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Evaluation of antioxidant enzymatic activity in plasma of the dromedary (*Camelus dromedarius*) in Tunisia

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Abstract – This study aimed to evaluate the antioxidant status in dromedary. The activity of some enzymes such as superoxide dismutase, catalase, peroxidase and some metal ions (calcium, magnesium, iron) were explored. A total of 40 dromedaries were divided into 4 groups: 10 young males (aged less than 1 year), 10 adult males (age varied from 3 to 5 years), 10 lactating females and 10 pregnant. The antioxidant status was assessed according to the age of the animal, the sex and the physiological situation of the female (pregnant, lactating). The results show an average activity in superoxide dismutase, catalase and peroxidase of 180.21 ± 8.21 kU/L, 105.84 ± 4.25 kU/L and 102.27 ± 4.75 mU/ L respectively. These enzymatic activities were more important in young animals (vs. adults) (p = 0.012, 0.025, 0.026 for superoxide dismutase, catalase and peroxidase respectively) and lactating (vs pregnant) females (p = 0.015, 0.028, 0.026 for superoxide dismutase, catalase and peroxidaserespectively). Besides, the variations of some metal ions were correlated positively; whereas no, statistically significant, variation was detected according to the sex of the animal (p = 0.07, 0.06, 0.12 for superoxide dismutase, catalase and peroxidase respectively).

Keywords: dromedary, superoxide dismutase, catalase, peroxidase, metal ions, plasma

1. Introduction

Dromedary hasseveral physiological, anatomical and behavioural mechanisms that help him to survive successfully in dry and hot areas. Water conservation ability, the unique features of blood, thermoregulation, and efficient digestion and metabolism are among these physiological adaptations (Abdel Gader and Alhaider, 2016). Anatomically, the nature of skin coat, eyes, nostril and lips, large body size and long height and large foot pads contribute to this adaptation capacity (Wu et al., 2015). The feeding, drinking and thermal behaviours of dromedary play also a major role in successful existence in the desert. On the other hand, the extreme environmental conditions tolerated by dromedary suppose that this species is physiologically well equipped to develop reduced oxidative stress.In fact, the continuous exposure of the dromedary to these extreme environmental conditions could break the balance between the production of reactive oxygen species (hydrogen peroxide (H_2O_2), superoxide ($^{\bullet}O^-_2$), hydroxyl radical ($^{\bullet}OH$), oxygen (O_2)) and the antioxidant defences, leading to oxidative stress (Birben et al., 2012) damaging several biomolecules such as deoxyribonucleic acid, proteins and lipids (Halliwell, 1991). On this side, superoxide dismutase, catalase, and peroxidase can play an important role, in dromedary, to prevent oxidative stress by neutralizing these free radicals already mentioned (Sheikhansari et al, 2019).Oxidative stress status was explored previously, in dromedary, especially in red blood cells (Bengoumi et al., 1998; Nazifi et al., 2009). However, only a few studies reported the activity of antioxidant enzymes in plasma and no results were reported in Tunisian dromedary. Thus, the main objective of the present study was to evaluate the plasmatic activity of antioxidant enzymes (superoxide dismutase, catalase, and peroxidase) as well as concentrations of metal ions (calcium, magnesium, iron)which can actas cofactors for these antioxidant enzymes.

2. Materials and methods

Animals and samples

The camels used in the experiment belonged to Office des Terres Domaniales d'El Alam (Sbikha,

Kairouan governorate). Blood samples were collected during the summer season from 40 camels belonging to 4 groups of 10 animals each: young males (less than 1-year-old), adult males (3 to 5 years old), pregnant females and lactating ones. The animals are raised semi-extensively in a region characterized by food scarcity and whose vegetation is dominated by grass plants.
Eight millilitres of blood were collected from the jugular vein of each camel, on a dry vacuum tube for the determination of serum parameters indicative of the antioxidant status (superoxide dismutase, catalase, peroxidase, iron, magnesium, calcium). The samples were sent under cold conditions to the laboratory. Blood samples were centrifuged at 3000 rpm for 15 minutes and sera were stored in Eppendorf tubes at -80°C until used.

Superoxide dismutase activity
Superoxide dismutase activity was determined by using a modified epinephrine assay (Misra and Fridovich 1972). Serum was added to a 2 mL reaction mixture consisting of 10 µL bovine catalase (0.4 U/µL), 20 µL epinephrine (5 mg/mL), and 62.5 mM sodium carbonate-sodium bicarbonate buffer (pH 10.2). Absorbance was recorded at 480 nm wavelength with Shimadzu UV-1800 spectrophotometer.

Catalase activity
Catalase activity was estimated by measuring the initial rate of H_2O_2 disappearance at 240 nm wavelength with Schimadzu UV-1800 spectrophotometer. The reaction mixture consisted of 33 mM (1000 µL) H_2O_2 in 50 mM (1995 µL) phosphate buffered saline (PBS) (pH 7.0) and 40 µL of serum. Catalase activity was calculated using an extinction coefficient of 40 $mM^{-1}cm^{-1}$ for H_2O_2 (Aebi, 1984).

Peroxidase activity
Peroxidase activity was measured at 25°C using guaiacol as the hydrogen donor. The reaction mixture contained 9 mM (25 µL) guaiacol, 19 mM (100 µL) H_2O_2 in 50 mM (870 µL) PBS (pH 7) and 40 µL of serum. The reaction was initiated by the addition of H_2O_2 and monitored by measuring the increase in absorbance at 470 nm wavelength every 30s for 3 min with Zuzi spectrophotometer (Model 4201/50). Peroxidase activity was expressed as nmols of guaiacol oxidized per min and calculated using a molecular extinction coefficient of 26.2 mM^{-1}.

Metal ions
Calcium measurement
Calcium was determined using a commercially available kit from Biomaghreb. At basic pH, calcium constitutes with cresolphtalein a purple colourful complex measurable at 570 nm. Briefly, 50 µLof plasma was added to 650 µL of reaction mixture containing 2-amino-2-methyl 1-propanol buffer (500 mmol/L), cresolphtalein (0.62 mmol/L), and hydroxy-8 quinoleine (69 mmol/L). The incubation was carried out at room temperature.

Magnesium measurement
Magnesium levels were determined using a commercially available kit from Biomaghreb, Tunisia. Magnesium forms a purple coloured complex when treated with calmagite in alkaline solution, measurable at 520 nm. The intensity of the purple colour is proportional to the magnesium concentration.

Free iron determination
Plasma free iron levels were determined using a commercially available kit from Biomaghreb. Briefly, at acidic pH 4.8 all Fe^{3+} released from transferrin were reduced by ascorbic acid into Fe^{2+}, which constitutes, with ferrozine, a purple colourful complex measurable at 560 nm. Briefly, 50 µL of plasma was added to 250 µL of reaction mixture containing ascorbic acid (5 g/L) and ferrozine (40 mM), and incubation was performed at 37°C for 10 min.

Statistical analyses
Three factors of variation were considered: age, sex and physiological situation of females. Results were expressed as means ± standard deviation (SD). Data were compared using unpaired Student's t-tests or one-way analysis of variance (ANOVA). All statistical tests were 2-tailed andwere considered significant at a level 0.05(a significant difference is marked with an asterisk *).

3. Results and discussion

3.1. Results

Considering all categories, mean activities of superoxide dismutase, catalase and peroxidase were 181.00 ± 6.01 kU/L, 102.86 ± 5.48 kU/L and 102.72 ± 5.27 mU/L respectively.

Young animals, whose age is less than one year, have a greater antioxidant activity than adult ones, considering superoxide dismutase (Figure 1) (214.11 ± 6.21 vs 160.79 ± 4.57 kU/L; p = 0.012), catalase (Figure 2) (117.46 ± 4.24 vs 92.72±5.27 kU/L; p = 0.025) and peroxidase (Figure 3) (119.40 ± 5.14 vs 93.36 ± 6.12; p =0.026). Besides, suckling females, compared to pregnant ones, have a better antioxidant status, materialized by higher activities of superoxide dismutase (Figure 1) (207.08 ± 5.68 kU/L vs 142.02 ± 4.18 kU/L; p = 0.015), catalase (Figure 2) (109.09 ± 5.67 vs 92.16 ± 4.65 kU/L; p = 0.028) and peroxidase (Figure 3) (112.17 ± 3.54 vs 85.95 ± 5.24 mU/L; p = 0.026). However, no significant difference, depending on the sex of the animal, was noticed for all enzyme's activities (table 1, figures 1, 2, 3 and 4).

Table 1. Mean enzymatic activity and concentration of metal ions

	Mean enzymatic activity ± SD			Mean concentration metal ions ± SD		
Animal category	**Superoxide dismutase (kU/L)**	**Catalase (kU/L)**	**Peroxidase (mU/L)**	**Calcium (mmol/L)**	**Magnesium (mmol/L)**	**Iron (µmol/L)**
Young animals	214.11* ±6.21	117.46* ± 4.24	119.40* ± 5.14	3.35* ± 0.15	2.84* ± 0.22	10.74* ± 0.94
Adults	160.79 ± 4.57	92.72 ± 5.27	93.36 ± 6.12	2.74 ± 0.16	2.03 ± 0.17	8.64 ± 0.92
Females	174.55 ± 6.65	100.63 ± 6.78	99.06 ± 7.14	3.30±0.22	2.40±0.14	11.02 ± 1.11
Males	187.45 ± 7.25	105.09 ± 6.26	106.38 ± 4.45	3.03 ± 0.22	2.43 ± 0.18	9.63 ± 1.11
Pregnant	142.02 ± 4.18	92.16 ± 4.65	85.95 ± 5.24	2.89 ± 0.19	2.19 ± 0.19	10.10 ± 1.28
Lactating	207.08* ± 5.68	109.09* ± 5.67	112.17* ± 3.54	3.75* ± 0.19	2.62* ± 0.17	12.03* ± 1.34

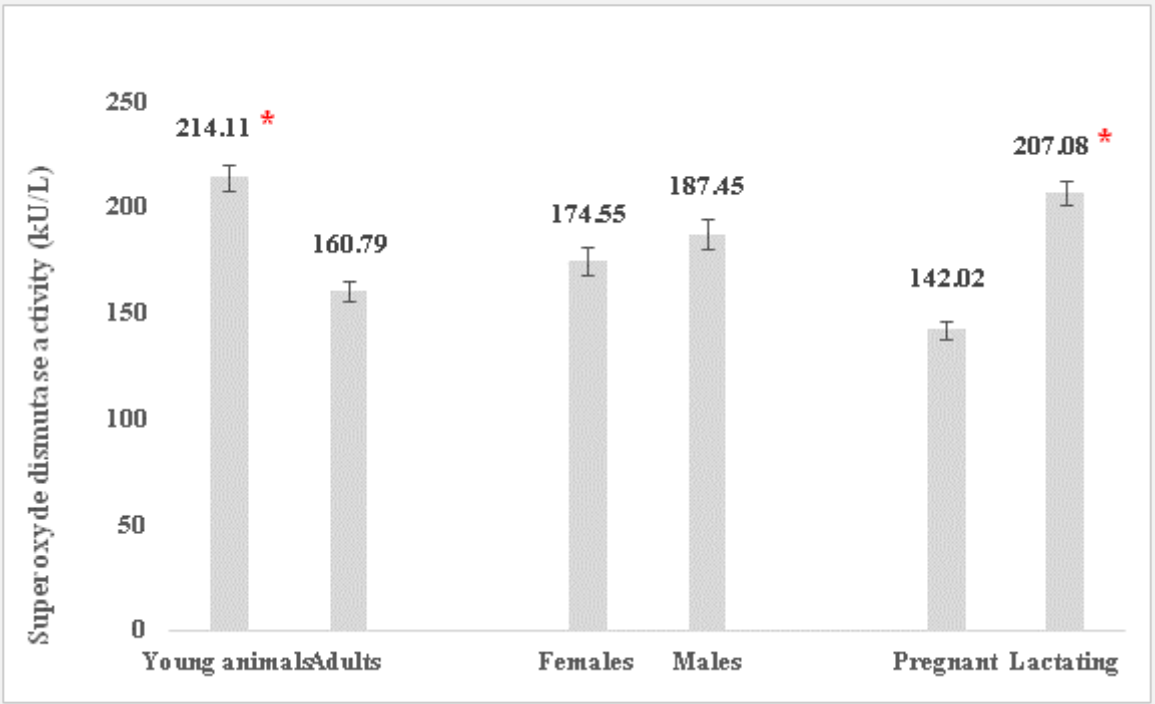

Figure 1. Superoxide dismutase activity in dromedary's plasma (n = 40). Significant difference was noted as a function of animal age (p = 0.012) and reproduction situation (pregnant, lactating) (p = 0.015).

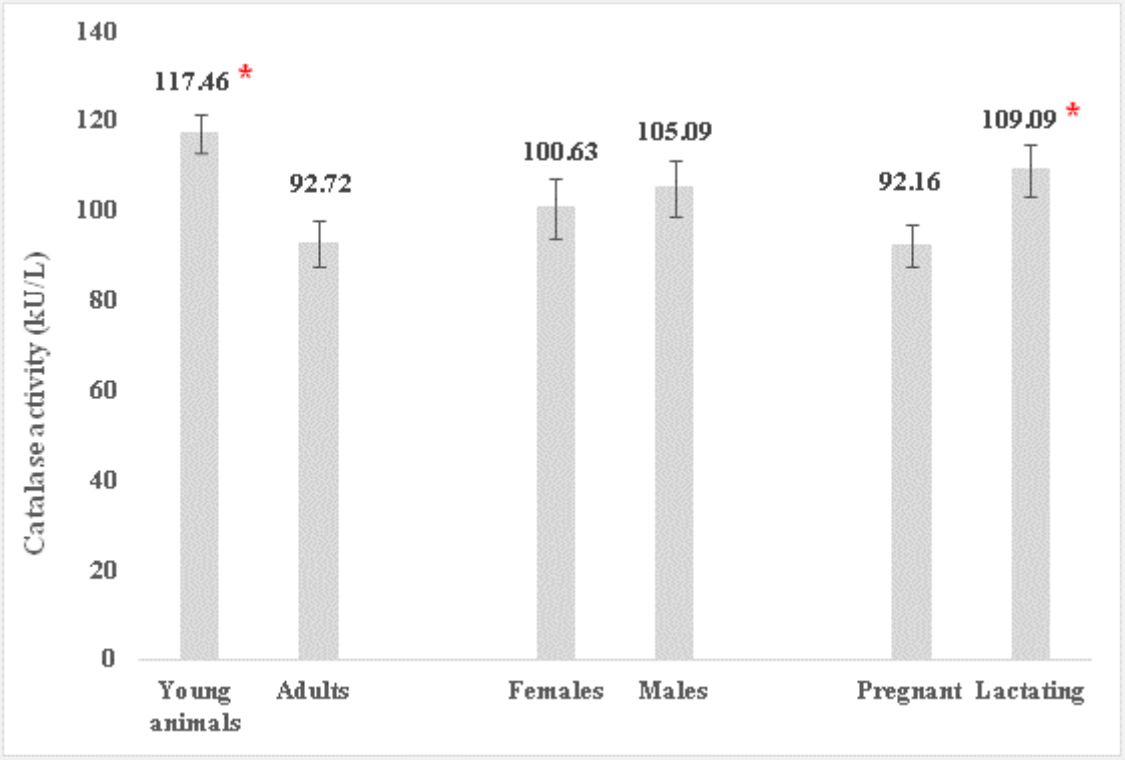

Figure 2. Catalase activity in dromedary's plasma (n = 40). Significant difference was noted as a function of animal age (p = 0.025) and reproduction situation (pregnant, lactating) (p = 0.028).

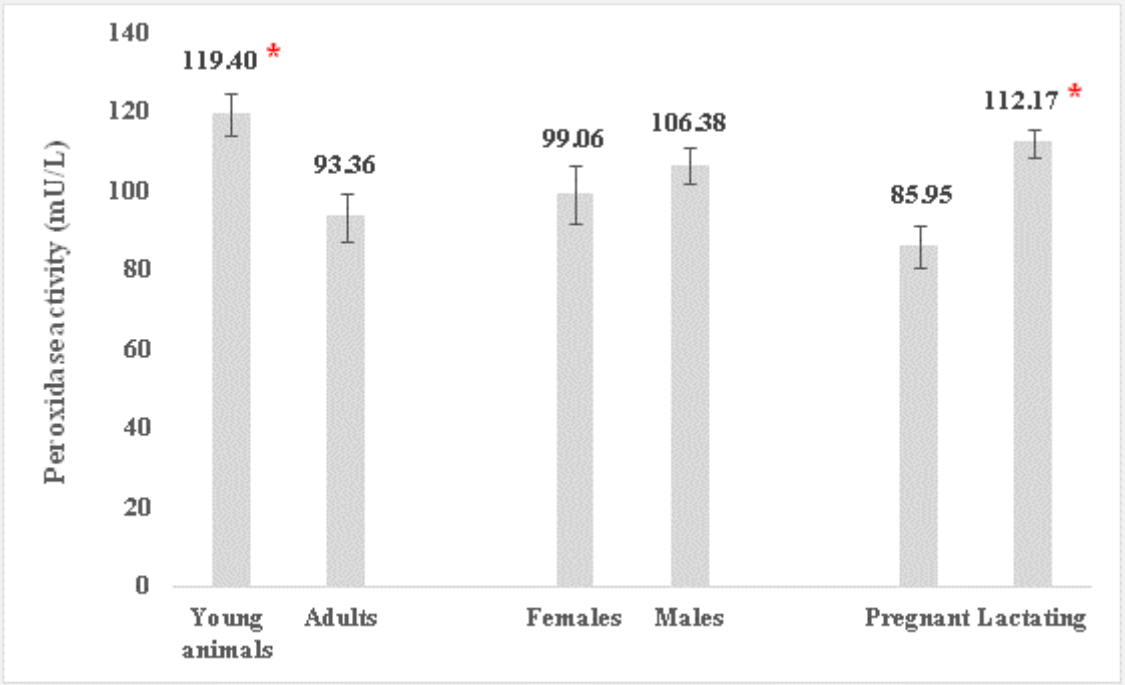

Figure 3. Peroxidase activity in dromedary's plasma (n = 40). Significant difference was noted as a function of animal age (p = 0.026) and reproduction situation (pregnant, lactating) (p = 0.026).

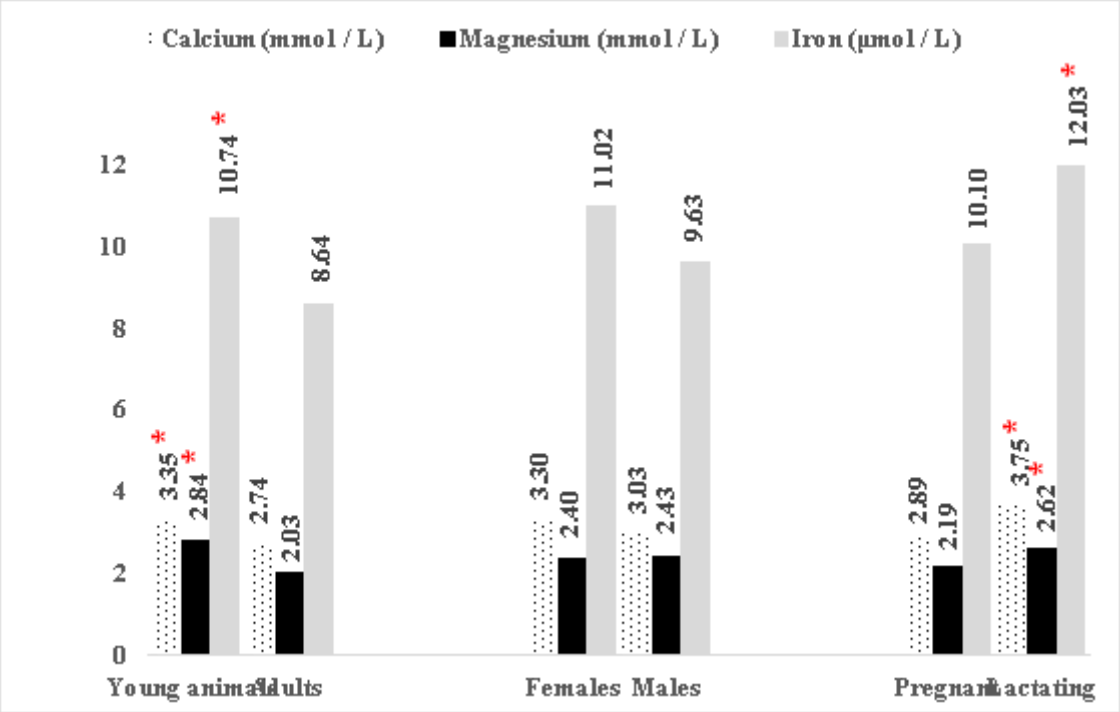

Figure 4. Concentration of metal ions (calcium, magnesium, iron). In young animals, greater antioxidant activity was confirmed by mineral cofactors assays: calcium ($p = 0.021$), magnesium ($p = 0.015$), iron ($p = 0.031$). A superior antioxidant activity was s also established, for lactating females compared to pregnant ones, by high values of calcium ($p = 0.004$), magnesium ($p = 0.042$) and iron ($p = 0.032$). However, no significant difference, depending on the sex of the animal, was noticed.

3.2. Discussion

The dromedary is a species of the desert environment which is characterized by extreme temperatures with a difference of up to 8°C between day and night; such deviations are fatal for most non-adapted mammals (Schmidt-Nielsen, 1997). This constant thermal stress should be associated with optimal antioxidant activities to counteract the deleterious effects of reactive oxygen species that can be produced consequently.

Superoxide dismutases belong to the family of non-heme metalloenzymes. The first enzyme of this kind, superoxide dismutase 1, was isolated in 1939 from bovine erythrocytes by Mann and Keilin. Its activity was discovered only 30 years later (Miller, 2012), yet its biological role is essentialsince it consists of detoxifying the body from the superoxide radical ($O_2^{\cdot-}$), catalyzing the following reaction: $O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$. Considering its major antioxidant activity, superoxide dismutase is a fundamental element in the fight against oxidative stress. For this reason, it isone of the most conserved enzymes in the alive reign. In our study, the hypothesis, supporting optimal antioxidant activity seems to be confirmed by a superiority of superoxide dismutase activity in dromedary (181.00 ± 6.01 kU/L). Indeed, several authors report lower serum superoxide dismutase activities in sheep (150.88 ± 3.28 kU/L) (Suzuki and Agar, 1983), goats (126.28 ± 5.7 kU/L) (Jarikre et al, 2017), cattle (104.14 ± 2.48 kU/L) (Vu et al, 2012) and desert gazelles (134.48 ± 0.21 kU/L) (Pospisil and al, 1984). However, in red blood cells, the activity of superoxide dismutase is lower in dromedary than in cows under similar feeding conditions: between 1474 ± 252 and 1813 ± 352 U/100g Hb (hemoglobin) in dromedary by level of mineral supplementation vs 2254 ± 205 to 2436 ± 237 U/100g Hb in cows (Bengoumi et al., 1998). Different values with low daily variability have been reported in Iran (Nazifi et al., 2009): from 1323.4 ± 37.2 to 1412.2 ± 41.6 U/g Hb (100 times more than the previous reference). Bengoumi et al. in 1998, report that copper-zinc supplementation has no significant effect on dromedary superoxide dismutase activity, unlike cow. In dromedaries with urinary tract infections, the activity of the superoxide dismutase decreased significantly from 4.98 in healthy dromedaries to 3.73 U/g Hb in affected ones; values return to normal after treatment (El-Deeb and Buczinski, 2015). A lower activity of superoxide dismutase has also been demonstrated in Bactrian dromedaries with an advanced cachectic state (Shen and Li, 2010): 14.3 ± 1.9 (cachexia) vs 18.5 ± 2.3 µmol/L (normal animals).Hydrogen peroxide (H_2O_2), a deleterious intermediate for the cell, formed, following

metabolic activities, is partially removed by catalases according to the following reaction: $2H_2O_2 \rightarrow 2 H_2O + O_2$. Catalases are strategically located in peroxisomes since flavin enzymes, urate oxidase, glucose oxidase, and D-amino oxidases produce H_2O_2 (Sheikhansari et al, 2019). In our work, serum catalase activity was elevated in dromedary (102.86 ± 5.48 kU/ L) compared to sheep (78.25 ± 7.23 kU/L) (Suzuki and Agar, 1983), cattle (65.25 ± 5.27 kU/L) (Vu et al, 2012) and goats (72.25 ± 6.14 kU/L) (Jarikre et al, 2017). The tissue activity of catalase in dromedary has, also, been determined in the liver (Al-Bar, 2012), which contains a high level (32.22 U/g). A seasonal study showed that serum catalase activity was lower in winter (41 ± 3 kU/L) than in summer (52 ± 5 kU/L) (Lektib et al., 2016). Similar values have already been reported by Kataria et al. (2010). As a stress marker, the activity of catalase increases considerably with the transport distance varying from 60 ± 3 kU/L after 72 to 80 km of truck transport to 94 ± 4 kU/L after 350 to 360 km (El-Khasmi et al., 2015).

Peroxidases are selenium enzymes that catalyze the reduction of hydroperoxides and hydrogen peroxide by reduced glutathione. The permanent recycling of the latter is provided by glutathione reductase. The role of peroxidase is very important in most tissues, for example in red blood cells and platelets where it carries out the total elimination of H_2O_2(Sheikhansari et al., 2019). The serum activity of peroxidase, in our work (102.72 ± 5.27 mU/L) is greater than that reported in sheep (85.25 ± 2.24 mU/L) (Suzuki and Agar, 1983) and goats (74.29 ± 4.62 mU/L) (Shi et al, 2010) and lower than cattle (112.25 ± 2.78 mU/L) (Vu et al, 2012). Glutathione peroxidase activity is generally correlated with the selenium status of animals, including dromedary (El-Magawry et al., 1988). This correlation is stronger in dromedary than in cow (Bengoumi et al., 1998). The determination of glutathione peroxidase activity was performed in red blood cells and the results are expressed in U/g Hb. The first reference for glutathione peroxidase is probably El-Magawry et al. (1988) who found no significant difference between mean values of 25 ± 0.64 U/g Hb in males and 23.7 ± 0.17 U/g Hb in females.

These higher values of enzymatic antioxidant activities in dromedary, compared with those reported in other ruminants (sheep, goats, cattle), make dromedary less susceptible to oxidative stress, which can be generated by high temperatures in the desert. Thus, another feature of dromedary metabolism is added to many other characteristics of this species making dromedary a legend in the resistance to drought, thirst and high temperatures that are lethal for other non-adapted animals.

In young animals, compared with adults, the high level of superoxide dismutase (214.11 ± 6.21 vs 160.79 ± 4.57 kU/L), catalase (117.46 ± 4.24 vs 92.72 ± 5.27 kU/L) and peroxidase (119.40 ± 5.14 vs 93.36 ± 6.12 mU/L) are associated with growth stress and metabolic stimulation during this period (Cai et al., 2019). Moreover, Mousa et al., (2006), reported that, in red blood cells, catalase activity is slightly higher in young dromedaries than in adult ones: 4 ± 0.04 vs 3 ± 0.05 U/mg Hb. However, in the human species, elevated levels of the above-mentioned enzymes are generally found in the elderly following senescence and progressive cell death as a function of age resulting in the release of reactive oxygen species (Chi et al, 2019).

During the lactation period, suckling females are over-exploited to feed theiroffspring, whose survival and growth depend on mother's milk production. Thus, lactating females are prone to decreases in blood levels of glucose, fat, and proteins. This export to milkcould stimulate the activity of superoxide dismutase, catalase and peroxidase to avoid the deleterious effects of free radicals which can accumulate under these conditions of the stress of production (Hamzaoui, 2013). Generally, dromedary females are often confronted with a scarcity of food and water in their environment, but the female is able, nevertheless, to maintain a satisfactory milk production to ensure the survival of her offspring. In dromedaries subjected to water restrictions for 15 days, the liquid content in milk remains equivalent to normo-hydrated females (Bengoumi, 2005). This process of conservation of equivalent water composition in milk would form stress generating deleterious metabolites that can be neutralized by this higher activity of enzymes involved in oxidative stress, in suckling dromedary's females. Besides, worksfocusing on antioxidant enzymatic activity in dromedary milk, reported that catalase activity was also very high compared to cow and buffalo milk: 6 ± 0, 27 U/L vs. 1 ± 0.41 U/L and 1 ± 0.23 U/L, respectively (Yoganandi et al., 2014).

4. Conclusions

This study showed an antioxidant superiority of dromedary metabolism, considering the first line of defense; superoxide dismutase and the role played by catalase and peroxidase to neutralize free radicals formed following extreme environmental conditions of the desert. Besides, the investigation, by animal category, shows an antioxidant advantage for young animals and lactating females. These

results obtained could be useful for the estimation of the appropriate supplementation in mineral elements (calcium, magnesium, iron, copper, zinc, selenium...) acting as antioxidant cofactors and this according to the age of the animal and the stage of reproduction of the female for a possible adapted need, comfort and well-being of the animal in its environment. However, additional research is needed to improve our knowledge of this topic within the dromedary species.

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