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ESTABLISHMENT OF IRAQI CAMELS REFERENCE INTERVALS OF IRON STATUS PARAMETERS

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Abstract

Iron is necessary for many iron-based enzymes such as cytochromes, catalase, hydrogenase, as well as the essential oxygen storage molecule myoglobin and oxygen transporter hemoglobin. Reference interval (RI) calculation of iron status parameters in Iraqi camels have not been done yet. For this reason, the present study was designed. The current study carried out by blood sample collection from 122 healthy camels from Al-Kut & Al-Najaf provinces. According to the age, camels were divided in two groups, less than 2 years (<2 year) and more than 2 years (>2 year) for both sexes (103 male & 19 female). Results were analyzed using Roc analysis at 90% confidence interval and expressed as median and the lower and upper limit. Total serum iron, total iron binding capacity, unsaturated iron binding capacity, transferrin saturation and ferritin lower and upper limits were (66.25:104.81); (288.38:390.07); (194.02: 316.92); (19.55:33.14); (255.60: 521.68) respectively. There were no significant influences of sex and age less than two years on iron status parameters. Adult male and female showed significant differences in ferritin concentration, which was not observed in young male and female. In conclusion The present fixed data can play very important role to estimate the healthy state of Iraqi camels.

***Keywords*:** Reference interval, Iron status parameters, and Iraqi dromedary camels.

Introduction

In recent years, there has been a growing interest in breeding and producing of camels in different Arab countries as a result of their economic important and characteristics as tolerate the hard environmental conditions. Iraq is one of the most Arab countries, that have a high number of camels, but these numbers began to decrease in a way that need to investigate the main causes and find the necessary solutions to limit and prevent this animals from reaching the extinction (Montaser *et al*., 2018). Serum iron biochemical parameters are vital indicator of the animals' health status especially since there are many diseases associated with the level of iron in the blood or with iron status. Anemia due to iron status imbalance accompanying with many infectious diseases caused by bacterial, viral, parasitic agent or due to nutritional factors. There are researches traded the influence of different season, age, health status, lactation stage on hematological and biochemical profile in many countries that are considered breeding grounds for camels, in Saudi-Arabia, Sudan, Iran, and Bangladesh (Al- Busadah and Osman, 2000; Babeker *et al*., 2013; Islam *et al*., 2019). In Iraq, The hematological and biochemical references interval values of camels have been less documented and rare. However, there are studies established database of iron parameters values of male and female camels in Iraq (Al-Dhalimy and Al-Hadithy, 2016) and other hematological and some liver function enzymes (Ayoub *et al*., 2003; Alzubidi *et al*., 2019). But to date, no research findings is available on health camel serum Iron status parameters references interval values in Iraq. Therefore, this study was carried out to establishing reference intervals of Iraqi dromedary camels to be a useful guide for veterinarians to monitor the health status of animals along with investigation of the effect of age and gender on studied parameters.

Materials and Methods

Study design and samples collection

Animal included in the present study were (122) camels that obtained to slaughtering house in AL-Najaf city and from outdoor farming camels in Al-Kut city. A registry was opened for each animal that included confirmation of age and sex. Animals were distinguished according to age, in to two groups, less than two years (<2 year) and more than two years (>2 year), and to sex into Male & Female (Table 1).

Table 1: Origin samples collection and distribution according to sex and age.

Origin (City)	No. of samples according to age	No. of samples according to sex	Percent (%)
Al-Najaf City	≤ 2 year N= 59	Male = 55	45.0 %
		Female = 4	3.27 %
	≥ 2 year N=34	Male = 32	26.22 %
		Female = 2	1.63 %
Al-Kut City	≤ 2 year N=8	Male = 5	4.09 %
		Female = 3	2.45 %
	≥ 2 year N=21	Male = 11	9.0 %
		Female =10	8.19 %
	Total	122	100%

20 ml were collected from jugular vein of camels by using disposable syringes and serum was collected after centrifugation of blood samples 3500 round / minute for 15 minute and then serum sample was stored in freezer at -18˚C for used to determine Iron Status parameters.

Iron Status Measurements:

1. Total Serum Iron Concentration (TSI) µg/dl.

Serum iron concentration measured by spectrophotometric method using enzymatic assay kit (human, Germany). And calculated by equation one.

$$\text{Total serum Iron concentration (µg/dl)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ standard}} \times 100 \quad \ldots(1)$$

2. Total Iron Binding Capacity (TIBC) µg/dl:

Total iron binding capacity (TIBC) is amounting of iron that serum transferrin bind when excess of Fe^{+3}. A special

iron kit (human, Germany) was used for determination TIBC by colometeric method at 600 nm according to company recommendation, and calculated by the equation

TIBC = Iron binding to transferrin concentration × 3 ...(2)

3. Unsaturated Iron Binding Capacity (UIBC) µg /dl:

Unsaturated Iron Binding Capacity calculated from the following equation

UIBC (µg/dl) =Total serum Iron concentration (µg/dl)

-TIBC (µg/dl) ...(3)

4. Transferrin Saturation % (TfS):

Transferrin saturation calculated by equation 4:

$$TfS\ (\%) = \frac{\text{Total serum iron}}{\text{Total iron binding capacity}} \times 100 \quad ...(4)$$

5. Serum Ferritin Concentration µg/l:

Total serum ferritin measured routinely by using commercial Ferritin kit, provided by Spectrum Company, Egyptian. Calculated according to standard curve equideral equation.

Statistical analysis

Reference intervals values (RI) of parameters were estimated using reference value advisor (Geffre *et al*., 2011) at confidence interval (CI) 90% RI was calculated. The nested model was used to study the effect of sex and age on some parameters as shown below:

$Y_{ijkl} = \mu + Sij + A_{ik} + e_{ijkl}$

Y_{ijkl}= The observation

µ=Overall mean

S_j= The effect of J^{th} sex (male or female) within status i

A_{ik}= The effect of age (<2 or ≥2 year) within status i

e_{ijkl}= The random error

Data were analyzed using SAS software version 2010. Means were compared as pairwise comparison. Comparisons among proportions were performed using Chi-Square test, P≤ 0.05 is considered a significant. For non-normal TSI a box cox transformation used, for ferritin and transferring saturation non-parametric analysis, TIBC analyzed by standard method, finally UBIC by robust analysis

Results

Iron statue profiles of 122 healthy local breed Iraqi camels are listed in Table 2. Based on age and sex there were no significant differences in TSI, TIBC, UBIC, and TS. Adult male and female showed significant differences in ferritin concentration, which was not observed in young male and female. The highest level of ferritin recorded in healthy adult male (371.53) with a statistic significant (p<0.05).

Table 2: Estimated values of TSI, TIBC, UIBC, TS, and Ferritin in the health camels' males and females of different ages.

Age	Sex	TSI (µg/dl)	TIBC (µg/dl)	UIBC (µg/dl)	TS (%)	Ferritin (µg/L)
≤ 2 year N=67	Male N= 60	84.246±1.11 A	338.16±3.39 A	253.91±3.82 A	25.11±0.46 A	363.10±7.41 AB
	Female N= 7	86.687±2.76 A	335.71±7.43 A	249.02±9.20 A	25.95±1.20 A	343.71±11.08 AB
≥ 2 year N=55	Male N= 43	84.043±1.74 A	341.30±3.77 A	257.25±4.87 A	24.86±0.68 A	371.53±5.34 A
	Female N=12	79.985±2.83 A	339.19±8.62 A	259.21±11.08 A	23.96±1.46 A	328.16±14.58 B
LSD		**7.691**	**21.317**	**25.429**	**3.180**	**17.764**

The references intervals were listed in Table 3 the iron statues parameters using Roc analysis at 90% confidence interval in different analysis methods. Results revealed that some iron profile parameters are non- normally distributed (Figure-1). The data represent the median means with ±SE in addition to the lower and upper limit of RI 90 % confidence intervals, According to the type of data distribution different types of tests were used. Total serum iron data analyzed by converting data to Box-Cox conversion, to gate more accurate analysis. Results revealed a wide variation in the range. Also the Roc curves showed that data of the present study were abnormal distribution with the exception of TIBS & Ferritin.

Table 3: Reference intervals values and distribution of TSI, TIBC, UIBC, TS, and Ferritin in the health camels' males and females at 90% confidence interval.

Test	Mean±SD	LLRI	ULRI	90%CILL	90%CIUL	Method	Dist
TSI (µg/dl)	83.89±9.76	66.25	104.81	64.33-68.28	101.70-107.83	BCS	Non
TIBC (µg/dl)	339.23±25.57	288.38	390.07	282.45-294.5	383.75-396.32	S	normal
UIBC (µg/dl)	255.33±30.85	194.02	316.92	186.08-202.4	309.69-324.06	R	Non
TS (%)	24.96±4.47	19.55	33.14	18.88-19.26	31.23-33.68	NP	Non
Ferritin(µg/l)	351.62±35.41	255.60	521.68	281.25-298.6	457.17-493.46	NP	normal

LLRI= Low limit, ULRI= Upper limit,
CIUL= Confident interval, BCS=Box-Cox standard,
S=Standard, R= Robust,
NP=Non parametric Dist=Distribution

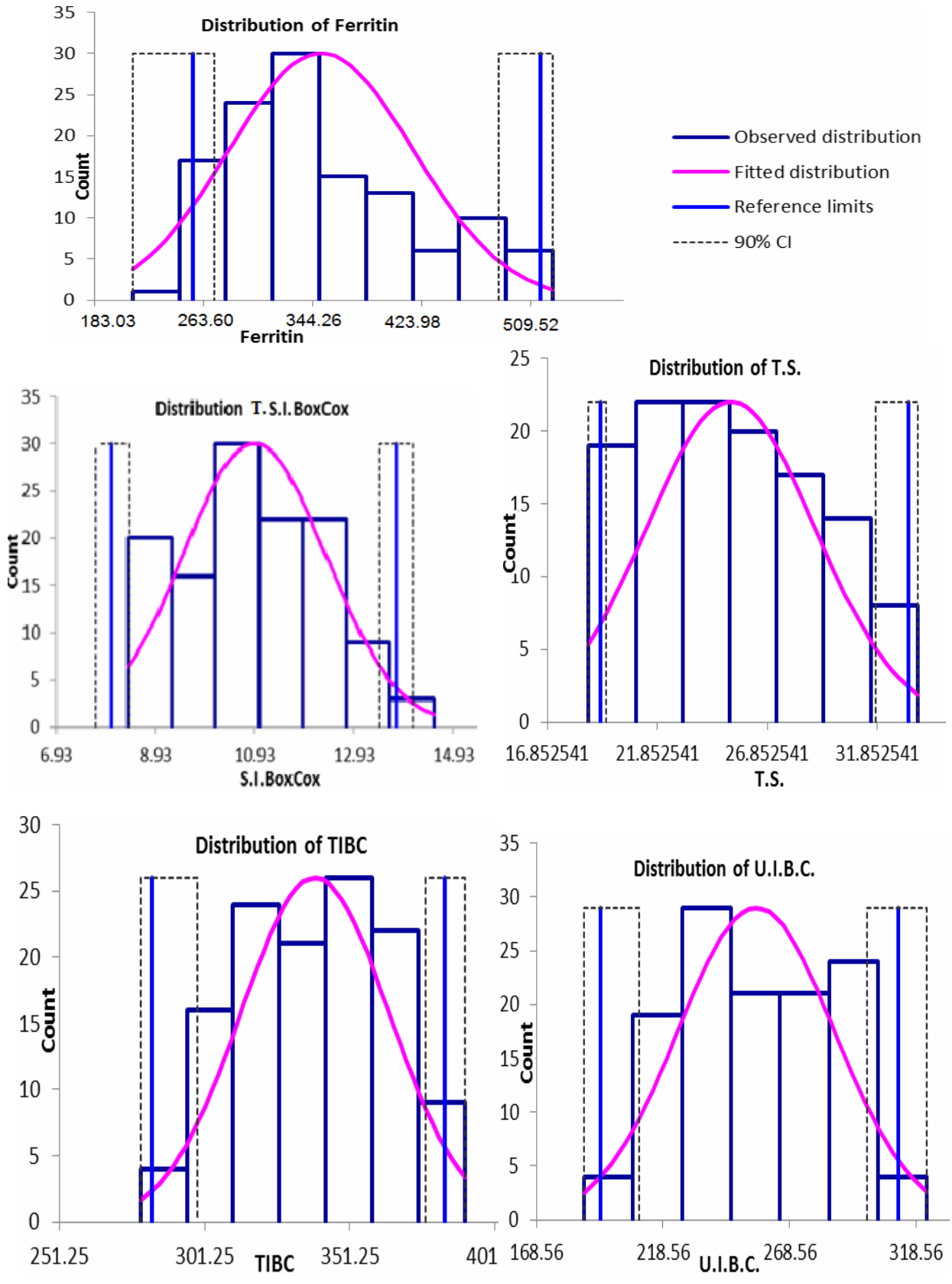

Fig. 1 : Distribution of observed (blue boxes) and fitted (purple) curves of iron status parameters for 122 clinically healthy Iraqi camels by using Roc analysis at 90% confidence interval.

Discussion

Stabilization of health and production standards for the camels attract the attention of researchers, such as hematological and liver function enzymes (Islam *et al.*, 2019; Waziri *et al.*, 2019), some heavy metals and fertility (Ali *et al.*, 2019), and cardia markers (Tharwat *et al.*, 2020). Regardless of the importance of reference interval (RI) for iron status of dromedary camels in Iraq, there are a few reports on this field which were published, and the estimations of values about the iron status in the other studied and are usually presented as the range or as the mean ± standard deviation. For this reason the current study in our knowledge represented the first work to establish the reference interval for Iraqi dromedary camels iron parameters, and assists the clinician to reach a definitive diagnosis. The present results determined the reference intervals for iron and other related parameters using

reference interval advisor estimated by parametric and nonparametric method tend to convert the non- normally distributed to normal or close to normal distribution. At the local level of Iraq the present results revealed that normal and healthy Iraqi camels had a median level of serum iron in a consistency with previous published data presented as mean and range of iron in Iraqi camels (Al-Dhalimy and Al-Hadithy, 2016). At regional level the present data agree with the results of (Al-Kasmi, 1989; Al-busadah, 2003; Waheed *et al*., 2018). Present study disagrees with the results obtained by (Hussein *et al*., 1997; Wernery *et al*., 2009). The statistical analysis of the present data revealed that neither age nor sex had significant interference with TSI, TIBS, UBIC and TS of local Iraqi camels. With the exception of Ferritin concentration, Sex affects on serum ferritin which increased significantly in adult male camels than female which is agree with (Mohammed, 2006; Faye *et al*., 2005; Eltohamy *et al*., 1986). Ferritin is the storage protein for iron, in case of excess iron ferritin bound to and storage it in different cell in particular hepatocytes and macrophages (Skjørringe *et al*., 2015). Females in general are suffering from iron deficiency may be due to the lactation and pregnancy periods, during this times the fetus consume a high level of iron in different form that led to decreased iron storage in pregnant female when compared with male. The established current reference intervals showed acceptable range between upper (ULRI) and lower limits (LLRI) for each parameter related with iron status (TSI, TIBC, UIBC, TS and Ferritin concentration) for the different parameters studied. This mentioned rang will be a standard reference to increase the precision diagnosis. In Iraq Similar results fixed in other farm animals done by Haydar and Al-Samarai, (2019) on Iraqi local horses. The results of iron status parameters showed that only TIBC & Ferritin are normally distributed, many researchers reported that the some parameters are most often not normally distributed in animals (Aros *et al*., 2017; Al-Samarai and Mohammad, 2017; Cywińska *et al*., 2015). Camels had lower of serum iron concentration than other farm animals. The animals requirement for microelements depends on variable factors some physiological (age, sex, breed, and production), environmental (seasonal weather and nutritional), and health conditions. Such results was noticed by (Al-Busadah, 2003; Osman and Al-Busadah, 2003; Khamis *et al*., 2011), where they attributed this to differences in breed, husbandry, environment and the major cause nutrition. In the camels iron homeostasis is well controlled process attributed to many factors including increasing capacity of absorption within limited time (Faye and Bengoumi, 2018). In this regard it could be postulated that intestinal mucosal epithelial cell shedding in camels is more prolonged which reduce iron loosing and keep stable storage. Red blood cells of camels characterized by their membrane protein "spectrin" (Ghoke *et al*., 2013), and phospholipids constituents (Al-Qarawi and Mousa 2004) responsible for the high resistance to osmotic pressure changes during dehydration- rehydration cycle with less deformability and no any hemolysis (Windberger *et al*., 2018) and has long survival half life time (Amin *et al*., 2007), this might be one of the causes for less iron demined in camels. Iron homeostasis dysregulation caused imbalance between erythrophgocytosis and erythropoiesis signs for anemia (Joao *et al*., 2020). Decrease in serum iron of copper deficiency camels resulted from a decrease in Fe transportation, and it might be a cause of decrease in Fe amount (Nazifi *et al*., 2009).

Conclusion

The present study perhaps it came to be the basis for determining the iron imbalance in camel, as which helps in determining health status and the values were presented as lower and upper limits Setting a reference values for the iron status in healthy Iraqi dromedary camels. Efficient concentration of serum Iron is an important fertile, health and productive factor in camels, affected by disease specially blood parasite disease. Iron supplement is recommended for adult females because of low storage ferritin.

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