



## EVALUATION OF SPECIFIC BIOCHEMICAL VALUES IN CLINICALLY NORMAL AND ANEMIC AWASSI SHEEP

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### ABSTRACT

The study was conducted on 200 healthy and 66 affected with iron deficiency anemia (IDA) Awassi sheep to determine serum iron, total iron binding capacity (TIBC), unbound iron binding capacity (UIBC), transferrin saturation (TS%) and copper concentration. The 200 normal sheep (80 males; 40 ram lambs and 40 rams and 120 females; 40 ewe lambs, 40 pregnant and 40 lactating ewes), while 66 diagnosed iron deficient anemic sheep (19 males and 47 females), both aged 7 months-4 years old in Baghdad governorate. The samples were collected from October 2011 to January 2012, and the tests were done in the Clinical Pathology Laboratory in College of Veterinary Medicine-Baghdad University - Iraq. The result showed that the range and mean  $\pm$  standard error(SE) of serum iron, TIBC, UIBC, TS%, and copper in clinically normal and anemic Awassi sheep were as follows ; serum iron 17.1-46.8  $\mu\text{mol/L}$  and 27.2 $\pm$ 0.40  $\mu\text{mol/L}$ , 3.5-17.1  $\mu\text{mol/L}$  and 11.4 $\pm$ 0.45  $\mu\text{mol/L}$ , serum TIBC 35-78.1  $\mu\text{mol/L}$  and 52.9 $\pm$ 0.68  $\mu\text{mol/L}$ , 63.4-112.5  $\mu\text{mol/L}$  and 80.5  $\pm$ 1.40  $\mu\text{mol/L}$ , UIBC 4.1-56.1  $\mu\text{mol/L}$  and 25.7 $\pm$ 0.77  $\mu\text{mol/L}$ , 47.7-105.3  $\mu\text{mol/L}$  and 69.1 $\pm$ 1.51  $\mu\text{mol/L}$ , TS% 28.1-90.6 and 52.9 $\pm$ 0.99, 5.1-25.1 and 14.4 $\pm$ 0.66 and serum copper 7-33.8  $\mu\text{mol/L}$  and 16.5 $\pm$ 0.37  $\mu\text{mol/L}$ , 3.3-21.1  $\mu\text{mol/L}$  and 11.5 $\pm$ 0.48  $\mu\text{mol/L}$  respectively. However, significant difference's ( $P < 0.05$ ) were recorded in serum iron, UIBC, TS% and copper in subgroups of normal Awassi sheep, as well as a significant difference was found between males and females in serum copper ( $P < 0.05$ ). On other hand results revealed significant difference ( $P < 0.05$ ) between normal and anemic values. In conclusion, present data recoded normal range reference and mean  $\pm$  SE of studied biochemical in Iraqi Awassi sheep, significant differences recorded between clinically normal and anemic animals, as well as between normal groups.

**KEY WORDS:** Serum Iron, TIBC, UIBC, TS%, Copper and Iraqi Awassi sheep.

### INTRODUCTION

The role of iron was a major structural component of hemoglobin and directly required for erythropoiesis, while other elements were indirectly required for interaction, metabolism and utilization iron (Mullally *et al.*, 2004). Moreover during pregnancy, the requirement for iron is reported to increase for fetus growth, continued gestation and parturition (Tapiero *et al.*, 2001). Furthermore, the connection between anemia, length of gestation and low birth weight has been described in the majority of studies. Iron deficiency anemia could cause low birth weight, preterm delivery and increased oxidative damage to erythrocytes in placenta – fetus unites (Lindsay, 2001). Iron deficiency (ID) was the most common nutritional worldwide disorder (Özahi *et al.*, 2011). It's deficiency initiated with normal serum iron level, which reduced due to low dietary iron intake, insufficient intestinal iron absorption or increased iron losses and resulting in decreased hemoglobin synthesis (Haas and Brownlie, 2001). Although iron deficiency caused by chronic blood loss, gastro-intestinal blood loss (parasitism or ulceration) and cutaneous blood loss caused by external parasites – fleas, lice (Barnes *et al.*, 2007) (AWI, 2012). However iron deficiency can be divided into three different phases: storage iron deficiency, iron deficient erythropoiesis, and iron deficiency anemia. (Welles, 2012). Serum iron, TIBC, TS%, UIBC and Ferritin consider important biochemical tests for diagnosis iron deficiency anemia (Munoz *et al.*, 2011). Many researchers evaluated or

recorded some of biochemical related to anemia in sheep, serum iron, TIBC, UIBC, TS% and serum copper were studied in normal and infected with internal parasite sheep Kozat *et al.*, (2006). In Jordan Abdelrahman *et al.*, (2006) studied the effect of gestation stage on copper and iron level in fetus and Awassi ewes, also serum copper and iron levels evaluated in growing Awassi lambs (Abdelrahman, 2012). These values documented by (Radostits *et al.*, 2007 ; Kaneko, 2008). Many of the above mentioned studies were conducted on small number, therefore this study was carried out on a large number to measure some of biochemical including serum iron, TIBC, UIBC, TS% and copper which have not been measured previously in clinically healthy and iron deficient anemic Awassi sheep in Iraq.

### MATERIALS AND METHODS

Blood samples were collected into plain tubes from jugular vein of 200 sheep clinically normal (80 males and 120 females) and 66 sheep diagnosed clinically suffering from iron deficiency anemia in Baghdad governorate- Iraq. Normal males was divided into two groups according to aged (40) ram lambs aged between (7-12 months) and (40) rams aged (1.5-4 years) and normal females divided into ewe lambs (40) aged (7-12 months) , pregnant ewes (40) aged (1-4 years) and lactating ewes (40) aged (1.5-4 years). On other hand 66 anemic sheep (19 males and 47 females) aged (7 month-4 years). The blood centrifuged for 5-10 minutes at 3000

rpm (Coles, 1986). The separated sera used directly for measurement of iron, TIBC and copper. The serum iron and TIBC were measured according to colorimetric method by (Young, 1995), serum copper was assayed according to colorimetric method by (Abe *et al.*, 1989). While TS and UIBC according to the following formula:

TS%= serum iron / TIBC × 100 and UIBC=TIBC-Serum iron (Weiss and Wardrop, 2010).

SAS program was used for statistical analysis. Data were subjected to Analysis of Variance (ANOVA) and significant means were compared by T-test at a level (P<0.05).

**RESULTS AND DISCUSSION**

The values of the measured serum iron, TIBC, UIBC, TS% and copper for sheep independent of any subdivisions are presented in (table 1), according to sex in (table 2) and the physiologic status in (table 3).

**TABLE 1:** The biochemical values in clinically normal and iron deficient anemic Awassi sheep; range and mean ±SE.

Groups	No.	Iron μmol/L	TIBC μmol/L	UIBC μmol/L	TS%	Copper μmol/L
Normal sheep	200	17.1-46.8	35-78.1	4.1-56.1	28.1-90.6	7-33.8
		27.2±0.40a	52.9±0.68b	25.7±0.77b	52.9±0.99a	16.5±0.37a
IDA sheep	66	3.5-17.1	63.4-112.5	47.7-105.3	5.1-25.1	3.3-21.1
		11.4±0.45b	80.5±1.40a	69.1±1.51a	14.4±0.66b	11.5±0.48 b

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

**TABLE 2:** The biochemical values of males and females in normal and iron deficient anemic Awassi sheep; range and mean± SE

Gender	No.	Iron μmol/L	TIBC μmol/L	UIBC μmol/L	TS%	Copper μmol/L
males	80	17.8-46.8	37.5-78.1	4.1-56.1	28.1-90.6	10.7-33.8
		28.3± 0.72a	52.4±1.05b	24.1±1.23b	55.5±1.7a	18.8± 0.62a
females	120	17.1-40	35-75.1	6-49.8	28.5-84.7	7-28.5
		26.4 ±0.45a	53.2± 0.90b	26.8 ±0.98b	51.2 ±1.1a	14.9± 0.42b
IDA males	19	5-15.7	65-102.7	49.3-93.8	6.4-24.1	4.8-21.1
		10.1± 0.76b	80.4± 2.75a	70.3± 2.87a	12.8±1.1b	14.3±1.04b
IDA females	47	3.5-17.1	63.4-112.5	47.7-105.3	5.1-25.1	3.3-15.6
		11.9± 0.54b	80.5± 1.65a	68.6± 1.79a	15.1±0.8b	10.3± 0.44c

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

**TABLE 3:** The biochemical values are presented according to physiologic status in normal and iron deficient anemic Awassi sheep; range and mean± SE

Groups	No	Iron μmol/L	TIBC μmol/L	UIBC μmol/L	TS%	Copper μmol/L
Ram lambs	40	17.8 – 42.9	37.5 – 67.1	6 – 40.8	31.1 – 90.6	10.7 – 33.1
		AB 27.4±0.99a	B 48.1±1.26b	B 20.7±1.33b	A 57.9±2.3a	A 19.3±0.89a
IDA ram lambs	12	9.65±1.04b	83.6±3.63a	73.9±3.54a	11.6±1.3b	15.8±1.03b
Rams	40	21 – 46.8	43.8 – 78.1	5.1 – 56.1	28.1 – 90.1	11.5 – 33.8
		A 29.2±1.04a	A 56.7±1.39b	AB 7.5±1.95b	AB 53±2.4a	AB 8.3±0.88a
IDA rams	7	10.9±1.06b	75.1±3.36a	64.1±3.9a	14.9±1.8b	11.7±1.9b
Ewe lambs	40	20.1 – 40	35 – 75.1	8.1 – 47.9	29.5 – 80.9	11.7 – 28.5
		A 29 ±0.83a	AB50.3±1.69b	B 21.3±1.74b	A 59.4 ±2a	AB 7.4±0.67a
IDA ewe lambs	5	11.8±1.39b	88.02±6.6a	76.1± 7.93a	14.1±2.3b	11.7±1.37b
Pregnant ewes	40	17.1 – 31.6	42.9 – 74.1	16.9 – 46.6	30.3 – 60.7	7 – 21.1
		B 24.6 ±0.67a	A 56.1±1.42b	A 31.5 ±1.34b	C 44.5±1.3a	C 11.7±0.52a
IDA pregnant ewes	21	11.8± 0.88b	79.8± 2.65a	68± 2.93a	15.2± 1.3b	10.5±0.61a
Lactating ewes	40	17.9 – 33.7	37.5 – 75	6 – 49.8	28.5 – 84.7	7.1 – 27.2
		B 25.7±0.70a	AB 53.3 ±1.5b	AB27.6±1.68b	BC 49.6±1.9a	B 15.7±0.67a
IDA lactating ewes	21	12.1± 0.80b	79.5± 2.04a	67.4±1.97a	15.4± 1.1b	9.9± 0.72b

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

Serum iron in normal Awassi sheep was found to be 27.2±0.40 μmol/L range 17.1-46.8 μmol/L, it was 28.3± 0.72 μmol/L in males and 26.4 ±0.45 μmol/L in females with no significant differences between them, this agreed with Nazifi *et al.* (2005), they revealed that sex had no significant effect on serum iron concentration. Serum iron

in iron deficient anemic Awassi sheep was 11.4±0.45 μmol/L, in males 10.1± 0.76 μmol/L and in females 11.9± 0.54 μmol/L with a significant decrease compared to normal sheep (tables 1 and 2). The serum iron concentration of this study was close to the range (18-48 μmol/L) of Aitken (2007), there were only 6 values out of

200 (3%) in the present study lower than the lowest limit reported by them.

However, the reference of serum iron concentration reported by Radostits *et al.* (2007) and Kaneko (2008) were 30 – 40  $\mu\text{mol/L}$  and 29.7 – 39.7  $\mu\text{mol/L}$  respectively. Also Kaneko (2008) documented a higher mean of (34.5 $\pm$ 1.25  $\mu\text{mol/L}$ ) compared to our data. The serum iron concentration in normal showed significant decrease in pregnant and lactating ewes compared to rams and ewe lambs (table 3). This did agree with Tapiero *et al.* (2001) whom suggested that the pregnant ewes are more susceptible to iron deficiency due to fetus growth requirement, while Goran *et al.* (2010) recorded that iron shading in colostrums could be 10 – 17 times more than in ordinary milk and lactating ewes secreted about 1.5  $\mu\text{mol/L}$  copper daily in colostrum. Also this study showed serum iron mean  $\pm$  SE in pregnant ewes (24.6  $\pm$  0.67 $\mu\text{mol/L}$ ), which was lower than the mean of 28.7  $\mu\text{mol/L}$  reported by Kozat *et al.* (2006). Moreover, in pregnant ewes the mean value of our study was similar to large extent, to that of Abdrahman *et al.* (2007), who reported a serum iron mean  $\pm$  SE of 25.9 $\pm$ 1.1 $\mu\text{mol/L}$  in pregnant ewes. However, the difference between the serum iron concentration of the present study and other researches could be attributed to the genetic factors or the type of feeding. On the other hand, recorded mean values of serum TIBC in normal Awassi sheep 52.9 $\pm$ 0.68  $\mu\text{mol/L}$  and ranged 35-78.1  $\mu\text{mol/L}$ , it was 52.4 $\pm$ 1.05  $\mu\text{mol/L}$  in males and 53.2 $\pm$  0.90  $\mu\text{mol/L}$  in females, with no significant differences between them. TIBC in IDA Awassi sheep was found to be 80.5  $\pm$ 1.40  $\mu\text{mol/L}$ . It was 80.4 $\pm$  2.75  $\mu\text{mol/L}$  in males and 80.5 $\pm$  1.65  $\mu\text{mol/L}$  in females with a significant increase compared to normal Awassi sheep (tables 1 and 2). The TIBC concentration in normal showed a significant decrease in ram lambs compared to rams and pregnant ewes (table 3). Also, this study showed a serum mean  $\pm$  SE of TIBC in pregnant normal sheep to be 56.1 $\pm$ 1.42  $\mu\text{mol/L}$  (table 3), which was higher than the mean of 50.9  $\mu\text{mol/L}$  reported by Kozat *et al.* (2006) in pregnant ewes. The higher values in this study perhaps it refers to the type of feeding program and breeding.

Moreover, serum UIBC was 25.7 $\pm$ 0.77  $\mu\text{mol/L}$  ranged from 4.1-56.1  $\mu\text{mol/L}$  in normal healthy sheep of our study. It was 24.1 $\pm$ 1.23  $\mu\text{mol/L}$  in males and 26.8  $\pm$ 0.98  $\mu\text{mol/L}$  in females with no significant differences between them. On the other hand the UIBC in IDA Awassi sheep was 69.1 $\pm$ 1.51 ranged 47.7-105.3  $\mu\text{mol/L}$ . It was 70.3 $\pm$  2.87 in males and 68.6 $\pm$  1.79  $\mu\text{mol/L}$  in females (tables 1 and 2). There was significant increase in UIBC values of IDA compared to normal sheep which may be due to the increase of transferrin binding site unbound with iron (Yamanishi *et al.* 1997). Furthermore, serum UIBC was significantly higher in pregnant ewes than in ram lambs and ewe lambs (table 3). However, the mean of serum UIBC in pregnant ewes of this study was 31.5 $\pm$ 1.34  $\mu\text{mol/L}$  and was higher than the mean (28.1  $\mu\text{mol/L}$ ) reported by Kozat *et al.* (2006). This is possibly due to the absence of scientific feeding program. Also, the TS% was found 52.9 $\pm$ 0.99 and ranged 28.1-90.6 in normal healthy sheep. It was 55.5 $\pm$ 1.7in males and 51.2  $\pm$ 1.1in females with no significant difference between them. The TS%

was 14.5 $\pm$ 0.66 in sheep affected with IDA. It was 12.8 $\pm$ 1.1and 15.2 $\pm$ 0.8 in males and females respectively (tables 1 and 2). Moreover it was significantly higher ( $P<0.05$ ) in normal compared to iron deficient sheep, this decreased TS% in IDA may indicate that the binding site of transferrin was unsaturated (Huebers *et al.*, 1987). In addition the TS% in normal was significantly higher in ram lambs and ewe lambs compared to pregnant and lactating ewes (table 3). However, the mean value of TS% in pregnant ewes of this work was (44.5 $\pm$ 1.3) and was lower than the mean documented by Kozat *et al.* (2006) which was 50. This difference may be attributed to the type of feed or living in hot area. In normal Awassi sheep, serum copper was 16.5 $\pm$ 0.37  $\mu\text{mol/L}$  ranged 7-33.8  $\mu\text{mol/L}$ , 18.8 $\pm$  0.62  $\mu\text{mol/L}$  in males and 14.9 $\pm$  0.42  $\mu\text{mol/L}$  in females. While serum copper in IDA sheep was 11.5 $\pm$ 0.48  $\mu\text{mol/L}$ , with significant decrease compared to normal Awassi sheep. It was 14.3 $\pm$ 1.04  $\mu\text{mol/L}$  in males and 10.3 $\pm$ 0.44  $\mu\text{mol/L}$  in females. There was a significant difference between males and females in both groups (tables 1 and 2). Moreover, ram lambs showed a significantly higher value compared to normal lactating ewes, while healthy pregnant ewes showed a significant decrease compared to all normal groups. Moreover serum copper in pregnant ewes showed no significant difference between normal and anemic (table 3). Many researchers studied or reported serum copper concentration, Aitken (2007) reported arrange of 9.4-23.6  $\mu\text{mol/L}$ , while Kaneko (2008) documented arrange of 9.13-25.2  $\mu\text{mol/L}$ , both ranges almost did agree with our results (more than 85% within these ranges). However, in the present study the serum copper in pregnant ewes was 11.7 $\pm$ 0.52  $\mu\text{mol/L}$  and ranged 7-21.1  $\mu\text{mol/L}$ . It was lower than the mean of 20.5  $\mu\text{mol/L}$  reported by Kozat *et al.* (2006), while it was close to the mean 10.9 $\pm$ 0.05 reported by Abdelrahman *et al.* (2006). Also, did agree with Khan *et al.* (2006), which recorded 7  $\mu\text{mol/L}$  as minimal value, as well as with Joseph *et al.* (2007), which stated serum copper level of 7.8  $\mu\text{mol/L}$  as safe in pregnant sheep. While Laven and Smith (2008) suggest that a range of 4.5-7.3  $\mu\text{mol/L}$  in serum copper may be used to define copper deficiency status in sheep. Moreover, Abdelrahman (2012) reported arrange of 16.9-18  $\mu\text{mol/L}$ , the present data did not agree with this narrow range. The difference in serum copper concentration of this study compared to other researchers may be attributed to one or more of the followings: absence of scientific feeding program, type of feed and breeding, living in hot areas or genetic factors.

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