IJRES 3 (2016) 101-108 ISSN 2059-1977

International Journal of Research in Environmental Studies
www.bluepenjournals.org/ijres

Blood oxidant stress in relation to seasonal activity of thyroid and adrenal glands in *Camelus dromedarius*

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Article History
Received 31 March, 2016
Received in revised form 02 May, 2016
Accepted 06 May, 2016

Keywords:
Camel,
Catalase,
Cortisol,
Malondialdehyde,
Oxidant stress,
Thyroid hormones.

Article Type:
Full Length Research Article

ABSTRACT

It is a well-established fact that thyroid and adrenal gland activities show seasonal variations that are considered crucial to sustain the productive performance in domestic animals. Thyroid hormones (TH) and cortisol (COR) are physiologic modulators of tissue oxidation by regulating the expression of enzymes involved in all steps of oxidative stress (OS). The study was carried out to investigate the effects of seasonal variations on serum concentrations of total thyroxine (T_4), total triiodothyronine (T_3), COR, malondialdehyde (MDA) and catalase activity (CAT); and their correlations together in dromedarian camels (*Camelus dromedarius*). T_4 only and T_4 and COR levels showed marked seasonal variation with a significant ($P<0.05$) increase during winter and a significant ($P<0.05$) decrease during summer. Comparison to summer, MDA levels were significantly ($P<0.05$) higher in winter, while, CAT activity was significantly lower in winter. A correlation between serum levels of TH and COR, as well as MAD levels and CAT was observed, suggesting that there is an over production of reactive oxygen species (ROS) in camels during winter. The relationships between TH and COR to OS and other circulating hormones, should be identified by further analysis.

INTRODUCTION

It is largely known that hormones secreted by the adrenal and thyroid glands are able to play major roles in thermoregulation, metabolic adjustments and reproduction in livestock in general (McDonald, 1980; Romero, 2002; Todini, 2007; Roth, 2008) and in camel in particular (El Khasmi et al., 2005; Zia-ur-Rahman et al., 2007) and these have been found to increase as a result of exposure of the animals to low ambient temperature (Kamal et al., 1989; Romero, 2002). In dromedary camel (*Camelus dromedarius*), the thyroid and adrenal gland functions show an important adaptability to desert conditions (El Khasmi et al., 2005) and are mainly affected by seasonal variations (Abdel-Magied et al., 2000; Zia-ur-Rahman et al., 2007; Tajik et al., 2013). Thyroid hormones (TH) regulate oxygen use and basal metabolic rates, cellular metabolism and growth and development to a number of environmental and physiological factors. They are also implicated in the physiological regulation of mitochondrial respiration and oxidative phosphorylation (Banks, 1993; Todini, 2007).

Free radicals or ROS are generated in the cell during

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stepwise reduction of molecular oxygen (Singh et al., 1999). But an excessive generation and/or inadequate removal of these radicals results in destructive and irreversible damage to the cell (Lopaczyski and Zeisel, 2001). ROS including superoxide radical, hydrogen peroxide and hydroxyl radical have a great impact on the normal function of biomolecules like nucleic acids, proteins and cell membrane phospholipids. The role and beneficial effects of antioxidants against various disorders and diseases induced by oxidant stress (OS) have received much attention (Niki, 2010). Enzymes with important antioxidant functions include superoxide dismutase, catalase (CAT) and glutathione peroxidase. The quantization of Malondialdehyde (MDA) is widely used as an indicator of polyunsaturated fatty acids peroxidation which results in disorganization of cell membrane framework and function (Simsek et al., 2006). Furthermore, the mitochondrial antioxidant defense system is considerably influenced by the thyroid status in many species such as camel (Zia-ur-Rahman et al., 2007) and rat (Varghese et al., 2001; Sawant et al., 2003). In fact, TH might be able to regulate the activities of superoxide dismutase, CAT and glutathione peroxidase in the lymphoid organs and skeletal muscles in rat (Pereira et al., 1994). Additionally, TH and cortisol (COR) are the hormones which may be regarded as indicators of stress in several species, such as cattle (Grandon, 1997), wildlife (Knowles et al., 1995), pigs (Warriss et al., 1992; Madej et al., 1998), alpacas (Anderson et al., 1999) and camel (Nazifi et al., 2009a; El Khasmi et al., 2011; Saeb et al., 2010; Baraka, 2012; El Khasmi et al., 2013, 2015).

To our best knowledge, there is no report describing probable relationship between the seasonal variation of circulating TH and COR, and OS enzymes in camels. Therefore, this study was undertaken to evaluate the effects of season on serum concentrations of some hormones (TH and COR) and SO indicators (MDA and CAT), and their correlations together in *C. dromedarius*.

MATERIALS AND METHODS

Meteorological conditions

The ambient temperature and relative humidity registered in the study area during experimentation ranged from 8-10°C and 80-88% in January respectively, and these parameters fluctuated between 17 and 19°C and 76 and 90% in the month of June. While, the wind speed during these two seasons ranged between 7 and 8 Km/h.

Experimental subjects

In this study, 22 adult one-humped male camels (*C. dromedarius*) were used, with age ranged between 3–9 years and slaughtered at the Casablanca Municipality slaughterhouse in Morocco. Camels were divided into 2 groups of 11 camels each. The first group was slaughtered during winter season (January) and the second group was slaughtered during summer season (June). All animals were clinically healthy and feed deprived overnight. According to the meteorological data of the study period, the camels have been exposed to marked seasonal changes in ambient temperature (cold or heat), relative humidity and rainfall.

Collection of blood samples

About 20 h after the arrival of *C. dromedarius* samples to the slaughterhousse and before slaughter, blood samples were collected from each animal at 7 o'clock in the morning by venipuncture from the left jugular vein. These samples were taken under aseptic conditions directly in dry tubes without anticoagulant for the determination of serum levels of total thyroxine (T_4), total triiodothyronine (T_3), cortisol (COR), malondialdehyde (MDA) and catalase activity (CAT). 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.

Malondialdehyde level and catalase activity determination

Serum thiobarbituric acid-reactive substances (TBARS) were measured by a colorimetric method based on a previously described method (Satho, 1978). TBARS values were expressed in nmol/mL MDA equivalents. Serum samples were homogenized with cold and were mixed with trichloroacetic acid (20%) and the precipitate was dispersed in H_2SO_4 (0.05 M). TBA (0.2% in sodium sulfate 2 M) was added and heated for 30 min in boiling water bath. TBARS adducts were extracted by n-butanol and measured at 532 nm.

The CAT activity was measured using the method of Aebi (1974). The disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm for 5 min. A molar extinction coefficient of 0.041/mM/cm was used to determine the CAT activity. The activity was defined as the µmol decreased H_2O_2/min/L.

Thyroid hormones and cortisol analysis

Serum T_4, 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

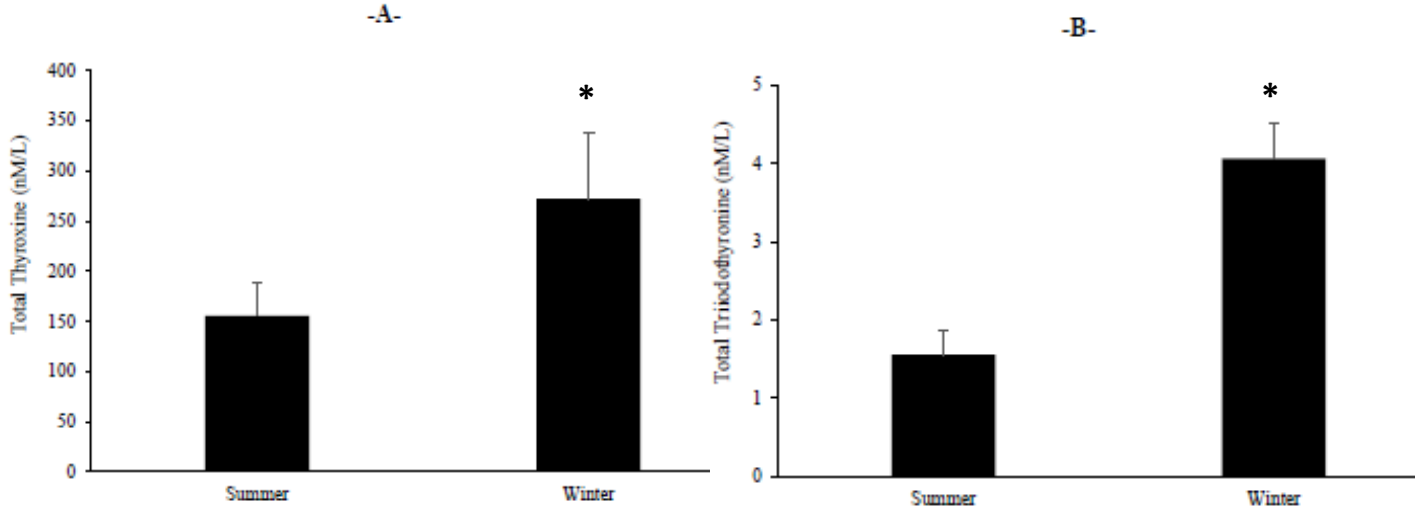

Figure 1. Seasonal variation of serum levels of T_4 and T_3 in Dromedary camels of Casablanca Municipality Slaughterhouse (M±ET, *P<0.001, comparisonbetweensummer and winter values).

previous experiments in dromedary camels (El Khasmi et al., 1999, 2010, 2015), and was purchased from DIA source (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.

Statistical analyses

The data obtained 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.

RESULTS

Total thyroid hormones and cortisol

Significant (P<0.001) seasonal differences were observed for all hormonal parameters studied. Serum T_4 and T_3 levels (nM/L) were higher in winter (271.91±65.35 and 4.06±0.45) than in summer (155.68±33.10 and 1.55±0.31, respectively) (Figure 1). Serum COR levels (ng/mL) showed a significant (P<0.001) increase during winter when compared with those measured during summer (135.43±17.17 vs 93.92±18.19) (Figure 2). The T_4:T_3 ratio was significantly lower during winter by comparison to that observed during summer (67.85±17.51 vs 100.04±6.10; P<0.001).

Malondialdehyde and catalase activity

Significant (P<0.001) seasonal changes in serum levels of MDA (nM/L) and CAT activity were reported. MDA (KU/L) was higher in winter (3.39±0.44) and lower in summer (1.16±0.22) (Figure 3). However, CAT activity was lower during winter (40.79±2.73) and higher during summer (52.21±5.34) (Figure 3).

The results showed a significant correlation between serum TH levels and COR and between these hormones and the serum levels of MDA and CAT (Table 1).

DISCUSSION

In this study, the seasonal variations of serum TH, COR, MDA and CAT activity and their correlation together in camel were evaluated. The results obtained indicate that the season of the year have a significant effect on these parameters with significant correlations between the hormones and OS indicators.

Hormonal parameters

The serum concentrations of T_3 and T_4 in *C. dromedarius* used in this experiment were in accordance with those reported earlier in literature (Nazifi et al., 2009a; Saeb et al., 2010; El Khasmi et al., 2010) but higher than values reported by Bengoumi et al. (1999) and lower than values reported by Zia-ur-Rahman et al. (2007). The results obtained also shows significant difference between seasons, and the highest values were observed during the winter season. In *C. dromedarius*, the circulating

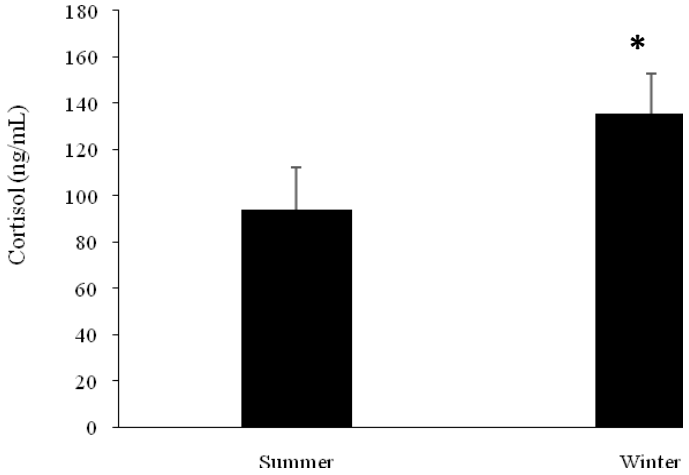

Figure 2. Seasonal variation of serum levels of cortisol in *C. dromedarius* of Casablanca Municipality Slaughterhouse (M±ET, *P<0.001, comparison between summer and winter values).

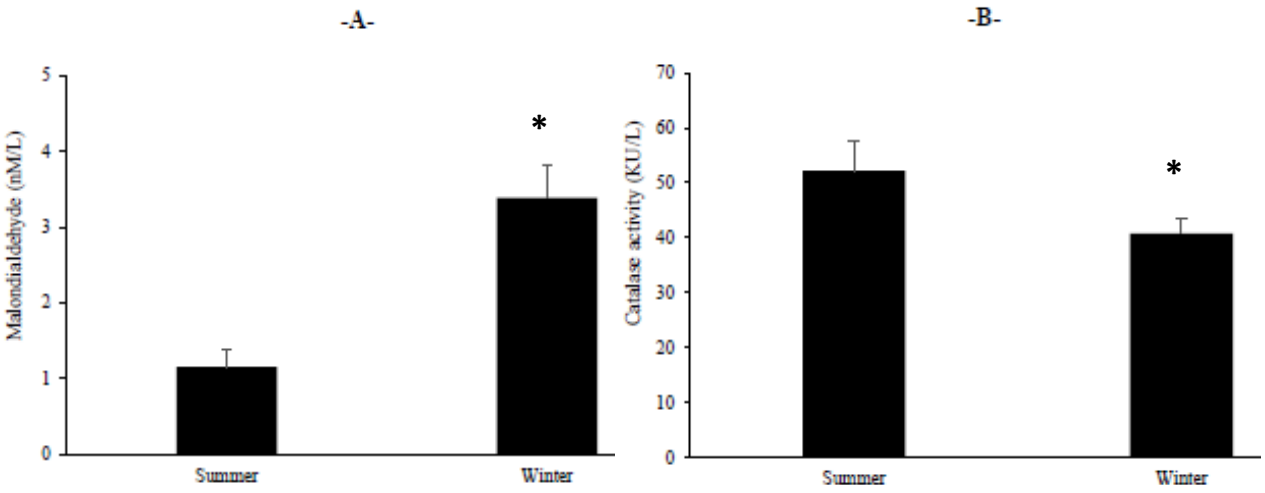

Figure 3. Seasonal variation of serum malondialdehyde and catalase activity in *C. dromedarius* of Casablanca Municipality Slaughterhousse (M±ET, *P<0.001, comparison between summer and winter values).

Table 1. Correlation coefficients between hormones and stress oxidant markers in *C. dromedarius*.

	T_4	T_3	COR	MDA	CAT
T_4	1.0000	r = 0.7810 p = 0.000	r = 0.4713 p = 0.018	r = 0.9118 p = 0.000	r = -0.917 p = 0.000
T_3		1.0000	r = 0.6714 p = 0.000	r = 0.9230 p = 0.000	r = -0.8621 p = 0.000
COR			1.0000	r = 0.8233 p = 0.000	r = -0.8645 p = 0.000
MDA				1.0000	r = -0.9038 p = 0.000
CAT					1.0000

T_4, total thyroxine ; T_3, total triiodothyronine; COR, cortisol; MDA, malondialdehyde; CAT, catalase activity.

levels of TH are lower in female (Bengoumi et al., 1999) and higher during gestation (6 months), in foetus and in newborn (El Khasmi et al., 1999).

On the other hand, normal thyroid status is dependent on the presence of many trace elements such as Selenium for both the synthesis and metabolism of TH in camel (Nazifi et al., 2009a) and sheep (Bik, 2003). In addition, the serum levels of TH in camel are mainly affected with general body metabolism, the water availability (Yagil et al., 1978) and season (Nazifi et al., 1999; Abdel-Magied et al., 2000).

Changes of circulating TH levels may be considered as indicators of the metabolic and nutritional status of the animals (Todini, 2007). Thus, higher thyroid gland activity that accompanies a low environmental temperature during winter, may aid in thermoregulation by increasing the internal production of heat. In the Indian sheep, seasonal changes had showed higher circulating levels of T_3 and COR levels in the winter and the rains than in the summer and spring seasons (Chahal and Rattan, 1983; Nazki et al., 1986; Nazki and Rattan, 1991). Furthermore, El-Nouty and Hassan (1983) and Salem et al. (1991) from Egypt reported that exposure of animals to heat stress caused a significant decrease in plasma T_4 and T_3 levels.

Glucocorticoids and TH play a pivotal role in affecting homeostatic adjustments necessary for adaptation and reproductive efficiency which may be affected by stress (Roth, 2008). The dromedary camel is largely known to be a seasonal breeder and its reproduction is characterized by a seasonal activity where the breeding season is confined to the cool winter months of the year. Thus, in the Moroccan male camel, the breeding activity maximizes in the rutting period occurring during winter and spring seasons, where the circulating levels of testosterone are very high than those analyzed in summer (El Khasmi et al., 2011). This rutting period might be considered as stresfull situation. 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 (Zarrouk et al., 2003; Ouajid and Kamel, 2009; Marai et al., 2009). In addition, during the breeding season, salivary COR concentrations increased significantly and are correlated with circulating levels of testosterone in horses (Aurich et al., 2015).

Appropriate thyroid gland function and TH activity show seasonal variations and are considered crucial to sustain the productive performance and heat production in domestic animals (Singh et al., 2005; Todini, 2007) and in *C. dromedarius* in particular (Abdel-Magied et al., 2000; Tajik et al., 2013).

In mammals, COR is one of the hormones secreted by the adrenal gland in response to a stressor (Oki and Atkinson, 2004; Sheriff et al., 2010). The increased serum levels of COR observed in the experimental *C. dromedarius* samples during winter season are in agreement with those reported in goat (Alila-Johansson et al., 2003). Contrarily, COR level in camels was higher in summer than winter (Baraka, 2012) and has been explained by the stress of climate and dehydration (Kataria et al., 2000). The COR levels in females are higher than males (Baraka, 2012) and in young more than adult camels (El Khasmi et al., 2009; Baraka, 2012).

COR is the principle effector in the hypothalamic-pituitary-adrenocorticol axis which affects both neurotransmission and neuroendocrine control (Fuch et al, 2001; Oberoi et 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 COR secretion. Increase in plasma concentration of TH and/or COR has been used as an indicator of stress in horses (Fazio et al., 2008; 2015), cattle (Odore et al., 2004; Gupta et al., 2007), sheep (Cockram et al., 1997), goat (Aoyama et al., 2005; Kadim et al., 2006) and dromedary camel (Chakir et al., 2013; Baraka, 2012; El Khasmi et al., 2009, 2013, 2015).

Oxidant stress parameters

An increase in free-radical generation as a result of stress may be responsible of the high levels of MDA and CAT observed, resulting in oxidative damage of the erythrocytes membrane (Ramnath et al., 2008; Nazifi et al., 2009a). The ROS are compounds with high potential to damage almost all types of cellular constituents by increasing lipid peroxidation, resulting in an induction and/or amplification of a number of tissular lesions (Bernabucci et al., 2002).

In this work, cold stress during winter season was associated to an activation of thyroid and adrenal glands which might induce OS resulting in higher serum levels MDA and lower CAT activity as a potents biomarkers of OS. In fact, in camel, serum CAT activity increased significantly, and serum vitamin A, vitamin C, vitamin E and glutathione levels were lower during cold environmental periods which indicating a depletion in the process to prevent OS (Kataria et al., 2010). In camel, stress induced by dehydration (20 days) caused an increase of circulating levels of norepinephrine, dopamine, COR and MDA levels in plasma, liver and kidney homogenates (Ali et al., 2013).

Additionally, a stress induced hypercortisoleamia (Russella et al., 2002) or a treatment with dexamethasone in dog (Hatamoto et al., 2006) might be widely associated to ROS formation.

Relationship between hormones and oxidant stress

The study shows a positive correlation between T_4, and

T_3 and COR, and these hormones were positively correlated with MDA levels, and were negatively correlated with serum CAT activity. These results may explain the high thyroid and adrenal activity during winter in relation to a lipid peroxidation of cell membrane. One of the most prominent actions of TH is the regulation of mitochondrial function which is the major site of oxidative processes that lead to heat production and to generation of ROS (Mookerjee et al., 2010; Zambrano et al., 2014). Additionally, the mitochondrial antioxidant defense system is considerably influenced by the thyroid status of the body (Das and Chainy, 2001). TH might be able to regulate the activities of Superoxide Dismutase (SOD), CAT and Glutathione Peroxidase (GPX) in the lymphoid organs and skeletal muscles (Pereira et al., 1994; Das and Chainy, 2001). The role of TH in metabolic pathways and antioxidant enzyme activities are well known in many species such as rat (Asayama et al., 1987) and camel (Zia-ur-Rahman et al., 2007).

Furthermore, thyroid disease, namely hypothyroidism and hyperthyroidism, constitutes the most common endocrine abnormality associated with various metabolic dysregulations (Kim, 2008), decreased CAT activity (Nazifi et al., 2009a) in camels, and decreased SOD activity in rats (Sawant et al., 2003).

A positive significant correlation between TH and antioxidant enzymes (SOD, CAT and GPX) had been reported in both hypo- and hyperthyroidism in rats (Shinohara et al., 2000; Sawant et al., 2003; Poncin et al., 2008), Ostriche (Khoshvaghti et al., 2012) and goats (Nazifi et al., 2010). However, there was no significant correlation between TH and antioxidant enzymes in dromedary camels (Nazifi et al., 2009a) and Iranian ewes (Nazifi et al., 2009b).

Several studies have found a positive correlation between hyperthyroidism and OS in rats. In fact, the mitochondrial levels of hydroperoxides, protein-bound carbonyls, and Coenzyme Q9 decreased significantly in liver and heart (Venditti et al., 2003). In addition, an increase of SOD, CAT, GPX activities and a decline in glutathione (GSH) content in both blood and heart were detected (Messarah et al., 2010). Furthermore, Venditti et al. (2015) have found an increase of the rate of H_2O_2 removal by both non-respiring and respiring liver mitochondria, leading to more ROS, resulting in harmful effect on such organelles. On other hand, a damage was observed in liver and kidney after TH administration to mice (Zambrano et al., 2014).

The increase in plasma COR in response to cold stress in winter could be an additional factor responsible for increasing the OS in the *C. dromedarius* samples in this study, as reflected by its positive correlation with MDA and CAT activity. According to Orzechowski et al. (2000), high glucocorticoid concentrations have been reported to decrease blood glutathione concentrations and erythrocyte SOD activity in rats.

Conclusion

On the basis of the present findings, it may be concluded that, seasonal variation of serum TH, COR, MDA and CAT activity in the *C. dromedarius* may contribute greatly to the understanding of the chronophysiology of this species. In addition, it seems that TH and COR may prove to be useful for studying the seasonal mechanisms mediating oxidant stress interactions in camels. It is recommended that camels be supplied with trace minerals, vitamin E and ascorbic acid during the winter season. However, it remains to be investigated whether administration of COR and/or T_4 affects oxidant stress indicators in *C. dromedarius*.

ACKNOWLEDGEMENTS

The authors are grateful to the president of Urban Municipalities of Casablanca and Dr. ABOUHAFS Rachid who is responsible for prefectural veterinary service of Casablanca, for the help to carry out this research.

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