Ameliorative effects of *Moringa oleifera* extract, Sodium Bicarbonate, and Vitamin C on heat Stress-induced reproductive dysfunction and testicular degeneration in male rabbits

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Abstract

Heat stress (HS) critically impairs rabbit growth and reproductive performance in tropical and subtropical regions. This study aimed to evaluate chronic HS effects on male V-line rabbits and investigate the potential protective roles of *Moringa oleifera* aqueous extract (MOE), sodium bicarbonate (SB), and vitamin C (VC). 50 male rabbits were split into 5 groups: a normothermic group served as a negative control (NC), and four groups were exposed to daily heat stress (27–39°C). The HS groups included an untreated control (PC) and three groups supplemented with either 100 mg/kg MOE, 300 mg/kg SB, or 200 mg/kg VC in drinking water for 10 weeks. HS (temperature-humidity index (THI) = 31.13) significantly reduced growth performance, increased mortality (50%), and caused severe testicular oxidative stress, elevated nitric oxide (NO) and malondialdehyde (MDA), and reduced activities of catalase (CAT) and superoxide dismutase (SOD). Furthermore, steroidogenic genes of cytochrome P450 family 11 subfamily A member 1 (CYP17A1), and 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2) were downregulated by HS and reduced levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and serum testosterone and led to profound histopathological degeneration of the seminiferous tubules. Supplementation with MOE, SB, and VC significantly mitigated these effects. MOE and VC demonstrated superior efficacy in restoring testicular architecture, germ cell counts, and daily sperm production. While SB improved tubular physical dimensions, it was less effective at protecting cellular populations. Supplementation with antioxidants, particularly MOE and VC, effectively protects the male rabbit reproductive system from the deleterious effects of severe heat stress by maintaining hormonal balance, gene expression, and testicular integrity.

Keywords: Heat stress, Male rabbits, Moringa oleifera, Vitamin C, Sodium bicarbonate, Testicular function, and Steroidogenic genes

Introduction

HS represents a critical environmental constraint that severely impairs the reproductive efficiency of various livestock species, with rabbits being particularly susceptible when reared in arid and tropical regions. Physiological manifestations of HS in male rabbits include decreased libido, impaired spermatogenesis, and significant pathological changes in testicular structure (Hosny *et al*., 2025; Ding *et al.,* 2025; Khalifa *et al*., 2026). Exposure to elevated ambient temperatures (AT), particularly during peak summer months, results in reduced ejaculate volume and sperm concentration, alongside a higher incidence of morphological abnormalities (Ebeid *et al*., 2023). Furthermore, HS disrupts the metabolic profile of semen, deteriorating sperm motility, viability, and overall fertilizing potential (Abdelnour *et al*., 2021).

At the cellular level, the pathogenesis of heat-induced infertility is primarily driven by the excessive output of reactive oxygen species (ROS) and the activation of oxidative stress within the male reproductive tract (Nowicka-Bauer & Nixon, 2020). This biochemical imbalance is characterized by an elevation in lipid peroxidation markers, such as MDA, and decrease in the activity of essential antioxidants enzymes, including glutathione peroxidase (GPx) and CAT (Hanafi *et al*., 2025). Such oxidative damage not only compromises the integrity of the seminiferous tubules, reducing the population and functional capacity of Sertoli and Leydig cells, but also triggers apoptotic pathways that halt the stages of spermatocytogenesis and spermiogenesis (Sui *et al*., 2019; Shahat *et al*., 2020). Histologically, these changes manifest as reduced epithelial thickness and mitochondrial degeneration, with research indicating that the rate of spermatogenesis may decline by 14% for every 1 °C rise in testicular temperature (Gao *et al*., 2012; Durairajanayagam *et al*., 2015).

Beyond direct tissue injury, HS exerts a profound inhibitory effect on the hypothalamic-pituitary-gonadal (HPG) axis. This endocrine disruption leads to suppressed secretion of FSH, LH, and progesterone (Sirotkin *et al*., 2021; Abdelnour *et al*., 2022). The subsequent reduction in testosterone levels by HS effect was attributed to the downregulation of steroidogenic enzymes, specifically cytochrome P450 isozymes such as *CYP11A1* and 17β-hydroxysteroid dehydrogenase (17β-HSD) (Bui-Le *et al*., 2023; Sheweita *et al*., 2025).

To mitigate these adverse effects, nutritional interventions involving antioxidants and electrolytes have gained significant attention (Vaghamashi et al., 2025). Supplementation with MOE has been shown to enhance the reproductive hormonal profile, improve semen quality, and bolster the total antioxidant capacity in goats (Ukey et al., 2025) and rabbits (El-Desoky *et al*., 2017; Mutwedu *et al.*, 2022). Similarly, the administration of antioxidant VC, VE and SB has been documented to reduce lipid peroxidation, enhance seminal total antioxidant, reproductive outcomes in both males and females and support acid-base balance under thermal challenge (Ewuola *et al*., 2022; Khaleel *et al*., 2025).

The present study was designed to evaluate the comparative effectiveness of MOE, VC, and SB in ameliorating heat stress-induced reproductive and physiological dysfunction in rabbits. By examining growth parameters, antioxidant defense mechanisms, and gene expression related to reproductive health.

Methods and Material

Experimental design, housing, and animals

An artificial climate-controlled chambers was used to conduct the experiment located at Sids research station of the Animal Production Research Institute (APRI), affiliated with the Agricultural Research Center (ARC). Fifty male V-line rabbits, old approximately 3 months and with an average of body weight (BW) 1.49 ± 0.050 kg, were employed as experimental animals during the period from May to August, 2025, it should be noted that the oral administration period for the treatments was only 10 weeks, within a total trial duration of 12 weeks. Rabbits were randomly distributed separately into equal five groups (10 each). The first group (normothermic group) served as a negative control (NC), maintained under thermal comfort conditions, with relative humidity (RH) of 55% and an average AT of 24°C regulated using artificial ventilation or air conditioning systems. The second group (PC) was housed under temperature regimen programmed to fluctuate daily between 27 °C and 39 °C, with an average RH of 58%. Animals were exposed to the peak temperature (39 °C) for a duration of 4 hours during the central part of the day to mimic natural HS conditions. The third group (MOE) were treated daily for 10 weeks with 100 mg/Kg BW of the MOE in drinking water (Abdel-Latif *et al*., 2018). The fourth group (SB) was treated daily for 10 weeks with 300 mg / Kg BW of SB solution (Sigma Aldrich in Egypt) in drinking water (Abdel-Latif *et al*., 2018). The fifth group (VC) was treated daily for 10 weeks with 200 mg / Kg BW of VC (Sigma Aldrich in Egypt) in drinking water (Abdel-Latif *et al*., 2018). The three remaining treated groups were maintained under identical environmental conditions to those of the PC group.

Rabbits were protected under observation for around ten days before the commencement of the experiment to exclude any underlying infections. During period of adaptation, the rabbits were maintained at 22℃ and RH 50±5%. The animals′ diet was formulated as described by Al-Sagheer *et al*. (2017). Diets were available in pellet form *ad libitum* and fresh water was supplied at all times from automatic nipple drinkers. Chemical analysis of the diet was done according to AOAC (2007).

The experiment was conducted in two artificial climatic-controlled chambers. Daily, a photoperiod of 12 hours of light and 12 hours of darkness (12L:12D) was maintained. In this study, THI was calculated using an equation specifically developed for rabbits (Abdel-Latif *et al*., 2018).

Quantitative real-time PCR and Total RNA extraction

Total RNA was extracted from cryopreserved testicular tissue used the TRIzol reagent in strict accordance with the manufacturer's protocols. The extracted mRNA was reverse transcribed using the Omniscript® Reverse Transcription kit (TaKaRa Biotechnology, China) in a 20 µl reaction volume. Quantitative real-time PCR (qRT-PCR) employed the resultant cDNA as a template to evaluate the expression profiles of the CYP17A1, HSD3B2, and Hydroxysteroid 17-beta dehydrogenase 4(HSD17B4) genes within the rabbit testes. The gene of β-actin was employed as an internal endogenous control. All specific primer sequences utilized in this study are detailed in Table 1.

Oxidative and antioxidant status in testes

To assess antioxidant defense mechanisms and oxidative biomarkers in testicular tissue, the method explained by Shawky *et al*. (2014). After centrifuging the homogenate, the supernatant was put into sterile tubes and maintained at -80°C until biochemical examination (Magdy *et al*., 2016). Hormonal assays

At the end of the trial, rabbits were euthanized after a 12-hour fast, with blood collection occurring between 7:00 and 8:00 in the morning. Testosterone concentrations were determined according by Feldman *et al*. (2002). Quantitative analysis of serum LH and FSH was performed by (Pagana and Pagana, 2007).

Epididymal sperm preparation

Briefly, the caput, corpus and cauda epididymis were excised, minced and immersed in 1 mL of PBS to facilitate the release of spermatozoa into the medium, as previously described by Talebi *et al*. (2007).

Testicular histology and Morphometric parameters

Testicular tissues were dehydrated in graded ethanol (70% to 100%), cleared with xylene, and embedded in paraffin. Histological evaluation was performed using haematoxylin and eosin (H&E) staining.

Quantitative morphometric analyses of seminiferous tubules, including relative area, tubule and lumen diameters, and differential cell counts, were performed on hematoxylin and eosin-stained histological sections, following the protocol used by Shabana *et al*. (2024).

Statistical Analysis

Data were analyzed using the General Linear Model (GLM) procedure in SPSS Statistics (version 20 for Windows, SPSS Inc., Chicago, IL). The statistical model included the treatment group as a fixed effect. For parameters measured over time, specifically weekly body weight (BW) and feed intake (FI), a repeated-measures ANOVA was employed to evaluate the main effects of treatment, time, and their interaction. Prior to analysis, the assumptions of normality and homoscedasticity (variance homogeneity) were verified using the Shapiro-Wilk test and Levene's test, respectively. Outliers were assessed for data entry errors, and no missing data were found. Differences among groups were determined using one-way ANOVA followed by Dunnett's t-test for pairwise comparisons. Mortality rate statistics were evaluated using the Chi-square test. All values are presented as least-squares means ± standard error (SE), and statistical significance was set at $P < 0.05$.

Results

Thermal stress indices

The environmental parameters monitored during The duration of the experiment are summarized in Figure 1. To evaluate the level of thermal stress imposed on the subjects, AT, RH, and THI were recorded for both the normothermic and heat-stressed groups. AT for the heat-stressed group was significantly higher (33.63 °C, $P < 0.001$) compared to the normothermic group (23.97 °C), representing a substantial thermal challenge. Interestingly, the RH remained nearly identical ($P > 0.05$) between the two environments, with values of 54.94% and 55.28%, respectively, ensuring that the primary variable of stress was temperature-driven rather than humidity-driven.

The THI, which is considered a comprehensive indicator of thermal comfort, reflected the disparity in environmental conditions. The normothermic group was maintained at a THI of 22.63, whereas the heat-stressed group reached a THI of 31.13. This marked increase in THI confirms that the experimental model successfully induced a state of HS in the treatment groups, as a THI exceeding 28-30 is typically associated with moderate to severe heat strain in biological models.

Growth performance and body weight gain

The effects of HS and various supplements (MOE, SB, and VC) on growth performance are illustrated in Figure 2. Exposure to chronic HS resulted in a severe decline in growth performance. The PC group exhibited a significant reduction ($P < 0.001$) in both growth rate and body gain (kg) when compared to the negative control (NC) group. However, the administration of therapeutic supplements significantly mitigated these adverse effects. MOE group showed the most robust recovery in growth metrics, with growth rate and body gain significantly higher ($P < 0.001$) than the PC group. SB and VC treatments successfully improved growth performance compared to the untreated heat-stressed rabbits ($P < 0.01$), although values stayed below those of the thermoneutral NC group.

Mortality rates

Mortality data (Figure 3) reflected growth performance trends, highlighting the lethal effect of a high THI on rabbit survivability. The PC group suffered a significantly higher mortality rate (approximately 50%) compared to the NC group (approximately 10%; $P < 0.05$). All three treatments effectively reduced the mortality rate compared to the PC group ($P < 0.05$). Specifically, MOE and VC groups demonstrated the lowest mortality among the heat-stressed group (approx. 20%), while the SB group showed a slightly higher but still improved rate (approx. 30%).

Testicular mRNA expression of steroidogenic enzymes

The relative mRNA expression levels of key enzymes involved in testicular function (CYP17A1, HSD3B2, and HSD17B4) were analyzed to evaluate the impact of thermal stress and the efficacy of various treatments (Figure 4). Exposure to high environmental temperatures caused a significant disruption in the steroidogenic pathway. In the heat-stressed control group, there was a marked ($P < 0.001$) downregulation of CYP17A1 and HSD3B2 expression from 1.272 and 1.118 in NC group to 0.825 and 0.624 in PC group, respectively. Conversely, HSD17B4 expression was significantly ($P < 0.001$) upregulated in the PC group in (1.111) compared with the negative control (0.890), suggesting a compensatory or stress-induced shift in enzyme activity. Administration of *MOE*, SB, and VC significantly ($P < 0.001$ or $P < 0.01$) mitigated the effects of HS, steering expression levels back toward normothermic values. MOE demonstrated the most potent restorative effect, achieving the highest recovery levels for CYP17A1 (1.194) and HSD3B2 (0.965) among all treated groups. In the VC group, VC significantly recovered CYP17A1 expression (1.105) and downregulated the stress-induced HSD17B4 (0.950), showing an ameliorative profile second only to the MOE group. While SB was significantly better than the PC group, SB showed a more moderate recovery in expression levels compared to the other two supplements.

Testicular oxidative biomarker and antioxidant incidence

Oxidative stress biomarkers confirmed the damaging effect of HS (Table 2). PC rabbits markedly ($P < 0.001$) increased oxidative biomarkers, with MDA rising six-fold (13.683 vs. 2.100 nmol/g tissue in NC) and NO more than doubling (53.012 vs. 21.659 nmol/g tissue in NC), indicating lipid peroxidation and nitrosative stress. Concurrently, antioxidant defenses, represented by SOD and CAT were significantly ($P < 0.001$) reduced in PC (1.021 and 62.782, respectively) compared to NC (5.951 and 161.656, respectively). Supplemented groups displayed lower MDA and NO and significantly ($P < 0.001$) higher SOD and CAT activities compared to heat-stressed rabbits, with VC showed the most pronounced antioxidant protection. Indicating restored antioxidant defense.

Sexual hormone levels

HS significantly disrupted reproductive endocrine function as presented in Table 3. Serum testosterone, FSH, and LH levels were significantly ($P < 0.001$) reduced in the PC group contrasted to NC, consistent with suppressed reproductive function. Serum testosterone levels dropped from 0.538 ng/mL in the NC to 0.271 ng/mL in the PC, while FSH and LH levels were also reduced, indicating impaired hypothalamic-pituitary-gonadal (HPG axis) regulation. Treatment with MOE extract, SB, and VC significantly improved hormone levels, with VC showing the most notable recovery in testosterone and LH levels, while MOE also demonstrated substantial improvement, which restored hormonal levels closer to those of the negative control group and helped restore normal endocrine activity.

Reproductive traits and testicular development

Sexual activity, testicular development, and gonadal sperm reserves (GSR) were severely impaired by HS (Table 4). PC rabbits exhibited delayed sexual maturity throughout reached maturity later (164.33 days vs. 151.33 in NC), reduced BW at maturity, and prolonged reaction time and latency period. Testicular weights and paired testes index were also diminished in PC. GSR were also significantly diminished, with both right and left testicular sperm counts reduced compared to NC, as reflected by reduced testes weight and paired testes index. Supplementation with MOE, SB, and VC significantly improved these reproductive traits. Among treatments, MOE and VC groups showed near-complete restoration of testicular weight and sperm reserves close to control values, while SB produced moderate improvement, indicating strong protection of reproductive function under heat stress-induced reproductive dysfunction.

Changes in epididymal sperm reserves (ESR)

As shown in Table 5, HS significantly ($P < 0.001$) decreased the weight of the right, left, and paired epididymis in all regions (caput, corpus, and cauda) in the heat-stressed control group compared to the negative control group. For instance, the right epididymis weight dropped from 0.640 g in NC to 0.460 g in PC, indicating severe reproductive tissue degeneration due to HS. However, treatment with extract MOE, SB, and VC improved epididymal weights, with VC showing the most pronounced improvement, with the right epididymis weight reaching 0.590 g, approaching the normal level.

Similarly, total and segmental epididymal sperm reserves (caput, corpus, cauda, and total epididymis) were significantly reduced in the PC group. The total ESR dropped from 15.446×10^6 in NC to 11.331×10^6 in PC. Similarly, ESR per gram of epididymis insignificant ($P > 0.05$) increased from 11.809×10^6/g in NC to 11.927×10^6/g in PC. Treatment with MOE, SB, and VC markedly restored ESR values. The VC group exhibited the highest recovery in sperm reserves per epididymis and per gram of epididymal tissue, with a total ESR of 14.813×10^6, indicating its potent role in protecting epididymal function under HS conditions.

Table 1. Primers used in quantitative real time PCR

Name of primers	Primer sequences (5, to 3,)	Product length, (bp)
β-actin	F: 5'- GAAATCGTGCGTGACATTAAG -3' R: 5′- CTAGAAGCATTTGCGGTGGAC -3'	154
HSD3B2	F: 5′- TCCTCACATGAGCTACGCAGAC-3′ R: 5′- CAGGTGGCGGTTGGTTGA-3′	178
HSD17B4	F: 5′- TTTGCCATGAGACCTGTA-3′ R: 5′- GTCTATCTCAGTGTTCCCTC-3′	247

F, forward; R, reverse; HSD3B2, 3 β –hydroxysteroid dehydrogenase type 2; CYP17A1, Cytochrome P450 17A1; HSD17B4, 17 β – hydroxysteroid dehydrogenase type 4.

Table 2. Effect of Moringa extract, sodium bicarbonate and vitamin C on testicular oxidative biomarker and antioxidant incidence of heat-stressed V-line rabbits.

Items	**Experimental groups**					**±SEM**
	NC	**PC**	**MOE**	**SB**	**VC**	
MDA (nmol/g tissue)	2.100	13.683$^{\#\#\#}$	4.334***	5.470***	3.633***	0.219
NO (mmol/g tissue)	21.659	53.012$^{\#\#\#}$	35.487***	37.271***	30.882***	1.723
CAT (U/g tissue)	5.951	1.021$^{\#\#\#}$	4.222***	3.028***	4.872***	0.066
SOD (U/g tissue)	161.656	62.782$^{\#\#\#}$	124.876***	105.503***	125.958***	1.917

Degree of statistical difference between heat stress and control groups. *Degree of statistical difference between treated and heat stress groups. **NC**=negative control group; **PC**= heat-stressed control; **MOE**= heat-stressed group treated with moringa extract; **SB**= heat-stressed group treated with $NaHCO_3$; **VC**= heat-stressed group treated with vitamin C.

Table 3. Effect of Moringa extract, sodium bicarbonate and vitamin C on sexual hormones level of heat-stressed V-line rabbits.

Items	**Experimental groups**					**±SEM**
	NC	**PC**	**MOE**	**SB**	**VC**	
Testosterone (ng/ml)	0.538	0.271$^{\#\#\#}$	0.420***	0.329***	0.442***	0.007
FSH (mIU/ml)	0.519	0.280$^{\#\#\#}$	0.406***	0.333***	0.413***	0.003
LH (mIU/ml)	5.543	2.089$^{\#\#\#}$	4.021***	3.721***	4.524***	0101

Degree of statistical difference between heat stress and control groups. *Degree of statistical difference between treated and heat stress groups. **NC**=negative control group; **PC**= heat-stressed control; **MOE**= heat-stressed group treated with moringa extract; **SB**= heat-stressed group treated with $NaHCO_3$; **VC**= heat-stressed group treated with vitamin C; **FSH**= Follicle stimulating hormone; **LH**= Luteinizing hormone

Table 4. Effect of Moringa extract, sodium bicarbonate and vitamin C on sexual activity at maturity, testicular weights, and gonadal sperm reserves of heat-stressed V-line rabbits.

Items	**Experimental groups**					**±SEM**
	NC	**PC**	**MOE**	**SB**	**VC**	
Age at maturity (day)	151.333	164.333$^{\#\#\#}$	150.668***	163.333^{ns}	155.668*	1.350
BW at maturity (Kg)	3.168	2.483$^{\#\#\#}$	3.003***	3.008***	3.028***	0.019
Reaction time (second)	37.668	94.333$^{\#\#\#}$	38.668***	68.333***	36.000***	2.494
Latency period (second)	48.333	117.000$^{\#\#\#}$	68.333***	76.333***	62.000***	2.429
Slaughter weight (kg)	3.300	2.668$^{\#\#\#}$	3.094***	3.058***	3.074***	0.048
Testicular weights (g)						
Right testis	1.193	0.855$^{\#\#\#}$	1.030***	0.910^{ns}	1.153***	0.014
Left testis	1.190	0.808$^{\#\#\#}$	1.113***	0.958^{ns}	1.153***	0.008
Paired testes	2.383	1.663$^{\#\#\#}$	2.143***	1.868^{ns}	2.306***	0.019
Testes mass index [1]	0.722	0.623$^{\#\#\#}$	0.693***	0.611^{ns}	0.750***	0.006
GSR (×10^6)						
Right testis per g testis	1.940	1.038$^{\#\#\#}$	1.540***	1.243^{ns}	1.720***	0.037
per testis	2.314	0.887$^{\#\#\#}$	1.586***	1.131^{ns}	1.983***	0.045
Left testis per g testis	0.903	0.488$^{\#\#\#}$	0.678***	0.540^{ns}	0.828***	0.017
per testis	1.075	0.394$^{\#\#\#}$	0.755***	0.517^{ns}	0.955***	0.020
Paired testes per g testis	2.843	1.526$^{\#\#\#}$	2.218***	1.783^{ns}	2.548***	0.043
per testis	6.580	1.354$^{\#\#\#}$	3.518***	2.017*	5.053***	0.107

Degree of statistical difference between heat stress and control groups. *Degree of statistical difference between treated and heat stress groups, ns=not significant between them. [1] Testes mass index= testes weight (g) / slaughter weight (kg); **GSR**= Gonadal sperm reserves. **NC**=negative control group; **PC**= heat-stressed control; **MOE**= heat-stressed group treated with moringa extract; **SB**= heat-stressed group treated with $NaHCO_3$; **VC**= heat-stressed group treated with vitamin C.

Table 5. Effect of Moringa extract, sodium bicarbonate and vitamin C on epididymal weights and epididymal sperm reserves of heat-stressed V-line rabbits.

Items	Experimental groups					±SEM
	NC	PC	MOE	SB	VC	
Epididymal wt. (g)						
Right Epididymis	0.640	$0.460^{###}$	0.530^{*}	0.478^{ns}	0.590^{***}	0.010
Left Epididymis	0.668	$0.490^{###}$	0.578^{*}	0.498^{ns}	0.600^{***}	0.010
Paired Epididymis	1.308	$0.950^{###}$	1.108^{*}	0.976^{*}	1.190^{***}	0.018
ESR ($\times 10^6$)						
Total Caput	3.950	$2.970^{###}$	3.560^{**}	3.340	3.770^{***}	0.029
Total Corpus	4.478	$3.423^{###}$	4.083^{**}	3.680^{**}	4.260^{***}	0.089
Total Cauda	7.018	$4.938^{###}$	6.333^{**}	6.030^{*}	6.783^{***}	0.128
Right Epididymis	7.368	$5.313^{###}$	6.623^{**}	5.968^{*}	7.058^{***}	0.101
Left Epididymis	8.078	$6.018^{###}$	7.353^{**}	7.082^{*}	7.755^{***}	0.105
ESR	15.446	$11.331^{###}$	13.976^{**}	13.050^{*}	14.813^{***}	0.206
ESR / g Epididymis	11.809	11.927^{NS}	12.614^{*}	13.371^{*}	12.448^{*}	0.148

Degree of statistical difference between heat stress and control groups, NS=Not significant between them. Degree of statistical difference between treated and heat stress groups, ns=not significant between them.**NC**=negative control group; **PC**= heat-stressed control; **MOE**= heat-stressed group treated with moringa extract; **SB**= heat-stressed group treated with $NaHCO_3$; **VC**= heat-stressed group treated with vitamin C; **ESR**= epididymal sperm reserves

Table 6. Effect of Moringa extract, sodium bicarbonate and vitamin C on daily sperm production and testicular sperm reserves of heat-stressed V-line rabbits.

Items	Experimental groups					±SEM
	NC	PC	MOE	SB	VC	
DSP (per testis $\times 10^6$) [1]	1.918	$0.395^{###}$	1.026^{***}	0.588^{ns}	1.473^{***}	0.031
SPE (DSP / g testis $\times 10^6$) [2]	0.805	$0.237^{###}$	0.479^{***}	0.315^{*}	0.639^{***}	0.013

Degree of statistical difference between heat stress and control groups.*Degree of statistical difference between treated and heat stress groups, ns=not significant between them.**NC**=negative control group; **PC**= heat-stressed control; **MOE**= heat-stressed group treated with moringa extract; **SB**= heat-stressed group treated with $NaHCO_3$; **VC**= heat-stressed group treated with vitamin C.[1] **DSP** = Daily Sperm Production (Testicular sperm count / Time divisor (3.43).[2] **SPE** = Sperm Production Efficiency

Table 7. Effect of Moringa extract, sodium bicarbonate and vitamin C on testicle histomorphometric changes of heat-stressed V-line rabbits.

Items	Experimental groups					±SEM
	NC	PC	MOE	SB	VC	
Area of tubules (μm^2)	28664.93	$13299.29^{###}$	25691.11^{***}	22195.83^{***}	24840.20^{***}	968.26
Area % of seminiferous tubules	12.12	$5.32^{###}$	9.57^{**}	9.05^{**}	10.03^{**}	0.61
Tubular diameter (µm)	183.41	$96.44^{###}$	156.05^{***}	142.89^{***}	134.45^{***}	3.59
Luminal diameter (µm)	30.74	$84.59^{###}$	52.81^{***}	66.14^{***}	61.15^{***}	3.16
Leydig cells count	30.80	$5.80^{###}$	22.40^{***}	4.80^{ns}	17.60^{**}	1.87
Sertoli cells count	76.40	$35.20^{###}$	74.40^{***}	32.60^{ns}	78.60^{***}	3.70
Spermatogonia count	97.00	$45.00^{###}$	91.20^{***}	43.40^{ns}	87.60^{***}	4.36
Spermatid count	70.40	$46.20^{###}$	73.20^{**}	41.00^{ns}	66.20^{*}	3.52
Spermatocyte count	97.40	$50.20^{###}$	94.40^{***}	$41.2\ 0^{ns}$	90.40^{***}	3.99

Degree of statistical difference between heat stress and control groups.*Degree of statistical difference between treated and heat stress groups. **NC**=negative control group; **PC**= heat-stressed control; **MOE**= heat-stressed group treated with moringa extract; **SB**= heat-stressed group treated with $NaHCO_3$; **VC**= heat-stressed group treated with vitamin C.

Changes in daily sperm production and efficiency

According to Table 6, HS severely impaired spermatogenic activity. Daily sperm production (DSP) per gram of testis and per testis was significantly ($P < 0.001$) impaired by more than half in heat-stressed rabbits. DSP per testis significantly dropped from 1.918×10^6 in the NC group to 0.395×10^6 in the PC group ($P < 0.001$), reflecting compromised testicular function. Among the treatment groups, the VC group recorded the highest improvement, followed by MOE and SB, indicating their protective roles against heat stress-induced testicular damage. The VC group recorded the highest DSP value (1.473×10^6), followed by MOE (1.026×10^6), suggesting partial restoration of testicular function. Similarly, sperm production efficiency (SPE) per gram of testis was significantly decreased in the PC group (0.237×10^6) compared to NC (0.805×10^6), while treatments, especially VC, significantly improved this parameter (0.639×10^6), nearly doubling the efficiency observed in the PC group.

Histopathological and histomorphometric analysis of the testicular tissues

The histopathological and histomorphometric analysis of the testicular tissues reveals the severe impact of HS on reproductive architecture and the varying degrees of efficacy among the therapeutic interventions. The following results characterize the transition from normal physiology to pathological degeneration and subsequent recovery.

Integrating the quantitative data with the histological evidence is shown in Table 7 and Graphs 1, 2, 3, 4, and 5. HS induces testicular degeneration and varying degrees of recovery offered by therapeutic interventions. Exposure to HS resulted in a profound collapse of the testicular architecture, evidenced by a nearly 54% reduction in tubular area and a sharp decline in all cellular populations, most notably Leydig cells, which dropped from 30.80 to 5.80. This architectural damage is further highlighted by the significant expansion of the luminal diameter, suggesting a depletion of the germinal epithelial layers. The photomicrographs verify these metrics, showing irregular tubular shapes, widespread vacuolar degeneration, and the sloughing of spermatogenic cells into a widened, empty lumen. Moreover, exposure to HS induced significant degenerative changes in the testicular architecture compared to the negative control. There was tubular atrophy, including a marked reduction in the area of tubules (13299.29 vs. 28664.93 μm^2) and tubular diameter (96.44 vs. 183.41 μm). Interestingly, the luminal diameter increased significantly (84.59 vs. 30.74 μm), likely indicating a depletion of the germinal epithelium, leading to a wider, emptier lumen. Also, data showed that cellular depletion, including all cellular counts such as Leydig, Sertoli, and germ cells (spermatogonia, spermatids, and spermatocytes), showed a sharp decline ($P < 0.001$), confirming that HS severely impairs spermatogenesis and endocrine function. While sodium bicarbonate (SB) offered some structural restoration of tubular dimensions, it failed to significantly protect the cellular counts, indicating that systemic pH buffering alone is insufficient to safeguard the spermatogenic niche against thermal insult.

In contrast, MR extract and VC demonstrated potent cytoprotective capabilities, with MR emerging as the superior intervention and providing the most significant morphological and functional rescue. The MOE group showed a remarkable recovery in Sertoli and spermatogonia counts, effectively returning them to levels statistically comparable to the negative control. This suggests that Moringa's bioactive compounds provide superior protection to the blood-testis barrier and the foundational germ cell stock. VC also showed significant efficacy, particularly in preserving the spermatocyte and spermatid populations, though it was slightly less effective than MR in restoring overall tubular area. Furthermore, the three treatments showed varying degrees of success in rescuing the testicular tissue from heat-induced damage. MOE demonstrated the most robust protective effect across almost all parameters. It restored the area of tubules to 25691.11 μm^2, nearly reaching the control levels. Most notably, it maintained Sertoli cell counts (74.40) and spermatogonia counts (91.20) at levels statistically similar to the non-stressed control, which is histologically supported by the appearance of intact spermatogenic layers and orderly cell maturation. VC followed closely behind MR. It was particularly effective in preserving spermatocyte and spermatid counts. SB improved the physical dimensions of the tubules (area and diameter) and a relatively preserved architectural outline in the micrographs compared to the heat-stressed control, but it failed to significantly improve cellular counts. The counts for Leydig, Sertoli, and germ cells remained statistically non-significant compared to the PC group.

Discussion

The present study demonstrated that HS in V-line male rabbits had broad deleterious effects on growth, gene expression, oxidative stress biomarkers, reproductive hormones, and testicular function, and that MOE, SB, and VC significantly mitigated many of these adverse effects.

Effect of heat stress

Under thermal stress, animal genetics and environmental factors affect the intensity and duration of physiological and behavioral responses, such as the immunological, cardiorespiratory, endocrine, and behavioral systems (Al-Dawood, 2017). Zhai *et al*. (2026) reported that rabbits with chronic HS led to disruption of reproductive tissue, endocrine disruption, and inflammation.

The current results illustrated that environmental monitoring confirmed that rabbits in the heat-stressed group were exposed to significantly higher AT and a higher THI. Rabbits have body temperatures (BT) between 38.5 and 39.5°C, with individual differences between 0.5 and 1.2°C. Rabbits' ideal temperature range is between 15 and 25°C, while their ideal humidity range is between 55 and 65%. HS occurs when AT is greater than 30°C and when BT exceeds 35°C, which causes heat failure (Nielsen *et al.,* 2020).

In the current results, growth performance was negatively influenced by HS. Farghly *et al.* (2020) revealed that rabbits were exposed to HS, which led to reduced growth performance measures (BW and BWG).

In the present study, heat-stressed rabbits showed a pronounced suppression of CYP17A1 and HSD3B2 mRNA expression, contrasted by a significant upregulation of HSD17B4. The reduction in CYP17A1, a key enzyme in glucocorticoid synthesis, likely hinders the production of stress-related steroids. Conversely, the upregulation of HSD17B4, which is essential for the conversion of pregnenolone to testosterone, indicates a potential bottleneck in the synthesis of downstream metabolic and reproductive hormones, potentially contributing to reduced performance observed in heat-stressed rabbits (Ko, 2024). HS negatively impacts testicular function, potentially impairing reproductive

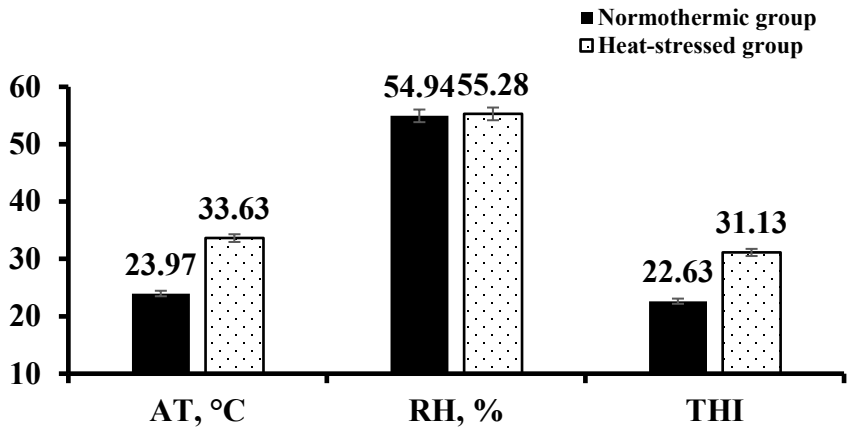


Figure 1. Mean of ambient temperature (AT, °C), relative humidity (RH, %) and temperature-humidity index (THI) during experimental period.THI = *AT* (°C) - {(0.31 - 0.31 *RH*) × [*AT* (°C) - 14.4]}

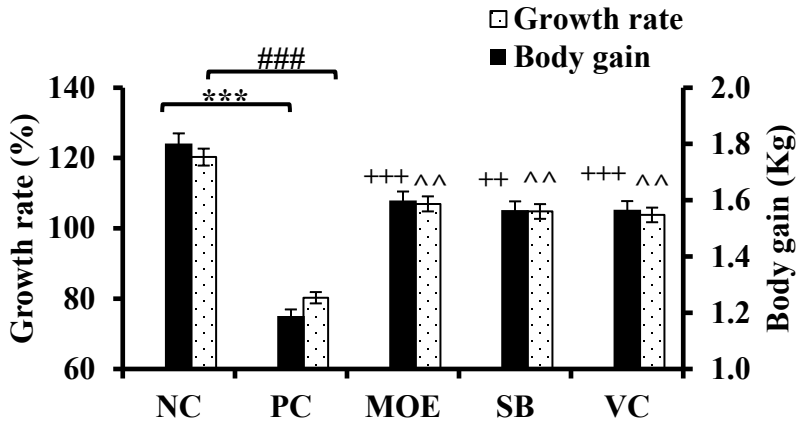


Figure 2. Effect of Moringa extract, sodium bicarbonate, and vitamin C on growth performance of heat-stressed V-line rabbits. Symbols # and * indicate significant differences between the negative control (NC) and heat-stressed control (PC) groups, while + and ^ denote significant differences between treated groups and the PC group. Group abbreviations: NC = negative control (non-heat-stressed); PC = heat-stressed control; MOE = heat-stressed group treated with Moringa extract; SB = heat-stressed group treated with sodium bicarbonate; VC = heat-stressed group treated with vitamin C.

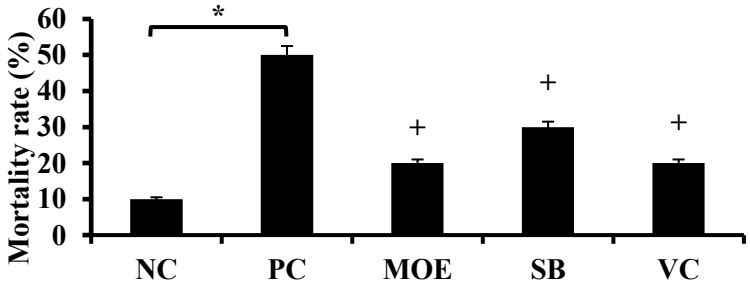
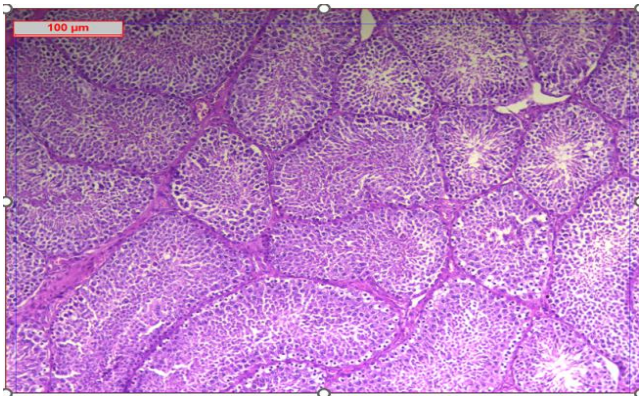
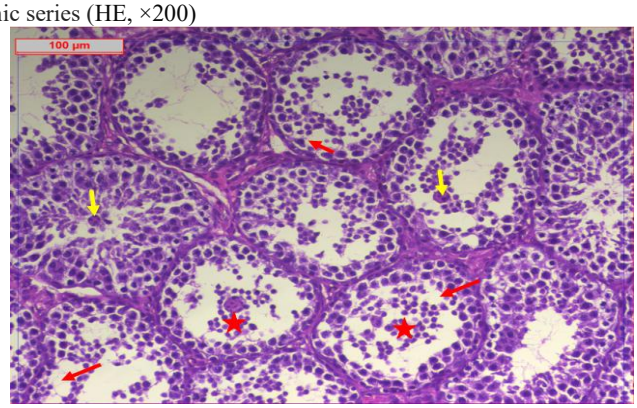


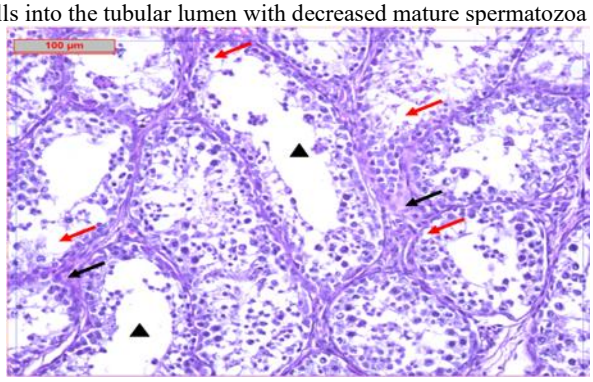
Figure 3. Effect of Moringa extract, sodium bicarbonate, and vitamin C on mortality rate of heat-stressed V-line rabbits. Symbols * indicate significant differences between the negative control (NC) and heat-stressed control (PC) groups, while + denote significant differences between treated groups and the PC group. Group abbreviations: NC = negative control (non-heat-stressed); PC = heat-stressed control; MOE = heat-stressed group treated with Moringa extract; SB = heat-stressed group treated with sodium bicarbonate; VC = heat-stressed group treated with vitamin C.



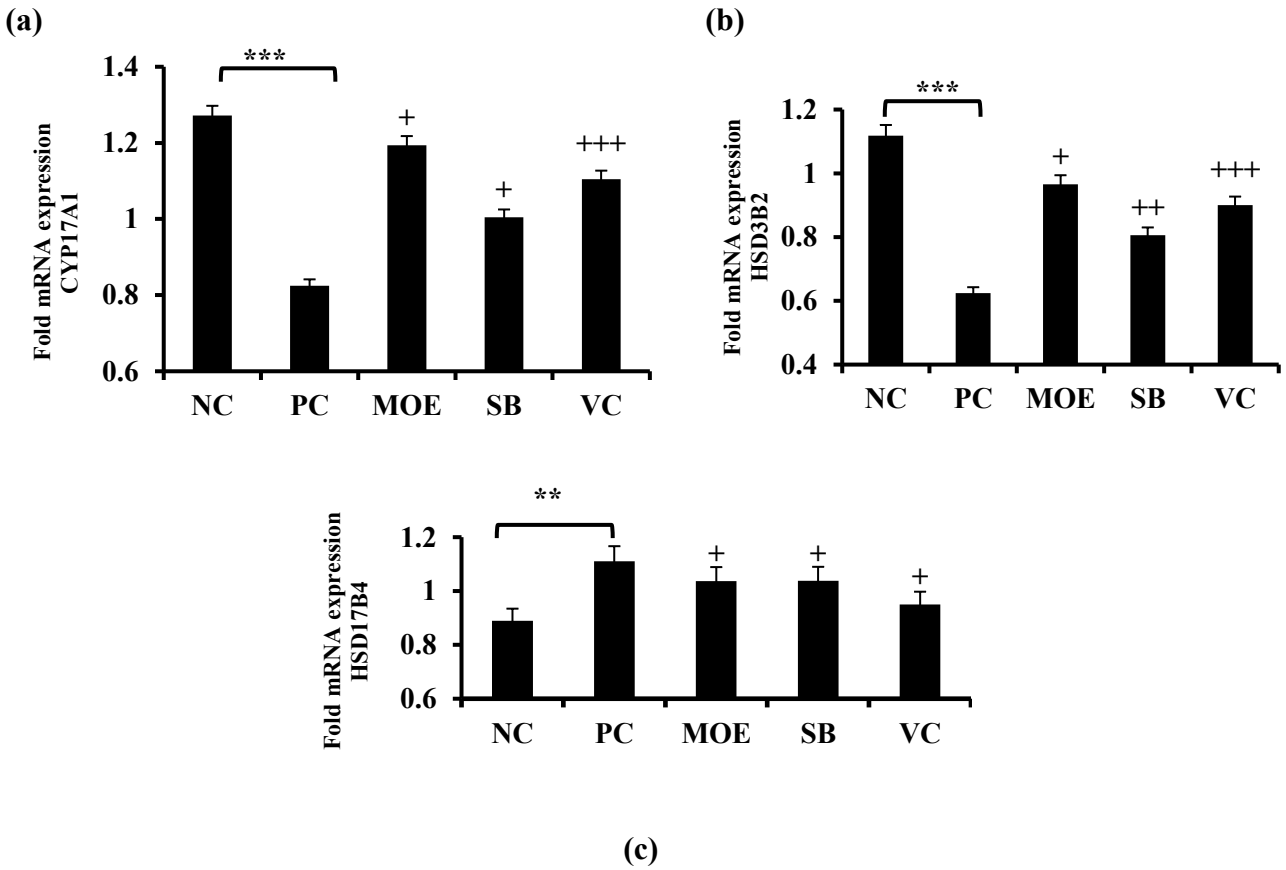
Graph 1. A photomicrograph of normal control testicular tissue revealed closely packed seminiferous tubules with normal histological structure, regular basement membrane, normal thickness of germinal epithelium, narrow lumen filled with spermatozoa associated and complete spermatogenic series (HE, ×200)



Graph 2a. A photomicrograph of heat-exposed testicular tissue revealed degenerative changes in most of the seminiferous tubules characterized by disorganization of the germinal epithelium, vacuolar degeneration (red arrow), germinal cell atrophy (yellow arrow) and sloughing of spermatogenic cells into the tubular lumen with decreased mature spermatozoa (star) (HE, ×200)

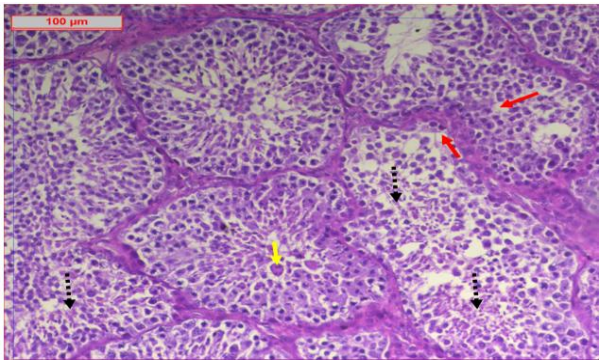


Graph 2b. A photomicrograph of heat-exposed testicular tissue revealed marked degenerative changes in seminiferous tubules. The tubules appear irregular in shape with thinning and disorganization of the germinal epithelium, accompanied by reduction in spermatogenic cell layers. There is prominent vacuolar cell degeneration (red arrow), and wide lumen with absence of spermatozoa (arrowhead), decrease Leydig cells (black arrow) (HE, ×200)

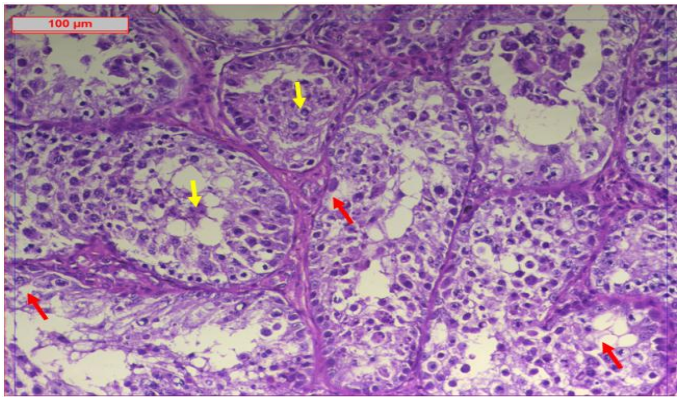


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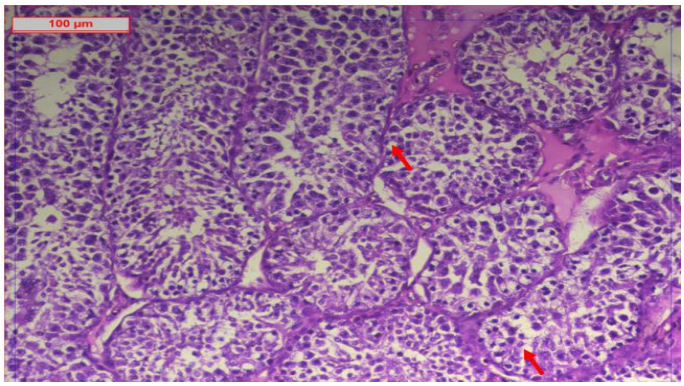
Figure 4. Effect of Moringa extract, sodium bicarbonate, and vitamin C on relative mRNA expression levels in testicular tissues of heat-stressed V-line rabbits, including (a) CYP17A1, (b) HSD3B2, and (c) HSD17B4. Symbol * indicates significant differences between the negative control (NC) and heat-stressed control (PC) groups, while + denotes significant differences between treated groups and the PC group. Group abbreviations: NC = negative control (non-heat-stressed); PC = heat-stressed control; MOE = heat-stressed group treated with Moringa extract; SB = heat-stressed group treated with sodium bicarbonate; VC = heat-stressed group treated with vitamin C.



Graph 3. A photomicrograph of the Moringa-treated group revealed a nearly normal appearance of most of the seminiferous tubules with germinal cell atrophy (yellow arrow), others are characterized by vacuolar cell degeneration (red arrow), and desquamated cells (dashed arrow). Intact spermatogenic cell layers with orderly maturation from spermatogonia to more differentiated forms toward the lumen. (HE, ×200).



Graph 4. A photomicrograph of Na bicarbonate-treated group revealed marked degenerative changes of seminiferous tubules, disorganization of germinal epithelium, prominent vacuolar degeneration (red arrow) and germinal cell atrophy (yellow arrow) (HE, ×200)



Graph 5. A photomicrograph of Vitamin C-treated group revealed relatively preserved architecture of the seminiferous tubules with nearly normal tubular outlines and maintained germinal epithelium. The spermatogenic cell layers are largely intact, demonstrating orderly maturation from spermatogonia to more differentiated forms toward the lumen. There are mild degenerative changes in the form of scattered cytoplasmic vacuolization (red arrow) (HE, ×200).

performance through both direct cellular stress and inhibition of hormone production (Oka *et al*., 2017; Shahat *et al*., 2020). CYP17A1 was essential for regulating testosterone biosynthesis (Xiao *et al.,* 2021). This agrees with the current study, which showed that testicular gene expression (CYP17A1) was significantly suppressed and serum testosterone level was significantly reduced. The formation of estradiol depends on the alteration of intermediates into androgens and estrogens, respectively, by CYP17A1 and CYP19A1 (aromatase). Ding *et al*. (2025) reported that the testes' mRNA expression profiles detected functional genes and pathways that regulate reproductive responses to HS. Congenital adrenal hyperplasia (CAH) due to HSD3B2 deficiency is important for the synthesis of gonadal androgens. Moreover, HS led to oxidative stress in the testicles, which enhanced ROS and lipid peroxidation production while reducing antioxidant defenses (Monageng *et al*., 2023). HS-induced oxidative stress may impair Leydig cells and reduce testosterone production. Especially, HS diminished the expression of key hormones that are crucial for the production of testosterone, like 3β-HSD, CYP11A1, HSD3B1, and CYP17A1 (Cai *et al*., 2021).

Saghir *et al.* (2023) stated that exposure to HS enhanced serum oxidative markers like protein carbonyl (an indicator of amino acid oxidation) and MDA (an indicator of lipid peroxidation) in growing rabbits while lowering the activity of antioxidative enzymes like GSH-Px, SOD, and CAT. Madkour *et al.* (2023) detected that HS at 36 °C enhanced the concentration of MDA in the blood plasma and muscle of broiler rabbits while diminishing the levels of SOD, GSH, and CAT. Jaswal *et al.* (2025) confirmed that heat exposure elevated NO production as part of an inflammatory oxidative response.

Then, HS also converted the levels of serum metabolites MDA that led to organ injury in rabbits (Garner *et al.,* 2020).

On the other hand, serum concentrations of testosterone, FSH, and LH were significantly reduced in the PC group compared to NC group, showing reductions of 49.63%, 46.05%, and 62.31%, respectively, indicating a pronounced suppression of reproductive endocrine function under HS conditionsCortisol is produced in reaction to stress, and raised

cortisol levels may lower testosterone levels (Khan *et al*., 2023). HS significantly lowers progesterone, LH, and FSH levels, according to prior research (Sirotkin *et al*., 2021). By adversely affecting the hypothalamic-pituitary-gonadal (HPG) axis, HS can diminish reproductive function by lowering the production of these vital reproductive hormones in rabbits. (Abdelnour *et al*., 2022). Also, due to the production of reactive oxygen free radicals, HS during the summer stimulated the elevation of lipid peroxidation, which injured the testes (Ahmad *et al*., 2022).

In the current study, exposure to HS significantly hindered male reproductive development and performance. It delayed puberty (164 vs. 151 days) in the control group and impaired sexual behavior, evidenced by prolonged reaction time and latency period. Furthermore, HS adversely affected physiological markers, resulting in lower testicular weight, a reduced testes mass index, and diminished GSR. HS profoundly compromised both the storage capacity and the generative potential of the reproductive tract. Male rabbits' epididymis is injured by HS, which decreases fructose and α-Glu in the seminal plasma.

Ding *et al*. (2025) reported that severe HS reduced sperm density and semen volume in the summer more than in the spring and fall. Additionally, a testicular morphological study demonstrated that throughout the summer, the seminiferous tubules manifested atrophy and that the distance between them and a few germ cells increased. One hypothesis for summer azoospermia could be that HS negatively impacted spermatogenesis by restricting spermatocyte proliferation (Mohanty *et al*., 2019). Serag El Din (2025) found that all species showed decrease in seminiferous tubule size with no spermatozoa (azoospermia) over the winter and summer.

HS varies an animal's body weight and testicular activity, leding to bodily malfunction, and reduces testosterone levels, sperm quality, and quantity (Huang *et al*., 2023). El-Emam *et al*. (2023) stated that HS destroys sperm quality and testicular function, posing a serious risk to male reproductive health. Male rabbits are more vulnerable to high temperatures than female rabbits; high temperatures delay GnRH production and secretion, which has a major influence on testicular function and reduces the quality of male rabbit semen (Durairajanayagam *et al*., 2015; Daader *et al*., 2016). Due to the accumulation of free radicals in male rabbits' gonads, which destroy the antioxidant system, HS can have an influence on the quality of semen (Kuang *et al*., 2021). Cao *et al*. (2023) stated that HS primarily diminished sperm quality due to the alteration of retinol metabolism and disruption of the testicular microenvironment.

Ameloirate effect of treatments

Considering the multifaceted response to MR, VC, and SB treatments, the evidence suggests that MR extract contains high concentrations of bioactive compounds, such as quercetin and kaempferol, which act as potent antioxidants to neutralize these radicals and improve immune response in growing rabbits (El-Desoky *et al*., 2017). MR has flavonoids, phenolic compounds, and natural vitamins (Assaye *et al.*, 2025). During exposure to HS, the rabbit's internal stores of VC are rapidly depleted as the body attempts to manage elevated cortisol levels (Abu Hafsa *et al*., 2024).

The histomorphometric data and histopathological observations provide HS-induced testicular damage and the varying protective effects of the treatments. HS induced testicular atrophy, reducing seminiferous tubule area (13,299.29 μm²) and diameter (96.44 μm), and causing a dramatic increase in luminal diameter (from 30.74 to 84.59 μm), indicating germ cell loss and impaired spermatogenesis. HS disrupts cell adhesions within the seminiferous epithelium, leading to the premature release of immature germ cells into the lumen. The decline in Leydig and Sertoli cell counts indicates that HS targets both the endocrine support system and the structural niche required for sperm maturation. Heat-induced oxidative stress, which triggers apoptotic pathways in sensitive germ cell populations like spermatogonia and spermatocytes. Reduced Sertoli and Leydig cell counts impaired spermatogenesis and steroidogenesis. The presence of prominent vacuolar degeneration and germinal cell atrophy in the PC and SB groups suggests that oxidative stress-induced apoptosis is the primary driver of this cellular loss.

In the present study, supplementation with MOE, SB, or VC markedly restored these gene expression levels toward normal, improved growth performance, significantly lower levels of mortality and increased sexual hormone levels, with values approaching those of the normothermic control group. Supplemented groups showed reduced MDA and NO and significantly higher CAT and SOD activities compared with HS-rabbits, indicating restored antioxidant defense. MR extract and VC supplementation restored sexual maturity and testicular parameters close to control values, while SB produced moderate improvement. Treated groups had significantly higher epididymal weights and sperm reserves, with MR and VC showing the greatest recovery.

The essential chemical components in MR leaf extract were glycerin, diethyl phthalate, ascorbic acid, and α-linolenic acid (Hashem *et al*., 2019). Treatments with MR, VC, and SB diminish the subsequent reactions due to a fall in stress hormone levels. El-Ratel *et al*. (2025) showed that the addition of different natural components allicin or lycopene or vitamins (E or C), significantly ameliorated the final BW in growing rabbits exposed to HS in contrast to the HS group (El-Gindy *et al*., 2023; Bashar *et al*., 2024). Falemara *et al*. (2020) indicated that adding MR leaves to the experimental diets ameliorated the growth performance of rabbits. Additionally, rabbits receiving MR leaves exhibited superior growth metrics, overall weight gain and performance index (El-Adawy *et al*., 2020). VC administration can ameliorate rabbit growth performance and minimize HS (Anoh *et al*., 2020).

Consistent with these findings, Mohamed *et al*. (2019) stated that supplementation with MR significantly elevated serum testosterone concentrations by upregulating testicular steroidogenic enzymes. Furthermore, 3β-HSD is an essential enzyme in the formation of androgens and other active steroids.

MOE supplementation improved hormonal concentrations to control values. Alkafafy *et al*. (2021) stated that serum FSH, LH, testosterone, sperm count, and sperm motility were all raised by MR leaf extract. In rabbit bucks, MR markedly enhanced serum testosterone levels and LH and FSH gene expressions (Khalifa *et al*., 2016). El-Kashef (2022) indicated that adding more MR leaves to male rabbits' diets boosted the quality of their semen, and blood components, mainly serum testosterone, FSH and LH levels. Gouda *et al*. (2020) claimed that using MR resulted in a significant rise in testosterone hormone levels in the blood of buck's rabbits. These bioactive constituents alleviate testicular oxidative stress, reduce inflammatory damage, and enhance the expression of steroidogenic genes such as StAR, HSD3B, and CYP17A1 (Ragab *et al*., 2024).

Moreovere, Mthiyane *et al*. (2022) reported that MR significantly enhanced CAT, GSH, and SOD activities while reducing MDA concentrations. Collectively, these data suggest that the treatments successfully restored the antioxidant defense system and reduced heat stress-induced cellular damage. MR extract consists of antioxidants known as alkaloids, flavonoids and tannins that protect cells from oxidative damage and prevent the production of new free radicals (Widiastini *et al*., 2022). Alkafafy *et al*. (2021) reported that MR leaf extracts decreased the harmful consequences of oxidative stress by enhancing antioxidant enzymes such as SOD, CAT, and GSH and decreasing MDA concentration, a sign of lipid peroxidation. By acting on the HPG axis and lowering the anterior pituitary gland's release of LH and FSH, high levels of ROS may affect the hormonal balance that regulates male reproductive activities (Spiers *et al*., 2015). Mohlala *et al*. (2023) reported that MR can improve all the effects of ROS on the male reproductive system and elevate semen quality, ameliorating libido, erection, and ejaculatory function through its antioxidant, anti-inflammatory, and anti-apoptotic properties. Hassan *et al*. (2023) reported that VC enhanced FI and FCR. Improved serum concentrations of SOD and TAC.

Monageng *et al*. (2023) reported that Leydig cell function is essential for steroidogenesis and spermatogenesis. An imbalance of ROS results in Leydig cell dysfunction and male infertility. MR may protect Leydig cells through its antioxidant properties. Furthermore, after MR therapy, male rats demonstrated a significant elevation in the width of seminiferous tubule, height and epithelial area, type a spermatogonia and spermatogenic effectiveness, as well as a rise in Sertoli cells and total number of spermatogenic cells (Laoung-On *et al*., 2021).

Mohammed *et al*. (2023) reported that bucks fed *Moringa oleifera* leaf meal had significantly high testis weight and volume, right caput epididymis length, complete caudal epididymis sperm reserves, caput epididymis sperm reserves. Fathy *et al.* (2022) stated that administration of MR leaves and MR seeds to rams' diet can ameliorate the quality of their semen; this could be because they are abundant in minerals including iron and vitamins A, B complex, C, K, and E, which are essential for increasing semen quality and reaction time.

The therapeutic efficacy of MOE stands out as the most comprehensive rescue strategy, achieving over 95% recovery in spermatocyte and spermatid counts and restoring Sertoli cells to levels significantly comparable to the negative control (74.40 vs. 76.40). Histological observations of nearly normal tubular appearance in the MOE group suggest that its rich profile of flavonoids and phenolic compounds effectively neutralizes ROS, thereby stabilizing the germinal epithelium against thermal degradation. This suggests that Moringa's bioactive compounds effectively stabilize the blood-testis barrier and germ cell niche. VC followed closely behind MOE. It was particularly effective in preserving spermatocyte and spermatid counts. This indicates that as a classic antioxidant, VC mitigates the oxidative stress that typically triggers apoptosis in developing sperm cells during heat exposure. While SB improved the physical dimensions of the tubules (area and diameter) compared to the heat-stressed control, it failed to significantly improve cellular counts. This suggests that while SB may help with systemic acid-base balance under HS, it does not provide the specific cytoprotective or antioxidant support needed to maintain the seminiferous epithelium. Interestingly, while SB provided a relatively preserved architectural outline and improved tubular area, it failed to significantly restore germ cell counts. This suggests that while pH buffering can mitigate the systemic metabolic acidosis associated with heat stress to maintain physical tubular structure, it lacks the specific antioxidant mechanisms necessary to protect the DNA integrity of developing germ cells. Conversely, the poor performance of VC in maintaining epithelial organization suggests that at the administered dose, a single-source antioxidant may be insufficient compared to the multi-functional bioactive compounds found in MOE.

Abdelhamid *et al*. (2019) stated that water-soluble VC is essential for controlling the immunological response and protection of cells from oxidative damage. The treatments in the VC group significantly improved testosterone and LH levels. This effect is due to the antioxidant properties of VC, which effectively scavenge ROS, preserve mitochondrial integrity, and protect steroidogenic machinery within Leydig cells (Rochdi *et al*., 2024).

Yin *et al*. (2018) stated that using VC or VC-SB additives significantly raised exogenous antioxidants, lowering the requirement for endogenous antioxidants. Ascorbic acid was a crucial nutrient for preserving homeostasis. Also, Ewuola *et al*. (2022) found that giving heat-stressed bucks ascorbic acid, SB, or a mixture of ascorbic acid and SB boosted antioxidant status and prevented seminal and serum oxidative stress by depressing lipid peroxidation.

Tran *et al*. (2025) reported that rabbit spermatozoa can be effectively preserved using a medium containing a bicarbonate buffer system (HCO_3^-), which helped maintain a physiological pH between 7.2 and 7.4.

By maintaining redox balance, VC showed the greatest protective effect against heat stress, preserving epididymal integrity and improving daily sperm production (1.473×10^6) and sperm production efficiency (0.639×10^6). Although MR supplementation has a protective effect through its antioxidant and anti-inflammatory phytochemicals, and SB provided partial improvement via correction of acid–base imbalance, VC is an effective treatment in restoring ESR.

Conclusion

This study concluded that chronic heat stress (THI = 31.13) severely impairs growth and male reproductive performance in V-line rabbits, resulting in 50% mortality, profound testicular degeneration, endocrine failure, and marked downregulation of key steroidogenic genes (CYP17A1, HSD3B2, and HSD17B4). The supplementation with *Moringa oleifera* extract or Vitamin C significantly counteracted these deleterious effects. Both interventions effectively restored serum gonadotropin (FSH, LH) and testosterone levels, upregulated steroidogenic gene expression, enhanced endogenous antioxidant defenses (SOD, CAT), and reduced oxidative markers (MDA, NO). Furthermore, MOE and VC preserved seminiferous tubule architecture, germ cell populations, and daily sperm production, outperforming sodium bicarbonate in cytoprotection. Overall, antioxidant supplementation, particularly MOE represents a vital management strategy to safeguard male rabbit fertility and farm profitability in heat-stressed regions.

Declaration of interest The study's authors Verify that they have no Conflicts of interest

Ethics statement The Animal Ethics Committee of the Zoology Department in the College of Science at Beni-Suef University established criteria for the care and use of experimental animals, which were followed in all research methods. The Institutional Animal Care and Use Committee (IACUC) protocol Number (BSU/FS/024-090) for the protection of animals used for scientific purposes was met by the experimental activities of this study.

References

1) Abdelhamid, F., El-Gohary, F. and El-Maaty, H. A. (2019). Ameliorative effects of dietary vitamin C and E supplementation on growth performance, hemato-biochemical, antioxidant and non-specific immune parameters in heat-stressed rabbits. *Annals of Veterinary and Animal Science, 6*(6), 108–119. Doi.org/10.5455/ijlr.20200605.
2) Abdel-Latif, M., Sakran, T., Badawi, Y. K. and Abdel-Hady, D. S. (2018). Influence of *Moringa oleifera* extract, vitamin C, and sodium bicarbonate on heat stress-induced HSP70 expression and cellular immune response in rabbits. *Cell stress & chaperones*, *23*(5), 975–984. Doi.org/10.1007/s12192-018-0906-1.
3) Abdelnour, S. A., Al-Gabri, N. A., Hashem, N. M. and Gonzalez-Bulnes, A. (2021). Supplementation with Proline Improves Haemato-Biochemical and Reproductive Indicators in Male Rabbits Affected by Environmental Heat-Stress. *Animals: an open access journal from MDPI*, *11*(2), 373. Doi.org/10.3390/ani11020373.
4) Abdelnour, S. A., El-Ratel, I. T., Peris, S. I., El-Raghi, A. A. and Fouda, S. F. (2022). Effects of dietary thyme essential oil on blood haematobiochemical, redox status, immunological and reproductive variables of rabbit does exposed to high environmental temperature. *Italian Journal of Animal Science, 21*(1), 51–61. DOI: 10.1080/1828051X.2021.2006807.
5) Abu Hafsa, S. H., Centoducati, G., Hassan, A. A., Maggiolino, A., Elghandour, M. M. and Salem, A. Z. (2024). Effects of dietary supplementations of vitamin C, organic selenium, betaine, and pomegranate peel on alleviating the effect of heat stress on growing rabbits. *Animals*, 14(6), 950. DOI: 10.3390/ani14060950.
6) Ahmad, R., Yu, Y.-H., Hsiao, F. S.-H., Su, C.-H., Liu, H.-C., Tobin, I., Zhang, G. and Cheng, Y.-H. (2022). Influence of Heat Stress on Poultry Growth Performance, Intestinal Inflammation, and Immune Function and Potential Mitigation by Probiotics. *Animals*, *12*(17), 2297. Doi.org/10.3390/ani12172297.
7) Al-Dawood, A. (2017): Towards heat stress management in small ruminants-a review. *Annals of Animal Science*, *17*(1), 59-88. DOI: 10.1515/aoas-2016-0068.
8) Alkafafy, M. E., Sayed, S. M., El-Shehawi, A. M., El-Shazly, S., Farouk, S., Alotaibi, S. S., Madkour, D. A., Orabi, S. H., Elbaz, H. T. and Ahmed, M. M. (2021). *Moringa oleifera ethanolic* extract ameliorates the testicular dysfunction resulted from HFD-induced obesity rat model. *Andrologia*, *53*(8), e14126. Doi.org/10.1111/and.14126.
9) Al-Sagheer, A. A., Daader, A. H., Gabr, H. A. and Abd El-Moniem, E. A. (2017). Palliative effects of extra virgin olive oil, gallic acid, and lemongrass oil dietary supplementation on growth performance, digestibility, carcass traits, and antioxidant status of heat-stressed growing New Zealand White rabbits. *Environmental Science and Pollution Research*, *24*(7), 6807-6818. Doi.org/10.1007/s11356-017-8396-8
10) Anoh, K. U., Barje, P. P., Iyeghe-Erakpotobor, G. T. and Akpa, G. N. (2020). Growth performance of heat stressed rabbits fed diets supplemented with synthetic and organic antioxidants. *Nigerian Journal of Animal Production*, *44*(5), 177–180. Doi.org/10.51791/njap.v44i5.1349.
11) AOAC (Association of official analytical chemists), 2007. *Official methods of analysis* (18th Ed) Washington, D.C., U.S.A.
12) Assaye, M. A., De Leo, M., Volterrani, D., Tesfay, H., Teka, F., Debebe, E. and Gebre, S. G. (2025). Phytochemical profiling, antioxidant and anti-inflammatory potential of methanolic extracts of *Moringa oleifera* (L.) Lam. and Moringa stenopetala (Bak.) Cufod. leaves grown in Arba Minch, Ethiopia. *RSC advances*, *15*(51), 43818-43829. Doi.org/10.1039/d5ra05914c.
13) Bashar, A. M., Abdelnour, S. A., El-Darawany, A. A. and Sheiha, A. M. (2024). Dietary supplementation of microalgae and/or nanominerals mitigate the negative effects of heat stress in growing rabbits. *Biological Trace Element Research*, 202(8), 3639-3652. DOI: 10.1007/s12011-023-03953-0.

14) Bui-Le, T. N., Hoang-Tan, Q., Hoang-Viet, H., Truong-Thi, B. P. and Nguyen-Thanh, T. (2023). Protective effect of Curculigo orchioides Gaertn. Extract on heat stress-induced spermatogenesis complications in murine model. *Current Issues in Molecular Biology*, *45*(4), 3255-3267. DOI: 10.3390/cimb45040212.
15) Cai, H., Qin, D. and Peng, S.(2021). Responses and coping methods of different testicular cell types to heat stress: overview and perspectives. *Bioscience reports*, 41(6), BSR20210443. Doi.org/10.1042/BSR20210443.
16) Cao, K. X., Deng, Z. C., Liu, M., Huang, Y. X., Yang, J. C. and Sun, L. H. 2023. Heat Stress Impairs Male Reproductive System with Potential Disruption of Retinol Metabolism and Microbial Balance in the Testis of Mice. *The Journal of nutrition*, 153(12), 3373–3381. Doi.org/10.1016/j.tjnut.2023.10.017.
17) Daader, A. H., Yousef, M. K. and Abdel-Samee, A. M. (2016). Recent trends in rabbit does reproductive management: Special reference to hot regions. In *Proceedings of the 1st World Rabbit Congress* (Qingdao, China) (pp. 149–166).
18) Ding, H., Wang, Y., Zhao, H., Wang, J. and Huang, D. (2025). Negative effect of seasonal heat stress on testis morphology and transcriptomes in Angora rabbit. *BMC Genomics, 26*, 478. https://doi.org/10.1186/s12864-025-11659-x.
19) Durairajanayagam, D., Agarwal, A. and Ong, C. (2015). Causes, effects and molecular mechanisms of testicular heat stress. *Reproductive Biomedicine Online, 30*(1), 14–27. https://doi.org/10.1016/j.rbmo.2014.09.018.
20) Ebeid, T. A., Aljabeili, H. S., Al-Homidan, I. H., Volek, Z. and Barakat, H. (2023). Ramifications of heat stress on rabbit production and role of nutraceuticals in alleviating its negative impacts: an updated review. *Antioxidants (Basel, Switzerland)*, 12(7), 1407. DOI: 10.3390/antiox12071407.
21) El-Adawy, M. M., El-Komy, A. M., Rashad, A. M., Fahmy, W. G. and Abd El-Aziz, N. A. (2020). The influence of dried *Moringa oleifera* leaves in feeding of growing rabbits: 1—Growth performance, nutrients digestibility, nitrogen utilization, and economical efficiency. *Egyptian Poultry Science Journal,* 40(4), 753–768. DOI: 10.21608/epsj.2020.134962.
22) El-Desoky, N. I., Hashem, N. M., Elkomy, A. and Abo-Elezz, Z. R. (2017). Physiological response and semen quality of rabbit bucks supplemented with *Moringa leaves* ethanolic extract during summer season. *Animal*, 11(9), 1549-1557. DOI: 10.1017/S1751731117000088.
23) El-Emam, M. M. A., Ray, M. N., Ozono, M. and Kogure, K. (2023). Heat stress disrupts spermatogenesis via modulation of sperm-specific calcium channels in rats. *Journal of Thermal Biology*, 112, 103465. DOI: 10.1016/j.jtherbio.2023.103465.
24) El-Gindy, Y. M., Abu Hafsa, S. H. and El-Deeb, N. M. (2023). The expression of liver TNF-α gene, liver and small intestine histology of thermal stressed growing rabbits affected by allicin and lycopene. *Journal of thermal biology*, *113*, 103521. https://doi.org/10.1016/j.jtherbio.2023.103521.
25) El-Kashef, M. M. (2022). Effect of *Moringa oleifera* leaves on physiological response, hormonal changes and semen quality of male rabbits under North Sinai conditions. *Egyptian Journal of Rabbit Science, 32*(2), 105–119. Doi.org/10.21608/ejrs.2022.249692
26) El-Ratel, I. T., Mekawy, A., Hassab, S. H. and Abdelnour, S. (2025). Enhancing growing rabbit heat stress resilience through dietary supplementation with natural antioxidants. *BMC Veterinary Research, 21*, 28. Doi.org/10.1186/s12917-024-04466-1.
27) Ewuola, E. O., Sanni, R. O., Aina, T., Oni, O. and Jimoh, O. A. (2022). Thermoregulatory response and oxidative stress indices of rabbit bucks administered ascorbic acid and sodium bicarbonate in a humid tropical environment. *The Journal of Basic and Applied Zoology*, 83(1), 55. Doi.org/10.1186/s41936-022-00307-5.
28) Falemara, B. C., Aina, O. O., Shittu, S. and Usman, H. S. (2020). Growth performance of kit rabbits fed concentrate diet supplemented with varying foliage leaf meals. *Animal Review, 7*, 1–13. DOI: 10.18488/journal.ar.2020.71.1.13.
29) Farghly, M. F. A., Mahrose, K. M., Mahmoud, G. B., Ali, R. M., Daghash, W., Metwally, K. A. and Abougaba, M. S. (2020). Lighting programs as an appliance to improve growing New Zealand White rabbit's performance. *International Journal of Biometeorology*, 64, 1295–1303. DOI: 10.1007/s00484-020-01906-z.
30) Fathy, A. A., Nebar, A. F., Ahmed, B. M., Temraz, N. S. and Nayel, U. A. (2022). Effect of *Moringa Olifera* Leaves and Seeds Supplementation on Semen Characteristics, testes and epididymis biometry and some blood paramaters in Barki Rams. *Menoufia Journal of Animal Poultry and Fish Production*, *6*(5), 105-120. DOI:10.21608/mjapfp.2022.247531
31) Feldman, H. A., Longcope, C., Derby, C. A., Johannes, C. B., Araujo, A. B., Coviello, A. D., Bremner, W. J. and McKinlay, J. B. (2002). Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *The Journal of Clinical Endocrinology and Metabolism*, 87(2), 589-598. Doi.org/10.1210/jcem.87.2.8201.
32) Gao, J., Zuo, Y., So, K. H., Yeung, W. S., Ng, E. H. and Lee, K. F. (2012). Electroacupuncture enhances spermatogenesis in rats after scrotal heat treatment. *Spermatogenesis*, *2*(1), 53–62. https://doi.org/10.4161/spmg.19282.
33) Garner, J. B., Chamberlain, A. J., Jagt, C. V., Nguyen, T. T. T., Mason, B. A., Marett, L. C., Leury, B. J., Wales, W. J. and Hayes, B. J. (2020). Gene expression of the heat stress response in bovine peripheral white blood cells and milk somatic cells in vivo. *Scientific Reports, 10*, 19181. Doi.org/10.1038/s41598-020-75438-2.
34) Gouda, N., Elkelawy, H., El-Gafaary, M. and Ibrahim, H. (2020). Utilization of moringa oleifera as a new rabbits feed. 3-effect of dietary inclusion of moringa oleifera hay on reproductive performance of buck rabbits. *Egyptian Journal of Rabbit Science*, *30*(1), 61-73. Doi.org/10.21608/ejrs.2020.131965.
35) Hanafi, E., Khalifa, W., I. A., E., G., N., Danial, E., Ramadan, M., Ezzo, O., Aboelmaaty, A. M. and Mdboli, A. E.-. A. (2025). The Role of Nano-Spirulina platensis in Enhancement of Female Rabbit Puberty and Amelioration of the Heat Stress Adverse Effects.. *Egyptian Journal of Chemistry*, *68*(5), 515-526. Doi: 10.21608/ejchem.2024.316799.10302.
36) Hashem, N. M., Soltan, Y. A., El-Desoky, N. I., Morsy, A. S. and Sallam, S. M. A. (2019). Effects of *Moringa oleifera* extracts and monensin on performance of growing rabbits. *Livestock Science, 228*, 136–143. https://doi.org/10.1016/j.livsci.2019.05.008.

37) Hassan, F. A., Shalaby, A. G., Elkassas, N. E. M., El-Medany, S. A., Hamdi Rabie, A., Mahrose, K., Abd El-Aziz, A. and Bassiony, S. (2023). Efficacy of ascorbic acid and different sources of orange peel on growth performance, gene expression, anti-oxidant status and microbial activity of growing rabbits under hot conditions. *Animal biotechnology*, 34(7), 2480–2491. Doi.org/10.1080/10495398.2022.2101114.

38) Hosny, N. S., Morsy, A. S., Abo-Elezz, Z. R. and Hashem, N. M. (2025). Physiological responses and reproductive performance of naturally heat-stressed rabbit does treated with postbiotic of *Bacillus subtilis* and *Saccharomyces* cerevisiae in free and nano-encapsulated forms. *BMC Veterinary Research*, *21*(1), 288. Doi.org/10.1186/s12917-025-04728-6.

39) Jaswal, P., Bansal, S., Chaudhary, R., Basu, J., Bansal, N. and Kumar, S. (2025). Nitric oxide: Potential therapeutic target in Heat Stress-induced Multiple Organ Dysfunction. *Naunyn-Schmiedeberg's archives of pharmacology*, 398(3), 2535–2546. Doi.org/10.1007/s00210-024-03556-z

40) Khaleel, H. K., Abdelnour, S. A., Osailan, R., El-Kholy, K. H., El-Haroun, E., El-Nagar, H. A., Mehilp, S.T., El-Raghi, A. A., Hassan, M. A., Moustafa, M. and El-Ratel, I. T. (2025). Improving heat resilience in fattening rabbits: nutritional strategies for mitigation via regulating blood physiology, inflammation and antioxidant pathways. *Frontiers in Veterinary Science*, *12*, 1677144. Doi.org/10.1007/s00210-024-03556-z.

41) Khalifa, W. H. K., Abusinna, G., Maghraby, N. and Hanafi, E. (2026). Impact of heat stress on rabbit reproductive performance and physiology: mechanisms, consequences, and mitigation strategies. *Egyptian Pharmaceutical Journal*, 25(2), 177-185. Doi: 10.21608/epj.2026.442606.1256.

42) Khalifa, W. H., Ibrahim, F. M., El Makawy, A. I., Sharaf, H. A., Khalil, W. K. and Maghraby, N. A. (2016). Safety and fertility enhancing role of *Moringa oleifera* leaves aqueous extract in New Zealand rabbit bucks. *International Journal of Pharmaceutical Research, 6*(1), 56–168.

43) Khan, S. U., Jannat, S., Shaukat, H., Unab, S., Tanzeela, Akram, M., Khan Khattak, M. N., Soto, M. V., Khan, M. F., Ali, A. Rizvi, S. S. R. (2023). Stress Induced Cortisol Release Depresses The Secretion of Testosterone in Patients With Type 2 Diabetes Mellitus. Clinical medicine insights. Endocrinology and diabetes, 16, 11795514221145841. Doi.org/10.1177/11795514221145841.

44) Ko, S.-H. (2024). Effects of heat stress-induced sex hormone dysregulation on reproduction and growth in male adolescents and beneficial foods. *Nutrients, 16*(17), 3032. Doi.org/10.3390/nu16173032.

45) Kuang, L. D., Li, C. Y., Guo, Z. Q., Ren, Y. J., Zheng, J. and Mei, X. L. (2021). Effects of heat stress on reproductive performance, serum biochemical indexes and reproductive hormones in female rabbit of Qixing. *Southwest China Journal of Agricultural Sciences, 34*, 1323–1329. Doi.org/10.16213/j.cnki.scjas.2021.1323.

46) Laoung-on, J., Saenphet, K., Jaikang, C. and Sudwan, P. (2021). Effect of *Moringa oleifera* Lam. Leaf Tea on Sexual Behavior and Reproductive Function in Male Rats. *Plants*, *10*(10), 2019. Doi.org/10.3390/plants10102019.

47) Madkour, M., Shakweer, W. M. E., Hashem, N. M., Aboelazab, O., Younis, E., El-Azeem, N. A. and Shourrap, M. (2023). Antioxidants status and physiological responses to early and late heat stress in two rabbit breeds. *Journal of Animal Physiology and Animal Nutrition, 107*(1), 298–307. Doi.org/10.1111/jpn.13781.

48) Magdy, B. W., Mohamed, F. E., Amin, A. S. and Rana, S. S. (2016). Ameliorative effect of antioxidants (vitamins C and E) against abamectin toxicity in liver, kidney and testis of male albino rats. *The Journal of Basic and Applied Zoology*, *77*, 69-82. DOI: 10.1016/j.jobaz.2016.10.002

49) Mohamed, M. A., Ahmed, M. A. and El Sayed, R. A. (2019). Molecular effects of Moringa leaf extract on insulin resistance and reproductive function in hyperinsulinemic male rats. *Journal of diabetes and metabolic disorders*, *18*(2), 487–494. Doi.org/10.1007/s40200-019-00454-7.

50) Mohammed, A. A., Iyeghe-Erakpotobor, G. T., Zahraddeen, D., Barje, P. P. and Samuel, F. (2023). Testicular and epididymal anatomy and spermatozoa reserves of rabbit bucks fed Moringa oleifera leaf meal-based diets supplemented with mixtures of garlic, ginger or black pepper. *Ghana Journal of Agricultural Science*, 58(1), 35-48. DOI: 10.4314/gjas.v58i1.4

51) Mohanty, T., Bhakat, M., Yadav, H., Kumar, R., Sinha, R., Lone, S., Singh, A. and Shah, N. (2019). Impact of climate change on reproductive efficiency of indigenous animals. In Management of animal genetic resources under climate change and socio-economic perspectives (p. 238). ICAR–*National Bureau of Animal Genetic Resources.*

52) Mohlala, K., Offor, U., Monageng, E., Takalani, N. B. and Opuwari, C. S. (2023). Overview of the effects of *Moringa oleifera* leaf extract on oxidative stress and male infertility: a review. *Applied Sciences*, *13*(7), 4387. Doi.org/10.3390/app13074387.

53) Monageng, E., Offor, U., Takalani, N. B., Mohlala, K. and Opuwari, C. S. (2023). A review on the impact of oxidative stress and medicinal plants on Leydig cells. *Antioxidants, 12*(8), 1559. Doi.org/10.3390/antiox12081559.

54) Mthiyane, F. T., Dludla, P. V., Ziqubu, K., Mthembu, S. X. H., Muvhulawa, N., Hlengwa, N., Nkambule, B. B. and Mazibuko-Mbeje, S. E. (2022). A Review on the Antidiabetic Properties of *Moringa oleifera* Extracts: Focusing on Oxidative Stress and Inflammation as Main Therapeutic Targets. *Frontiers in pharmacology*, *13*, 940572. Doi.org/10.3389/fphar.2022.940572.

55) Mutwedu, V. B., Nyongesa, A. W., Kitaa, J. M., Ayagirwe, R. B., Baharanyi, C. and Mbaria, J. M. (2022). Effects of *Moringa oleifera* aqueous seed extracts on reproductive traits of heat-stressed New Zealand white female rabbits. *Frontiers in veterinary science*, *9*, 883976. Doi.org/10.3389/fvets.2022.883976.

56) Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Depner, K., Drewe, J. A., Garin-Bastuji, B., Gonzales Rojas, J. L., Gortázar Schmidt, C., Michel, V., Miranda Chueca, M. Á., Roberts, H. C., Sihvonen, L. H., Stahl, K., Velarde Calvo, A., Viltrop, A., Winckler, C., Candiani, D., Fabris, C., Mosbach-Schulz, O., Van der Stede, Y. and Spoolder, H. (2020). Scientific opinion on stunning methods and slaughter of rabbits for human consumption. *EFSA Journal, 18*(1), 5927. Doi.org/10.2903/j.efsa.2020.5927.

57) Nowicka-Bauer, K. and Nixon, B. (2020). Molecular Changes Induced by Oxidative Stress that Impair Human Sperm Motility. *Antioxidants*, *9*(2), 134. Doi.org/10.3390/antiox9020134.
58) Oka, S., Shiraishi, K., Fujimoto, M., Katiyar, A., Takii, R., Nakai, A., & Matsuyama, H. (2017). Role of heat shock factor 1 in conserving cholesterol transportation in Leydig cell steroidogenesis via steroidogenic acute regulatory protein. *Endocrinology, 158*(8), 2648–2658. Doi.org/10.1210/en.2017-00132
59) Pagana, K. D. and Pagana, T. J. (2007). Mosby's Diagnostic and Laboratory Test Reference 8th Edition: Mosby, Inc., Saint Louis, MO. 629-631.
60) Ragab, S. M., Almohaimeed, H. M., Alghriany, A. A., Khalil, N. S. A. and Abd-Allah, E. A. (2024). Protective effect of Moringa oleifera leaf ethanolic extract against uranyl acetate-induced testicular dysfunction in rats. *Scientific Reports*, *14*(1), 932. Doi: 10.1038/s41598-023-50854-2.
61) Rochdi, C., Ouadrhiri, M., Allai, L., Bellajdel, I., Mamri, S., Taheri, H., Saadi, H., Mimouni, A. and Choukri, M. (2024). Beneficial effects of oral antioxidant supplementation on semen quality parameters, reproductive hormones, and sperm DNA integrity in men with idiopathic oligoasthenoteratozoospermia. *Clinical and experimental reproductive medicine*, *51*(2), 135–141. Doi.org/10.5653/cerm.2023.06555.
62) Saghir, S. A. M., Hroob, A. M., Majrashi, K. A., Jaber, F. A., Abduh, M. S., Al-Gabri, N., Albaqami, N. M., Abdelnour, S. A., Alqhtani, A. H., Abd El-Hack, M. E., Swelum, A. A. and Simal-Gandara, J. (2023). Effects of alginates on the growth, haematological, immunity, antioxidant and pro-inflammatory responses of rabbits under high temperature. *Research in Veterinary Science, 155*, 36–43. Doi.org/10.1016/j.rvsc.2023.01.002
63) Serag El Din, O. S., Abd El Fattah, N. and Salama, M. A. (2025). Effect of seasonal climatic changes on fertility in different species of male mammals and birds. *The Journal of Basic and Applied Zoology*, *86*(1), 1-15. Doi.org/10.1186/s41936-025-00501-1.
64) Shabana, M. E., Soliman, A. S., Abouelfadl, D. M. and Yassen, N. N. (2024). Immunomorphometric Analysis of CD82 in Colorectal Carcinoma and its Prognostic Significance. *Disease and Diagnosis*, 13(2):69-74. DOI: 10.34172/ddj.1564.
65) Shahat, A. M., Rizzoto, G. and Kastelic, J. P. (2020). Amelioration of heat stress-induced damage to testes and sperm quality. *Theriogenology*, *158*, 84–96. Doi.org/10.1016/j.theriogenology.2020.08.034.
66) Shawky, S. M., Ramadan, S. G., and Orabi, S. H. (2014). Hemato-biochemical, behavioral and neurological effects of vitamin C administration against lead exposure in Mice. Int J Adv Res, 2(12), 418-429.
67) Sheweita, S. A., Al-Qahtani, S. M., Ahmed, R. M., Sheweita, M. S. and Atta, A. (2025). Molecular mechanisms contributing to the impairment of steroid hormones, sperm characteristics, and testicular architecture in male rabbits after chronic exposure to cadmium: role of gallic acid and selenium as antioxidants. *Toxics*, *13*(4), 323. Doi.org/10.3390/toxics13040323.
68) Sirotkin, A. V., Parkanyi, V. and Pivko, J. (2021). High temperature impairs rabbit viability, feed consumption, growth and fecundity: examination of endocrine mechanisms. *Domestic animal endocrinology*, *74*, 106478. Doi.org/10.1016/j.domaniend.2020.106478
69) Spiers, J. G., Chen, H. J., Sernia, C. and Lavidis, N. A. (2015). Activation of the hypothalamic-pituitary-adrenal stress axis induces cellular oxidative stress. *Frontiers in neuroscience*, *8*, 456. Doi.org/10.3389/fnins.2014.00456.
70) Sui, J., Feng, Y., Li, H., Cao, R., Tian, W. and Jiang, Z. (2019). Baicalin protects mouse testis from injury induced by heat stress. *Journal of thermal biology*, *82*, 63–69. Doi.org/10.1016/j.jtherbio.2019.03.009.
71) Talebi, A. R., Khalili, M. A., Nahangi, H., ABBASI, S. A. A. and Anvari, M. (2007). Evaluation of epididymal necrospermia following experimental chronic spinal cord injury in rat. *International Journal of Reproductive BioMedicine* 5 (4), 171-176.
72) Tran, T. T. K., Lam, N. A., Nguyen, T. N., & Nguyen, L. K. D. (2025). The role of sodium bicarbonate in improving cold storage preservation of indigenous black rabbit spermatozoa. *CTU Journal of Innovation and Sustainable Development*, *17*(1), 54-59. Doi.org/10.22144/ctujoisd.2025.021.
73) Ukey, A., Singh, S. V., Biswal, S., Misra, A., Gujjalkar, P., Kumar, G., Vaidya, M., & Bhalakiya, N. 2025. Moringa oleifera as a concentrate replacer for enhancing immune status and ameliorating stress of Barbari goats during thermal stress Journal of Livestock Science 16: 536-544 doi. 10.33259/JLivestSci.2025.536-544
74) Vaghamashi, D. G., Garg, D. D., Sabapara, G. P., Dangar, N. S., Chavda, J. A., Patel, A. S., Bariya, A. R., Ribadiya, N. K., & Karangiya, V. K. 2025. Effects on egg quality attributes of RIR hens fed with Moringa oleifera leaf meal-containing diet. Journal of Livestock Science 16: 516-523 doi. 10.33259/JLivestSci.2025.516-523
75) Widiastini, L. P., Karuniadi, I. A. M., Karmaya, I. N. M. and Widhiantara, I. G. (2022). The potential of a Balinese traditional medicine kelor leaves (*Moringa oleifera*) for male infertility treatment: A mini review. *Indian Journal of Forensic Medicine & Toxicology,* 16(1), 734–742. Doi.org/10.37506/ijfmt.v16i1.17579.
76) Xiao, Y., Zhao, L., Li, W., Wang, X., Ma, T., Yang, L., Gao, L., Li, C., Zhang, M., Yang, D., Zhang, J., Jiang, H., Zhao, H., Wang, Y., Chao, H. W., Wang, A., Jin, Y. and Chen, H. (2021). Circadian clock gene BMAL1 controls testosterone production by regulating steroidogenesis-related gene transcription in goat Leydig cells. *Journal of cellular physiology*, 236(9), 6706–6725. Doi.org/10.1002/jcp.30334.
77) Yin, B., Tang, S., Sun, J., Zhang, X., Xu, J., Di, L., Li, Z., Hu, Y. and Bao, E. (2018). Vitamin C and sodium bicarbonate enhance the antioxidant ability of H9C2 cells and induce HSPs to relieve heat stress. *Cell Stress and Chaperones, 23*(4), 735–748. Doi.org/10.1007/s12192-018-0885-2.
78) Zhai, P., Yang, J., Qin, F., Yao, Y., Shao, L., Li, J. and Zhang, X. (2026). Effects of N-carbamylglutamate supplementation on growth, serum biochemistry, antioxidant capacity, immune function, hormonal profile, and reproductive performance in rabbits under chronic heat stress conditions. *Animal reproduction science*, *291*, 108208. Advance online publication. Doi.org/10.1016/j.anireprosci.2026.108208.