

Breed variation in blood constituents of the one-humped camel (*Camelus dromedaries*) in Algeria

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Abstract

Normal hematological (RBC: erythrocyte count, PCV: packed cell volume, Hb: hemoglobin concentration, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin and MCHC: mean corpuscular hemoglobin concentration) and biochemical parameters (mineral indices: calcium "Ca", sodium "Na", potassium "K", magnesium "Mg" and phosphorous "P" and organic indices: glucose "Glu", triglycerides "Tri", cholesterol "Chol", urea "urea" and creatinine "crea" and enzymes: aspartate aminotransferase "AST", creatinine kinase "CK", lactate dehydrogenase "LDH", phosphatase alkaline "PAL", gamma glutamyl tranferase "GGT" and alanine amino transferase "ALAT") have been determined in three breeds of Algerian camels (*Camelus dromedaries*). Statistical analysis showed no differences between breeds. Compared to other farm animals lymphocytes in the camel were the predominant leucocytes, MCHC's were in excess and PCV was lower.

Keywords: Camel, blood constituents, breeds.

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1. Introduction

The dromedary camel is distributed in Africa, Middle East and Indian sub-continent (Jeblawi, 2005). There are 24,246,291 one humped camels in the world (FAO, 2009) with 80% of them in Africa and the highest population in Somalia (7 million) and Sudan (4, 25 million). There are about 160, 000 in Algeria (FAO, 2009) with highest population in the southern part of the country (Aichouni and Jeblawi, 2007).

Skeletal muscles are the richest source of serum CK. Therefore it is the most widely used serum enzyme determination in muscular disease of large animals (kaneko, 1989). Normal LDH values reported here was similar to that reported by other workers (Nyang'ao et al., 1997; Benromdhan et al., 2003 and AL busadah, 2007). LDH is not organ-specific and

may be of value in conjunction with other enzymes (Coodley, 1970).

Like other animals the serum level of ALT in conjunction with other enzymes may be useful indicator for hepatic or muscular damage (Kaneko, 1989), but Kerr (1989) considers ALT as nonspecific index for liver investigations.

Haematological and biochemical analysis of blood can often provide valuable information regarding heath and sickness of animals. The standard of this parameters in camels were determined in Tunisia (Benromdhan et al., 2003); Moroccan (2003), in Morocco camels (Bengoumi, 1999); Iranian (Ghodsian et al., 1978 ; Badiei et al., 2006 and Mohri et al., 2008); Turkmen (Rezakhari et al., 1997); Pakistani (Majeed et al., 1980; Ziar-ur-Rahman et al., 2007); Kenyan (Nyang'ao et al., 1997 and Kuria et al., 2006); Sudanese (Damir et al., 2008; Muna et al., 2003 and Mohamed, 2004);,

Kuwaitian (Mohamed and Hussein, 1999); Emiratian (Faye et al., 2008); Omani (Yasmin et al., 2010); European (Faye et al., 1995) and in Saoudian camels (Osman and Al busadah, 2003; Al shami, 2009 and Al busadah, 2007). Thus the values obtained in one country could not be taken as standard in other countries having different climate.

There is no published information on the Algerian camel's hematological and biochemical values. This study is designed to investigate the haematological and biochemical indices in some breeds of Algerian one-humped camels.

2. Materials and methods

Animals: This study was conducted in El Bayed in the South of Algeria, where the climate is subtropical with mild winter and hot summer (Chehma, 2002). The physical environment of south Algeria has been described (Dellal, 2006). The camel breeds used in the study have been described (Benaissa, 1989). Sixteen healthy camels (*Camelus dromedarius*) of 1 to 14 years old of each, Chaambi, Ouled sid cheikh and Ouled naiel breeds, were used in this study. The selection of these breeds was based on the finding that they are the most numerous and widely distributed in this area (Aichouni and Jeblawi, 2007). Animals living under similar conditions of management (natural pasture) were healthy, routinely dewormed and dipped against endo- and ectoparasites.

Collection of blood samples: Blood samples were collected from a camel's jugular vein by venipuncture. 20 milliliter blood samples were collected from each camel using plastic disposable syringes. 10 milliliter of the blood sample were immediately transferred to capped and heparinized tubes (medical disposable industrial complex, (MDIC). These samples were used for the

hematological analysis. The rest of the samples were reported in two sets:

- One containing fluoride- oxalate for urea and glucose analysis;
- One without anticoagulant for other biochemical analyses.

Allowed to clot for 2 h at room temperature, the sera were then separated by centrifugation at 3000 g for 10 min.

The blood samples were subsequently transported in a cool box to the laboratory, where the serum was stored under -15 °C, for no longer than 1 month while waiting for analysis, and the blood samples were immediately used to determine the hematological indices.

Biochemical parameters: The automate biochemical analyzer (SYNCHRON CX 9PRO) was used to determine the serum concentration of:

- **Organic indices;** Glucose, Triglycerides, Cholesterol, Urea and Creatinine;

- **Enzymes:** Aspartate Amino transferase (AST), Creatinine kinase (CK), Lactic Dehydrogenase (LDH), Alkaline Phosphatase (PAL), Gamma Glutamyl Transferase (GGT) and Alanine Amino Transferase (ALAT).

- **Mineral indices:** Calcium (Ca), Phosphore (P), Sodium (Na), Magnesium (Mg) and Potassium (K).

Haematological parameters: Erythrocytic indices were determined according to the methods described in Shalm's Veterinary Hematology (Jain, 1986). The Packed Cell Volume of erythrocytes (PCV) was determined by the micro-haematocrit method using a special centrifuge. Hemoglobin concentration was determined by the cyano-methaemoglobin method as described by Van kampan and Zijlstra (1961). Differential Leukocyte Count (DLC) was determined microscopically from counts of 200 leucocytes in thin May-Grunwald-Giemsa stained blood smears (Kelly, 1984).

Statistical analysis: Data were analyzed by student t-test using GLM procedure of SAS (Goodnight et al., 1986) and Duncan's multiple range test was used to detect significant difference among means.

3. Results

In the three studied breeds no significant differences were observed in blood mineral parameters (Table 1), enzymes (Table 2) and organic parameters (Table3).

Table 1. Mean ($\pm$ SD) and ranges of serum mineral parameters values in different breeds of dromedary camels.

Mineral parameters (mmol/l)	Chaambi (n =16)	Ouled sid cheikh (n =16)	Ouled naiel (n =16)	Mean of all camels (n = 48)	Range (n=48)
Calcium	2.3 $\pm$ 0.3	1.93 $\pm$ 0.3	2.21 $\pm$ 0.5	2.24 $\pm$ 0.6	2.07-2.65
Phosphorus	1.50 $\pm$ 0.6	1.82 $\pm$ 0.7	1.68 $\pm$ 0.6	1.58 $\pm$ 0.7	0.75-2.11
Potassium	5.72 $\pm$ 0.7	4.98 $\pm$ 0.5	5.38 $\pm$ 0.6	5.72 $\pm$ 0.8	4.55-5.99
Sodium	163.00 $\pm$ 12.1	160.0 $\pm$ 11.9	162.8 $\pm$ 12	159.00 $\pm$ 14	151-177.01
Magnésium	1.12 $\pm$ 0.2	0.95 $\pm$ 0.2	1.47 $\pm$ 0.1	1.12 $\pm$ 0.3	0.76-1.8

n=Number of animals

Table 2. Mean ($\pm$ SD) and ranges of serum enzyme parameters values in different breeds of dromedary camels.

Enzyme ui/l	Chaambi (n =16)	Ouled sid cheikh (n = 16)	Ouled naiel (n =16)	Mean (n=48)	Range (n=48)
AST	74.5 $\pm$ 15	78.2 $\pm$ 16	76.38 $\pm$ 16	73.3 $\pm$ 17	29-122
ALT	4.18 $\pm$ 3.5	4.22 $\pm$ 4	4.81 $\pm$ 4	4.42 $\pm$ 6	3.01-6.91
LDH	385.4 $\pm$ 70	411.46 $\pm$ 68	399.6 $\pm$ 71	331 $\pm$ 77	303-561
CK	47.25 $\pm$ 23	59.01 $\pm$ 22	61.51 $\pm$ 24	44.78 $\pm$ 26	40-77.42

n=Number of animals.

Table 3. Mean ($\pm$ SD) and Ranges of serum organic parameters values in different breeds of dromedary camels.

Organic parameters	Chaambi (n =16)	Ouled sid cheikh (n =16)	Ouled naiel (n =16)	Mean (n=48)	Range (n=48)
Glucose (mmol/l)	5.25 $\pm$ 1.1	4.82 $\pm$ 1	5.95 $\pm$ 1.2	5.01 $\pm$ 2	4.45-6.94
Urea (mmol/l)	5.59 $\pm$ 1.4	6.00 $\pm$ 1.2	5.42 $\pm$ 1.5	5.59 $\pm$ 2.1	4.65-6.77
Triglycérides (mmol/l)	0.67 $\pm$ 0.2	0.74 $\pm$ 0.3	0.69 $\pm$ 0.2	0.642 $\pm$ 0.4	0.5-0.7
Créatinine (μ mol/l)	96.05 $\pm$ 2.3	97.85 $\pm$ 23.7	96.67 $\pm$ 23.2	96.05 $\pm$ 2.51	93.1-98.35

Sodium and potassium concentrations were similar to those obtained by Rezakhan et al. (1997) in Turkman camels, Ben romdhan et al.(2003) in Tunisian camels, and Al-busadh (2007) in Saoudian camels, but higher than those

obtained by Al ani et al. (1992) in Iraqi camels. Results of erythrocytic indices and leukocytic series are shown in Table 4. Statistical analysis showed no-significant breed effect (P varied between 0.1 to 08) therefore breed results for

each parameter were pooled and one mean for all camels is given in Table 4. Lymphocytes were the predominant cells of total leucocytes count ($46.58 \pm 5.7\%$) followed by the neutrophils ($41.74 \pm 0.71\%$), few monocytes ($7.73 \pm 0.65\%$)

and eosinophils ($3.92 \pm 1.22\%$) and rarely basophils ($0.03 \pm 0.19\%$) were the main feature of leukocytic series. The PCV was $30.85 \pm 1.2\%$, RBC 7.95 ± 0.51 . $106/\text{mm}^3$ and HB $14.5 \pm 0.51\text{g/dl}$.

Table 4. Mean ($\pm$ SD) and ranges of haematological values in different breeds of dromedary camels.

Item	Chaambi (n=16)	Ouled sid cheikh (n=16)	Ouled naiel (n=16)	Mean (n=48)	Range (n=48)
RBC ($10^6/\text{mm}^3$)	8.25 ± 0.15	7.95 ± 0.22	8.11 ± 0.19	7.95 ± 0.51	7-9
PCV(%)	29.88 ± 0.23	30 ± 0.21	31.22 ± 0.25	30.85 ± 1.2	27-35
Hb (g/dl)	14.54 ± 0.14	14.55 ± 0.15	14.81 ± 0.17	14.5 ± 0.51	14-15
MCV (μm^3)	36.66 ± 0.73	38.1 ± 0.62	39.41 ± 0.59	37.95 ± 0.61	31.5-48.5
MCH (pg)	17.92 ± 0.38	18.9 ± 0.32	18.55 ± 0.36	18.01 ± 0.5	15.5-21.4
MCHC (%)	48.87 ± 1.58	49.99 ± 1.4	47.78 ± 1.6	48.2 ± 2.1	40-57.6
WBC ($10^3/\text{mm}^3$)	15.59 ± 0.32	16 ± 0.31	16 ± 0.33	15.51 ± 0.3	13-18
Lymphocytes (%)	44.39 ± 1.6	46.58 ± 1.5	43.08 ± 1.7	46.58 ± 5.7	41.14-47.4
Neutrophils (%)	$42.45 \pm .50$	41.74 ± 1.3	42.77 ± 1	41.74 ± 0.71	40.15-46.32
Monocytes (%)	8.67 ± 0.51	7.73 ± 0.42	8.9 ± 0.39	7.73 ± 0.65	0.02-0.07
Eosinophils (%)	4.42 ± 0.52	3.92 ± 0.49	4.97 ± 0.48	3.92 ± 1.22	3.52-6.06
Basophils (%)	0.04 ± 0.2	0.03 ± 0.1	0.04 ± 0.1	0.03 ± 0.19	7.4-10.01

4. Discussion

The mean values of serum calcium in this study are in agreement with those reported by Soliman and Ahaker (1967), Al ani et al. (1992), Rezakhani et al. (1997), Ben romdhan et al. (2003) and Al-busadh (2007).

Similar values of AST have been established by several workers (Boid et al., 1980; Eldiridiri et al., 1987; Ben romdhan et al., 200 and Al busadah, 2007). AST lacks organ specificity but is present in skeletal muscle, cardiac muscle and liver of large animals and pathological changes in these organs elevate the activity of AST in the blood (Kaneko, 1989).

The values of CK presented here was lower than values reported elsewhere (Al ani et al., 1988; Beaunoyer, 1992 and Nyang'ao et al., 1997).

The blood urea, creatinine, cholesterol and enzymes were similar to the reference values for cattle (Zongping,

2003) and the dromedary camels (Abdelgadir et al., 1984; Wahbi et al., 1984; Eldiridiri et al., 1987; Bengoumi et al., 1999; Benromdhan et al., 2003 and Al busadah, 2007). However, at times of poor grazing or water deprivation exceptionally high level urea was reported in camels in comparison to other livestock in view of the camel's ability to utilize urinary nitrogen (Kandil, 1984).

The haematological values presented in this study were within the reference ranges to those reported elsewhere for the dromedary (Abdelgadir et al., 1984; Mehrotra and Gupta, 1997 and Al-busadah, 2007). Compared to other species like horse and ruminants, camels have more RBC but lower PCV (Schalm et al., 1975). Consequently the MCHC was also higher than in any other species as the PCV is the denominator in the formula which determines MCHC (Jain, 1986). The finding that RBC count was higher and PCV value was lower in the camel compared to other species is

because the smaller elliptical cells pack tighter in the camel. The RBC count was inversely proportional to MCV, the indicator of red cell size. This is, the smaller the size of red cells the greater their number per unit volume of blood (Kerr, 1989 and Al-busadah, 2007).

The values obtained in this study for WBC count is comparable to values reported in other studies (Lakhotia et al., 1964; Soliman, Shaker, 1967; Al ani et al., 1992 and Al- busadh, 2007). However the most frequent white cells are not Neutrophils but Lymphocytes. In this studies the percentage of Lymphocytes was $46, 58 \pm 1, 7\%$ and Neutrophils was $41, 74 \pm 0, 71\%$.

Corresponding values of lymphocyte and Neutrophil counts were 29% and 58% in Iranian camels (Ghodsian et al., 1978), 45,9% and 48,11% in Turkman camels and 50% and 36,6% in Pakistani camels (Majeed et al., 1980) (Rezakhari et al., 1997) 56% and 38% respectively in Kenyan camels (Nyang et al., 1997). As the camels belonged and lived in the same region, these differences could be due to the different breeds used. This in part confirms Majeed et al. (1980) finding that Lymphocytes and Eosinophils appear to reciprocate the Neutrophils in different seasons.

Since the camel is an adaptable species, standard hematological and serum biochemical values need to be determined in a number of animals in variable environmental and physical conditions.

Conclusion

There are few variations between the present findings and those from previous workers that may be attributed to the breed differences, nutrition, and husbandry or assay methodology. Findings of the current study provide baseline values that may be used by clinicians for the main breeds of camels in Algeria. Values recorded for hematological and biochemical

parameters were within the ranges reported for camels in the Maghreb and Gulf region.

References

- Abdelgadir S.E, Wahbi A.G.A. and Idris O.F., 1984. Some blood and plasma constituents of the camel. In: Al busadah K.A 2007. Some biochemical and haematological indices in different breeds of camels in Saudi Arabia. Sci. J. King Faisal Univ. 8: 131-142.
- Aichouni A., Jeblawi R.J., 2007. A study of breeding and reproduction of camels in the Algerian south west. Tishreen University Journal for Study and Scientific Research, Biological Sciences Series. 29(1): 51-62.
- Al ali A. K., Husayni H. A. and Power D. M., 1988. A comprehensive biochemical analysis of the blood of the camel. Comparative Biochemistry and Physiology. B(89): 35-37.
- Al ani F. K., Al azawi, W.A.R.A. Jermukly M.S. and Razzaq, K.K., 1992. Studies on some haematological parameters of camel and llama in Iraq. Bulletin of Animal Health and Production in Africa. 40: 103-106.
- Al busadah K.A., 2007. Some biochemical and haematological indices in different breeds of camels in Saudi Arabia. Sci. J. King Faisal Univ. 8: 131-142.
- Al schami S.A., 2009. Comparative determination of serobiochemical constituents in in-door and free grazing camels. J. Anim.Vet. Adv. 8: 896-908.
- Barakat M. Z. and Abdel-fettah M., 1971. Seasonal and sexual variations of certain constituents of normal camel blood. Zentralblatt for Veterinarmedizin. A(18): 174-178.
- Bdiei K. K., Mostaghni M., Pourjafar H. and Parchami A., 2006. Serum and tissue trace elements in Iranian camels. Comp.Clin. Pathol. 15: 58-61.
- Beaunoyer D.E., 1992. Changes in serum enzyme activities after maximal exercise. In Proceedings of first international camel conference. Eds. Allen W. K., Higgins A. J., Mayhew L. G., Snow D. H. and Wade J. F. 331-333.
- Benaissa. R., 1986. Le dromadaire en Algérie : Perspectives d'avenir. Magister thesis. Institut National d'Agronomie, Algiers. 234pp.
- Bengoumi M., Moutaoukil F., Farge F.D.L. and Faye B., 1999. Thyroidal status of the dromedary camel. Effect of some physiological factors. Journal of camel Practice and Research. 6: 41-43.

- Ben romdhane S.M., Romdhan N., Feki M, Sanhagi H. and Kaabachi N., 2003. Blood biochemistry in dromedary. *Revue Med. Vet.*, 154: 695-702.
- Boid R, Mohamed M. M. and Gray A.R., 1980. Changes in the levels of some serum enzymes in dromedary camels infected with *Trypanosoma evansi*. *Research in Veterinary Science*. 28 (3): 336-340.
- Chehma A., 2002. Etude floristique des parcours du dromadaire. PhD Thesis. Badj Mokhtar University, Batna. Algeria. 302 pp.
- Coodley E.L., 1970. *Diagnostic Enzymology* 4th ed. WB Saunders Co. Philadelphia, USA.
- Damir H.A, Abbas T.A. and Ali A.M., 2008. Copper status in breeding and racing camels and response to cupric oxide needle capsules. *Trop. Anim. Health Prod.* 40:643-648.
- Dellal A., 2006. Le sol et le climat de la région Sud Ouest de l'Algérie. *Ibn Khaldoun Journal*. 15: 32-47.
- Eldiridiri K. L, Suliman H. B. and Shommein A. M., 1987. Normal serum activities of some diagnostic enzymes in dromedary camel in Sudan. *Veterinary research communication*. 11: 201-203.
- FAO. 2009. Food and agriculture organization of the united nation. <http://fao.org/>.
- Faye B., Rotovonahary M., Chacornac J.P. and Soubre P., 1995. Metabolic profiles and risks of diseases in camels in temperate conditions. *Comp. Biochem. Physiol.* 112: 67-73.
- Faye B., Rabiha M. and Askar M., 2008. Trace elements and heavy metals status in Arabia camels. In: Yassmin et al., 2010; Serum mineral contents of the Omani racing Arabian camels. *Journal of Animal and Veterinary Advances*. 9 (4): 764-770.
- Ghodsian I., Nowrouzian I. and Schels H. F., 1978. A study of some haematological parameters in the Iranian camel. *Tropical Animal Health and Production*. 10: 109-110.
23. Goodnight J. H, Sal J.P. and Sarie R. F., 1986. In *SAS User's Guide*. Statistics SAS Institute Inc., Cary, North Carolina, USA.
- Ghosal A.K, Dwarkanath P.K., and. Appana T.C., 1975. A study on the changes of the blood electrolyte of camel during water deprivation. *Indian Journal of Animal health*. 14: 113-115.
- Jain N., 1986. *Essentials of Veterinary Haematology*. Lea & Febiger, Philadelphia. 65-66.
- Jebrawi R. J., 2005. Horse and camels. publication of Tishreen University, Ladiquia, Syria, 237-331
- Kandil.H.M, (1984): Studies on camel nutrition. Ph.D.Thesis. Fac. Agri. Ain Shams.Univ. 115 pages.
- Kaneko J. J., 1989. *Clinical biochemistry of domestic Animals*. 3rd Ed. New York, USA, Academic Press.
- Kelly W.R., 1984. The blood and blood forming organs. In: Bailliere Tindal, London. *Veterinary Clinical Diagnosis*, Kelly, E. R. Ed. 312-337.
- Kerr M.G., 1989. *Veterinary Laboratory Medicine, Clinical Biochemistry and Haematology*. Oxford, Blackwell Scientific.
- Kuria S.G., Wahome R.G, Wanyoike M. M. and Gchur C.K., 2006. Effect of mineral supplement on plasma mineral concentration of camels in Kenya. *Int. Agric. Biol.* 8:168-171.
- Lakhotia R. L., Bhargava A. K. and Mehrotra P. N., 1964. Normal range for some blood constituents of the Indian camel. *Vet. Res.* 76, 121-122.
- Majeed M., Hur G., Raman Z. and Ahmed A., 1980. Effect of sex and season on 10 haematological values of normal adult humped camels. *Rev. Elev. Med. Vet. Pays. Trop.* 33: 135-141.
- Mehrotra V. and Gupta M. L., 1989. Seasonal variations in certain blood constituents in camel. *Indian Journal of Animal Sciences*. 59: 1559-1561.
- Mohamed H. A., 2004. The zinc and copper content of the plasma biochemistry of one-humped camel: effects of anticoagulants and comparison with serum. *Rs. Vet. Sci.*, 85:554-558.
- Mohamed H. A. and Hussein A. N., 1999. Studies on normal haematological and serum biochemical values of racing camels in Kuwait. *Vet. Res. Commun.* 23: 241-248.
- Mohri M., Moosayian H. R and Hadian M. J., 2008. Plasma biochemistry of one humped camel: effect of anticoagulant and comparison with serum. *Res. Vet. Sci.*, 85: 554-558.
- Muna Ahmed M. M., Awad M.E. and Barri M.E.S., 2003. Some micromineral profile in the serum of female camels as affected by the physiological state. *Pak. J. Biol. Sci.*, 6:1853-1855.
- Nyang'ao j M., Olaho-Mukani N., Maribei J. M. and Omuse J. K., 1997. *Journal of Camel Practice and Research* 31-33.
- Osman T. E. A and Al busadah K. A., 2000. Effect of age and lactation as some biochemical constituents of camel blood in Saudi Arabia. *J. of Camel Practice* 7, 149-152.
- Rezakhani A., Habibabadi S.N., Ghogh M. M., 1997. Studies on normal haematological and biochemical parameters of Turkmen camel in Iran. *Journal of Camel Practice and Research* 4, 41-44.

- Schalm O. W., Jain N.C. and Carrol W. J. 1975. Veterinary Haematology (3rd.ed), Lea and Febiger, Philadelphia, USA. Pp. 807.
- Soliman M. K. and Shaker M., 1967. Cytological and biochemical studies on the blood of adult she-camels. Indian Veterinary Journal 44:989-995.
- Van Kampen E. J. and Zijlstra 1961. Standarization of HaematolobinometryII. The haemoglobinocyanide method. Clin. Chem. Acta. 6:538-544.
- Wahbi A. G. A, Abdelgadir S. E, and Idris O. F., 1984. The plasma electrolytes and minerals of nomad camels in the Sudan. In: Al Busadah K. A., 2007. Some biochemical and haematological indices in different breeds of camels in Saudi Arabia. Sci. J. King Faisal Univ. 8: 131-142.
- Yassmin E. E., Hassin M. A., Mansour M.H. and Mahgoub O., 2010. Serum mineral contents of the Omani racing Arabian camel (*Camelus dromaderius*). J. Anim. Vet. Adv. 9(4): 764-770.
- Zia-ur-Rahman N., Bukhari S.A, Akhtar N. and Haq I.U., 2007. Serum hormonal, electrolytes and trace elements profiles in the rutting and non-rutting one humped male camel. Anim. Repro. Sci. 101:172-178.