

DYNAMICS OF SERUM BIOCHEMICAL ATTRIBUTES IN INDIGENOUS SIPLI SHEEP BREED KEPT UNDER INTENSIVE FARMING SYSTEM

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ABSTRACT

The present study is the first record of deducing age-wise and sex-wise normal reference intervals (RIs) for various serum biochemical attributes in apparently healthy Sipli sheep being reared under intensive farming system at Livestock Farm of the Faculty of Veterinary and Animal Sciences (FV&AS), The Islamia University of Bahawalpur (IUB). Blood samples were aseptically collected, serum was harvested and analyzed for serum chemistry analytes through semi-automatic chemistry analyzer using commercial kits. For the sake of analysis, groups were assigned as per age *i.e.* G1= up till 1 year (n= 41), G2= from 1 to 2 years (n= 46), G3= from 2 to 3 years (n= 43) and as per sex *i.e.* females (n= 79) and males (n= 51). All the overall mean values and RIs for all the studied attributes were within the reference ranges provided in earlier studies for sheep. Bilirubin, Alkaline Phosphatase (ALP) and creatinine were significantly ($P \leq 0.05$) lower in females as compared to those in males. However, uric acid was significantly ($P \leq 0.05$) higher for females 52.8 ± 2.6 mmols/L as compared to 44.5 ± 2.9 mmols/L for males. The remaining attributes (Alanine Transaminase, Aspartate Aminotransferase, Total Proteins, Albumin, Glucose, Low Density Lipoproteins, High Density Lipoproteins and Urea) did not differ -significantly ($P \geq 0.05$) between male and female sheep under study. For age-related results, only ALP, glucose and HDL were significantly ($P \leq 0.05$) different between the three studied age groups of Sipli sheep. The ALP and glucose were higher in G1 animals (up till 1 year) whereas HDL was lower in G2 animals (from 1 to 2 years) as compared to their counter-part groups 1 and 3. The data can be beneficial in diagnostic/prognostic purpose for veterinarians, academicians, researchers and all other stakeholders of sheep-rearing sector in Pakistan as well as for other indigenous sheep breeds being reared under similar climatic conditions throughout the world.

Keywords: Sipli sheep, Serum chemistry, Biochemical analytes

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Published first online November 15, 2023

Published final January 20, 2024

INTRODUCTION

Reference intervals (RIs) are vital component of laboratory diagnostic testing and clinical decision-making. These values are typical indicative of reference values from healthy populations of comparable individuals. Ideally, the values comprise of 95% of a healthy reference population (Friedrichs *et al.*, 2012). Globally, it has been endorsed that the RIs for a specific population in a specific geo-location cannot normally be implied for same species in another geo-location. Considering this variance, it is imperative that the RIs of a population may be determined for that specific area in order to enhance clinical decision-making.

Pakistan is endowed with numerous indigenous livestock breeds of cattle, sheep, goats and camels which have been reviewed earlier (Ali *et al.*, 2009; Farooq *et al.*, 2010). The indigenous sheep of Pakistan belonging to 17 different breeds probably originated from urial (*Ovis vignei*), the wild sheep of Afghanistan, Baluchistan and

Central Asia (Khan *et al.*, 2007). The livestock census of Pakistan, 2006 incorporated Lohi, Kajli, Thalli, Buchi, Bekerre, Harnai, Balochi, Bibrik, Damini, Waziri, Hasht Nagri, Balkhi, Kaghani, Rakhshani, Kooka, Kachii and Kail breeds of sheep, however, Sipli breed went missing (Livestock & Dairy Development Department, 2018). Sipli is a thin-tailed indigenous sheep breed of Pakistan with a relatively long tail. Small number of this breed (n=260) is being maintained at two institutional farms in South Punjab. It is a medium-sized sheep breed with an average body weight of 32.8kg for males and 29.2kg for females, and has a daily milk yield of 0.2-0.4 L (Khan *et al.*, 2007; Jaffar *et al.*, 2015). It has white body coat with white or light brown head/ears. Its head is medium sized and has a flat nose with ears reaching about 15 cm long (Figure 1). It is mostly reared for mutton and wool purposes by the nomadic herders of Bahawalpur, Bahawalnagar and Rahim-Yar-Khan- the three cities which lay in the middle of the Cholistan desert, (Southern Punjab) Pakistan. Scanty work on leptin

gene and some productive attributes has been reported from Pakistan (Qureshi *et al.*, 2015; Jaffar *et al.*, 2015) however, there is no study on blood chemistry of this indigenous sheep breed. Furthermore, the RIs for serum biochemical attributes published earlier for various sheep breeds globally cannot be used as a diagnostic/prognostic tool for other indigenous sheep owing to difference in breed and geo-location. Therefore, the present study is the first record of deducing age-wise and sex-wise normal reference intervals (RIs) for various serum biochemical attributes in apparently health Sipli breed of sheep (n= 130) kept under intensive farming system.

MATERIALS AND METHODS

Geo-location of the study: The blood sampling for this study was carried out at the Livestock Farm of the FV&AS, IUB, Pakistan whereas the laboratory protocol was conducted at the post-graduate lab Physiology, IUB. The facility is located on the outskirts of Cholistan desert of Pakistan which is located at latitudes 27°42' and 29°45' North and longitudes 69°52' and 75°24' East and at an altitude of 112m above the sea level. The climate of this area is arid, hot subtropical and monsoonal with the average annual rainfall of 180 mm. The mean annual temperature is 28.33°C, with the month of June being the hottest when the daily maximum temperature normally exceeds 45°C. Its geo-location and livestock pastoral systems have been reviewed extensively in earlier studies (Ali *et al.*, 2009).

Study animals: Apparently healthy Sipli breed of sheep (n= 130) being reared under intensive farming system at Livestock Farm of the university was incorporated in the study. The study was conducted from August to September, 2022 apropos the ethical approval provided by the Departmental Research Ethics Committee, Department of Physiology, The Islamia University of Bahawalpur, Pakistan vide PHYSIO-77/2023-18 dated 23rd of March, 2023. The animals were stall-fed, and the feeding included freshly cut/chopped seasonal fodder along with concentrate ration containing about 15 percent crude protein. In addition, maize silage and wheat straw were being offered on the need base. Fresh, clean drinking water was provided *ad libitum*. The data regarding productive/reproductive attributes, feeding regimen, breeding and health control measures was recorded properly. The weight and growth parameters for lambs were being recorded fortnightly on digital weighing scale in kilograms for further analysis and selection.

Blood collection and analyses: Sheep were restrained by trained personnel and blood (5 mL) was collected aseptically from the jugular vein of each animal in serum collecting vacutainers containing thixotropic gel for serum extraction (BD vacutainers®, Becton Dickinson,

USA). The collection method, timing, personnel and restraint were kept constant for least stress upon the study animals. Samples were transported to the laboratory in ice-packs for further analyses within 1 hour. Blood in serum tubes was allowed to clot for 30 minutes before serum extraction through centrifugation (2000G for 10 minutes). Samples were refrigerated at -20°C till further analyzed for serum chemistry attributes.

Serum chemistry attributes: Serum samples were thawed at room temperature and various serum chemistry attributes were analyzed through chemistry analyzer (Semi-auto Chemistry Analyzer RT-9600, China) using commercially available kits as per following detail:

- Uric acid, alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and creatinine by Human Diagnostica, Germany
- Albumin (ALB), low-density-lipoproteins (LDL), total protein (TP), glucose and high-density-lipoproteins (HDL) by Centronic GmbH, Germany
- Urea by TNMC Devices Ltd. Croatia

Statistical analysis: Statistical Package for Social Science (SPSS for Windows version 12, SPSS Inc., Chicago, IL, USA) was used for data analysis. For the sake of analysis, groups were assigned as per age keeping in view the data provided by the farm *i.e.* G1= up till 1 year (n= 41), G2= from 1 to 2 years (n= 46), G3= from 2 to 3 years (n= 43) and as per sex *i.e.* females (n= 79) and males (n= 51). Confirmation of this grouping was attained through k-clustering technique. Normality of attained data was tested for each parameter and for each group through visual inspection as well as through Shapiro-Wilk test. Considering the Gaussian nature of data, the difference between sex groups and age groups was deduced through independent t-test and ANOVA (followed by Duncan's post-hoc test), respectively. Significance was considered at $P \leq 0.05$. The overall, age-wise and sex-wise RIs for the studied chemistry attributes were determined keeping in view the guidelines provided by the American Society for Veterinary Clinical Pathology (ASVCP) (Friedrichs *et al.*, 2012). The mean (\pm SE), median, range and reference intervals (25th to 95th percentile) were accordingly deduced.

RESULTS

Over all mean (\pm SE) values and RIs for serum biochemical attributes in apparently healthy Sipli sheep (n= 130) of the present study are given in Table 1. All the values for all studied attributes were within the reference range provided in earlier studies for sheep.

Sex-wise results for the studied serum biochemistry attributes are given in Table 2. Bilirubin, ALP and creatinine were significantly ($P \leq 0.05$) lower in females as compared to that in males. However, uric acid was significantly ($P \leq 0.05$) higher for females being

Table 1: Overall mean (±SE), median, range, minimum, maximum, 25th to 95th percentile of reference interval (RI) and 95% confidence interval (CI) for serum biochemistry attributes in apparently healthy Sipli sheep (n= 130).

Attributes	Mean±SE	Median	(Min-Max)	Range	RI (25 th to 95 th)	95% CI
Alanine transaminase (U/L)	29.3±0.5	29.0	19-45	26.0	26-38	28.1- 30.5
Aspartate aminotransferase (U/L)	89.5±3.3	88.0	36-218	182.0	71-135.9	82.8- 96.1
Alkaline phosphatase (U/L)	160.7±9.7	137.5	56-411	355.0	89.7-354.5	141.4- 180.0
Total protein (g/L)	67.6±0.7	65.0	58-81	23.0	62.2-79.9	66.1- 69.1
Albumin (g/L)	27.7±0.3	28.0	21-34	13.0	25-32.9	27.0- 28.4
Glucose (mmol/L)	3.2±0.05	3.2	2.1-4.4	2.3	2.8-4.0	3.1- 3.3
High density lipoprotein (mmol/L)	0.8±0.01	0.9	0.1-1.1	1.0	0.7-1.0	0.8- 0.8
Low density lipoprotein (mmol/L)	1.8±0.04	1.9	0.8-2.4	1.6	1.6-2.3	1.7- 1.9
Creatinine (µmol/L)	55.0±1.4	51.5	35-99	64.0	43.2-76.9	52.0- 57.9
Urea (mmol/L)	5.1±0.2	4.4	2.9-11.8	8.9	4-10.1	4.7- 5.5
Uric Acid (mmol/L)	49.6±2.0	43.0	18-109	91.0	39-81	45.5- 53.6

Table 2: Overall mean (±SE), median, range, minimum, maximum, 25th to 95th percentile of reference interval (RI) and 95% confidence interval (CI) for serum biochemistry attributes as affected by sex in apparently healthy Sipli sheep (n= 130).

Groups	Mean±SE	Median	(Min-Max)	Range	RI (25 th to 95 th)	95% CI
Alanine Transaminase (U/L)						
Females (n= 79)	29.2±0.7	29	19-45	26	26-38	27.8-30.8
Males (n= 51)	29.5±0.9	29	21-39	18	26-40.8	27.3-31.3
Aspartate Aminotransferase (U/L)						
Females (n= 79)	85.4±3.1	82	36-218	182	69-133.5	81-99.2
Males (n= 51)	95.9±6.9	89	42-187	145	71-199.4	78.5-98.4
Alkaline Phosphatase (U/L)						
Females (n= 79)	141.4±9.7*	151	58-411	353	85-282.5	151.5-207.5
Males (n= 51)	191.1±18.6	124	56-281	225	103-405	110.4-151.6
Total Protein (g/L)						
Females (n= 79)	68.0±0.9	69	58-80	22	63-80	66.5-70.7
Males (n= 51)	66.9±1.2	64	60-81	21	62-79.4	63.9-68.0
Albumin (g/L)						
Females (n= 79)	27.5±0.5	29	21-34	13	25-32.5	27.0-28.9
Males (n= 51)	28.0±0.4	27	21-34	13	26-33.4	26.1-28.4
Glucose (mmol/L)						
Females (n= 79)	3.1±0.06	3.2	2.3-4.4	2.1	2.9-4.0	3.0-3.3
Males (n= 51)	3.2±0.08	3.2	2.1-4.1	2	2.8-4.2	3.0-3.3
High Density Lipoproteins (mmol/L)						
Females (n= 79)	0.8±0.02	0.9	0.1-1.1	1	0.7-1.0	0.7-0.8
Males (n= 51)	0.8±0.02	0.9	0.6-1.1	0.5	0.7-1.0	0.7-0.9
Low density Lipoproteins (mmol/L)						
Females (n= 79)	1.9±0.05	1.8	0.8-2.4	1.6	1.7-2.4	1.6-1.8
Males (n= 51)	1.7±0.07	1.9	1.1-2.4	1.3	1.4-2.3	1.8-2.0
Creatinine (µmol/L)						
Females (n= 79)	52.6±1.8*	52	38-99	61	42-75.5	52.1-60.2
Males (n= 51)	58.7±2.2	51	35-70	35	49-85.8	48.9-57.3
Urea (mmol/L)						
Females (n= 79)	5.0±0.2	4.5	2.9-11.8	8.9	4-9.9	4.7-6.0
Males (n= 51)	5.3±0.4	4.3	3.5-9.9	6.4	4-10.8	4.2-5.3
Uric Acid (mmol/L)						
Females (n= 79)	52.8±2.6*	42	18-109	91	40-91	43.6-54.7
Males (n= 51)	44.5±2.9	46	18-81	63	36-76.2	44.3-56.1

*significant at P≤0.05 within the column between females and males

52.8±2.6mmols/L as compared to 44.5±2.9mmols/L for males. The remaining attributes (ALT, AST, TP, Albumin, glucose, LDL, HDL and Urea) were statistically non-significant (P≥0.05) within male and female sheep under study.

For age-related results, only ALP, glucose and HDL were significantly (P≤0.05) different between the three studied age groups of Sipli sheep (Table 3). The ALP and glucose were higher in G1 animals (up till 1 year) whereas HDL was lower in G2 animals (from 1 to 2 years) as compared to the counter-part groups G1 and G3.

Table 3: Overall mean (±SE), median, range, minimum, maximum, 25th to 95th percentile of reference interval (RI) and 95% confidence interval (CI) for serum biochemistry attributes as affected by age in apparently healthy Sipli sheep (n= 130).

Groups	Mean±SE	Median	(Min-Max)	Range	RI (25 th to 95 th)	95% CI
Alanine Transaminase (U/L)						
G1 (n= 41)	31±1.1 ^a	30	22-45	23	27- 44.3	28.5-33.4
G2 (n= 46)	28±0.8 ^a	27.5	19-38	19	24.2- 37.1	26.3-29.6
G3 (n= 43)	29.9±1.1 ^a	30	21-39	18	26- 38.8	27.6-32.2
Aspartate Aminotransferase (U/L)						
G1 (n= 41)	85.4±6.6 ^a	82	36-158	122	69.5- 155.8	71.5-99.3
G2 (n= 46)	94.6±5.7 ^a	91.5	42-218	176	71- 191.6	83.0-106.2
G3 (n= 43)	85.2±19.7 ^a	85	54-126	72	72- 124.2	76.6-93.7
Alkaline Phosphatase (U/L)						
G1 (n= 41)	213.1±21.4 ^b	185	60-411	351	141- 410	168.4-257.9
G2 (n= 46)	151.9±12.6 ^a	137.5	56-357	301	89- 292.4	126.2-177.5
G3 (n= 43)	126.6±14.9 ^a	116	61-387	326	76- 352.2	95.5-157.6
Total Protein (g/L)						
G1 (n= 41)	65.6±1.1 ^a	63	59-78	19	62- 77.6	63.2-67.9
G2 (n= 46)	67.8±1.1 ^a	64.5	58-81	23	62.2- 80.1	65.3-70.2
G3 (n= 43)	69.1±1.4 ^a	68	60-79	19	63- 79	66.1-72.1
Albumin (g/L)						
G1 (n= 41)	27.8±0.6 ^a	29	21-33	12	26- 32.8	26.4-29.2
G2 (n= 46)	27.9±0.5 ^a	28	23-34	11	25- 33.1	26.8-28.9
G3 (n= 43)	27.3±0.7 ^a	27	21-34	13	25- 33.6	25.7-28.8
Glucose (mmol/L)						
G1 (n= 41)	3.3±0.09 ^b	3.2	2.6-4.4	1.8	3.2- 4.36	3.1-3.5
G2 (n= 46)	3.2±0.08 ^{ab}	3.2	2.1-4.1	2	2.8- 4.1	3.06-3.4
G3 (n= 43)	3.0±0.07 ^a	3	2.3-4.1	1.8	2.8- 4.0	2.8-3.2
High Density Lipoproteins (mmol/L)						
G1 (n= 41)	0.8±0.03 ^{ab}	0.8	0.5-1.1	0.6	0.7- 1.0	0.7-0.9
G2 (n= 46)	0.8±0.03 ^a	0.8	0.1-1.1	1	0.7- 1.0	0.7-0.8
G3 (n= 43)	0.9±0.02 ^b	0.9	0.6-1.1	0.5	0.9- 1.0	0.8-0.9
Low density Lipoproteins (mmol/L)						
G1 (n= 41)	1.9±0.07 ^a	1.9	1.2-2.4	1.2	1.7- 2.4	1.7-2.0
G2 (n= 46)	1.7±0.07 ^a	1.8	0.8-2.4	1.6	1.4- 2.4	1.5-1.8
G3 (n= 43)	1.9±0.08 ^a	2.1	1-2.3	1.3	1.8- 2.3	1.7-2.0
Creatinine (µmol/L)						
G1 (n= 41)	49.7±2.1 ^a	49	35-69	34	41.5- 68.4	45.2-54.2
G2 (n= 46)	56.9±2.1 ^a	55	38-96	58	46- 81.5	52.5-61.3
G3 (n= 43)	56.9±3.1 ^a	49	40-99	59	43- 94.6	50.3-63.4
Urea (mmol/L)						
G1 (n= 41)	5.3±0.5 ^a	4.3	2.9-10.2	7.3	3.9- 10.2	4.2-6.4
G2 (n= 46)	5.1±0.3 ^a	4.4	3.5-10.2	6.7	4- 9.9	4.5-5.7
G3 (n= 43)	4.9±0.3 ^a	4.5	3.3-11.8	8.5	4- 11.3	4.1-5.7
Uric Acid (mmol/L)						
G1 (n= 41)	49.8±3.0 ^a	54	18-81	63	41- 80.2	43.4-56.2
G2 (n= 46)	50.9±3.3 ^a	44	18-109	91	39- 93.7	44.1-57.8
G3 (n= 43)	47.2±3.7 ^a	42	18-91	73	37- 87.4	39.4-55.0

^{a,b}significant at P≤0.05 within columns for age-based groups; G1= up till 1 year, G2= from 1 to 2 years, G3= from 2 to 3 years



Figure 1. An elite specimen of Sipli male sheep being reared at the Livestock Farm of the university

DISCUSSION

The present study is the first record of indigenous Sipli breed of sheep regarding their overall, sex-wise and age-wise RIs for various serum biochemical attributes. The biochemical attributes *i.e.* bilirubin, ALT, AST, ALP, TP, Glucose, ALB, HDL, LDL, creatinine, urea and uric acid are presently in vogue for attaining a diagnostic/prognostic approach towards animal health. As the work has been conducted on an indigenous sheep breed (Sipli) of Pakistan, hence the results can be implied for other indigenous and native sheep breeds being reared in the global settings having similar geo-location as to Pakistan specifically those located in Asia and Africa. Though the RIs attained in the present study for all the biochemistry analytes were within the normal range provided earlier for ovine, however variation was noticed for overall mean results.

The ALT, AST and ALP are known as the ‘liver panel’ or the Liver Function Test (LFT) enzymes which are measured as an indicative of hepatic pathophysiology. These enzymes, apart from liver, are normally localized in the skeletal muscles, RBCs, kidneys and cardiac muscles of the body (Britti *et al.*, 2005). The value of AST (89.5 ± 3.3 U/L) in the present study is higher than that of 79.2 ± 16.3 U/L reported for British Suffolk breed of sheep (de Souza *et al.*, 2020). For free-ranging desert Bighorn sheep, a RI of 71-318U/L has been reported for serum AST as compared to the RIs of 71.0-136.0U/L for the present study (Borjesson *et al.*, 2000). The overall mean value of ALT in present study (29.3 ± 0.5 U/L) is in line with a previous work from Pakistan conducted on sheep which reported 30.4 ± 18.0 U/L. This study also had revealed that goats have significantly higher values for ALT as compared to that in sheep (Kiran *et al.*, 2012). Similarly, it is also in concordance with the work conducted on wild sheep of Iran which have reported a close value of 29.1 ± 3.2 U/L (Mostaghni *et al.*, 2005). Lower values of ALT (10.0 ± 1.0 U/L) have been reported

for African sheep (Soch *et al.*, 2011). The comparisons acknowledge the prior reports that the three LFT enzymes exist within a wide range for sheep breeds across different geological distribution which could be due to difference in breed, feeding regimen, husbandry practices or environment (Alonso *et al.*, 1997; Borjesson *et al.*, 2000; de Souza *et al.*, 2020; Lepherd *et al.*, 2009).

The value of ALP in the present study was lower for females (141.4 ± 9.7 U/L) as compared to that in males (191.1 ± 18.6 U/L). Similarly, G1 (up till 1 year) had a higher value (213.1 ± 21.4 U/L) as compared to that in other two study groups. However, the ALT and AST were not different within the age-based and sex-based groups of the study. These results are in line with various previous studies which have shown a positive correlation between age and LFT enzymes in various species. It has also been ascertained that after birth, there is a gradual increase in these enzymes which is due to a gradual increase in mass and activity of the muscles. Furthermore, increased body mass leads to enhance hepatic activity and hence an increased production of these enzymes (de Souza *et al.*, 2020; Mohri *et al.*, 2007; Santo da Cruz *et al.*, 2017).

Creatinine, urea and uric acid (renal function test parameters) were included in the present study and the RIs were within the ranges presented earlier for sheep. However, variations were noticed for overall mean values. Our overall mean value for creatinine in the present study (55.0 ± 1.42 μmol/L) is lower than earlier reported higher values for British Suffolk sheep (62.0 ± 5.2 μmol/L) (de Souza *et al.*, 2020), desert Bighorn sheep (176.0 ± 18.2 μmol/L) (Borjesson *et al.*, 2000) and for South African Dorper sheep (97.2 ± 4.2 μmol/L) (Santo da Cruz *et al.*, 2017). Mean value for urea in present study (5.1 ± 0.2 mmol/L or 91.2mg/dL) is higher than most of the earlier reported values for British Suffolk sheep (54.2 ± 1.5 mg/dL) (de Souza *et al.*, 2020), indigenous Iraqi sheep (41.7 ± 9.8 mg/dL) (Oramari *et al.*, 2014), South African Dorper sheep (38.8 ± 7.2 mg/dL) (Santo da Cruz *et*

al., 2017). These differences could plausibly be attributed to difference in geography, dietary patterns and breeds or can be considered as an adaptive physiological mechanism.

In the present study, creatinine was significantly lower in females ($52.6 \pm 1.8 \mu\text{mol/L}$) as compared to males ($58.7 \pm 2.2 \mu\text{mol/L}$) whereas urea and uric acid were non-significantly different within both sex groups. Age-based groups had no difference in these attributes for the present study. Group-based results of the present study are not in line with previous studies on sheep and as well as on other ruminants which have indicated that both the age and sex have significant effect on creatinine, urea and uric acid. In all the ruminants, creatinine is considered as a main filtration biomarker of glomerular filtration and is not affected by exogenous factors (Mohri *et al.*, 2007). Previous studies have reported that with the advancing age, the ruminants tend to have higher serum creatinine levels which is due to increased musculature. This increased musculature, in turn, increases the creatinine storages and reservoirs in various organs of the body (Santo da Cruz *et al.*, 2017). Furthermore, a decreased thyroxine level in growing animals could also be the cause, as elaborated earlier. Some reports have mentioned very high values of creatinine in first few days of birth, with a subsequent decrease in growing age which may be due to enhanced renal function with the growing age (Allaoua *et al.*, 2021; Borjesson *et al.*, 2000; de Souza *et al.*, 2020; Frye *et al.*, 2022).

The overall mean values of the present study for TP ($67.6 \pm 0.7 \text{g/L}$) and ALB ($27.7 \pm 0.3 \text{g/L}$) as well as their RIs are in concordance to the previous studies conducted on indigenous Iraqi sheep (Oramari *et al.*, 2014). However, higher values for TP ($58.0 \pm 0.7 \text{g/L}$) and ALB ($34.8 \pm 0.3 \text{g/L}$) have been recorded for British Suffolk sheep (de Souza *et al.*, 2020). In our results, no significant difference was noticed for these parameters in age-based or sex-based groups. This is not in line with previous studies on sheep as well as on other ruminants which have presented strong correlation of age and sex with TP and ALP.

Regarding glucose, the overall value for present study ($3.2 \pm 0.05 \text{mmol/L}$ or 57.6mg/dL) is in line with most of the previous work reported for various sheep breeds. A comparable value of $60.3 \pm 15.2 \text{mg/dL}$ has been reported for indigenous Iraqi Awassi sheep breed (Badawi and AL-Hadithy, 2014). However, a higher RI of $80.2\text{--}86.2 \text{mg/dL}$ has been reported for European Suffolk sheep (de Souza *et al.*, 2020). Difference in breed and geo-location of the study could be a few factors responsible for these differences.

Sex had no effect on glucose level in our study though the G1 animals (up till 1 year) had higher glucose level of $3.3 \pm 0.09 \text{mmol/L}$ as compared to other two study groups. These results are in line with previous works conducted both for indigenous Asian and European sheep

breeds which have elaborated that glucose levels are higher from birth till about 60 days and then they show a sharp deflection (Badawi and AL-Hadithy, 2014; Borjesson *et al.*, 2000; Britti *et al.*, 2005; de Souza *et al.*, 2020). Higher glucose level in early life of sheep has been deputed towards intake of milk/colostrum, gradual growth in liver and allied hepatic functions, and enhanced enzymatic activities (Britti *et al.*, 2005; de Souza *et al.*, 2020).

In a nutshell, the present study had incorporated serum biochemical attributes which are in vogue for diagnostic/prognostic purposes in sheep. And all the studied attributes were within the normal reference ranges of sheep and are affected by age and sex. The minor deflections in the RIs may be due to immunological, nutritional or adaptation-related phenomena. It is recommended that interpretation of tests may be based on specific RIs for the animals in that specific geo-location. Similar studies with higher sample size and animal number on hematological attributes are recommended for future.

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