



# Modulation Impacts of *Moringa oleifera* on Thermo Tolerance Parameters and Blood Indices in Subtropical Ewes under Heat Stress

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**Abstract** | Appropriate strategies are required for the alleviation of heat stress resulting from global warming contributing to impaired metabolism and dysregulated immune and reproductive functions in animals. The aims of the current study were to investigate modulation impacts of *moringa oleifera* on thermo-tolerance parameters and blood indices in subtropical ewes under heat stress. Eighteen ewes with an average weight of  $50.3 \pm 1.80$  kg and aged 2.5 - 3.0 years were randomly allocated into three equal groups (six/group). The 1<sup>st</sup> and 2<sup>nd</sup> groups were assigned to *Moringa oleifera* treatments up to 50 and 100 g respectively and the 3<sup>rd</sup> group was used as a negative control. The 1<sup>st</sup> and 2<sup>nd</sup> groups were subjected to heat stress for three hours from 12:00 to 3:00 pm for three consecutive days. Before and after heat stress; thermo-tolerance parameters (rectal temperature, respiration rate, pulse rate, and partial pressure of oxygen (SPO<sub>2</sub>)) were recorded, as well as, blood samples were collected and analyzed for hematological parameters and plasma biochemistry parameters. The results indicated that heat stress negatively influenced the thermo-tolerance, hematological, and plasma biochemistry parameters. *M. oleifera* alleviated the negative effects of heat stress, as well as modulated thermo-tolerance responses, metabolites, and liver and kidney functions. *M. oleifera* supplementation with a rate of 50 and 100 g to stressed ewes especially during high ambient temperatures and in subtropics is recommended.

**Keywords** | Blood, Heat stress, *Moringa oleifera*, Metabolites, Plasma

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## INTRODUCTION

The climatic conditions of the Eastern Province of Saudi Arabia provide sheep and goats with extreme heat during the summer season and lower rates of heat stress during the spring and fall seasons. Temperature humidity index (THI) is the most common parameter describing the level of heat stress in animals (Bohmanova et al., 2006). THI of small ruminants when recording

more than 82, means that animals are suffering from heat stress (THI Levels: moderate 82 to < 84°F; severe 84 to < 86°F; extreme >86°F) (Froehlich et al., 2021). Heat stress negatively influences productive and reproductive performances by decreasing dry matter intake (DM), weight gain, ovarian follicle development, and milk production in livestock species. Therefore, it is important to adopt specific and profitable strategies for alleviation of heat stress when animals experience stress.

Feed additives and supplementation for their antioxidant contents are used for alleviation of heat stress in small ruminants (Kassab and Mohammed 2014a, b; Kassab et al., 2017). Recent interest has grown in the use of *M. oleifera* for the modulation of body functions because of its macro- and micro-nutrient contents (Fahey, 2005; Razis et al., 2014; Vongsak et al., 2014; Lin et al., 2018; Afzal et al., 2020, 2021; Abdel-Raheem and Hassan, 2021; Giuberti et al., 2021; Al-Mufarji and Mohammed, 2022a, b). *M. oleifera* is an important plant rich in nutrients that are important in medicine and nutrition for both humans and animals (Oyeyinka and Oyeyinka, 2018; Gupta et al., 2018). *M. oleifera* leaves are reported to contain substantial amounts of nutrients such as protein, fiber, carotenoids, and tocopherols (Jongrungruangchok et al., 2010; Moyo et al., 2011, 2012; Saini et al., 2014a, b); vitamins as A, C, and E (Hekmat et al., 2015); and minerals as potassium, calcium, sodium, magnesium, iron, copper, zinc, selenium, and manganese (Hekmat et al., 2015; Al-Mufarji and Mohammed, 2022a, b). The *M. oleifera* contains beta-carotene and other phytochemicals known for their antioxidant abilities (quercetin, kaempferol, rutin, and caffeoylquinic acids), antioxidant vitamins (A, C, and E), and antioxidant minerals (zinc and selenium) which can play important roles as anti-stress (Jaiswal et al., 2009; Vongsak et al., 2014; Afzal et al., 2021). The current study aims to evaluate the modulatory influences of *M. oleifera* at rates of 50 and 100 g on thermo-tolerance parameters, blood, and metabolic status of ewes before subjecting to heat stress in arid subtropical areas.

## MATERIALS AND METHODS

### ETHICAL APPROVAL

The experimental procedures were approved by the Ethical Clearance of the deanship of scientific research, the vice presidency for graduate studies and scientific research, King Faisal University, Saudi Arabia [Ref. No. KFUREC-2022-MAR-EA000532].

### MORINGA OLEIFERA SOURCE

The organic *M. oleifera* leaves were obtained from Nadawy Farm, Gizan, Kingdom of Saudi Arabia (053/SA). The farm has got a certificate according to Saudi Organic law and By Law (OSKSA) valid from 30<sup>th</sup> November 2021 until 29<sup>th</sup> November 2022.

### LOCATION OF THE STUDY

This experiment was carried out in the Research and Training Station of King Faisal University during the fall season. King Faisal University locates in the Eastern Province of King Saudi between 25°15'58" N Latitude and 49°41'45" E longitude and lies at 154 m altitude above the sea level. The climate of this area is dry, rainfall is almost

negligible (0.9 mm), the ambient temperature ranges from 45.4°C during the summer days to 8.5°C on the winter nights, and the relative humidity ranges from 22.0% during summer days to 56.0% in the winter nights.

### CLIMATIC CONDITIONS AND THERMO-TOLERANCE RESPONSES

Relative humidity and ambient temperature were recorded simultaneously at 12.00 and 3.00 pm. A hygrometer (AcuRite Digital Hygrometer, USA) was used to measure relative humidity (%), while a mercury centigrade thermometer was used to measure ambient temperature. THI values for the experimental site were calculated using the equation of Mader et al. (2006).  $THI = [0.8 \times \text{air temperature}] + [(\% \text{ relative humidity}/100) \times (\text{air temperature} - 14.4)] + 46.4$ . Rectal temperatures were measured using a clinical thermometer (Citizen Flex Digital Thermometer CTA303). Respiration rates were counted through inward and outward movement of the flank per minute (breaths/minute) (Brandt et al., 2022). The pulse rates and partial pressure of oxygen (PO<sub>2</sub>) were measured through a pulse oximeter and heart rate monitor (CMS60D-VET Handheld Veterinary Pulse Oximeter).

### ANIMALS AND MANAGEMENT

Eighteen healthy ewes (local breed in the Al-Ahssa) weighing  $50.3 \pm 1.80$  kg and aged 2.5 - 3.0 years were used in this experiment, which provided farm vaccination routine protocols. The ewes were living in a standard pen of semi-close housing system at a stocking rate of 1.75 m<sup>2</sup>/ewe. The ewes were kept free inside the pen designed to allow ewes the opportunity for social interaction. The designed floor surface covered with bedding were cleaned weekly. The ewes were daily fed a 1 kg basal concentrate diet for the control group and a basal concentrate diet supplemented with 50 and 100 g *M. oleifera*, as well as *ad-libitum* berseem hay. The concentrate diet was offered twice at 08:00 am and 4:00 pm. Ewes were given free access to automatic clean drinking water *ad-libitum*. The heat stress exposures were performed from 12:00 to 3:00 pm for three consecutive days.

### THERMO-TOLERANCE PARAMETERS, BLOOD AND PLASMA ANALYSIS

On heat stress days, rectal temperatures, respiration and pulse rates, and partial pressure of oxygen (SPO<sub>2</sub>) were recorded before and after heat stress as thermal responses. Rectal temperature was recorded using a digital centigrade thermometer (Citizen CTA-303). Flank movement was used for recording respiration rates. The pulse rates and partial pressure of oxygen (PO<sub>2</sub>) were measured through a pulse oximeter and heart rate monitor (CMS60D-VET Handheld Veterinary Pulse Oximeter). Six blood samples were collected before and after heat stress from each group

in three consecutive days. The obtained blood samples were analyzed for hematological and biochemistry parameters through hematology analyzers (Abaxis Vetscan HM5) and chemistry analyzers (Skyla VB1; <http://www.skyla.com/page/about/index.aspx?kind=103>). The measured hematological parameters included blood cells, hematocrit, and hemoglobin values. The measured plasma parameters included total proteins, glucose, liver and kidney functions, and mineral values.

### STATISTICAL ANALYSIS

Thermo-tolerance, blood, and plasma biochemistry values were statistically analyzed using the General Linear Model (GLM) procedure of SAS (SAS, 2008) according to the following model:  $Y_{ij} = \mu + T_i + e_{ij}$  Where:  $\mu$  = Mean,  $T_i$  = Effect of *M. oleifera* and  $e_{ij}$  = Standard error.

Duncan's Multiple Range Test (1955) was used to compare the means of the control and treated groups.

## RESULTS AND DISCUSSION

Thermo-tolerance response parameters in addition to hematological and biochemical parameters are presented in Tables 1, 2, 3. Hematological parameters, after the 3-h, revealed that heat stress contributed to changes in the control and *Moringa* groups with an increase in the red and white blood cells of the treated groups compared to the control group (Table 2). The neutrophils/lymphocyte ratios were non-significantly decreased upon *Moringa* supplementation. Ewes after heat stress had higher total protein and albumin and lower concentrations of AST, ALT, and glucose (Table 3). Generally, the data indicated that the values of blood and metabolites in *Moringa* groups were modulated positively compared to the control. Thus, *Moringa* supplementation (50 and 100 g) alleviated stress

effects and contributed to maintaining serum metabolites and mineral concentrations (Table 3).

### BODYWEIGHT LOSS AND THERMO-TOLERANCE RESPONSES

Bodyweight loss and thermo-tolerance responses revealed in Table 1 bodyweight loss up to  $-0.86 \pm 0.13$ ,  $-0.70 \pm 0.15$ , and  $-0.60 \pm 0.05$  in the control and the two *Moringa* treated groups, respectively. *Moringa* supplementation alleviated live weight loss from 1.71% in control to 1.37 and 1.2% in the treated groups respectively, but the differences among the groups were not significant. Rectal temperature, respiration, and pulse rates were increased, while  $SPO_2$  was decreased as a result of heat stress. The changes were more pronounced in the control group compared to the two *Moringa* groups.

High ambient temperature is a major stumbling block of animal production, such as sheep maintained in subtropics and tropics environments (Marai et al., 2007). High ambient temperature leads to stress-induced hyperthermia and increased metabolic heat production. The negative effects of high temperature are triggered when it is accompanied by high ambient humidity (Abdel-Hafez, 2002). The extreme temperature and humidity rates negatively affected animal health and diseases, growth, and reproduction (Kassab et al., 2017). The rectal temperature has been used to determine core temperature and to evaluate heat stress response in farm animals (Lefcourt et al., 1986; Alhidary et al., 2014). Core animal temperature reflects the temperatures of the main internal organs such as the brain, heart, and viscera (Sellier et al., 2014). Core body temperature is the most important value for evaluating heat stress in farm animals and is associated with an inverse relationship with health, growth, and reproductive performance.

**Table 1:** Effects of *M. oleifera* on body weight and thermo-tolerance parameters of ewes over heat stress.

Parameters	Before and after heat stress	Treatments		
		Control	<i>M. oleifera</i> 50.0g	<i>M. oleifera</i> 100g
Body weight, kg	Before	50.20 $\pm$ 1.74	50.80 $\pm$ 1.73	49.86 $\pm$ 1.17
	After	49.30 $\pm$ 1.74	50.16 $\pm$ 0.06	49.26 $\pm$ 0.07
Rectal temperature, °C	Before	38.9 $\pm$ 0.24	39.10 $\pm$ 0.06	39.10 $\pm$ 0.07
	After	39.5 $\pm$ 0.06	39.40 $\pm$ 0.06	39.4 $\pm$ 0.07
Respiration rate/min.	Before	29.0 $\pm$ 0.50	27.0 $\pm$ 0.57	27.3 $\pm$ 0.88
	After	38.0 $\pm$ 0.15	35.0 $\pm$ 0.89	37.0 $\pm$ 0.58
Pulse rate/min.	Before	89.0 <sup>a</sup> $\pm$ 2.30	86.0 <sup>ab</sup> $\pm$ 0.33	81.0 <sup>b</sup> $\pm$ 0.67
	After	97.0 $\pm$ 2.30	93.0 $\pm$ 0.33	95.5 $\pm$ 0.67
Partial pressure of oxygen, $SPO_2$	Before	96.0 $\pm$ 0.88	97.0 $\pm$ 1.45	98.0 $\pm$ 2.03
	After	93.0 <sup>b</sup> $\pm$ 1.50	95.0 <sup>a</sup> $\pm$ 1.45	96.0 <sup>a</sup> $\pm$ 2.03

<sup>a,b</sup> Values with different superscripts between groups significantly differ at  $P < 0.05$ .

**Table 2:** Effects of *M. oliefera* on blood indices of ewes over heat stress.

Parameters	Before and after heat stress	Treatments		
		Control	<i>M. oliefera</i> 50g	<i>M. oliefera</i> 100g
Red blood cells, 10 <sup>12</sup> /L	Before	12.24 <sup>b</sup> ± 0.64	13.18 <sup>a</sup> ± 0.17	13.11 <sup>a</sup> ± 0.43
	After	12.49 <sup>b</sup> ± 0.77	14.05 <sup>a</sup> ± 0.16	13.480 <sup>a</sup> ± 0.48
Hemoglobin, g/dl	Before	13.07 <sup>b</sup> ± 0.18	13.63 <sup>a</sup> ± 0.49	14.03 <sup>a</sup> ± 0.66
	After	13.57 <sup>b</sup> ± 0.54	14.63 <sup>a</sup> ± 0.74	14.27 <sup>a</sup> ± 0.52
Hematocrit, %	Before	34.39 ± 1.32	33.02 ± 0.77	37.11 ± 2.06
	After	34.13 ± 0.70	36.54 ± 1.25	37.18 ± 2.02
MCV, fl or µm <sup>3</sup>	Before	28.0 ± 2.31	25.00 ± 0.58	28.3 ± 2.40
	After	27.6 ± 2.19	26.00 ± 0.58	27.6 ± 2.67
MCH, pg/cell	Before	10.77 ± 0.50	10.37 ± 0.23	10.73 ± 0.74
	After	10.93 ± 0.41	10.40 ± 0.40	10.63 ± 0.78
MCHC, g/dl or %	Before	38.23 ± 1.34	41.30 ± 0.56	37.90 ± 0.57
	After	39.87 ± 1.71	40.00 ± 0.60	38.50 ± 0.71
RDWc, %	Before	26.17 ± 1.42	26.97 ± 0.85	26.73 ± 1.41
	After	25.40 ± 1.19	27.13 ± 0.78	27.03 ± 1.52
RDWs, fl	Before	30.43 ± 1.19	27.60 ± 0.93	31.23 ± 0.90
	After	28.64 ± 1.56	28.67 ± 1.13	30.47 ± 1.19
White blood cells, 10 <sup>9</sup> /l	Before	9.83 <sup>b</sup> ± 0.60	11.82 <sup>a</sup> ± 0.43	13.36 <sup>a</sup> ± 1.13
	After	11.46 <sup>b</sup> ± 0.78	11.46 <sup>b</sup> ± 0.23	13.41 <sup>a</sup> ± 1.24
Lymphocytes, 10 <sup>9</sup> /l	Before	5.62 <sup>b</sup> ± 0.44	6.72 <sup>a</sup> ± 0.33	7.047 <sup>a</sup> ± 0.37
	After	6.35 ± 0.58	6.10 ± 0.22	6.71 ± 0.25
Monocytes, 10 <sup>9</sup> /l	Before	0.06 ± 0.006	0.06 ± 0.005	0.07 ± 0.007
	After	0.06 ± 0.003	0.057 ± 0.003	0.07 ± 0.006
Neutrophils, 10 <sup>9</sup> /l	Before	2.97 <sup>b</sup> ± 0.20	3.85 <sup>a</sup> ± 0.34	4.97 <sup>a</sup> ± 0.87
	After	3.88 <sup>b</sup> ± 0.31	4.07 <sup>b</sup> ± 0.39	5.28 <sup>b</sup> ± 0.10
Eosinophils, 10 <sup>9</sup> /l	Before	1.04 ± 0.14	1.07 ± 0.02	1.15 ± 0.08
	After	1.05 ± 0.03	1.12 ± 0.04	1.22 ± 0.10
Basophils, 10 <sup>9</sup> /l	Before	0.15 ± 0.018	0.12 ± 0.03	0.12 ± 0.006
	After	0.12 ± 0.009	0.11 ± 0.012	0.13 ± 0.01
Neutrophils/ Lymphocytes ratio	Before	0.53 <sup>b</sup> ± 0.04	0.58 <sup>ab</sup> ± 0.70	0.70 <sup>a</sup> ± 0.11
	After	0.62 <sup>b</sup> ± 0.06	0.67 <sup>ab</sup> ± 0.09	0.78 <sup>a</sup> ± 0.14
Platelet, 10 <sup>9</sup> /l	Before	434.0 <sup>a</sup> ± 134.8	235.0 <sup>b</sup> ± 78.03	260.3 <sup>b</sup> ± 57.38
	After	414.0 <sup>a</sup> ± 101.30	354.6 <sup>b</sup> ± 166.97	289.3 <sup>b</sup> ± 48.53
Mean platelet volume, fl	Before	5.73 ± 0.22	5.97 ± 0.36	6.10 ± 0.45
	After	5.47 ± 0.34	6.00 ± 0.06	6.13 ± 0.43
Platelet mass, %	Before	0.25 ± 0.08	0.130 ± 0.04	0.16 ± 0.05
	After	0.22 ± 0.04	0.21 ± 0.10	0.18 ± 0.04
Platelet distribution width PDWc, %	Before	21.27 ± 0.37	26.47 ± 2.28	26.90 ± 2.70
	After	22.70 ± 0.81	23.77 ± 2.87	26.90 ± 2.70
Platelet distribution width PDWs, fl	Before	4.60 ± 0.10	6.07 ± 0.84	6.33 ± 1.13
	After	4.67 ± 0.39	5.470 ± 0.77	6.33 ± 1.13

<sup>a,b</sup> Values with different superscripts between groups significantly differ at P < 0.05. MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; MCHC, Mean corpuscular hemoglobin concentration; RDW, Red cell distribution width; fl, femtoliters (10<sup>-15</sup>).



**Table 3:** Effects of *M. oleifera* on blood biochemistry of ewes over heat stress.

Parameters	Before and after heat stress	Treatments		
		Control	<i>M. oleifera</i> 50g	<i>M. oleifera</i> 100g
Total protein, g/dl	Before	7.10 <sup>b</sup> ± 0.02	8.10 <sup>a</sup> ± 0.15	8.00 <sup>a</sup> ± 0.12
	After	7.23 <sup>b</sup> ± 0.20	8.23 <sup>a</sup> ± 0.15	8.07 <sup>a</sup> ± 0.07
Albumin g/dl	Before	3.26 <sup>b</sup> ± 0.08	3.45 <sup>a</sup> ± 0.08	3.37 <sup>a</sup> ± 0.09
	After	3.30 <sup>b</sup> ± 0.20	3.50 <sup>a</sup> ± 0.12	3.40 <sup>ab</sup> ± 0.03
Globulin g/dl	Before	3.84 <sup>b</sup> ± 0.10	4.65 <sup>a</sup> ± 0.20	4.63 <sup>a</sup> ± 0.17
	After	3.93 <sup>b</sup> ± 0.30	4.73 <sup>a</sup> ± 0.15	4.67 <sup>a</sup> ± 0.03
Albumin/Globulin	Before	0.85 <sup>a</sup> ± 0.04	0.75 <sup>b</sup> ± 0.05	0.73 <sup>b</sup> ± 0.04
	After	0.85 <sup>a</sup> ± 0.11	0.74 <sup>b</sup> ± 0.04	0.73 <sup>b</sup> ± 0.00
Glucose mg/dl	Before	60.33 <sup>a</sup> ± 1.46	52.0 <sup>b</sup> ± 2.65	51.0 <sup>b</sup> ± 1.53
	After	56.33 <sup>a</sup> ± 5.50	48.67 <sup>b</sup> ± 1.86	49.33 <sup>b</sup> ± 3.48
Alkaline phosphatase, U/L	Before	124.0 <sup>a</sup> ± 3.21	108.67 <sup>b</sup> ± 5.81	115.33 <sup>ab</sup> ± 4.33
	After	126.33 ± 12.39	118.0 ± 6.0	119.67 ± 2.60
Aspartate aminotransferase, U/L	Before	103.33 ± 4.41	98.67 ± 5.21	95.67 ± 4.41
	After	104.0 ± 1.73	106.33 ± 4.50	103.0 ± 4.36
Gamma-glutamyl transferase, U/L	Before	74.33 <sup>a</sup> ± 2.60	64.67 <sup>ab</sup> ± 4.10	53.67 <sup>b</sup> ± 4.33
	After	75.00 <sup>a</sup> ± 1.53	58.33 <sup>b</sup> ± 8.74	52.0 <sup>b</sup> ± 7.00
Creatine phosphokinase, U/L	Before	265.67 ± 12.86	263.67 ± 4.10	260.0 ± 5.77
	After	296.67 ± 7.80	285.33 ± 13.62	280.67 ± 10.27
Blood urea nitrogen, mg/dl	Before	17.67 <sup>a</sup> ± 0.88	14.33 <sup>b</sup> ± 1.45	14.40 <sup>b</sup> ± 2.65
	After	17.33 <sup>a</sup> ± 0.84	14.67 <sup>b</sup> ± 1.96	15.03 <sup>b</sup> ± 0.15
Calcium, mg/dl	Before	10.57 <sup>b</sup> ± 0.17	11.73 <sup>a</sup> ± 0.29	11.93 <sup>a</sup> ± 0.18
	After	10.03 <sup>b</sup> ± 0.42	11.80 <sup>a</sup> ± 0.4	12.07 <sup>a</sup> ± 0.23
Phosphorus, mg/dl	Before	6.07 <sup>b</sup> ± 0.15	6.40 <sup>a</sup> ± 0.32	6.43 <sup>a</sup> ± 0.27
	After	5.63 <sup>b</sup> ± 0.32	6.03 <sup>a</sup> ± 0.55	6.40 <sup>a</sup> ± 0.31
Sodium, mmol/L	Before	144.67 ± 2.33	157.33 ± 3.93	159.67 ± 7.31
	After	137.67 <sup>b</sup> ± 4.84	144.0 <sup>b</sup> ± 8.0	153.67 <sup>a</sup> ± 3.28
Potassium, mmol/L	Before	7.20 ± 0.23	7.30 ± 0.15	7.630 ± 0.12
	After	6.87 ± 0.19	7.80 ± 0.20	7.40 ± 0.32
Chloride, mmol/L	Before	114.0 ± 2.08	117.67 ± 4.33	116.33 ± 3.48
	After	111.33 ± 4.91	112.67 ± 4.84	115.0 ± 2.52
Magnesium, mg/dl	Before	0.647 ± 0.04	0.69 ± 0.04	0.65 ± 0.03
	After	0.64 ± 0.04	0.71 ± 0.05	0.62 ± 0.02
Urea, mg/dL	Before	37.57 <sup>a</sup> ± 3.87	29.13 <sup>b</sup> ± 2.60	30.83 <sup>b</sup> ± 5.67
	After	39.00 <sup>a</sup> ± 2.31	30.47 <sup>b</sup> ± 2.27	31.00 <sup>b</sup> ± 3.21
Corrected calcium, mg/dl	Before	11.23 ± 0.19	12.10 ± 1.50	12.03 ± 0.09
	After	10.73 ± 0.37	12.10 ± 0.10	12.23 ± 0.23
Sodium/potassium	Before	20.11 ± 0.33	20.64 ± 0.83	21.88 ± 0.86
	After	20.10 ± 1.11	18.43 ± 0.57	20.47 ± 0.77

<sup>a,b</sup> Values with different superscripts between groups significantly differ at P < 0.05.

The alleviation effects of *M. oleifera* on heat stress parameters might be due to several factors including antioxidative properties, nutrient digestibility, rumen fermentation, and regulating pathways involved in the metabolism (Elghandour et al., 2017; Roshdy et al., 2021;

Abdel-Raheem and Hassan, 2021). *M. oleifera* leaf meal supplemented with goat and steer resulted in a significant decrease in CH<sub>4</sub>, ruminal ammonia-N, and total protozoal number, while an increase in CO<sub>2</sub> production, fermentation pH, and total bacterial counts (Elghandour et al., 2017).

Abdel-Raheem and Hassan (2021) experimented with *M. oleifera* dietary inclusion in growing buffalo calves on nutrient digestibility, rumen fermentation, and growth performance. They found improvements in rumen fermentation, growth performance, blood metabolites, and mitigated ammonia and methane values.

It seems that the antioxidative properties of *M. oleifera* might help to maintain homeostasis under heat stress conditions (Yasoob et al., 2022). The partial pressure of oxygen was significantly increased in the *Moringa* groups compared to the control. This could be attributed to the significant increase of RBCs, hematocrit, and hemoglobin values in *Moringa* treated groups. Antioxidants in *Moringa* are free radical scavengers, which protect the body defense system against excessively produced free radicals and stabilize the health status of the stressed animal. Such natural antioxidants might involve some oxidation and reduction reactions in the body. *Moringa* is rich in antioxidant vitamins and minerals, particularly vitamin C and selenium. Selenium, as an antioxidant element, helps in detoxification and immune health. Vitamin C directly alters the thermal set point by decreasing prostaglandin output. Prostaglandin turnover increases during stress (Hadden et al., 1987) and has a direct effect on the hypothalamic thermoregulatory zone (Ganong, 2001).

The great protective effects of *M. oleifera* leaves extract against many diseases and the widely persistent environmental toxins, which disrupted cellular metabolic function have been confirmed (Hassan et al., 2021). Up-regulation of genes for thermo-tolerance, antioxidation, and immunity over supplementing rabbits with oral *M. oleifera* leaf powder under heat stress was recorded indicating beneficial aspects on liver functions in heat-stressed rabbits (Yasoob et al., 2022). Moreover, up-regulation of the antiapoptotic BCL2A1 gene by *M. oleifera* leaves extract may suggest protection against apoptosis induced due to heat stress.

#### HEMATOLOGY AND BLOOD BIOCHEMISTRY

The results indicated significant ( $P < 0.05$ ) improvements in red and white blood cells, packed cell volume, hemoglobin values, plasma metabolites (glucose and urea), liver enzymes, and minerals (Tables 2 and 3). Blood indices and plasma metabolites are indicative of the body's health and thermal responses in mammalian species. Inclusion of 25, 50, 75, and 100% *M. oleifera* in the African ewe diets improved blood indices (RBCs, WBCs, Hb, and PCV) (Fadiyimu et al., 2010, 2016, 2017). *M. oleifera* leaves that were supplemented at rates of 25, 50, 75, and 100% to Sirohi goat kids' diets resulted in significantly increased RBCs, Hb, total protein, and albumin in the 100% treatment, while the white blood cells significantly

decreased (Meel et al., 2018). *M. oleifera* extracts given to lambs as an anti-methane additive were proven to be effective in reducing intestinal methane emission (Akanmu et al., 2020). *M. oleifera* leaves powder when supplemented with rabbits during heat stress, resulting in reduced glucose, total cholesterol, low-density lipoprotein cholesterol, and triglycerides (Yasoob et al., 2022). Therefore, these plant extracts can be safely used as alternative food additives to reduce intestinal methane emissions and alleviate heat stress. Due to the numerous nutritional benefits, *M. oleifera* leaves can improve physiological parameters, blood, and plasma values. Because of *M. oleifera* lipid contents, they might be considered key constituents of the plasma membrane and they are essential for the functionality of all cellular membranes. In addition, lipids form membrane vesicles or lipid droplets (LDs) that are involved in the transport of proteins, hormones, or fat-soluble vitamins (A, D, E, and K) in cells and extracellularly, for example in the bloodstream (Vachier et al., 2002).

Liver enzymes (alkaline phosphatase, aspartate aminotransferase, and gamma-glutamyl transferase) in addition to creatine phosphokinase (CPK) were improved in *M. oleifera* treated groups compared to the control. CPK is found mainly in the skeletal muscle, heart, and brain. One of the conditions that may contribute to higher CPK values is hyperthyroidism (McCullough, 2019), which is expected to increase in the control group compared to *M. oleifera* groups due to higher thermo-tolerance responses. In addition, Yasoob et al. (2022) indicated beneficial aspects of liver functions in heat-stressed rabbits upon *M. oleifera* supplementation. The antioxidant activity of *M. oleifera* leaf extract on the enzymatic activity of the liver in goats has been confirmed (Moyo et al., 2012). The effects of *Moringa* leaf and its extracts on immunity functions and antioxidant activity are due to *Moringa* polyphenols extract, which might have immunomodulatory properties (Adjei-Fremah et al., 2019).

#### CONCLUSIONS AND RECOMMENDATIONS

The results concluded that adding 50.0 and 100.0 g *M. oleifera* to the diets of heat stressed ewes resulted in alleviation of heat stress influences, as well as modulating thermo-tolerance responses, metabolites, and liver and kidney functions. This could be attributed to the bioactive compounds of *M. oleifera* as promising protectors of inflammation and oxidative stress processes. Moreover, *in-vivo* and *in-vitro* studies should be carried out on *M. oleifera* bioactive compounds to authenticate their possible applications over a wide range of dysregulation causing impaired metabolism of different species.

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## NOVELTY STATEMENT

The article presented for the first time modulation impacts of *Moringa oleifera* on thermo-tolerance parameters and blood indices in subtropical ewes under heat stress

## AUTHORS CONTRIBUTION

Aiman Al Mufarji, Rashid Al Zeidi and Haitham Al Masruri carried out the study and Abd El-Nasser Ahmed Mohammed carried out the experimental design and statistical analysis of data, wrote the manuscript for publication.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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