

Original article

Blood Profile in Normal One Humped Dromedary (*Camelus Dromedarius*) Camels in Libya. Part 4: Effect of Age Variation on Biochemical and Haematological Blood Profile

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ABSTRACT

Background and aims. As little is known about the blood profile of camels in Libya, this article is the last of a four-part series describing the biochemical and hematological blood profile in Libyan camels. In part one of these manuscripts, the overall blood biochemical and hematological mean values of camels in Libya were determined. Parts two and three evaluated the effects of breed and gender on these values respectively. In this forth and last part, the effect of age was examined. **Methods.** Blood samples were collected from eight young and fifty-eight adult apparently healthy camels and the levels of enzymes, metabolites, electrolytes and hematological indices were measured. **Results.** The blood of the young camels showed higher values of glucose, urea, triglycerides, total cholesterol, Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), Sodium (Na), Potassium (K), Phosphorus (Ph), Calcium (Ca) than the adult camels which on the other hand showed higher values of Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), High Density Lipoprotein (HDL) and Magnesium (Mg). **Conclusion.** This study has shown significant age differences between young and adult Libyan camels in many biochemical but not hematological parameters.

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INTRODUCTION

Age is one of the endogenous parameters that has an influence on many haematological and biochemical blood parameters in human as well as animals [1]. Young animals showed high Hemoglobin (Hb), hematocrit, Red Blood Cell (RBC) count, ALP activity and VLDL levels in sheep [2], high ALP activity, direct bilirubin, and Ph levels in horse

[3,4], high Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) in Buffalo [5], high ALP, LDH, Creatine Kinase (CK), Aspartate Aminotransferase (AST), Gamma Glutamyl Transferase (GGT) activities, glucose, cholesterol, Mg and Ph in cats [6] and high RBC count and Hb levels in dairy cows calves [7]. Meanwhile, adult animals showed high serum protein and globulin concentrations, cholesterol and bilirubin levels in sheep [2], high urea, creatinine, total protein, total bilirubin, Ca and Hb levels in horse [3,4], high RBC count, Hb, and PCV in Buffalo [5], high MCV and monocyte number in cats [6], high RBC counts, PCV and Hb in dogs [8].

The overall blood profile's mean of sixty six Libyan camels was recorded in the first part of this series [9], the effects of breed and gender variation on the measured biochemical and haematological blood parameters in the participants three selected Libyan breeds was evaluated in the second and third parts respectively [10,11]. The blood profile's data that was generated in the first part of this series was subdivided in this fourth and last part and the effect of age variation on the measured blood parameters was investigated and compared with similar studies performed elsewhere.

METHODS

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. Eight camels were suckling calves under six months of age and fifty-eight camels were non-suckling, over one year of age camels.

Blood collection

Blood samples were collected in the summer time of the year. Thirteen milliliter of blood were collected from the jugular vein of each animal by disposable plastic syringe and a 19G needle. Three milliliter of blood were distributed into EDTA anti-coagulant containing tubes for hematological analysis while the remained ten milliliter 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 -80° C for later analysis.

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.

Hematological 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, $\times 10^6/\text{mm}^3$) and white blood cells (WBC, $\times 10^3/\text{mm}^3$). Haemoglobin concentration was determined following Sahli's method [12]. 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 *et al.* [13]. Red blood cells and white blood cells were counted using haemocytometer and counted at x40 objective of phase contrast microscope according to Schalm *et al.* [13]. 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 *et al.* [13]. Erythrocyte sedimentation rate (ESR) was determined by Westergren method according to Bull *et al.* [14]. Erythrocyte osmotic fragility was determined according to Benson and Swallen [15].

Statistical analysis

Results are expressed as mean \pm 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 $p < 0.05$.

RESULTS

The serum enzyme activity 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 ALP and LDH activities were higher in the serum of adult camels than the young ones. However, the enzymatic activities of ALT, AST, GGT and AMS were not significantly different between the young and adult camel groups.

Table 1. Mean \pm S.E. of activity of ALT, AST, ALP, LDH, GGT and AMS enzymes in the serum of young (no=8) and adult (no=58) Libyan camels.

Parameter	Unit	Young camels (< 6 months)	Adult camels (> 1 year)
ALT	UL ⁻¹	5.68 \pm 1.99a	5.33 \pm 0.66a
AST	UL ⁻¹	10.88 \pm 4.18a	12.11 \pm 1.59a
ALP	UL ⁻¹	1.85 \pm 0.64a	4.59 \pm 0.53b
LDH	UL ⁻¹	7.08 \pm 1.67a	37.66 \pm 6.52b
GGT	UL ⁻¹	2.58 \pm 0.55a	1.67 \pm 0.12a
AMS	UL ⁻¹	1.43 \pm 0.65a	1.83 \pm 0.31a

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

The mean \pm 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 glucose, urea, triglycerides, total cholesterol, LDL and VLDL values were significantly higher in the serum of young camels than the adult ones while only the HDL values were higher in the serum of adult camels when compared to the young ones. The total proteins, albumin, globulin, A/G and creatinine levels did not show significant differences between the two camel groups.

Table 2. The Mean \pm SEM concentration of glucose, total proteins, albumin, globulin, urea, creatinine, triglycerides, cholesterol and lipoproteins in the serum of young (no=8) and adult (no=58) Libyan camels.

Parameter	Unit	Young camels (< 6 months)	Adult camels (> 1 year)
Glucose	mg dl ⁻¹	145.7 \pm 7.59a	107.1 \pm 5.75b
Total proteins	g l ⁻¹	49.14 \pm 1.06a	51.24 \pm 1.02a
Albumin	g l ⁻¹	31.87 \pm 0.65a	30.40 \pm 0.71a
Globulin	g l ⁻¹	17.27 \pm 1.39a	20.83 \pm 0.92a
A/G	g l ⁻¹	1.93 \pm 0.15a	1.66 \pm 0.10a
Urea	mg dl ⁻¹	47.29 \pm 1.61a	36.35 \pm 1.93b
Creatinine	mg dl ⁻¹	1.54 \pm 0.06a	1.49 \pm 0.02a
Triglycerides	mg dl ⁻¹	48.43 \pm 4.76a	29.28 \pm 1.75b
Total cholesterol	mg dl ⁻¹	44.66 \pm 2.41a	35.25 \pm 1.88b
HDL-cholesterol	mg dl ⁻¹	6.12 \pm 2.14a	17.26 \pm 1.27b
LDL-cholesterol	mg dl ⁻¹	28.86 \pm 3.66a	12.13 \pm 2.06b
VLDL-cholesterol	mg dl ⁻¹	9.68 \pm 0.95a	5.85 \pm 0.35b

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

The mean \pm 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 Na, K, Ph, and Ca were significantly higher in the serum of the young camels than the adult ones in contrast to the Mg levels which were higher in the serum of the adult camels when compared to the young ones. The difference in Fe levels among young and adult camels was not significant.

Table 3. The Mean \pm SEM concentration of Na, K, Ph, Fe, Ca and Mg in the serum of young (no=8) and adult (no=58) Libyan camels.

Parameter	Unit	Young camels (< 6 months)	Adult camels (> 1 year)
Na	mmol/l	153.3 \pm 1.30a	147.7 \pm 0.75b
K	mmol/l	5.73 \pm 0.36a	4.87 \pm 0.09b
Ph	mg dl ⁻¹	7.26 \pm 0.35a	4.91 \pm 0.25b
Fe	mg l ⁻¹	1.52 \pm 0.50a	0.74 \pm 0.11a
Ca	mg dl ⁻¹	10.31 \pm 0.15a	9.81 \pm 0.09b
Mg	mg dl ⁻¹	2.16 \pm 0.11a	2.56 \pm 0.06b

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

The mean \pm SEM values of various haematological parameters are shown in tables 4 and 5. The sera of the young and adult camels did not significantly differ regarding any of the measured parameters.

Table 4. Mean \pm S.E. of red blood cell values in the blood of young (no=8) and adult (no=58) Libyan camels.

Parameter	Unit	Young camels (< 6 months)	Adult camels (> 1 year)
PCV	%	30.88 \pm 1.65a	33.83 \pm 1.12a
Hb	g/dl	13.11 \pm 0.52a	12.47 \pm 0.30a
Fragility	%	0.80 \pm 0.00a	0.77 \pm 0.14a
ESR	mm/hr	33.25 \pm 9.76a	30.37 \pm 3.08a
RBC count	10 ⁶ /mm ³	11.47 \pm 0.61a	11.84 \pm 0.40a
MCV	fL	27.68 \pm 2.48a	29.46 \pm 1.00a
MCH	pg	11.59 \pm 0.68a	10.98 \pm 0.35a
MCHC	g/dl	43.26 \pm 2.75a	38.84 \pm 1.42a

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 \pm S.E. of white blood cell values in the blood of young (no=8) and adult (no=58) Libyan camels.

Parameter	Unit	Young camels (< 6 months)	Adult camels (> 1 year)
Total WBC count	10 ³ /ml	12.03 \pm 0.81a	10.83 \pm 0.56a
Lymphocytes	10 ³ /ml	6.67 \pm 0.85a	6.73 \pm 0.42a
Neutrophils	10 ³ /ml	4.07 \pm 0.83a	2.81 \pm 0.23a
Monocytes	10 ³ /ml	1.19 \pm 0.14a	1.14 \pm 0.08a
Eosinophils	10 ³ /ml	0.02 \pm 0.01a	0.03 \pm 0.00a
Basophils	10 ³ /ml	0.02 \pm 0.01a	0.03 \pm 0.00a

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

DISCUSSION

The non-significant differences in serum enzyme activities of ALT, AST, GGT and amylase enzymes between the two camel groups reported in this study was comparable with the findings of [16] for ALT, [16,17] for AST and [17] for

GGT. However, the high levels of ALP and LDH in adult camels reported here is not in consistent with other reports. It is known that ALP is related to the osteogenesis in the growing young camels and its level is decreasing with age as reported by [17,18]. Also the high LDH plasma activity in young camels was attributed to the higher muscular activity of young camels [17].

The higher levels of glucose, triglycerides, urea measured in the sera of young camels in this study were in accordance with the findings of [19-23]. On the other hand, other researchers reported lower levels of glucose [24], triglycerides [25,26] and urea [21,25,26] in young camels. Also, the high levels of cholesterol in young camels found here were not in accordance with many authors who did not find an age effect on the plasma cholesterol level [26-28].

With the exception of Fe levels which did not significantly differ between the two camel groups in this study and; which was in accordance with the finding of [29] who reported increasing Fe with age and stabilize from the fifth month of age, the young camels showed high serum levels of Na, K, Ph and Ca and low level of Mg when compared with the adult camels. This finding was in accordance with [30] for Na, [31] for K, [32] for Ph and [33] for Ca. However, other researchers documented no age effect on Na levels [34]. Magnesium is the only electrolyte that was lower in young camels and supported by the finding of [32] who reported increasing Mg levels with age. However, [35] reported an opposite finding regarding Mg.

Age did not affect any of the haematological parameters determined in this work. Similar findings were also reported regarding RBC count and PCV [36]. However, according to some studies, young camels showed higher indices of PCV and WBC count [21,37-39] and lower indices of Hb, PCV, RBC, MCV, MCH, MCHC and ESR [21,40 41].

CONCLUSION

This study has proved the effect of age on the blood profile of Libyan dromedaries and ended this series that has determined the blood profile and; investigated the effects of breed, gender and age in three different Libyan camel breeds. These investigations could be used as reference values in clinical disease diagnosis, prognosis as well as in preventive programs conducted in Libya.

Conflict of Interest

There are no financial, personal, professional conflicts of interest to declare

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