Pakistan J. Zool., pp 1-9, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230819131031

Natural Heat Stress Affects Physiological, Hematological and Hormonal Parameters in North African Ram Breeds

Walid Mâaoui[1,2], Mohamed Amine Ferchichi[2], Jihen Toumi[3], Bayrem Jemmali[2]*, Naceur M'hamdi[1] and Abderrahmen Ben Gara[2]

[1]*National Agronomic Institute of Tunisia, Carthage University, 43 Avenue Charles Nicolle, Tunis 1082, Tunisia*
[2]*University of Carthage, LR13AGR02, Mateur Higher School of Agriculture, Mateur, Tunisia*
[3]*Central Analytical Laboratory of Animal Feeds, Tunisia*

Article Information
Received 19 August 2023
Revised 20 November 2023
Accepted 04 December 2023
Available online 14 March 2024 (early access)

Authors' Contribution
MW, MAF and JT designed the study and wrote the manuscript. MW, MAF, JT, NM and BJ performed the field work. ABG, MW and BJ analyzed the data.

Key words
Heart rate, Heat stress, Hematological parameters, Rams, Rectal temperature, Temperature humidity index, Thyroxine

ABSTRACT

To study the ability of sheep to maintain their homeothermy in a North African natural heat stress condition, three Tunisian sheep breeds the Barbarin (BTR), Queue Fine de l'Ouest (QFO) and Noire de Thibar (NTB) were used. A total of twelve (4 per breed) rams were used in this study, for the period of 3 months from March to June. During this period, the ambient temperature varied from 12.5 °C to 32 °C, relative humidity from 49% to 94% and the temperature humidity index (THI) from 50 units to 85 units. The THI values exceeded the borderline of thermal stress, resulting a higher rectal and skin temperature, heart and respiratory rates (P<0.05). The rectal temperature (RT) varied from 39.41 °C to 39.38 °C for BTR, from 39.20 °C to 39.52 °C for QFO and from 39.51 °C to 39.72 °C for NTB. A significant (P<0.05) decrease in thyroxine (T4) from 16.58 pmol/l to 7.52 pmol/l for BTR rams, from 10.99 pmol/l to 6.34 pmol/l for QFO rams and from 12.43 pmol/l to 6.91 pmol/l for NTB rams. TSH hormone decrease significantly from a comfort situation to a stressful situation for BTR (0.124 mUI/l to 0.039 mUI/l), QFO (0.132 mUI/l to 0.065 mUI/l) and NTB (0.113 mUI/l to 0.057 mUI/l). No change in tri-iododthyronine (T_3) and a no significantly decrease for cortisol hormone, from 6.91 ng/l to 2.83 ng/l for BTR, from 9.69 ng/l to 4.20 ng/l for QFO and from 7.65 ng/l to 2.42 ng/l for NTB. These responses allowed animals to efficiently dissipate heat, maintain a physiological rectal temperature, and avoid thermal stress. Blood samples were taken and analyzed during two different periods: no stress and heat stress to study the relationship between homeothermy and hematological (RBC, WBC, Hb, Ht, Pl, PCV, MCH, and MCHC), biochemical (glucose, cholesterol, total protein) and hormonal (T_4, T_3, Cortisol and TSH) parameters responses.

INTRODUCTION

To feed the world's rising population, crop and animal production must become more intensive and efficient globally, particularly in the intertropical zone (Renaudeau *et al*., 2012; Scholtz *et al*., 2013). However, the climate change anticipated the raise of the mean temperatures in temperate regions. Agriculture, biodiversity, energy sector, hydrology, human and animal health could all suffer as a result of the rising temperature (Solymosi *et al*., 2010). Negative impacts of climate change on animal production parameters are: (1) An increase in atmospheric CO_2 levels and temperatures will have a major impact on feed quantity and quality (Chapman *et al*., 2012). (2) A water scarcity will have an impact on the livestock industry, which needs water for animal drinking, feed crops, and manufacturing activities (Thornton *et al*., 2009). (3) A higher temperature may accelerate the development of diseases and parasites that spend part of their life cycle outside of their host, putting animals at risk (Patz *et al*., 2000; Harvell *et al*., 2002; Karl *et al*., 2009). (4) Temperature, humidity, species, genetic potential, life stage, and nutritional status all influence heat stress in livestock. Because livestock in lower latitudes are generally better acclimated to high temperatures and drought, livestock in higher latitudes will be more affected by higher temperatures than livestock in lower latitudes (Thornton *et al*., 2009).

The core temperature of sheep range between 38.3

* Corresponding author: jemmali.bayrem@gmail.com
0030-9923/2024/0001-0001 $ 9.00/0

and 39.9 °C. They are able to maintain their temperature in this range even the environmental conditions are disadvantageous. On the other hand, the range of thermal settings in which the maximum of the welfare and productivity of agricultural animals like ewes and rams is quite narrow, so sheep's physiology, behavior, and energy expenditure are altered in order to maintain a stable core temperature in this restricted range (Silanikove, 2000; Al-Dawood, 2017; Johnson, 2018). Non-essential functions such as growth, reproduction, and milk production might be affected by the environmental conditions and the productivity can be restricted in thermal conditions. Depending of breed age and physiological state, the top threshold temperature from which thermoregulatory mechanisms are substantially tested and begin to involve significant energy expenditure ranges from 25 to 31 °C (Hopkins *et al.*, 1978; Silanikove, 2000; Al-Dawood, 2017).

Many parameters can be controlled to study the effect of environmental conditions on sheep and specially rams, but very little is known about this subject. The most important parameter to follow, is the testicular temperature, which must be maintained below core body temperature for most mammals, including sheep, in order to produce motile and morphologically normal spermatozoa (Setchell, 1998). At ambient temperatures of 20-30°C, the ram has intra-testicular temperatures range between 33-35°C, indicating a rectal-testicular temperature gradient of 4-6°C (Marai *et al.*, 2008, 2009; Kahwage *et al.*, 2018; Barragán Sierra *et al.*, 2021; Van Wettere *et al.*, 2021). Multiple studies have examined the effects of higher testicular temperature on ram ejaculation output and quality using local or whole-body heating. However, few research have looked at the impact of temperature on sperm quality across North African meteorological condition. In the other hand, earlier research has found that both absolute temperature in different regions of the testes and the testicular thermal gradient are useful predictors of semen quality (Menegassi *et al.*, 2015) and fertility in pasture-raised animals (Lunstra and Coulter, 1997).

The temperature humidity index (THI) is a heat stress index that takes account of both temperature and relative humidity. It is commonly used to evaluate the severity of heat stress in livestock animals. This indicator was created as a weather safety measure in order to control and reduce heat stress-related losses. The THI includes the impacts of both ambient temperature and relative humidity in one index, that's why it is widely used in hot places around the world as a realistic indicator for the degree of stress on livestock animals caused by weather conditions (Habeeb *et al.*, 2018; Thom, 1959). Some authors have recently employed this parameter to study the influence of heat stress on animal physiological parameters like rectal temperature, heart rate, respiration rate, skin temperature (Sejian *et al.*, 2012), and blood parameters (Sejian *et al.*, 2012).

The objectives of this paper were to study physiological, blood, biochemical and hormonal parameters of rams when they were exposed to heat stress conditions, to fined crucial threshold values, and to determine the most affected markers.

MATERIALS AND METHODS

In this trial, The Experimental Animal Use Ethics Committee of National Agronomic Institute of Tunisia has previously approved (protocol No 05/15). It took place in the farm of the Heigh School of Agriculture of Mateur in North of Tunisia (latitudes 37° 04' North and longitudes 9° 62' East), between March and June 2021. This subhumid area region shows a typical Mediterranean climate, the annual average rainfall is between 400-500 mm. A hot and extremely dry air from the Sahara Desert characterize the dry season, which extend from June to September. Nonetheless, when subtropical marine air masses converge in the region during the dry season, some convective clouds develop occasionally. Summer is usually hot and dry season in Tunisia. The continental zones are characterized by daily maximum temperatures surpassing 40°C (Verner *et al.*, 2018). An automated meteorological station 300 meters away from the animal allows the recording of climatic parameters such as ambient temperature (T_a, °C) and relative air humidity (RH, percent). In addition of the climatic data provided by the automatic weather station and to have more exactitude, temperature and humidity were controlled in the feeding area by a thermos hygrometer (Testo 608-H1, Entech Industrial Solution Co.,Ltd, Thailand). The THI was calculated according to the formula described by Thom (1959).

$$THI = 0.8*T_a + ((HR/100) * (T_a-14.3)) + 46.4$$

With T_a is the daily ambient temperature in °C, HR is the daily relative air humidity in %.

According to Moran (2005), the THI values obtained indicate: < 72 = thermal comfort zone (TCZ); > 72 = thermal stressful zone (TSZ). In addition, the THI was calculated at the time of physiological data collection to allow for real-time assessment of environmental circumstances.

A total number of twelve clinically healthy mature Tunisian breed rams aged between 3 and 4 years were used in this study. The breeds were divided in three groups, 4 animals for each group. The first one is composed by Tunisian Barbarin sheep (BTR, n = 4; BW= 67 ± 7.81 Kg; Age = 3.2 years) with a white fat-tailed breeds. The second one is composed by Queue Fine de l'Ouest Tunisian sheep, with a white breed (QFO, n = 4; BW = 56 ± 2.00 Kg; Age = 3.5 years), the last one composed by the Noire de Thibar Tunisian sheep with a black breed (NTB, n = 4; BW= 64 ± 9.16 Kg; Age = 3.4 years).

All of the rams were confined on a single lot of 25 m^2 (L =5 x l=4.5 m, 1.8 m^2/animal) without pasture and no exposition to direct solar radiation. The animals were maintained in well-ventilated sheds with a one open side and asbestos roofing at 3.0 meters height, with a normal hygienic condition.

Oat hay (3 Kg/animal/day) and 350 g/animal/day of a commercial concentrate were included in the diet (ground corn, crushed barley, bran, soybean meal, calcitic lime, and mineralized salt). The animals were watered and fed twice a day (0800 h and 1800 h), and they had unlimited access to the feeding trough.

The variables: respiration rate (RR), heart rate (HR) and rectal temperature (RT) were measured weekly in the afternoon (from 1100h-1400h). The RR (*breath/min) was measured by counting flank movements for one minute by an observer positioned beside the animals. The HR (beats/min) was monitored with a veterinary stethoscope, counting the number of hart battements for 15 sec then multiplied by four. A transrectal digital thermometer was used to measure the RT (°C) after 15 sec of its introduction, the thermometer beeped and displayed the temperature reading to one decimal point.

The skin temperatures monitored were forehead temperature (FOT, °C), right shoulder temperature (RST, °C), left shoulder temperature (LST, °C), top of shoulder temperature (TST, °C), rump temperature (RUT, °C), chest floor temperature (CFT, °C) and scrotal temperature (SCT, °C). An infrared thermometer (Berrcom JXB-178, Berrcom Factory, China) was used to measure skin temperatures in various body locations.

Blood samples were obtained from the jugular vein at the same time of day in two periods (no stress and heat stress), into 5-ml vacutainer tubes, three tubes per animal and period (two vacutainer tubes with Lithium Heparin and one tube with EDTA). Due to a time restriction, this blood was delivered to the Physiology Laboratory in an ice carrier and preserved at a low temperature. Firstly, with Auto Hematology Analyzer (Rayto RT-7600; Rayto Life and Analytical Sciences Co., Ltd.; China), EDTA blood samples were analyzed to determinate blood metabolites such as red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), hematocrit (Ht), platelets (Pl), packed cell volume (PCV), mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). Secondly, the heparinized blood samples were centrifugated during 5 to 10 min with 3000 rpm to recover plasma. Glucose, total protein, cholesterol and alkaline phosphatase (ALP) were measured using an (RT-2100 C. Microplate reader; Rayto Life and Analytical Sciences Co., Ltd.; China). Finally, cortisol and free tri-iodothyronine (T_3) concentrations were determined using an enzyme linked fluorescence assay (ELFA) equipment, while the concentrations of thyrotropin (TSH) and free thyroxine (T_4) in blood plasma were determined using (Simens IMMLITE 1000 Immunoassay equipment; Siemens Healthineers Headquarters; Germany).

The following formula was the statistical model used to examine the effects of heat stress on ram breeds, as well as the interactions, on ram physiological parameters, skin temperatures, blood components, biochemical and hormonal compositions:

$$Y_{ijkl} = \mu + HSC_i + Br_j + Age_k + HSC_i * Br_j + e_{ijkl}$$

Were Y_{ijkl} is the observation for the physiological parameters, μ, overall mean, HSC_i, heat stress classes (thermal comfort zone, thermal stressful zone), Br_j Breed of the rams (BTR, NTB and QFO), HSC_i*Br_j Interaction heat stress classes and breed, e_{ijkl} is Random error.

RESULTS

Climate variables

Figure 1 present the changes of the climatic elements during the experiment period registered with the automatic weather station. The period from March to June is the changing period from spring to summer. The Ta varied from 12.5°C to 32°C, RH from 49% to 94% and the THI from 50 units to 85 units.

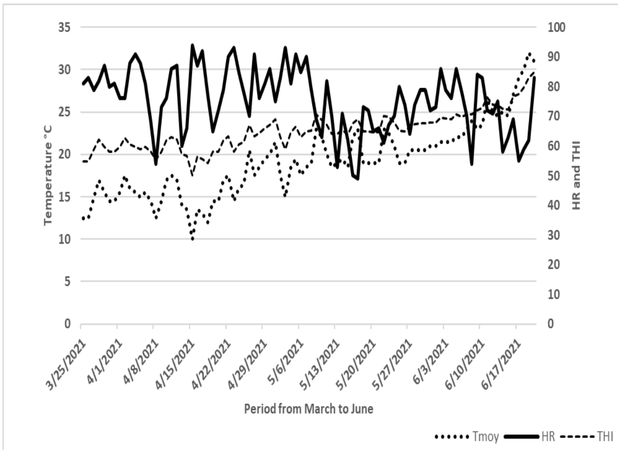

Fig. 1. Daily evolution of ambient temperature (°C), relative humidity (%) and THI values during period March-June in the North of Tunisia.

Table I. Least square means of heat stress effect on skin temperatures, physiological, hematological and biochemical parameters of three Tunisian rams breed (BTR, QFO, NTB).

Variables	Thermal comfort zone (TCZ) (T_a= 18.27±3.67 °C; THI= 62.77 ± 4.52)			Thermal stress zone (TSZ) (Ta= 33.41±4.54°C; THI= 77.66 ± 3.93)			Significance		
	BTR	QFO	NTB	BTR	QFO	NTB	C1	C2	C3
Phycological parameters									
RT (°C)	39.41	39.20	39.51[c]	39.38	39.52	39.72[b]	NS	0.024	NS
RR (breath/min)	44.59	44.88	42.81	56.00	60.22	57.00	0.0026	<.0001	<.0001
HR (beat/min)	87.70	88.29	89.03	102.57	104.22	105.88	<.0001	<.0001	<.0001
Skin temperature									
FOT	33.07	33.11	33.71	37.55	37.98	37.70	<.0001	<.0001	<.0001
TST	35.20	35.29	35.06	37.61	38.26	38.25	<.0001	<.0001	<.0001
RST	34.78	35.31	35.27	37.21	37.85	37.93	<.0001	<.0001	<.0001
LST	35.04	35.48	35.26	36.89	37.68	37.41	0.0042	0.0003	0.0004
RUT	35.32	35.02	35.26	37.12	38.22	38.18	0.0042	<.0001	<.0001
CFT	33.62	34.04	33.78	37.12	37.51	37.47	0.0003	0.0001	<.0001
SCT	33.55	33.90	33.21	36.17	37.08	37.11	0.0145	0.0014	0.0001
Hematological parameters									
RBC (nb x 10^9/l)	10.05	8.75	7.92	11.40	10.30	11.35	NS	NS	NS
WBC (nbx 10^{12}/l)	8.40	7.95	7.65	7.45	7.37	6.72	NS	NS	NS
Hb (g/dl)	11.00	11.05	9.70	13.80	11.72	11.67	NS	NS	NS
Ht (%)	25.20	25.40	22.20	34.87	30.80	30.07	0.0030	NS	0.0120
Pl (nb x 10^9/l)	323.50	338.75	308.00	290.75	251.00	265.25	NS	0.0007	0.0163
PCV (%)	29.90	30.02	27.90	35.82	34.67	30.92[bc]	0.0018	0.0102	NS
MCH (pg)	13.07	13.32	12.75	9.12	8.95	9.37	<.0001	<.0001	<.0001
MCHC (g/dl)	43.75	44.00	44.00	32.55	33.62	32.67	<.0001	<.0001	<.0001
Biochemical parameters									
Glucose (g/l)	0.45	0.41	0.25	0.38	0.29	0.32	NS	NS	NS
Cholesterol (g/l)	0.41	0.33	0.42	0.64	0.64	0.52	0.0464	0.0095	NS
Total protein (g/l)	62.96	71.68	72.63	73.00	84.25	57.90	NS	NS	NS
ALP (UI/l)	188.27	197.19	184.87	96.25	117.25	84.00	0.0057	0.0139	0.0029
Hormonal composition									
Thyroxine (pmol/l)	16.58	10.99[a]	12.43[b]	7.52	6.34	6.91	<.0001	0.0004	<.0001
Tri-iodothyronine (pmol/l)	2.61	4.54	2.85	2.76	2.91	3.15	NS	NS	NS
Cortisol (ng/l)	6.91	9.69	7.65	2.83	4.20	2.42	NS	NS	NS
TSH (mUI/l)	0.124	0.132	0.113	0.039	0.065	0.057	0.0046	0.0206	0.0464

RR, respiration rate, HR, heart rate; RT, rectal temperature; FOT, forehead temperature; RST, right shoulder temperature; LST, left shoulder temperature; TST, top of shoulder temperature; RUT, rump temperature; CFT, chest floor temperature; SCT, scrotal temperature.
Hb, hemoglobin; Ht, hematocrit; Pl, platelets; PCV, packed cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; ALP, alakaline phosphate; TSH, thyrotropin.
a, difference is statistically significant between BTR and QFO for the same parameter in the same thermal situation; b, difference is statistically significant between BTR and NTB for the same parameter in the same thermal situation; c, difference is statistically significant between QFO and NTB for the same parameter in the same thermal situation; C1, Difference between BTR for the same parameters in TCZ and TSZ conditions; C2, Difference between QFO for the same parameters in TCZ and TSZ conditions; C3, Difference between NTB for the same parameters in TCZ and TSZ condition.

Physiological parameters

In this study, according to Table I, only the RT for NTB rams has a significant difference between breed in thermal stress conditions. No changes are observed between RT breeds in a comfort situation and a stressful situation, except for QFO rams (39.20 °C with TCZ conditions; 39.52 °C with TSZ conditions). For the RR (BTR = 56.00 breath/min; QFO = 60.22 breath/min; NTB = 57 breath/min) and HR (BTR = 102.57 beat/min; QFO = 104.22 beat/min; NTB = 105.88 beat/min) no significant difference between breeds in a thermal stress condition have been reported.

In the other hand, skin temperatures: FOT, RST, LST, TST, RUT, CFT and SCT were higher, moving from spring to summer for all genotypes (Table I). The SCT had the lowest skin temperature for all the ram's breed.

Hematological parameters

Some changes in the blood parameters of animals exposed to high temperatures have been recorded (Table

I). In fact, RBC and Hb increase no significantly from TCZ to TSZ situation and no change was registered in WBC parameters during the experiment. However, Ht increase significantly for BTR rams from 25.20 % to 34.87 % and NTB rams from 22.20 % to 30.07 %. Contrary, Pl decrease significantly for QFO rams (338.75 10^9/l in TCZ conditions vs 251.00 10^9/l in TSZ conditions) and NTB rams (308.00 10^9/l in TCZ conditions vs 265.25 10^9/l in TSZ conditions). On the other hand, we found that PCV increase with a significative difference with BTR (29.90 % vs 34.67 %) and QFO (30.02 % vs 34.67 %). Finally, MCH and MCHC decrease significantly for the three types of breeds from a comfort to a stressful situation.

Biochemical parameters

Minor changes in the biochemical parameters of rams from a TCZ to TSZ situation (Table I). The glucose decreases non significantly for all the ram breeds, form 0.45 g/l to 0.38 g/l for BTR, from 0.41 g/l to 0.29 g/l for QFO and from 0.25 g/l to 0.32 g/l for NTB. Cholesterol increases significantly ($P<0.05$) only for BTR and QFO rams. No changes in total protein and a significantly decrease for ALP parameters from 188.27 10^9/l to 96.25 10^9/l for BTR, from 197.19 10^9/l to 117.25 10^9/l for QFO and from 184.87 10^9/l to 84.00 10^9/l.

Hormonal parameters

Hormonal response of animals to high temperature (Table I) was examined. A significant ($P<0.05$) decrease in thyroxine (T4) from 16.58 pmol/l to 7.52 pmol/l for BTR rams, from 10.99 pmol/l to 6.34 pmol/l for QFO rams and from 12.43 pmol/l to 6.91 pmol/l for NTB rams. TSH hormone decrease significantly from a comfort situation to a stressful situation for BTR (0.124 mUI/l to 0.039 mUI/l), QFO (0.132 mUI/l to 0.065 mUI/l) and NTB (0.113 mUI/l to 0.057 mUI/l). No change in tri-iododthyronine (T_3) and a no significantly decrease for cortisol hormone, from 6.91 ng/l to 2.83 ng/l for BTR, from 9.69 ng/l to 4.20 ng/l for QFO and from 7.65 ng/l to 2.42 ng/l for NTB.

DISCUSSION

According to temperature and humidity conditions (temperature between 13 to 38.5°C and humidity between 18 to 64.5%, THI between 56.1 to 37.1). Bouraoui *et al.* (2002), in an arid zone, had found difference in the averages of THI values (68±3.75 units vs. 78±3.23 units for the spring and summer periods, respectively). In comparative research, Bellagi *et al.* (2017) studied the effect of heat stress on Holstein and Tarentaise dairy cows and showed that the average values per month between 2009 and 2014 of T_a, RH, and THI from five public weather stations in the North of Tunisia were 18.8°C, 68.9%, and 64.0 units, respectively. The monthly means of T_a and THI reached their highest points in August (28.1°C and 76.6 units, respectively) and their lowest points in February (10.7°C and 52.1 units, respectively). The standard deviations of the three parameters were 0.81°C, 2.30%, and 1.22 units, respectively. In conclusion, there were no significant climatic difference amongst the five locations. According to Djelailia *et al.* (2020), Holstein dairy cows raised in the south arid zone of Tunisia were subject of heat stress from June to September with monthly THI values exceeding the critical threshold which is 72 units. During this period, THI ranged from 73±1.74 to 79±3.01. In the feeding area, the temperature and the THI were characterized by TCZ conditions equal to 18.27±3.67°C and 62.77±4.52 units respectively. Whereas, the TSZ conditions were: 33.41±4.54°C for temperature and 77.66±3.93 units for THI.

The mean THI recorded in the hottest seasons of the year, spring and summer, can moderate stress of the rams (Kahwage *et al.*, 2018). During physiological evaluations, Marai *et al.* (2007), indicated that the THI is an environmental condition that help the setting up of severe to very severe stress, which increase in the afternoon, when T_a rise.

In high ambient temperature, animals are forced to activate many thermoregulatory mechanisms. The physiological processes of the body are greatly influenced by the changes of environmental temperature (Wojtas *et al*., 2014). According to this study RT varied from 39.41 °C to 39.38 °C for BTR, from 39.20 °C to 39.52 °C for QFO and from 39.51 °C to 39.72 °C for NTB (Table I). The increase in the RT by around 1°C above the physiological comfort limits (20 to 30°C) may hinder vital function in animal production, essential functions, necessitating more investigation (Silanikove, 2000). the capacity of Morada Nova and Santa Inês hair sheep breeds to maintain homeothermy was studied by Kahwage *et al.* (2018). It showed that Santa Inès animals exhibited somewhat higher RT (0.2-0.3 °C) than Morada Nova animals. In the other hand, the physiological adaptation shown by both breeds favored the action of heat dissipation mechanisms and the rams were able to sustain homeothermy even in the seasons when the heat load was the most intense. In our study, from comfort to a stressful situation, the respiration rate (RR) varied from 44.59 to 56.00 breaths/min for BTR, from 44.88 to 60.22 breaths/min for QFO and from 42.81 to 57.00 breaths/min for NTB (Table I). Generally, in sheep the normal respiratory rate (RR) varied between 25 and 30 breaths/min (Arruda and Pant, 1984). The animals intensify thermolysis by the respiratory system with the increasing of the RR from 40 to 60 breaths/min (Silanikove, 2000). Kahwege *et al.* (2018) indicated that the RR in Morada Nova and in Santa Inès rams had higher values moving from spring to summer. It increases from 58.4±3.2 breath/min to 62.6±3.7 breath/min for the firth breeds and from 57.9 ± 2.8 breath/min vs 56.3±3.2 breath/min for the second one, indicating that the respiratory evaporative mechanism had been activated to prevent hyperthermia.

Heart rate (HR) varied with the seasons and times of day, with greater values in the afternoon for both breeds (Table I), especially from spring to summer, NTB animals had higher HR (105.88 beats/min) than QFO animals (104.22 beats/min) and then BTR animals (102.57 beats/min). A compensatory reaction to the requirement to redirect blood flow to the capillaries in order to promote heat dissipation following peripheral vasodilatation, HR also increases in the afternoons in the spring and summer (Fadare *et al.*, 2013). Due to this, the animals of both genotypes were able to keep their RT temperatures within the species' physiological range, which has a maximum threshold of 39.9 °C (Marai *et al.*, 2007). Various study indicated an increase in heart rate from comfort to heat stress conditions: Wojtas *et al.* (2014) with Merinos rams noted 90.23 beats/min in comfort conditions and 107.79 beats/min in heat stress conditions, Kahwege *et al.* (2018) reported higher HR in summer than in winter for Santa Inès (85.5±1.2 vs 84.8±1.3 beats/min) and Morada Nova (91.2±1.3 vs 92.2±1.5 beats/min).

In the present experiment, the increase in FOT, RST, LST, TST, RUT, CFT and SCT were observed from spring to summer (Table I). The highest levels of skin temperatures seen during the hottest seasons of the year were registered in the afternoon and they are linked to the higher thermal load that happens naturally during that period of time (Barros *et al.*, 2015), causing rams of both genotypes to cumulate body thermal energy (Kahwege *et al.*, 2018).

A correlation between environmental temperature and blood parameters was confirmed in many study (Srikandakumar *et al.*, 2003; Cwynar *et al.*, 2014; Wojtas *et al.*, 2014). The hematological results obtained in the study of Srikandakumar *et al.* (2003) showed that heat stress in sheep caused an increase in Hb and MCHC, a decrease in RBC and WBC and no change in PCV and MCV parameters. During another experiment and in order to examine heat stress effect and air flow on hematological parameters of Merino Rams, Wojtas *et al.* (2014) found that only WBC decrease significantly.

The exhibition to heat stress affects negatively blood metabolites (Marai *et al.*, 2008, 2009; Barragán Sierra *et al.*, 2021). In this study (Table I). Firstly, serum glucose decreases non significatively in the blood of the three genotypes, this result is conformed with many studies (Nazifi *et al.*, 2003; Marai *et al.*, 2009; Cwynar *et al.*, 2014) and in contradiction with others (Al-Haidary *et al.*, 2012; Wojtas *et al.*, 2014). Secondly, the cholesterol increases significantly for BTR (0.41 vs 0.64 g/l) and QFO rams (0.33 vs 0.64 g/l) this result is in conflict with Shaffer *et al.* (1981), Marai *et al.* (1995) and Habeeb *et al.* (1996) studies. Furthermore, when the glucose-saving system is activated, a reduction in blood concentrations of these lipid metabolites is partially related to the mobilization of fatty acids to meet energy requirements (Baumgard and Rohads, 2013; Sejian *et al.*, 2017). Thirdly, in this study no significative change in serum total protein was observed from spring to summer. However, Baumgartner and Parnthaner (1994) observed that the serum total protein levels were significantly ($P<0.05$) lower in summer than in winter in Karakul sheep. In turn, a significative difference was found with ALP ($P<0.05$), alkaline phosphatase enzyme activities decrease significantly ($P<0.05$) in summer compared to winter with Egyptian Suffolk rams (Marai *et al.*, 2009).

Finally, all species, including rams, use their thyroid glands to regulate their body temperatures (Binsiya *et al.*, 2017). Thyroid hormones are secreted less frequently as a result of the heat stress, which leads lower metabolic heat generation and body heat load (Nazifi *et al.*, 2003; De *et al.*, 2017). According to Table I, T_4 hormone decrease significantly for the three Tunisian ram breeds form a comfortable situation to heat stress situation (BTR: 16.58 vs 7.52 pmol/l; QFO: 10.99 vs 6.34 pmol/l; NTB: 12.43 vs 6.91 pmol/l) likewise Shalaby (1996) discovered that the exposure of Finn sheep to direct solar radiation in summer decrease the T_4 level. Marai *et al.* (1997) showed that the level of T_4 was higher during summer than in winter season in Barki and Ossimi x Suffolk crossbred sheep and finally, no change was founded by De *et al.* (2017) in T_4 concentrations from a comfort to a stressful situation with crossbred rams (Garole x Malpura x Malpura). No change in T_3 level hormone and a no significant decrease in cortisol hormone have been observed in this study, which is agree with the finds of Marai *et al.* (2009). However, a decrease in T_3 level was observed with Youssef and Johnson's (1985) and Marai *et al.* (1997). In conclusion, TSH secretion decrease significantly with heat stress conditions. Thyroid hormones in the blood are also regarded to be good indicators of an animal's nutritional status (Riis and Madsen, 1985; Rhoads *et al.*, 2009). It's seems that thermal stress have a direct effect on the hypothalamus-pituitary axis, resulting the reduction of TSH secretion (Rasooli *et al.*, 2004; Sejian and Srivastava, 2010).

CONCLUSION

The physiological adaptations showed by all genotypes boosted the activity of heat dissipation mechanisms, permitting the Tunisian rams breed to maintain their homeothermy during the hottest season of the year. During the spring-summer transition period, physiological parameters such as RR and HR are affected by heat stress, the RT for NTB rams, the rectal temperature for NTB rams were the most affected by the rise of temperature, which shows that BTR and QFO rams have better adaptative capability. As a result, BTR and QFO males have an adaptative capability to fight the thermal challenges imposed by Noth African climate. Those genotypes have an advantageous physiological characteristic, they can resist to the environmental adversity. The THI elevation also had an effect on hemato-biochemical parameters. Some of them increase, such as RBC, Ht, PCV and cholesterol, while others decreasing, such as Pl, MCH, MCHC and ALP.

ACKNOWLEDGEMENT

The authors are grateful to the Coordination for the Improvement of "High School of Agriculture of Mateur" for the financial support for carrying out the research.

Ethical statements and IRB approval

This study was approved by the Official Animal Care and Use Committee of the National Institute of Agronomy of Tunisia (protocol No 05/15).

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Al-Dawood, A., 2017. Towards heat stress management in small ruminants. A review. *Annls Anim. Sci.*, **17**: 59-88. https://doi.org/10.1515/aoas-2016-0068

Al-Haidary, A., Aljumaah, R., Alshaikh, M., Abdoun, K., Samara, E. and Okab, A., 2012. Thermoregulatory and physiological responses of Najdi sheep exposed to environmental heat load prevailing in Saudi Arabia. *Pak. Vet. J.*, **32**: 515-519.

Arruda, F.A.V. and Pant, K.P., 1984. Tolerância ao calor de caprinos e ovinos sem lã em Sobral. *Pesqui. Agropecu. Bras.*, **19**: 379–385.

Barragán Sierra, A., Avendaño-Reyes, L., Hernández Rivera, J.A., Vicente-Pérez, R., Correa-Calderón, A., Mellado, M., Meza-Herrera, C.A. and Macías-Cruz, U., 2021. Thermoregulation and reproductive responses of rams under heat stress. A review. *Rev. Mex. Cien. Pecuarias*, **12** : 910-931. https://doi.org/10.22319/rmcp.v12i3.5624

Barros, D.V., Silva, L.K.X., Lourenço, J.J.B., Silva, A.O.A., Silva, A.G.M., Franco, I.M., Oliveira, C.M.C., Tholon, P., Martorano, L.G. and Garcia, A.R., 2015. Evaluation of thermal comfort, physiological, hematological, and seminal features of buffalo bulls in an artificial insemination station in a tropical environment. *Trop. Anim. Hlth. Prod.*, **47**: 805–813. https://doi.org/10.1007/s11250-015-0792-9

Baumgard, L.H. and Rhoads, R.P.J., 2013. Effects of heat stress on postabsorptive metabolism and energetics. *Annu. Rev. Anim. Biosci.*, **1**: 311-337. https://doi.org/10.1146/annurev-animal-031412-103644

Baumgartner, W. and Parnthaner, A., 1994. Influence of age, season and pregnancy upon blood parameters in Austrian Karakul sheep. *Small Ruminant Res.*, **11**: 147-151. https://doi.org/10.1016/0921-4488(94)90090-6

Bellagi, R., Martin, B., Chassaing, C., Najar, T. and Pomiès, D., 2017. Evaluation of heat stress on Tarentaise and Holstein cow performance in the Mediterranean climate. *Int. J. Biometeorol.*, **61**: 1371–1379. https://doi.org/10.1007/s00484-017-1314-4

Binsiya, T., Sejian, V., Bagath, M., Krishnan, G., Hyder, I. and Manimaran, A., 2017. Significance of hypothalamic-pituitary-adrenal axis to adapt to climate change in livestock. *Int. Res. J. Agric. Fd. Sci.*, **2**: 1-20.

Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, M. and Belyea, R., 2002. The relationship of the temperature-humidity index with milk production of dairy cows in a Mediterranean climate. *J. Anim Res.,* **51**: 479–491.

Chapman, S.C., Chakraborty, S., Dreccer, M.F. and Howden, S.M., 2012. Plant adaptation to climate change: opportunities and priorities in breeding. *Crop Pasture Sci.*, **63**: 251–268. https://doi.org/10.1071/CP11303

Cwynar, P., Kolacz, R. and Czerski, A., 2014. Effect of heat stress on physiological parameters and blood composition in Polish Merino rams. *Berl. Munch. Tierarztl. Wochenschr.*, **127**: 177-182.

De, K., Kumar, D., Balaganur, K., Kumar Saxena, V., Thirumurugan, P. and Naqvi, K.S.M., 2017. Effect of thermal exposure on physiological adaptability and seminal attributes of rams under semi-arid environment. *J. Therm. Biol.*, **65**: 113-118. https://doi.org/10.1016/j.jtherbio.2017.02.020

Djelailia, H., Bouraoui, R., Jemmali, B. and Najar, T., 2020. Effects of heat stress on reproductive efficiency in Holstein dairy cattle in the North African arid region. *Reprod. Domest. Anim.*, **55**.

https://doi.org/10.1111/rda.13772

Fadare, A.O., Sunday, O.P., Yakubu, A., Sonibare, A.O., Adekayode, O., Adeleke, M.A., Ozoje, M.O. and Imumorin, I.G., 2013. Physiological and haematological indices suggest superior heat tolerance of white-coloured West African Dwarf sheep in the hot humid tropics. *Trop. Anim. Hlth. Prod*., **45**: 157–165. https://doi.org/10.1007/s11250-012-0187-0

Habeeb, A.A., El-Masry, K.A., Aboul-Naga, A.I. and Kamal, T.H., 1996. The effect of hot summer climate and level of milk yield on blood biochemistry and circulating thyroid and progesterone hormones in Friesian cows. *Arab J. Nucl. Sci. Appl*., **29**: 161-173.

Habeeb, A.A., Gad, A.E. and Atta, M.A., 2018. Temperature-humidity indices as indicators to heat stress of climatic conditions with relation to production and reproduction of farm animals. *Int. J. Biotechnol. Recent Adv*., **1**: 35-50. https://doi.org/10.18689/ijbr-1000107

Harvell, C.D., Mitchell, C.E., Ward, J.R., Altizer, S., Dobson, A.P., Ostfeld, R.S. and Samuel, M.D., 2002. Climate warming and disease risks for terrestrial and marine biota. *Science*, **296**: 2158–2162. https://doi.org/10.1126/science.1063699

Hopkins, P.S., Knights, G.I. and Le Feuvre, A.S., 1978. Studies of the environmental physiology of tropical Merinos (responses to heat stress sheep). *Austral. J. agric. Res*., **29**: 161-171. https://doi.org/10.1071/AR9780161

Johnson, J.S., 2018. Heat stress: Impact on livestock well-being and productivity and mitigation strategies to alleviate the negative effects. *Anim. Prod. Sci*., **58**: 1404-1413. https://doi.org/10.1071/AN17725

Kahwage, P.R., Esteves, S.N., Jacinto, M.A.C., Barioni, J.W., Machado, R., Romanello, N., Passeri, L.F., Laurindo de Mendonça, K. and Rossetto, G.A., 2018. Assessment of body and scrotal thermoregulation and semen quality of hair sheep rams throughout the year in a tropical environment. *Small Rumin. Res*., **160**: 72-80. https://doi.org/10.1016/j.smallrumres.2018.01.015

Karl, T.R., Melillo, J.M. and Peterson, T.C., 2009. *Global climate change impacts in the United States*. U.S. Global Change Research Programme. Cambridge University Press.

Lunstra, D.D. and Coulter, G.H., 1997. Relationship between scrotal infrared temperature patterns and natural-mating fertility in beef bulls. *J. Anim. Sci*., **75**: 767–774. https://doi.org/10.2527/1997.753767x

Marai, I.F.M., El-Darawany, A.A., Fadiel, A. and Abdel-Hafez, M.A.M., 2008. Reproductive performance traits as affected by heat stress and its alleviation in sheep tropical and subtropical agroecosystems. *Univ. Autónoma de Yucatán Mérida, Yucatán, México*, **8**: 209-234.

Marai, I.F.M., El-Darawany, A.A., Abou-Fandoud, E.I. and Abdel-Hafez, M.A.M., 2009. Reproductive and physiological traits of Egyptian Suffolk rams as affected by selenium dietary supplementation during the sub-tropical environment of Egypt. *Livest. Res. Rural Dev.,* **21***:* 157. https://doi.org/10.5194/aab-52-402-2009

Marai, I.F.M., El-Darawany, A.A., Fadiel, A. and Abdel-Hafez, M.A.M., 2007. Physiological traits as affected by heat stress in sheep. A review. *Small. Rumin. Res*., **71**: 1–12. https://doi.org/10.1016/j.smallrumres.2006.10.003

Marai, I.F.M., Habeeb, A.A.M., Daader, A.H. and Yousef, H.M., 1995. Effects of Egyptian subtropical conditions and the heat stress alleviation techniques of water spray and diaphoretics on the growth and physiological functions of Friesian calves. *J. Arid Environ*., **30**: 219-225. https://doi.org/10.1016/S0140-1963(05)80073-4

Marai, I.F.M., Shalaby, T.H., Bahgat, L.B. and Abdel-Hafez, M.A.M., 1997. Fattening of lambs on concentrates mixture di*et al*one without roughages or with addition of natural clay under subtropical conditions of Egypt, physiological reactions. *Proc. Int. Conf. Anim. Prod. Hlth*., pp. 351-360.

Menegassi, S.R.O., Barcellos, J.O.J., Dias, E.A., Koetz Jr, C., Pereira, G.R., Peripolli, V., McManus, C., Canozzi, M.E.A. and Lopes, F.G., 2015. Scrotal infrared digital thermography as a predictor of seasonal effects on sperm traits in Braford bulls. *Int. J. Biometeorol*., **59**: 357–364. https://doi.org/10.1007/s00484-014-0847-z

Moran, J., 2005. *Tropical dairy farming: Feeding management for smallholder dairy farmers in the humid tropics*. Landlinks Press, Collingwood, pp. 312. https://doi.org/10.1071/9780643093133

Nazifi, S., Saeb, M., Rowghani, E. and Kaveh, K., 2003. The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine (T_3), thyroxine (T_4) and cortisol concentrations. *Comp. clin. Pathol*., **12**: 135-139. https://doi.org/10.1007/s00580-003-0487-x

Patz, J.A., Graczyk, T.K., Geller, N. and Vittor, A.Y., 2000. Effects of environmental change on emerging parasitic diseases. *Int. J. Parasitol*., **30**: 1395–1405.

https://doi.org/10.1016/S0020-7519(00)00141-7

Rasooli, A., Nouri, M., Khadjeh, G.H. and Rasekh, A., 2004. The influence of seasonal variations on thyroid activity and some biochemical parameters of cattle. *Iran. J. Vet. Res*., **5**: 1383–1391.

Renaudeau, D., Collin, A., Yahav, S., De Basilio, V., Gourdine, J.L. and Collier, R.J., 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal*, **6**: 707-728. https://doi.org/10.1017/S1751731111002448

Rhoads, M.L., Rhoads, R.P., VanBaale, M.J., Collier, R.J., Sanders, S.R., Weber, W., Crooker, B.A. and Baumgard, L.H., 2009. Effect of heat stress and plane of nutrition on lactating Holstein cows: Production, metabolism and aspects of circulating somatotropin. *J. Dairy Sci*., **92**: 1986-1997. https://doi.org/10.3168/jds.2008-1641

Riis, P.M. and Madsen, A., 1985. Thyroxine concentration and secretion rates in relation to pregnancy, lactation and energy balance in goats. *J. Endocrinol*., **107**: 421-427. https://doi.org/10.1677/joe.0.1070421

Scholtz, M.M., McManus, C., Leeuw, K.J., Louvandini, H., Seixas, L., De Melo, C.B., Theunissen, A. and Neser, F.W.C., 2013. The effect of global warming on beef production in developing countries of the southern hemisphere. *Nat. Sci*., **5**: 106–119. https://doi.org/10.4236/ns.2013.51A017

Sejian, V. and Srivastava, R.S., 2010. Pineal-adrenal-immune system relationship under thermal stress: Effect on physiological, endocrine, and non-specific immune response in goats. *J. Physiol. Biochem*., **66**: 339–349. https://doi.org/10.1007/s13105-010-0040-8

Sejian, V., Bhatta, R., Gaughan, J., Malik, P.K., Naqvi, S. and Lal, R., 2017. *Sheep production adapting to climate change*. Springer Nature Singapore Pte Ltd. Singapore, https://doi.org/10.1007/978-981-10-4714-5

Sejian, V., Maurya, V.P., Kumar, K. and Naqvi, S.M.K., 2012. Effect of multiple stresses on growth and adaptive capability of Malpura ewes under semi-arid tropical environment. *Trop. Anim. Hlth. Prod*., **45**: 107-116. https://doi.org/10.1007/s11250-012-0180-7

Setchell, B.P., 1998. The Parkes Lecture: Heat and the testis. *J. Reprod. Fertil.*, pp. 114. https://doi.org/10.1530/jrf.0.1140179

Shaffer, L., Roussel, J.D. and Koonce, K.L., 1981. Effect of age, temperature, season and breed on blood characteristics of dairy cattle. *J. Dairy Sci*., **64**: 62-70. https://doi.org/10.3168/jds.S0022-0302(81)82529-5

Shalaby, T.H., 1996. *Physiological responses of Finn sheep and their crosses to subtropical environmental conditions of Egypt.* Proceedings of Symposium on use of Exotic Breeds in Improving Sheep Production, Finn Sheep as a Case Study. Cairo Egypt. pp. 131– 139.Silanikove, N., 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest. Prod. Sci*., https://doi.org/10.1016/S0301-6226(00)00162-7

Solymosi, N., Torma, C., Kern, A., Maróti-Agóts, A., Barcza, Z., Könyves, L., Berke, O. and Reiczigel, J., 2010. Changing climate in Hungary and trends in the annual number of heat stress days. *Int. J. Biometeorol*., **54**: 423-431. https://doi.org/10.1007/s00484-009-0293-5

Srikandakumar, A., Johnson, E.H. and Mahgoub, O., 2003. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Rumin. Res*., **49**: 193-198. https://doi.org/10.1016/S0921-4488(03)00097-X

Thom, E.C., 1959. The discomfort index. *Weatherwise*, **12**: 57-61. https://doi.org/10.1080/00431672.1959.9926960

Thornton, P.K., Van de Steeg, J., Notenbaert, A. and Herrrero, M., 2009. The impacts of climate change on livestock and livestock systems in developing countries: A review of what we know and what we need to know. *Agric. Syst*., **101**: 113–127. https://doi.org/10.1016/j.agsy.2009.05.002

Van Wettere, W., Kind, K.L., Gatford, K.L., Swinbourne, A.M., Leu, S.T., Hayman, P.T., Kelly, J.M., Weaver, A.C., Kleemann, D.O. and Walker, S.K., 2021. Review of the impact of heat stress on reproductive performance of sheep. *J. Anim. Sci. Biotechnol*., **12**: 26. https://doi.org/10.1186/s40104-020-00537-z

Verner, D., Tréguer, D., Redwood, J., Christensen, J., Mcdonnell, R., Elbert, C. and Konishi, Y., 2018. *Climate variability, drought, and drought management in Tunisia's agricultural sector*. World Bank Group. https://doi.org/10.1596/30604

Wojtas, K., Cwynar, P. and Kołacz, R., 2014. Effect of thermal stress on physiological and blood parameters in merino sheep. *Bull. Vet. Inst. Pulawy,* **58**: 283-288.

Yousef, M.K. and Johnson, H.D., 1985. Endocrine system and thermal environment. In: *Stress physiology in livestock* (ed. M.K. Yousef). CRC Press, Inc. Boca Raton, Florida, U.S.A., **1**: 133-154.