

E-ISSN: 2320-7078 P-ISSN: 2349-6800 JEZS 2018; 6(1): 1304-1306 © 2018 JEZS Received: 15-11-2017 Accepted: 16-12-2017

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# Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com



### Effect of sex factor on blood ceruloplasmin, copper level of serum and liver in Karadi sheep, Sulaimani province

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#### Abstract

The objective of this study was to survey the serum ceruloplasmin enzyme, serum copper and hepatic copper in male and female Karadi sheep. The study was carried out on 80 Karadi sheep at the Slaughterhouse in the province of Sulaimani - Iraq for a period from November of 2009 to June 2010. Results indicated that the overall mean serum ceruloplasmin, serum copper and hepatic copper level were 112.81 $\pm$ 2.10 mg/dl, 112.40 $\pm$ 0.71 ug/dl and 124.37 $\pm$ 0.93 ug/dl, respectively. Ceruloplasmin concentration was slightly higher among the female (114.36 $\pm$ 20.63 mg/L) than that in male (111.28  $\pm$  17.01 mg/dl); serum concentration was higher (p<0.05) for female (122.39 $\pm$ 7.13 ug/dl) than those for males (102.42 $\pm$ 5.70 ug/dl). Serum activity of ALT was higher (p<0.05) in the female (36.97 $\pm$ 3.66 U/L) compared to the males (25.42 $\pm$ 2.04 U/L). It can be concluded that the sex, overall, has no significant effects on Karadi sheep in Sulaimani province (north of Iraq) regarding blood ceruloplasmin, copper level of serum and liver.

Keywords: Karadi sheep, ceruloplasmin enzyme, serum copper, hepatic copper

#### 1. Introduction

Ceruloplasmin (Cp) is a copper-binding protein that is expressed in several tissues, including liver, brain, retina and lung <sup>[1-3]</sup>. Cp is known as a multifunctional protein <sup>[4]</sup>. During development, Cp expression is rapidly increased in the liver and lungs, and Cp becomes the major copper-binding protein in plasma after birth <sup>[1, 5]</sup>.

High levels of ceruploplasmins occur in pregnancy due to high estrogens, and with oral contraceptives when the agent contains estrogen as well as progesterone <sup>[6, 7]</sup>. As an essential component of several enzymes, Cu functions in numerous physiological processes <sup>[8]</sup>. Age, sex, the amount of dietary copper, and overall health determine the amount of copper distributed to the various tissues <sup>[9]</sup>.

Copper deficiency has been linked to a variety of clinical signs, including anemia, poor growth, pale hair coat, spontaneous fractures, myocardial degeneration, hypomyelinisation of spinal cord, decreased resistance to infectious diseases, diarrhea and general ill-thrift <sup>[8, 10-12]</sup>. Ewes suffering from an induced copper deficiency showed a rise in plasma copper during the luteal phase of the estrous cycle. This rise in plasma copper corresponded with the rise in progesterone concentrations <sup>[13]</sup>. Copper is also known to act as a catalyst in the production of prostaglandin F2a <sup>[14]</sup>.

Since very few investigations on the ceruloplasmin and copper levels in sheep have been done, therefore, the present study was undertaken to evaluate the effect of animal sex on the concentration of ceruloplasmin in the blood serum and the concentration of copper in the blood serum and liver.

#### 2. Materials and methods

The present survey was conducted with collaboration between the Department of Animal Production of Faculty of Agriculture - University of Sulaimani and the Department of Slaughterhouse in the Sulaimani (one of the provinces north of Iraq) to detect the concentrations of each enzyme ceruloplasmin and copper in the blood of sheep and liver, from November 2009 to June 2010.

#### **2.1 Experimental animals**

Eighty Karadi sheep (males and females) aged between six months and five years and weighting less 30- 60 kg were used to draw the samples.

#### 2.2 Collection of blood samples

Blood samples from the jugular vein were withdrawn by sterile syringes prior to slaughter then the samples were kept in reefer containers. After there, a blood serum was separated using a centrifuge (3000 RPM/min) for a period of 15 minutes. One part of the sample was sent to a laboratory for determination the concentration of copper and ceruloplasmin and the second sample (5ml) was frozen (-20 C°) in reserve for the purpose of analysis for the future <sup>[10]</sup>.

#### 2.3 Collection of liver samples

After Labelling or marking the Animals in the Slaughterhouse, samples of 10 - 20g of liver were taken from each animal and immediately transferred to the laboratory of animal production. 2g of each liver sample was crushed manually with 5ml of 0.9% Nacl and immigrations for about 15 min, then centrifuged (6000 RPM / min) for about 15 min. Later, the samples were kept in the deep freezer (-20 C°) until analysis have been done for determination of copper in the liver.

## 2.4 Determination of ceruloplasmin enzyme in the blood serum, copper in blood serum, liver, AST and ALT.

Series of laboratory tests were conducted which include:

## 1. Determination of ceruloplasmin enzyme in the blood serum

It was carried out by a radioisotope laboratory the capital Baghdad, and make a careful analysis using several specials called (Accet zoo ceruloplasmin from the production company (NN cormay, Italy)<sup>[11]</sup>.

#### 2. Determination of copper in blood serum and liver

Concentration of copper in serum and liver was determined by using a spectrum Spectrophotometer (wavelength of 550 nm) (Bussero-(Milan) ITALY)<sup>[14]</sup>.

#### **3.** Determination of AST and ALT

(AST and ALT) was determined according to Reitman and Frankle<sup>[15]</sup>.

Information that was recorded at the Slaughterhouse:

1. Animal weight.

2. Estimate the age of the animal depending on the animal owner.

3. Sex (male and female).

#### 2.5 Statistical analysis

Statistical Analysis system SAS <sup>[16]</sup> was used to test the significant differences of ceruloplasmin and copper in the serum and liver of Karadi sheep with different sex. Furthermore, Duncan's multiple range test <sup>[17]</sup> has been applied to determine the differences between means.

#### 3. Results and discussion

Effect of sex on the concentration of serum ceruloplasmin, copper in the serum and hepatic copper are mentioned below:

#### 3.1 Effect of sex on serum ceruloplasmin

Ceruloplasmin concentrations were slightly higher among the female (114.36  $\pm$  20.63 mg/ L) than that in male (111.28  $\pm$  17.01 mg/ dl) (Table 1). Johnson *et al.* <sup>[18]</sup> reported that plasma copper and ceruloplasmin activity were greater in women than in men. Estradiol-17 $\beta$  appears to stimulate the ceruloplasmin synthesis.

#### 3.2 Effect of sex on serum copper

Serum copper concentration was higher (p<0.05) for female (122.39 ± 7.13 ug/dl) than those for males (102.42 ± 5.70 ug / dl) (Table 1).

Higher serum copper and ceruloplasmin concentrations have been reported in females than in males <sup>[19-24]</sup>.

Sex, hormonal state, diet and geographical differences are known to affect serum copper levels <sup>[18]</sup>. A study demonstrates that female rats were susceptible to cardiac hypertrophy, anemia and decreased body weight induced by copper deficiency. The severity of these effects was, however, found to be to a lesser extent than in males, and estrogens did not influence the susceptibility of female rats to copper deficiency <sup>[25]</sup>.

#### 3.3 Effect of sex on hepatic copper

Table 1 shows that the concentrations of hepatic copper were  $123.12\pm5.46$  and  $133.87\pm6.65$  ug/dl for females and males, respectively, with no significant different between them. Lynch and Klevay <sup>[26]</sup> reported that hepatic copper was significantly reduced in copper-deficient females, but not in males.

Sex	No. of samples	Ceruloplasmin conc. (mg/L)	Copper conc. in serum (ug/dl)	Copper conc. in liver (ug/dl)
Over all mean	80	$112.81 \pm 2.10$	$112.40 \pm 0.71$	$124.37 \pm 0.93$
Female	33	114.36±20.63 <sup>a</sup>	122.39 ±7.13 <sup>a</sup>	123.12 ±5.46 <sup>a</sup>
Male	47	111.28±17.01 <sup>a</sup>	102.42 ±5.70 <sup>b</sup>	133.87±6.65 <sup>a</sup>

**Table 1**: Effect of sex on the concentration of ceruloplasmin, copper in the serum and liver (Mean ±SE) of Karadi Sheep.

Means with different letters for column differ significantly (p < 0.05).

## **3.4** Effect of sex on the concentration of the aminotransferase

Serum activity of ALT was higher (p<0.05) in females (36.97±3.66 U/L) compared with the males (25.42±2.04 U/L). While, no significant different was reported in the concentration of AST for females and males Karadi sheep (Table 2).

Gender has a serious role in directing the levels of several biochemical compounds <sup>[27]</sup>. Physiological changes may be involved in such difference <sup>[28]</sup>.

<b>Table 2</b> : Effect of sex on the concentration of aminotransferase
(Mean ±SE) of Karadi Sheep.

Sex	No. of samples	ALT (U/ L)	AST (U/ L)
Over all mean	80	31.19±0.32	144.17±0.99
Female	33	$36.97 \pm 3.66^{a}$	142.73±8.43 <sup>a</sup>
Male	47	$25.42 \pm 2.04^{b}$	145.59±9.43 <sup>a</sup>

Means with different letters for column differ significantly (p < 0.05)

#### 4. Conclusion

Overall, the sex did not affect significantly on Karadi sheep in Sulaimani province regarding blood ceruloplasmin, copper level of serum and liver. Even though, significant differences have been reported in copper level and ALT aminotransferase for females than males.

#### 5. Acknowledgment

This paper was supported by University of Sulaimani at 2010. We thank all the staff in the Department of Animal Production in the Agricultural Faculty for the technical assistance.

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