

The concentration of serum proteins and protein fractions during the transition period in camels (*Camelus dromedarius*)

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Abstract

This study aimed to evaluate the changes in serum protein fractions during the transition period in camels. Forty-five non-pregnant and non-lactating Arabian camels (age: 11–16 years, body weight: 600–700 kg, number of parities: 2–3) were used to provide the reference and percentile ranges of serum protein fractions. In the experiments, twenty-two time-mated multiparous pregnant camels of the same age and weight were monitored from one month prepartum (late pregnancy) to one month postpartum (early lactation). Blood samples were collected by jugular venipuncture four times: -30 and -15, and +15 and +30 days pre- and postpartum, respectively, and the serum harvested was used to determine the concentrations of total protein (TP) and protein fractions. Capillary electrophoresis of serum proteins revealed one albumin band and five globulin bands: α_1 , α_2 , β_1 , β_2 and γ -fractions. Serum TP and albumin and β_2 obtained on days -30 and -15 did not differ significantly from the corresponding values on +15 and +30 days; however, α_1 , α_2 , β_1 and γ -fractions, and A/G ratio showed significant differences ($P \leq 0.05$) during late pregnancy and early lactation. The overall mean of TP showed significantly ($P \leq 0.05$) higher values during late pregnancy and early lactation. At the same time, only albumin concentration (g/L) was significantly higher ($P < 0.05$) during late pregnancy compared to the control. The concentrations of α_1 (g/L and %) were significantly ($P < 0.01$) lower during late pregnancy and early lactation compared to the control; the significant lowest value was observed during late pregnancy. Significantly lower concentrations of α_2 ($P < 0.0001$) were observed during late pregnancy compared to the control, whereas α_2 values were higher ($P < 0.0001$) during early lactation compared to the control and late pregnancy. The β_1 concentrations (g/L and %) were significantly ($P < 0.01$) higher during early lactation compared to the control and late pregnancy. Transition period had no significant effect on β_2 concentrations; however, significantly higher ($P < 0.01$) values were observed during late pregnancy and early lactation compared to the control. The concentrations of γ -globulins (g/L and %) showed a gradual significant increase ($P < 0.01$) during late pregnancy and early lactation compared to the control. The A/G ratio was significantly higher ($P < 0.01$) during late pregnancy compared to the control; however, it showed a lower value ($P < 0.01$) during early lactation compared to the control and late pregnancy. The considerable alterations in α -, β - and γ -globulins fractions during the transition period constituted an adaptive physiological response to immunoglobulins transfer, colostrum, and milk synthesis.

Keywords: Camel, transition period, serum protein fractions

Introduction

Camels (*Camelus dromedarius*) are well adapted to harsh climatic conditions; therefore, they are recommended as suitable species for sustainable livestock production (Schwartz, 1992; Faye, 2016). However, one of the limitations and constraints in the camel industry is the low reproductive efficiency and calving rate that causes infertility and reproductive loss (Tibary et al., 2006). Reproductive diseases, infections, repeated breeding, ovulation failure, early embryonic death and fetal loss, and abortion are the most common problems facing productivity in camels (Ali et al., 2010; Khalafalla et al., 2017; El-Malky et al., 2018; Bello and Bodinga 2020; Elshazly et al., 2020; Belina et al., 2021).

The transition period from late pregnancy to early lactation is a physiological state that has been demonstrated to modify protein metabolism in camels (Ahmed and Elkhair, 2019). Other investigators stated that female camels during the transition period experience substantial oxidative stress, as indicated by the increased per-oxidative by-products of proteins in parallel to the enhanced production of pro-oxidative stress biomarkers (Saleh et al., 2021). It is well documented that the demands of proteins increased during the transition period to maintain the mother's well-being, rapid fetal growth, and for milk and immunoglobulins production (Kaneko et al., 2008). Therefore, serum protein fractions have been successfully used to assess and evaluate the changes in protein metabolism in animals (Tóthová et al., 2018). Many investigators

observed significant alterations in serum proteins pattern during the transition period in camels (Kelanemer et al., 2015; Tharwat et al., 2015; Elkhair et al., 2018; Ebissy et al., 2019).

Few data are available concerning the changes in serum protein fractions during the transition period in camels. Therefore, the study aimed to assess the changes in the serum protein profile in periparturient camels, which can help to identify the possible risks associated with protein metabolism.

Materials and Methods

Ethical approval

The study was approved by The Sudan Veterinary Council (EA/0030/2018). All experimental procedures, including animal care, were conducted in accordance with the standards established by the Faculty of Veterinary Medicine Research Ethics Committee (University of Khartoum, Sudan).

Animals and management

A total number of sixty-seven clinically healthy female camels were selected from the herd of the Camel Research Centre of the University of Khartoum, Sudan. Forty-five non-pregnant and non-lactating Arabian camels (age: 11–16 years, body weight: 600–700 kg, number of parities: 2–3) were used to provide the reference and percentile ranges of serum protein parameters (control animals). In the experiments, twenty-two time-mated multiparous pregnant camels of the same age and weight were monitored from one month prepartum (late pregnancy) to one month postpartum (early lactation). The

animals were reared under a semi-intensive system and maintained on grazing and browsing trees for 2 hours daily, in addition to a daily concentrate ration, which consisted of a mixture of 25% groundnuts cake, 35% sorghum feterita (*Sorghum bicolor* L), 25% wheat bran, 3% molasses, 1% limestone, 1% NaCl salt and Sorghum Abu 70 (*Sorghum bicolor* L) (10%), which was offered twice, at 7:00 am and 18:00, with free access to fresh water. The ration provided to the experimental camels was composed of 93.78% dry matter intake, 14.95% crude protein, 9.32% crude fibre, 5.25% ash, 1.47% fat, 62.80% nitrogen free extract and 11.41 MJ/kg metabolizable energy.

Blood collection and laboratory analysis

The blood samples were collected once from the control animals and four (4) times from the experimental animals at two week intervals (-30 and -15, and +15 and +30 days pre- and postpartum, respectively) in the morning before feeding by jugular venipuncture using plastic disposable syringes (7.5 mL, Pirmvetta®, Laboratory Technique, GmbH, Germany). The blood was drawn into clean, dry plain vacutainer tubes and allowed to stand at room temperature for 3 hours, then centrifuged at 3000 rpm for 15 min using a bench centrifuge (2002, Germany). The serum samples were stored in clean Eppendorf tubes and frozen at -20°C until further analysis.

Serum total protein and capillary electrophoresis:

Serum total protein (TP) was determined by the Biuret method according to Weichselbaum (1946) using commercial kits

(Bio-systems, S. A. Barcelona, Spain).

Serum protein fractions (albumin, α_1 , α_2 , β_1 , β_2 and γ -globulins) were analyzed using a capillary electrophoresis technique (Automated MINICAP-SEBIA Instrument, France) using the MINICAP CDT kits (France). The automated system was regularly monitored for accuracy and precision in accordance with MINICAP Manual (Sebia-MINICAP CDT, Ref. 2208) guidelines.

Statistical analysis

The sample size calculation for this study was established using the Resource Equation Approach (Mead, 1988; Arifin and Zahiruddin, 2017). Statistical analysis was performed using IBM SPSS Statistics 22 (Chicago, USA). The One-Sample Kolmogorov-Smirnov test for normality was applied to estimate the normal distribution of the values; the data were normally distributed. Descriptive statistics procedures, One-way ANOVA and Pearson correlation were used to assess the possible significant differences between late pregnant and lactating camels at $P \leq 0.05$.

Results

Table 1 shows the reference intervals for serum TP, albumin and globulin fraction concentrations, and A/G ratio in 45 control females raised under the same conditions as late pregnant and early lactating camels.

Table 1. Reference intervals for the concentrations of serum total protein (TP), albumin and globulins fractions, and A/G ratio of control female camels (*Camelus dromedarius*) (n=45).

Parameter	Mean $\pm$ SD	Reference range ¹	Median	(1.-3. Quartile)
TP (g/L)	42 $\pm$ 4.6	38–47	41	41–47
Albumin (g/L)	27 $\pm$ 8.2	28–33	28	28–40
Albumin (%)	65 $\pm$ 17.5	47–82	66	66–82
α -fraction				
α_1 (g/L)	1.2 $\pm$ 1.3	0.3–2.7	0.3	0.6–2.7
α_1 (%)	3 $\pm$ 3.5	1–7	1	1–8
α_2 (g/L)	2.1 $\pm$ 1.4	0.8–3.6	0.8	0.8
α_2 (%)	5.7 $\pm$ 4.0	2–10	5	5–10
β -fraction				
β_1 (g/L)	3.6 $\pm$ 1.5	2.5–5.3	2.5	2.9–5.3
β_1 (%)	8.7 $\pm$ 4.7	5–14	7	7–14
β_2 (g/L)	1.6 $\pm$ 1.1	0.7–2.8	0.7	1.4–2.8
β_2 (%)	4 $\pm$ 2	2–6	4	4–6
γ -fraction (g/L)	6.1 $\pm$ 3.8	1.9–9.4	1.9	7–9.4
γ -fraction (%)	14.33 $\pm$ 8.15	5–20	18	18–20
A/G ratio	2.33 $\pm$ 1.53	1–4	2	2–4

¹ Mean $\pm$ SD $\times$ 1.96 indicated the lower and the upper limits, SD: Standard deviation

Capillary electrophoresis of serum proteins from the control, late pregnant, and early lactating camels revealed one albumin band and five globulin bands: α_1 , α_2 , β_1 , β_2 and γ fractions, which varied substantially from the control regardless of late pregnancy or early lactation.

Table 2 reveals that serum TP, albumin and globulins fractions obtained on days -30 and -15 did not differ significantly from the

corresponding values on +15 and +30 days, except β_2 - fractions and A/G ratio showed significant differences ($P \leq 0.05$) during late pregnancy and early lactation. The overall mean of TP showed significantly higher values during late pregnancy ($P < 0.01$) and early lactation ($P < 0.05$) compared to the control (Table 2). The overall mean of albumin fraction concentration (g/L) was significantly higher during late pregnancy

($P < 0.05$) compared to the control, while early lactation had no significant effect on albumin concentration (Table 2).

Table 2 demonstrates that the concentrations of α_1 and α_2 (% and g/L) were higher ($P < 0.01$) on days -30 and -15 compared to the values obtained on days +15 and +30. The overall mean values of α_1 -fraction concentration were significantly ($P < 0.01$) lower during late pregnancy and early lactation compared to the control; the significantly lowest value was observed during late pregnancy (Table 2 and Figure 3). The overall mean values of α_2 -fraction concentration were significantly ($P < 0.0001$) lower during late pregnancy compared to the control, whereas α_2 -fraction values were significantly ($P < 0.0001$) higher during early lactation compared to the control and late pregnancy (Table 2).

The concentration of β_1 values (% and g/L) were higher ($P < 0.01$) on days -30 and -15 compared to those obtained on days +15 and +30. The overall mean values of β_1 -fraction

concentration were significantly ($P < 0.01$) higher during early lactation compared to the control and late pregnancy (Table 2). The transition period had no significant effect on β_2 -fraction concentration; however, significantly ($P < 0.01$) higher values were observed during late pregnancy and early lactation compared to the control.

The concentration of γ -fraction and A/G ratio values were higher ($P < 0.01$) on days -30 and -15 compared to those obtained on days +15 and +30 (Table 2). The overall mean values of γ -fraction concentration (% and g/L) showed a gradual significant ($P < 0.01$) increase during late pregnancy and early lactation compared to the control (Table 2). The mean values of A/G ratio were significantly ($P < 0.01$) higher during late pregnancy compared to the control; however, A/G ratio showed lower values during early lactation compared to the control and late pregnancy.

Table 2. The concentrations of serum total protein (TP), albumin and globulins fractions, and A/G ratio in female camels (*Camelus dromedarius*).

Parameter	Control (n = 45)	Late pregnancy (n = 11)			Early lactation (n=11)		
		- 30	- 15	Overall mean	+ 15	+ 30	Overall mean
TP (g/L)	42 ± 4.6 ^{A**}	60.8 ± 7 ^b	61.2 ± 3.5 ^b	60.1 ± 10.5 ^{B**}	62.4 ± 3.8 ^b	60.2 ± 4.4 ^b	61.5 ± 8.8 ^{B**}
Albumin (g/L)	27 ± 8.2 ^{B*}	26.3 ± 2.3 ^a	33.4 ± 2 ^a	37.2 ± 6.6 ^{A*}	32.7 ± 5.7 ^a	30.3 ± 4.5 ^a	31.7 ± 4.3 ^{AB*}
Albumin (%)	65 ± 17.5 ^A	62.7 ± 11.4 ^a	64.3 ± 10 ^a	63.6 ± 15.4 ^A	54.1 ± 2.7 ^a	51.6 ± 7.4 ^a	52.1 ± 7.4 ^A
α-fraction:							
α ₁ (g/L)	1.2 ± 1.3 ^{B**}	2.0 ± 0.4 ^c	1.6 ± 0.3 ^c	0.7 ± 0.4 ^{C**}	2.0 ± 0.6 ^a	2.8 ± 0.1 ^a	1.6 ± 0.3 ^{A**}
α ₁ (%)	3.0±3.5 ^{A**}	1.1±0.3 ^c	1.4±0.6 ^c	1.6±0.5 ^{C**}	2.5±0.4 ^b	2.8±0.1 ^b	2.6±0.6 ^{B**}
α ₂ (g/L)	2.1±1.4 ^{A***}	2.0±0.1 ^a	2.5±0.8 ^a	1.7±0.4 ^{A***}	5.7 ± 1.7 ^b	6.3 ± 0.9 ^b	5.6±1.2 ^{B***}
α ₂ (%)	5.7±4.0 ^{B***}	3.0±1.1 ^c	2.8±1.0 ^c	3.1±1.0 ^{C***}	8.9 ± 1.1 ^a	9.0±1.3 ^a	9.0±1.6 ^{A***}
β-fraction:							
β ₁ (g/L)	3.6 ± 1.5 ^{B**}	3.4 ± 1.0 ^b	5.8 ± 1.3 ^b	4.9 ± 1.6 ^{B**}	7.7 ± 0.5 ^a	8.1 ± 1.3 ^a	7.1 ± 1.6 ^{A**}
β ₁ (%)	8.7±4.7 ^{B**}	8.1±1.1 ^b	8.0 ± 1.5 ^b	8.0±1.6 ^{B**}	10.5±0.9 ^a	12.2±1.1 ^a	11.5±1.9 ^{A**}
β ₂ (g/L)	1.6±1.1 ^{B**}	6.9±1.6 ^a	7.7±3.7 ^a	7.6±3.1 ^{A**}	5.7±0.5 ^a	7.4±0.3 ^a	6.5±2.4 ^{A**}
β ₂ (%)	4.0±2.0 ^{B**}	11.2±4.7 ^a	12.0±4.7 ^a	12.2±4.7 ^{A**}	8.0±0.3 ^a	11.1±0.1 ^a	10.3±3.5 ^{A**}
γ-fraction (g/L)	6.1 ± 3.8 ^{C**}	9.6 ± 1.8 ^b	11.1 ± 3.1 ^b	10.3 ± 6.7 ^{B**}	15.4 ± 5.3 ^a	16.1 ± 3.4 ^a	15.1 ± 5.4 ^{A**}
γ-fraction (%)	14.3 ± 8.2 ^{B**}	15.9 ± 8.9 ^c	16.1 ± 8.1 ^c	16.0 ± 9.6 ^{C**}	24.3 ± 5 ^a	24.1 ± 3 ^a	24.1 ± 7 ^{A**}
A/G ratio	2.43 ± 1.5 ^{B*}	2.49 ± 1.3 ^a	2.39 ± 1.1 ^a	3.28 ± 1.8 ^{A*}	1.1 ± 0.5 ^c	1.4 ± 0.4 ^c	1.15 ± 0.4 ^{C*}

A, B, C, a, b, c Means within the same row bearing different superscripts (Capital letters between groups and Small letters within the same group) are different at *P<0.05, **P<0.01, ***P<0.0001; n: number of animals; SD standard deviation.

Discussion

The results obtained in the present study showed that the reference range for the concentrations of serum TP, albumin, and globulin fractions and A/G ratio for the control data were similar to the values reported previously for camels (Elkhair and Hartmann, 2014; Faye and Bengoumi, 2018).

The data obtained in the present study demonstrate that the transition period in dromedary camels influenced serum total protein and protein fractions as adaptive physiological mechanisms associated with rapid foetal growth and lactogenesis and the dynamic changes in the concentration of serum protein fractions observed during late pregnancy compared to early lactation.

In the current study, the higher concentration of TP observed during late pregnancy and early lactation compared to the control group is probably due to the increase in protein demands required for rapid foetal growth accompanied by higher utilization of amino acids from the maternal circulation for colostrum and milk synthesis (Jainudeen and Hafez, 1994). On the other hand, serum TP did not change significantly during late pregnancy compared to early lactation. Similar results have been reported previously by investigators during the transition period in camels (Ahmed and Elkhair, 2019; El-Sayed, 2020), whereas other researchers have reported a significant decrease in TP during the same period (Tharwat et al., 2015; El Zahar et al., 2017; Abd-El-Rahman et al., 2017; Elkhair et al., 2018). The considerable decrease in serum TP during the transition period accounted for the increased protein intake by the mammary glands for milk and immunoglobulin synthesis, as demonstrated

by the positive connection between TP and γ -globulins. Bell et al. (2000) and Kaneko et al. (2008) attributed the considerable drop in serum TP during the transition period to increased demand for proteins required for lactogenesis.

In the present study, the slight increase in albumin concentration reported during the transition period compared to the control data is most likely due to the significant influence of peripartum metabolic stress. Many researchers have speculated that albumin might counteract its consumption during such stressful conditions to maintain redox homeostasis or act as a powerful antioxidant to protect vital organs from the free radicals produced by iron/copper cations and hydrogen peroxide reaction (Belinskaia et al., 2020; Saleh et al., 2021). Many investigators concluded that serum albumin does not change significantly during the transition period in camels (Tharwat et al., 2015; Saleh et al., 2021). However, the non-significant decrease in albumin concentration during early lactation compared to late pregnancy is most likely due to the increased utilization of proteins for milk synthesis associated with the onset of milk production. Moreover, many researchers observed a significant decrease in albumin concentration during the transition period in camels (Abd-El-Rahman et al., 2017; Elkhair et al., 2018; Ahmed and Elkhair, 2019). According to Kaneko et al. (2008) and Bobbo et al. (2017), increased utilization of proteins by the mammary glands was accompanied by a significant decrease in albumin concentration.

The present results indicated that the α_1 -globulin fraction concentration recorded during the transition period was of similar value to the control data; however, α_2 -

globulins increased. The concentration of α_1 and α_2 -globulins increased significantly during early lactation compared to late pregnancy. The increase in α -globulin fractions during early lactation agrees with the findings of Piccione et al. (2011, 2012) in ewes and dairy cows. Tóthová et al. (2018) reported that α_1 - and α_2 -globulins increased markedly in periparturient dairy cows. According to Bobbo et al. (2017), the considerable increase in serum globulins concentration during early lactation in dairy cows was linked to the significant increase in α -globulins fraction. Kaneko et al. (2008) stated that the increase in α -fraction early lactation is most likely due to an increase in various acute-phase protein molecules. The significant increase in α_1 and α_2 -globulins during early lactation has been reported previously by many researchers in camels (Elkhair and Hartmann, 2014; Adam and Elkhair, 2023).

The present results indicated that the β_1 and β_2 -globulins fraction concentration recorded during the transition period was higher than the control data value and is most likely due to the significant influence of peripartum metabolic stress. In the present study, the significant decrease in β -globulins during late pregnancy could be explained by the transport of transferrin, plasminogen, fibrinogen, transcobalamin and low-density lipoproteins from the plasma to the mammary gland for colostrum and milk synthesis (Tóthová et al., 2018; Adam and Elkhair, 2023). On the other hand, β -globulins are well known to include several classes of proteins with various functions, including immunoglobulins IgA and IgM, which transport to the mammary glands for colostrum synthesis and proteins involved in inflammatory and stress

responses (Kaneko et al., 2008). Therefore, the decrease in β -globulins fraction observed during late pregnancy explained the translocation of immunoglobulins into the mammary glands and increased stress biomarker responses to late pregnancy and early lactation.

The present results indicated that the γ -globulin fraction concentration recorded during the transition period was of similar value to the control data. The significant decrease in γ -globulins during late pregnancy compared to early lactation explained the translocation of immunoglobulins (IgG, IgM, IgE) into the mammary glands required for passive immunity in neonates (Eckersall, 2008). Furthermore, the significant decrease in γ -globulins during late pregnancy associated with a significant increase in β -globulins fraction explained the translocation of immunoglobulins into the mammary glands. A similar increase in the γ -globulins fraction has been observed during early lactation in camels and dairy cows (Elkhair and Hartmann, 2014; Tóthová et al., 2018; Adam and Elkhair, 2023). In contrast, Piccione et al. (2012) reported that γ -globulins decreased significantly in early lactating ewes.

Moreover, the correlation between globulins fractions (α_1 , α_2 , β_1 and β_2 - and γ -globulins, the main contributors to stress responses and immunoglobulin production) was reported as being correlated positively during late pregnancy and early lactation. This pattern of globulin fractions response could reflect their synergic role in providing immunoglobulins and as stress biomarkers in camels during the transition period. On the other hand, the positive correlation between α_1 and α_2 , β_1 - and γ -globulins could also reflect that the

physiological adaptation to pregnancy and lactation depends mainly on α_1 , β_1 and γ globulins changes and less on TP, albumin and α_2 changes. Therefore, critical attention to this situation should be considered during the critical transition period in camels.

The results obtained in the present study clearly indicated that the shift in albumin and globulin fraction concentrations resulted in marked changes in the A/G ratio, with higher values during late pregnancy followed by lower values during early lactation. The increase in the A/G ratio reported during late pregnancy was attributed to the increased albumin fraction observed and the provision of immunoglobulins. A similar lower A/G ratio has been reported previously during early lactation in camels and dairy cows (Elkhair and Hartmann, 2014; Tóthová et al., 2018; Adam and Elkhair, 2023). Štolcová et al. (2020) observed that the A/G ratio was lower in periparturient Holstein cows. The same authors concluded that the considerable changes in the protein status of periparturient cows generally reflected the development in energy metabolism parameters. Furthermore, the higher A/G ratio observed during late pregnancy demonstrates an adaptive physiological response to the increased demands of circulatory water mobilization to the mammary glands for milk production under semi-arid environments.

In conclusion, serum protein fractions changed significantly in periparturient camels; the considerable alterations in α -, β - and γ -globulins constituted an adaptive physiological response to immunoglobulin translocation and colostrum and milk synthesis. Further studies on protein metabolism during the critical transition period in camels are required.

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Conflict of interest

The author declares that there is no conflict of interest.

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