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CLINICAL REFERENCE VALUES FOR BLOOD SERUM PROTEINS IN NOMADIC BEDOUIN CAMELS (CAMELUS DROMEDARIUS) IN THE EGYPTIAN OASIS

(With 5 Tables and 3 Figures)

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القيم المرجعية الإكلينيكية لبروتينيات مصل الدم في الجمال البدوية الرحالة (وحيدة السنم) في الواحات المصرية

مصطفى أحمد صالح ، جمال حسن السكري

وضع قيم مرجعية لنمط التحليل الكهربي الطبيعي لبروتينيات مصل الدم في الجمال البدوية باستخدام رقائق أسيتات السلولوز ، تم اختيار عدد ١٦٠ من الجمال السليمة ظاهريا من بيئتها الطبيعية في ضواحي واحة الخارجة. قسمت هذه الحيوانات بالتساوي بين موسمي الشتاء (الاعتدال المناخي) والصيف (الإجهاد الحراري الجاف) وأيضا طبقا للجنس وكذلك العمر (٨٠ حدث عمر ٢-٤ سنوات و٨٠ ناضج عمر ٧-١٠ سنوات) . أظهرت النتائج انقسام البروتينيات إلي خمسة أحزمة واضحة هي الألبومين والألفا جلوبيولين والبيتا ١ جلوبيولين والبيتا ٢جلوبيولين والجاما جلوبيولين في ٩٧,٥ في المائة من العينات بينما لم تنقسم باقي العينات (٢,٥ في المائة) في منطقة البيتاجلوبيولين إلى بيتا ١ وبيتا ٢ وأظهرت مايطلق عليه زيادة الجلوبيولين متعدد الارتجاف، وكان ذلك في الجمال المعمرة. كان متوسط القيم المسجلة (جم / ١٠٠ ملي) والانحراف المعياري للبروتينيات الكلية والألبومين والألفا جلوبيولين والبيتا جلوبيولين والجاما جلوبيولين هي ٦,٨٤٢ (٠,٠٦٧) ،٣,٤ (٠,٠٣٢) ، ٠,٨١ (٠,٠١٧) ، ١,٠١٦ (٠,٠٢٥) ، ١,٦١٦ (٠,٠٤١) على الترتيب وهذه المؤشرات يمكن استخدامها كقيم مرجعية للجمال البدوية التي ترعى بنظام الرحالة. وأظهرت النتائج أيضا أن متوسطات القيم كانت أعلى في الجمال البالغة عنها في الأحداث ولكن لم تختلف بين الذكور والإناث. كما إن أحزمة الجلوبيولينيات والجلوبيولينيات الكلية لم تتأثر بالإجهاد الحراري البيئي بينما نقص الألبومين بدون تأثير على متوسط قيم البروتينيات الكلية. كما استنتجت الدراسة أن نمط التحليل الكهربي لزلال مصل الدم يمكن أن يكون منذرا ويعطي دلالة على الأمراض غير الواضحة في الجمال كما هو الحال في السلالات الأخرى.

SUMMARY

To setup reference values for the normal patterns of blood serum protein electrophoretogram in nomadic Bedouin camels, a total number of 160 apparently healthy camels were selected from their natural nomadic habitat in suburban areas at El-kharga Oases for blood sampling. The selection of these animals was designed to obtain equal distribution of sex, age (80 juvenile, 2-4 years and 80 mature, 7-10 years) and seasons (thermoneutral and hot dry). By cellulose acetate electrophoresis, blood serum proteins were separated into 5 distinct bands, namely: albumin, α-globulin, β_1-globulin, β_2-globulin and γ-globulin in 97.5% of the examined samples. β-Globulin of the remainder 4 samples (2.5%) was not sub-fractionated into β_1 and β_2 and showed polyclonal hyperglobulinemia in older camels. The registered all mean values ±SE for total proteins, albumin, α, β and γ globulin were 6.842 ± 0.067, 3.4 ± 0.032, 0.81 ± 0.017, 1.016 ± 0.025 and 1.616 ± 0.041 g/dl respectively. These indices can serve as reference values for Bedouin camels reared under nomadic system. Electrophoretic data showed that mature camels had higher mean values than juveniles did, while males did not differ from females. Globulin fractions and total globulin did not differ by environmental heat stress but albumin decreased during hot dry conditions without effect on the mean values of total proteins. As in other species, electrophoretic pattern of serum proteins may be prognostic and gives an indication for inapparent diseases in camel.

Key words: *Camel - Serum protein - Electrophoresis.*

INTRODUCTION

Camel is preferable and multipurpose animal in Bedouin regions. The maintenance of good health and productivity of camel is of vital importance to the owner, particularly perhaps for those who exist under nomadic farming systems. The concentration of serum proteins reflects the health status because they are nutritive, and they are carrier and transport component for most of the plasma constituents in addition to their function in body defenses (Kaneko, 1997). Individually, in other species rather than camel, α-globulin fraction consisted mainly of α_1-antitrypsin, α_1-acid glycoprotein, α_1- lipoproteins, α_2-lipoproteins, α_2-macroglobulin, ceruloplasmin, haptoglobin and serum amyloid A. β-Region is subfractionated into β_2-lipoprotein, ferritin, C-creative protein, fibrinogen, transferrin and some immunoglobulins. γ-Globulin

represents immunoglobulins A, G, M and E (Thompson *et al.,* 1992; Gruys *et al.,* 1994; Kaneko, 1997 and Thomas 2000 a,b). It seems that there are no reports on individual classification of serum proteins in camel.

From a clinical perspective, the evaluation of plasma proteins provides a valuable diagnostic tool in monitoring clinical progress and as a disease marker (Kushner and Mackiewic, 1987). These proteins can react in response to disturbances in animal's homeostasis caused by infection or tissue injury (Thompson *et al.,* 1992 and Gruys *et al.,* 1994) and their variations may indicate that tissue damage has occurred at the time when there are no clinical signs of the process in question (Batamuzi *et al.,* 1996).

For a diagnostic and or prognostic clinicopathological test report to be meaningful for abnormality detection in camel, there must be a standard of familiar reference values regarding the management and environmental factors in which the animal acclimatized (Higgins et al, 1992). Reports on normal hematology and biochemistry in racing and indoor reared camel were recently published (Rezakhani et al, 1997; Sarwar and Majeed (1997), Mohamed and Hussein, 1999). Serum protein electrophoretogram was also reported in indoor reared Egyptian riverine camels (El-Sepai, 1989), Iranian camels (Khadjeh, 1998) and during the course of pregnancy in Egyptian Oasis camels (Saleh *et al.*, 2000). The critical aim of this study was to identify clinical reference values for blood serum total protein concentrations and serum protein fractionation patterns under the effect of age, gender and environmental stress categories in Bedouin camels reared in their nomadic habitat in the Egyptian Oasis.

MATERIALS and METHODS

Study area and meteorological data:

The Egyptian Oasis (New-valley area) covers most of the western Egyptian desert (Fig. 1) There are no rainfall, surface water or rivers. The study was carried out during February (thermoneutral) and July (mid-summer) 2000. The meteorological data are shown in table (I). During summer, this area is characterized by intensified ambient temperature, low relative humidity, high wind speed and long duration of solar radiation time.

Fig. 1 Egypt map showing Kharga Oases.

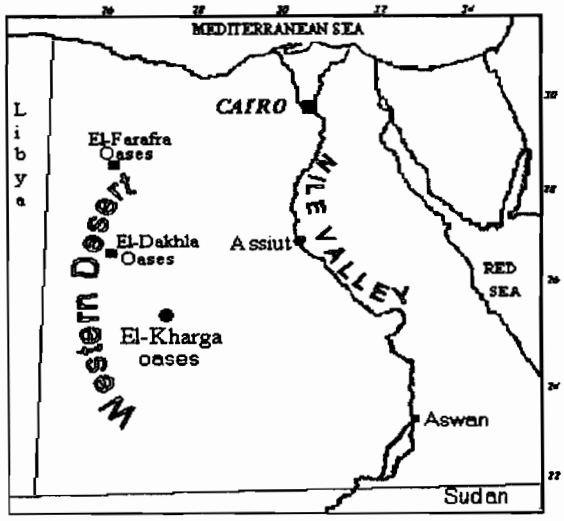

Table I: Meteorological data (Mean ±SD) during winter (February) and summer (July) 2000 at El-Kharga Oases.

	Winter	Summer
Air temperature (°C)	18.1 ±4.2	44.8 ±1.7
Relative humidity (%)	61.2 ±7.2	18.3 ±1.2
Wind speed (m/sec)	1.81 ±0.87	2.04 ±0.71
Sunshine duration (h)	08.91 ±0.33	11.94 ±0.21

Animals:

The nature of camels in Egyptian Oasis is nomadic. They browse freely on perennial vegetation. The chance of watering depends on untreated water pumped from the few scattering ground wells or water passages when available. A total number of 160 apparently healthy camels were followed up by the help of their owners and selected from their natural habitat. They were equally distributed regarding to age (80 juvenile, 3-4 years and 80 mature, 7-10 years), sex (80 male and 80 non-pregnant females) and season (80 camels during themoneutral and 80 during hot dry environment). These camels were parasite free either by testing or they were collectively treated for blood and gastrointestinal parasites in request of their owners or for other researches.

Sampling and biochemical analysis:

Blood samples were drained from each camel by jugular vein-puncture to obtain serum for the biochemical determination of total protein and protein electrophoretogram. Total serum protein was measured after the methods described by Henry *et al.* (1974). Protein electrophoretogram was carried out by using Titan III cellulose acetate plate at pH 8.8 at ionic strength of 0.067, stained with Ponceau S dye and scanned by autodensitometer (Helena Laboratories, Cat. 1023) at absorption peak of 525 nm according to manufacture instructions.

Statistical analysis:

Obtained data were subjected to a software program (SPSS, Ver. 10) according to Borenstein *et al.* (1997). Values of total serum protein and the absolute values of electrophoretic pattern were evaluated dependently in all samples by use of linear general model anova to asses the all mean values and the effect of age, sex and stress factors on the measured variables. Differences between means were compared independently using paired t-test *[P* (T</= t) *two-tail]* with unequal variance.

RESULTS

By cellulose acetate electrophoresis, dromedary serum protein was separated into 5 distinct bands, namely: albumin, α-globulin, β_1-globulin, β_2-globulin and γ-globulin (Fig.2). It was noticed that β-region was not sub-fractionated into β_1 and β_2 in 4 samples (2.5 %). However, from a practical point of view, the values of β_1 and β_2 subfractions were pooled into one value (β fraction) to facilitate calculations and statistics.

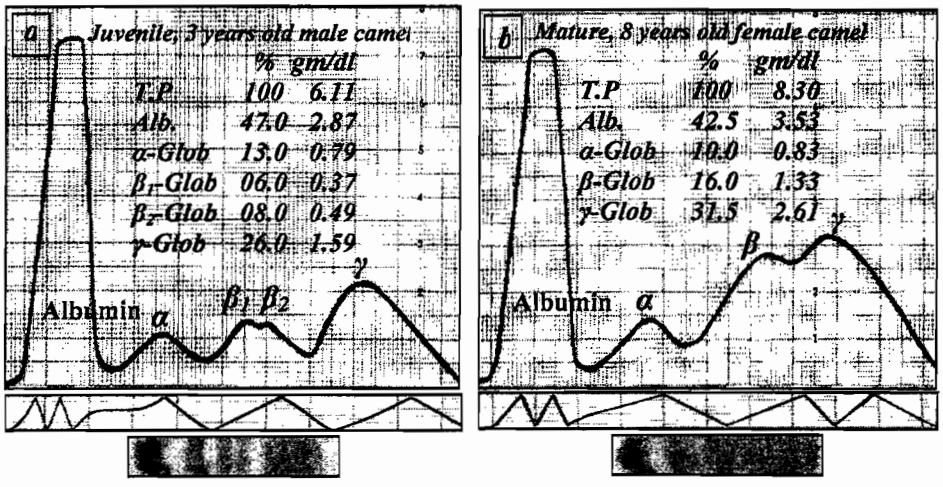

Fig 2: Patterns of cellulose acetate electrophoretogram of camel serum protein.
a- Normal pattern shown in 96.67 % of samples, b- Abnormal pattern (polyclonal hyperglobulinemia) was shown in 3.33 % of samples, all of them old animals. α, β and γ are globulin fractions.

This study was conducted during hot dry and temperate seasons on four categories (40 each), namely: immature female, immature male, mature non-pregnant female and mature male camels. The registered all mean values (total 160 samples), ranges, standard error of means, F

value within treatments and sample variance of total serum protein and absolute values of serum protein fraction concentrations are shown in Table II.

Table II: Linear model ANOVA and F value of serum proteins (g/dl) under the effect of age, gender and stress factors variability in Egyptian Oasis camel.

	All Mean (n =160)	Range Min.	Max.	Standard Error	F value	Sample Variance
Total protein	6.842	5.31	8.30	0.067	43.1^{***}	0.549
Albumin	3.400	2.81	4.20	0.032	14.2^{***}	0.125
T. Globulin	3.442	2.44	4.49	0.071	21.3^{***}	0.306
α-Globulin	0.810	0.48	1.16	0.017	0.42^{ns}	0.031
β-Globulin	1.016	0.57	1.41	0.025	6.73^{***}	0.039
γ-Globulin	1.616	0.72	2.61	0.041	20.7^{***}	0.204
A/G ratio	0.992	0.68	1.31	0.018	3.20^{*}	0.020

ns: not significant, *, * significant at p <0.05, 0.001 respectively.**

The overall means under the effect of age, gender and stress factors (Table II) showed a narrow standard error but sample variance differed according to protein fraction. F value of investigated samples (Table II) indicate highly significant variations (P<0.001) in the mean values of total protein, albumin, γ-globulin and total globulin. The results showed also significant variation (P<0.05) in the mean values of β-globulin and non-significant effect on the mean values of α-globulin.

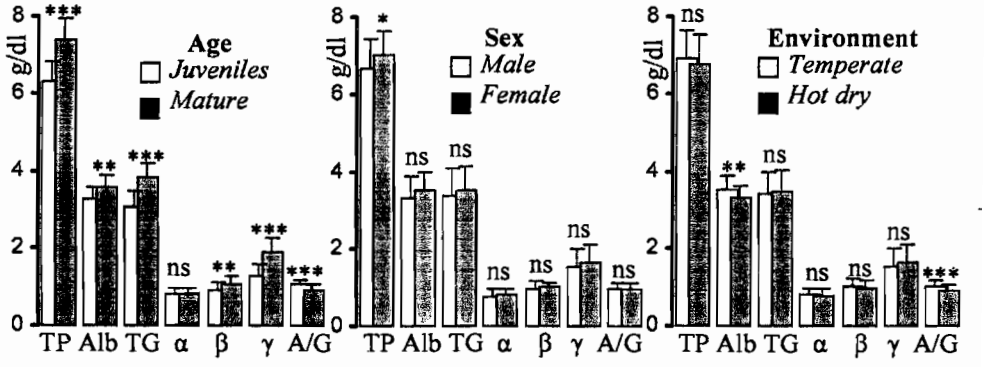

Fig. 3: The effect of age, sex and stress on dromedary blood serum proteins (TP), albumin (Alb), globulin (TG), globulin fractions (α, β, γ) and albumin/globulin ratio (A/G). Error bar indicates SD. *ns: non-significant.* *, ** *and* *** *are the statistic significance within each pair at P<0.05, 0.01 and 0.001 respectively.*

Source of this variation is clear in Tables III, IV & V and Fig. 3. The factor having the greatest influence on serum protein concentrations was the age category, where it had affected six of seven variables, while the contrast was shown in sex and stress factors, where each one had affected only one of seven variables. Age (Table III) had a highly significant positive effect (P<0.001) on the mean values of total protein (14.5 %), γ-globulin (32.97 %), total globulins (20.73 %), in addition to significant positive effect (P<0.01) on the mean values of albumin (7.5%) and β-globulin (14.26 %) but it had no significant effect on the mean values of α-globulin concentration (0.25 %). Meanwhile, serum protein electrophoretic pattern was not affected by gender factor (Table IV) but females showed higher mean values (p<0.05) in serum total protein concentration (5.15 %) when compared with males. On the other hand environmental stress resulted in reduction in albumin fraction (P<0.01, 6.82 %) without significant alteration in the mean values of total serum proteins.

Table III: Mean values ±SD and (ranges) of blood serum proteins (g/dl) and variation (V, %) in camels under the effect of age category.

	Juvenile (n =80)		Mature (n =80)		V %	P
	MEAN ±SD	RANGE	MEAN ±SD	RANGE		
T. protein	6.307 ±0.487	(*5.31-7.11*)	7.377 ±0.551	(*6.34-8.30*)	14.50	<0.001
Albumin	3.263 ±0.317	(*2.81-3.84*)	3.537 ±0.335	(*2.92-4.20*)	07.75	0.002
T. Globulin	3.044 ±0.404	(*2.44-3.65*)	3.840 ±0.361	(*3.34-4.49*)	20.73	<0.001
α -Globulin	0.809 ±0.188	(0.48-1.16)	0.811 ±0.163	(*0.53-1.09*)	00.25	0.953
β -Globulin	0.938 ±0.174	(*0.57-1.29*)	1.094 ±0.187	(*0.72-1.41*)	14.26	0.002
γ -Globulin	1.297 ±0.306	(*0.72-1.89*)	1.935 ±0.334	(*1.37-2.61*)	32.97	<0.001
A/G ratio	1.072 ±0.119	(*0.87-1.31*)	0.921 ±0.141	(*0.68-1.19*)	-14.09	<0.001

Table IV: The effect of gender factor [Mean values ±SD and (range)] on serum proteins (g/dl) in camels.

	Male (n =80)		Female (n =80)		V %	P
	MEAN ±SD	RANGE	MEAN ±SD	RANGE		
T. protein	6.661 ±0.746	(*5.31-8.19*)	7.023 ±0.593	(*6.48-8.30*)	5.15	0.042
Albumin	3.310 ±0.536	(*2.81-4.20*)	3.489 ±0.494	(*3.09-4.11*)	5.13	0.065
T. Globulin	3.351 ±0.707	(*2.44-4.32*)	3.534 ±0.582	(*2.91-4.49*)	5.18	0.124
α -Globulin	0.793 ±0.201	(*0.48-1.13*)	0.827 ±0.147	(*0.61-1.16*)	4.11	0.281
β -Globulin	1.002 ±0.203	(*0.70-1.34*)	1.031 ±0.113	(*0.57-1.41*)	2.81	0.425
γ -Globulin	1.556 ±0.446	(*0.72-2.54*)	1.676 ±0.454	(*0.87-2.61*)	7.16	0.143
A/G ratio	0.988 ±0.151	(*0.78-1.21*)	0.987 ±0.130	(*0.68-1.31*)	0.10	0.887

Table V: The effect of dry thermal stress (Mean values ± SD) on serum total proteins (g/dl) serum proteins electrophoresis (g/dl) and variation (%) than normal in camels.

	Temperate (n =80)		Hot dry (n =80)		V %	P
	MEAN ±SD	RANGE	MEAN ±SD	RANGE		
T. protein	6.934 ±0.725	(*5.31-8.30*)	6.751 ±0.766	(*5.47-8.17*)	-2.64	0.380
Albumin	3.520 ±0.344	(*2.98-4.20*)	3.280 ±0.327	(*2.81-3.98*)	-6.82	0.008
T. Globulin	3.414 ±0.556	(*2.64-4.28*)	3.471 ±0.557	(*2.44-4.49*)	1.64	0.692
α -Globulin	0.827 ±0.149	(*0.87-1.16*)	0.793 ±0.201	(*0.48-1.01*)	-4.11	0.451
β -Globulin	1.031 ±0.193	(*0.57-1.41*)	1.002 ±0.204	(*0.69-1.29*)	-2.81	0.577
γ -Globulin	1.556 ±0.450	(*0.84-2.61*)	1.676 ±0.454	(*0.72-2.47*)	7.16	0.305
A/G ratio	1.031 ±0.140	(*0.76-1.31*)	0.945 ±0.143	(*0.68-1.24*)	-8.34	<0.001

DISCUSSION

The electrophoretic pattern of dromedary camel serum protein in this study resulted in 5 distinct peaks (albumin, α-globulin, β_1-globulin, β_2-globulin and γ-globulin) in 156 (97.5 %) of the examined samples (Fig. 1a). The same pattern was obtained in Egyptian riverine camels (El-Sepai, 1989). However, these results revealed more peaks than those previously reported for cattle (Tumbleson *et al.,* 1973) and sheep (Alonso *et al.,* 1997) by using cellulose acetate plate, in which only 4 bands were obtained (albumin, α, β, and γ-globulin) indicating species differences in serum protein fractions as early reported by Irfan (1967).
Inspection of electrophoretic scans revealed that, although the absolute values of protein in each fraction varied between camels (Table I), all samples had a similar pattern with only slight variation in β-globulin region. Four samples (2.5 %) were not sub-fractionated into β_1 and β_2 and broadly diffused along with γ-globulin (Fig. 1b). This phenomenon is difficult to be discussed in normal apparently healthy animal, but by tracing the obtained data, it was noticed that these 4 samples pertained to mature animals (3 male and one female). These individuals were having a noticeable higher value of β (1.32-1.41 g/dl) and γ-globulin (2.34-2.61 g/dl) and lower A/G ratio (0.68-0.76) when compared with other mature camels. Batamuzi et al (1996) obtained similar results in apparently healthy old dogs and found that the variations in these fractions indicate the occurrence of tissue damage at the time when there are no clinical signs of the process in question. The authors interpreted their results by the probability of a lifetime exposure to foreign antigens, exposure of these animals to clinical disease in the past or they are having a current subclinical or inapparent chronic disease resulting in higher β and γ-

globulin, which may be acceptable in this work. Camels are susceptible to many viral, bacterial and parasitic diseases along their lifetime (Higgins, *et al.*, 1992 and Wernery and Kaaden, 1995). Camels in this study are familiar with parasitic diseases especially trypanosomiasis and filariasis (personal observation). The production of large amounts of non specific immunoglobulins in such diseases may reach up to 10 times that of normal cases and may persist for long time even after treatment (Boid *et al.*, 1980, 1996) due to the polyclonal stimulation of β-lymphocytes (Anosa, 1988). During hyperglobulinemia, some immunoglobulins, especially IgA, IgM or both migrate in the β-globulin region during electrophoresis (Kaneko, 1997). This migration resulted in a diffuse or broad increase in this region (polyclonal gammopathy) or what is called β-γ bridging (Meyer and Harvey, 1998 and Thomas, 2000b), which may interfere with β-globulin sub-fractionation in the present cases.

The over all mean values of serum protein and protein fractions concentrations in this study lie within the ranges cited by Kaneko (1997) for other ruminants. Instead, the observed result for total serum protein concentration was lower than the value (8.87 g/dl) reported by El-Sepai, (1989) on the Egyptian reverine camels. These differences may be related to nutritional, management and environmental factors. Under arid conditions of Egyptian Oasis, camels are nomadic and rear freely in their natural habitat, depend on browsing unmanaged perennial feeding resources which differ from indoor and riverine feedings.

The present study showed that age has a large effect on the mean values of serum protein concentrations and its electrophoretogram (except α globulin). These results are in agreement with the reports of Tumbleson *et al.* (1973) and Thomas (2000a) for other ruminants. Rezakhani *et al.* (1997) and Khadjeh (1998) obtained similar trend in Turkmen and Iranian camels. Payne and Wilson (1999) reported that both male and female camels are not fully fertile until 5 years of age in unmanaged herds. Some anabolic hormones as testosterone and estrogens, which are highly excreted after puberty (Kaneko, 1997), may be responsible for these variations. So that, age categorization should be considered when evaluating serum protein electrophoresis in camels.

Sex has no significant effect on the mean values of electrophoretic pattern of serum protein, but females tend to have higher values than males especially in albumin ($P = 0.065$). The coalition of these non-significant increase in protein fractions resulted in significant ($P = 0.042$) higher mean values of total serum protein in females. El-Sepai (1989) noticed absence of variation of total serum protein and

serum protein electrophoretogram under the effect of sex in riverine indoor reared camels. Sex differences of total protein in nomadic camels in this study may be related to the social and behavioral differences between the two sexes. In open rearing, Metwally *et al.* (1998) found more time and frequency of eating in female camels, while males showed more frequency of restlessness. These differences may result in higher feed intake with a concomitant increase in serum protein in females. Furthermore, it was found that thyroid hormones (T_3 & T_4) were higher in male camels than females (Bengoumi *et al.;* 1999). These hormones have a catabolic effect (Kaneko, 1997) and may be responsible for the reduction of total protein in males.

Livestock populate their natural habitat, arid or semi-arid zones are stressed by complex interactions between the environment and animal health. The physiologists usually define such stress as a biological coast of adaptation (Willmer *et al.*, 2000), while in view of the pathologists, it is considered an environmental disease (Radostits *et al.*, 2000). The camels under the study were thermally stressed during summer and undergo water scarcity, where watering is accidental and depends on untreated water directly pumped from the scattering ground wells or water passages when available. In contrast to other ruminants in which their serum albumin and total proteins are increased, the present work showed that the only major variation that appears to be of potential clinical significance in stressed camels was reduction in the mean values of serum albumin concentrations. Firstly, under opened tropical summer environment, animals are naturally ventilated (Igbokwe, 1997). Concomitantly, water is lost throughout the higher sweating rate and rapid evaporation in the presence of high environmental temperature, low humidity and high wind velocity especially in camels, where sweating contributes to 95 % of there evaporative cooling (Finch, 1973 and Willmer *et al.*, 2000). So that, camels can lose body water exceeding a third of their body weight without suffering ill effect (Gauthier-Pilters and Dagg, 1981). In other tropical ruminants, such cases of dehydration resulted in a functional rise in serum albumin and total serum proteins (Collier *et al.*, 1982 and Igbokwe, 1997). In camel, however, water is stored in the rumen-like fore-stomach for few days (Payne and Wilson, 1999), so that it can substitute water loss from the ruminal reservoir, and initially it frustrate the rise of serum albumin during water deprivation, contrasting other ruminants. Secondly, Schmidt-Nielsen (1965) reported that camel temperature range is 7°C (34.5-41.5). There is an apparent disadvantage of this elevation of temperature during the day, and it has

an effect on increasing of the basal metabolic rate (Schmidt-Nielsen *et al.*, 1967). The increased metabolic demands lead to a state of negative nitrogen balance, with excessive albumin breakdown to provide amino acids as substrates for energy production (Willmer *et al.*, 2000). Thirdly, due to its high osmotic sensitivity and its relatively lower molecular mass and size than other protein fractions, albumin may be filtered and redistributed into the extravascular spaces during thermal stress (Kaneko, 1997 and Thomas, 2000a) resulting in reduction of its entravascular concentrations.

Finally, the over all mean values of total serum protein and its electrophoretic pattern in this study were obtained from apparently clinically healthy camels reared under nomadic system and can serve as reference values. These values may be an aid in setting a preliminary data for future works on individual serum proteins in the camel. Age categorization should be considered when evaluating serum protein electrophoretic data in camels. As in other species, a simple electrophoretic pattern of serum protein may be prognostic and gives an indication on chronic, inapparent or subclinical diseases in camel.

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