DETERMINATION CONCENTRATIONS OF SERUM CERULOPLASMIN, COPPER, ENZYMES ACTIVITY AND LIVER COPPER CONCENTRATION OF DIFFERENT AGES OF KARADI SHEEP AT IN SULAIMANI PROVINCE

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ABSTRACT

The objectives of this study was to measure the Concentrations of serum ceruloplasmin, Copper, enzymes activity and liver copper in Karadi sheep at different ages. The study was carried out on eighty Karadi sheep(47 male and 33 female) were used and taken from the slaughterhouse, the province of Sulaimani during period November 2009 to June 2010. Blood samples (10 ml) were collected from each animal before slaughtering and the serum were separated. Results indicated that the overall mean serum ceruloplasmin , serum copper and liver copper concentration l were 112.55 ± 13.047 mg/dl, 110.66 ± 4.56 ug\dl and 129.43 ± 4.52 ug\dl, respectively. Serum ALT and AST activity were 30.18 ± 2.018 U\L and 144.40 ± 6.50 U\L, respectively. The correlation coefficients between age and each of serum ceruloplasmin, serum copper and hepatic copper were not significant. Data revealed highly ($P \leq 0.01$) positive correlations between age of Karadi sheep and the activity of AST (0.246) and ALT (0.267) enzymes, was found. Data also indicate ($P \leq 0.01$) negative correlation between the concentrations of ceruloplasmin in serum and the liver copper concentrations (- 0.294). Other wise the correlations coefficient between other parameters were not significant ($P < 0.05$).

Key words : Ceruloplasmin , Copper , enzymes activity , blood serum , liver, Karadi sheep .

تقدير السيروبلازمين ومستوى النحاس وفعالية الانزيمات في مصل الدم وتركيز الثاني في كبد الاغنام الكرادية بالاعمار المختلفة في محافظة السليمانية

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المستخلص

هدف هذه الدراسة قياس تراكيز السيروبلازمين، انحاس وفعالية انزيمات ALT و AST في مصل الدم و تركيز النحاس في الكبد باعمار مختلفة في الاغنام الكرادية. تم اجراء هذه الدراسة على 80 راس من الاغنام كرادية (ذكور واناث) فى مجزرة السليمانية وذلك للفترة من تشرين الثانى لعام 2009 ولغاية حزيران 2010. تم جمع عينات الدم (10 مللتر) من كل حيوان قبل الذبح. اشارت النتائج الى ان المتوسط العام لتركيز السيروبلازمين وانحاس فى مصل الدم والنحاس فى الكبد بلغ 13.047 mg/L± 112.55، 4.56 ± 110.66 ug\dl و4.52 ± 129.43 ug\dl، على التوالي. بلغ المتوسط العام لمستوى انزيمي ALT وAST فى مصل الدم 30.18 ± 2.018 U/L و 6.50 ± 144.40 U\L، على التوالي. كان معامل الارتباط بين العمر وكل من السيروبلازمين والنحاس فى المصل و نحاس الكبد غير معنوى. لكن البيانات كشفت وجود ارتباط عالي المعنوية ($P \leq 0.01$) وموجب بين عمر اغنام الكرادي و تركيز انزيمي AST (0.246) و ALT (0.267).

كلمات مفتاحية: سيروبلازمين، النحاس، فعالية الانزيمات، مصل الدم، الكبد، الاغنام الكرادي.

INTRODUCTION

Ceruloplasmin, a serum glycoprotein is synthesized predominantly in the liver and its major function is a carrier for copper in the blood. It is certainly involved in iron metabolism, since it possesses a ferroxidase activity (1), and individuals lacking a functional ceruloplasmin gene have an impaired iron metabolism (2). Kurdistan is considered to be rich in mineral resources and these are some of them: copper, iron, Chrome, Magnesium , gold, silver, zinc and (3). A strong correlation between low concentrations of Cu, Co and Zn in the soil samples are known as trace element deficiency in grazing livestock and wildlife is demonstrated in many regions of the world (4 , 5). Copper is an essential micronutrient, its deficiency has been associated with disease state that decrease the production of livestock (6, 7). Enzymes are produced in liver cells that release into the general circulation when liver cells are injured.

AST is mainly contained in striated muscle, myocardium, and liver, its serum activity has been found to rise in diseases of these organs (8, 9, 10). Serum ALT level increase rapidly when the liver is damaged by any cause including: hepatitis, hepatic cirrhosis, liver tumor, obstructive jaundice, Reye's syndrome or hepatotoxicity of certain drugs (11). The purpose of this study was to study the effect of age of karadi sheep upon concentrations of ceruloplasmin, copper, enzymes activity and liver copper.

MATERIALS AND METHODS

This study was conducted in collaboration between the Department of Animal Production at the Faculty of Agricultural Sciences / University of Sulaimani and the Department of Slaughterhouse in the Province of Sulaimani during the period from November 2009 to June 2010.

Experimental animals:

Eighty Karadi sheep (47 males and 33 females) aged between 6 months and 5 years and weighting 30- 60 Kg were used to collect the samples.

Blood sampling and assay

Blood samples from the jugular vein were taken by sterile syringes prior to slaughter then the samples were kept in ice containers. Thereafter , a blood serum was separated using a centrifuge (3000 rpm / min) for 15 minutes. One part of the sample was sent to a laboratory for copper and ceruloplasmin assay and the second sample (5ml) was frozen (-20 C°) for other future assays.

Collection of liver samples:

After labelling or marking the animals in the slaughterhouse, a sample of 10 – 20 gm of liver was taken from each animal and immediately transferred to the laboratory of Animal Production Department. Thereafter an a mount of about 2 gm of each liver sample were minced manually with 5 ml 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.

Determination of ceruloplasmin , copper, AST and ALT in serum and copper in the liver**.** A series of laboratory tests was conducted which include:

1. Determination of ceruloplasmin in the blood serum: This was carried out by a Radioisotope Laboratory,Harthiyia, Baghdad , the kit (Accet zoo ceruloplasmin from the production company(NN cormay,Italy)(12).
2. Determination of copper in blood serum and liver: Concentration of copper in serum and liver was determined using a spectrum spectrophotometer (wavelength of 550 nm)using kit (Bussero-(Milan) Italy)(13).

Determination of AST and ALT**:** (AST and ALT) was determined according to Reitman and Frankel (14). Information that was recorded at the Slaughterhouse:

Animal weight, Estimate the age of the animal depending on the animal owner, and Sex (male or female).

Statistic analysis:

The significant differences of ceruloplasmin and copper in the serum and liver of Karadi sheep with different ages were analyzed . The Statistical Analysis System, SAS (15) was used. Duncan multiple range test (16) was used to determine the differences among means.

RESULTS AND DISCUSSION

1. Effect of age on serum ceruloplasmin: Ceruloplasmin concentration was higher ($P \leq 0.05$) in Karadi sheep aged 6 month (162.25 ± 12.37 mg/ L) in comparision with age of 11

month (74.53 ± 13.03 mg/ L) , 1-2 years (71.44 ± 11.67 mg/ L) , 2.1-3 years (114.50 ± 27.75 mg/ L) and 3.1 - 5 years (104.81 ± 30.60 mg/ L) (Table 1). Other studies found no such effect.(17). Also Paynter(18)says some biological or environmental variables such as age or molybdenum status may not influence ceruloplasmin activity directly.

2. Effect of age on serum copper: Serum copper concentration was higher ($P \leq 0.05$) in Karadi sheep aged 6 month (136.00 ± 7.62 ug / dl) in comparision with the 11 month (82.37 ± 6.28 ug / dl) , 1-2 years (122.11 ± 6.78 ug / dl) , 2.1-3 years (83.92 ± 7.74 ug / dl) and 3.1 - 5 years (119.88 ± 11.78 ug / dl) (Table 1) . These data were unagree with other results reported by other studies(19), several factors can affect serum copper including inflammation, infectious diseases and age, they reported that plasma Cu level increased with age whereas liver and bone Cu levels and urinary Cu excretion remained unchanged (20,21). In our study the increase of copper in the serum in animal with 6 month of age may indicated that they were in growing state and may be need more copper.

3. Effect of age on hepatic copper: Table 1 revealed a non-significant effect of age on the liver copper concentration. The values were 133.29 ± 6.18 , 133.29 ± 14.00, 114.89 ± 9.99, 142.25 ± 11.88 and 117.38 ± 6.00 ug / dl for sheeps aged 6 month ,11 month , 1-2 years , 2.1-3 years and 3.1 - 5 years ages respectively. iver copper concentrations decline with age in red deer (*Cervus elaphus*) (22, 23).

Table 1. Effect of age on the concentrations of ceruloplasmin, copper in the serum and liver (Mean ±SE) of Karadi sheep

Age	No. of samples	Serum ceruloplasmin conc. (mg / L)	Serum copper conc. (µg/ dl)	Liver copper conc. (µg / dl)
Over all mean	80	112.55 ± 13.05	110.66 ± 4.47	129.43 ± 4.46
6 month	24	162.25 ± 12.37[a]	136.00 ±7.62[a]	133.29±6.18[a]
11 month	19	74.53 ± 13.30[b]	82.37 ±6.28[b]	133.29±14.00[a]
1- 2 years	9	71.44 ± 11.67[b]	122.11 ±6.78[a]	114.89 ±9.99[a]
2.1 - 3 years	12	114.50 ± 27.75[b]	83.92 ±7.74[b]	142.25 ±11.88[a]
3.1 - 5 years	16	104.81 ± 30.60	119. 88 ±11.78[a]	117.38 ±6.00[a]

Means with different superscripts for each column differ significantly ($P \leq 0.05$)

4. Effect of age on the concentration of aminotransferases : Serum activities of ALT was higher ($p < 0.05$) in Karadi sheep with advancing age 3.1 - 5 years (40.00 ± 4.57 U/L) as compared 11 month (25.10 ± 3.73 U/L), while it is not significant between other age groups (Table 2). Serum activities of AST was higher ($p \leq 0.05$) in Karadi sheep 2.1-3 years old (196.42 ± 28.69) as compared with 6 month old (119.07 ± 4.24) , 11 month old (139.68 ± 10.62 U/L), 1-2 years old (155.33 ± 15.69 U/L) and 3.1 - 5 years old (142.88 ± 28.69 U/L) (Table 2) . Kohn et.al. (24) reported that the peak values of ALT occurred in the third decade of life for males and between 50-60 y of ages in women.

Table 2. Effect of age on the serum activity of aminotransferase enzymes of Karadi sheep (Mean ±SE)

Age	No. of samples	ALT (U/ L)	AST (U/ L)
Over all mean	80	30.18± 2.01	144.40±6.51
6 month	24	27.08±2.63[ab]	119.07±4.24[b]
11 month	19	25.10±3.73[b]	139.68±10.62[b]
1 - 2 years	9	34.22 ±5.25[ab]	155.33±15.69[b]
2.1 - 3 years	12	28.33±4.57[ab]	196.42±28.69[a]
3.1 - 5 years	16	40.00±4.57[a]	142.88±28.69[b]

Means with different superscripts for each column differ significantly ($p \leq 0.05$)

Simple correlation coefficient among different parameters The correlations coefficients among ages with serum ceruloplasmin activity, serum copper concentrations and liver copper concentrations, were summarized in Table 3. Serum copper level is not significantly correlated with age .Such no significant correlation was also reported earlier (25 , 26). There was a significant ($P \leq 0.01$) negative correlation between the concentra-

tions of ceruloplasmin and weight of sheep (-0. 286) (Table 3). A non- significant correlation (-0.014) between serum ceruloplasmin activity and serum copper was observed. However, a significant correlation was found between plasma Cu and ceruloplasmin (27). In addition, in sheep, the relationship between serum ceruloplasmin and whole blood copper concentrations was positive (R = 0.75) (28). The increase in serum copper is mostly due to increased hepatic synthesis and release of ceruplasmin (29). Results revealed a highly significant (P < 0.01) positive correlation between age of Karadi sheep and the concentration of AST (0.246) and ALT enzyme (0.267) respectively. (30) stated the association of ALT values with ages was a positive. In our study a highly significant (P < 0.01) negative correlation between the concentrations of ceruloplasmin and the liver copper concentrations (- 0.294) was found. In contrast, other studies (31 , 32) have indicated that liver Cu and CP concentrations are not correlated at either normal or high amounts of hepatic Cu accumulation. A non-significant correlation between serum copper and hepatic copper was found. Plasma Cu often does not reflect liver Cu stores (33, 34). But, In cattle with moderate hepatic Cu accumulation, neither the serum Cu nor ceruloplasmin concentrations are significantly associated with liver Cu concentration. (35, 36) Furthermore a highly significant (P ≤ 0.01) negative correlation between liver copper with ALT (-0.359) was found, but the correlation between liver copper and AST lacked significance. However, other authors (37, 38) have found only weak correlation between serum enzyme activities and hepatic Cu values or clinical manifestations of hepatic insufficiency.

Table 3. Simple correlation coefficient between different parameters

	Age	Ceruloplasmin	Copper in serum	Copper in liver	ALT enzyme	AST enzyme
Age	—	**N.S. - 0.134**	**N.S. - 0.142**	**N.S. - 0.101**	*** 0.246**	**** 0.267**
Ceruloplasmin		—	**N.S. -0.014**	**** - 0.294**	**N.S. 0.344**	**N.S. - 0.146**
Copper in serum			-------	**N.S. 0.027**	**N.S. - 0.119**	*** - 0.219**
Copper in liver				—	**** - 0.359**	**N.S. - 0.049**
ALT enzyme					—	**N.S. 0.013**
AST enzyme						—

***= (p<0.05) , ** = (p<0.01) , N.S. = Non significant**

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