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Some Biomarkers in Barki and Rahmani sheep to heat stress challenge under the effect of hot dry conditions of the Egyptian oasis

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Abstract

This study was carried out to investigate the physiological response of Barki and Rahmani sheep under the effect of the hot dry conditions in El-Dakhla oasis, New-valley governorate, Egypt. A total of 60 (6-9 months) clinically healthy male Barki and Rahmani sheep were randomly selected from their natural habitat (15 of each in winter and summer season). Blood was collected from the jugular vein for haematological and some biochemical assay. Results revealed that both rectal temperature and respiration rate increased ($P<0.5$) in summer than winter season, and the increase was more pronounced ($P<0.5$) in Rahmani breed compared to Barki breed, whereas ruminal contraction was not affected by season or breed. In contrast, hematological parameters in sheep revealed a decrease ($P<0.5$) in RBCs, Hb and PCV in summer than winter season and the reduction was more clear ($P<0.5$) in Rahmani breed, but methemoglobin concentration was not affected by season or breed. Corpuscular osmotic fragility increased ($P<0.5$) in Rahmani sheep in summer, while it did not change in Barki breed. On the other hand, plasma proteins and albumin decreased ($P<0.5$) in summer compared to winter season in Rahmani breed and not affected by season in Barki breed but plasma globulins not affected by season or breed. Furthermore, plasma glucose and cholesterol decreased but blood urea, Na and K increased ($P<0.5$) in summer than winter season and the change was more pronounced ($P<0.5$) in Rahmani breed than Barki breed. As a conclusion, Barki sheep seem to perform better than the Rahmani which still having a thermoregulatory mechanism in hot dry desert areas. Therefore, their health and production under such conditions would require alleviation of the impact of heat stress.

Keywords: *Sheep, biomarker, heat stress.*

Introduction

The variation in climatic variables like temperature, humidity and radiations were recognized as the potential hazards in the growth and production of all domestic livestock species. Thermal environment is a major factor that can negatively affect production and reproduction in animals **(Dobson and Smith, 2000).**

Livestock populate their natural habitat, arid or semi-arid zones are stressed by complex interactions between the environment and animal health **(Burgos *et al.,* 2001).** In this respect, **Bauman and Currie (1980)** introduced the term homeostasis as a reaction of the internal environment for the nutrient partitions and metabolism regulation in a coordinated and orchestrated mechanism for the priorities and to support the body against the stress stimulus.

Despite having well developed mechanisms of thermoregulation, ruminants do not maintain strict homeothermy under heat stress **(NRC, 1986; St-Pierre, *et al.,* 2003).** Such stress is usually defined by the physiologists as a biological coast of adaptation against the stressor **(Willmer *et al.,* 2000).** On the other hand, there is an evidence of the deleterious effects of heat stress on animal health. These effects are manifested by clinical symptoms (hyperthermia, panting, reduced feed intake and interrupted rumination), in addition to hormonal (cortisol and thyroidal activity), metabolic and immune disorders **(Nienaber, *et al.* 1999; Srikandakumar *et al.,* 2003 and Zamiri and Khodaei 2005).** In view of the pathologists, these deleterious changes caused by thermal stress are considered deviation than normal levels so that thermal stress is considered as an environmental disease **(Martin and Aitken, 2000 and Radostits *et al.,* 2000).** In despite, mild heat stress does have some beneficial role via positively regulating cell proliferation and differentiation, and immune response in mammalian cells **(Park, *et al.* 2005).** Sheep farming is a very important animal production activity in tropical countries **(Baker and Gray, 2003).** The changes in the biological functions of sheep due to exposure to heat stress include the depression in feed intake and utilization, disturbance in the metabolism of water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites **(Habeeb *et al.,* 1992).** The thermal environment is a major factor that negatively affects sheep performance. Increased body temperature and respiration rate are the most important signs for heat stress in sheep. The increase in body temperature is associated with marked reduction in feed intake, redistribution in blood flow and changes in endocrine functions that will affect negatively the productive and reproductive performance of the sheep **(Marai *et al.,* 2001).** These physiological adjustments are essential to maintain normal body temperature and to prevent hyperthermia **(Lowe *et al.,* 2001).** Moreover, under these conditions the animal's productivity severely affected that result in a tremendous economic loss for the sheep industry **(Al-Haidary, 2004)**

The importance of heat stress to livestock industries is increasing with time because of the long-term trend shift in the location where animal agriculture is primarily located **(St-Pierre, *et al.* 2003).** New-Valley is considered one of the most promising areas for agricultural expansion in Egypt. In fact, veterinary studies on animals, especially sheep in this area are almost considered virgin. There are scarce published data on the indices of general health of normal sheep in such areas. This study was carried out to investigate the physiological response of Barki and Rahmani sheep under the effect of the hot dry conditions in El-Dakhla oasis, New-valley governorate.

Materials and Methods

Study area:

This study was carried out in El-Dakhla oasis, New-valley governorate, Egypt. The climate in this area is arid, essentially that of the desert. The temperature ranges from 49°C during summer days to 2°C in the chilly winter nights. Rainfall is almost negligible and the average precipitation is 0.3mm. Watering and irrigation depend absolutely on the ground wells. This study was carried out during January and feburary 2018 (winter season) and July 2018 (summer season). Weather data were available from an on-site weather station (Tab.1).

Table (1). Average values of meteorological data in El-Dakhla oasis during the study period

Month	Ambient Temperature (˚C)	Relative humidity (%)	Wind speed (m/sec)	Sunshine duration (h)
Winter	15.9	44.5	2.3	10.4
Summer	44.9	24.8	2.2	13.5

Animals:
A total of 60 (6-9 months) male Barki and Rahmani sheep were randomly selected from their natural habitat (15 of each breed during winter and the same number during summer season). All animals were apparently healthy and in good physical condition. These animals were located in closed neighboring areas. They were allowed to graze with their herds on the free range outdoors in their natural habitat. The grazing was mainly on perennial vegetation and Barseem Hegazi (Medicago sativa). Animals were shaded during the summer midday times under the available trees, bushes, shrub, and hedge or even under artificial shades made of palm leaves.

Clinical investigations and sampling: Sheep were selected after they had been subjected to careful clinical examinations to prove their fitness and to reject any health abnormality. Standard methods were used for specific clinical investigations including measurement of respiratory rate, ruminal contractions using stethoscope and rectal temperature using a digital thermometer. These investigations were carried out at 16 h. The selected sheep were bled by Jugular vein puncture directly after clinical examination (at 16 h). Blood was collected from the jugular vein (10 ml) into heparinized sterile tubes. One ml of each blood sample was separated for immediate hematological studies, and the remainder was centrifuged to obtain plasma, which was kept at –20°C until be used for biochemical assay.

Haematological and biochemical investigations:
Haematological studies including red blood cell count (RBC), haemoglobin concentration (Hb) and packed cell volume (PCV) were carried out using standard techniques of hematology after **Schalm *et al.* (1975).** Plasma protein, albumin, cholesterol, glucose and blood urea were carried out using commercial test kits (Sclavo diagnostics, 53100 Sienna, Italy) described by **Henry *et al.* (1974).** Spectrophotometer—UNICAM, Helios Gamma, No. UVG 060609, England, was used for all biochemical assays. Globulins were calculated by the difference between total protein and albumin **(Henry *et al.*, 1974).** Plasma sodium and potassium were measured by using flame photometer, Corning 40 **(Henry *et al.*, 1974).** Blood methemoglobin (MetHb; as percentage of total Hb) was measured spectrophotometrically by using its absorbance spectrums and Allen correction **(Fairbanks and Klee, 1994).**

Corpuscular osmotic fragility (COF):
COF was determined by the methods of **Luzzatto and Roper (1995).** Briefly, 50 µl of well-mixed fresh blood was added into tubes with increasing concentration of buffered saline ranging from 0% (distilled water) to 0.9% NaCl at pH 7.4. The tubes were well-mixed and incubated at 25 °C for 30 min. Then, the samples were centrifuged at 1000 rpm for 15 min. Absorbance was measured at 540 nm. The 0.9% NaCl was used as a negative control and the distilled water as a positive control. Haemolysis in each NaCl concentration was ex-

pressed as percentage of the absorbance in distilled water. A fragiligram of haemolysis percent against NaCl concentrations was plotted. The effective concentration of the NaCl solution inducing 50% haemolysis (COF_{50}) of RBCs was calculated from the haemolysis curve.

Statistical analysis:

Data were analyzed using the packaged SPSS program for windows version 20.0.1 (SPSS Inc., Chicago, IL.) according SP**SS (1999).** The results were subjected to ANOVA and expressed as mean ± SE to differentiate between groups. The interactions between the groups were included by the pair-wise multiple comparison procedures (Duncan's new multiple range test).

Results

Table (1) and Fig (1) showed that both rectal temperature and respiration rate increased ($P < 0.5$) in summer than winter season, and the increase was more pronounced ($P < 0.5$) in Rahmani breed compared to Barki breed, whereas ruminal contraction was not affected by season or breed. In contrast, hematological parameters in sheep revealed a decrease ($P < 0.5$) in RBCs, Hb and PCV in summer than winter season and the reduction was more pronounced ($P < 0.5$) in Rahmani breed, but methemoglobin concentration was not affected by season or breed. On the other hand, COF increased ($P < 0.5$) in Rahmani sheep in summer, while it did not change in Barki breed.

Table (2) and Fig. (2) showed that plasma proteins and albumin decreased ($P < 0.5$) in summer compared to winter season in Rahmani breed but not affected by season in Barki breed. Plasma globulins not affected by season or breed. On the other hand, plasma glucose and cholesterol decreased but blood urea, Na and K increased ($P < 0.5$) in summer than winter season and the change was more pronounced ($P < 0.5$) in Rahmani breed than Barki breed.

Corpuscular osmotic fragility curves (hemolysis percentage of erythrocytes against varying NaCl concentrations) in Rahmani and Barki in winter and summer are presented in fragillograms (Fig. 3). It is clear from the hemolysis curves of erythrocytes where Rahmani sheep reared in summer had the higher COF during summer season.

Table (1). Rectal temperature (RT), Respiratory rate (RR), Ruminal contraction (RC) and some hematological parameters in Rahmani and Barki sheep during winter and summer season

	Rahmani		Barki	
	Winter	**Summer**	**Winter**	**Summer**
RT (°C)	39.11±0.061^{ab}	39.92±0.058^{c}	38.76±0.063^{a}	39.41±0.049^{b}
RR (/min)	37.4±1.35^{a}	54.5±1.44b^{b}	36.9±1.51^{a}	45.6±1.28^{c}
RC (/2min)	4.11±0.093^{a}	3.93±0.082^{a}	3.81±0.84^{a}	3.86±0.91^{a}
RBC (x10^{6}/ul)	9.54±0.41^{a}	8.07±0.32^{b}	9.61±0.36^{a}	8.89±0.36^{ac}
Hb (g/dl)	11.96±0.34^{a}	10.31±0.39^{b}	12.22±0.29^{a}	11.14±0.29^{c}
PCV (%)	33.61±0.84a^{b}	28.01±0.91^{b}	34.54±0.73^{a}	31.11±0.31^{bc}
MetHb %	3.99±0.33^{a}	4.21±0.41^{a}	3.84±0.24^{a}	4.12±0.31^{a}
COF_{50} (g/dl)	0.581±0.001^{a}	0.629±0.002^{b}	0.576±0.002^{a}	0.592±0.001^{a}

^{a,b,c}: Values in the same row with unlike descriptive superscript letters are significantly different ($P < 0.05$).

Table (2). Biochemiical parameters in Rahmani and Barki sheep during winter and summer season

	Rahmani		Barki	
Parameter (Unit)	**Winter**	**Summer**	**Winter**	**Summer**
T. protein (g/dl)	6.87±0.46[a]	5.14±0.39[b]	7.01±0.41[a]	6.91±0.43[a]
Albumin (g/dl)	3.81±0.12[a]	2.24±0.09[b]	3.90±0.16[a]	3.76±0.14[a]
Globulin (g/dl)	3.06±0.16[a]	2.90±0.11[a]	3.11±0.19[a]	3.15±0.18[a]
Glucose (mg/dl)	71.1±1.55[ab]	54.3±1.21[c]	74.9±1.67[a]	66.2±1.25[b]
Cholesterol (mg/dl)	61.1±1.14[a]	51.2±1.34[b]	58.8±1.46[a]	56.9±1.42[a]
Blood urea (mg/dl)	25.1±0.84[ab]	33.8±0.67c	23.4±0.54[a]	28.6±0.51[b]
Na (mmol/l)	135.9±1.05[a]	149.5±1.11[b]	134.1±1.24[a]	141.2±1.17[c]
K (mmol/l)	4.61±0.16[a]	5.86±0.14[b]	4.43±0.11[a]	5.19±0.12[c]

[a,b,c]: Values in the same row with unlike descriptive superscript letters are significantly different ($P < 0.05$).

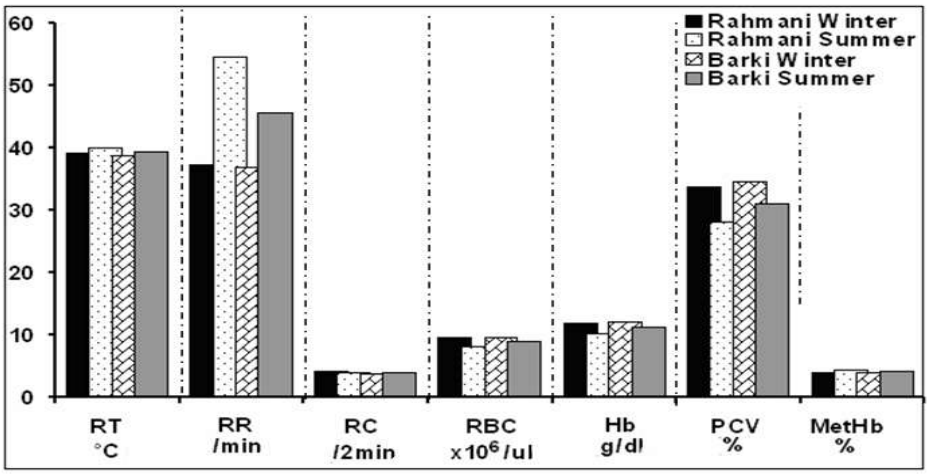

Fig. (1): Rectal temperature (RT), Respiratory rate (RR), Ruminal contraction (RC) and some hematological parameters in Rahmani and Barki sheep during winter and summer season

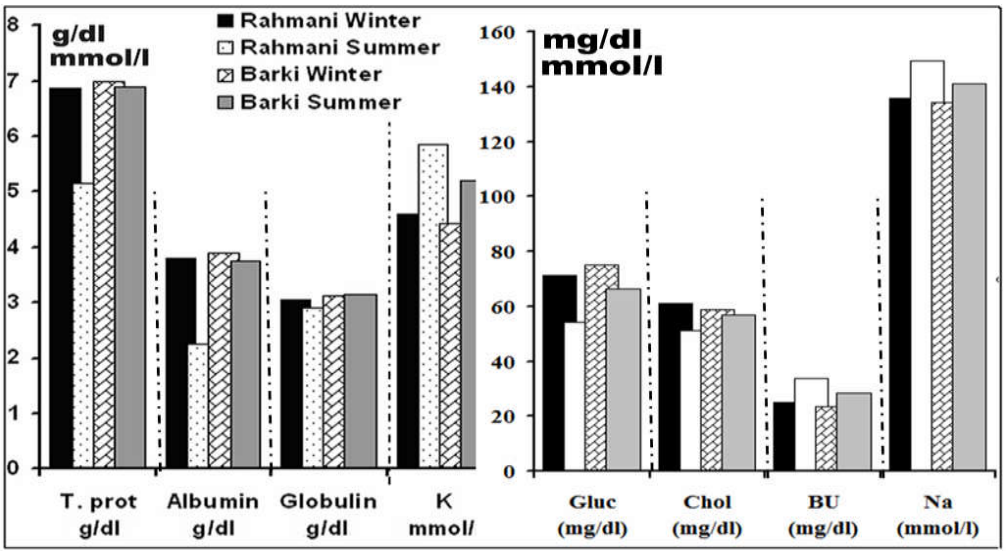

Fig. (2): Biochemiical parameters in Rahmani and Barki sheep during winter and summer season

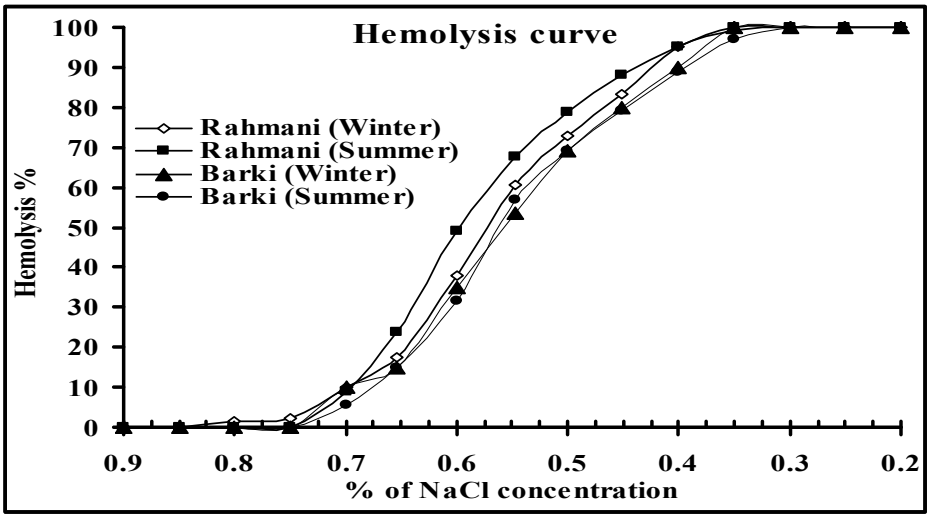

Fig. (3): Corpuscular osmotic fragility curves (hemolysis percentage of erythrocytes against varying NaCl concentrations) in Rahmani and Barki in winter and summer.

Discussion

Under thermal stress, a number of physiological and behavioral responses vary in intensity and duration in relation to the animal's genetic make-up and environmental factors that are associated with negative impacts of heat stress on production and reproduction in dairy animals **(West, 2003).**

The increase of rectal temperature and respiration rate has been considered as good indicators to the level of heat stress upon animals **(Alamer and Al-Hozab, 2004).** Therefore, the observed elevation in rectal temperature and respiration rate under hot summer conditions indicate that the animals were heat stressed. Furthermore, the elevation in rectal temperature and respiration rate in Rahmani than Barki sheep indicates that Rahmani sheep are more stressed than Barki sheep. Hyperpnoea develop when heat production exceeds heat loss or when the evaporative heat loss mechanisms becomes impaired due to excessive loss of body fluids and reduced blood volume. This response is in part due to direct stimulation of peripheral temperature receptors that transmit nervous impulses to the heat and respiratory centers in the hypothalamus **(Habeeb *et al.*, 1992).**

Our results revealed a decrease in RBCs, Hb and PCV in summer than winter season. During hot-dry climatic exposure, water intake increased between 37% and 45% **(Guerrini, 1981).** Therefore, this reduction in haematological indices is concordant with the haemodiulution occurred in stressed sheep **(Kuselo, *et al.* 2005)**. The reduction in oxygen requirements to minimize the metabolic heat load, the depression of haematopoiesis, and the sequestration of erythrocytes in the capillary beds **(Igbokwe, 1997)** might be additional factors responsible for reduction in hematological indices. This reduction in the hematological indices was more pronounced in Rahmani breed, indicating that Barki breed is well tolerated to heat stress than Rahmani breed.

The observed variation in the thermophysiological parameters under hot summer conditions was associated with alterations in some plasma biochemical parameters (Table 2). Plasma albumin was decreased in Rahmani sheep with a consequent reduction in total protein levels without breed difference. The significant reduction in these proteins in Rahmani sheep seems to be due to dilution of these proteins, the decrease in protein synthesis as a result of the depression of anabolic hormonal secretion and the increase in the catabolic hormones as glucocorticoids and catecholamins (**Habeeb *et al,* 1992).** Also, albumin might be filtered and redistributed into the extravascular

spaces during thermal stress due to its high osmotic sensitivity and its relatively lower molecular mass and size than other protein fractions, **(Kerr, 2002)** resulting in reduction in the circulating portion.

The current results revealed a decrease in plasma glucose, cholesterol and an increase in blood urea in summer than winter season in sheep. It is possible that sheep had decreased blood glucose in summer because of the elevated energy cost that occurs with the rise in respiration rate as a thermoregulatory mechanism **(Srikandakumar *et al*. 2003; Sejian *et al.* 2010).** At the same time, the reduction in glucose levels may have caused the drop in blood cholesterol **(Rasooli *et al.* 2004 and Indu *et al.* 2015).** Moreover, blood urea results are attributed to the low blood flow directed to kidneys when heat stress is experimented in this breed, since most blood is redirected to skin and muscles associated with breathing in order to dissipate heat **(Srikandakumar *et al.* 2003).** Thus, urea release from the body in the urine was low. Other studies in sheep adapted to heat stress are consistent with this hypothesis **(Srikandakumar *et al.* 2003 and Indu *et al.* 2015).**

In our study, heat stress increased plasma sodium and potassium concentration in sheep. The increase in plasma electrolytes might be a functional compensatory mechanism for retention of body water to insure efficient evaporative cooling because plasma osmolality and blood volume depend mainly on sodium concentration than other osmotic ingredients **(Collier *et al.* 1982).** The increase of electrolytes in combination with the result of increased blood urea can be an indicative that sheep maintained their water balance through reduction of fecal and urinary water losses **(Piccione *et al.* 2012).** The differences between breeds in these parameters indicate that Barki sheep are more homoeothermic and cope with the hot arid environment of the desert in the New-Valley area. **El-Nouty *et al.* (1988)** reported that Barki sheep seem to perform better than the Rahmani in newly reclaimed desert areas.

The COF test is a sensitive marker of osmotic stress, membrane stability and sensitivity to hemolysis **(Harvey, 2008).** In the present study, the increased COF_{50} values in Rahmani sheep during summer indicates that erythrocytes of heat stressed sheep are under osmotic stress and provide evidence that stability of the erythrocyte membrane is dropped and the erythrocytes are susceptible to hemolysis during heat stress. **Utoh *et al.* (1992)** reported an increase of membrane fragility in erythrocytes of calves exposed to high in vitro temperatures. COF of the desert sheep RBCs was enhanced and values for 50% lyses in hypotonic saline decreased from 77 mM NaCl to 68 mM NaCl after dehydration **(Turner, 1979).** Furthermore, COF increased due to lowered glucose uptake and red cell ATP level in heat stressed hamsters **(Meyerstein and Cassuto, 1970).**

As a conclusion, the results of this study indicate that Rahmani sheep are more prone to heat stress than Barki sheep under hot dry summer conditions and still having a thermoregulatory mechanism. Therefore, their health and production under such conditions would require environmental and/or nutritional modification to alleviate the impact of heat stress.

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