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INVESTIGATIONS ON SERUM COPPER VALUES IN HEALTHY AND COPPER DEFICIENT NAJDI SHEEP IN THE EASTERN REGION OF SAUDI ARABIA

BY

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ABSTRACT

Copper deficiency in sheep pose major challenge to producers in Saudi Arabia. To uncover clinical impact of this diseases, a total of 26 animals (13 copper- deficient lambs, six months of age and 13 adult diseased ewes, 3-4 years old) were subjected to this study. In addition 10 apparently healthy animals (5 lambs six months old and 5 adult ewes 3-4 years old) were used as control groups. Blood samples were obtained from all animals under the study and were subjected to cellular and biochemical analysis. The obtained results of blood cellular examination revealed significant ($P<0.05$) decrease of Hb concentrations, PCV%, and total RBCs counts in copper deficient animals when compared to their values in the control ones. Meanwhile, the results of serum biochemical analysis revealed significant decrease ($P<0.05$) of serum copper values and albumin concentrations in copper deficient animals when compared to their corresponding values in control ones. Moreover, total protein showed the same trend in lambs only. On the contrary, glucose level response to copper deficiency revealed a reverse effect, It was higher in diseased lambs and ewes than their control ones.

Key words: copper, deficiency, ewes, lambs.

INTRODUCTION

Copper is required for tissue oxidation in relation with cytochrome oxidase systems and for the iron mobilization in hemoglobin formation. Copper deficiency occurs either because of an absolute lack of copper in the diet (primary deficiency) or when the pasture molybdenum content is high (secondary deficiency).

Copper plays an important role mainly in hematopoiesis of both animals and human **(O'Dell,1976),** although its relation to it and to iron metabolism has not been completely elucidated **(Bencko et al., 1995).** Blood hemoglobin volumes depend on many external factors. The most important ones include nutrition and the hygienic conditions of the animal husbandry system. The amount of hemoglobin in blood is directly dependent on the presence of its initial building components. They are, first and foremost, proteins with amino acids (glycine and histidine), Fe, Cu, Co, vitamin B12 and other substances necessary for the synthesis and renewal of hemoglobin in the organism. Copper deficiency in the animal reduces hemoglobin production **(Shenck and Kolb, 1991).** Blood hemoglobin concentrations also depend on the animals age, weight, sex, yield, nutrition and health and also on the altitude above sea level and health status **(Sova et al., 1981).**

It is essential to correlate the clinical signs of deficiency with the serum copper levels because these levels fluctuate from week to week, fall with increasing age and are affected by diet and low values can be obtained from apparently healthy animals **(Suttle et al., 1980).** Serum copper levels will fall only when the liver copper has been markedly reduced, so normal serum copper levels do not always indicate a normal copper status. Despite these drawbacks it is generally agreed that serum levels of less than 10 µmol/liter are suspicious and values below 5 µmol/liter indicate deficiency **(Irwin et al., 1979; White-law et al., 1979; Humphries, 1980).** In clinically apparent deficiency in calves plasma copper was below 8.8 µmol/liter **(Smart et al., 1980)**. Cows had even lower levels, while cattle with severe deficiency had levels as low as 1.6 µmol/litre and sheep 3.1 µmol/litre **(Suttle, 1981)**. Liver levels in deficiency are in the range of less than 5 mg/kg .The copper levels in hair may also be used as a guide to deficiency. Normal levels in cattle are given as 6-7.5 mg/kg **(Kellaway et al., 1978).**

There were several studies concerning the effect of age on the liver (Robert, 1971). The author concluded that the effects of cow age on plasma and liver content can only manifest itself when the cattle are fed copper deficient ration and when there is increased copper

demand of growth and pregnancy. It has been reported that the high copper content in the liver increases with age, indicating an alteration in copper metabolism **(Hoag et al., 1977).** Copper deficiency can be detected by profile test especially when animals rely on pasture grazing for their sole intake; concentrate usually contains sufficient copper **(Payne and Payne, 1987).**

Reports on the factors influence on copper values in Najdi sheep in Saudi Arabia are scanty. Therefore, the aim of this study is to throw the light on the copper status in healthy and diseased Najdi sheep, native breed, in the eastern region of Saudi Arabia.

MATERIALS AND METHODS

Animals:

A total of 13 lamb six months with signs of copper deficiency were admitted to the Veterinary Teaching Hospital at King Faisal University (VTHKFU). These lambs were presented with major clinical signs of incoordination and weakness. In addition, 13 adult ewes 3-4 years old with signs of copper deficiency were admitted with clinical signs of weakness and steely wool. In addition 10 apparently healthy animals (5 lambs and5 adult sheep) were belonging to the Agriculture and Veterinary Training and Research Center, King Faisal University were used as control groups. Those healthy animals were housed in yard and fed green fodder ration and water was given ad labium. The animals were also offered salt lick with copper content.

Blood Samples:

Two blood samples were obtained from all animals under the study through jugular vein puncture. The first blood samples were obtained in vacutainer tubes with EDTA as anticoagulant and were used for cellular evaluation. The second blood samples were collected via the jugular vein puncture using evacuated blood collection tubes without anticoagulant. The samples were clotted and then centrifuged at 3000rpm for 15 minutes. Only clear non-hemolyzed serum was obtained and kept frozen at -20ºC until used for biochemical analysis.

Hematological and Biochemical analysis:

Cellular evaluation of the selected parameters was carried out using the electronic cell counter (UDIHEM-UDI). The measured parameters include Total erythrocytic count

(TRBCs), Hemoglobin concentration (Hb), Packed cell volume (PCV), total leucocytic count (TWBCs) and differential leucocytic count.

The concentrations of the selected biochemical parameters particularly, total protein (TP), albumin (Alb), glucose (Glu), calcium (Ca), magnesium (Mg), blood urea nitrogen (BUN), creatinine (Cr) and alanine aminotranseferase (ALT) were measured calorimetrically with auto analyzer (Ellipse-UDI) machine. Serum copper (Cu) concentrations were measured by Atomic Absorption Spectrophotometry, 2380.

Statistical Analysis:

Data were analyzed by the General Linear Model (GLM) procedure for unequal numbers (SAS, Institute, Inc, 2002). The Least Square Mean (LSM) ± standard errors were calculated for diseased and healthy lambs and ewes and tested for significances using the student "t" test **(Steel and Torrie, 1960).**

RESULTS

The obtained results of blood cellular examination in diseased and control lambs and adult ewes (Table 1) revealed significant ($P<0.05$) decrease of Hb concentrations, PCV%, and total RBCs counts in copper deficient-animals when compared to their values in the control ones. The obtained values were 8.27 ± 0.28 and 9.07 ± 0.32 for diseased lambs and ewes compared to their corresponding control ones (14.8 ± 0.61 & 14.2 ± 0.64 g/dl), respectively. Similar findings were noticed for PCV%. The obtained values being 29.57 ± 0.35 vs. 41.71 ± 2.73 in lambs and 31.61 ± 0.55 vs. 41.71 ± 2.73 in ewes. The mean values of RBCs counts in lambs were (7.12 ± 0.28 vs. 13.72 ± 0.54) compared to the values of in ewes (7.83 ± 0.18 vs. 13.32 ± 0.69).

Meanwhile, the results of serum biochemical analysis revealed that deficient lambs and ewes showed significant decrease ($P<0.05$) of serum copper values (11.11 ± 0.45 and 16.26 ± 0.32), ALT (19.9 ± 1.39 & 18.75 ± 1.27), and Albumin (28.46 ± 1.38 & 29.15 ± 1.42g/dL) concentrations compared to their corresponding values in control ones (Table 2). Moreover, total protein showed the same trend in lambs only (7.5 ± 0.03 vs. 7.8 ± 0.32). On the contrary, glucose level response to copper deficiency revealed a revers effect, It was higher in diseased lambs (63.75 ± 232) and ewes (65.14 ± 2.37) than their control ones (48.2 ± 1.8 & 48 ± 0.11).

Table 1: Means ± standard errors (SE) for the effect of copper deficiency on Blood cellular elements of adult ewes and lambs .

Blood cellular elements	Health status	N	LAMBS			ADULT EWES		
			Mean ± SE			Mean	SE	
MONO X10^3	DISEASED	13	5.24	1.37		7.79	1.69	
	CONTROL	5	3.90	0.81		3.90	0.81	
GRANUIO X10^3	DISEASED	13	57.63	4.01		53.53	2.63	
	CONTROL	5	61.36	4.64		61.36	4.64	
HB g/dl	DISEASED	13	8.27	0.28	ax	9.07	0.32	ax
	CONTROL	5	14.80	0.61	bx	14.20	0.64	bx
WBCS X10^3	DISEASE	13	14.55	2.53		13.62	1.56	
	CONTROL	5	14.38	3.23		14.38	3.23	
LYMPHO X10^3	DISEASED	13	37.12	3.56		38.68	2.01	
	CONTROL	5	34.74	4.56		34.74	4.56	
PCV%	DISEASED	13	29.57	0.35	ax	31.61	0.51	ax
	CONTROL	5	41.71	2.73	bx	41.71	2.73	bx
RBCS X10^6	DISEASED	13	7.12	0.28	ax	7.83	0.18	ax
	CONTROL	5	13.72	0.54	bx	13.32	0.69	bx

$^{a-c}$ different letters between sheep condition (column) are significant ($P<0.05$)

$^{x-y}$ different letters between sheep (row) within condition are significant ($P<0.05$)

Table 2: Means ± standard errors (SE) for the Serum biochemical analysis of adult sheep and lambs.

SERUM parameters	Health status		LAMBS			ADULT EWES		
	TYPE	N	Mean	SE		Mean	SE	
COPPER mg/dl	DISEASED	13	11.11	0.45	ax	16.27	0.32	ay
	CONTROL	5	26.20	0.58	bx	20.80	0.86	by
BUN mg/dl	DISEASED	13	16.01	0.90		16.20	0.91	
	CONTROL	5	18.00	0.82		18.16	0.70	
MAGNESIUM mg/dl	DISEASED	13	2.95	0.20		2.90	0.19	
	CONTROL	5	3.32	0.15		3.32	0.15	

ax	2.37	65.14	ax	2.32	63.75	13	DISEASED	GLUCOSE mg/dl
bx	1.10	48.00	bx	1.80	48.20	5	CONTROL	
ax	0.23	7.6	ax	2.03	7.5	13	DISEASED	TP g/dl
ax	0.37	7.6	bx	0.32	7.8	5	CONTROL	
	0.20	1.06		0.19	1.01	13	DISEASED	CREATININ mg/dl
	0.12	0.80		0.19	0.70	5	CONTROL	
ax	1.27	18.75	ax	1.39	19.19	13	DISEASED	ALT µ/l
bx	0.55	27.72	bx	0.55	27.72	5	CONTROL	
ax	0.24	7.06	ax	0.26	7.23	13	DISEASED	CLACIUM mg/dl
ax	0.25	8.20	ax	0.39	8.04	5	CONTROL	
ax	1.42	2.91	ax	1.38	2.86	13	DISEASED	ALBUMIN g/dl
bx	0.37	4.62	bx	0.24	4.76	5	CONTROL	

$^{a-c}$ different letters between sheep condition (column) are significant ($P<0.05$)

$^{x-y}$ different letters between sheep (row) within condition are significant ($P<0.05$)

Fig1: A demonstration figure of clinical case of copper deficiency in a lamb showing weakness, front and back leg paresis and recumbancy.

Fig 2: A demonstration figure of another clinical case of copper deficiency in a lamb showing severe weakness, leg paresis and recumbancy.

DISCUSSION

In the present study there was significant reduction in the mean values of Hb, PCV % and RBCs of diseased lambs and ewes which could be attributed to the role of the copper in hematopoesis and its importance in iron metabolism. As copper is considered as one of initial building components and could be necessary for the synthesis and renewal of hemoglobin. **(Shenck and Kolb, 1991).** These findings are in concern with those obtained by **O'Dell (1976)** and **Bencko et al (1995)**. In the same concern **Shenk and Kolb** (1991) stated that copper deficiency in the organism reduces hemoglobin production. Moreover, The reductions could be attributed to the reduced bioavailability of Cu which was inadequate for the formation of red cells **(Draksler et al., 2002).**

Cu deficiency has been reported in sheep and goats **(Fouda et al, 2012).** Copper deficiency may be due to its deficiency in feed or soil (dietary), however other factors can contribute to Cu deficiency most important of which is excessive intake of molybdenum (Mo) and sulphur. Cu and Mo affect the absorption and excretion of each other **(Raczykowski, 1995).**

The clinical signs presented by examined animals in this study were typical of Cu deficiency specially the in-coordination signs. Changes in severity may be attributed to individual variation **(Sherry, 2004).**

The blood copper concentrations in lambs was 11.11 mg/dl in lambs and 16.27mg/dl while in adult sheep. Generally the concentrations below 54 mg/dl indicating depletion of liver reserves. It is reported that lamb growth rates are not adversely affected until plasma concentrations fall below 18 mg/dl. The concentration of examined animals specially lamb was under this concentration **(Scott, 2012).**

Total serum proteins and albumin values in diseased lambs were lower than those of control animals; this may be a result of inappetence and low feed intake and impaired protein synthesis in the liver **(Cerone, et al, 1998).** It could be concluded that cu deficiency in investigated ewes and lambs present obvious clinical signs with detectable changes in blood chemistry and cellular content.

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الملخص العربي

دراسة عن مستوى النحاس في دم النعاج النجدية السليمة والتي بها عجز النحاس في شرق المملة العربية السعودية

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أجريت هذه الدراسة علي عدد ٢٦ حيوان (١٣ حمل & ١٣ نعجة مصابون بنقص النحاس). بلاضافة إلي مجموعتان ضابطتان (٥حملان وخمس نعاج). وكان الهدف من هذه الدراسة هو إلقاء الضوء على حالة مستوي عنصر النحاس في الأغنام والحملان المريضة ومقارنتها بالمستويات الطبيعية في الحملان والنعاج السليمة في المنطقة الشرقية من المملكة العربية السعودية. وقد تم الحصول على عينات من الدم من جميع الحيوانات تحت الدراسة وتم عمل التحاليل الخلوية والكيميائية الحيوية. وكشفت النتائج التي تم الحصول عليها من الفحص الخلوي للدم إلي انخفاض تركيز الهيموغلوبين،وحجم الخلايا المضغوطة، و العدد الكلي لكرات الدم الحمراء في الحيوانات التي تعاني من نقص مستوي عنصر النحاس في الحيوانات المريضة بالمقارنة مع قيمها في المجموعات الضابطة. ، كشفت نتائج التحليل الكيميائي الحيوي لمصل الدم إلي انخفاض كبير في قيم عنصر النحاس في الحيوانات المريضة مع انخفاض في مستوي إنزيمات الكبد ومستوي بروتينات الدم والجلوكوز عند مقارنتها بمستويات هذه العناصر في المجموعات الضابطة.