Clinical and hematological study of experimentally induced secondary copper deficiency in sheep

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Abstract

The study was conducted on ten male Awassi sheep to evaluate the clinical and hematological changes in addition to measure serum copper level of sheep suffering from secondary copper deficiency during 4 months period. Animals were divided randomly into two equal groups; One group was drenched with a mixture of ammonium molybdate 100 mg with 1g of sodium sulfate in 100 ml of water daily for induction of secondary copper deficiency. The second group left without treatment as control group. The serum copper level, and complete blood picture (Red blood cells, hemoglobin, packed cell volume, total white blood cells, granulocytes %, lymphocyte %, and monocytes %), were estimated on day zero and repeated every two weeks. Results revealed appearance of clinical signs of secondary copper deficiency in deficient (treated) group; including emaciation of all 5 animals, loss of wool and easily to detached, bleaching around eye in one animal, change in wool color, and increased in respiratory and pulse rate compared with control group. The clinical examination of both groups revealed no significant differences in temperature, while there was a significant ($P \leq 0.05$) difference in pulse rate, and respiratory rate between treated (37.6 ± 3.07) and control (27.4 ± 1.53) group. Copper level decreased gradually to reach (0.64 ± 0.06 ppm) which regard subnormal level with statistical significant decrease (after 2 months of treatment) in treated compared with control group. Blood parameters included (RBC, Hb, PCV, MCV, MCH and MCHC) were recorded non significant differences along the experiment period in treated compared with control group. Total WBC in treated group were recorded variation in the values with presence of significant gradual decrease in the treated compared with control group with non-significant differences in granulocyte %, monocyte % and lymphocyte %.

Key words: Secondary copper deficiency, copper, hematology, sheep.

دراسة سريرية ودمية لنقص النحاس الثانوي المستحدث تجريبيا في الاغنام

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الخلاصة

اجريت الدراسة على 10 من ذكور الاغنام العواسية لغرض دراسة التغيرات السريرية والدمية أضافه إلى قياس مستوى النحاس في الأغنام التي تعاني من نقص النحاس الثانوي المستحدث خلال فترة 4 أشهر. قسمت الحيوانات عشوائيا إلى مجموعتين متساويتين؛ جرعت احداهما بواسطة معلق من موليبدات الامونيا 100 ملغم مع 1غم من كبريتات الصوديوم مذابة في 100 مل من الماء يوميا لغرض استحداث نقص النحاس الثانوي ، وتركت الثانية بدون معالجة كمجموعة سيطرة. تم قياس مستوى النحاس في مصل الدم والصورة الدموية الكاملة والتي شملت العدد الكلي لكريات الدم الحمراء ، كمية الهيموكلوبين ، حجم الخلايا المضغوطة ، العدد الكلي لكريات الدم البيضاء ، نسبة الخلايا الحبيبية ، نسبة الخلايا اللمفية ونسبة الخلايا وحيدة النواة في اليوم الاول والإعادة لهذه الفحوصات بصورة دورية كل أسبوعين. ظهرت العلامات السريرية لنقص النحاس الثانوي على مجموعه النقص والتي تضمنت الهزال لكل حيوانات المجموعة ، فقدان وسهوله أزاله الصوف وتغيير لونه ، تفتيح اللون حول العين في واحد من الحيوانات وزيادة في معدلات النبض والتنفس بالمقارنة مع المجموعة الاخرى. قل مستوى النحاس بشكل تدريجي ليصل (0,06±0,64) جزء من المليون والذي يعتبر

تحت المستوى الطبيعي مع قلة معنوية عند($p\leq0.05$) بعد شهرين من البدء بالتجريع الفموي بالمقارنة مع مجموعة السيطرة. شملت الفحوصات الدموية قياس (RBC, Hb, PCV, MCV, MCH & MCHC) ولم تسجل فرقا معنويا طيلة فترة التجربة في مجموعه المعالجة بالمقارنة مع السيطرة عند مستوى احتمال ($p\leq0.05$). سجل العدد الكلي في كريات الدم البيضاء تنوعا في مجموعه المعالجة مع وجود قله تدريجية معنوية في مجموعه المعالجة مقارنة مع السيطرة ولم تسجل فروقا معنوية إحصائية في نسب الخلايا الحبيبية ، واللمفية ووحيدة النواة عند ($p\leq0.05$). أشار الفحص السريري لكلا المجموعتين في التجربة إلى عدم وجود فرق معنوي في درجة الحرارة بينما كان هنالك فرق معنوي في معدل التنفس بين مجموعة المعالجة (3,07±37,6) ومجموعة السيطرة (1,53±27,4) كما اظهر قياس معدل النبض أيضا وجود فرق معنوي بين المجموعتين.

الكلمات المفتاحية: النحاس ، نقص النحاس الثانوي ، الدم ، الاغنام.

Introduction

Copper deficiency is endemic in ruminants worldwide and causes disease of economic importance (1). Copper (Cu) is an essential microelement that presents a variety of function in animals, it plays a part in the active role in more than 20 metallo-enzymes, cofactor and metalloproteinase that are connected with destruction of free radicals, synthesis of connective tissue, formation of myelin and bones, pigmentation and formation of fur and wool, it is also acts indirectly in hematopoiesis (2, 3 and 4). Copper deficiency can be either primary due to low content in forage, or secondary to an excess of antagonists, mainly sulfur and Molybdenum (5). Molybdenum and sulfur can form strong Cu chelating complexes known as thiomolybdates, and the reducing environment in rumen potentiates the formation of these insoluble complexes. Thiomolybdate greatly affect Cu homeostasis in ruminant by decreasing Cu absorption, increasing biliary exertion of Cu and also removing Cu from vital cupro-enzymes (6). Symptoms of Cu deficiency in sheep include limp and glossy wool and losses its crimps, depigmentation, anemia, scouring, swayback and bone deformities. Also cause decrease in humeral and cell mediated immunity and decrease non-specific immunity regulated by phagocytic cells such as: macrophages and neutrophils (1). Therefore the study was suggested to record the clinical signs and hematological changes which occur with secondary copper deficiency.

Materials and methods

Animals: Ten male awassi lambs (3.5 - 4 months aged), were used through a 4 months period in sheep field of Veterinary Medicine College, University of AL-Qadissiya. All lambs stayed for 45 days before beginning of experiment for adaptation and received enterotoxaemia and F.M.D. vaccines and ivermactin with drenched of Albendazole and Ectopour to treated and protected the animals from internal and external parasites through the adaptation periods. Animals were divided randomly into two equal groups (Cu deficient (treated), and control groups). Both groups fed on integrated diet which contain 5.2 mg Cu in all period of study according to (7). Early in the morning and before feeding, treated group drenching with a mixture of ammonium molybdate 100 mg and sodium sulfate 1gm dissolved in 100 ml of water and given to each animals (7). Control group left without treatment.

Copper level: The level of copper was determined in serum of all experimental animals at two weeks intervals by using atomic absorption spectrophotometer (EnGineeRX; British) (8). Five ml of blood were obtained from jugular vein into test tube without anticoagulant left to clot and centri-fuged to separate serum.

Hematological tests: Five ml of blood obtained from jugular vein with anticoagulant tubes to evaluate the blood parameters; RBCs X 10^6 corpuscle /ml^3, Hemoglobin concentration g\100 ml, packed cell volume %, mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), in addition to total WBC cell/ml^3 and differential leukocyte counts include granulocytes %, lymphocytes % and monocytes %. These parameters were

performed by using hemo-analyzer HORIBA ABX diagnostics (French).

Clinical examination: Clinical signs of Cu deficiency was observed during induction of secondary Cu deficiency and clinical examination which included: temperature (C°), pulse and respiratory rates/minute were recorded.

Statistical analysis: To determine statistical differences between the two groups, two way analysis of variance ANOVA in the SPSS windows program statistical package for social science were used.

Results

Induced secondary copper deficiency

Gradual decrease of serum Cu level were seen through the continuous observation (every 2 weeks intervals), until become (0.64 ± 0.06ppm) where recorded a significant (P≤0.05) decrease after 2 months from beginning of drenching (Table 1). While in control group, the Cu level was still at normal level, ranged about (1.18 ± 0.04 to 1.28 ± 0.14) in the same period.

The clinical finding of secondary Cu deficiency

All five animals in treated group were appeared emaciated with decreased in body weight and retardation of growth compared with control. Moreover depigmentation of color around eyes (Fig. 1), and wool (Fig. 2). Alopecia and easily removed of the wool also were seen (Fig. 3). While there were no any abnormalities in control group. The clinical examination of both groups show no significant differences in temperature while there was significant differences in the respiratory rate between treated (37.6 ± 3.07 /min.) and control (27.4 ± 1.53 /min.) group. The pulse rate show significant differences between treated group and control group. Also a significant increase in pulse rate of treated sheep compared with control were seen (Table 2).

Table (1): Serum Cu level during the experimental period (Mean±SE)

Group \ Date	3\3	20\5
Treated	1.28±0.16Aa	0.58±0.03Ab
Control	1.20±0.13Aa	1.21±0.1Ba

Table (2):The clinical examinations during experimental period (Mean±SE), T= treated, C=control.

Group	Date 26/5		
	Temp. (C°)	Respiration (breath/min.)	Pulse (beat/min.)
T	39.14±0.17Aa	37.6±3.07Aa	80.6±1.07Aa
C	39.04±0.12Aa	27.2±0.86Ba	76.4±1.74ABa

Different letters referred to significant (P≤0.05) differences between groups. Similar letters represent no significant differences. Capital letters referred to vertical compression, small letters referred to horizontal compression.

Fig. (1): Bleaching around the eye in treated group.

Fig. (2): Depigmentation of the wool in treated group

Fig. (3): Loss of wool in treated group

Blood parameters

The study revealed no statistical significant (P≤0.05) differences in hematological parameters between treated and control groups (Table 3). Total WBCs in the period of inducing Cu deficiency were recorded a significant gradual decrease in the treated group compared with control group. Also there were non-significant differences in GRN%, Mon%, and LYM% between groups (Table 3).

Table(3): The blood parameters during experimental period (Mean±SE).

Groups / Blood parameters	Treated		Control	
	Day zero	Cu deficiency period	Day zero	Cu deficiency period
RBC (corpuscle\ml^3)	3.85±0.23Aa	4.01±0.47Aa	4.04±0.4Aa	3.55±0.34Aa
Hb g/100ml	8.56±0.48Aa	7.46±0.38Aa	8.76±0.14Aa	7.84±0.3Aa
PCV(%)	12.9±1.04Aa	12.98±1.32Aa	14.26±1.36Aa	13.08±0.75Aa
MCV(fl)	35.2±0.37Aa	35.4±0.24Aa	35.6±0.24Aa	35.4±0.24Aa
MCH(pg/cell)	23.88±1.92Aa	20.96±2.37Aab	22.36±1.99Aa	21.54±1.57Aa
MCHC(g/dl)	67.78±5.27Aa	59.48±6.39Aab	66.87±5.3Aa	60.74±4.11Aa
WBC cell/ml^3	67.08±11.48Aa	48.86±6.88Ab	52.56±4.98Ba	57.7±11.43ABa
GRN%	7.12±1.2Aa	7.4±1.13Aa	7.1±0.3Aa	6.98±0.78Aa
LYM%	90.06±1.82Aa	85.66±3.63Ab	88.76±0.1Aa	86.72±1.01Aa
MON%	6.68±0.16Aa	6.24±0.71Aa	6.54±0.34Aa	6.56±0.6Aa

Different letters referred to significant (P≤0.05) differences between groups, similar letters represent no significant differences.

Discussion

Significant decrease of serum Cu level is seen after 2 months from beginning of drenching, similar finding grasped by (9) who observed appearance of Cu deficiency after daily drenching with molybdenum sulfate after 6 week of drenching. The results agreed with (10) who referred to normal value of serum Cu concentration in sheep ranged from (0.7-2 ppm) which regard adequate for animals maintenance while the Cu concentration (0.1 and 0.4 to 1 ppm) regarded deficient and marginal concentrations for sheep. Also this results of normal values of serum Cu level were accepted by (11) who showed the normal level of serum copper about (0.75-1.7 ppm). The emaciation and loss of condition result agree with (12,13 and 14) in ruminants and this signs attributed to reduction of the Cu activity enzymes such Cytochrome C oxidase which is important in energy production (15) and in late stage this impairment of oxidation tissue lead to interference with metabolism and loss of condition and failure to growth (1). This results were agreed with (7) who showed signs of emaciation and weakness in the experimental animals which suffered from Cu deficiency. Retardation growth of sheep suffer from Cu deficiency may be related to localized depletion of Cu in the mucosa of the intestine which influence digestion, motility and inflammatory responses (16). The emaciation occurred in Cu deficient group due to biochemical relation to disturbance in cross-linked protein of connective tissue caused by Lysal oxidase deficiency due to Cu deficiency (17, 18). Copper deficiency was stimulate catabolism

of protein of connective tissue result in affecting on the tissue and growing (19). The result also agree with (20) whom reported that decreases in body mass with alopecia and wool production in the secondary Cu deficiency in merino sheep. (21) showed the depigmentation of hair in Cu deficient cattle regard that signs is the earliest visual signs (22, 23, and 24) showed decrement in the activity of tyrosinase in Cu deficient animals which regarded important enzyme to conform tyrosine to melanin which lead to depigmentation of color of the wool in case of copper deficiency disease. The result accepted with (25, 26, and 27) whom showed loss of the wool and appearance of alopecia in sheep suffering from Cu deficiency. The result of body temperature agree with (27, 28). While the results of (28) showed no significant change in the pulse rate and respiratory rate in between Cu deficiency animals. (27) accepted with our study by the respiratory rate, whom found significant increase in Cu deficiency sheep compared with control**.** The results of blood parameters agreed with (4, 20, and 29) whom showed no significant differences in the hematological parameters in secondary Cu deficiency while disagree with (27, 30, and 31) whom referred to significant differences without referred to Cu deficiency if it primary or secondary. Also disagree with (7) whose referred to significant effect on blood parameters of experimental secondary Cu deficiency. (1) confirmed that anemia occurred in the late stage of primary Cu deficiency and not remarkable signs in secondary Cu deficiency. The results of WBC picture agreed with (7) who recorded significant (gradual) decrease in total WBC counts in sheep experimentally suffered from secondary Cu deficiency. The results also agreed with another study by (32) in buffalo calves. The decreased total leukocyte count could be contributed to stress of malnutrition which cause secretion of adrenocortico-trophic hormone from adenohypophysis and resultant increased in blood cortisol concentration (33). While the result disagree with (27) who referred to non-significant differences occurred in WBC counts. (34) also referred to significant increase in monocytes% in Cu deficiency sheep. While (35) in cattle referred to total WBCs not affected with Cu deficient and increased in Mon% and decreased in B lymphocytes.

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