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Blood Profile in Normal One Humped Dromedary (Camelus Dromedarius) Camels in Libya. Part 3: Effect of Sex Variation on Biochemical and Haematological Blood Profile

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Abstract

As little is known about the blood profile of camels in Libya, this article is the third of a 4-part series describing the biochemical and haematological blood profile in Libyan camels. In part 1 of these manuscripts, the overall blood biochemical and haematological mean values of camels in Libya were determined, parts 2-4 evaluate the effects of breed, gender and age respectively on these values. Blood samples were collected from 24 male and 42 female apparently healthy camels and the levels of enzymes, metabolites, electrolytes and haematological indices were measured. The blood of the male camels showed higher values of aspartate aminotransferase (AST), Lactate dehydrogenase (LDH), Amylase (AMS), total proteins, globulin and Phosphorus (Ph), than the

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female camels which showed higher values of glucose, Albumin/Globulin (A/G) ratio, urea, Iron (Fe), Calcium (Ca), Packed Cell volume (PCV), Haemoglobin (Hb), erythrocyte osmotic fragility, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), neutrophil and monocyte numbers. This study shows significant sex differences between male and female Libyan camels in many haematological and biochemical analytes.

Keywords: Gender; camel breeds; blood profile; biochemistry; haematology; Libya.

1. Introduction

Gender is one of the endogenous parameters that has an influence on many haematological and biochemical blood parameters in human as well as animals [1]. This physiological factor, in addition to the reproductive cycle, age and season, should be considered in order to ensure accurate interpretation of the blood parameters, clinical diagnosis and prognosis in domestic animals [2, 3]. The effect of sex on blood parameters has been studied in many animal species such as horses [4-9], donkeys [8], cattles [10-14], sheep [3, 15], goats [1, 16-21], dogs [22-24], cats [25, 26], rabbits [27-29], deers [30], primates [31-35], rats [36, 37], chickens [38-40], ducks [41], pigeons [42], guinea fowls [43], doves [44] and bats [45]. Literature reports show variable outcomes when the gender effect on blood parameters was examined between or within animal species. Higher values in males than females were documented in blood parameters relating to albumin, cholesterol, creatinine, bilirubin, total proteins, AST, Ca, Na, Ph, MCHC and urea in horses [5, 7, 9], erythrocyte osmotic fragility, MCH and MCHC in cattles [13, 14], glucose and ALP in sheep [3, 15], ALP, PCV, WBC, lymphocytes and fibrinogen in goats [2, 16-20], PCV, Hb and RBC values in rabbits [27, 28], RBC counts, Hb, PCV, creatinine, total protein and globulin in monkeys [33-35], RBC, PCV, MCV values in chicken [38, 39] and total leukocyte in African giant rats [36]. On the other hand, higher values in females than males were documented in blood parameters relating to uric acid, HDL-cholesterol, Creatine Kinase (CK), Ca, Mg, Hb, PCV and WBC count in horses [4, 5, 9], RBC counts, PCV, MCV and neutrophils proportion in cattles [11, 13], Hb and PCV, Na and Cl in sheep [15], cholesterol, RBC and WBC counts, Hb, PCV, neutrophils proportions in goats [16, 17, 19], erythrocyte osmotic fragility in dogs and bats [22, 45], Hb and WBC counts in rabbits [27, 29], total proteins in guinea fowls [43] and total proteins, MCHC and WBC counts in chicken [38-40]. Other reports presented similar effects in males and females in parameters relating to WBC counts in horses [6], Hb and WBC counts in cattles [12, 13], total proteins in sheep [3], glucose, albumin, globulin, cholesterol, ALT, AST, LDH, Hb and PCV in goats [2, 17, 18, 21], triglycerides, cholesterol, lipoproteins, urea, creatinine, bilirubin, ALT, AST, CK, LDH, AMY, PCV, RBC counts and erythrocytic indices in African fruit bat [44, 45], Nigerian laughing doves [44], ducks [41], pigeons and peafowls [42], total protein in ducks and pigeons [46, 47]. The differences in the values of blood parameters observed between the male and female animals were attributed in literature to many reasons such as the effect of androgen which activates erythropoiesis by stimulating erythropoietin production and thus increasing the number of circulating WBC, RBCs, PCV and Hb concentration in males [5, 48, 49], the effect of sex hormones in males which elevate the transaminases (ALT and AST) levels involved in storage of the excess fat into the intra-abdominal and perivascular cells, skeletal muscles and liver instead of subcutaneous tissues [50], the cholesterol lowering effects of testosterone in males [51], the higher muscular mass and activity that raise creatinine , ALP and CK levels in males [19] and the capture stress and catecholamine release during blood

sampling which alter glucose, muscle and liver associated enzymes concentration in both sexes [15, 52]. The overall blood profile's mean of sixty six Libyan camels was recorded in the first part of this series [53] and the effect of breed variation on the measured biochemical and haematological blood parameters in the participants three selected Libyan breeds was evaluated in the second part [54]. To the best of the authors' knowledge, there is no literature report the influence of sex on haematological or biochemical parameters in Libyan camels. Therefore, the blood profile's data that was generated in the first part of this series was subdivided in this third part and the effect of sex variation on the measured blood parameters was investigated and compared with similar studies performed elsewhere.

2. Materials and Methods

2.1 Animals

Camels were chosen randomly and based on their availability from three different breeds, Fakhreya, Sirtaweya and Mahari breeds, with different ages and of both sexes with a total of sixty six apparently healthy camels. Twenty four camels were males and forty two camels were females.

2.2 Blood collection

Blood samples were collected in the summer time of the year. Thirteen millilitre of blood were collected from the jugular vein of each animal by disposable plastic syringe and a 19G needle. Three millilitre of blood were distributed into EDTA anti coagulant containing tubes for haematological analysis while the remained ten millilitre of blood were distributed into clean dry plain tubes for serum analysis. All blood samples were transferred on ice to laboratory at the Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya. The blood allowed to clot and after centrifugation at 5000rpm for 15 min, the serum samples were aliquoted in dry clean Eppendorf capped tubes and stored at -80º C for later analysis.

2.3 Biochemical analysis

The serum activity of aspartate aminotransferase (AST, L-aspartate/2-oxoglutarate as a substrate), alanine aminotransferase (ALT, L-alanine/2-oxoglutarate as a substrate), lactate dehydrogenase (LDH, Pyruvate/NADH+H^+ as a substrate), alkaline phosphatase (ALP, p-nitrophenylphosphate as a substrate), gamma glutamyl transferase (GGT, Gulpa Carboxy/glycyglycine as a substrate), amylase (AMS, 2-chloro-4-nitrophenyl α-D-maltotriose as a substrate) and the concentration of glucose (glucose oxidase method, GOD-PAP), cholesterol (cholesterol oxidase method, CHOD-PAP), cholesterol-High Density Lipoprotein (HDL, cholesterol oxidase method after precipitation by phosphotungstic acid/magnesium chloride, CHOD-PAP), triglyceride (glycerol-3-phosphate oxidase method, GPO-PAP), urea (Berthelot modified method), creatinine (kinetic test without deproteinization), total protein (biuret method), albumin (bromocresol green method), calcium (Ca, O-cresolphtaleine method), inorganic phosphorus (Ph, ammonium molybdate method), magnesium (Mg, calmagite method) and iron (Fe, ferrozine method) were measured by commercial kits (Biomaghreb, Ariana, Tunisia) and the values were calculated according to the manufacturer instructions using Jenway spectrophotometer, Model 6500 (Bibby Scientific Ltd, Stone, Staffordshire, United Kingdom). Sodium (Na) and potassium (K) were

measured using EasyLyte analyser that uses ion selective electrode technology. Globulin levels were calculated by subtraction of albumin content from the total protein value, cholesterol-Very Low Density Lipoprotein (VLDL) level was calculated by dividing triglyceride level on 5 while cholesterol- Low Density Lipoprotein (LDL) level was calculated by subtraction of the cholesterol-VLDL and cholesterol-HDL from the total cholesterol value.

2.4 Haematological analysis

The EDTA- anti coagulated blood was used to determine the haemoglobin concentration (Hb, g/dl), packed-cell volume (%), Fragility (% of haemolysis), Erythrocyte sedimentation rate (ESR, mm/hr), counts of red blood cells (RBC, $x10^6/mm^3$) and white blood cells (WBC, $x10^3/mm^3$). Haemoglobin concentration was determined following Sahli's method [55]. Packed–cell volume was estimated by haematocrit capillary tube and centrifuged at 600 *g* for 20 minutes. Haematocrit value was read and recorded according to Schalm and his colleagues [56]. Red blood cells and white blood cells were counted using haemocytometer and counted at x40 objective of phase contrast microscope according to Schalm and his colleagues [56]. The haematological indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were calculated from the erythrocytic series values. The differential cell count was enumerated on slides with Giemsa stain and performed counting a minimum of 100 cells under a light microscope according to Schalm and his colleagues [56]. Erythrocyte sedimentation rate (ESR) was determined by Westergren method according to Bull and his colleagues [57]. Erythrocyte osmotic fragility was determined according to Benson and Swallen [58].

2.5 Statistical analysis

Results are expressed as mean ± SEM. Data were analyzed using GraphPad Prism statistical software (version 6.0b; GraphPad Software Inc, La Jolla, CA, USA). Analysis of data between groups was performed using Mann Whitney test and statistical significance between groups was accepted at $p < 0.05$.

3. Results

The serum enzyme activities of ALT, AST, ALP, LDH, GGT and AMS measured in the serum of the camels involved in this study are shown in table 1. The AST, LDH and AMS activities were higher in the serum of male camels than the female ones. ALT, ALP and GGT enzymes did not show significantly different activities in the serum of two camel groups.

The mean ± SEM concentrations of glucose, total proteins, albumin, globulin, urea, creatinine, triglycerides, cholesterol and lipoproteins measured in the serum of the camels involved in this study are shown in table 2. The total proteins and globulin values were significantly higher in the serum of male camels than the female ones while the glucose, urea and A/G values were higher in the serum of female camels when compared to the male ones. Albumin, creatinine, triglycerides, total cholesterol and lipoproteins levels did not show significant differences between the two camels groups.

Table 1: Mean ± S.E. of activity of ALT, AST, ALP, LDH, GGT and AMS enzymes in the serum of males (no=24) and females (no=42) Libyan camels

Parameter	Unit	Males	Females
ALT	UL^{-1}	5.34±1.02a	5.39±0.79a
AST	UL^{-1}	16.39 ±2.93a	9.42±1.50b
ALP	UL^{-1}	4.57±0.97a	4.08±0.52a
LDH	UL^{-1}	53.70±13.31a	22.68±4.51b
GGT	UL^{-1}	1.82±0.24a	1.76±0.15a
AMS	UL^{-1}	3.23±0.56a	0.95±0.22b

Values were analysed using Mann Whitney test and values with different letters in the same row are significantly different with $p \leq 0.05$

Table 2: The Mean ± SEM concentration of glucose, total proteins, albumin, globulin, urea, creatinine, triglycerides, cholesterol and lipoproteins in the serum of males (no=24) and females (no=42) Libyan camels

Parameter	Unit	Males	Females
Glucose	mg dl^{-1}	87.83±9.95a	125.5±5.21b
Total proteins	g l^{-1}	54.67±1.36a	48.87±1.09b
Albumin	g l^{-1}	30.80±1.38a	30.45±0.61a
Globulin	g l^{-1}	23.87±1.70a	18.42±0.74b
A/G	g l^{-1}	1.49±0.14a	1.81±0.12b
Urea	mg dl^{-1}	36.35±1.93a	47.29±1.61b
Creatinine	mg dl^{-1}	1.52±0.04a	1.48±0.03a
Triglycerides	mg dl^{-1}	29.41±2.00a	32.86±2.60a
Total cholesterol	mg dl^{-1}	34.81±2.88a	37.29±2.15a
HDL-cholesterol	mg dl^{-1}	17.84±2.14a	14.81±1.49a
LDL-cholesterol	mg dl^{-1}	11.09±3.38a	15.91±2.42a
VLDL-cholesterol	mg dl^{-1}	5.88±0.40a	6.57±0.52a

Values were analysed using Mann Whitney test and values with different letters in the same row are significantly different with $p \leq 0.05$

The mean ± SEM concentrations Na, K, Ph, Ca, Mg and Fe measured in the serum of the camels involved in this study are shown in table 3. The values of Ph were significantly higher in the serum of the male camels than the female ones in contrast to the Fe and Ca levels which were higher in the serum of the female camels when compared to the male ones. The Na, K and Mg levels did not show significantly different values between the two camel groups.

Table 3: The Mean ± SEM concentration of Na, K, Ph, Fe, Ca and Mg in the serum of males (no=24) and females (no=42) Libyan camels

Parameter	Unit	Males	Females
Na	mmol/l	146.6±1.51a	149.5±0.67a
K	mmol/l	5.11±0.16a	4.90±0.12a
Ph	mg dl^{-1}	6.56±0.32a	4.42±0.26b
Fe	mg l^{-1}	0.41±0.16a	1.08±0.14b
Ca	mg dl^{-}	9.56±0.18a	10.05±0.08b
Mg	mg dl^{-1}	2.61±0.10a	2.45±0.06a

Values were analysed using Mann Whitney test and values with different letters in the same row are significantly different with $p \leq 0.05$

The mean ± SEM values of various haematological parameters are shown in tables 4 and 5. The sera of the female camels showed significantly higher values of haemoglobin, PCV, erythrocyte osmotic fragility, MCV, MCH and higher numbers of neutrophils and monocytes than the male camels. No significant differences were observed between the two camel groups relating to the values of ESR, MCHC, counts of RBC and WBC, and the number of lymphocytes, eosinophils and basophils.

Table 4: Mean ± S.E. of red blood cell values in the blood of males (no=24) and females (no=42) Libyan camels

Parameter	Unit	Males	Females
PCV	%	29.71±1.67a	35.62±1.15b
Hb	g/dl	11.00±0.41a	13.44±0.27b
Fragility	%	0.73±0.03a	0.80±0.00b
ESR	mm/hr	36.18±6.53a	27.87±2.85a
RBC count	10^6/ mm^3	12.27±0.89a	11.52±0.25a
MCV	fL	25.45±1.37a	31.41±1.11b
MCH	pg	9.68±0.61a	11.84±0.30b
MCHC	g/dl	39.76±2.76a	39.16±1.32a

Values were analysed using Mann Whitney test and values with different letters in the same row are significantly different with $p \leq 0.05$

Table 5: Mean ± S.E. of white blood cell values in the blood of males (no=24) and females (no=42) Libyan camels

Parameter	Unit	Males	Females
Total WBC count	10^3/ mm^3	10.45±0.86a	11.28±0.61a
Lymphocytes	10^3/ mm^3	7.52±0.77a	6.27±0.40a
Neutrophils	10^3/ mm^3	1.85±0.15a	3.60±0.31b
Monocytes	10^3/ mm^3	0.95±0.11a	1.25±0.09b
Eosinophils	10^3/ mm^3	0.03±0.01a	0.03±0.00a
Basophils	10^3/ml	0.03±0.01a	0.03±0.00a

Values were analysed using Mann Whitney test and values with different letters in the same row are significantly different with $p \leq 0.05$

4. Discussion

In general, the serum enzyme activities reported in the two camel groups of this study was lower than the normal ranges of ALT (6-25U/l), AST (37-131U/l), ALP (32-110U/l), LDH (337-2620U/l), GGT (8-28U/l) and AMS (2325 U/l) [59]. The high AST, LDH and AMS serum activities observed in the male camels of this work were not observed in studies performed by [60, 61], which concluded no sex effect on the AST, LDH and AMS activities. The ALT, ALP and GGT serum activities did not significantly differ between the males and females in this work supporting the findings of [60-62] but disagree with other works that show high ALT activity in males [63, 64], high ALP activity in males [65, 66] and females camels [67]. With the exception of urea (5-40mg/dl) and total proteins (63-83g/l), all of the measured metabolites in both sex camel groups of the present study showed values fall within the normal ranges of glucose (60-140mg/dl), albumin (25-45g/l), globulin (20-50g/l), creatinine (0.8-2mg/dl), triglycerides (10-80mg/dl) and total cholesterol (18-150mg/dl) [59]. The higher female levels of glucose and urea reported here were in accordance with the findings of [68] for glucose and those reported by [62] in Djibouti, [69] in Tunisia, [70, 71] in India and [72] in Nigeria for urea. However, other researchers did not find sex impact on the levels of glucose and urea [60, 72-74]. The total proteins and globulin values were higher in the serum of male camels while the A/G ratio was higher in the female ones. This finding was in agreement with those cited previously by [75] but in contrary to the finding of [72] who reported higher protein and albumin values in the female camels, against the findings of [76] who recorded higher γ-globulin values in the female camels; and in opposite to the finding of [70] who reported higher A/G ratio in male camels. Moreover, many researchers found no sex effect on the total proteins and their fractions [60, 70, 72, 77, 78]. Creatinine, triglycerides, cholesterol and lipoproteins values were not significantly different between the two camel sexes. Similar trend was recorded by [72, 73, 77, 79, 80]. However, creatinine levels were significantly higher in female [70] and male [72] camels in other studies. The values of electrolytes in the serum of both camel sexes in the current study were within the normal ranges of Na (140-178mmol/l), K (3.6-6.0mmol/l), Ph (3.8-6.8mg/dl), Ca (8.4-12.4mg/dl), Mg (1.8-2.8mg/dl) and Fe (0.7-1.2mg/l) [59]. The sex effect

was observed in the case of Fe, Ca and Ph with the females had higher Fe and Ca but lower Ph values compared to the male camels. The high female Fe and Ca values were in parallel with the finding reported by [68, 81]. However, the Fe levels were higher in males than females according to some authors [82] and were not different between both sexes according to others [83]. In addition, no sex differences were reported in the case of Ca [70, 77, 80, 84, 85] or Ph [70]. The present study did not reveal sex effect regarding Na, K or Mg serum values in camels. Similar trend was recorded by [62, 80, 84, 85]. However, [72, 75] reported higher male K levels than females. The haematological indices investigated in this research fall within the normal ranges of RBC counts ($5\text{-}12.5x10^6/ mm^3$), WBC counts ($10.5\text{-}15.5x10^3/ mm^3$), PCV (22-43%), Hb (9.3-15.5g/dl), MCV (28.5-60fL), MCH (9-38.5pg), and MCHC (27.1-54.4g/dl) [59]. However, with the exception of monocytes proportion (11.1% for females and 9.1% for males) that was within the normal ranges of (1-11.6%) [59], the leukocyte formula was different where the lymphocytes proportion (55.5% for females and 71.9% for males), neutrophils proportion (31.9% for females and 17.7% for males) and eosinophils proportions (0.28% for both males and females) recorded in this study were higher than the normal range of lymphocytes (29-63%) and lower than the normal range of neutrophils (37-60%) and eosinophiles (1.5-13.8%) [59]. The haematological parameters determined in this work were mostly higher in the female camels compared to the male ones. The values of PCV, Hb, erythrocyte osmotic fragility, MCV, MCH and the number of neutrophils and monocytes were higher in female camels than the male ones while the values of ESR, MCHC and counts of RBC, WBC, lymphocytes, eosinophils and basophiles did not show significant differences between the two camel sexes. The female camel erythrocytes' in this study showed more resistance for haemolysis when immersed in NaCl solution (0.80%) than the male ones (0.73%). The neutrophils numbers were also higher in females than males in this work similar to what documented by [86, 87]. However, some references did not report any sex effect in the leukocyte formula [73, 88, 89] while others found higher eosinophils numbers in females [90] and higher lymphocytes numbers in males [87, 90]. Furthermore, some studies showed higher PCV and Hb levels in males compared to the female ones [86, 91]. In the current study, neither RBC counts nor WBC counts were significantly different between the two camel groups. This is in accordance with the findings of many authors [73, 89, 92, 93] for RBC and [73, 89, 90, 94] for WBC, although others reported a lower counts of RBC [89, 95] and WBC [86, 95] to the male. The erythrocyte sedimentation rate of 36.18mm/hr and 27.87mm/hr reported here did not significantly differ for the male and female camels respectively. This finding was in line with [73, 95] but in contrast with the finding cited by [90], which assumed that the sedimentation rate would be faster in females. In conclusion, the present study has proved the influence of gender on blood haematological and serum biochemical parameters in Libyan camels. The blood values determined in this investigation may serve as reference values for male and female Libyan camels and could be used in clinical disease diagnosis, prognosis as well as in preventive programs conducted in Libya. The effect of age on blood parameters in Libyan camels is to be investigated in the following last part of this series.

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