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# EFFECT OF HOT DRY ENVIRONMENT ON THE OXIDATIVE STRESS INDICES IN MALE BARKI LAMBS

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## ABSTRACT

Heat stress (HS) is the main challenge facing livestock health. The present work aimed to study the oxidative stress status in the blood of desert Barki lambs during the hot dry season in El-Kharga oasis in the western Egyptian desert. Twenty-four male Barki lambs (4-5 months) were included in this study. Twelve of them were selected during July (hot dry, HS group) and the remaining 12 animals were selected during December (thermoneutral, TN-group) as controls. Temperature humidity index (THI) registered 65.4 (satisfactory) in winter and 89.6 (risky HS) in summer**.** Compared to controls, increased rectal temperature ($P < 0.001$) and respiration rate ($P < 0.001$) were detected in the HS group. Red blood cells count ($P < 0.001$), packed cell volume ($P < 0.01$) and hemoglobin concentration ($P < 0.001$) were reduced in HS group. Increased total peroxides (TPx, $P < 0.05$), the free radical superoxide anion ($P < 0.05$) and decreased total antioxidant capacity (TAC, $P < 0.05$) were shown in plasma of the HS group. Malondialdehyde concentration as an indicator of lipid peroxidation was increased ($P < 0.05$) but superoxide dismutase activity was decreased ($P < 0.05$) in the erythrocyte of the HS group. Oxidative stress index (OSI, TPx/TAC ratio) increased ($P < 0.05$) in HS group. In conclusion, HS is associated with oxidative stress and corpuscular redox imbalance in lambs under tropical conditions.

***Keywords:*** Erythrocyte; Hot dry heat stress; Lambs; Oxidative stress

## INTRODUCTION

Heat stress (HS) is basically described as a biological response to thermal stimuli in the environment (Constable *et al.*, 2017). Seasonal changes in ambient temperature and relative humidity (RH %), can affect the thermal equilibrium between the animal and its environment, and exacerbate the severity of heat stress (Habeeb *et al.*, 2018). The temperature-humidity index (THI) combines the ambient temperature with the RH %. It has become a method of choice and a metric guide for the heat stress caused by thermal environmental circumstances (Ravagnolo *et al.*, 2000).

Ruminants have well-developed thermo-regulation mechanisms, however, they are not capable to maintain adequate homeothermy under severe HS, so they

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display physiological and metabolic adaptation such as hyperthermia, panting and reduced feed intake, to withstand heat stress (Constable *et al.,* 2017).

Heat stress can disrupt the innate immune response's equilibrium and affect metabolism and gene expression, resulting in imbalanced cellular, immunological, and physiological responses (Habeeb *et al.,* 2018). Heat stress increases components of systemic inflammation, and enhances leukocytes to produce inflammatory cytokines and free radicals with a subsequent increase in prooxidants (Gutteridge and Halliwell, 2018). Total oxidant status (TOS) is a metric that indicates the burden of total peroxides in a given sample (Erel, 2005).

The body, on the other hand, contains sufficient non-enzymatic and enzymatic antioxidant capabilities that work in concert to neutralize the increased prooxidant generation (Gutteridge and Halliwell, 2018). The total antioxidant capacity (TAC) is defined as the sum of all antioxidants' potential (Erel, 2005). When the increased prooxidants exceed the antioxidant state, oxidative stress (OS) develops (Sies *et al.,* 2017). Pathological processes such as DNA damage and cytotoxicity are induced by the increased OS (Gutteridge and Halliwell, 2018). The ratio of TOS to TAC is proposed as a new way to quantify the oxidative stress index (OSI), and it may be used to investigate the homeostatic status of the redox system (Abuelo *et al.,* 2013).

The erythrocyte is highly vulnerable to free radicals, but it also contains a strong antioxidant mechanism that can scavenge these radicals (Fujii *et al.,* 2021). When the TOS surpasses the TAC, the triggered OS can cause erythrocytic oxidative injury and cytotoxicity, including damage to membrane lipids and proteins, as well as hemolytic injuries (Fujii *et al.,* 2021).

The goal of this study was to estimate the response of some physiological, hematological, and erythrocytic redox status, such as TOS, lipid peroxides, TAC, the antioxidant superoxide dismutase, and OSI in desert male Barki lambs during the hot dry season (summer HS) environment of the western Egyptian desert (El-Kharga oasis).

## MATERIALS AND METHODS

**Study area:**

This research was conducted in El-Kharga oasis in the western Egyptian desert. This oasis lies between 25° 30′ N and 27° 30′ E as a depression area. This region's climate is defined by hot, dry summers and temperate winters. There is no rainfall, and watering and irrigation are solely dependent on ground wells.

**Meteorological data:**

This research was conducted in December (mid-winter, Thermoneutral period) and July (mid-summer, HS period), El-Kharga weather station provided the weather data. Air temperature (Td, C) and relative humidity (RH %) were used to calculate the temperature-humidity index (THI) using the following formula (Lallo *et al.,* 2018): THI =Td - (0.55 - 0.55RH "as a decimal") (Td - 14.4).

THI readings between 74 and 75 are regarded as comfortable, 75 to 78 is considered moderate stress, and THI values between 79 and 83 are considered serious heat stress. When THI reaches 84, the exposed animals are regarded to be in danger of succumbing to heat stress (Lallo *et al.,* 2018).

**Animals:**

Sheep in small herds are constrained to limit grazing grounds in the morning. They browse vegetation that grows around groundwater in the morning. They are shaded in the afternoon by available trees. clover (Hegazi) was admitted without any further supplements at night. In this study, twenty-four, apparently healthy male Barki lambs (ages 4-5 months) were chosen. The

first 12 animals (TN-group) were chosen in December (thermoneutral period), whereas the remaining 12 animals (HS-group) were chosen in July (Hot dry period).

**Clinical investigations:**
Clinical and analytical investigations were performed on these animals. Standard clinical examination methods (Constable *et al.*, 2017) at 1600 h during mid-December (TN group) and mid-July (HS group) were used to evaluate respiratory rate (RR) and rectal temperature (RT). Standard parasitological examinations (Soulsby, 1982; Urquhart *et al.*, 1996) on fecal and blood samples from each lamb were done to confirm that the examined lambs were free from internal and blood parasitic infections. The examined lambs were also free from external parasites and skin lesions.

**Blood sampling**:
From each lamb, blood was sampled by jugular vein puncture (at 1600 h) into 10-ml heparinized sterile tubes. Samples were sent on crushed ice to the laboratory immediately (within 2 h). Two ml of blood was separated for immediate hematological studies and preparation of erythrocyte hemolysate. Hemolysate was used for determinations of lipid peroxides concentrations and superoxide dismutase activity. The rest of the blood samples were used for separation of plasma by centrifugation at 1500 rpm for 15 min at 4°C. Plasma was stored at -20 ºC until it was utilized to measure TOS, superoxide anion ($^{\bullet}\bar{O}_2$) concentration, and total antioxidant capacity (TAC).

**Hematological investigations**
Standard hematological methods (Kramer, 2000) were used for the determination of the values of red blood cell count (RBC) and hemoglobin concentration (Hb) in addition to packed cell volume (PCV).

**Preparation of erythrocyte hemolysate**
At 4°C, one ml of blood was centrifuged at 650 g for 15 minutes. The packed erythrocytes in the pellet were washed three times in buffered saline after aspiration of the plasma and buffy coat. The crude erythrocytes were lysed by hypotonic shock using nine ml of cold bi-distilled water to obtain a 10% hemolysate (Kramer, 2000).

**Biochemical analysis**
**Total peroxides (TPx)**
The concentration of plasma TPx, or so-called total oxidant status (TOS) (as a characteristic marker of ROS), was measured using the method of Erel (2005). The oxidative molecules oxidize the $Fe^{+2}$-o-dianisidine complex to $Fe^{+3}$, resulting in a colored complex with xylenol orange that can be read at 560 nm. TPx was expressed as µmol $H_2O_2$ Equiv/L.

**Superoxide anion ($^{\bullet}\bar{O}_2$) concentration**
Plasma concentration of superoxide anion ($^{\bullet}\bar{O}_2$) was measured according to Podczasy and Wei (1988). The reddish-pink color originated from the reduction of p-iodonitrotetrazolium by $^{\bullet}\bar{O}_2$ that generated by xanthine/xanthine oxidase can be read at 505 nm. The method of Henry *et al.* (1974) was used for the determination of plasma total protein (TP), and $^{\bullet}\bar{O}_2$ concentration was adjusted to TP and represented as µmol /mg protein.

**Total antioxidant capacity (TAC)**
Plasma TAC was measured after the method of Miller *et al.* (1993). A colorimetric ABTS assay kit (Beyotime Institute of Biotechnology, Haimen, China) was used. TAC was expressed as mmol Trolox equiv/L.

**Lipid peroxides:**
The concentration of erythrocytic lipid peroxide was evaluated using thiobarbituric acid reactive compounds, the most prevalent of which being malondialdehyde (MDA) concentration (Tsikas, 2017). The resultant color complex can be read at 548 nm and quantified as nmol MDA/g Hb.

**Superoxide dismutase**
The activity of Erythrocytic SOD was determined by the method of Sirota (2011). At pH 10.2, the oxidation of epinephrine to

adrenochrome is inhibited by SOD and the color was read at 480 nm. SOD activity was expressed as U/mg Hb.

**Oxidative stress index (OSI)**

The OSI value was derived using the following equation (Abuelo *et al.,* 2013): OSI (Arbitrary unit) == (TOS, µmol/L / TAC, µmol Trolox equv/L) ×100.

**Statistical analysis**

Statistical analysis was done by using the SPSS program for windows, v. 21 (SPSS, Chicago, IL, USA). After testing the normal distribution by using the Shapiro-Wilk test, and the data were shown to be normally distributed, analysis of variance (ANOVA) was used for statistical analysis. The student's t-test was used to compare the means of data between the two groups. Data were tabulated as mean ± standard error. The significant difference was set at $P < 0.05$.

## RESULTS

The recorded ambient temperature and RH % in the study area were 17.9 ˚C, 44.2 % in January and 44.8 ˚C, 21.3 % in August, respectively. Accordingly, the calculated THI was 65.4 in December and 89.6 in July**.** The HS group showed a significantly higher mean value of RR ($P < 0.001$) and RT ($P < 0.001$) when compared with the NT group (Table 1).

Hematological indices (Table 1) showed lower mean values of RBC ($P < 0.001$), Hb ($P < 0.001$) and PCV ($P < 0.01$) in HS group when compared with TN group.

**Table 1:** Mean values (±SE) of rectal temperature (RT), respiration rate (RR), red blood cell count (RBC), packed cell volume (PCV) and hemoglobin concentration (Hb) in thermoneutral (TN) and heat-stressed lambs (HS).

| | TN group (n=12) | HS group (n=12) |
|---|---|---|
| RT (°C) | 38.30 ± 0.07 | 39.5 ± 0.14 *** |
| RR (breath/min) | 32.40 ± 0.83 | 64.8 ± 1.67*** |
| RBC ($x10^{12}$/L) | 7.37 ± 0.122 | 6.57 ± 0.151*** |
| Hb (g/L) | 100.53 ± 2.041 | 88.93 ± 1.872*** |
| PCV (L/L) | 33.07 ± 0.743 | 29.53 ± 0.952** |

**, *** difference between means of thermoneutral (TN) and heat-stressed lambs (HS) is significant at P<0.01 and P<0.001, respectively.

Oxidative stress biomarkers in plasma and erythrocytes are presented in table 2. A significant increase in the mean value of plasma TPx and superoxide anion was detected in the HS lambs ($P < 0.05$) compared to the control value. On the contrary, a significant reduction in the mean concentration of plasma TAC was noticed in the HS lambs ($P < 0.05$) compared to the control value. Compared to the control results, the mean value of erythrocytic MDA as an indicator of lipid peroxidation was enhanced ($P < 0.05$), whereas, the value of erythrocytic SOD was lower ($P < 0.05$) in HS lambs compared with the NS group. Accordingly, the calculated OSI value as a marker of oxidative stress was higher ($P < 0.05$) in HS lambs compared with the corresponding result of the NS group.

**Table 2**: Mean values (± SE) of pro-oxidants and antioxidants in plasma (p) and erythrocytes (e) in thermoneutral (TN) and heat-stressed lambs (HS).

| Parameter | TN group (n=12) | HS group (n=12) |
|---|---|---|
| p TPx (µmol $H_2O_2$ Equiv/L) | 11.680 ± 1.026 | 17.213 ± 0.767* |
| p $\dot{O}2$ (µmol/mg protein) | 0.211 ± 0.022 | 0.298 ± 0.019* |
| p TAC (mmol Trolox equiv/L) | 1.566 ± 0.170 | 1.065 ± 0.096* |
| e SOD (U/mg Hb) | 8.55 ± 0.99 | 5.65 ± 0.65* |
| e MDA (nmol MDA/g Hb) | 4.05 ± 0.54 | 5.72 ± 0.53* |
| OSI (Arbitrary unit) | 0.746 ±0.124 | 1.61 ± 0.172* |

TOS: total oxidant status, $\dot{O}_2$: Superoxide anion TAC: total antioxidant capacity, MDA: malondialdehyde, SOD: superoxide dismutase. OSI: oxidative stress index.
* difference between means in thermoneutral (TN) and heat-stressed lambs (HS)is significant at $P<0.05$.

## DISCUSSION

Ruminants in the open pasture are exposed to direct contact with the environment so that they suffer from the dangerous stress of uncontrolled environmental changes. THI is a scale for expressing the ranges of thermal comfort for animals (Lallo *et al.,* 2018).

The threshold or critical THI value for optimum sheep health was estimated to be 81 (Bhattacharya and Uwayjan, 1975). The THI in the current study was inside the thermoneutral zone in the winter, but it was beyond the thermoneutral zone in the summer, indicating that the HS group was reared in a severe HS environment (risky zone).

The rise in RR and RT in this research was expected and consistent with prior results for sheep (Bhattacharya and Uwayjan, 1975; Ellamie *et al.,* 2020), which could be due to direct stimulation of the hypothalamus' respiratory and heat centers (Habeeb *et al.,* 2018).

Higher HS can induce systemic inflammation and trigger proinflammatory cytokine concentrations with a subsequent enhancement of reactive oxygen species (ROS) generation (Constable *et al.,* 2017). Increased plasmatic TPx and $\dot{O}2$ in the current study indicate overproduction of ROS in heat-stressed lambs. Similar results were reported in a previous study that found increased TPx in sheep plasma (Chauhan *et al.,* 2015).

The TAC assesses antioxidant power against increased free radicals by measuring the collective functions of enzymatic and non-enzymatic antioxidants (Erel, 2005). The decreased TAC in the plasma of HS lambs in the current study suggests that the antioxidant potential as a free radical scavenger has been exhausted. Similarly, the observed decrease of SOD in the heat-stressed lambs reveals also endogenous antioxidant mobilization to neutralize free radicals (Ellamie *et al.,* 2020). SOD functions to enhance the dismutation of superoxide anion to water (Gutteridge and Halliwell, 2018). Ellamie *et al.* (2020) found similar results in heat-challenged sheep, with diminished TAC and suppressed SOD activity in plasma.

The elevated TOS and lowered TAC in the current study suggest a disruption in the redox equilibrium in the heat-stressed lambs. Previous studies have shown that environmental HS contributes to the induction of oxidative stress in farm animals by activating ROS or impairing the animal's antioxidant capacity (Chauhan *et al.,* 2015). OS can result in tissue injury in the form of peroxidation of macromolecules (Valacchi *et al.,* 2018). Peroxidation of lipids especially polyunsaturated fatty acids produces lipid

peroxides, the most prevalent of which are the malondialdehyde (MDA) macromolecules (Gutteridge and Halliwell, 2018).

In a recent study (Ellamie *et al.,* 2020), OS produced by environmental HS causes enhanced indicators of tissue injury in the form of increased lipid peroxidation as detected by the enhanced MDA in Barki sheep plasma. MDA is a good biomarker for determining the severity of lipid oxidative damage and oxidative stress in the erythrocytic cell membrane (Gutteridge and Halliwell, 2018). The increased corpuscular MDA in the present study suggests increased lipid peroxidation of the erythrocytic membrane of heat-stressed lambs.

The severity of OS is governed by increased TPx, impaired TAC or even both (Gutteridge and Halliwell, 2018). Therefore, TOS and TAC are the main actors in the redox equilibrium (Valacchi *et al.,* 2018). The estimation of OSI can provide a satisfactory finding of the relationship between TOS and TAC that is not evident when both substances are separately estimated (Abuelo *et al.,* 2013). In the current study, OSI was shown to be higher in lambs reared during the thermos-stress season than in lambs reared during the thermoneutral season indicating that the animals were at risk during the thermos-stress season. This shows that a competent antioxidant power could not control the increased burden of prooxidants, resulting in high OS in HS lambs. Our results agree with the findings of Chauhan *et al.* (2015) who showed activated TOS and inhibited TAC accompanied by increased OSI in experimentally induced HS in sheep.

HS reduced RBC count, PCV, and Hb concentration in the current research, which is consistent with recent results on heat stress in sheep (Ellamie *et al.,* 2020; Nicolás-López *et al.,* 2021). Although these findings could be explained by hemodilution, the current research suggests that HS may also enhance erythrocytic OS, which can lead to erythrocytic damage. Furthermore, OS can cause cytotoxicity, apoptosis, and an increase in the spleen's clearance of these cells (Fujii *et al.,* 2021).

In conclusion, HS is associated with redox imbalance and enhanced erythrocytic oxidation.

## DECLARATIONS OF INTEREST

The authors have no competing interests to declare

## ETHICS APPROVAL

This study was approved by the ethical rules of the Animal Health Research Institute, Agriculture research center, Egypt.

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## تأثير البيئة الحارة الجافة على مؤشرات الاجهاد الحراري في ذكور الحملان البرقي

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الإجهاد الحراري (HS) هو التحدي الرئيسي الذي يواجه الصحة الحيوانية . يهدف البحث الحالي إلى دراسة حالة الإجهاد التأكسدي في دم ذكور حملان البرقي الصحراوية خلال موسم الإجهاد الحراري الحار الجاف في الصحراء الغربية (الخارجة) . اختير في هذه الدراسة اربعة وعشرون ذكر من الحملان البرقي السليمة (٤-٥ أشهر). تم اختيار ١٢ منهم خلال شهر يوليو (اجهاد حراري - HS) وتم اختيار الحيوانات الاثنى عشر المتبقية خلال شهر ديسمبر (متعادلة حراريًا - TN)كمجموعة ضابطة . سجل مؤشر درجة الحرارة والرطوبة (THI) 65.4 في الشتاء (متعادل حراريا) و ٨٩,٦ في الصيف (خطير) . بالمقارنة مع المجموعة الضابطة ، لوحظ زيادة درجة حرارة الجسم ($P < 0.001$) ومعدل التنفس ($P < 0.001$)في مجموعة HS . انخفض عدد خلايا الدم الحمراء ($P < 0.001$) ، وحجم الخلايا المصمت ($P < 0.01$) وتركيز الهيموجلوبين ($P < 0.001$) في المجموعة HS . لوحظ زيادة البيروكسيدات الكلي (TPx- $P < 0.05$) و أنيون السوبراكسيد ($P < 0.05$) وانخفاض السعة الكلية المضادة للأكسدة ($P < 0.05$, TAC) في بلازما المجموعة HS . لوحظ ايضا زيادة تركيز المالونالدهيد كمؤشر على أكسدة الدهون ($P < 0.05$) ولكن نشاط انزيم السوبرديسموتاز انخفض ($P < 0.05$)في كريات الدم الحمراء لمجموعة HS . لذلك زاد مؤشر الإجهاد التأكسدي (OSI, $P < 0.05$) وهو نسبة TPx /TAC) في مجموعة HS . يمكن ان نستنتج من هذه الدراسة ان الاجهاد الحراري يتسبب في الإجهاد التأكسدي وعدم توازن الأكسدة والاختزال في كريات الدم الحمراء قي الحملان في ظل الظروف الصحراوية.