

Effect of temperature on the physiological characteristics of Awassi and crossbred sheep



Osama Anwer Saeed^a   | Rawaa Turk Abdulghafoor^b | Sawsan Sabir Al-Salmany^a |
Faris Mahmoud Ali^c | Anjas Asmara Samsudin^d | Elham Khalifa Mahmood^b 

^aDepartment of Animal Production, College of Agriculture, University of Anbar, Ramadi, 31001, Iraq.

^bGeneral Directorate for Education of Anbar, Ministry of Education, Anbar, Ramadi, 31001, Iraq.

^cDivision of Al-Qaim Agriculture, Directory of Al-Anbar Agriculture, Ministry of Agriculture, Ramadi, 31001, Iraq.

^dDepartment of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.

Abstract This research was carried out to analyze the hematological, biochemical and physiological characteristics correlated with climate temperature in Awassi and crossbred sheep. Two hundred fifty adult Awassi and crossbred sheep (Arabi x Hamadani) were used. Three times weekly, ten times for each breed, vital signs, including heart rate (HR), respiratory rate (RR), rectal temperature (RT), and skin temperature (ST), were recorded. The heat tolerance indices (HT) and the heat tolerance coefficient (HTC) were evaluated. SAS was used to conduct the analyses, which included variance and correlation analyses. Awassi had a significantly higher ($P < 0.01$) RR than crossbred in the summer under low stress conditions. Awassi HR was significantly lower than that of crossbred sheep, which recorded the highest value in summer. There were no significant differences in RT, HT and HTC between Awassi and crossbred sheep. MCH, MCV, and RDW increased substantially in Awassi sheep exposed to colder winter temperatures. In summer, Awassi's liver enzyme and triglyceride levels significantly decreased, while albumin levels increased. Both HT and HTC had negative correlations with RR. Therefore, it can be concluded that the Awassi breed is equally adapted to the region's hot and arid climate.

Keywords: adaptation, awassi, infrared thermography, physiological response, temperature

1. Introduction

The vast majority of sheep farms in Central-Western Iraq, where pastures lack shade, are extensive. The sheep are subject to high solar radiation and low relative humidity during the dry season that runs from the end of May to early September, and in December, the temperature falls to the lowest point of the month. The future climate is predicted to be more volatile, with more frequent and severe heat waves, droughts, and floods (Ouarda et al. 2019). However, ruminant livestock raised outdoors in natural environments are well-suited to adapt to a changing climate, but this resilience may be tested by the rapidity with which climate change is occurring. Because of this, it would be helpful to determine how sensitive today's livestock resources are to stresses that are expected to worsen as the climate changes in the future (McManus et al. 2009). To determine physiological adaptation, multiple traits are used. The most commonly used characteristics are respiratory rate, heart rate, surface temperatures of the rectum and body, and cutaneous evaporation, although there is no consensus among the authors on which characteristics should be utilized. Other measurements include air inhalation volume, sweating rate, activity level, shade type, rumen movement frequency, and hematological and physiological characteristics (Habeeb et al. 2018). The temperature-humidity index (THI) is an alternative measure

of heat stress that takes into account the impact of both air temperature and humidity. The THI is a helpful measure of heat stress in sheep because it takes into account both temperature and humidity. Awassi sheep accounted for 58.2% of the indigenous breeds in Iraq, while Arabi sheep accounted for 21.8% (Al-Barzinji and Othman 2013). All of these breeds have the potential to produce milk and have fat-tailed, carpet-wool-producing tails. Farmers have a significant economic interest in these breeds due to their high milk and wool production, year-round sexual activity, and adaptability to harsh conditions. We would therefore hypothesize that variations in the genetic backgrounds of the breeds of sheep studied would impact the efficacy of their heat resistance and sensitivity to Iraq's prevalent hot subtropical conditions. Therefore, the purpose of this study was to investigate the thermophysiological responses and heat resistance of native sheep breeds that have adapted to arid climates.

2. Materials and Methods

2.1. Location of the study

Experimentation was performed in the city of Al-Qaim (longitude: 41° 05' 24.06" E and latitude: 34° 22' 4.59" N), which is located in the far west of the province of Anbar. Hot, dry summers and cold, wet winters characterize the BWh climate, as described by the Köppen climate



classification system (Table 1). There are two distinct times of year that were used for this study: winter (from November to March) and summer (May-September).

2.2. Study design

A total of 250 sheep from two genetic groups were selected randomly and were clinically healthy. The sheep were characterized based on morphological traits of the sheep breeds that were assigned afterward to two groups of each. The animals belonged to the Awassi breed

and crossbred sheep involved (Arabi x Hamadani), whose average initial live weight was 46 ± 2 kg at a mean age of 2-3 years. The animals were kept in a loose housing system and were fed on two types of feeding, grazing and feeding concentrate, throughout the year at the same time. The animals were given *ad libitum* access to water. Data were collected three times per week (Saturday, Tuesday, and Thursday), with an average of ten samples per breed collected on each sampling day.

Table 1 Climate changes at the study site.

Item	Al-Qaim City
Summer season	
Average temperature (°C)	31.96
Mean of maximum temperature (°C)	39.46
Mean of minimum temperature (°C)	24.34
Humidity (%)	24.54
Wind speed (kh/h)	16.37
Winter season	
Average temperature (°C)	12.29
Mean of maximum temperature (°C)	18.85
Mean of minimum temperature (°C)	7.13
Humidity (%)	55.02
Wind speed (kh/h)	10.96

2.3. Data collection

The data consisted of 2400 records from 250 sheep belonging to two flocks (Awassi and crossbred sheep). Rectal temperature, skin temperature, respiratory rate, and heart rate were collected in the shade (14:00). The rectal temperature was taken using a digital thermometer (USA, Model V900G). Head, neck, rump, and rib temperatures (STs) were taken with a noncontact infrared thermometer (Model 610, USA). Using a modified version of Silanikove's times of breath followed scale, the respiration rate was calculated by first counting flank movements for 15 s before converting the data to a 1 min scale (Silanikove 2000). The individual's heart rate was determined by placing their fingertips on the femoral arteries of the hind limb for a period of 1 min.

Blood samples were harvested in the middle of the experimental period of each season. A total of 40 samples were collected from two groups: crossbred ($n = 10$) and Awassi ($n = 10$). Each of the ten samples for each breed was combined and made into a single sample. After collecting 10 ml of blood via venipuncture, the sample was separated into 2 ml in vacutainer tubes containing ethylene diamine tetra-acetic acid (EDTA) and 8 ml in plain tubes for the measurements of physiological traits. After that, the serum was separated by centrifugation at 4 °C and $2000 \times g$ for 10 minutes. The serum was stored at -20 °C for later biochemical analysis.

The Meteorology Office in AL-Qaim city provided information on the local temperature, humidity, and wind speed. Morning and afternoon ambient temperatures that were measured throughout the experiment.

The temperature-humidity index (THI) was calculated using the method (Habeeb et al. 2018) of the following formula:

$$THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)$$

where RH is a fraction of the unit representing relative humidity and T is the degree Celsius value of the ambient temperature.

However, the heat tolerance index (HT) and calculation of heat tolerance coefficient (HTC) were calculated according to (Gaughan et al. 2012):

$$HT = \frac{RT}{RR} + \frac{39.1}{27}$$

Rectal temperature (RT) is expressed in °C; respiratory rate (RR) is expressed in breaths per minute; normal RT (°C) for sheep is 39.1; and normal RR (breaths/minute) for sheep is 27.

The calculation of the heat tolerance coefficient (HTC) followed the following formula:

$$HTC = 100 - (18 \times (RT - 39.1))$$

The optimal average rectal temperature for sheep is 39.1 °C, where HTC is the heat tolerance coefficient, RT is the rectal temperature, and 100 is the maximum effectiveness in maintaining body temperature at 39.1°C.

2.4. Analysis of samples

A hematology analyzer was utilized to assess the hematological values (CELL- DYN 3700 Abbott, USA). Biochemical indices were assessed by measuring serum concentrations of triglycerides, cholesterol, creatinine, urea, total protein, globulin, and urea (Beckman-Coulter, AU5400,

Portugal). Roche Diagnostics GmbH's commercially available diagnostic kits were used in the Roche Hitachi Diagnostic Modular Analyzer P-800 (D-68298, Germany) to determine aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) concentrations.

2.5. Statistical analysis

Heat tolerance indices and physiological, physical thermographic, and hematological parameters were analyzed by breed using SAS version 9.4. (Statistical Analysis Institute, Cary, North Carolina, United States). The collected data were analyzed using an analysis of variance (ANOVA) with repeated measures so that comparisons could be made between the various breeds. Methods such as analysis of variance (GLM) and principal and component analysis (PRINCOMP) were used to look for patterns in the data and determine where the differences between breeds lay, while correlation analysis, exploratory factor analysis, and

exploratory factor analysis were also employed. Means were considered significant if they had a probability of being less than 5% ($p < 0.05$).

3. Results

3.1. Environmental parameters

Climate variables and bioclimatic indices are described for the whole study area using data from meteorological stations (Table 1 and Figure 1). The highest temperature degree during the summer season was 38.55 °C, and the humidity index was 19.81%.

During the winter season, the temperatures declined to 4.52 °C, and the humidity index fell to 48.63%. However, from March to September, summer had a rise in the THI compared to the winter, with the highest THI of 81.54 recorded in July. Furthermore, the average THI in summer was 76.35, and in winter, it was 55.05.

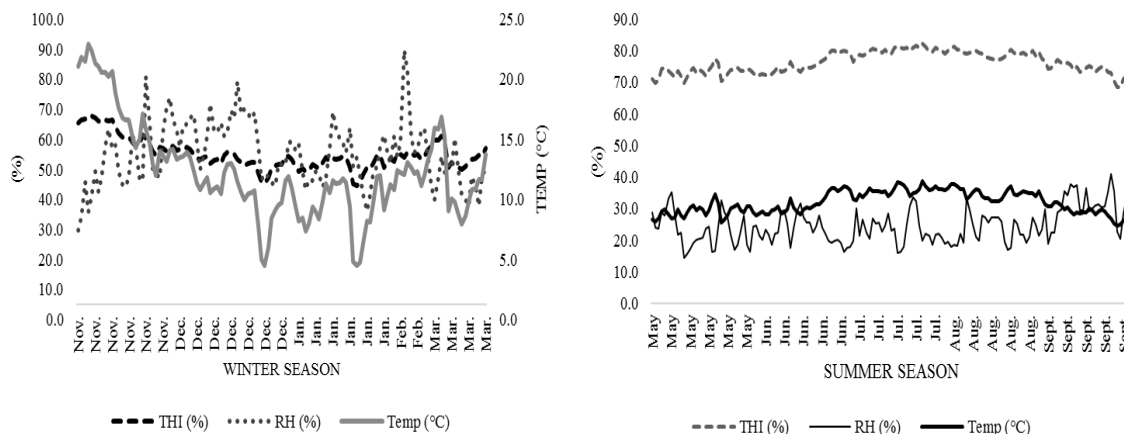


Figure 1 THI based on the average temperature and relative humidity during two seasons (winter and summer).

3.2. Physiology response

The variation in the average respiratory values during the day is presented in Table 2. The daily average values of RR were higher than normal RR in sheep and peaked in the summer season when THI was maximal. The average RR was significantly different ($P < 0.01$) at low stress levels, which were higher in Awassi during the hot summer than in the crossbreeds (58.00 and 55.00 breaths/min, respectively). However, RR did not differ significantly ($P > 0.05$) for other stress levels during the winter and summer seasons between these two breeds. Interestingly, the RR was increased in the level of stress from medium to higher during the winter season in crossbred compared to Awassi sheep.

The experimental data collected on the HR of Awassi and crossbred sheep over the course of two seasons are presented in Figure 2. Heart rate increased from 74.41 to 76.48 beats per minute in crossbred sheep, while HR recorded a slight increase in Awassi sheep from winter to summer seasons. Further examination revealed a statistically significant ($P < 0.05$) decrease in HR between Awassi and the crossbred sheep that had the highest value

during the summer (73.72 and 76.48 beats/min, respectively). However, no significant difference between the two breeds was found in the HR value in the winter season.

The rectal and skin temperatures of Awassi and crossbred sheep are shown in Table 3. The RT was registered as 39.15 and 39.16 °C during winter and 39.27 °C for both breeds in summer. However, no significant changes in RT were detected in Awassi and crossbred sheep. Overall, skin temperatures (including the head, neck, ribs, and rump) in winter were lower ($P < 0.01$) than those in summer in both breeds. The highest ($P < 0.01$) skin temperature in the different regions of the body was found in Awassi sheep during winter. However, during summer, the differences in skin temperature regions were statistically increased in crossbred sheep but reduced in Awassi sheep.

3.3. Heat tolerance of sheep

The results obtained from the preliminary analysis of the heat tolerance of sheep are presented in Table 4. The study found that neither the heat tolerance index nor the heat tolerance coefficient were statistically significant during seasonal changes.



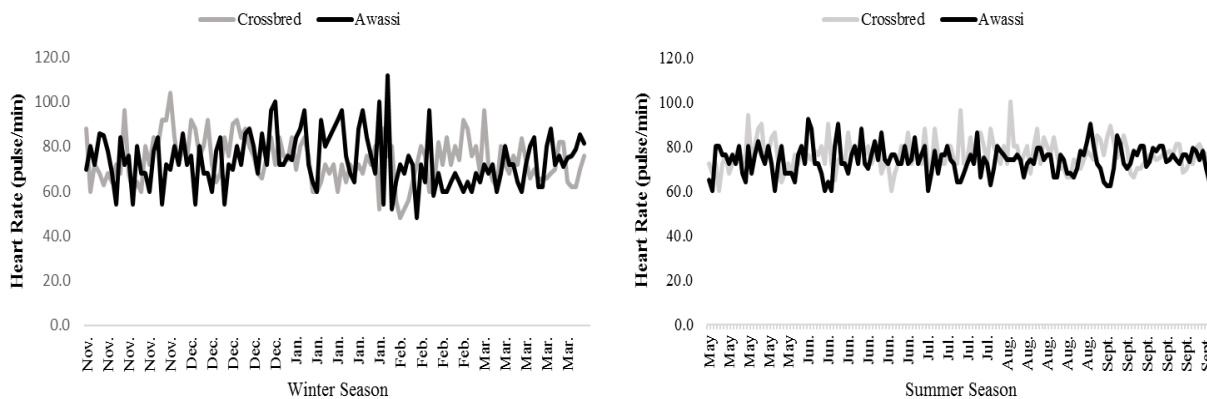


Figure 2 Heart rate of crossbred and Awassi sheep during winter and summer seasons.

Table 2 Classification of respiratory rate in sheep during the winter and summer seasons.

RR scale	Stress level	Breed		SEM	p value
		Awassi	Crossbred		
Winter season					
< 40 breaths/min	No stress	--	--	--	--
40 – 60 breaths/min	Low	58.00 ^a	55.00 ^b	0.506	0.01
61 – 80 breaths/min	Medium - High	71.80	72.03	0.306	0.71
81 – 120 breaths/min	High	91.52	90.18	0.492	0.18
Above 200 breaths/min	Severe	--	--	--	--
Summer season					
< 40 breaths/min	No stress	37.31	37.68	0.210	0.39
40 – 60 breaths/min	Low	48.32	48.65	0.275	0.55
61 – 80 breaths/min	Medium - High	67.05 ^b	69.69 ^a	0.478	0.01
81 – 120 breaths/min	High	--	--	--	--
Above 200 breaths/min	Severe	--	--	--	--

Table 3 Rectal temperature and skin temperature of local sheep during winter and summer seasons.

Variables	Rectal Temperature (°C)	Skin Temperature (°C)			
		Head	Neck	Rump	Ribs
Winter season					
Awassi	39.15	28.89 ^a	33.66 ^a	34.91 ^a	34.06 ^a
Crossbred	39.16	26.07 ^b	32.47 ^b	33.01 ^b	33.50 ^b
SEM	0.01	0.14	0.10	0.11	0.01
p value	0.79	0.01	0.01	0.01	0.01
Summer season					
Awassi	39.27	39.38 ^b	38.91 ^b	39.68 ^b	39.05 ^b
Crossbred	39.27	40.69 ^a	40.32 ^a	41.71 ^a	40.93 ^a
SEM	0.02	0.12	0.09	0.14	0.13
p value	0.97	0.01	0.01	0.01	0.01

3.4. Blood hematological parameters

Hematological parameters are presented for different sheep breeds and their average values under cold and heat stress in Table 5. It is apparent from this table that several hematological parameters showed that MCH, MCV, and RDW increased significantly ($P < 0.01$) in Awassi sheep when exposed to colder temperatures in the winter, whereas RBC, WBC, lymph, Hb, PCV, and MPV were not significantly altered in either breed. However, PCT and PLT were significantly reduced in Awassi and increased in other breeds in the winter season. However, there was a statistically significant difference ($P < 0.01$) between the breeds in the summer, when Hb, PCV, and MPV levels

appeared to decrease in Awassi but increase in the crossbreeds.

3.5. Blood biochemical parameters

The exposure of sheep to colder temperatures resulted in slightly lower ($P < 0.01$) ALT and AST levels in Awassi sheep than in crossbred sheep (Table 6) and a higher level of urea in the same winter season. However, regarding the biochemical values, ALT and TG were significantly decreased while albumin levels increased in summer for Awassi ($P < 0.01$). On the other hand, the biochemical parameters that were measured in the winter and summer did not differ much between breeds.



3.6. Pearson correlation

Table 7 shows the correlations between physiological responses and blood characteristics. Both ST and HTC were negatively correlated ($P < 0.001$), while RT was positively correlated with RR ($P < 0.01$). More specifically, RT was positively correlated with HR ($P < 0.05$)

but not with HTC ($P < 0.001$). The RR was significantly ($P < 0.05$) higher in areas where HR was higher. HT and HTC were both negatively correlated with RR. Statistically significant negative correlations were found between cheek HR and HT. Nonetheless, a significant positive correlation between the ALT enzyme and PCV was observed.

Table 4 The heat tolerance in crossbred and Awassi sheep.

Variables	Awassi	Crossbred	SEM	p value
Winter Season				
heat tolerance index	2.23	2.24	0.01	0.39
heat tolerance coefficient	99.21	99.07	0.27	0.79
Summer Season				
heat tolerance index	1.98	1.99	0.03	0.50
heat tolerance coefficient	97.08	97.14	0.45	0.95

Table 5 The effect of seasonal changes on the hematological characteristics of sheep.

Variables	RBC ($10^{12}/L$)	WBC ($10^9/L$)	Lymph (%)	Hb (g/dL)	PCV (%)	MCH (pg)	MCV (fL)	MCHC (g/dL)	RDW (fL)	MPV (fL)	PCT (mL/L)	PLT ($10^9/L$)
Winter season												
Awassi	8.18	41.23	80.73	7.76	24.86	9.67 ^a	30.77 ^a	31.43	20.20 ^a	5.70	1.80 ^b	321.00 ^b
Crossbred	8.82	49.96	79.10	9.23	27.77	8.98 ^b	27.05 ^b	32.43	18.70 ^b	5.20	3.06 ^a	581.25 ^a
SEM	0.33	4.28	1.83	0.56	1.60	0.18	0.87	0.47	0.33	0.15	0.27	55.67
p value	0.42	0.36	0.70	0.22	0.41	0.03	0.01	0.32	0.01	0.11	0.01	0.01
Summer season												
Awassi	7.53 ^b	16.77	61.32	8.03 ^b	24.1 ^b	10.73	32.20	33.30	18.00	5.53 ^b	2.23	401.00 ^a
Crossbred	8.37 ^a	20.63	62.80	8.62 ^a	25.0 ^a	10.33	30.13	34.48	17.48	6.13 ^a	2.55	183.90 ^b
SEM	0.23	1.53	1.97	0.14	0.25	0.17	0.74	0.42	0.57	0.16	0.24	49.99
p value	0.05	0.24	0.74	0.01	0.04	0.28	0.17	0.17	0.68	0.04	0.59	0.01

RBC: red blood cell, WBC: white blood cell, Lymph: lymphocytes, Hb: hemoglobin, PCV: packed cell volume, MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume, MCHC: MCH concentration, RDW: RBC distribution width, MPV: mean platelet volume, PCT: procalcitonin and PLT: platelet count. ^{a, b} means with different superscripts in the same column in different treatment groups are significantly different.

Table 6 Biochemical variations of local sheep during winter and summer seasons.

Variables	Glob (g/dl)	ALP (U/L)	ALT (U/L)	AST (U/L)	Albumin (g/l)	TP (g/dl)	TG (mg/dl)	CL (mg/dl)	Cr (mg/dl)	Urea (mg/dl)
Winter season										
Awassi	3.70	184.85	11.00 ^b	77.00 ^b	3.03	6.73	18.23	77.32	0.83	39.67 ^a
Crossbred	370	172.90	15.33 ^a	117.00 ^a	3.13	6.72	13.56	72.00	0.80	23.66 ^b
SEM	0.05	13.03	1.05	10.56	0.08	0.09	1.68	2.52	0.02	3.35
p value	0.39	0.71	0.02	0.05	0.59	0.87	0.18	0.32	0.62	0.01
Summer season										
Awassi	4.02	179.93	12.33 ^b	115.33	2.60 ^a	6.63	18.97 ^b	75.32	0.87	46.32
Crossbred	3.97	176.87	48.33 ^a	91.33	2.23 ^b	6.20	25.10 ^a	80.00	0.93	48.32
SEM	0.17	8.54	6.85	7.99	0.08	0.17	1.32	1.95	0.02	0.89
p value	0.89	0.54	0.01	0.14	0.02	0.25	0.01	0.27	0.39	0.30

Glob: globulin, ALP: alkaline phosphatase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TP: total protein, TG: triglyceride, CL: cholesterol, Cr: creatinine. ^{a, b} means with different superscripts in the same column in different treatment groups are significantly different.

4. Discussion

4.1. Environmental parameters

The THI is a crucial indicator for classifying the degree of environmental heat stress because its calculation combines temperature and RT (Macías-Cruz et al. 2016). A significant unfavorable association was found between THI and RT in indigenous sheep, as shown by the findings presented in this study: when the THI rises (which indicates an increase in heat stress), there is an analogous decrease in the RT. Another important finding was that the lower THI

during the winter season was followed by rainy months compared to the summer study period. A previous study hypothesized that the lower THI during winter is due to lower ambient temperature and higher RT (Rathwa et al. 2017). The THI has been the most commonly utilized indicator for evaluating the thermal stress of animals. The literature classifies heat stress according to THI as mild (72 - 79), moderate (80 - 89), and severe (THI 90) stress levels (Pinto et al. 2020). The THI values during the winter are as follows: 22.2 indicates no thermal stress; 22.2–23.3 indicates mild thermal stress; 23.3–25.6 indicates severe thermal stress; and > 25.6 indicates extremely severe



thermal stress (de Andrade Pantoja et al. 2017). Our study results indicated that the THI was between moderate and mild thermal stress under the two climate conditions. Several prior investigations documented the correlation between high temperatures and stress responses in sheep (Lu et al. 2019). Physiological changes experienced by sheep during heat stress are not reflected by THI, although THI can

be useful as an environmental index for the evaluation of heat stress levels (Liu et al. 2019). In this particular research endeavor, the emphasis will be placed on the aforementioned physiological alterations. The temperatures that are either high or low for an animal's thermal comfort zone might cause heat stress in the animal.

Table 7 Pearson correlations between physiological and blood characteristics linked to heat tolerance in crossbred and Awassi sheep.

	ST	RT	RR	HR	HT	HTC	ALT	PLT
RT	0.17**							
RR	0.15*	0.01						
HR	0.05	0.13*	0.14*					
HT	-0.09	0.03	-0.78***	-0.12*				
HTC	-0.27***	-0.67***	-0.13*	-0.03	-0.05			
ALT	0.62	0.59	-0.35	-0.33	0.51	-0.43		
PLT	-0.14	-0.33	0.21	-0.01	-0.03	0.11	-0.47	
PCV	0.44	0.68	-0.21	-0.19	0.24	-0.57	0.91***	-0.54

ST: skin temperature, RT: rectal temperature, RT: respiratory rate, HT: heat tolerance index, HR: heart rate, HTC: heat tolerance coefficient, ALT: alanine aminotransferase, HCT: hematocrit, PLT: platelets. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

4.2. Physiology response

The RR showed a statistically significant increase throughout the heat exposure in both the summer (31.69 °C) and the winter (12.29 °C), which is consistent with earlier findings in sheep. As mentioned in the literature review, the average RR normal range was 20 – 30 breaths/min in sheep (Jackson and Cockcroft 2008). This finding is consistent with that of (Macías-Cruz et al. 2016), who reported that the increase in ambient temperature and relative humidity was associated with a rise in RR. The results demonstrated that season and breed had a highly significant effect on RR, with winter months exhibiting lower RR values than summer months. In a study conducted by (Al-Azzawi et al. 2017), the RR was lower in local Awassi than in Turkish Awassi (84 and 70.6 breaths/min, respectively) in summer. This finding was similar to ours in this study, which showed an increased RR in crossbred Awassi compared to indigenous Awassi. Thermoregulatory responses to heat stress include increased respiration by increasing the amount of water vapor in exhaled air, which helps the body remove excess heat more quickly. Therefore, lower RR values reflect greater thermotolerance (Sailo et al. 2017). Similarly, the increased RR in Awassi could be due to the increased oxygen demand of the tissues under situations of heat stress. However, native sheep breeds in arid and semiarid regions are ideally suited to life in such extreme environments. Although the values were comparable to those of the Awassi breed, the pattern of RR with the change in ambient temperature during winter in crossbred sheep was different, as shown by the significant decrease in RR at low stress levels in winter (Naqvi et al. 2013). In addition, the RR in both breeds during winter suggests that crossbred sheep employ the same advantageous adaptive strategy of heat loss during summer and winter to maintain their core body temperature, similar to the indigenous sheep breed.

For all breeds, the HR values were significantly greater under conditions of heat stress. This is in line with

the physiology of different types of sheep when exposed to heat stress in arid climates during the summer. These results are in agreement with (Farias Machado et al. 2020) findings, which showed that the increase in HR was a result of increased muscle activity to manage the simultaneous increase in RR and the decrease in peripheral vascular resistance, which may have promoted higher blood perfusion to remove heat from the skin. However, instead of the pulse rate, the respiratory rate is the primary indicator of the response. This is because the initial response of animals exposed to environmental temperature is an increase in respiratory rate. This is done to prevent a rise in RT in homoeothermic animals, such as sheep (Singh et al. 2016). The increase in HR in Awassi in our study was also documented by (Al-Azzawi et al. 2017), which revealed that the HR of local Awassi was lower than that of Turkish Awassi. A possible explanation may be that more heat was dissipated through the skin as a result of increased circulation from the heart and lungs.

The RT of the sheep did not show a significant difference in variation between the summer and winter months. These findings are consistent with (Gesualdi Júnior et al. 2014) findings, which showed that genetic factors had no effect on the sheep's physiological characteristics (RT, RR and HR). However, the RT was stable and within normal limits either in Awassi or crossbred during thermal stress. Previous research has established a normal RT for sheep to be between 38.3 and 39.9 °C, which is in agreement with the current results (Leite et al. 2017). On the other hand, the color of the coat may have affected the Head, Neck, Ribs, and Rump temperatures. The lower winter skin temperatures of crossbred sheep can be explained by the physical properties of the wool, which acts as a thermal insulator, reflects heat, and exhibits higher thermal resistance (de Andrade Pantoja et al. 2017). These results are in agreement with those obtained by (Titto et al. 2016), who stated that respirational evaporation is essential for

thermoregulation in these animals due to the substantial reduction in sweating afforded by the wool fleece's protective coat. To dissipate the body's excess heat into the air, the skin temperature of Awassi rose in the summer as a result of expanding the blood vessels at the periphery. The different colored coats of these studied breeds lead to the scientific truth that the color of an animal's coat indicates its genetic superiority in hot environments. In dry climates, animals with lighter coats have an advantage because their fur can reflect up to 60 percent of the sun's rays (Joy et al. 2020). However, lightly pigmented sheep breeds had lower RT, RR, and HR values than darkly pigmented sheep breeds.

4.3. Heat tolerance of sheep

The heat tolerance coefficient and heat tolerance index are two of the heat tolerance indices that are regarded as variables by RT. Since these indices are directly correlated with physiological responses such as RR and RT, it can be assumed that animals with lower RR and RT are better adapted (Seixas et al. 2017). It is encouraging to compare this figure with that found by (Al-Thuwaini et al. 2020), who found that the mean heat tolerance coefficient was higher in the summer than in the winter for Awassi, indicating that both populations were exposed to significant stress during the summer.

4.4. Blood hematological parameters

The results demonstrated that indigenous sheep were in a healthy physiological state and within normal ranges as the seasons changed. Blood components such as MCV, MCH, and RDW of Awassi were increased during the winter, while they were reduced in the crossbreeds. These results are in agreement with (Oikonomidis et al. 2018), who reported that the hemodilution effect, in which the circulatory system transports more water to aid in evaporative cooling, may cause a decrease in blood constituents in response to high temperatures. Some researchers have linked the increased water consumption seen in heat stress-exposed sheep to changes in the animals' hematological profile (Seixas et al. 2017). Regardless, the increased levels of PCT and PLT in crossbred sheep might be due to the bone marrow of sheep continuously producing and releasing platelets into circulation, possibly in response to the presence of a mild inflammatory process (Avendaño-Reyes et al. 2020). During summer, heat stress caused some changes in Hb, PCV and MPV, which were reduced in Awassi and increased in the crossbreeds. These results confirm the association between the hematological response and heat stress (Dhuha et al. 2021). The hemodilution effect in the summer was reflected in inverse relationships between temperature, Hb, and PCV levels, as well as between these hematological constituents and THI. This explanation is contrary to that of (Nedeva et al. 2019), who indicated that the reduced feed intake in the warmer months contributes to lower Hb levels. Most likely, the reduction in PCV and Hb under heat and the higher

temperature during the hot months of our experiment led to an increase in RR, which led to a greater oxygen intake and an increase in oxygen partial pressure in the blood (Singh et al. 2016). Consequently, this had an effect on erythropoiesis, reducing the number of PCV and Hb values in all sheep breeds.

4.5. Blood biochemical parameters

The reduction in AST and ALT levels in Awassi sheep as a result of the effect is consistent with the findings of (Attia 2016). The decreases in these enzymes suggest that the animals' exposure to heat stress did not cause liver damage but rather a decrease in liver function (Attia 2016; Saeed et al. 2021). However, the environment's high temperature contributes to the increase in cortisone in the circulatory system, which causes an increase in ALT and AST enzymes in the liver due to oxidative stress (Hussain et al. 2017). Similar results regarding a significant decrease in blood urea under conditions of heat stress were obtained by (Attia 2016). This may be due to the decreased ruminal ammonia being counterbalanced by the increased rumen absorption of urea, which in turn reduces blood urea and raises urinary nitrogen excretion. During thermal stress in Awassi, the level of albumin in the blood dropped, and our results were similar to those of (Rahman et al. 2018), which clarified that the variations in various biochemical parameters may be attributable to diet, body water content, stress, hormonal influence, and environmental conditions. The decrease in triglyceride levels in all three sheep breeds could be attributed to an increase in hormone-sensitive TG-lipase due to increased cortisol secretion during summer stress (Singh et al. 2016). It has been suggested that triglyceride levels were found to be higher in young sheep, which may be due to their higher body fat content (Rahman et al. 2018).

4.6. Pearson correlation

The current study found that the physiological parameters were positively correlated with each other. (Kim et al. 2018) estimated correlations of physiology and blood variables with THI and concluded that THI was highly and positively correlated with HR and RT, which is consistent with the correlation coefficients in our study. However, medium and negative correlations were observed between physiological characteristics and blood parameters. This finding was also reported by (Alfonzo et al. 2016). The RT is another important indicator for homeostasis regulation of the calves' body temperature. The existence of a metabolic connection between the levels of cortisol and RT may be supported by the fact that there is a positive correlation between the two variables (cortisol levels and RT) (Kim et al. 2018). The increase in ALT activity in the sheep's blood indicated a rise in hepatic metabolism. This increase in ALT has an effect on PCV, which is involved, and a higher PCV indicates more efficient transport (Rahman et al. 2018). However, this result has not previously been described by

(Umar et al. 2021), and there is evidence to suggest that an increase in ALT activity due to heat stress is due to a general slowing of liver function rather than direct liver damage.

5. Conclusion

This study evaluates and predicts climatic variables and bioclimatic indices for local sheep in an arid region of the province of Al-Anbar (Iraq). In general, the Awassi breed had the lowest ST and HR but a higher RR than crossbred animals. The Awassi breed had the lowest ALT and PCV, indicating that it was best adapted to heat stress. The thermography data demonstrated a strong correlation with the physiological indices, with HTC, HT, and ALT constituting the primary points.

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Ethical considerations

The experiment adhered to the rules for animal experiments given by the Institutional Animal Ethics Committee, which approved this work, and the ethical guidelines of the University of Anbar (Ref. No. 182/2023). All procedures used in the animal experiment were approved by the University of Anbar's ethical approval committee.

Conflict of interest

This article's authors report no conflicts of interest.

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References

Al-Azzawi SH, Khalil RI, Mohammed ZI (2017) Effect of Vitamin C administration on heat tolerance of local and Turkish Awassi sheep in Diyala Province of Iraq. *Basrah Journal of Veterinary Research* 16, 288-297.

Al-Barzinji YM, Othman GU (2013) Genetic polymorphism in FecB gene in Iraqi sheep breeds using RFLP-PCR technique. *IOSR Journal of Agriculture and Veterinary Science* 2, 46-48.

Al-Thuwaini TM, Al-Shuhaib MBS, Hussein ZM (2020) A novel T177P missense variant in the HSPA8 gene associated with the low tolerance of Awassi sheep to heat stress. *Tropical Animal Health and Production* 52, 2405-2416.

Alfonzo EPM, Barbosa da Silva MVG, dos Santos Daltro D, Stumpf MT, Dalcin VC, Kolling G, Fischer V, McManus CM (2016) Relationship between physical attributes and heat stress in dairy cattle from different genetic groups. *International Journal of Biometeorology* 60, 245-253.

Attia NE-S (2016) Physiological, hematological and biochemical alterations in heat stressed goats. *Benha Veterinary Medical Journal* 31, 56-62.

Avendaño-Reyes L, Macías-Cruz U, Correa-Calderón A, Mellado M, Corrales JL, Corrales G, Ramirez-Bribiesca E, Guerra-Liera JE (2020) Biological responses of hair sheep to a permanent shade during a short heat stress exposure in an arid region. *Small Ruminant Research* 189, 106146.

de Andrade Pantoja MH, Esteves SN, Jacinto MAC, Pezzopane JRM, de Paz CCP, da Silva JAR, Junior JdBL, Brandão FZ, Moura ABB, Romanello N (2017) Thermoregulation of male sheep of indigenous or exotic breeds in a tropical environment. *Journal of Thermal Biology* 69, 302-310.

Dhuha J, Muayad M, Saeed O, Al-Bayar M, Saeid Z, Al Bakri S, Musa HC, Safaa A, Abed Q, Kaabi S, (2021) Tropical seasonal changes impact on hematological parameters of goats. *Journal of the Indonesian Tropical Animal Agriculture* 46, 219-226.

Farias Machado NA, Filho JADB, de Oliveira KPL, Parente MdOM, de Siqueira JC, Pereira AM, Santos ARD, Sousa JMS, Rocha KS, Viveiros Kkds (2020) Biological rhythm of goats and sheep in response to heat stress. *Biological Rhythm Research* 51, 1044-1052.

Gaughan J, Mader T, Gebremedhin K, Collier R, Collier J (2012) *Environmental Physiology of Livestock*. pp. 243-65. Wiley-Blackwell, West Sussex, UK.

Gesualdi Júnior A, Sales ÉSV, Freitas RS, Henry FdC, Oliveira VdPsd, Gesualdi ACLdS (2014) Effects of heat stress on the physiological parameters and productivity of hair sheep in tropical and coastal environments. *Revista Brasileira de Zootecnia* 43, 556-560.

Habeeb AA, Gad AE, Atta MA (2018) Temperature-humidity indices as indicators to heat stress of climatic conditions with relation to production and reproduction of farm animals. *International Journal of Biotechnology and Recent Advances* 1, 35-50.

Hussain SO, Badry KA, Zalzal SJ, Zakri AM (2017) Activity of transaminase enzyme and testosterone hormone in blood of Awassi rams during different season. *Asian Pacific Journal of Reproduction* 6, 217.

Jackson P, Cockcroft P (2008) 'Clinical examination of farm animals.' (John Wiley & Sons:

Joy A, Dunshea FR, Leury BJ, Clarke IJ, DiGiacomo K, Chauhan SS (2020) Resilience of small ruminants to climate change and increased environmental temperature: a review. *Animals* 10, 867.

Kim WS, Lee J-S, Jeon SW, Peng DQ, Kim YS, Bae MH, Jo YH, Lee HG (2018) Correlation between blood, physiological and behavioral parameters in beef calves under heat stress. *Asian-Australasian Journal of Animal Sciences* 31, 919.

Leite JHGM, Da Silva RG, da Silva WST, da Silva WE, Asensio LAB, Façanha DAE (2017) Adaptive evaluation of different coat colour of Morada Nova sheep in an equatorial semiarid region. *Journal Contribution* 1, 5195125.

Liu J, Li L, Chen X, Lu Y, Wang D (2019) Effects of heat stress on body temperature, milk production, and reproduction in dairy cows: A novel idea for monitoring and evaluation of heat stress—A review. *Asian-Australasian Journal of Animal Sciences* 32, 1332.

Lu Z, Chu M, Li Q, Jin M, Fei X, Ma L, Zhang L, Wei C (2019) Transcriptomic analysis provides novel insights into heat stress responses in sheep. *Animals* 9, 387.

Macías-Cruz U, López-Baca M, Vicente R, Mejía A, Álvarez F, Correa-Calderón A, Meza-Herrera C, Mellado M, Guerra-Liera J, Avendaño-Reyes L (2016) Effects of seasonal ambient heat stress (spring vs. summer) on physiological and metabolic variables in hair sheep located in an arid region. *International Journal of Biometeorology* 60, 1279-1286.

Macías-Cruz U, Gastélum MA, Álvarez FD, Correa A, Díaz R, Meza-Herrera CA, Mellado M, Avendaño-Reyes L (2016) Effects of summer heat stress on physiological variables, ovulation and progesterone secretion in Pelibuey ewes under natural outdoor conditions in an arid region. *Animal Science Journal* 87, 354-360.

McManus C, Paludo GR, Louvandini H, Gugel R, Sasaki LCB, Paiva SR (2009) Heat tolerance in Brazilian sheep: physiological and blood parameters. *Tropical Animal Health and Production* 41, 95-101.

Naqvi SM, De K, Gowane G (2013) Sheep production system in arid and semi-arid regions of India. *Annals of Arid Zone* 52, 1-9.

Nedeva I, Slavov T, Varlyakov I, Radev V, Panayotov D (2019) Haematological and blood biochemical parameters in Lacaune dairy sheep. *Bulgarian Journal of Agricultural Science* 25, 91-95.

Oikonomidis IL, Brozos C, Kiossis E, Kritsepi-Konstantinou M (2018) Combined and breed-specific RIs for hematologic, biochemical, and



- hormonal analytes in Chios and Florina adult rams. *Veterinary Clinical Pathology* 47, 56-68.
- Ouarda TB, Charron C, Kumar KN, Phanikumar DV, Molini A, Basha G (2019) Nonstationary warm spell frequency analysis integrating climate variability and change with application to the Middle East. *Climate Dynamics* 53, 5329-5347.
- Pinto S, Hoffmann G, Ammon C, Amon T (2020) Critical THI thresholds based on the physiological parameters of lactating dairy cows. *Journal of Thermal Biology* 88, 102523.
- Rahman MK, Islam S, Ferdous J, Uddin MH, Hossain MB, Hassan MM, Islam A (2018) Determination of hematological and serum biochemical reference values for indigenous sheep (*Ovis aries*) in Dhaka and Chittagong Districts of Bangladesh. *Veterinary World* 11, 1089.
- Rathwa SD, Vasava A, Pathan M, Madhira S, Patel Y, Pande A (2017) Effect of season on physiological, biochemical, hormonal, and oxidative stress parameters of indigenous sheep. *Veterinary World* 10, 650.
- Saeed O, Jaber B, Mohammed M, Sani U, Ziara K, Saad H (2021) 'Impacts of heat stress on blood metabolic in different periods of lactation and pregnancy in Holstein cows, IOP Conference Series: Earth and Environmental Science.' (IOP Publishing:Vol. 779, No. 1, p. 012013).
- Sailo L, Gupta I, Das R, Chaudhari M (2017) Physiological response to thermal stress in Sahiwal and Karan Fries cows. *International Journal of Livestock Research* 7, 275-283.
- Seixas, L de Melo, CB Tanure, CB Peripolli, V, McManus, C (2017) Heat tolerance in Brazilian hair sheep. *Asian-Australasian Journal of Animal Sciences* 30, 593.
- Silanikove N (2000) Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science* 67, 1-18.
- Singh KM, Singh S, Ganguly I, Ganguly A, Nachiappan RK, Chopra A, Narula H (2016) Evaluation of Indian sheep breeds of arid zone under heat stress condition. *Small Ruminant Research* 141, 113-117.
- Titto CG, Veríssimo CJ, Pereira AMF, de Mira Geraldo A, Katiki LM, Titto EAL (2016) Thermoregulatory response in hair sheep and shorn wool sheep. *Small Ruminant Research* 144, 341-345.
- Umar SIU, Konwar D, Khan A, Bhat MA, Javid F, Jeelani R, Nabi B, Najjar AA, Kumar D, Brahma B (2021) Delineation of temperature-humidity index (THI) as indicator of heat stress in riverine buffaloes (*Bubalus bubalis*) of a sub-tropical Indian region. *Cell Stress and Chaperones* 26, 657-669.