



## **Comparative study of serum copper concentration in natural copper deficient and healthy Awassi sheep in Al-Najaf city\Iraq**

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### **Abstract**

This study was conducted on Awassi sheep in Al-Najaf city during October /2014 till January /2015 to detect the main clinical features and copper concentration associated with natural copper deficiency of sheep .

About one hundred clinically copper deficient sheep (50 male,50 female) character by important clinical copper deficient features as affected sheep group and control sheep group which apparent healthy was included one hundred sheep (50 male,50 female) both group sheep was selected randomly and blood samples were collected from each one to estimate serum copper concentration .

Clinical examination of deficient sheep was showed wool abnormalities 60% as higher percentage ,ill-thriftiness and retarding of growth 80%, Ataxia(incoordination gait-posterior paralysis) 54% so the predominant signs is paleness of mucous membrane 100% .

So, heart and respiratory rates were increase in deficient sheep ( $92.11\pm 0.35, 36.8\pm 0.8$ ) than in control group ( $75.8\pm 0.54, 29.6\pm 0.19$ ) respectively ,but temperature rate not changes in both animals groups.

Serum copper concentration was ( $5.58\pm 0.13$ ) $\mu\text{mol/L}$  in deficient sheep when compared with control sheep ( $15.61\pm 0.56$ ) $\mu\text{mol/L}$  and the serum copper was higher significantly in male than in female in both groups as ( $6.482\pm 0.21, 5.131\pm 0.12$ )  $\mu\text{mol/L}$  in deficient sheep , ( $18.21\pm 0.78, 13.014\pm 0.62$ )  $\mu\text{mol/L}$  in control group respectively, and copper concentration in adult examined sheep (1-4) years as ( $5.66\pm 0.68$ ) lower than yearling lamb ( $9.8\pm 1.03$  in deficient group).

This study was conducted to estimate serum copper concentration in clinically copper deficient sheep and the effect of gender and sex on their concentration in Awassi sheep.

**Keywords:** Copper ,Naturally deficient ,Awassi sheep ,Clinical features ,Al-Najaf

## دراسة مقارنة لقياس تركيز النحاس في أغنام العواسي المصابة طبيعياً بنقص النحاس والغير مصابة في مدينة النجف\العراق

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### الخلاصة:

أجريت هذه الدراسة في مدينة النجف خلال الفترة من شهر تشرين الأول / 2014 لغاية شهر كانون الثاني / 2015 للتعرف على العلامات السريرية المميزة لنقص النحاس الطبيعي الحدوث مع قياس تركيز النحاس في امصال الاغنام المفحوصة . شملت الدراسة فحص مائة من الاغنام التي تعاني من نقص النحاس سريريا (50 من الذكور و 50 من الإناث) ومائة من الاغنام السليمة كمجموعه سيطره وحيث اختيرت الاغنام من المجموعتين بشكل عشوائي وجمعت عينات الدم منها لغرض قياس تركيز النحاس . وقد أظهر الفحص السريري في الأغنام المصابة بنقص النحاس تغيرات في لون وقوام الصوف وبنسبة 60% ، واعتلال مع تاخر النمو 80%، وتأرجح وعدم الاستقامة في المشي 54% وإن من العلامة السائدة لنقص النحاس هو شحوب الأغشية المخاطية وبنسبه 100% في الاغنام المصابة بالنقص . كانت معدلات تردد القلب والجهاز التنفسي اكثر من الطبيعي في الأغنام المصابة (92.11 ± 0.35، 36.8 ± 0.8) على التوالي مما كانت عليه في المجموعة السليمة (75.8 ± 0.54، 29.6 ± 0.19) على التوالي، لكن معدل درجة الحرارة لم يتغير في كلا المجموعتين . كان تركيز النحاس في الدم (0.13 ± 5.58) ميكرومول /التر في الأغنام المصابة بالمقارنة مع الأغنام السليمة (0.56 ± 15.61) ميكرومول / لتر. وان تركيز النحاس في المصل عند الذكور اعلى منه في الإناث في كل من المجموعتين على التوالي (6.482 ± 0.21، 5.131 ± 0.12) ميكرومول / لتر في مجموعة الأغنام التي تعاني من نقص النحاس و (18.21 ± 0.78، 13.014 ± 0.62) ميكرومول / لتر في مجموعة السيطرة على التوالي وكان تركيز النحاس في الأغنام الكبيرة (1-4) سنوات هو (5.66 ± 0.68) أقل من الضأن الحولي الذي عمره سنة فما دون والذي اظهرت (9.8 ± 1.03) في مجموعة الاغنام التي كانت تعاني من النقص .  
الكلمات المفتاحية: النحاس، الاغنام العواسية، النقص الطبيعي، العلامات السريرية، النجف

### Introduction:

The Awassi sheep is one of the most popular, economically important sheep in Iraq that distributed in Iraq and neighboring countries which survive on the low nutrition level, due to decline of agriculture and expensive foodstuff, that lead to malnutrition and thriftiness in sheep, this danger may lead to halt breeding and loss the economic benefit (1, 2).

Copper deficiency is endemic in ruminants, worldwide occurrence and cause diseases which have economic importance of all ages, but primarily young and growing animals are affected (3), copper (Cu) deficiency in ruminants is a worldwide problem (4

) which can occur as a primary deficiency where Cu intake is inadequate, or as a secondary deficiency produced when copper of the ration is marginal but absorption and utilization of ingested copper is impaired by other minerals (5) and these antagonistic minerals include molybdenum, sulfur and iron (6). Hypocuprosis in sheep clinically character by different sings moderately as render of growth, emaciation, change in color and texture of coat, moreover, the black wool showed depigmentation. or severely as diarrhea, anemia and sway back disease (7, 3), copper is one of the

key trace minerals and is critical for life, copper is needed for a healthy nervous and immune system and healthy wool development and is stored in a sheep's liver (8 ). Copper is a cofactor of several metalloenzymes and other metalloprotein such as ceruloplasmin, superoxide dismutase, cytochrome oxidase, lysyl oxidase, and metallothione (9 ).

Copper deficiency induces hypochromic microcytic anemia (10 ). Hemoglobin level and erythrocytic count are depressed in advanced cases of primary copper deficiency ( 3 )

As well as, the physiological role of copper (Cu) in the body is related to several functions, which include cellular respiration, bone formation, connective tissue development, and essential catalytic cofactor of some metallo-enzymes (11 ). Copper is required for the activity of enzymes associated with ferrous metabolism, elastin and collagen formation, melanin production and integrity of central nervous system ( 12 ). Some studies were carried in Iraq as ( 13 ) about induce copper deficiency

experimentally in Baghdad city, so many researchers in Al-Basra studied mineral deficient in sheep ( 14 ,15 ).

**Materials and methods:**

Two hundred Awassi sheep with different ages and both sex that categorized into healthy and deficient sheep groups ,and the study was carried out in Al-Najaf city from ( 15/10/2014 to 15/1/2015)

**Clinical study**

One hundred (100) clinically naturally copper deficient sheep(affected ) showed clinical signs of ill-thrift , limp and steely wool with depigmentation and retardation of growth as well as paleness of mucous in addition of depression of animal viability and loss appetite and examined animals also involved another one hundred (100) healthy Awassi sheep (control).The selection of animals was randomly with different ages (1 month -4years) and both sex . Table ( 1 ). Physical examination including temperature ,respiration and heart rates of all animals in the present study was recorded according to (16) .

**Table(1) :The numbers of examined sheep in study**

Animal groups	Male		Female	
	1d-12 month	>12mon-4yrs	1d-12 month	>12mon-4yrs
Control	25	25	25	25
Affected	25	25	25	25

**Blood sampling:**

Five milliliters (5ml) of blood sample was collected from jugular vein by disposable then transferred into Eppendorf tubes and syringes , that blood was put in plain tubes storage under freezing until be exam . to separate serum after centrifuged at 3000 rpm \10 minutes(Coles, 1986) . Serum was

**Estimate serum Copper concentration :**

Copper kit by Biochain \ Italy was used to estimate serum copper concentration according to instruction by manufactured company .

**Statistical analysis:**

The obtained data was statically analyzed for means and significances between groups using ANOVA according to computerized SPSS program (version 7) .

**Results:**

**Clinical study :**

The results showed the naturally copper deficient sheep characterized by abnormalities in the wool of affected sheep in percentage as 60% and ataxia which

ranged from in coordination in gait into severe sign as posterior limbs paralysis in total percentage as 54% .Table ( 2) .

So ,that examined adult sheep were revealed ill-thriftiness (90%) and wool abnormalities signs (80%) as more severe that in lamb which show signs of swayback or difficulty in standing or walking ,but ataxia signs were mentioned severe in lamb 80% rather than in adult 28% table (2) .While the paleiness of mucous membrane was the predominant sign of all affected sheep in this study

**Table (2) : Apparent clinical signs of natural copper deficient sheep in the study .**

Copper deficient sheep n=100						
Clinical signs	Lambs n =50		Adults n 50=		Total	
	No.	%	No	%	No	%
Wool abnormalities (cooler and texture )	20	40 a	40	80 b	60	60
ill- thriftiness	35	70 a	45	90 b	80	80
Ataxia(incoordination gait-posterior paralysis)	40	80 a	14	28 b	54	54
Paleness of mucous membrane	50	100a	50	100a	100	100

The differences in small letters horizontally refer to presence of significant value at (p<0.05).

While, physical examination of affected and non affected (control )sheep appeared there was non- significantly(p>0.05) differences between sheep in two animal groups in temperature ,but the respiratory rate and heart rate showed significant (p<0.05) increase in affected sheep (36.8±0.8),(92.11±0.35) than in control study group as (29.6±0.19),(75. 8±0.54) respectively table (3).

**Table (3) :Physical examination (temperature ,respiratory and heart rates) in both control and affected sheep groups**

Parameters	Control group n=100 M±SE	Affected group n=100 M±SE
Temperature /C°	39.4±0.81 a	39.3±0.32 a
Heart rate / minute	75.8±0.54 a	92.11±0.35 b

Respiratory rate/ minute	29.6±0.19 a	36.8±0.8 b
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The differences in small letters horizontally refer to presence of significant value at (p<0.05).

**Serum copper concentration :**

There was significant (p<0.05) dropping of copper level in affected group as (5.58±0.13µmol/L) which lower than their value in control group as r (15.61±0.56µmol/L) table (4).

**Table(4): Serum copper concentration (µmol/L) of control and copper deficient sheep groups ; rans and means ± SE**

Biochemical parameters	Control group (n=100)	Affected group (n=100)
Copper µmol/L	5.8-32.8 15.61±0.56 a	3.3-8.80 5.58±0.13 b

The differences in small letters horizontal refer to presence of significant value at (p<0.05).

The values of serum copper in both sheep groups was affected by sheep gender and table(5) appeared significant(p<0.05) increase of copper 6.482±0.215 in male than in female 5.131± 0.121 in deficient sheep as well as in male 18.216± 0.78 higher than in female 13.014±0.621 in control groups

**Table (5): The effect of gender on serum copper concentration (µmol/L) of control and copper deficient Awassi sheep**

Serum copper Range and M± SE		
Sheep groups	Gender	Copper µmol/L
Control	Males (n=50)	10.3-32.8 18.216± 0.78 a
	Females (n=50)	6-27 13.014±0.621 b
Affected	Males (n=50)	4.2-8.8 6.482±0.215 c
	Females (n=50)	3.5-6.6 5.131± 0.121 d

The differences in small letters vertically refer to presence of significant value at (p<0.05).

According to table (6) ,there was no significant(p>0.05) effect of age on concentration of Cu in serum of control sheep ,whereas, in deficient copper sheep group a significant (p<0.05) drop of Cu in aged sheep (1-4 years ) as (5.66±0.68 ) than in younger sheep (under one year )as (9.8±1.03 ).

**Table (6): The effect of age on serum copper concentration(μmol/L) of control and copper deficient Awassi sheep**

serum copper M± SE		
Sheep groups	Age	Copper μmol/L
Control	Under 1year	19.3±0.89 a
	1-4years	17.164± 1.08 a
Affected	Under 1year	9.8±1.03 b
	1-4years	5.66±0.68 c

The differences in small letters vertically refer to presence of significant value at (p<0.05).

**Discussion :**

Clinical signs

In the present study ,the clinical signs in copper deficient sheep appeared as limp and glossy wool ,retarded growth ,depigmentation of wool and paleness of mucous membrane, and these clinical findings concern with those achieved by (18 , 3 ,9 ,20 ,21 ) .The ill-thriftiness or retardation of growth and un steady gait was described by ( 22 ) which attributed to interference of intermediary metabolism due to failure of tissue oxidation ,whereas the limp and glossy wool may be attributed to the requirement for copper to oxidized the SH into S-S disulphide bridge in the two adjacent cysteine molecules groups in keratin synthesis ,and appears depigmentation of wool may be due to deficiency of copper containing enzyme tyrosine( polyphenyloxidase) which catalyses

the conversion of L-tyrosine into melanin ( 23 , 3 )

Signs of ataxia like unsteady gait, incoordination were observed on copper deficient sheep that insimilar resultant obtained by ( 13 ) like lack of balance and atrophy of hind legs with the evidence of the neurological signs of sheep ,that copper deficient causes a damage of nerves due to its role in the synthesis of phospholipids and caused demyelination in the spinal cord ,so as nervous signs should be appeared .

Body temperature, heart rate and respiratory rate were recorded in examined sheep in this study, body temperature similar in both group (control and deficient sheep) , while respiratory and pulse rates were significantly increased in affected sheep which is in agreement with results of researchers in Basrah city by(15 ,24 , 25 ) .While ( 22 ) reported there were no significant

differences in the clinical parameters of temperature, pulse and respiration between copper deficient and healthy sheep in south west China.

The increase in respiratory and pulse rates with mineral deficiency occur to compensate the hypoxia and anemia which resulted from copper deficiency in order to supply body cells with oxygen which suffer from hypoxia because the decreased in RBCc ,Hb which lead to anemia associated with copper deficiency ( 26 ,27 )

### Copper concentration:

Serum copper in present study showed significant variation  $P < 0.05$  when compare control and affected sheep (  $15.61 \pm 0.56 \mu\text{mol/L}$ ;  $5.58 \pm 0.13 \mu\text{mol/L}$  ) , and the ranges ( $5.8-32.8 \mu\text{mol/L}$ ), ( $3.3-8.8 \mu\text{mol/L}$ ) respectively, this result was agree with those of ( Al Khalidi,(2004); Khan (2005) ; Smith (2002) which indicated that value of copper in serum decreased in animal which supported by a low concentration of copper in diet or soil.

The level of normal and deficient serum copper were closely to other results by Mohammed *et al* .(2013) as Iraqi study of normal Cu level( 0.977 ppm which equals in SI unit to  $15.22 \mu\text{mol/L}$  and diseased level 0.15 ppm =  $5.72 \mu\text{mol/L}$  and to resultant of Chinese researcher who revealed 0.95ppm =  $14.9 \mu\text{mol/L}$  in control sheep but the deficient Cu level 0.23ppm =  $3.6 \mu\text{mol/L}$  is lower than present result. But , the report by Abdelrahman (2012) which appeared the serum copper range of 16.9-18  $\mu\text{mol/L}$  in non-copper deficient sheep and the present data

did not agree with this narrow range which also reported by Underwood and Suttle ( 1999) as 9-15  $\mu\text{mol/L}$ .

Also, Aitken (2007) reported serum copper range of 9.4-23.6  $\mu\text{mol/L}$ , while Kaneko (2008) documented a range of 9.13-25.2  $\mu\text{mol/L}$ , both ranges almost did agree with present study results of control sheep (more than 90% within these ranges). The normal ranges of serum copper are wide and vary between sheep species (Radostits *et al.*,2007).

Serum copper levels between 3 - 9  $\mu\text{mol/L}$  represent marginal deficiency or hypocuprosis (Radostits *et al.*, 2007), therefore , according to present result of deficient sheep ( $5.58 \pm 0.13 \mu\text{mol/L}$  ) it suffering from hypocuprosis as indication of lost of liver Cu storage and drop the serum Cu in body sheep gradually .

On other hand, the marginal low value of serum copper was 7  $\mu\text{mol/L}$ , in Awassi sheep and the values lower than that minimal may be indicating serum copper deficiency.

While Khan *et al.*(2007) , Abdelrahman *et al.*(2007) reported the lowest values of Cu (7  $\mu\text{mol/L}$ ) was in agreement with present range without appearance clinical signs of copper deficiency. Also, Rodastits *et al.* (2007) and Joseph *et al.* (2007) reported the marginal value of serum copper without appearance of hypocuprosis was 7.8  $\mu\text{mol/L}$ . Furthermore, Laven and Smith (2008) suggest that a range of 4.5 to 7.3  $\mu\text{mol/L}$  in serum copper may be used to define copper deficiency status in sheep.

The younger examined sheep and males in control and copper deficiency sheep were character by high level of serum copper than aged and females in the present study which in harmony to those by Dar *et al* .(2004) in Kashmir /India whom showed that lamb had(1.02±0.9) than adult rams (0.88±0.11) as serum copper level ,and our result was nearest to finding by Al-Hadithy and Al- Badwi (2012) about gender and cu level as males with high cu level (18.8±0.62) than in female (14.9±0.42) in non-affected sheep ,as well as, the fall in serum cu with increasing age especially in natural rearing sheep (suttle *etal* .,1986), and that fact was ensured by Scott (2012).While ,the study in Sudia by Al-Mujalli(2012) showed that adult Najdi sheep had high cu level (16.27mg/dL) than in lamb (11.11mg/dL) .But according to Radostits *etal* .(2007) the serum cu in adult was more dropping than in lambs which that agreement with the present result and on other hands the newly lamb from cu deficient dam character by depletion cu from their liver storage as highly serum cu decline than in adult sheep (Pugh,2002) .Sheep depending on natural pasturing suffering from cu deficient with increase age due to natural rearing not supply the mineral body demanding as well as heavy parasitic infestation with aged exhausted different body mineral and cu is one these many minerals .

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