were calculated according to (20, 21).

The second sample (5 ml) was collected into a test tube without anticoagulant to obtain the value of zinc in serum.

Zinc deficiency was induced by increasing the calcium and phosphorus level in the ration (Table 1) as ground limestone and calcium diphosphate were added according to (22).

| Element | mg/100g |
|---------|---------|
| Ca      | 1349.6  |
| Mg      | 972.3   |
| K       | 58.12   |
| Zn      | 1.83    |
| Cu      | 1.52    |
| P       | 3.2     |

The level of serum Zinc was determined by the atomic absorption spectrophotometer as described by (23), using the commercial kit (LTA s.r.i 20060, Bussero (Milan), Italy.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) with 2x2 factorial in a RCBD according to (24). Quantitative data were presented as the mean with standard error (M±SE) and P≤ 0.05 was considered to be statistically significant.

Results and Discussion

Zinc is an essential trace element that is required by all cells in animals as well it has important roles in numerous enzymatic reactions, on the other hand, deficiency of Zn is associated with reduced growth rate, poor immune function, decreased reproductive performance, as well as affecting the skin in severe cases (25-27).
The important factors that predispose to zinc deficiency are increasing calcium and phosphorus (decrease zinc absorption), a diet rich in legume (high calcium) or high phosphorus grain supplement (corn-soybean, corn-oat-barley) with no added minerals. 

The legumes may contain a smaller amount of zinc than grasses grown on soil, and zinc concentration drops with mature of the plant (28), raise of soil pH above 6.5 and the use of fertilizers (29).

The main clinical signs appeared were blemish in the growth of hair, especially of legs, head, and rough hair coat, losing of hairs on head, limbs, and scrotum.

Rough skin, thickened, cracked, wrinkled with dandruff, alopecia, scaling, crusting, and hyperkeratosis. Swollen joint, poor growth, cracking of the hooves, and deformity with overgrowth of hooves.

The pale mucous membrane, itching, loss of appetite, and emaciation (Figures 1-4).

**Figure 1.** Goat on the 10th week (end of the study) with rough and hair loss in head

**Figure 2.** Goat on the 10th week (end of the study) with rough and hair loss and emaciation

**Figure 3.** Goat on the 10th week (end of the study) with rough and hair loss on face
The availability of zinc in ruminants may be affected by numerous factors and cause secondary zinc deficiency. These consist of the consumption of young grass, nourishing on late-cut hay, and high dietary sulphur, besides, the contamination of silage with soil at harvesting time can also affect the digestibility of zinc (18). Sheep and goats have a small zinc storage unit, for that reason, the clinical signs and laboratory abnormalities associated with zinc deficiency occur quickly after removal of zinc from diets and return to normal after supplementation because sheep and goats are able to absorb zinc very efficiently at low intake (30), therefore optimal zinc nutrition is required (31). The young rapidly growing ruminants achieve its high requirement from the dam’s milk, which is rich in zinc (32).

In another study on sheep, it was found that the decrease of appetite in Zn deficient sheep represented by significant decrease in the rumen movement may be due to reduced taste and smell foods (33), whereby changes in appetite were associated with changes in the concentration of amino acid-derived (neurotransmitters) in the brain.

Thus, some trace elements deficiency as Zn may reduce the appetite by impairing the taste because it is postulated that the sense of taste is mediated through the salivary zinc-dependent, therefore low salivary zinc concentration leads to a reduction of taste (34) and reduced appetite (35). On the other hand, reduced appetite has also been reported in buffalo calves affected with Zn deficiency (36), the reduced appetite and rumen movement induced reduction in the body weight (37), and reduced body gain weight in experimentally induced Zn deficient goats.

Thus, there was no significant increase in body weight over the study period. Alopecia was the frequent sign in goats with Zn deficiency. This finding was reported by other authors in calves (18,38, 39), buffalo calves (35), and sheep (21).

The skin alopecia and abnormality belonged to the zinc as an essential element of an inclusive range of metalloenzymes and acts as a cofactor for RNA and DNA polymerases (26). Zinc is of importance in rapidly-dividing cells, as well as those of the skin (40). The skin has the main richness of zinc in the body. The zinc concentration is higher in the epidermis than in the dermis of the skin, owing to a zinc requirement for the active proliferation and differentiation of epidermal keratinocytes (41) that may explain the dermatological variations observed in this study.

The results of the current study revealed that body weight was not significantly increased in the 2nd, 4th, 6th, and 8th weeks in comparison with the 1st week, the highest level was in the 6th week (18.02±1.56 Kg) in comparison with the 1st week (15.53±1.05 Kg). The results of heart rates were non significantly decreased in the 2nd and 4th week in comparison with the 1st, 6th and 8th week, the highest level was 129.55±5.52 beat/min in the 6th week, while the lowest was in the 2nd week (115.9±3.829 beat/min).

There were no significant changes in body temperature during the study, the lowest was in the 2nd week (38.40±0.09 °C) in comparison with the highest (38.62±0.12 °C). The respiration rate was significantly decreased (P≤0.05) in the 3rd and 6th weeks in comparison with the 1st week, the lowest was in the 2nd week (26.0±0.79) (Table 2).

In the present study, the decrease in respiration rate was different from that reported by (36) and might be due to the fact that animals suffering from zinc deficiency naturally take the chronic phase, and in this experiment, the breeding system was different (The animals were reserved in the barn), this reduces stress on the animal 1 and leads to unobvious respiratory signs.

The total red blood cells count significantly decreased in the 2nd week compared with the 1st week, then raised in the 4th, 6th, and 8th weeks. The
The highest level of erythrocyte counts was in the 6th week (14.06±0.80×10^6/µl) compared with the lowest in the 2nd week (9.5±0.54×10^6/µl). Hemoglobin concentration significantly increased in the 2nd, 4th, and 6th weeks in comparison with the 1st week, the highest level was 6.45±0.22 g/dl, while the lowest level was 5.19±0.06 g/dl. PCV percentage increased in the 2nd, 4th, 6th, and 8th weeks compared with the 1st week, the highest level was in the 6th week (19.0±0.63) in comparison with the lowest level in the 1st week (15.55±0.20).

MCV significantly increased in the 2nd, 4th, 6th, and 8th weeks in comparison with the 1st week, the highest was in the 2nd week (20.77±1.22 Fl) in comparison with 13.42±1.33 Fl in the 1st week. MCHC did not show significant changes, the lowest level was in the 2nd week (33.51±0.42 g/dl) in comparison with the highest level (34.23±0.23 g/dl) in the 1st week. MCH significantly increased in the 2nd, 4th, 6th and 8th weeks in comparison with the 1st week, the highest was in the 2nd week (6.88±0.46 Pg) in comparison with the lowest (4.33±0.38 Pg) in the 1st week. Serum zinc was significantly decreased, starting from the the 2nd till the end of the study, the lowest level was 7.61±0.28 µmol/L compared with the 1st week (11.34±0.70 µmol/L) (Table 3).

RBC, Hb, and PCV values showed differences in the current study than those previously reported (40 - 46). Age, breed, and environments have been reported to influence the hematological values of goat in the arid zone (47 - 49). The disease could also influence the hematological parameters in goats (50). Because rare research performed to provide information on zinc requirements for native Iraqi local breed of goats using the factorial approaches, it was challenging to compare these results with other values generated with similar animals.

So, new studies on the animals' nutrient should be undertaken, containing different breeds and different environments because of the influence of breed and environment on the requirements of the animal. Thus, these differences must be taken into account when a diet is formulated (51).

In the current study, serum zinc concentration was significantly decreased (P<0.05) from the 2nd week. Plasma zinc concentration can fall in response to another factors unconnected to Zn status or dietary Zn consumption, including infection, inflammation, stress, and trauma. On the other hand, tissue catabolism during starvation can release Zn into the circulation, causing a transient increase in circulating Zn levels (52).

Singer (53) found that the affected ram had been fed on a diet of alfalfa legume high in calcium which can block zinc uptake. So, it can be concluded that the main factor, which could block zinc absorption, was the high dietary calcium. Decrease in zinc absorption happens with increased calcium and phosphorus consumption. Genetic predisposition may be the cause behind decreased zinc absorption found in some breeds of goats. Goats with this genetic feature may require lifelong zinc supplementation in the appearance of high calcium (and other minerals) intake (54).

The total leucocyte counts were not significantly decreased in the 2nd week (5678.1±366.13) in comparison with 7079+953.56 in the 1st week, but increased to its highest level (6824.45±323.45) all were not-significantly changes.

Neutrophils showed no significant differences at all times, in which it increased not significantly in comparison with the 1st week (39.70±2.28), with the highest level was 53.75±3.06. Lymphocytes showed a significant decrease in the 4th week in comparisons with the 1st, 2nd, 6th, and 8th weeks, the highest level was in the 1st week (54.95±2.71). Eosinophils significantly decreased in the 2nd week (1.35±0.28) and the 6th week (1.80±0.38) in comparison with the 1st week (2.55±0.41) the 4th week (2.7±0.57) and the 8th week (2.3±0.59). Monocytes and Basophiles did not show significant differences (Table 4).

A significant decrease in lymphocytes in Zn deficient goats may indicate that Zn deficiency had an effect on cell-mediated immunity (55, 56).

A significant decrease in lymphocytes in Zn deficient goats in the current study may be attributed to the ability of Zinc deficiency to decrease the activity of serum thymine (a thymes hormone), which is required for maturation of T-helper cells, resulting in cell-mediated immune dysfunction (57).

The immune system function is impaired even in cases of moderate Zn deficiency (58). Severe Zn deficiency reduces immune system function. Eosinophils were also significantly decreased in the 2nd and 6th weeks compared with the 1st week, and increased during the 4th week and the 8th week compared with the 2nd and 6th respectively. Monocytes and Basophiles showed no significant differences.
In this study, when increasing the calcium and phosphorus level in the ration, pruritus occurred because of the imbalance of calcium, magnesium, and phosphorus in the body (59). Eosinophils are multifunctional leukocytes implicated in the pathogenesis of numerous inflammatory processes such as non-specific tissue injury (Pruritus) or response to a variety of stimuli, eosinophils are recruited from the circulation into the tissue where they modulate immune responses through multiple mechanisms. Previous studies have suggested that localized eosinophil-nerve interactions at sites of inflammation significantly alter tissue innervation. Thus, eosinophil-nerve interactions provide a potential mechanistic link between eosinophil-mediated events and neurosensory responses (60). Eosinophils may be decreased because of their migration from circulatory blood vessels to the skin tissue in response to stimuli of tissue injury (Pruritus).

It can be concluded from the results of this study that Zn deficiency causes some clinical and hematological alterations in Iraqi local breed goats.

### Table 2. Clinical parameters in experimentally induced zinc deficiency in goats

| Time     | 1-week    | 2-weeks   | 4-weeks   | 6-weeks   | 8-weeks   |
|----------|-----------|-----------|-----------|-----------|-----------|
| Weight/ Kg | 15.52±1.05 | 17±1.19   | 17.05±1.46 | 18±1.49   | 18.02±1.56 |
| Temperature°C | 38.58±0.13 | 38.40±0.09 | 38.55±0.48 | 38.54±0.15 | 38.62±0.12 |
| Hear rate/min | 123.7±2.80 | 115.9±3.289 | 116.65±4.65 | 129.55±5.52 | 123.9±5.33 |
| Respiration rate/min | 32.6±1.95 | 26.0±0.79** | 31.4±1.0 | 26.2±1.28a | 30.40±1.68 |

Values are Mean ± SE. a. The means significance in comparison with first-day, b. in comparison with 2nd week, c. in comparison with 4th week, d. in comparison with 6th week, * significance at P ≤ 0.05

### Table 3. Hematological parameters and serum Zn in experimentally induced zinc deficiency goats

| Time     | 1-week    | 2-weeks   | 4-weeks   | 6-weeks   |
|----------|-----------|-----------|-----------|-----------|
| RBC×10⁶/μl | 11.0±0.53 | 9.5±0.54a | 11.52±0.57ab* | 14.06±0.80ab* | 12.54±0.55ab* |
| Hb g/ dl | 5.19±0.06 | 6.3±0.26a | 6.07±0.22a* | 6.45±0.22a* | 5.96±0.19 |
| PCV % | 15.55±0.20 | 18.7±1.03a | 17.95±0.64a* | 19.0±0.63a* | 17.75±0.55a* |
| MCV Fl | 13.42±1.33 | 20.77±1.22a | 16.20±2.23a* | 15.14±1.71a* | 14.64±0.74a* |
| MCHC g/dl | 34.23±0.23 | 33.51±0.42 | 33.83±0.35 | 33.91±0.25 | 33.59±0.38 |
| MCH Pg | 4.33±0.38 | 6.88±0.46a | 5.44±0.27a* | 5.13±0.58a* | 4.90±0.24a* |
| Serum Zn μmol/L | 11.34±0.70 | 9.17±0.43a | 8.94±0.49a* | 7.62±0.17abc | 7.61±0.28abc* |

Values are Mean ± SE. a. The means significance in comparison with first-day, b. in comparison with 2nd week, c. in comparison with 4th week, d. in comparison with 6th week, * significance at P ≤ 0.05

### Table 4. Total leukocyte count and differential leukocytes in experimentally induced zinc deficiency in goats

| Time     | 1-week    | 2-weeks   | 4-weeks   | 6-weeks   | 8-weeks   |
|----------|-----------|-----------|-----------|-----------|-----------|
| WBC×10³/μl | 7079±953.56 | 5678.1±366.13 | 7480.66±246.76 | 6824.45±323.45 | 5824.5±559.32 |
| N % | 39.70±2.28 | 41.60±2.81 | 53.75±3.06 | 42.20±2.26 | 42.5±1.55 |
| L % | 54.95±2.71 | 54.60±2.99 | 38.95±2.99ab | 51.75±2.59 c | 52.65±3.81* |
| M % | 3.75±0.66 | 2.25±0.43 | 3.50±0.51 | 2.95±0.44 | 2.85±0.36 |
| E % | 2.55±0.41 | 1.35±0.28 ** | 2.7±0.57b | 1.80±0.38 ac | 2.3±0.59bd* |
| B % | 0.45±0.19 | 0.50±0.14 | 0.65±0.17 | 1.05±0.22 | 0.75±0.17 |

Values are Mean ± SE. a. The means significance in comparison with first-day, b. in comparison with 2nd week, c. in comparison with 4th week, d. in comparison with 6th week, * significance at P≤0.05
Conflict of Interest

The authors declare that there is no conflict of interest.

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دراسة النقص التجريبي للزنك في الماعز المحلي العراقي

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الخلاصة
أجريت الدراسة الحالية لدراسة نقص الزنك المستحث تجريبياً والكشف عن التغيرات في العلامات السريرية والدمية في الماعز المحلي العراقي. خمسة وعشرون رأس من الماعز، وبعمر 5 - 6 أشهر قد أستخدمت في هذه الدراسة، وامتدت الدراسة من كانون الأول 2018 واستمرت لمدة 10 أسابيع في حقل كلية الطب البيطري، جامعة ديالى. اعترضت الحيوانات على غذاء تحتوي نسبة عالية من الكالسيوم وبمعدل (400 - 500) غم / رأس / يوم، إضافة إلى النمط والماطر بصورة طيلة خلال الدراسة. إذ كانت العلامات السريرية الرئيسية هي التخلف في نمو الشعر على الرأس، تورم المفاصل، خشونة الشعر، فقدان الشعر على الرأس والأطراف، وتقرن وشفاف الالاف، ومصابات معنوية في الأسبوع 3 والثاني، ورماد، وحكة وتهيج. لم تظهر النتائج تغيرات معنوية في معدلات وزن الجسم، معدلات ضربات القلب ودرجة حرارة الجسم، واظهرت معدلات التنفس انخفاضاً معنيعاً في الأسبوع 3 والثاني، وهو الارتفاع في عدد الكريات الحمراء في الأسبوع 3 والثاني، وانخفاض الناتج الساحر في الأسبوع 3 والثاني، وارتفاع في القيمة المتوسطة لحجم الخلايا اللمفاوية في الأسبوع 3 والثاني، وارتفاع في القيمة المتوسطة لحجم الخلايا الصفائحية في الأسبوع 3 والثاني. إذ نقص الزنك يؤدي بصورة واضحة على حالة الصحة العامة وانهت في النهاية إلى خسائر اقتصادية في الماعز المحلي.

الكلمات المفتاحية: نقص الزنك، الماعز المحلي العراقي، التغيرات السريرية، العلامات السريرية