Level of zinc and some blood phenomena in sheep infested naturally with hard tick (Ixodidae) in Sulaimani province-Iraq

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Abstract

This study was aimed to determination relationship between zinc levels concentration in the blood serum (PCV, Haemoglobin concentration, differential count of WBC, total protein/dl, albumin g/dl and globulin g/dl) of twenty local breed (Karadi) grazing on pasture plants through April to July month of 2012 in Sulaimani province. Ten sheep were naturally infested with number of 16.9±3.6 hard tick (Ixodidae) and suffered from emaciation, alopecia and hyperkeratosis. The blood picture was estimated and their values were also significant lower $P<0.05$ in total erythrocyte count $7.8\pm0.8\times10^6$ c/mm^3, Hemoglobin concentration 7.3±1.1 g/dl, Packed cell volume 28.5%±2.5 in infested naturally sheep when compared with control in $9.5\pm0.7\times10^6$ c/mm^3, 9.92±1.1g/dl and 33.6%±3.6 respectively, and no statistically significant difference in mean corpuscular volume 37.4±5.5 fl, mean corpuscular hemoglobin 10±1.2 pg and mean corpuscular hemoglobin concentration 27.2±4.1g/dl in infested sheep when compared with control in 33.4±5.2 fl, 10.3±0.9 pg and 32±6.3g/dl respectively. Indicated normocytic hypochromic anemia, and there was leukocytosis $11.55\pm2.2\times10^3$ c/mm^3 and increase in percentage of neutrophils 52.4%±3.9,basophils 0.9%±0.2, monocytes 5.4%±0.4 and decrease in percentage of lymphocyte 37.1%±3.1 and eosinophils 1.3%±0.2. Biochemical analysis showed significant $P<0.01$ decreased in zinc level 56.31±3.58 µg/dl by an atomic absorption spectrophotometer (AAS), and significant increase in $P<0.05$ level in total protein 5.48±0.45 g/dl and albumin 2.31±0.61 g/dl values and increase in globulin values 3.17 ± 0.47 g/dl when compared with ten clinically healthy sheep (control group) 6.83±0.28 g/dl, 4.17±0.43 g/dl and 2.65±0.34g/dl respectively. During the result revealed two genus of Ixodidae (*Hyalomma* spp. and *Rhipicephalus* spp.) The objective of this study was to measure the zinc levels concentration and blood parameters in healthy and infested sheep and relationship between zinc level in sheep and hard tick infestation.

مستوى الزنك وبعض المعاييرالدمية في الأغنام المصابة طبيعياً بالقراد الصلب (Ixodidae) في محافظة السليمانية-العراق

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الخلاصة

أجريت الدراسة لتحديد العلاقة بين مستوى تركيز الزنك في مصل الدم وكذلك بعض المعلييير الدميـــة (حجـــم الخلايا المرصوصة، تركيز الهيمموكلوبين، والعد التفريقي لكريات الدم البيض ومستوى البروتين الكلي، الالبـــومين والكلوبيولين لعشرين رأس غنم محلية (كرادي)، ترعى جميعها على نباتات المراعـــي لحقـــول التابعـــة لمحافظـــة السليمانية من شهر نيسان الى شهر تموز سنة 2012. عشرة منها مصابة طبيعياً بالقراد الصلب Ixodidae وبمعدل

3.6 ± 16.9 قراد لكل رأس غنم، وتعاني من ضعف عام، فرط التقرن وفقدان الصوف وكانت القيم الدموية لكل من كريات الدم الحمراء 7.8 ± 0.8 ×10^6كرية/ ملم^3 ، وتركيز الهيموكلوبين 7.3 ± 1.1غرام/ديسي ليتر وحجم الكريات المتراصة 28.5% ± 2.5 لها تأثير معنوي منخفض وبمستوى P<0.05 مقارنتاً بالأغنام السيطرة وهي 9.5 ± 0.7 ×10^6 كرية/ ملم^3، 9.92 ± 1.1 غرام/ ديسي و33.6 % ± 3.6 على التوالي. بينما لم تشاهد أي تأثير معنوي على كل من معدل حجم الكرية 37.4 ± 5.5 فمتوليتر ومعدل هيموكلوبين الكرية 10 ± 1.2 بايكوغرام ومعدل تركيز هيموكلوبين الكرية 27.2±4.1 غرام/ ديسي ليتر في الأغنام المصابة مقارنتاً بالأغنام السيطرة والتي كانت 33.4 ± 5.2 فمتوليتر، 10.3 ± 0.9 بايكوغرام و 32 ± 6.3 غرام/ ديسي ليتر على التوالي. ونوع فقر الدم كانت من النوع normocytic hypochromic. وهناك ازدياد ملحوظ في عدد الكريات البيضاء ± 2.2×10^3 11.55 كرية/ ملم^3 والنسبة المئوية للعدلات 52.4% ± 3.9 والقاعدية 0.9 % ± 0.2 والمونوسايت5.4 %± 0.4 وانخفاض في النسبة المئوية للمفوسابت 37.1% ± 3.1 والحمضات1.3% ± 0.2. التحليل الكيمياوي لمصل الدم شوهدت انخفاض كبير في مستوى تركيز الزنك 56.31 ± 3.58 مايكوغرام/ ديسي ليتر والتي تم قياسها عن طريق جهازAtomic absorption spectrophotometer. وبمستوى (P<0.01) وانخفاض ملحوظ في تركيز الكلي للبروتين 5.48 ± 0.45 مايكوغرام/ ديسي ليتر والألبومين 2.31 ± 0.61 مايكوغرام/ ديسي ليتر وزيادة معنوية في تركيز الكلوبيولين 3.17 ± 0.47 مايكوغرام/ ديسي ليتر في الأغنام المصابة مقارنة بالأغنام السليمة كلبنيكيا والتي كانت 6.83 ± 0.28 مايكوغرام/ ديسي ليتر، 4.17 ±0.43 مايكوغرام/ ديسي ليتر و2.65± 0.34 مايكوغرام/ ديسي ليتر على التوالي. أظهرت نوعين من Ixodidae أثناء الدراسة وهما (*Hyalomma* spp and *Rhipicephalus* spp.).

Introduction

External parasitic infestation causes severe health problems in livestock that may be accompanied by a decrease in some blood biochemical parameters, blood trace elements and mineral levels (1, 2). Hard tick (Ixodidae) is obligatory blood sucking arachnid arthropoda; infesting mammals, birds, reptile and amphibian. They act as vectors of diseases, causing anemia, dermatitis, paralysis, otoacariasis as well as loss production (3). There are a few reports available on natural zinc deficiency in sheep, this could be due to the facts that pastures rarely contain <20 mg zinc kg DM and that that sheep are able to absorb zinc very efficiently at low intake (4). 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 (5). In ruminants, normal serum zinc levels are between 11 and 18 µmol/ L and animals with levels below 10.5 µmol/ L are considered deficient (6). Acidic soil PH may affect decomposition of added organic materials in humid tropical forest soils (7). Zinc deficiency was diagnosed in a sheep farm in Khartoum Province; the young sheep and lambs were mostly affected skin lesions, depression, wool eating and skin revealed mainly hyperkeratosis accompanied sometimes by parakeratosis (8). In Spain, values below 9.2 µmol/ L are considered low for ovine whereas levels about 12.2 µmol/ L are considered normal (9). (10) observed the parasitic infestation causes variations and changes in the blood picture as well as serum composition with reduce of zinc concentration level among infested calves. (11) observed that the serum

concentration of zinc in sheep naturally infested with lice was lower than in the healthy controls and played an important role in the formation of disease symptoms. Changes in some hematological parameters (erythrocyte count, mean cell volume, hematocrit, hemoglobin concentration, leukocyte counts, serum albumin and globulin concentration, total serum protein) in animals with lice were reported (12). Concentration of serum globulins increased in infested Guina pigs with hard tick (Ixodidae) (13).

Materials and Methods

Clinical pathology of 20 sheep which dependence mainly on grazing in nutrition, ten naturally highly infested sheep with hard ixodid (Ixodidae) and 10 healthy sheep were examined from Sulaimani province in 2012. Numerated hard ixodid (Ixodidae) in each sheep and kept in sterile classes to identification of by (14).

Blood sample collection: Ten milliliters of blood was collected (in both healthy and infested sheep) from jugular vein, 3 ml of blood were deposited into commercial blood vacutainers coated with Ethylene Diamine tetra Acetic acid (EDTA) for estimation of hematological parameters, total ($\times 10^3$ c/mm^3) and differential leucocytic count was carried out using stained blood film with wrights stain. Total red blood cells count ($\times 10^6$ c/mm^3), packed cell volume (PCV), hemoglobin concentration (Hg), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and blood smear for detection piroplasmosis in both healthy and infested sheep by using Gaimsa stain (15). Another blood sample, 7 ml centrifuged at 4000 rpm, serum of samples were stored at -20C° using for biochemical analysis Total protein, albumin and globulin by using spectrophotometer (Spectrophotometer, Apel, PD- 303, Japan), and estimation of zinc Concentration using atomic absorption spectrophotometer-china, were carried out in the department chemical, Faculty of Sciences- Sulaimani University.

Statistical analysis: The data generated an hematological biochemical and trace minerals were subjected to statistical analysis for test of significance by t-test were done to find the significant differences between healthy and infested sheep at level 5% and 1% using statistical tool (16).

Result

In the present study, the most investigated signs among the highly infested sheep with Ixodidae suffering among skin disorders (sever pruritus, hyperkeratosis, alopecia, pale mucous membranes, abscesses and hemorrhagic scar in the site of biting especially in ear and inguinal region which examined clinically in different region (district and subdistrict) and obvious flocks sheep in Sulaimani province. The concentration zinc levels in the infested sheep were highly significantly lower 56.31± 3. 58 µg/dl than in the healthy sheep 89.80± 6.80 µg/dl (Table 1).

Table (1) Levels of zinc (mean ± SE) in clinically sheep and infested sheep with Ixodidae sheep

Sheep groups	Zinc µg/dl
Healthy sheep	89.80± 6.80
Infested sheep with Ixodidae	56.31± 3.58
t (observed)	6.465*

*** Significant p<0.01.**

The total protein (6.83±0.28 g/dl) and Albumin concentration (4.17 ± 0.43 g/dl) in healthy sheep were dominant significantly (P< 0.05) in compare to the infested sheep with Ixodidae (5.48±0.45g/dl and 2.31±0.61g/dl for total protein and albumin concentration) respectively, while the globulin 3.17±0.47g/dl take an opposite direction which was more and significant (P<0.05) in infested sheep with Ixodidae in compare the healthy sheep (2.65 ± 0.34g/dl) (Table 2).

Table (2) Total protein, albumin and globulin concentration in clinically healthy sheep and infested sheep with Ixodidae sheep. (Mean ± SE)

Sheep groups	Total protein g/dl	Albumin g/dl	Globulin g/dl
Healthy Sheep	6.83 ± 0.28	4.17 ± 0.43	2.65 ± 0.34
Infested sheep with Ixodidae	5.48 ± 0.45	2.31 ± 0.61	3.17 ± 0.47
t (observed)	2.968*	3.173*	2.883*

***Significant p<0.05.**

The result of hematological examination showed that the mean values of hematological parameters in infested sheep with hard tick significantly p<0.05 reduction in Hemoglobin concentration, packed cell volume and total red blood cells (7.3 ± 1.1, 28.5 ± 2.5 and 7.8 ± 0.8) respectively, when compare to the healthy sheep (9.92 ± 1.1, 33.6 ± 3.6 and 9.5 ± 0.7) respectively. There was not significant in other parameters; mean corpuscular volume (33.4 ± 5.2/fl), mean corpuscular hemoglobin (10.3 ± 0.9/pg) and mean corpuscular hemoglobin concentration 32±6.3/ g/dl in healthy sheep, and infested sheep (Table 3). The type of anemia in infested sheep with Ixodidae as normocytic hypochromic.

Table (3) Hematological parameters in clinically healthy sheep and infested sheep with Ixodidae. (Mean ± SE)

Parameters	Healthy sheep (control group)	Infested sheep with Ixodidae	t (observed)
Hb g/dl	9.92 ± 1.1	7. 3 ± 1.1	5.055*
PCV%	33.6 ± 3.6	28.5 ± 2.5	4.168*
RBCs × 10^6	9.5 ± 0.7	7.8 ± 0.8	5.119*
MCV fl	33.4 ± 5.2	37.4 ± 5.5	$1.578^{N.s}$
MCH pg	10.3 ± 0.9	10 ± 1.2	$0.638^{N.s}$
MCHC g/dl	32 ± 6.3	27.2 ± 4.1	$1.913^{N.s}$

*** Significant p<0.05, N.s = non significant.**

Total and differential leukocyte were higher (p<0.05) in sheep infested naturally with Ixodidae. 11.55±2.2, and differential leukocyte count%; Neutrophils, Basophil and Monocyte in 52.4 ± 3.9, 0.9± 0.2 and 5.4±0.4 for leukocyte count, neutrophils, basophil and monocyte respectively, when compared with healthy sheep 9.38 ±1.8, 41.3 ±2.8, 0.00 and 3.7±1.4 respectively. Lymphocyte and Esonophil were statistically (p<0.05) decrease 37.1 ± 3.1 and 1.3±0.2 respectively, when compared with healthy sheep in Lymphocyte 49.9± 6.3 and Esonophil in, (2.5 ± 0.9).

Table (4) Total and differential count in clinically healthy sheep and infested sheep with Ixodidae. (Mean ± SE)

Leucogram	Healthy sheep	Infested sheep with Ixodidae	t (observed)
Total leucocytic count (× 10^3)	9.38 ± 1.8	11.55 ± 2.2	4.185*
Neutrophils%	41.3 ± 2.8	52.4 ± 3.9	6.847*
Lymphocytes%	49.9 ± 6.3	37.1 ± 3.1	5.412*
Esonophil	2.5 ± 0.9	1.3 ± 0.2	2.626*
Basophils	0.00	0.9 ± 0.2	2.894*
Monocyte	3.7 ± 1.4	5.4 ± 0.4	3.451*

*** Significant p<0.05.**

Discussion

In this study found that the all sheep which used was suffering from a lack of zinc concentration level when compared to the standard level of serum zinc concentration in sheep (15) may be its dependence on grazing. Drought and marginal or low Cu and Zn content in pasture may be the predisposing factors (17). The result indicated a highly significantly lower ($P<0.01$) in zinc level concentration in infested sheep when compare with healthy sheep. due to the sheep used in this study depending on grazing for feeding. Many investigators (18, 19, and 20) reported that the levels of serum Zn in sheep should be between80 and 120 µg/dl. In contrast to these findings our results were as 77.8 ±6.80 µg/dl (between 68-88 µg/dl) in healthy sheep, while 39.31 ± 3.58 µg/dl (34-46 µg/dl) in infested sheep. (21) observed in all Sulaimani region, soil PH above the 7 may be contain higher quantity of bicarbonate ions and show the alkalized PH affect of the optimum growth in plants by interfering with normal uptake of other ion such as zinc It associated with the soil organic matter; deficiency is more likely to occur on soils low in organic matter. This deficiency associated with skin disorders attributed to the role of zinc in several enzymes as carboxyl peptidase, alcoholic dehydrogenase, which is necessary for healthy skin (22, 23, and 24), while (25) observed Zinc deficiency leads to failure of keratinization parakeratosis, loss and failure of growth of wool and hair**.** (26, and 27) determined that serum copper, zinc, manganese and iron levels significantly decreased in sheep which had alopecia and wool eating habit. Weakening in muscle and decrease in wool quality in the infested goats may be depended on reduction in albumin level (2). Regarding to the hematological examination indicated that the values of total RBC count, Hb concentration and PCV% in infested sheep were significantly lower ($P<0.05$) than those of normal healthy sheep. The type of anemia in infested sheep (low Zn concentrations in sera) was of the normocytic hypochromic. These result were similar to those reported in hypozincemic in sheep (17, 28) 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 (29). Severe infestation with sucking lice may cause anemia, which was severe enough to be the cause of death (30). In the present study, total white blood cells, Monocyte, basophil and neutrophils significantly higher when compare with healthy sheep, while the lymphocytes, esonophil and basophil were significantly lower than in healthy sheep. This may be due to migration from blood stream to biting site of tick lesion causes degranulation and produced histamine. It has been shown that the skin reaction at the attachment site on resistance response appears as cutaneous basophil hypersensitivity reactions (31), and decreased lymphocytes with an increase in neutrophils can be an indication of an inflammatory or an immune response due to pathogen infection (32). (22) suggested that the thymic atrophy and lymphopenia that is associated with zinc deficiency in rodents resulted from chronic elevation of plasma corticosterone. In generally, tick infestation initiate induce a complex variety of immune responses, involving antigen presenter cells, T lymphocytes, B lymphocytes, antibodies, cytokins, complement, basophils, mastocytes, eosinophils, and number of bioactive molecules (33). In the present study, total protein and Albumin significantly lower in the infested sheep with Ixodidae than healthy sheep, while indicated the level of globulin is significantly increase in infested sheep than healthy sheep.. The decreased levels of total proteins and albumin 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 (34). The

decreased levels of protein 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 (35). In contrast (36) reported that acute alopecia and a marked reduction in serum albumin levels were seen in the sheep with zinc deficiency. (37) revealed that the parasitic infection production interleukin-4 in the spleen of zinc deficient mice is depressed, leading to depressed levels of IgE, IgG and eosinophils. Moreover, 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 (38). (39) concluded that the infection with intestinal parasites and mange mite especially when it is heavy and prolonged for long time has a marked stress effect on body immune efficiency.

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