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SEASONAL VARIATIONS OF BIOCHEMICAL PROFILE AND ITS CORRELATIONS WITH THYROID AND ADRENAL GLAND HORMONES IN MORROCAN CAMEL (*Camelus dromedarius*)

Running Title: Biochemical and hormonal profiles in camel.

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ABSTRACT

In domestic animals, it had been well established that thyroid and adrenal gland activities and biochemical profile show seasonal variations and are considered crucial to sustain the productive and reproductive performances. The study was carried out to investigate the effects of season on serum concentrations of total thyroxine (T_4), total triiodothyronine (T_3), cortisol (COR) and blood biochemical parameters [calcium, phosphorus, magnesium, glucose (Glu), cholesterol (CT), triglycerides (TG), total protein, urea, creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST)], and the correlations between these parameters in camels. T_4, T_3 and COR showed marked seasonal variation with a significant ($P<0.05$) increase during winter and a significant ($P<0.05$) decrease during summer. By comparison to summer, Glu, ALT and AST were significantly ($P<0.05$) higher in winter. However magnesium was significantly ($P<0.05$) lower in winter by comparison with summer. A significant correlation between thyroid hormones (TH) and COR, and between these hormones and magnesium, Glu, CT and TG was observed, suggesting that during winter the camel is submited to cold and rutt stress. We recommende supplying camels during the winter season by antioxidants.

Keywords: Biochemical profile, Camel, Cortisol, Thyroid hormones.

ISSN: 2455-6939

Volume:02, Issue:06

1. INTRODUCTION

Determination of baseline biochemical indices helps veterinarians to confirm clinical diagnoses, estimate the severity of cases and administer appropriate treatment in domestic animals (Daramolaet al., 2005; Faye et al., 2008;Babeker and Elmansoury, 2013). In fact, biochemical tests evaluate the function of different organs, so, concentration of glucose (Glu), cholesterol (CT) and triglyceride (TG) are considired an indicator of energy metabolism. The indicators of protein metabolism are urea, total protein (TP) and albumins. Liver condition is reflected in the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gammaglutamyl-transferase and total bilirubin concentration, while urea and creatinine are indicators of the basic parameter reflecting kidney function, nutritional status and stress responses (Ghada, 2014). However, season is known to affect these indices in several species like baffalo (Vermaet al., 2000), Holstein heifer (Rasooliet al., 2004) and camel (Babeker and Elmansoury, 2013; Badawy et al., 2008; El-Bahrawy and El Hassanein, 2011; Al-Harbi, 2012).

Male dromedary camels are seasonal breeders and its breeding season is confined to the cool winter months of the year with exhibition of endocrinological changes (Marai et al., 2009; El Khasmiet al., 2011). In domestic animals, thyroid and adrenal gland functions increase as a result of exposure of to cold (Romero, 2002) or heat (Nazifiet al., 2003) stress, and are mainly affected by season (Zia-ur-Rahman et al., 2007; Tajik et al., 2013). Higher circulating levels of TH and cortisol (COR) had been showen under cold stress in winter and the rains (Nazkiet al., 1986). It may be regarded as an indicators of stress in several species, such as cattle (Grandon, 1997), sheep (Ashutoshet al., 2001), wildlife (Knowles et al., 1995), pigs (Madejet al., 1998), alpacas (Anderson et al., 1999) and camel (Baraka, 2012; El Khasmiet al., 2015).

Many of the researches on blood biochemical parameters in Moroccan camel are incomplete or lack references to fluctuations in the parameters studied caused by environmental conditions or time of sampling during the day, and these researches had not been conducted in relation to the hormonal profile of this species. Therefore it was our intention to study the seasonal changes in the serum concentrations of some hormones (TH and COR) and blood biochemical indices (calcium, phosphorus, magnesium, CT, TG, Glu, TP, urea, creatinine, ALT and AST) and the correlations between these parameters in Moroccan dromedarian camels.

2. MATERIALS AND METHODS

2.1 Animals

In this study, 22 adult, one-humped male camels (*Camelusdromedarius*), aged 3–9 years, from Casablanca Municipality slaughterhouse in Morocco were used. The animals come from semi-

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Volume:02, Issue:06

extensive farming in the region of Essaouira, and were divided into 2 groups of 11 camels. The first group was salughetred during winter season (February) and the second one during summer season (June).

2.2 Blood sampling

Twinty hours after arrival of camels at slaughterhousse and before slaughter, blood samples were collected from each camel at 07 a.m by venipuncture from the left jugular vein in two dry tubes, and were transported to the laboratory for 10 min. Blood without anticoagulant was used for the determination of serum levels of, and COR. Following collection, the tubes were gently inverted to ensure mixing of the sample. The serum was separated by centrifugation at 750×g for 15 min at 4°C, pipetted into aliquots and then stored at -20°C until analysis of seum biochemical (Calcium, Phosphorus, Magnesium, Glu, TP, CT, TG, urea, creatinine, ALT and AST) and hormonal (total hormones thyroid and COR) profiles.

2.3 Thyroid hormones and cortisol analysis

Serum total thyroxine (T_4), total triiodothyronin (T_3) and COR levels were analyzed by radioimmunoassay (RIA) method in the National Center of Science and Nuclear Technical Energy in Maamoura, Morocco, by using commercially available coated RIA tubes. The hormones were quantified according to the manufacturer's instructions. These kits proved efficient in previous experiments in dromedary camels (El Khasmiet al., 2010; 2015), and was purchased from DIAsource (Immunoassays S.A., Nivelles, Belgium). The areas of validation for T_4, T_3 and COR assays included limits of detection, and precision in the standard curve following sample dilution, inter- and intra-assay coefficients of variation results were considered.

2.4 Biochemical indices analysis

Serum biochemical constituents including calcium (Ca), phosphorus (Pi), magnesium (Mg), CT, TG, Glu, TP, urea, creatinine, as well as liver function (ALT and AST) and kidney function (urea and creatinine) were determined spectrophotometrically using commercial kits produced by CHRONOLAB (AG Baarerstrasse 57, 6302 ZUG, Switzerland) according to the manufacture procedures.The amount of serum TPwas performed by the Biuret method (Gornallet al., 1949).

2.5 Statistical analysis

The data were expressed in SI units as mean and standard error (SE), and were analyzed by the Mann-Whitney U test for comparison between the winter and summer seasons. $P<0.05$ was seen as statistically significant. Correlations were analyzed by Pearson's correlation tests.

ISSN: 2455-6939

Volume:02, Issue:06

3. RESULTS

Significant (P<0.05) seasonal differences were observed for all hormonal parameters studied. Serum T_4 and T_3 levels were higher in winter than in summer (**table 1**). Serum COR levels showed a significant (P<0.001) increase during winter when compared with those measured during summer (**table 1**).

Table 1: Seasonal variation of serum creatinine, urea, alanine aminotransferase, aspartate aminotransferase, and thyroid and adrenal hormones in Dromedary camels of Casablanca Municipality Slaughterhousse.
(M±ET, *P<0.05, comparison between summer and winter values).

Parameters	Summer	Winter
Creatinine (mg/dL)	0.75±0.029	0.73±0.016
Urea (mg/dL)	32.54±2.56	29.67±1.83
Alanine aminotransferase (U/L)	13.12±0.45*	16.63±0.51
Aspartateaminotrnsferase (U/L)	2.78±0.13*	4.32±0.17
Thyroxine (nmole/L)	155.68±33.1*	271.9±65.35
Triiodothyronine(nmole/L)	1.55±0.31*	4.06±0.45
Cortisol (ng/mL)	93.92±18.19*	135.43±17.17

No significant difference was observed between the serum levels of Ca, Pi, CT, TG, TP, urea and creatinine measured during winter compared with those analyzed during summer (**table 2**). In winter, Glu (mM/L), ALT and AST (U/L) were significantly (P<0.05) higher (7.21±0.24; 16.63±0.51 and 4.32±0.17 respectively) than those observed in summer (6.46±0.33; 13.12±0.45 and 2.78±0.13 respectively). However, the Mg levels (mM/L) during winter were significantly (P<0.05) lower than those analyzed in summer (0.8±0.1 vs 1.2±0.1) (**tables1** and **2**).

ISSN: 2455-6939

Volume:02, Issue:06

Table 2: Seasonal variation of serum minerals, cholesterol, triglycerides, glucose and protein in Dromedary camels of Casablanca Municipality Slaughterhousse. (M±ET, *P<0.05, comparison between summer and winter values).

Parameters	Summer	Winter
Calcium (mmole/L)	2.3±0.1	2.6±0.2
Phosphorus (mmole/L)	2.3±0.2	2.4±0.2
Magnesium (mmole/L)	1.3±0.2	0.8±0.1
Cholesterol(mmole/L)	0.98±0.07	0.96±0.05
Triglycerides (mmole/L)	0.44±0.04	0.42±0.06
Glucose (mmole/L)	6.46±0.33*	7.21±0.24
Total protein (g/dL)	6.28±0.13	6.11±0.17

The results showed a significant correlation between serum levels of TH and COR, and between these hormones and Mg, Glu, CT, TG, ALT and AST (**table 3**).

Table 3: Correlation coefficients between hormones and biochemical parameters in camel.

	T_4	T_3	CT[(d)]	TG[(e)]	Glu[(f)]	TP[(g)]
T_4[(a)]	1.0000	r= 0.781 p= 0.000	r= - 0.511 p= 0.015	r= - 0.694 p= 0.000	r= 0.432 p= 0.044	r= - 0.293 p= 0.185
T_3[(b)]	1.0000	1.0000	r= - 0.422 p= 0.050	r= - 0.197 p= 0.380	r= 0.750 p= 0.000	r= - 0.020 p = 0.928
COR[(c)]	r= 0.471 p= 0.018	r= 0.671 p= 0.000	r= - 0.523 p= 0.017	r= - 0.678 p= 0.000	r= 0.461 p= 0.047	r= - 0.274 p= 0.216

a: total thyroxine, b: total triiodothyronine, c: Cortisol; d: cholesterol, e: triglyceride, f: glucose, g: total protein.

ISSN: 2455-6939

Volume:02, Issue:06

4. DISCUSSION

In this study we evaluated the seasonal variations of some biochemical (Ca, Pi, Mg, CT, TG, TP, Glu, urea, creatinine, ALT and AST) and and their correlations with TH and COR in camel.

4.1 Mineral status

An organism needs optimum concentrations of macroelements to maintain its life (Khan et al., 2009). Regarding Ca status in winter and summer seasons, our results agreed with our previous findings in camel (El Khasmiet al., 2011) but were not in accordance with those of Zia-Ur-Rahman et al. (2007) showing higher serum levels during winter. In domestic ruminants several factors such as animal physiological status, season, region, sex, year and forage species affect the mineral status of animal tissues or fluids (Khan et al., 2009; Abarghaniet al., 2013). The significant decrease of serum Mg levels observed in our study in winter might be explained by the cold stress during this season. In fact, the serum levels of Mg decreased immediately by transportation stress in camel (El Khasmiet al., 2011), cattle (Steinhardt, 2003) and goat (Ayo et al., 2009).

4.2 Hormonal parameters

In camel, the thyroid gland function and TH activity show seasonal variations depending to his productive performance and heat production (Katariaet al., 2000a; Abdel-Magiedet al., 2000; Tajik et al., 2013). In the Indian sheep, circulating levels of T_3 and COR were higher in the winter and the rains than in the summer and spring seasons (Nazkiet al., 1986). Accordig to our meteorological conditions, the ambient temperature ranged between 8°C and 10°C, in January (winter) and fluctuated between 17°C and 19°C in June (summer). So, the higher values of T_4, T_3 and COR observed in winter season may aid in thermoregulation by increasing the internal production of heat (Todini, 2007).

The dromedary camel's reproduction is characterized by a seasonal activity where the breeding season is confined to the cool winter months of the year and is associated with high circulating levels of testosterone (El Khasmiet al., 2013). This rutting period might be considered as a stressful situation involving TH and COR in the homeostatic adjustments necessary for adaptation and reproductive efficiency (Kim el al., 2005; Roth, 2008). In fact, during this period, the male is very agressive and presents some behavioural reactions like the extrusion of the soft palate and becomes very vocal (Ouajd and Kamel, 2009; Maraiet al., 2009). In addition, during the breeding season, salivary COR concentrations increased significantly and were weakly correlated with circulating levels of testosterone in horses (Aurichet al., 2015). On the other hand, Alonso-Alvarez et al. (2007) had repported that an experimental elevation of testosterone

ISSN: 2455-6939

Volume:02, Issue:06

levels in adult male zebra finches (*Taeniopygiaguttata*) had been able to increase oxidative damage and inhibit antioxidant activity during a free radical attack.

In mammals, COR is one of the hormones secreted by the adrenal gland in response to stress (Engelking, 2000). The increased serum levels of COR observed in our camels during winter season are in agreement with those reported in goat (Alila-Johansson et al., 2003), cow (Tittoet al., 2013) and human (Hadlowet al., 2014). Contrarily, COR level in camels was higher in summer than winter (Baraka, 2012) and has been explained by the stress of climate and dehydration (Katariaet al., 2000b; Alila-Johanssonet al., 2003). COR is the principle effector in the hypothalamic-pituitary-adrenocorticolaxis which affects both neurotransmission and neuroendocrine control (Oberoiset al., 2007). ACTH release can be triggered in conditions such as chemical, physical, and emotional stress, such as extreme external cold or heat (Engelking, 2000) leading to increase in cortisol secretion. Increase in plasma concentration of TH and/or COR has been used as an indicator of stress in horses (Fazio et al., 2015), cattle (Gupta et al., 2007), sheep (Cockramet al., 1997), goat (Kadimet al., 2006) and dromedary camel (El Khasmiet al., 2015).

4.3 Biochemical parameters

Our results showed no significant seasonal variation of serum CT, TG and total proteins. However, changes in CT and TG levels were reported by other studies indicating the metabolic status of the animals during extreme ambiences (Karapehlivanet al., 2007). CT values tende to decrease during winter by comparison to summer in camel (Badawyet al.; 2008) and dairy cattle (Shaffer et al., 1981). This decrease could be attributed to higher metabolic needs (Saeed et al., 2004). In contrary, Al-Harbi (2012) had reported that CT concentrations were higher in winter than in summer in dromedary camel. Higher serum CT and TG levels in cold ambience could be attributed to higher thyroid activity as increase in basal metabolism rate is required with the need for extra production of heat to maintain body temperature (Katariaet al., 2000a). The concentrations of TH do not correlate with CT levels in camel (Wasfiet al., 1987) as in goat (Nazifiet al., 2002). However, according to other studies, serum CT levels generally vary inversely with thyroid activity (Gueorguieva and Gueorguiev, 1997).

Our results showed no effect of season on serum levels of TP and are in accordance with those observed in camel by El-Bahrawy and El Hassanein (2011) and Al-Harbi (2012). However, other works had showed that plasma TP levels were higher during summer than winter in sheep (Moore, 2000), goat El-Noutyet al.(1983), cattle (Podar and Oroian, 2003) and dromedary (Kataria and Sharma, 2002; Badawyet al., 2008). Whereas, these levels were higher in winter season compared to those measured during summer in baffalo (Vermaet al., 2000), non-pregnant

ISSN: 2455-6939

Volume:02, Issue:06

Holstein heifer (Rasooliet al., 2004) and camel (Babekeret al., 2013). These observations could be explained by the stress conditions of cold or heat which may produce oxidative changes in blood proteins (Goswamiet al., 2000), suggesting that total serum proteins can also serve as marker of environment related stress (Nazifiet al., 2009).

In this work, the highest levels of Glu measured during winter compared to those observed during summer season, are in accordance with those reported in the literature (El-Bahrawy and El Hassanein, 2011; Al-Harbi, 2012; Babeker and Elmansoury, 2013). However, Nazifiet al. (1999) found that the concentration of serum Glu was significantly higher in summer than in winter. These differences may be due to breed, feeding and watering system differences, the basal metabolic rate and the use of Glu for energy production.

The plasma Glu concentration has been reported to be increased in stressed animal species such as gazelle, sheep (Hartmann, 1988) and camel (El Khasmiet al., 2015). Under cold conditions as stressor in winter, the hyperglyceamia observed in our camels might be due to an activation of the sympathetic nervous system and an increase of the adrenal cortical activity (Avciet al., 2008).So, catecholamines and glucocorticoids increase the glycogenolysis and the gluconeogenesis (Ganong, 1998) and secondarily the glyceamia. However, under heat stress during summer, feed consumption decreases, which comparatively lowers the blood Glu and the circulating TH and COR (Nath, 2006).

The results showed high levels of ALT and AST measured during winter when compared to those observed during summer season, suggesting that cold stress and rutting period during winter, could induce some oxidant stress alterations (Stojevićet al., 2005) which might increase serum concentrations of AST and ALT enzymes in camel.

5. CONCLUSION

In conclusion, seasonal variation of hemogram, blood metabolites and hormones in the dromedary camel may contribute greatly to the understanding of the adaptation of this species to its environment. In addition, it seems that thyroid hormones together with cortisol may prove to be useful for studying the haematological and biochemical profiles in camel.

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ISSN: 2455-6939

Volume:02, Issue:06

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Volume:02, Issue:06

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ISSN: 2455-6939

Volume:02, Issue:06

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ISSN: 2455-6939

Volume:02, Issue:06

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