

# DETERMINATION AND IDENTIFICATION OF REFERENCE INTERVALS FOR SELECTED BLOOD PARAMETERS DURING DIFFERENT PHYSIOLOGICAL STATES IN FEMALE DROMEDARY CAMELS

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## ABSTRACT

One hundred eighty female Arabian camels aged 3–16 years were used to determine the reference intervals for selected blood parameters during different physiological states. Venous blood was collected for determining erythrocyte and leukocyte parameters, blood glucose, protein fractions,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and Pi. The reference ranges for TEC, PCV, Hb, MCV, MCH, MCHC, TLC, neutrophils, eosinophils, basophils, lymphocytes and monocytes were  $4.06\text{--}5.03 \times 10^6/\mu\text{L}$ , 4.8–11.2 g/dL, 24.8–30.6%, 29.7–39.4 fl, 25.2–44.4 pg, 19.6–39.8 g/dL,  $11\text{--}13 \times 10^3/\mu\text{L}$ , 41–49%, 5–6%, 1–3%, 45–50% and 1–2%, respectively and for blood glucose, TP, albumin,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$  and  $\gamma$ -globulins, A/G ratio,  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and Pi were 67–100 mg/dL, 29–72 g/L, 46–78%, 1–7%, 2–10%, 5–14%, 3–14%, 10–27%, 1–15 and 5–9, 1–2, 4–6 mmol/L, respectively. The values of Hb were lower in the growing and early lactating camels than in dry ones, whereas TEC, monocytes percentage and serum  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , Pi were higher ( $P < 0.05$ ). The TEC, TP and albumin were higher in late pregnant camels than in dry and early lactating ones ( $P < 0.05$ ). The  $\alpha_1$ ,  $\alpha_2$  and  $\beta_1$  were lower in late pregnant camels than in dry females, whereas they showed higher values in early lactating ones ( $P < 0.05$ ). Neutrophil and lymphocyte percentages showed higher and lower values ( $P < 0.05$ ) in late pregnant and early lactating camels than in dry females, respectively. The blood parameters of female camels varied significantly depending on their physiological state. The findings are most likely applied to evaluate physiological and metabolic adaptations to growth, pregnancy and lactation.

**Key words:** Blood parameters, dromedary camel, physiological state, reference interval

Blood parameters are commonly utilised to assess the physiological state of the animals (Onasanya *et al*, 2015). Age, sex, breed, season, exercise, nutrition, stress, transport and diseases have been shown to alter haematological and serum biochemical parameters in all animal species (Jain, 1998; Faye and Bengoumi, 2018). In camels, significant differences have been found in these parameters in relation to age and during different physiological states (Tharwat *et al*, 2015; Elkhair and Elmgboul, 2015; Elkhair, 2016; Ahmed, 2017; Elkhair and Minawy, 2018; Ahmed and Elkhair, 2019; Islam *et al*, 2019; Waziri *et al*, 2019; Faraz *et al*, 2021; Mohamed *et al*, 2021; Martín-Barrasa *et al*, 2023).

It has been demonstrated that metabolic processes change significantly during pregnancy and lactation in camels; therefore, many investigators

used blood parameters to evaluate the metabolic profile of pregnant and lactating camels (Ayoub *et al*, 2003; Ahmed, 2017; Axay *et al*, 2017; El Zahar *et al*, 2017; Abd El-Salaam and Arafa, 2018; Jalali *et al*, 2018; Elkhair, 2019; Ebissy *et al*, 2019; Atta *et al*, 2021).

Age, growth phase, pregnancy and lactation are physiologically critical states associated with significant metabolic alterations in all animal species. Regarding female camels, few data are available concerning the reference intervals of blood parameters as influenced by age, growth phase, late pregnancy and early lactation and to describe the haematology and serum biochemical profile of growing, pregnant and lactating camels reared under semi-intensive system. Therefore, the study was aimed to establish reference ranges for selected blood parameters of female camels under various physiological states.

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## Materials and Methods

The study was conducted with utmost adherence to ethical guidelines, ensuring the welfare of the animals at all times. It was carried out at the Camel Research Centre of the Faculty of Veterinary Medicine, University of Khartoum (Sudan), after receiving approval from the Faculty Research Ethics Committee. The animal care protocol and sample procedures were conducted in strict accordance with the ethics regulations set by the same body and the guidelines established by the ARRIVE standards (<https://arriveguidelines.org>) for animal experiments.

### Animals and Management

The selection process for the animals was rigorous and thorough. One hundred eighty clinically healthy female Arabian camels (ages: 3-5 years for growing females and 8-16 years for dry, late pregnant and early lactating camels, number of parities: 2-3) were randomly divided into four groups of 45 each. The females were raised in a semi-intensive system, browsing and grazing shrubs and trees; water was offered *ad libitum*. The females were fed a daily ration of fresh Abu 70 (*Sorghum bicolor* L.) and concentrate supplements (a mixture of crushed *Sorghum bicolor* grains 30%, wheat bran 40%, groundnut cake 25% and NaCl salt 5%). The pregnant animals were meticulously observed one month *prepartum* up to one month *postpartum* and were chosen based on mating records for each individual participant in the trial, which showed the predicted delivery.

### Collection of blood sample

The experimental animals were sampled twice at two-week intervals in the morning before feeding. Approximately 7 mL of venous blood was obtained at 8:00 am via jugular venipuncture using disposable plastic syringes (Jiangsu Kangsu Medical Instrument Co., Ltd, China). A volume of 2 mL was placed into EDTA vacutainers for haematological analysis. Another 2 mL was drawn into sodium fluoride vacutainers for glucose determination. The remaining blood sample was drawn into plain plastic syringes (Pirmvetta®, Laboratory Technique, GmbH, Germany), centrifuged at 3000 rpm for 10 min (Gallenkamp Junior, UK). Free-hemolysis sera were placed into sterile vials (Eppendorf® Tube) and stored at -20 °C for serum biochemistry measurements.

### Laboratory analysis

**Haematological parameters:** The URIT-3010 VET Haematology Analyser (URIT medical

electronics) was employed to determine Hb, PCV, TEC, MCV, MCH, MCHC and TLC. The DLC was performed in thin Giemsa-Grunewald stained blood smears (Weiss and Wardrop, 2010).

**Blood glucose and serum Ca<sup>++</sup>, Mg<sup>++</sup> and Pi concentrations:** Standard spectrophotometric methods and Bio-systems kits (S. A. Barcelona, Spain) were used for determining blood glucose (Caraway, 1987), Ca<sup>++</sup> (Gindler and King, 1972), Mg<sup>++</sup> (Chromya *et al*, 1973) and Pi (Gamst, 1980).

**Total protein (TP) and capillary electrophoresis:** The Biuret standard spectrophotometric method described by Weichselbaum (1946) was performed for TP determination. Capillary electrophoresis was used to perform the percentages of albumin,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$  and  $\gamma$ -globulins using an Automated MINICAP-SEBIA and MINICAP CDT kits (MINICAP-SEBIA Instrument, France). The instrument was checked for precision and accuracy following the guidelines of Sebia-MINICAP manual, Ref. 2208.

### Statistical analysis

The IBM SPSS Statistics 20 (Inc. SPSS, 2011), a comprehensive and reliable tool, was used for data analysis. Resource Equation Approach was performed for computing the sample size (Mead, 1988; Arifin and Zahiruddin, 2017). The reference values for blood parameters were determined using the minimum sample size in the survey ( $n_{Min}$ ) according to the formula adopted by Werner (1992).

$$n_{Min} \geq \left( \frac{U_{1-\alpha/2}}{\Delta} \right) \cdot \delta^2 \quad (1)$$

The Kolmogorov-Smirnov test was applied to determine the distribution of the individual values; the data were normally distributed. General linear model procedures for analysis of variance ANOVA tests and descriptive statistics were applied to evaluate the differences between the groups; the differences were separated at  $P < 0.05$  using LSD.

## Results

### Overall reference range

Tables 1 and 2 demonstrate the overall reference ranges of 180 female camels, i.e. TEC (4.06-5.03×10<sup>6</sup>/μl), Hb (4.8-11.2 g/dL), PCV (24.8-30.6%), MCV (29.7-39.4 fl), MCH (25.2-44.4 pg), MCHC (19.6-39.8 g/dL), TLC (11-13×10<sup>3</sup>/μl), neutrophils (41-49%), lymphocytes (45-50%), monocytes (1-2%), eosinophils (5-6%) and basophils (1-3%). The established reference ranges of blood glucose, serum TP, albumin,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$ ,  $\gamma$ -globulins, A/G ratio,

Ca<sup>++</sup>, Mg<sup>++</sup> and Pi were 67–100 mg/dL, 29–72 g/dl, 46–78%, 1–7%, 2–10%, 5–14%, 3–14%, 10–27%, 1–15 and 5–9, 1–2 and 4–6 mmol/L, respectively.

**Blood parameters of the growing females**

The TEC, monocytes percentage, serum Ca<sup>++</sup>, Mg<sup>++</sup> and Pi in the growing females were significantly

**Table 1.** Statistical data of the haematological parameters in female camels (*Camelus dromedarius*) during different physiological states.

Parameter	Statistical values	Growing	Dry	Late pregnant	Early lactating
TEC (×10 <sup>6</sup> /μl)	$\bar{x} \pm s$	5.00±0.68 <sup>A</sup>	4.63±0.96 <sup>Bb</sup>	5.03±0.65 <sup>a</sup>	4.06±0.96
	Reference range <sup>1</sup>	4.2–5.5	3.6–5.5	4.4–5.7	3.8–4.9
	Median	5.2	4.8	5.0	4.7
	(1.-3. Quartile)	4.2–5.5	3.6–5.5	4.4–5.7	3.0–4.8
[Hb] (g/dl)	$\bar{x} \pm s$	7.80±2.7 <sup>B</sup>	9.40±1.9 <sup>Ab</sup>	9.90±0.89 <sup>a</sup>	8.02±1.2 <sup>c</sup>
	Reference range <sup>1</sup>	4.8–10.2	7.4–11.2	8.9–10.6	6.8–9.2
	Median	8.3	9.6	10.2	8.1
	(1.-3. Quartile)	4.8–10.2	7.4–11.2	8.9–10.6	8.9–9.2
PCV (%)	$\bar{x} \pm s$	27.76±4.7	29.4±4.9	29.9±4.89	28.02±4.82
	Reference range <sup>1</sup>	24.8–30.2	27.4–31.2	28.9–30.6	26.8–29.2
	Median	28.3	29.6	30.2	28.1
	(1.-3. Quartile)	24.8–30.2	27.4–31.2	28.9–30.6	26.9–29.2
MCV (fl)	$\bar{x} \pm s$	32.93±2.8	35.3±2.8	36.03±2.9	34.34±2.3
	Reference range <sup>1</sup>	29.7–34.7	32.7–38.2	34–39.4	32.5–38.2
	Median	14.4	15	14.7	13.7
	(1.-3. Quartile)	9.7–14.7	12.7–18.2	14–19.4	12.5–18.2
MCH (pg)	$\bar{x} \pm s$	21.67±4.35	20±0.62	28.67±0.6	20.76±2.7
	Reference range <sup>1</sup>	25.2–41.5	39.5–40.7	38.1–39.3	38.1–44.4
	Median	38.3	39.8	38.6	41.3
	(1.-3. Quartile)	15.2–41.5	39.5–40.7	38.7–39.7	38.2–43.1
MCHC (g/dl)	$\bar{x} \pm s$	26.47±11.5	20.27±0.3	19.63±0.9	21.10±1.88
	Reference range <sup>1</sup>	19.6–39.8	20–20.6	18.6–20.2	19.1–23.4 21.8
	Median	20	20.2	20.1	19.2–22.7
	(1.-3. Quartile)	19.6–39.8	20.0–20.6	18.6–20.2	
TLC (×10 <sup>3</sup> /μl)	$\bar{x} \pm s$	11.93±4.9	11.97±4.85	12.3±4.82	12.22±4.9
	Reference range <sup>1</sup>	11–12.8	11.8–12.1	12.1–12.5	11–13.3
	Median	12	12	12.3	12.5
	(1.-3. Quartile)	11–12.8	11.8–12.1	12.1–12.5	11.3–13
Neutrophils (%)	$\bar{x} \pm s$	42.5±2.1	41.67±2.5 <sup>b</sup>	41.5±0.7 <sup>c</sup>	47.5±2.1 <sup>a</sup>
	Reference range <sup>1</sup>	41–44	39–44	41–42	46–49
	Median	42.5	42	42	47.5
	(1.-3. Quartile)	30.8–33	39–44	30.8–31.5	34.5–36.8
Lymphocytes (%)	$\bar{x} \pm s$	48.50±2.1	46.50±2.1 <sup>c</sup>	50.33±1.5 <sup>a</sup>	49.50±2.1 <sup>b</sup>
	Reference range <sup>1</sup>	47–50	45–48	49–52	48–51
	Median	48.5	46.5	50	49.5
	(1.-3. Quartile)	35.25–37.5	33.75–33	49–52	36–38.5
Monocytes (%)	$\bar{x} \pm s$	2±0.02 <sup>A</sup>	1±0.01 <sup>B</sup>	1±0.01	1.33±0.58
	Reference range <sup>1</sup>	2–2.1	1–1.2	1–1.2	1–2
	Median	2	1	1	1
	(1.-3. Quartile)	1.5–1.5	0.75–0.75	0.75–0.75	1–2
Eosinophils (%)	$\bar{x} \pm s$	5±0.04	3.5±0.7	5±1.4	4.3±0.58
	Reference range <sup>1</sup>	5–5.2	3–4	4–6	4–5
	Median	5	3.5	5	4
	(1.-3. Quartile)	3.75–3.75	2.3–3.0	3–4.5	4–5
Basophils (%)	$\bar{x} \pm s$	2±0.03	1.5±0.7	3±1.4	2.67±0.58
	Reference range <sup>1</sup>	2–2.2	1–2	2–4	2–3
	Median	2	1.5	3	3
	(1.-3. Quartile)	1.5–1.5	0.75–1.5	1.5–3	1.5–3

<sup>1</sup>m± s.d.×1.96 indicated the lower and the upper limits, Brackets ( [ ] ) denote concentration.

Aa, Bb Overall means within the same row bearing different superscripts are significantly different at P≤0.05.

**Table 2.** Statistical data of selected serum biochemical parameters in female camels (*Camelus dromedarius*) during different physiological states.

Parameter	Statistical values	Growing	Dry	Late pregnant	Early lactating
Blood glucose (mg/dL)	$\bar{x} \pm s$	71.67±0.58	78.33±11	82.33±10.4	76.80±11.1
	Reference range <sup>1</sup>	71-72	67-89	72-100	66-97
	Median	72	79	75	73
	(1.-3. Quartile)	71-72	67-89	72-100	68-87
Serum-[TP] (g/l)	$\bar{x} \pm s$	42.67±10.8	43.67±5.1 <sup>c</sup>	59.67±8.5 <sup>a</sup>	50.40±8.8 <sup>b</sup>
	Reference range <sup>1</sup>	35-55	39-49	47-72	38-61
	Median	38	36	60	53
	(1.-3. Quartile)	35-55	29-39	47-72	40-59
Albumin (%)	$\bar{x} \pm s$	62.62±8.4	59.85±6.2 <sup>b</sup>	63.33±9 <sup>a</sup>	57.25±9.6 <sup>b</sup>
	Reference range <sup>1</sup>	46-70	47-77	46-78	47-70
	Median	63	58	66	56
	(1.-3. Quartile)	46-70	47-75	46-78	48.75-67
$\alpha_1$ -globulins (%)	$\bar{x} \pm s$	2.5±2.07	2.71±2.13 <sup>a</sup>	1.33±0.58 <sup>b</sup>	3.75±3.4 <sup>a</sup>
	Reference range <sup>1</sup>	2-3	2-3	1-2	2-7
	Median	2.5	2.5	1	3
	(1.-3. Quartile)	2-3	2-3	1-2	2-6.3
$\alpha_2$ -globulins (%)	$\bar{x} \pm s$	4.88±3.13	4.86±3.38 <sup>a</sup>	2.67±1.2 <sup>b</sup>	6.50±3.7 <sup>a</sup>
	Reference range <sup>1</sup>	2-9	2-9	2-4	2-10
	Median	5	5	2	7
	(1.-3. Quartile)	2-9	2-9	2-4	2.8-9.8
$\beta_1$ -globulins (%)	$\bar{x} \pm s$	9±3.2	9.29±3.4 <sup>a</sup>	7±2.6 <sup>b</sup>	11±2.9 <sup>a</sup>
	Reference range <sup>1</sup>	7-10	7-10	5-10	8-14
	Median	9	9	6	11
	(1.-3. Quartile)	7-10	7-10	5-10	8.3-13.8
$\beta_2$ -globulins (%)	$\bar{x} \pm s$	6.71±4.8	7.5±4.8	9.5±4.9	6.5±5.1
	Reference range <sup>1</sup>	4-12	6-14	6-13	3-14
	Median	6.5	7.5	9.5	4.5
	(1.-3. Quartile)	3.5-12.8	5.5-11.8	4.5-9.8	3.3-11.8
$\gamma$ -globulins (%)	$\bar{x} \pm s$	15.63±6.7	17.14±5.6	19.67±7.5	15.25±3.8
	Reference range <sup>1</sup>	11-18	11-19	12-27	10-18
	Median	16	17	20	16.5
	(1.-3. Quartile)	11-18	11-19	12-27	11.3-18
A/G ratio	$\bar{x} \pm s$	3.7±4.71	3.71±5.8	2.33±1.5	4.75±6.8
	Reference range <sup>1</sup>	3-4	3-4	1-4	1-15
	Median	3.5	3.5	2	1.5
	(1.-3. Quartile)	3-4	3-4	1-4	1-11.8
serum-[Ca <sup>++</sup> ] (mmol/l)	$\bar{x} \pm s$	8.33±1.2	5.67±1.2	5.67±0.58	7.2±1.8
	Reference range <sup>1</sup>	7-9	5-7	5-6	5-9
	Median	9	5	6	7
	(1.-3. Quartile)	7-9	5-7	5-6	5-9
serum-[Mg <sup>++</sup> ] (mmol/l)	$\bar{x} \pm s$	2±0.01	1.67±0.58	2±0.01	1.80±0.44
	Reference range <sup>1</sup>	2-2.1	1-2	2-2.1	1-2
	Median	2	2	2	2
	(1.-3. Quartile)	2-2.1	1-2	2-2.1	1.5-2
serum-[Pi] (mmol/l)	$\bar{x} \pm s$	6±0.04	5±1.0	5.67±0.58	4.8±0.45
	Reference range <sup>1</sup>	6-6	4-6	5-6	4-5
	Median	6	5	6	5
	(1.-3. Quartile)	6-6.2	4-6	5-6	4-5

<sup>1</sup>m± s.d.×1.96 indicated the lower and the upper limits, Brackets ([ ]) denote concentration.

Aa, Bb Overall means within the same row bearing different superscripts are significantly different at P≤0.05.

higher (P<0.05) than in dry adult ones, while Hb was lower (P<0.05). The PCV, MCV, MCH, MCHC, TLC, the percentage of neutrophils, lymphocytes, eosinophils and

basophils, the blood glucose, serum TP, albumin and  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$  and  $\gamma$ -globulins did not change significantly in the growing females compared to the dry ones.

Fig 1 illustrates a serum protein electrophoretic pattern with six fractions. Albumin fraction exhibited approximately a similar pattern in growing (a) and dry females (b), while  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$  and  $\gamma$  fractions differed from those in dry adults.

### **Blood parameters of late pregnant and early lactating camels**

The TEC and Hb were significantly higher ( $P<0.05$ ) in late pregnant camels than in dry ones, while Hb was lower ( $P<0.05$ ) in early lactating camels than in late pregnant and dry ones. Neutrophils percentage was significantly higher ( $P<0.05$ ) in early lactating camels than in dry females. In contrast, their percentage was lower ( $P<0.05$ ) in late pregnant camels than in early lactating and dry ones. Lymphocytes percentage was significantly higher ( $P<0.05$ ) in late pregnant camels than in early lactating and dry ones. The PCV, MCV, MCH, MCHC and TLC values and the percentage of eosinophils and basophils did not change significantly in late pregnant and early lactating camels.

Serum TP was significantly higher ( $P<0.05$ ) in late pregnant and early lactating camels than in dry ones (Table 2). The albumin fraction percentage was significantly higher ( $P<0.05$ ) in late pregnant camels than in dry and early lactating ones; however, the percentage was significantly lower ( $P<0.05$ ) in early lactating animals than in dry females. The percentage of  $\alpha_1$ ,  $\alpha_2$  and  $\beta_1$ -globulin fractions were lower ( $P<0.05$ ) in late pregnant than in dry and early

lactating camels, while the highest significant ( $P<0.05$ ) percentage was observed during early lactation. Camels in late pregnancy and early lactation revealed no substantial change in blood glucose,  $\beta_2$ - and  $\gamma$ -globulins, A/G ratio and serum  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and Pi.

Fig 2 shows albumin fraction exhibited approximately similar pattern in dry (a), late pregnancy (b) and early lactation (c). However,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$  and  $\gamma$ - globulins displayed different patterns in response to the physiological state.

### **Discussion**

Determination of the reference ranges for blood parameters is essential for monitoring health, prognosis, diagnosis and treatment of diseases. To the author's knowledge, there is relatively little published data on the reference intervals of blood parameters in female camels. Therefore, we established a reference range for these parameters during various physiological states, which might be utilised for the clinical monitoring of females health. In dromedary camels, most prior reference values were obtained on undefined and relatively small groups, with no consideration given to the physiological states (Faye and Bengoumi, 2018). In clinical practice, it is critical to assess the number of animals required for establishing representative reference ranges.

In this study, we applied equation (1), as established by Werner (1992), to determine the minimum number of samples ( $n_{Min}$ ) required. Equation (1) clearly demonstrated that the reference

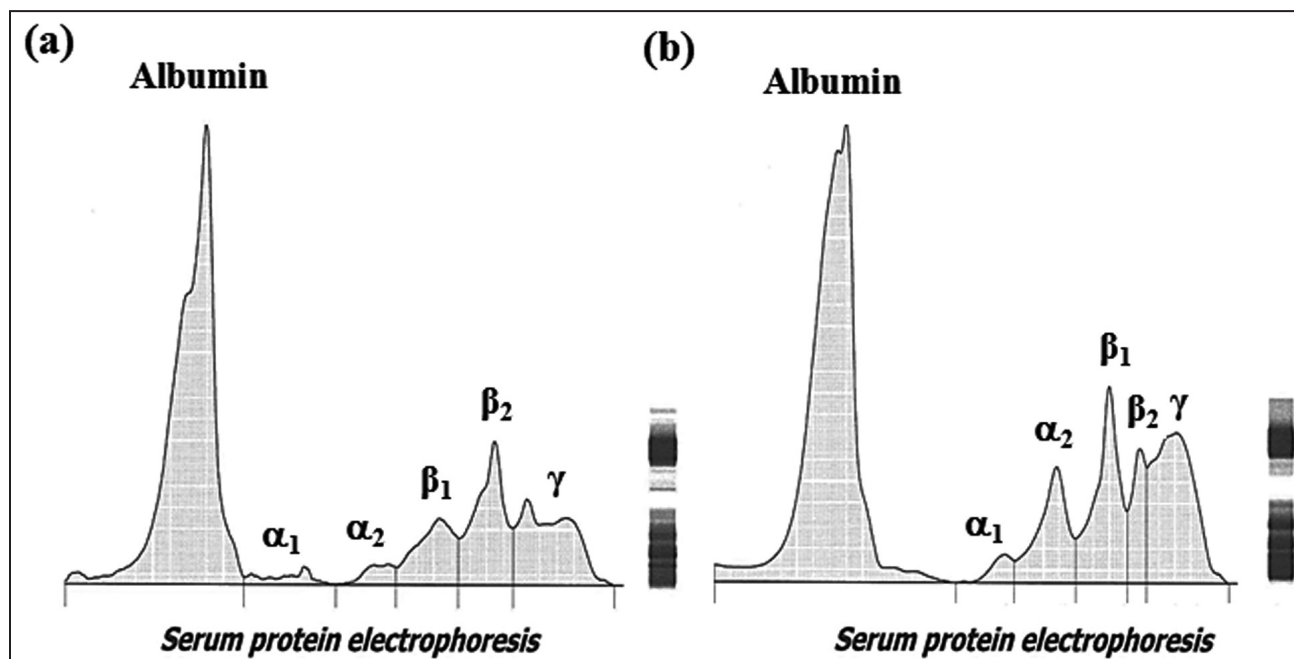


Fig 1. Serum protein capillary electrophoresis pattern of the growing (a) and dry adult female camels (b).

values for these parameters are mostly determined by the standard deviation of their individual values. The confidence level of  $\Delta = 1 \mu\text{l, g/dL, mmol/L}$  and  $\text{g/L}$  indicates that the average  $\delta$  of  $x$  values in the population with an error probability of  $\alpha = 0.05$  should not deviate more than  $1 \mu\text{l, g/dL, mmol/L}$ , or  $1 \text{g/L}$  from the mean value of the selected groups. The PCV, TLC and blood glucose demonstrated no significant differences between the experimental females (Tables 1 and 2); therefore, we computed a mean value for the standard deviation (s) of all groups for these parameters and substituted the estimated values of  $s = 4.8$  as  $\delta$  in Equation (1):

$$n_{\text{Min}} \geq \left(\frac{1.96}{1}\right) \cdot (4.8)^2 = 45 \text{ animals}$$

The determined minimum number of camels ( $n_{\text{Min}}$ ) equal to or less than 45 represents the precise number of females in each group; hence, the reference ranges for each parameter suggest reference values under defined physiological conditions. The  $n_{\text{Min}}$  for all parameters studied using the same equation was comparable to or less than the minimum number of animals employed (Tables 1 and 2).

The mean and reference interval for the erythrocytes parameters (PCV, Hb, TEC, MCV, MCH and MCHC) reported for the growing, dry, late pregnant and early lactating camels were similar to the reference range of dromedary camels (Faye and Bengoumi, 2018; Waziri *et al*, 2019; Islam *et al*, 2019; Martín-Barrasa *et al*, 2023).

The current study demonstrated significantly lower TEC and Hb in growing females than in dry ones, while PCV, MCH, MCV and MCHC exhibited no change. Conversely, many investigators reported significant changes in PCV, MCV, MCH and MCHC with age in camels (Al-Sultan, 2008; Elkhair and Elmgoboul, 2015; Tharwat *et al*, 2015). Significant lower

values of TEC and Hb have been reported by Yagoub (1988), who concluded that camels aged one to five years had lower TEC values than camels aged more than five years. Furthermore, Al-Busadah and Osman (2000) found that age influenced the erythrocytes parameters, with younger camels exhibiting lower TEC and Hb values than the older camels. In contrast, young camels showed higher TEC and Hb values than the older ones (Hussein *et al*, 1992; Martín-Barrasa *et al*, 2023; Monaco *et al*, 2024). Moreover, camel calves had significantly lower values for TEC and Hb than lactating females (Al-Rammahi *et al*, 2016).

The pattern of erythrocytes parameters (higher TEC and Hb) observed during late pregnancy could be explained by the increased metabolic demand for oxygen consumption required for rapid foetal growth and lactogenesis and suggesting a potential adaptation to the increased metabolic demands during pregnancy and redistribution of resources towards lactation. Conversely, TEC and Hb manifested significantly lower values in late pregnant and early lactating camels compared to early lactating and dry ones (Hussein *et al*, 1992; Al-Busadah and Osman, 2000; Elkhair, 2019; Mohamed *et al*, 2021). Saeed *et al* (2011) reported lower TEC, Hb and PCV in pregnant than those in non-pregnant camels. Other investigators reported higher values of Hb, PCV, TEC, MCV and MCH in late pregnant camels compared to non-pregnant (Abd El-Salaam and Arafa, 2018; Abdul-Rahaman *et al*, 2018), whereas TEC and MCV were significantly lower in lactating camels than those in non-lactating (Mohamed *et al*, 2021). Significantly higher values of PCV, Hb and TEC were recorded in pregnant and lactating camels and the transition period (Getnet and Abebe, 2005; Tharwat *et al*, 2015; Abd-El-Rahman *et al*, 2017). The non-significant variations in PCV and haematological induces (MCV,

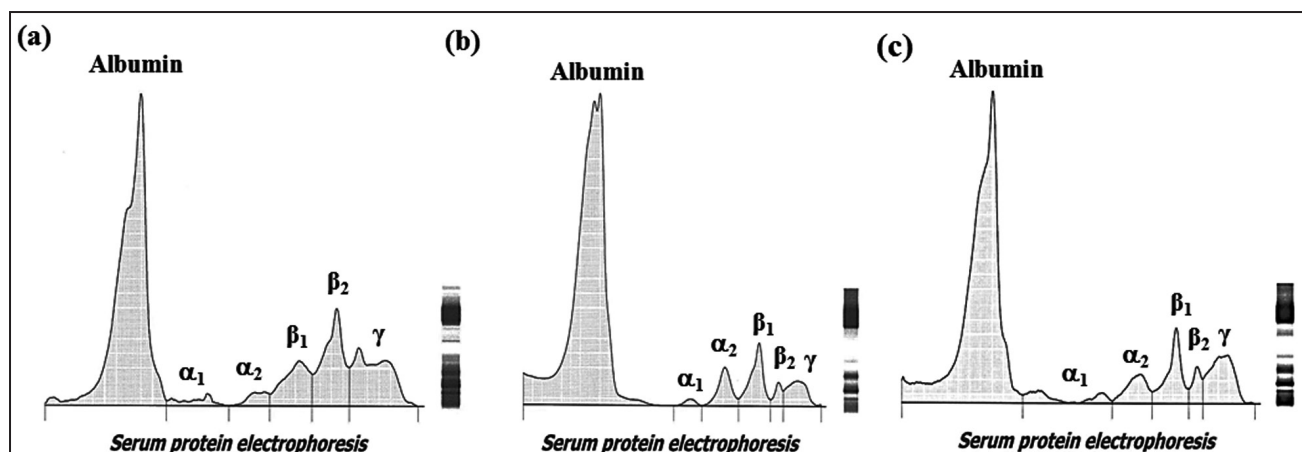


Fig 2. Serum protein capillary electrophoresis pattern of dry (a), late pregnant (b) and early lactating (c) camels.

MCH and MCHC) values recorded are comparable to those reported in late pregnant and early lactating camels and transition period (El Zahar *et al*, 2017; Elkhair and Minawy, 2018; Monaco *et al*, 2024).

The TLC in female camels corresponded with the reference range demonstrated in the literature for dromedary camels (Faye and Bengoumi, 2018; Waziri *et al*, 2019; Islam *et al*, 2019; Martín-Barrasa *et al*, 2023; Monaco *et al*, 2024). The TLC and eosinophils and basophils percentages did not change significantly in the growing females. The findings are comparable to previous results documented by many investigators in camels (Elkhair and Elmgbou, 2015; Omer *et al*, 2016; Ahmed, 2017). However, other investigators reported significant changes in TLC with age (Hussein *et al*, 1992; Al-Sultan 2008; Saeed and Hussein, 2008; Tharwat *et al*, 2015; Islam *et al*, 2019; Gaashan *et al*, 2020). Furthermore, TLC, eosinophils and basophils percentages did not differ significantly in late pregnant and early lactating animals; however, Axay *et al* (2017) reported marked leukocytosis in early lactating camels compared to late pregnant ones. Moreover, Al-Busadah and Osman (2000) reported that TLC of young camels was significantly higher compared to lactating camels. In other studies, TLC remained unchanged in late pregnant and lactating compared to dry camels (Ahmed, 2017; Elkhair and Minawy, 2018; Mohamed *et al*, 2021). The slight non-significant higher values of TLC recorded during early lactation compared to the dry females is attributed to neutrophilia recorded (Table 1).

The significant monocytosis obtained for the growing females contradicts the previous reports (Al-Sultan, 2008; Elkhair and Elmgbou, 2015; Omer *et al*, 2016). On the other hand, the significant neutrophilia and lymphocytosis observed during early lactation and late pregnancy, respectively, compared to the dry ones, potentially indicate an immune response to the stress of pregnancy, parturition and lactation, as well as the release of ACTH and cortisol (Getnet and Abebe, 2005; Omer *et al*, 2016; Ahmed, 2017; Mohamed *et al*, 2021). Neutrophilia and lymphocytosis during early lactation and late pregnancy were consistent with previous findings in camels (Muhammad *et al*, 2011; Tharwat *et al*, 2015; El Zahar *et al*, 2017; Ebissy *et al*, 2019; Elkhair, 2019).

The blood glucose concentration in camels, a key indicator of metabolic health, is comparable to the reference range established by Faye and Bengoumi (2018). The non-significant variation in glucose concentration in growing camels, specifically those between the ages of 1-5 years, is particularly

significant as it aligns with a previous study by Ghodsian *et al* (1978), which found no significant difference in camels up to five years old. However, it also revealed a contrasting trend, with many investigators reporting that young camels, typically under the age of 5 years, were more hyperglycemic than adults (Elias and Yagil, 1984; Faye and Mulato, 1991; Souilem *et al*, 1999; Ben Romdhane *et al*, 2003; Roba *et al*, 2023). These findings underscore the importance of age as a factor in blood glucose regulation in camels.

It is widely known that the blood glucose in camels increases with progressive pregnancy and decreases during the first two weeks of lactation (Kelanemer *et al*, 2015; Souilem *et al*, 1999). The current study found no significant differences in glucose concentration in late pregnant and early lactating camels. The results are consistent with previous findings reported in late pregnant, early lactating camels and the transition period, such as those by Tharwat *et al* (2015), El Zahar *et al* (2017), Ebissy *et al* (2019), Faraz *et al* (2021) and Mohamed *et al* (2021). The non-significant hypoglycemia reported in early lactating camels compared to late pregnant ones is most likely attributable to volume expansion combined with increased consumption of large amounts of blood glucose for lactose synthesis (Jainudeen and Hafez, 1992; Kaneko *et al*, 2008). Many investigators concluded that the potential synthesis of milk lactose was linked to increased glucose uptake by the mammary glands (Afshar and Fathi, 2012; Zhao, 2014).

It is well established that protein requirement increases with progressive pregnancy and the onset of lactation, as indicated by decreased maternal serum protein concentration (Muhammad *et al*, 2011; Saeed *et al*, 2011; Roba *et al*, 2023). Farm animals consume more amino acids from the maternal bloodstream for protein synthesis due to the progressive increase in foetal growth (Jainudeen and Hafez, 1994). Compared to dry ones, the considerable hyperproteinemia observed in late pregnant camels combined with marked variations in albumin and  $\alpha 1$ ,  $\alpha 2$  and  $\beta 1$ -globulins, indicates the critical metabolic changes during these stages required foetal growth and lactogenesis. The findings reveal unique metabolic changes in camels during late pregnancy and early lactation, such as hyperproteinemia and variations in albumin and globulin fractions, which are more pronounced in late pregnant and early lactating animals compared to dry females. These changes suggest a potential role of albumin and globulins in

regulating dynamic balance and immune response, respectively.

Camels in late pregnancy and early lactation revealed no substantial change in globulin fractions ( $\beta_2$ - and  $\gamma$ ) and A/G ratio, confirming the stability of these parameters during these stages. Generally, the significant changes in serum protein profile observed in late pregnancy ( $\uparrow$ TP and albumin ratio and  $\downarrow\alpha_1$ ,  $\alpha_2$  and  $\beta_1$ -globulins) followed by a marked hypoproteinemia in early lactating camels than those in the dry females reflects the maternal requirement of proteins for foetal growth or to cope with colostrum and milk synthesis and production and providing immunoglobulins. Many investigators explained the changes in serum protein profile during late pregnancy and early lactation by the increased maternal requirement of proteins for foetal growth, colostrum and immunoglobulins synthesis (Kaneko *et al*, 2008; Tharwat *et al*, 2015; Elkhair, 2019). In contrast, pregnant and lactating camels showed a marked decrease in serum protein parameters (Elkhair and Hartmann, 2014; Kelanemer *et al*, 2015; Tharwat *et al*, 2015; Elkhair, 2016; 2019; El Zahar *et al*, 2017; Elkhair and Minawy, 2018; Elkhair *et al*, 2018; Ebissy *et al*, 2019; Mohamed *et al*, 2021).

Serum protein capillary electrophoretic pattern is widely used to indicate health status and may act as crucial diagnostic and prognostic biomarkers for numerous pathogenic disorders (Alberghina *et al*, 2011; Yang *et al*, 2012). The current results validated the application of capillary electrophoresis for serum protein fractionation in female camels during different physiological states. Many researchers found that age and growth phase have a significant influence on serum proteins electrophoresis components: albumin,  $\alpha_1$ ,  $\alpha_2$ ,  $\beta_1$ ,  $\beta_2$ ,  $\gamma$ -globulins and A/G ratios in camels (Ahmadi-hamedani *et al*, 2014; Elkhair and Hartmann, 2014; Abdoslam *et al*, 2018). Serum protein capillary electrophoretic pattern obtained produced six fractions, including albumin and five globulin fractions, consistent with previous camel results (Chaudhary *et al*, 2003; Abdoslam *et al*, 2018). The progressive, non-significant increase in globulin fractions observed in dry adult females compared to the growing camels can provide an age-related explanation. The significant decrease in  $\alpha_1$ ,  $\alpha_2$  and  $\beta_1$  percentages during early lactation is probably due to the decrease in various  $\alpha$ -fraction molecules and the passage of  $\beta_1$ -fraction molecules from the bloodstream to the mammary glands for milk and colostrum biosynthesis (Kaneko *et al*, 2008). The significant increase in albumin fraction percentage combined

with a significant decrease in  $\alpha_1$ -,  $\alpha_2$ - and  $\beta_1$ -globulins has been observed in lactating camels, dairy cows and ewes (Piccione *et al*, 2011, 2012; Elkhair and Hartmann, 2014; Ebissy *et al*, 2019; Tóthová *et al*, 2018; Mohamed *et al*, 2021; Adam and Elkhair, 2023). In the present study,  $\gamma$ -globulins and A/G ratio showed non-significant changes during late pregnancy and early lactation. Conversely, significant higher values of  $\gamma$ -globulins accompanied by lower A/G ratio in lactating camels (Elkhair and Hartmann, 2014).

All animal species require mineral for growth, reproduction and lactation (Underwood and Suttle, 1999). The serum mineral profile obtained for  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and Pi concentrations is comparable to the reference values for camels (Elkhair, 2016; Faye and Bengoumi, 2018). In the growing females, the significant hypercalcemia, hypermagnesemia and hyperphosphatemia observed could be attributable to their higher mineral requirements for growth. This finding raises intriguing questions about the unique mineral needs of growing female dromedary camels and the potential implications for their health and development. Goff (2015) stated that  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and Pi were essential for blood and muscles functions, enzymatic reactions, cellular components synthesis and energy transferring molecules. Extensive research has consistently shown that young camels are hypercalcemic (Elias and Yagil, 1984; Rezakhani *et al*, 1997; Al-Busadah 2003, 2010; Ben Romdhane *et al*, 2003; Saeed *et al*, 2004; Barri *et al*, 2005; Tajik *et al*, 2015, Martín-Barrasa *et al*, 2023), hypermagnesemic (Barri *et al*, 2005; Tharwat *et al*, 2015; Elkhair, 2019; Martín-Barrasa *et al*, 2023) and hyperphosphatemic (Saeed *et al*, 2004; Al-Busadah, 2010; Elkhair, 2016; Martín-Barrasa *et al*, 2023) than adults. However, other researchers concluded that age had no remarkable influence on  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and Pi (Faye and Mulato, 1991; Saeed *et al*, 2004; Tajik *et al*, 2015; Tharwat *et al*, 2015; Elkhair, 2016).

The serum concentrations of  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$  and Pi in late pregnant and early lactating animals remained stable, a finding that aligns with previous reports in lactating camels (Singh *et al*, 2015; Tharwat *et al*, 2015; Ebissy *et al*, 2019). However, the observation of non-significant hypercalcemia and hypermagnesemia during these critical periods challenges the previously reported considerable hypocalcaemia and hypomagnesemia in camels. These conditions were attributed to excessive losses through urine and colostrum or impaired absorption of these minerals from the alimentary tract (Eltohamy *et al*, 1986; Kuria *et al*, 2006). The lack of statistical significance in serum



Pi during late pregnancy or early lactation also echoes the findings of several camel researchers (Khadjeh, 1998; Saeed *et al*, 2009; Ahmed, 2017; Faraz *et al*, 2021). However, a contrasting study by Mohamed *et al* (2021) suggests that hyperphosphatemia during early lactation is most likely caused by enhanced growth hormone (GH) activity combined with intestinal absorption and renal reabsorption of Pi, which stimulates GH synthesis by the alveolar cells of the mammary glands. This finding could have significant implications for our understanding of camel health and the management of late pregnancy and early lactation.

### Conclusion

Blood parameters were affected significantly by the age, growth phase, late pregnancy and early lactation in female camels reared under a semi-intensive system. Critical alterations in these parameters concerning the corresponding physiological state could be associated with growth requirements, intensive foetal growth and lactogenesis. The data could be employed to evaluate the metabolic profile of growing, pregnant and lactating camels and to develop efficient management strategies for females in a semi-intensive system.

### Abbreviations

Hb: Hemoglobin; PCV: Packed cell volume; TEC: Total erythrocytes count; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; TLC: Total leukocytes count; Serum-[TP]: Serum total protein concentration;  $[Ca^{++}]$ ,  $[Mg^{++}]$  and  $[Pi]$ : calcium and magnesium and inorganic phosphate concentration.

### Funding

The research project was funded by the Ministry of Higher Education and Scientific Research (Sudan) and the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (Grant No. 5, 960). Nawal M. Elkhair was a recipient of the research fund. The funder had no role in study design, data collection and analysis, publication decisions, or manuscript preparation.

### Acknowledgements

The author would like to thank and acknowledge the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University (Saudi Arabia) and

the technician team of the Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum (Sudan) for financial support and laboratory assistance.

### Conflict of Interest

The author declares no conflict of interest.

### Data Availability data Statement

The data is available in the manuscript and from the corresponding author upon reasonable request.

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