

THE EFFECT OF ZINC AND COPPER DEFICIENCY ON HEMATOLOGICAL PARAMETERS, OXIDATIVE STRESS AND ANTIOXIDANTS LEVELS IN THE SHEEP.

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(Received 10 May 2018, Accepted 12 June 2018)

Keywords: Zinc, Malondialdehyde, Sheep

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ABSTRACT

The objective of this study was to determine the levels of Zinc (Zn) and copper (Cu) concentration, as well as the clinical signs that may occur due to the Zinc and copper deficiency, in addition to the effect the two trace elements levels on the hematological levels, oxidative stress Malondialdehyde (MDA) and antioxidant levels glutathione and Catalase (CAT). This study carried out in Basra province, the study conducted on (75) sheep (male and female). About 60 sheep clinically have from zinc and copper deficiency (hypozincemia) and 15 clinically apparently healthy served as a control group. The clinical signs occur due to the two trace elements deficiency include: loss of appetite, depression, pica, repeatedly bites off the wool of other sheep, loss of the wool, loss of hair around the eyes, ear and nose, alopecia, parakeratosis, stiff in gait and some animals suffering from diarrhea, pale of the mucous membrane, decrease the rumenal contraction, and significant ($P < 0.05$) increase the heart and respiratory rate with normal body temperature. Also the results show significant increase ($P < 0.05$) of MDA concentration, in addition to significant decrease ($P < 0.05$) in glutathione and CAT levels due to significant decrease ($P < 0.05$) in both Zn and Cu concentration levels, this point the Zn and Cu play important role in prevent damage occur due to the oxidative stress via decrease the free radicals, also it has role in antioxidant synthesis.

INTRODUCTION

Zinc (Zn) and Copper (Cu) as an important trace metals in all living organisms and all tissues. It is essential for the normal growth and the reproduction of all higher plants and animals, and of humans (1). In addition, it plays a key role during physiological growth and fulfills an immune function. It is vital for the functionality of more than 300 enzymes, for the stabilization of DNA, and for gene expression (2). The systematic availability of Zn and Cu in tissues is highly influenced by the balance of the anabolic process regulating the renewal of soft and skeletal tissues (3, 4). The Zn and Cu deficiency occurs due to food stuff or hay contains insufficient quantities of dietary on this elements, Zn and Cu failure absorption and body stress (5). In addition to the two elements play important role as antioxidant and decrease or prevent the oxidative stress(6).The trace elements deficiency caused varies of clinical signs in sheep such as hair loss, parakeratosis, growth retardation, delayed sexual and maturation period, loss of appetite and delayed wound healing (7).

Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses (8). The Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their main risk comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defense system of antioxidants (9).

Malondialdehyde is a naturally occurring product of lipid peroxidation. Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress in cells and tissues (10).

Free radical reaction have been associated with pathogenesis of several diseases such as Cardiovascular diseases, Neurodegenerative diseases, Pulmonary diseases, Renal disorder, Gastrointestinal disorders, Skin diseases and Immunodepression (11).

Antioxidants have important role to prevent or repair the damage caused by reactive oxygen species (ROS), as well as to regulate redox-sensitive signaling pathways (12). Antioxidants such as glutathione and catalase, glutathione is soluble antioxidant which has a central protective antioxidant against both endogenous and exogenous ROS produced from a variety source, also glutathione (GSH) has important role in converting vitamins from inactive form to active forms such Vit C and Vit E (13). And the catalase consider enzymetic antioxidant which converted the hydrogen peroxide to oxygen and water.

This study was designed to find out a possible relationship between zinc and copper levels and clinical findings and hematological, in addition to the effect the trace elements difference on oxidative stress (MDA) and level the antioxidant (glutathione and catalase).

MATERIAL AND METHODS

Animals and study design: The study was conducted on (75) sheep (male and female) in deferent ages. This study carried out in Basra province, 60 sheep clinically have zinc (hypo zincemia) and copper deficiency and 15 clinically healthy animals served as a control group.

The clinical examination had been carried out in all animals, the clinical signs were recorded in special card.

Blood samples: Blood was collected aseptically from jugular vein by 10 ml disposable syringe, serum were separated by centrifugation at 3000 rpm and kept at -20 °C.

Determination the zinc and copper concentration

The serum concentrations of Zn and Cu determine by spectrophotometers via used special kit (Spectrum Co , Egypt).

Determination of malondialdehyde (MDA):

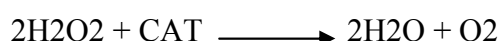
MDA was determined according to(14), thiobarbituric acid (TBA) reacts with MDA to form thiobarbituric acid reactive substance (TBARs) and the absorbance of this resultant was measured by spectrophotometer at 535 nm (Albasheer office- Iraq).

Determination of glutathione concentration

The glutathione concentration of the serum was determined according to the method described by (15), the method based on the reduction of 5,5-dithio-bis (2-nitrobenzoic acid – DTNB) with glutathione (GSH) to product a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 412 nm wave length(Albasheer office- Iraq).

Determination of catalase activity determination

Catalase was determined by colorimetric method according to (16). It catalyzes the divalent reduction of hydrogen peroxide (at high concentration) to water and free oxygen.



Consequently absorbance was decrease due to H₂O₂ consumption ($\epsilon = 0.04\text{mmol}^{-1} \text{cm}^{-1}$) (17). The activity determined by reading the initial and final absorbance at 240 nm (Albasheer office- Iraq).

RESULTS

The clinical signs of zinc and copper deficiency in sheep were included loss of appetite 38 (63.3%), depression 22 (36.6%), pica 20 (33.5%), repeatedly bites off the wool of other sheep 8 (13.3%), loss of the wool 41 (68.5%), loss of hair around the eyes, ear and nose 27 (45%), alopecia 16 (26.5%), parakeratosis 7 (11.5%) (Fig 1-1), stiff in gait 8 (20%) and some animals suffering from diarrhea 14 (23.3%), pale of the mucous membrane 45 (75%), significantly decrease the rumenal contraction 36 (60%) (Table 1), and significant increase the heart and respiratory rate with normal body temperature (Table 2).

The hematological parameters showed significant decrease $P < (0.05)$ in the red blood cells count (RBCs count), hemoglobin concentration (Hb) and packed cell volume, in other hand the result show in significant difference of the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of serum in sheep which suffering from Zn and Cu deficiency signs when compared with control group that mean the type of anemia was normocytic normochromic (Table 3) .

The result show significant decrease $P<(0.05)$ of the zinc and copper concentration of serum in sheep which suffering from Zn and Cu deficiency signs when compared with control group.

In additions to the result show significant increase $P<(0.05)$ in the MDA concentration while significant decrease $P<(0.05)$ of glutathione and catalase concentration in animals which suffering from Zn and Cu deficiency when compared with control group (Table 4).



Table (1) The clinical signs due to copper and zinc deficiency.

Clinical signs	Numbers & Percent%
Loss of apatite	38 (63.3%)
Depression	22 (36.6%)
Pica	20 (33.5%)
Wool loss	41 (68.5%)
loss of hair around the eyes, ear and nose	27 (45%)
Alopecia	16 (26.5%)
Parakeratosis	7 (11.5%)
Stiff in gait	8 (20%)
Icterus of mucous M.M	45 (75%)
Decrease of rumen contraction	36 (60%)
Weight loss	18 (45%)
Diarrhea	14 (23.3%)

Table (2) the physiological parameters in sheep suffered from zinc and copper deficiency compared with control group.

Parameters	Control group	Sheep have of Zn&Cu deficiency
Body Temperature	39.21 ± 0.72	39.37 ± 0.28
Respiratory Rate	34 ± 2.2	41 ± 3.1*
Heart Rate	86.2 ± 4.7	101.6 ± 4.6*

* P<(0.05) values are mean± stander error of mean.

Table (3) the Hematological parameters in sheep suffered from zinc and copper deficiency compared with control group.

Parameters	Control group	Sheep have Zn&Cu deficiency
RBCs count	10.6 ± 1.95	6.8 ± 1.42*
PCV	35.2 ± 1.64	24.3 ± 3.05*
Hb	11.3 ± 1.48	7.66 ± 1.03*
MCV	34.3 ± 1.81	35.7 ± 2.82
MCHC	32.1 ± 2.05	33.41 ± 3.65

* P<(0.05) values are mean± stander error of mean.

Table (4) Zn, Cu, MDA, GHT and CAT concentration in ewe suffered from Zinc and copper deficiency compared with control group.

Parameters	Control group	Sheep suffering of Zn&Cu deficiency
Zn µg/dl	62.51 ± 2.55	31.25 ± 5.43*
Cu µg/dl	48.3 ± 3.62	27.26 ± 4.88
MDA µ mol/L	3.62 ± 0.484	9.65 ± 2.17*
Glutathione µmol/L	6.73 ± 0.984	3.51 ± 1.028*
Catalase u/l	64.32 ± 3.75	33.16 ± 4.35*

* P<(0.05) values are mean± stander error of mean.

DISCUSSION

Zinc and copper considered essential trace element. They are as co factor in many enzymes (Cu/Zn SOD), and acts as proton donor at the active site of enzyme and bridging atom between the substrate and enzyme, The clinical signs such as weakness, decrease ruminal contraction, loss of hair around the eyes, ear and nose, alopecia and parakeratosis these occur may be due to the decrease both elements (Zn&Cu) levels lead to protein synthesis this result is agreement with (3,4) they refers to zinc and copper deficiency results in a decreased feed intake in all species and is probably the reason for the growth depression in growing animals and decrease body weight in mature animals, in addition to the Stiff in gait that recorded in this study

might be occurred due to abnormal mineralization due to the zinc play important role in bone mineralization these result in is compatible with finding of (18).

The significant ($P<0.05$) decrease in hemogram values might be due the decrease in the trace elements that subsequently to interference with RBCs synthesis, However, the accumulation the oxidative stress and lipid peroxidation lead to increase production of the free radicals and caused bone marrow depression this supported by previous studies (19,20) explained that depression of hemograms result resulted from the hypo function of erythropoietin du to the accumulation the free radicals.

On other hand the increase oxidative stress (MDA lvels) lead to production free radicals which caused damage to vital organs such as liver and kidneys which lead to liver disease and effected in protein synthesis caused decrease hypoprotienemia resulting in decreased body weight, easily detached and loss the hair attributed to zinc and copper deficiencies according to the finding of (6,22, 24).

(25) Refers to lipid peroxidation levels increase with trace elements deficiency and caused biochemical changes and some clinical signs such as loss of hair, decrease of appetite and eye and skin lesions.

Significant increase ($P<0.05$) in respiratory and heart rates occurred as a compensatory mechanism against hypoxia (Anemic hypoxia). Because the decrease RBCs in count and Hb concentration affected the oxygen transmitted to body tissues ,therefore a failure of tissues to receive an adequate supply of oxygen will occur and such interpretation was in accordance with those of (4,19,26) they refers to Zn and Cu are a components of enzyme carbonic anhydrase, which is located in the red blood cells, Zinc deficiency lead to reduced the RBCs due to decrease enzyme carbonic anhydrase which impair respiratory functions.

The current study show significant increase in MDA level and significant decrease in GSH and CAT level considered an indicator for lipid peroxidation and oxidative stress indicating imbalance between oxidant and antioxidant system this result agreement with (9, 27) these might be due to the Zinc and copper and iron are classified as a part of the antioxidant defense system of cells such as copper/zinc superoxide dismutase (Cu/Zn SOD) and ceruloplasmin enzymes(3, 4) so the element were consumed in the synthesis of an antioxidant enzymes (6, 21).

(22) concluded that the antioxidant enzymes (CAT and GSH) are depended on various essential trace elements for proper molecular structure and activity. Both

elements are important for the activity of SOD molecule and activity CAT and GSH. . Also (23) considered the high level of the CAT indicated the activity defense against free radicals while the low levels of the CAT indicated low cellular resistance against oxidative stress.

تأثير نقص الزنك و النحاس على القيم الدموية، العوامل الأوكسدة و مضادات الأوكسدة في الأغنام

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الخلاصة

كان الهدف من هذه الدراسة قياس مستويات الزنك و النحاس في الأغنام و كذلك دراسة العلامات السريرية و التغيرات الدموية التي تحدث نتيجة نقص الزنك في الأغنام. بالإضافة إلى تأثير الزنك على عوامل الأوكسدة من خلال قياس مادة المالدوهايد و تأثيره على مضادات الأوكسدة من خلال قياس الكلوتاثايون و مادة الكتلينز. أجريت هذه الدراسة في محافظة البصرة ، و شملت الدراسة ٧٥ رأس من الأغنام من كلا الجنسين. ٦٠ رأس من هذه الأغنام كانت تعاني من علامات نقص الزنك و النحاس بينما اعتبرت ١٥ رؤوس طبيعية كمجموعة سيطرة. و تمثلت العلامات السريرية التي لوحظت فقدان للشهية، خمول، شهية منحرفة، أكل و عض صوف الحيوانات الأخرى، فقدان الشعر حول العينين و الإذنين و الأنف ، تساقط و فقدان الصوف، تقرن الجلد، صعوبة في المشي و عانت بعض الحيوانات من الإسهال، شحوب في الأنسجة، و انخفاض معدل تقلص الكرش، و كذلك لوحظ زيادة معنوية ($P<0.05$) في معدلات النبض و التنفس و تركيز مادة المالدوهايد بالإضافة إلى انخفاض معنوي ($P<0.05$) في العدد الكلي الكريات الدم الحمراء، و تركيز خضاب الدم، حجم كريات الدم المرصوصة، الكلوتاثايون و الكتلينز و الزنك و النحاس و كان نوع فقر الدم سوية الحجم سوية صباغ في الحيوانات التي كانت تعاني من نقص علامات نقص الزنك و النحاس. أثبتت هذه النتائج أن الزنك و النحاس لهما دور مهم في الصحة العامة للحيوانات و الحفاظ على القيم الدموية ضمن المعدلات الطبيعية في منع التلف الحاصل نتيجة عوامل الكرب من خلال تقليل كميات الجذور الحرة و كذلك من خلال دورة في عميلة تصنيع مضادات الأوكسدة.

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