J I T A A
Journal of the Indonesian Tropical Animal Agriculture
Accredited by Ditjen Riset, Teknologi dan Pengabdian kepada Masyarakat No. 164/E/KPT/2021

J. Indonesian Trop. Anim. Agric.
pISSN 2087-8273 eISSN 2460-6278
http://ejournal.undip.ac.id/index.php/jitaa
51(2)83-93, June 2026
DOI: 10.14710/jitaa.51.2. 83-93

Effect of heat shock program on physical semen characteristics and blood constituents of buck rabbits under hot conditions

H. Z. Amer[1] and A. S. Morsy [2*]
[1]Deparment of Biology, College of Science, Jazan University, P.O.Box 114, Jazan 45142, Kingdom of Saudi Arabia.
[2]Animal and Poultry Physiology Department, Desert Research Center, Egypt.
[*]Corresponding E-mail: alisaber_drc@yahoo.com

Received January 19, 2026; Accepted April 09, 2026

ABSTRACT

This study aimed to release the heat shock protein 70 by applying heat shock exposure programs at early ages on physical semen characteristics and blood constituents of Hi-Plus buck rabbits under hot conditions. A total number of 40 Hi-Plus buck rabbits were divided into four equals treatments: the 1st treatment (C), bucks served as control. Rabbits of 2nd, 3rd and 4th treatments were exposed to heat shock program. The 2nd treatment (HSE30) exposed at 30 days of age, the 3rd treatment (HSE60) exposed at 60 days of age and the 4th treatment (HSE30+60) exposed at 30+60 days of age. HSP70 and blood metabolites were increased (P≤ 0.05) in the rabbits of HSE groups compared to control group. Triiodothyronine hormone was increased (P≤0.05) in HSE groups as compared to control group. Corticosterone hormone decreased (P≤0.05) in the rabbits of HSE groups as compared to control group. Physical semen quality was improved (P≤0.05) in rabbits of HSE groups as compared to control group. In conclusion, early heat shock exposure programs for buck rabbits might increase release of HSP70 which positive reflect on enhance physiological responses and physical semen characteristics under severe heat stress conditions.

Keywords: Buck rabbits, Heat stress, HSP70, Physiological parameters, Semen quality.

INTRODUCTION

In Egypt, high relative humidity and ambient temperatures have detrimental effects on rabbits. Heat stress conditions lead to deleterious effects on thermo-respiratory responses, hematological parameters, immune responses and semen quality characteristics (Sakr *et al.*, 2019). Fertility is one of the detrimental factors of economical income of rabbit farms. Hence, mating by subfertile males has an adverse effect on fertilization rate, embryo production (Fadl *et al.*, 2024) early or late embryonic mortality. Furthermore, hot conditions unfavorably affect semen quality, by reducing the ability of leydig and Sertoli cells to respond to LH and the diameter of the seminiferous tubules (Ding *et al.*, 2025). In addition, hot conditions caused an increase in oxidative stress and increase the production of reactive oxygen species, which can be detrimental to semen characteristics (Huang *et al.*, 2023 and Akiyama, 1999).

Heat shock programs during earlier age are alternative practice to increase thermo-tolerance and acclimate rabbits to heat stress conditions thereby minimizing heat-related mortality and enhance reproductive performance (Faisal *et al.*, 2008; Ahmed *et al.*, 2012; Morsy, 2013; Morsy, 2018; Sakr *et al.*, 2019; Ezzat *et al.*, 2020 and Madkour *et al.*, 2021). Heat shock proteins have

been detected in every cell type and tissues. Exposure of rabbits to heat stress conditions during growth period induces HSP70. HSP70 protein acts as chaperone, which assists in the folding, transport and assembly of protein in cytoplasm, mitochondria and endoplasmic reticulum or appears to play a critical role in protecting cells against the adverse effects of hyperthermia, helps newly synthesized proteins fold (Morimoto *et al.*, 1990). As a molecular chaperone, HSP70 limits oxidative damage and death in germ cells, stabilizes membranes, and prevents protein misfolding (Rosyada *et al.*, 2022). In sperm, HSP70 supports motility, capacitation, sperm–egg recognition, acrosome integrity, and mitochondrial function (Pardede *et al.*, 2023).

Therefore, this study aimed to evaluate the effects of early-age heat shock exposure programs on semen physical characteristics and blood constituents in Hi-Plus buck rabbits under hot environmental conditions.

MATERIALS AND METHODS

Study Region and Ethical Approval

This study was conducted in South Sinai Research Station, located in Ras Suder that belongs to the Desert Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Laboratory work was carried out in the laboratories of Desert Research Center. This research was carried out in accordance with the guidelines laid down by the Institute of Animal Ethics Committee for the use of animals (2010/63/EU of the European Parliament and of the Council of September 22, 2010).

Animals and Experimental Design

A total of 40 Hi-Plus buck rabbits, aged 6 months old with an average body weight of 2728.8 ± 58.1 g were used. The study was conducted from June to August, 2021. The rabbits were housed in standard dimensions (50×60×40 cm) wired metallic cages. All buck rabbits were fed a commercial concentrate pelleted diet containing 18.0% crude protein, 14.0% crude fiber, 2.5% fat, 0.6% minerals mixture and 2600 kcal/kg digestible energy according to NRC (1994) Fresh water was available all days through nipples drinking system. Buck rabbits were randomly assigned to four equal treatments: the first treatment (C), bucks served as control (non-exposure to heat shock program). The buck rabbits in the second, third, and fourth treatments were subjected to a heat shock program (36±1°C for 3 hours from 12:00 to 15:00 for three consecutive days). The second treatment (HSE30) was administered at 30 days of age, the third (HSE60) at 60 days of age, and the fourth (HSE30+60) at 30+60 days.

Meteorological Data

Ambient temperature (AT, °C), relative humidity (RH, %) were recorded using a hygrothermometer (Table 1). According to Marai *et al.* (2001), temperature-humidity index (THI) was calculated using the following equation:

$$THI = AT^{o}C - [(0.31 - 0.31 \times RH)] \times [(AT^{o}C - 14.4)]$$

Where, AT°C = Ambient temperature in centigrade and RH = relative humidity %. The THI values were classified as absence of heat stress (≤27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

Table 1. Indoor Ambient Temperature, Relative Humidity and Temperature-Humidity Index Throughout Experimental Period under South Sinai Conditions, Egypt

Months	Min. AT (°C)	Max. AT (°C)	Min. RH (%)	Max. RH (%)	Min. THI	Max. THI
June	24.4	33.7	27.9	43.6	22.7	29.4
July	23.5	34.3	27.1	41.1	21.9	29.8
August	26.2	34.7	30.2	50.8	24.4	30.3
Overall mean	24.7	34.2	29.1	45.2	23.0	29.8

AT= ambient temperature; RH= relative humidity; THI= temperature humidity index

Blood Samples

Every two weeks, blood samples were collected from each buck rabbit's ear vein and placed in tubes coated with ethylenediamin tetra-acetic acid (EDTA). The coulter (HA-VET, Clinding, Belgium) quickly measured the concentration of hemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs), and packed cell volume (PCV%). The following formulas were used to determine mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC): MCH (in picogram, pg) = (Hb content g/dl × 10) / RBCs in million. MCV (femtoliter, fl) = (PCV % × 10)/RBCs in million; MCHC (%) = (Hb content × 100) / PCV %. After centrifuging blood samples for 15 minutes at 3500 rpm, the plasma was separated and stored at -70 °C for hormonal assays, biochemical measurements, and HSP70.

Commercial kits (Biomed Diagnostics, Egypt) were used to test plasma biochemical markers, including total protein, albumin, glucose, and cholesterol. The formula globulin = (total protein - albumin) was used to determine the globulin concentration. Commercial kits (Biodiagnostic Research, Egypt) were used for the calorimetric measurement of total antioxidant capacity (TAC). ELISA kits were used to measure the hormones corticosterone, testosterone, and triiodothyronine (T3) (Boston, MA 02134). The ELISA kit from Uscn Life Science Inc. Wuhan, China was used to measure the concentration of HSP70. This assay's sensitivity and specificity for HSP70 detection are both quite good.

Semen Collection

Semen was collected biweekly (six times during experimental period) from 7 bucks/ treatment using a clean, dried and sterilized standard artificial vagina of rabbits and a teaser doe according to Mocé *et al.* (2000).

Buck Reaction Time

Reaction time was calculated in seconds as the time from introducing the doe to the buck and incidence of complete intercourse and ejaculation using stopwatch (Luzi *et al.*, 1996).

Physical Semen Characteristics

The ejaculate volume without gel fraction was measured by graduated test tube and recorded in milliliter. Sperm concentration was determined by the haemocytometer according to (Smith and Mayer, 1955). Total sperm output was calculated by multiplying ejaculate volume and sperm concentration. The percentages of live and abnormal sperm were determined after staining with eosin and nigrosine and then calculated as a percentage out of 100 randomly chosen sperm counted. The percentage of motile sperm was estimated with a phase-contrast microscope. Total motile sperm was estimated by multiplying the progressive motility percentage by the total sperm output. Semen quality factor = (ejaculate volume × sperm concentration × live spermatozoa) / 100. The concentration of hydrogen ions (pH) of semen was immediately determined after collection by using Whatman pH indicator paper. Acrosomal integrity was determined by using a Giemsa stain procedure as described by Watson (1975).

Statistical Analysis

Data was analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004). The model was as follows:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where, Y_{ij}= any observations of i^{th} buck rabbit within j^{th} treatment, μ = overall mean, G_i = effect of i^{th} group, (i: 1-4), e_{ij} = standard error

All statements of significance are based a probability of less than 0.05. Significant differences among means were tested using Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Heat Shock Protein 70 and Thermo-respiratory Responses

Early thermal conditioning at 36 °C for 6 hours at 42 days of age in rabbits induces over-expression of hepatic HspA9, a member of the HSP70 family, and reduces plasma and hepatic MDA later in life. This is an indicator for improving mitochondrial function to prevent oxidative damage in the liver; additionally, overexpression of hepatic HSPs later in life may be an indicator for improving thermotolerance acquisi-

Table 2. Effect of Heat Shock Programs on Heat Shock Protein 70 and Thermo-Respiratory Responses of Buck Hi-Plus Rabbits

Items	C	HSE30	HSE60	HSE30+60	±SE	P value
HSP70	1.70[b]	5.21[a]	4.83[a]	4.94[a]	0.14	0.001
RT (°C)	39.65[a]	39.17[b]	39.43[ab]	39.14[b]	0.11	0.011
RR (breath/min.)	182.31[a]	162.66[ab]	177.70[ab]	159.22[b]	6.84	0.052

C = control (non-exposure to heat shock program); HSE30, HSE60, and HSE30+60 = buck rabbits were subjected to a heat shock program (36±1°C for 3 hours from 12:00 to 15:00 for three days). The second treatment (HSE30) was administered at 30 days of age; the third (HSE60) was administrated at 60 days of age; and the fourth (HSE30+60) was administrated at 30+60 days. HSP70 = heat shock protein 70; RT = rectal temperature and RR = respiration rate. [a-b] Means bearing different superscripts within the same row are significantly different (P≤ 0.05).

Table 3. Effect of heat shock programs on hematological parameters of buck Hi-Plus rabbits

Items	C	HSE30	HSE60	HSE30+60	±SE	P value
WBC's ($\times10^3$/mm^3)	9.63[a]	7.12[b]	7.27[b]	7.77[b]	0.47	0.001
RBC's ($\times10^6$/mm^3)	3.80[c]	5.02[b]	5.69[a]	5.38[ab]	0.20	0.001
Hb (g/dl)	10.22[b]	13.59[a]	12.76[a]	13.68[a]	0.44	0.001
PCV (%)	30.08[c]	35.96[b]	38.64[a]	39.45[a]	0.92	0.001
MCV (fl)	88.54	73.49	68.67	74.73	5.39	0.075
MCH (pg)	29.02[a]	28.04[a]	22.73[b]	25.92[ab]	1.70	0.054
MCHC (%)	34.10	38.08	33.53	35.12	1.51	0.156

C = control (non-exposure to heat shock program); HSE30, HSE60, and HSE30+60 = buck rabbits were subjected to a heat shock program (36±1°C for 3 hours from 12:00 to 15:00 for three days). The second treatment (HSE30) was administered at 30 days of age; the third (HSE60) was administrated at 60 days of age; and the fourth (HSE30+60) was administrated at 30+60 days. WBC's = white blood cells; RBC's = red blood cells; Hb =hemoglobin; PCV= packed cell volume; MCV= mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration. [a-c] Means bearing different superscripts within the same row are significantly different (P≤ 0.05).

tion in thermally conditioned rabbits (Madkour *et al.*, 2021).

In the current study, heat exposure program was increased (P≤ 0.05) HSP70 level (Table 2) in the buck rabbits of HSE30, HSE60 and HSE30+60 compared to the rabbits of control group (C). This result revealed that early heat exposure program increased HSP70 level and suggest that this protein involved in the stress caused by heat shock exposure in the rabbits (Ahmed, *et al.*, 2012, Morsy, 2018 and Sakr *et al.*, 2019).

Release of HSP70 during heat stress conditions may play an important role in protecting stressed cells and reversing disorders caused by stress through its function as a molecular chaperone by binding to other cellular proteins, assisting intracellular transport and folding into the proper secondary structures and thus preventing aggregation of protein during stress (Aldahhan *et al.*, 2021) and hence it may positively return on rabbit's performance (Abd El-Kafy *et al.*, 2008).

Rectal temperature decreased (P≤0.05) in the HSE30 and HSE30+60 treatments by about 1.21 and 1.28 %, respectively as compared with control group (Table 2). Indeed, respiration rate decreased (P≤0.05) in the buck rabbits of HSE30+60 group by 12.66 % as compared to control group. No significant differences were observed among control and HSE30 and HSE60 groups in respiration rate. These results may suggest that heat shock exposure enhances thermotolerance of buck rabbits exposed to hot conditions at later age (Ahmed, *et al.*, 2012; Morsy, 2013 and Morsy, 2018). However, heat shock exposure physiologically controlled to better tolerate heat stress by thermal conditioning, thermal conditioning process is to combine starting changes that enable rabbits to cope, within certain limits, with acute exposure to unexpected heat waves (Yahav and Mc-Murtry, 2001). The HSP play a vital role in cellular homeostasis during development of thermotolerance (Hahn and Li, 1990). Meanwhile, Abd El-Kafy *et al.* (2008) reported that the reduction of the rectal temperature in heat group may be due to the fact that rab-

bits were able to maintain constant rectal temperature during heat exposure by low metabolic rate when previously acclimated to high temperature. These results agree with the results of Sakr *et al.* (2019); Ahmed *et al.* (2012) and Morsy (2013) and Morsy (2018).

Hematological Parameters

White blood cells (WBC's) were decreased (P≤0.05) in the bucks of HSE30, HSE60 and HSE30+60 by 26.06, 24.50 and 19.31 %, respectively compared with the control group. Exposure to high ambient temperatures (≈31–36 °C) often increases total WBC counts compared with thermoneutral controls, alongside oxidative stress and organ dysfunction (Mutwedu *et al.*, 2021). Since HSP70 is a component of leukocyte stress responses that are created in leukocytes themselves and the degree of leukocyte HSP70 elevation increases with stress, the decreased WBC count in treated groups may be the result of decreased stress and enhanced physiological stability (Mine *et al.*, 2019).

In contrast, red blood cells count (RBC's), hemoglobin (Hb) concentration and packed cell volume (PCV) were increased (P≤0.05) in the buck rabbits of HSE30, HSE60 and HSE30+60 compared with control group (Table 3).

On the other hand, bucks in HSE60 had significantly lower MCH compared with the bucks in control and the HSE30 groups. There are no significant effects of heat shock exposure on

Table 4. Effect of heat shock programs on blood metabolites parameters of buck Hi-Plus rabbits

Items	C	HSE30	HSE60	HSE30+60	±SE	P value
Total protein (g/dl)	6.79^{c}	7.92^{b}	8.53^{a}	8.28^{ab}	0.18	0.001
Albumin (g/dl)	4.95	4.90	4.83	4.77	0.14	0.849
Globulin (g/dl)	1.84^{c}	3.01^{b}	3.69^{a}	3.50^{ab}	0.19	0.001
Glucose (mg/dl)	150.01^{b}	169.02^{a}	172.71^{a}	170.56^{a}	6.06	0.037
Cholesterol (mg/dl)	62.03^{a}	45.84^{b}	50.83^{b}	45.97^{b}	3.48	0.004
TAC (mM/l)	0.21^{b}	0.42^{a}	0.41^{a}	0.49^{a}	0.05	0.008

C = control (non-exposure to heat shock program); HSE30, HSE60, and HSE30+60 = buck rabbits were subjected to a heat shock program (36±1°C for 3 hours from 12:00 to 15:00 for three days). The second treatment (HSE30) was administered at 30 days of age; the third (HSE60) was administrated at 60 days of age; and the fourth (HSE30+60) was administrated at 30+60 days. TAC = total antioxidant capacity. $^{a-c}$ Means bearing different superscripts within the same row are significantly different (P≤ 0.05).

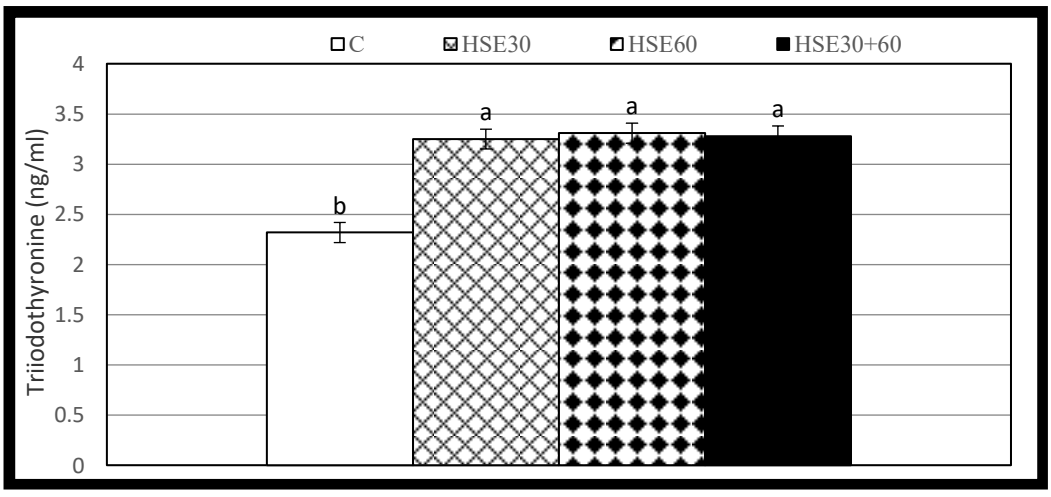

Figure 1. Effect of heat shock programs on blood triiodothyronine hormone of buck Hi-Plus rabbits.
C = control (non-exposure to heat shock program); HSE30, HSE60, and HSE30+60 = buck rabbits were subjected to a heat shock program (36±1°C for 3 hours from 12:00 to 15:00 for three days). The second treatment (HSE30) was administered at 30 days of age; the third (HSE60) was administrated at 60 days of age; and the fourth (HSE30+60) was administrated at 30+60 days. $^{a-b}$ Means bearing different superscripts are significantly different (P≤ 0.05).

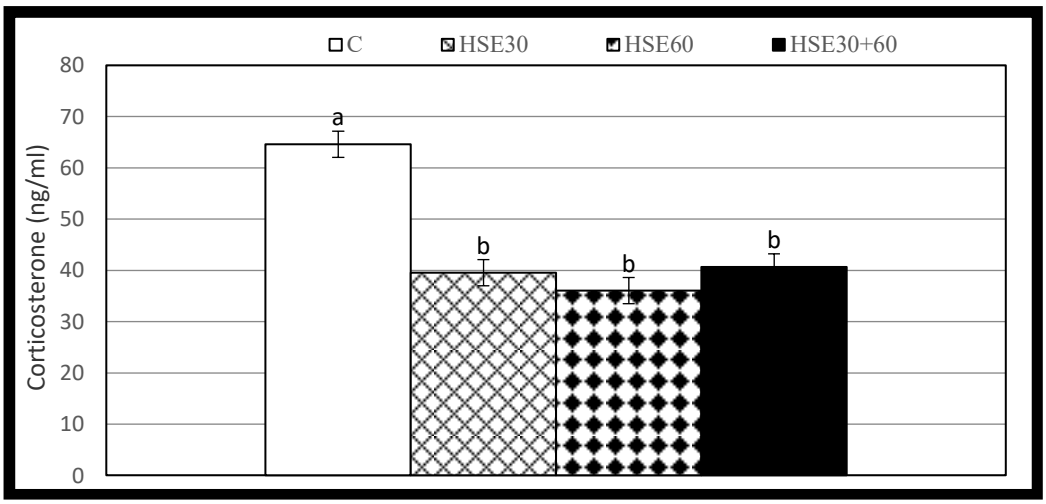

Figure 2. Effect of heat shock programs on blood corticosterone hormone of buck Hi-Plus rabbits
C = control (non-exposure to heat shock program); HSE30, HSE60, and HSE30+60 = buck rabbits were subjected to a heat shock program (36±1°C for 3 hours from 12:00 to 15:00 for three days). The second treatment (HSE30) was administered at 30 days of age; the third (HSE60) was administrated at 60 days of age; and the fourth (HSE30+60) was administrated at 30+60 days. [a-b] Means bearing different superscripts are significantly different (P≤ 0.05).

MCV and MCHC. These results may be due to enhance in the thermotolerance responses in the groups of heat shock exposure which might be revealed on enhanced hematological parameters. These results were agreed with Ahmed *et al.* (2012), Morsy (2013), Morsy (2018) and Sakr *et al.* (2019), they found that Hb concentrations decreased under heat stress and increased in acclimated animals. However, the decrease in the values of Hb and PCV (%) in the control group (non-exposure to heat shock program) might attributed to heat stress, which impair the synthesis of blood cells (Oladele *et al.*, 2001).

Blood Metabolites

The results in Table (4) showed that total protein, globulin, glucose and total antioxidant capacity (TAC) concentrations increased (P≤0.05) in the buck rabbits of the HSE30, HSE60 and HSE30+60 groups when compared to the bucks of control group. However, cholesterol concentration was decreased (P<0.05) in the bucks of heat shock exposure groups as compared to the rabbits of control group. These results might reflect the improvement of the immune responses of rabbits that exposed to heat shock exposure compared to control group.

The increase in globulin and TAC concentrations and decrease cholesterol level might be an indicator of immune responses and antibody production in the heat shock exposure groups (El -Kaiaty and Hassan, 2004). These results agree with those of Mashaly *et al.* (2004), Ahmed *et al.* (2012), Morsy (2018) and Sakr *et al.* (2019). Notably, heat stress increases reactive oxygen species (ROS), lowers endogenous antioxidants, and raises lipid peroxidation products in rabbits (Liang *et al.*, 2022). This is consistently accompanied by reduced TAC and antioxidant enzymes (SOD, CAT, GSH-Px) in plasma, liver, kidney and muscle, indicating systemic oxidative stress and organ damage (Ebeid *et al.*, 2023). TAC therefore serves as an integrated index of overall redox status, declining when oxidative load exceeds antioxidant defenses (El-Ratel *et al.*, 2025).

Hormonal Profile

Tri-iodothyronine (T_3) hormone was increased (P≤0.05) in the HSE30, HSE60 and HSE30+60 treatments by 40.08, 42.67 and 41.37 %, respectively as compared to control group (Figure 1). These results agree with Ahmed *et al.* (2012), Morsy (2013), Morsy (2018) and Sakr *et al.* (2019).

T_3 hormone play important role in regulating metabolism and thermogenesis in animals (Tao *et al.*, 2006). Concentration of T_3 hormone is highly correlated to decrease of feed intake and heat stress conditions (Yahav *et al.* 1995).

Table 5. Effect of heat shock programs on reaction time and physical semen characteristics of buck Hi-Plus rabbits

Items	C	HSE30	HSE60	HSE30+60	±SE	P value
Reaction time (seconds)	49.33[a]	16.88[b]	17.89[b]	18.38[b]	2.22	0.001
Ejaculate volume (ml)	0.38[b]	0.70[a]	0.64[a]	0.76[a]	0.04	0.001
Progressive motility (%)	71.66[b]	81.94[a]	82.22[a]	88.33[a]	2.50	0.002
Sperm conc. ($\times 10^6$/ml)	413.33[b]	522.77[a]	519.44[a]	552.77[a]	33.53	0.047
Total sperm output ($\times 10^6$)	176.05[c]	381.97[ab]	305.00[b]	415.13[a]	33.45	0.001
Total motile sperm ($\times 10^6$)	123.73[c]	324.47[ab]	260.22[b]	363.58[a]	29.99	0.001
Live Spermatozoa (%)	77.33[b]	86.55[a]	85.44[a]	87.11[a]	0.95	0.001
Dead sperm (%)	22.67[a]	13.45[b]	14.56[b]	12.89[b]	0.95	0.001
Sperm abnormalities (%)	13.77[a]	9.38[b]	9.44[b]	9.00[b]	0.55	0.001
Semen quality factor	132.12[c]	331.34[ab]	261.78[b]	360.71[a]	28.11	0.001
pH	7.23[a]	6.97[b]	7.02[b]	7.01[b]	0.04	0.003
Acrosome damage (%)	14.54[a]	10.33[c]	11.70[b]	9.83[c]	0.34	0.001

C = control (non-exposure to heat shock program); HSE30, HSE60, and HSE30+60 = buck rabbits were subjected to a heat shock program (36±1°C for 3 hours from 12:00 to 15:00 for three days). The second treatment (HSE30) was administered at 30 days of age; the third (HSE60) was administrated at 60 days of age; and the fourth (HSE30+60) was administrated at 30+60 days. [a-c] Means bearing different superscripts within the same row are significantly different (P≤ 0.05).

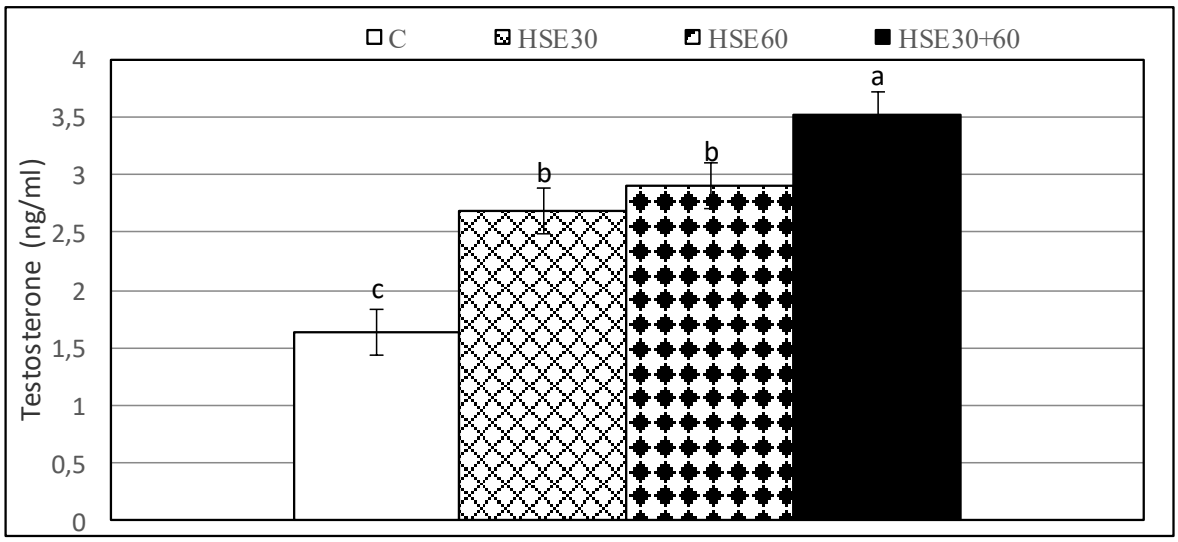

Figure 3. Effect of heat shock programs on blood testosterone hormone of buck Hi-Plus rabbits.
C = control (non-exposure to heat shock program); HSE30, HSE60, and HSE30+60 = buck rabbits were subjected to a heat shock program (36±1°C for 3 hours from 12:00 to 15:00 for three days). The second treatment (HSE30) was administered at 30 days of age; the third (HSE60) was administrated at 60 days of age; and the fourth (HSE30+60) was administrated at 30+60 days. [a-c] Means bearing different superscripts are significantly different (P≤ 0.05).

So, exposure of rabbits to heat stress conditions caused decrease of T_3 level and decreased heat production and sustain homoeothermic (Attia *et al.*, 2016). However, the increase in T_3 hormone of the buck rabbits exposed to heat shock programs might clarified the release of HSP70 which might play an important role to maintaining metabolic rate and reducing the harmful effects of stress (Yahav and Mc-Murtry, 2001).

Corticosterone hormone decreased (P≤0.05) in the buck rabbits of heat shock exposure (HSE30, HSE60 and HSE30+60) by 38.75, 44.17 and 37.02 %, respectively as compared to control group (Figure 2). These results agree with the results of Ahmed–*et al.* (2012), Morsy (2013) and Morsy (2018)and Sakr *et al.* (2019). Corticosterone hormone is more effective biological indicator of acute stress response (Siegel,

1995), evidently shows induction of the physiological stress response. Decrease the corticosterone hormone level in the groups of heat shock exposure might suggest that the buck rabbits achievement to tolerate to the heat stress conditions.

Semen Characteristics

Physical semen quality, reaction time and testosterone hormone were improved (P≤0.05) in buck rabbits of HSE30, HSE60 and HSE30+60 as compared to the bucks in control group (Table 5 and Figure 3). Buck rabbits of HSE30+60 showed the highest values of ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm, semen quality factor and testosterone hormone. However, it recorded the lowest values of dead spermatozoa, sperm abnormalities and acrosome damage. The significant improvement in semen characteristics in heat shock exposure groups may be attributed to increase expression of HSP70 which stimulates testicular growth in the early phase and promotes increased semen volume and sperm concentration (Morsy, 2013). Furthermore, HSP70 protects the seminiferous epithelial cell differentiation against heat stress damage, which is demonstrated in increase semen quality characteristics and/ or maintain homeostasis under the stress conditions (Eddy, 1999, Solanki *et al.*, 2023 and Obidi *et al.*, 2008). In addition, Garcia-Cardena *et al.* (1998) reported that HSE could activate nitric oxide synthase, which is beneficial to sperm motility.

More generally, HSP70 helps sustain spermatogenesis under stressful circumstances by shielding germ cells from oxidative stress and death. Cells are shielded by HSPs from both internal and external stresses. The molecular and cellular aspects of HSPs several essential functions in fertility and reproduction are only now being studied. Fertilization is hampered by impaired HSP70 expression in spermatozoa. Nevertheless, more studies on HSP70 as a sperm quality molecular biomarker are required due to the lack of understanding regarding its molecular foundations and mechanisms (Rosyada *et al.*, 2022).

On the other hand, testosterone hormone was increased (P≤0.05) in the buck rabbits of HSE30, HSE60 and HSE30+60 by 64.02, 76.82 and 115.24 %, respectively as compared with control group.

Testosterone hormone is essential for spermatogenesis (Castro *et al.*, 2002). High amounts of HSP70 are produced by the testis germ cells to provide protection against damage and maintain homeostasis under the stress condition (Morsy, 2013). The result of the present study may be attributed to heat shock exposure program induces cells to synthesize a group of polypeptides known as heat shock protein (Morimoto *et al.,* 1990). HSP70 is a potential protein to protect sertoli and leydig cells against heat stress, and may function as a marker of thermotolerance in cells (Parminder and Bansal, 2003). However, the reduction in testosterone hormone level in control group might be attributed to the decerased ability of leydig and sertoli cells to respond to LH leading to reduced biosynthesis of testosterone (Gomes *et al.*, 1971).

HSP70 is highly elevated in rabbit testes during heat exposure; this is thought to be a defensive response against heat-induced germ cell damage and apoptosis rather than a sign of healthy reproductive state (Pei *et al.*, 2011). A structural foundation for the function of HSP70 family proteins in spermatogenesis and stress responses is provided by developmental work mapping the expression of these proteins in rabbit testes, primarily germ cells (Wu *et al.*, 2011).

CONCLUSION

Early-age heat shock exposure programs enhanced thermotolerance in buck rabbits by increasing HSP70 expression, which was associated with improved physiological responses, hematological parameters, hormonal balance, and semen quality under hot environmental conditions. These findings suggest that early thermal conditioning may be a useful management strategy to improve the reproductive performance of rabbits raised in hot climates.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

Abd El-Kafy E.M., W.A. Ali, A.S. Hoda, and A.A Azoz. 2008. Effect of short heat exposure, balanced feed restriction and acetic acid supplement at post weaning on growth and thermoregulation in growing rabbits during hot season. 9th World Rabbit Congress, June 10,13, Verona, Italy.

Aldahhan, R., & Stanton, P. (2021). Heat stress response of somatic cells in the testis. Mol. Cel. Endocrinol. 527. https://doi.org/10.1016/j.mce.2021.111216.

Ahmed, N. A., M.H. Amal, G.M. Mehaisen and K.R. Emam. 2012. Effect of using heat shock programs on thermoregulation responses and performance of laying hens under desert conditions. Egyp. Poul. Sci., 32 (4): 777-790.

Akiyama, M. 1999. In vivo scavenging effect of ethylcysteine on reactive oxygen species in human semen. Nippon Hinyokika Gakkai Zasshi, 90 (3), 421-428. https://doi.org/10.1111/j.1439-0272.1997.tb00305.x.

Attia, Y.A., A.E. Abd El-Hamid, A.A. Abedalla, M.A. Berika, M.A. Al-Harthi, O. Kucuk, K. Sahin, and B.M. Abou-Shehema. 2016. Laying performance, digestibility and plasma hormones in laying hens exposed to chronic heat stress as affected by betaine, vitamin C, and/or vitamin E supplementation. Sprin. Plus, 5: 1619: 2-12. https://doi.org/1010.1186/s40064-016-3304-0.

Castro A.C.S., W.E. Berndtson, and F.M. Cardoso. 2002. Plasma and testicular testosterone levels, volume density and number of Leydig cells and spermatogenic efficiency of rabbits. Braz. J. Med. Biol. Res. 35:493–498. https://doi.org/10.1590/S0100-879X2002000400014

Ding, H., Y. Wang, H. Zhao, J. Wang and D. Huang. 2025. Negative effect of seasonal heat stress on testis morphology and transcriptomes in Angora rabbit. BMC Genomics, 26. https://doi.org/10.1186/s12864-025-11659-x.

Duncan, D.B. 1955. Multiple range and F. test. Biometric., 11:1-42. https://www.jstor.org/stable/3001478.

Ebeid, T., H. Aljabeili, I. Al-Homidan, Z. Volek and H. Barakat. 2023. Ramifications of heat stress on rabbit production and role of nutraceuticals in alleviating its negative impacts: an updated review. Antioxidants, 12. https://doi.org/10.3390/antiox12071407.

Eddy, E. 1999. Role of heat shock protein HSP70-2 in spermatogenesis. Rev. of reprod., 4 1, 23-30. https://doi.org/10.1530/ror.0.0040023.

El-Kaiaty, A.M. and M.S.H. Hassan. 2004. Some physiological and immunological parameters in the female of local chicken strains. Egyp. Poul. Sci., 24: 901- 916.

El-Ratel, I., A. Mekawy, S. Hassab and S. Abdelnour. 2025. Enhancing growing rabbit heat stress resilience through dietary supplementation with natural antioxidants. BMC Vet. Res., 21. https://doi.org/10.1186/s12917-024-04466-1.

Ezzat, S., I. Elwerdany, A. Galal and M. Madkour. 2020. Effect of early age heat stress on growth performance and some blood parameters of two strains of rabbits. Arab Univ. J. of Agric. Sci., 27, 2621-2629. https://doi.org/10.21608/ajs.2019.18072.1101.

Fadl, A., H. Samir and A. Shahat. 2024. Repeated injections of vitamin E and Se improves testicular morphology, testosterone and in vitro and in vivo sperm fertility in subfertile rabbits. Vet. Res. Commun., 48, 3157 - 3166. https://doi.org/10.1007/s11259-024-10439-4.

Faisal, B.A., S.A. Abdel–Fattah, Y.M. El–Hommosany and F. M. Maie. 2008. Immunocompetence, hepatic heat shock protein 70 and physiological responses to feed restriction and heat stress in two body weight lines of Japanese quail. Int. J. Poul. Sci., 7 (2): 174-183 https://doi.org/10.3923/ijps.2008.174.183.

Garcia-Gardena, G., R. Fan, V. Shah, R. Sorrentino, G. Cirino and A. Papapetropoulos 1998. Dynamic activation of endothelial nitric oxide synthase by HSP90. Nature, 392: 821-4. https://doi.org/10.1038/33934.

Gomes, W.R., W.R. Butler and A.D. Johnson. 1971. Effects of elevated ambient temperature on testis and blood levels and In vitro biosynthesis of testosterone in the ram. J. of Anim. Sci., 33: 804-807. https://doi.org/10.2527/jas1971.334804x.

Hahn, G.M., and G.C. Li. 1990. Thermotolerance, thermoresistance, and thermosensiti-

zation. In: Stress Proteins in Biology and Medicine. (Eds. Morimoto, R.I.; Tissieres, A. and Geogopoulos, C.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, p. 79-100. https://doi.org/10.1101/0.79-100.

Huang, D., J. Cai, C. Zhang, R. Jin, S. Bai, F. Yao, H. Ding, B. Zhao, Y. Chen, X. Wu, and H. Zhao. 2023. Semen quality and seminal plasma metabolites in male rabbits (*Oryctolagus cuniculus*) under heat stress. PeerJ, 11. https://doi.org/10.7717/peerj.15112.

Liang, Z., F. Chen, S. Park, B. Balasubramanian and W. Liu. 2022. Impacts of heat stress on rabbit immune function, endocrine, blood biochemical changes, antioxidant capacity and production performance, and the potential mitigation strategies of nutritional intervention. Frontiers in Vet. Sci., 9. https://doi.org/10.3389/fvets.2022.906084.

Luzi, F., L. Maertens, P. Mijtem and F. Pizzi. 1996. Effect of feeding level and dietary protein content on libido and semen characteristics of bucks. 6th World Rabbit Congress, Toulouse, France, 2: 87-92.

Madkour, M., M. Aboelenin, W. Shakweer, S. Alfarraj, S. Alharbi, S. Abdel-Fattah and M. Alagawany. 2021. Early life thermal stress modulates hepatic expression of thermotolerance related genes and physiological responses in two rabbit breeds. Ital. J. of Ani. Sci., 20, 736 - 748. https://doi.org/10.1080/1828051x.2021.1914207.

Marai, I.F., M.S. Ayyat and U.M. Abdel-Monem. 2001. Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. J. Trop. Anim. Heal. Prod., 33:1-12. https://doi.org/10.1023/A:1012772311177.

Mashaly., M.M., G.L. Hendricks, M.A. Kalama, A.E. Gehad, A O. Abbas and P.H. Patterson. 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poul. Sci., 83:889-894. https://doi.org/10.1093/ps/83.6.889.

Mine, Y., F. Fujita, T. Murase, S. Ito, M. Takatsuki, K. Ikematsu and S. Eguchi. 2019. Heat shock protein 70 messenger RNA in rat leukocytes elevates after severe intestinal ischemia-reperfusion. The J. of surg. Res., 242, 342-348. https://doi.org/10.1016/j.jss.2019.04.074.

Mocé E., R. Lavara, F. Lavara and J.S. Vicente. 2000. Effect of reproductive rhythm on seminal parameters from a rabbit line selected with high growth rate. In Proc. 7th World Rabbit Congress, Valencia, Spain, Vol. A, 197-201.

Morimoto, R.I., A. Tissieres and C. Georgopoulos. 1990. The stress response, function of the proteins, and perspectives. In: Stress Proteins in Biology and Medicine. (Eds. Morimoto, R.I., Tissieres, A. and Georgopoulos, C.) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 1-36. https://doi.org/10.1126/science.572-a.

Morsy, A.S. 2013. Effect of heat shock exposure on the physiological responses and semen quality of male chickens under heat stress conditions. Egyp. Poul. Sci., 33, (1): 143-161.

Morsy, A.S. 2018. Hematological parameters and productive performance of heat-stressed laying hens as influenced by early heat shock program, sodium bicarbonate and/or vitamin C supplementation. Res. J. Anim. and Vet. Sci., 10(2): 1-8. https://doi.org/10.22587/rjavs.2018.10.2.1

Mutwedu, V., A. Nyongesa, J. Oduma, J. Kitaa and J. Mbaria. 2021. Thermal stress causes oxidative stress and physiological changes in female rabbits. J. of therm. Bio., 95, 102780. https://doi.org/10.1016/j.jtherbio.2020.102780.

NRC, 1994. Nutrient Requirements of Poultry. 9th edition. National Academic Press. Washington, DC. Availabe at: Chttps://www.scirp.org/(S(oyulxb452alnt1aej1nfow45))/reference/ReferencesPapers.aspx? ReferenceID=1955728.

Obidi, J. A., B.I. Onyeanusi, P.I. Rekwot, J.O. Ayo and T. Dzenda. 2008. Seasonal variations in seminal characteristics of Shika-brown breeder cocks. Int. J. of Pou. Sci., 7, 12:1219–1223. https://doi.org/10.3923/IJPS.2008.1219.1223.

Oladele, S.B., S. Ogundipe, J.O. AYO and K.A.N. Esievo. 2001. Effect of season and

sex on packed cell volume, haemoglobin and total proteins of indigeneous pigeons in Zaria, Northern Nigeria. Veterinarski Arhiv., 71: 277-286.

Pardede, B., A. Kusumawati, M. Pangestu and B. Purwantara 2023. Bovine sperm HSP-70 molecules: a potential cryo-tolerance marker associated with semen quality and fertility rate. Frontiers in Vet. Sci., 10. https://doi.org/10.3389/fvets.2023.1167594.

Parminder, K. and M.P. Bansal. 2003. Effect of oxidative stress on the spermatogenic process and hsp70 expressions in mice testes. Ind. J. of Bioch. & Biophy., 40: 246-251. https://doi.org/10.1002/jbt.20276.

Pei, Y., Y. Wu and Y. Qin. 2011. Effects of chronic heat stress on the expressions of heat shock proteins 60, 70, 90, A2, and HSC70 in the rabbit testis. Cell Stress and Chaperones, 17, 81-87. https://doi.org/10.1007/s12192-011-0287-1.

Rosyada, Z., M. Ulum, L. Tumbelaka, D. Solihin, B. Purwantara and E. Memili. 2022. Implications of sperm heat shock protein 70 -2 in bull fertility. Vet. World, 15, 1456 - 1466. https://doi.org/10.14202/vetworld.2022.1456-1466.

Sakr, O.G., B.H. Mousa, K.R.S. Emam, A.S. Morsy, A. Nagwa. 2019. Effect of Early Heat Shock Exposure on Physiological Responses and Reproduction of Rabbits under Hot Desert Conditions. World's Vet. J. 9(2): 90-101. https://dx.doi. org/10.36380/scil.2019.wvj13.

SAS, 2004. SAS/STAT User's Guide (Version 8.2). SAS Inst. Inc., Cary NC, USA.

Siegel, H.S. 1995. Stress, strains and resistance. Brit. Poul. Sci., 36: 3-22. https://doi.org/10.1080/00071669508417748

Smith, J.T. and D.T. Mayer. 1955. Evaluation of sperm concentration by the haemocytometer method. Fert. and Ster., 6: 271-275. https://doi.org/10.1016/S0015-0282(16)31987-2.

Solanki, G., Singh, V., Kavani, F., Dhami, A., Savsani, H., Kumar, B., and Patbandha, T. (2023). Seasonal influence on expression of heat shock proteins (HSP70 and HSP90) vis -à-vis functional competence of Gir bull semen. Anim. Biotechnol, 34, 3739 - 3748. https://doi.org/10.1080/10495398.2023.2218453.

Tao, X., Z.Y. Zhang, H. Dong, H. Zhang and H. Xin. 2006. Responses of thyroid hormones of market-size broilers to thermoneutral constant and warm cyclic temperatures. Poul. Sci., 85:1520-1528. https://doi.org/10.1093/ps/85.9.1520.

Watson, P.F. 1975. Use of Giemsa stain to detect changes in acrosomes of frozen ram spermatozoa. Vet. Res., 97: 12-15. https://doi.org/10.1136/vr.97.1.12.

Wu, Y., Y. Pei and Y. Qin 2011. Developmental expression of heat shock proteins 60, 70, 90, and A2 in rabbit testis. Cell and Tissue Res., 344, 355-363. https://doi.org/10.1007/s00441-011-1151-4.

Yahav, S. and J.P. Mcmurtry. 2001. Thermotolerance acquisition in broiler chickens by temperature conditioning early in life-the effect of timing and ambient temperature. Poul. Sci., 80: 1662–1666. https://doi.org/10.1093/ps/80.12.1662.

Yahav, S., S. Goldfeld, I. Plavnik and S. Hurwitz. 1995. Physiological response of chickens and turkey to relative humidity during exposure to high ambient temperature. J. Biol. Thermo., 20: 245–253. https://doi.org/10.1016/0306-4565(94)00046-L.