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Effect of Early Heat Shock Exposure on Physiological Responses and Reproduction of Rabbits under Hot Desert Conditions

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ABSTRACT

This study aimed to apply early heat shock exposure programs for releasing HSP70 gene expression to improve production of rabbits reared under hot desert conditions at Egypt. 120 Hi-Plus rabbits, one-day old were randomly divided into six equal treatments (20 rabbits/ treatment), namely T1, T2, T3, T4, T5 and T6. T1 served as control. The rabbits of second, third, fourth, fifth and sixth treatments were exposed to heat shock (36±1 °C for 3 hours from 12:00 - 15:00 for three successive days). Rabbits of T2, T3, T4, T5 and T6 were exposed to heat shock at 3, 25, 60, 3+25 and 3+25+60 days of age, respectively. HSP70 expression and tri-iodothyronine hormone in the rabbits of T2, T3, T4, T5 and T6 were significantly increased. Rectal and fur temperatures, respiration rate, alanine transaminase, corticosterone hormone levels and overall mortality rate significantly decreased in the rabbits exposed to heat shock programs. Red blood cells count, packed cell volume and hemoglobin concentration increased in the rabbits of T2, T3 and T4. Total protein and globulin concentrations increased in the rabbits of T5 when compared to the rabbits of T1, T2 and T6. However, rabbits of T2 and T4 showed an increase in total antioxidant capacity when compared to the rabbits of T1. Conception rate was higher in the does of T5 than that in T3, T4 and T6. Litter traits, productive efficiency index, feed conversion and cost of feeding improved in the rabbits exposed to heat shock programs. In conclusion, applying heat shock exposure programs of rabbits especially T3 treatment, might increase HSP70 gene expression, this led to enhance immunity responses and production under severe heat stress conditions.

Key words: Heat stress, HSP70, Physiological responses, Productive and reproductive performance, Rabbits

INTRODUCTION

In Egypt, rabbit production suffered from heat stress conditions which considered as one of the important environmental stressors challenging rabbit production especially in desert areas (Morsy et al., 2011 and Nagwa et al., 2012). Many authors reported the deleterious effects of heat stress on physiological responses, productive and reproductive performance of rabbits (Sakr, 2003; Abdel-Samee et al., 2005 and Morsy, 2007).

Exposure of rabbits to heat stress conditions led to modulate their physiological responses to dissipate the latent heat (Morsy, 2007). Marai et al. (1994 and 1996) and Lebas et al. (1997) reported that rabbit's heat production and losses vary to maintain body temperature by using three devices for heat loss; general body position, respiration rate and peripheral temperature (temperature of ear). Under severe heat stress, rabbits use strategies that include; depression in feed intake, efficiency of feed utilization, disturbances in water, protein, energy and mineral metabolism balances, enzymatic reactions, hormonal secretions, blood metabolites and depressed immune function (Nagwa et al., 2005; Morsy, 2007 and Morsy et al., 2011).

Heat stress conditions accompanied with a peak of mortality rate in rabbits. Many authors reported 25-75 % increase of mortality rate in rabbits reared under hot weather conditions of Egypt and may be attributed this higher percentage to inability of regulate body temperature under heat stress conditions (Nagwa et al., 2005; Morsy, 2007 and Morsy et al., 2011). Currently, heat shock exposure programs during earlier age are alternative practice to increase thermo-tolerance and acclimate rabbits to heat stress conditions which led to minimize heat-related mortality, enhance productive and reproductive performance, feed conversion and enhance immune responses (Yalcin et al., 2001; De Basilio et al., 2003; Rahimi, 2005; Hanan, 2006; Faisal et al., 2008; El-Badry et al., 2009; Star et al., 2009; Zulkifli et al., 2009; El-Moniary et al., 2010; Nagwa et al., 2012; Emam, 2013; Morsy, 2013 and Morsy, 2018). Heat Shock Proteins (HSP's) have been noticed in every cell type and tissues. Exposure of rabbits to heat stress conditions during growth

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period led to induce HSP70. Members of HSP70 protein family act 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). Therefore, this study aimed to release the HSP70 gene expression by applying heat shock exposure programs at early ages and investigate its effects on physiological responses, productive and reproductive performance of Hi-Plus rabbits under hot desert conditions of South Sinai, Egypt during production period.

MATERIALS AND METHODS

Experimental region

The present study was carried out in South Sinai research station, located at Ras Suder that belongs to the desert research center, ministry of agriculture and land reclamation, Egypt. The experiment started at January 2016 up to August 2016. Laboratory work was carried out in the laboratories compound of desert research center.

Ethical approval

This experiment was performed according to all ethics and animal rights (desert research center, Egypt). As much as this work had considering all rules and regulations in conformity with the European union directive for the protection of experimental animals (2010/63/EU).

Experimental animals and feeding and management

The experimental rabbits were kept under the same managerial, hygienic conditions and examined clinically safe and free from internal and external parasites. Rabbits were vaccinned to keep them healthy. At production period, rabbits were individually housed in standard dimensions (50×60×40 cm) wired metallic cages attached with nest box (40×30×27 cm) for kindling and nursing. Cages were equipped with feeding hoppers. The rabbits fed, ad-libitum, a commercial concentrate pelleted diet containing 18.0% crude protein, 16.0% crude fiber, 2.5% fat, 0.6% minerals mixture and 2600 kcal/kg digestible energy according to NRC (1994). Fresh water was available all day through nipples drinker system.

Experimental design

120 one-day old of Hi-Plus rabbits were randomly divided into six equal treatments (20 rabbits/treatment). The first treatment (T1) served as control (non-exposure to heat shock program). The rabbits of second, third, fourth, fifth and sixth treatments were exposed to heat shock program (36±1 °C for 3 hours from 12:00 to 15:00 for three successive days). The second treatment (T2) exposed at three days of age, the third treatment (T3) exposed at 25 days of age, the fourth treatment (T4) exposed at 60 days of age, the fifth treatment (T5) was exposed at 3 and 25 days of age and the sixth treatment (T6) exposed at 3, 25 and 60 days of age. After the end of heat shock exposure all treatments returned to be reared under natural conditions. During productive period, all treatments reared under heat stress conditions.

Ambient temperature and relative humidity

Table 1 indicated monthly indoor climatic conditions recorded during the experimental period using electronic digital thermo-hygrometer (Model 303, China). The relationship between ambient temperature and relative humidity was termed as Temperature-Humidity Index (THI) and calculated according to Marai et al. (2001).

$$THI = db°C – [(0.31 – 0.31 × RH) × (db°C – 14.4)]$$

Where, db°C = dry bulb 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 conditions of South Sinai, Egypt

Months	Minimum AT (0C)	Maximum AT(0C)	Minimum RH (%)	Maximum RH (%)	Minimum THI	Maximum THI
January	9.3±0.54	18.1±0.38	34.2±1.24	58.3±2.30	10.3±0.54	17.6±0.82
February	10.6±0.88	17.9±0.66	31.2±1.09	57.2±1.11	11.4±0.57	17.4±0.63
March	12.2±0.50	21.1±0.55	33.1±1.53	53.6±0.99	12.7±0.53	20.1±0.33
April	13.4±0.87	26.0±1.11	31.0±1.88	43.9±1.65	13.6±0.87	24.0±1.1
May	19.4±0.63	30.2±0.44	27.2±2.11	46.5±1.32	18.3±0.52	27.6±0.38
June	24.1±0.24	33.9±0.48	25.9±1.22	41.6±1.43	21.9±0.22	30.4±0.35
July	23.8±0.81	35.6±0.78	25.3±1.98	42.1±2.10	21.6±0.55	31.8±0.66
August	25.1±0.61	35.9±0.71	29.8±1.65	49.6±1.60	22.8±0.37	32.5±0.81

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

Thermo-respiratory responses

Thermo-respiratory responses of rabbits were randomly measured on 10 rabbits / treatment during production period. The measurements were monthly recorded at 2:00 pm. Rectal Temperature (RT) was measured by inserting a clinical thermometer two cm in the rectum for one minute. Temperatures of Skin (ST), Fur Temperatures (FT) and Ear Temperatures (ET) were recorded using digital tele-thermometer Digitemp D2000/D2010 (Morsy et al., 2011). Respiration rate (breaths/min) was determined by counting the frequency of flank movements/minute (Nagwa et al., 2012).

Blood samples

Monthly blood samples were taken from the marginal ear vein into EDTA treated tubes (five rabbits/treatment). Hemoglobin (Hb) concentration, Red Blood Cells (RBC's) count and Packed Cell Volume (PCV%) were determined immediately by the coulter (HA-VET, Clinding, Belgium). Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated by the following equations: MCH (in pico gram, pg) = (Hb content g/dl × 10) / RBCs in million. MCHC (%) = (Hb content × 100) / PCV %. MCV (femtoliter, fl) = (PCV % × 10)/ RBCs in million. The rest of the blood was centrifuged for 15 minutes at 3500 rpm to collect plasma before being stored at -70^o Celesius for determination of HSP70, blood hormones (progesterone, corticosterone and tri-iodothyronine) and blood metabolites (total protein, albumin, glucose, cholesterol, alanine transaminase, aspartic transaminase and total antioxidant capacity). Blood metabolites were determined calorimetrically by using commercial kits. Concentration of HSP70 was determined by ELISA kit of Uscn Life Science Inc. Wuhan, Chain. Specificity of this assay has high sensitivity and excellent specificity for detection of gallinaceous HSP70. Concentrations of tri-iodothyronine, corticosterone and progesterone hormones were determined by ELISA kits of Monobind Inc. Lake Forest, USA.

Reproductive and productive performance

Conception rate (%), number of services per conception were calculated. Gestation period (day) and litter traits were recorded. Milk yield of the doe was estimated by deprivation of the litters from suckling for 24 hours. The litters were weighed before and after suckling and the increase in litters' weight was used as the doe milk yield. Stillbirth (%), pre-weaning mortality (%) and overall of mortality rate (%) were recorded.

Daily feed intake (g) was measured (offered diet–residual diet), total feed intake (Kg)= daily feed intake×123 days. Productive efficiency index (kg, live weight) = litter size at weaning× number of parities × total weaning weight (kg), cost of feed for producing one Kg live weight of rabbit = feed conversion × price of one kg feed and feed conversion of doe = total feed intake (kg) of doe / productive efficiency index (kg, live weight) of doe were calculated.

Average Daily Gain (ADG) and Relative Growth Rate (RGR) were calculated throughout the suckling period (28 days). ADG = (weaning weight – birth weight) /28 days. RGR = [(weaning weight – birth weight)/ 0.5× (weaning weight +birth weight)] × 100.

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: $Y_{ij}= \mu + T_i + e_{ij}$

Y_{ij}= Any observations of i^{th} rabbit within j^{th} treatment

μ = Overall mean

T_i = Effect of i^{th} treatment, (i: 1-6)

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). Mortality rate of does was analyzed by Chi square analysis.

RESULTS AND DISCUSSION

Heat shock protein 70

Early heat exposure program significantly (P< 0.05) increased HSP70 expression (Figure 1) in the rabbits of T2, T3, T4, T5 and T6 by (27.5-42.5 %) as compared to the rabbits of T1 (control group). This result exhibited that early heat exposure program may increase HSP70 expression and might suggesting the proteins involved in the stress caused by heat shock exposure in the rabbits (Maak et al., 2003; Hanan, 2006; Nagwa et al., 2012; Emam, 2013 and Morsy, 2018). Release of HSP70 during heat stress conditions may play an important role in protecting stressed cells and reversing disorders caused by stress through acts 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

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stress (Chirico et al., 1988 and Li and Werb, 1982) and hence it may positively return on rabbit's performance (Abd El-Kafy et al., 2008).

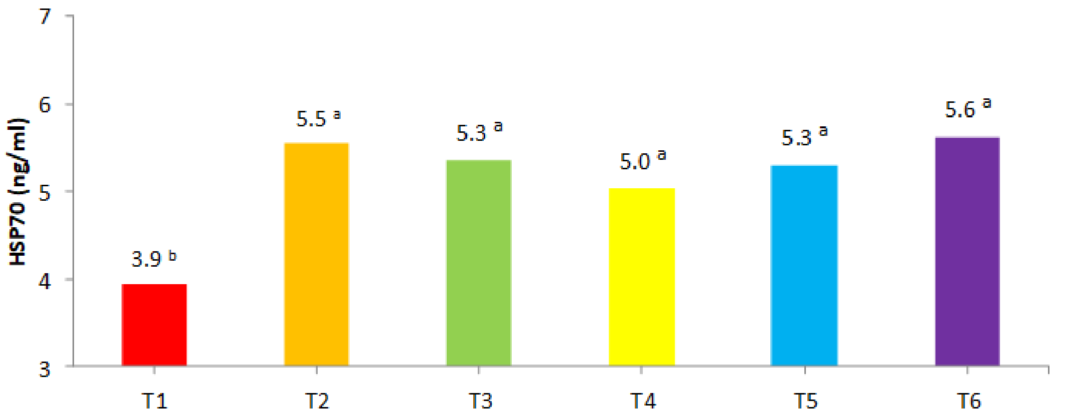

Figure 1. Effect of heat shock programs on formation of heat shock proteins 70 in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, HSP70 = heat shock protein 70, $^{a, b}$ different superscripts among groups means significant (P< 0.05).

Thermo-respiratory responses

RT decreased (P< 0.05) in the rabbits of T3, T5 and T6 by about 0.63, 0.57 and 0.75 ^{o}C, respectively as compared with control group (Table 2). Indeed, FT and RR significantly (P< 0.05) decreased in the rabbits exposed to heat shock programs (T2, T3, T4, T5 and T6) by about 1.1 – 1.5 ^{o}C and 8.7 – 16.7 %, respectively as compared to the rabbits in T1. However, no significant (P> 0.05) differences were detected between treatments in ST and ET.

These results advice that early heat exposure and release HSP70 may enhance thermo-tolerance of rabbits exposed to heat stress at later age (Nagwa et al., 2012 and Morsy, 2013) that enable rabbits to cope any exposure to unexpected heat waves at later ages. Also, the HSP70 may play a critical role in cellular homeostasis during development of thermo-tolerance (Hahn and Li, 1990; Yahav and Hurwitz, 1996; Zhou et al., 1996; Yahav and Mc-Murtry, 2001; Morsy, 2013 and 2018). On the other hand, Abdel-Kafy et al. (2008) reported that the reduction of the RT in the rabbits exposed to heat shock program (34^{o}C) may be due to the fact that rabbits were able to maintain constant RT during heat exposure by low metabolic rate when previously acclimated to high temperature. These results agree with the results of Faisal et al. (2008); El-Badry et al. (2009); Star et al. (2009); Zulkifli et al. 2009 and El-Moniary et al. (2010), they used heat shock exposure programs to acclimate birds to heat stress and enhance some physiological responses of birds.

Table 2. Effect of heat shock programs on thermo-respiratory responses of Hi-Plus rabbits in South Sinai, Egypt.

Items	T1	T2	T3	T4	T5	T6	±SE
Rectal temperature (^{o}C)	39.5^{a}	39.2^{abc}	38.9^{c}	39.4^{ab}	39.0^{bc}	38.8^{c}	0.13
Fur temperature (^{o}C)	34.5^{a}	33.3^{b}	33.3^{b}	33.4^{b}	33.2^{b}	33.0^{b}	0.32
Skin temperature (^{o}C)	36.5	35.7	35.5	35.7	35.5	35.8	0.32
Ear temperature (^{o}C)	37.8	37.5	37.5	37.4	37.5	37.2	0.22
Respiration rate (breath/min.)	185.3^{a}	169.2^{b}	165.1^{bc}	154.2^{c}	162.5^{bc}	163.7^{bc}	3.8

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error, $^{a, b, c}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

Hematological parameters

WBC’s count decreased (P< 0.05) in the rabbits of T2, T3 and T4 by 29.9, 37.6 and 25.1%, respectively as compared to the rabbits of T1 (Table 3). However, RBC's count and PCV increased (P<0.05) in the rabbits of T2 (by 15.5 and 19.8%, respectively), T3 (by 12.2 and 15.2%, respectively) and T4 (by 15.0 and 19.4%, respectively) when compared to the rabbits of T1. In addition, Hb concentration increased (P<0.05) in the rabbits of T2, T3, T4 and T6 by 14.6, 13.4, 14.8 and 15.1%, respectively as compared to the rabbits of T1. However, MCH increased (P<0.05) in the rabbits of T6 by (10.9%) compared with control group (Table 3). There are insignificant (P<0.05) effects of treatments on MCV and MCHC. Enhancement in hematological parameters might attribute to enhancement in the thermo-respiratory responses of rabbits exposed to heat shock exposure (Morsy, 2013). On the other hand, Nagwa et al. (2012); Emam (2013) and Morsy (2018) demonstrated that exposure of hens to heat stress conditions might impair the synthesis of blood cells. Yahav et al. (1997a) reported that hemoglobin increased in acclimated chickens compared with un-acclimated chickens.

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Table 3. Effect of heat shock programs on hematological parameters of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
WBC's (×10^3/mm^3)	10.2^a	7.1^{bc}	6.4^c	7.6^{bc}	9.9^{ab}	8.2^{abc}	0.66
RBC's (×10^6/mm^3)	4.8^b	5.6^a	5.4^a	5.5^a	5.0^{ab}	5.0^{ab}	0.22
Hb (g/dl)	9.9^b	11.4^a	11.3^a	11.4^a	10.7^{ab}	11.4^a	0.36
PCV (%)	31.0^b	37.1^a	35.7^a	37.0^a	33.3^{ab}	33.0^{ab}	1.7
MCV (fl)	64.2	66.8	66.3	66.4	65.9	65.4	1.9
MCH (pg)	20.7^b	21.1^{ab}	21.6^{ab}	20.9^{ab}	21.3^{ab}	23.0^a	0.64
MCHC (%)	33.1	31.9	32.6	31.9	32.8	35.4	1.2

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively ±SE = standard error, 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, b, c] Means bearing different superscripts within the same row are significantly different (P< 0.05).

Metabolites parameters

Total protein and globulin concentrations increased (P< 0.05) in the rabbits of T5 group when compared to the rabbits of T1, T2 and T6 groups (Table 4). Cholesterol concentration significantly (P< 0.05) decreased in the rabbits of T6 group as compared to the rabbits of T4 group. Alanine transaminase concentration decreased (P< 0.05) in the rabbits of T2, T3, T4, T5 and T6 groups (11.9, 12.5, 14.4, 17.1 and 17.7 %, respectively) as compared to the rabbits of T1 (control group). This increase of liver enzyme in the rabbits of T1 might indicate that rabbits were not capable to defeat the heat stress conditions and hence led to adverse effect on liver function (Faisal et al., 2008). Meanwhile, using early heat shock programs and release HSP70 might cause the reductions in the severity of histopathological degeneration in the liver which may occur resulting from exposure of rabbits to severe heat stress (Okolie and Ivoanya, 2003).

However, rabbits of T2 and T4 showed increase (P< 0.05) in Total Antioxidant Capacity (TAC) (70.0 and 73.3%, respectively) when compared to the rabbits of control group (Figure 2). The significant (P< 0.05) increase of TAC and globulin concentrations used as an indicator of immune responses and source of antibodies production (El-Kaiaty and Hassan, 2004; Morsy, 2018). These results agree with findings of Mashaly et al. (2004) and Nagwa et al. (2012). The differences were not significant (P> 0.05) among groups in albumin, glucose and aspartic transaminase concentrations (Table 4). Although, aspartic transaminase concentration insignificantly (P> 0.05) decreased in the heat shock exposure treatments compared with control group.

Table 4. Effect of heat shock programs on biochemical parameters of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
TP (g/dl)	6.2^b	6.1^b	6.7^{ab}	6.7^{ab}	7.1^a	6.3^b	0.18
Albumin (g/dl)	4.1	3.9	4.1	4.1	4.1	4.05	0.11
Globulin (g/dl)	2.1^b	2.2^b	2.5^{ab}	2.5^{ab}	3.0^a	2.2^b	0.20
Glucose (mg/dl)	169.1	161.3	168.9	161.7	169.4	162.6	9.0
Chol (mg/dl)	62.5^{ab}	61.7^{ab}	58.3^{ab}	67.3^a	64.4^{ab}	53.2^b	5.5
ALT (i.u./l)	9.6^a	8.5^b	8.4^b	8.2^b	8.0^b	7.9^b	0.27
AST (i.u./l)	20.6	19.6	20.1	20.0	20.8	18.3	0.85

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error. TP = total protein, Chol = cholesterol, ALT =alanine transaminase, AST=aspartate transaminase, [a, b] Means bearing different superscripts within the same row are significantly different (P< 0.05).

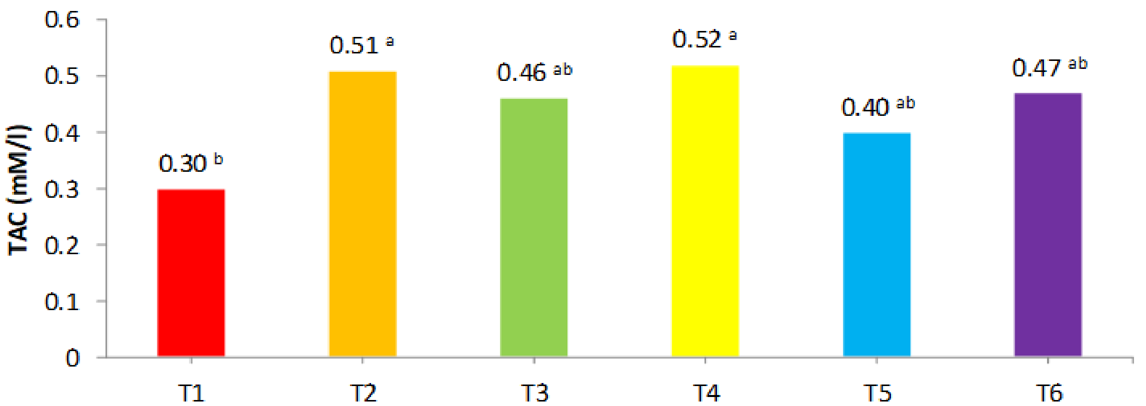

Figure 2. Effect of heat shock programs on total antioxidant capacity in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, TAC = total antioxidant capacity, [a, b] different superscripts among groups means significant (P< 0.05).

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Hormonal profile

Tri-iodothyronine (T_3) hormone increased (P<0.05) in the rabbits of T2, T3, T4, T5 and T6 (7.8, 9.4, 7.5, 6.8 and 7.5 % respectively) as compared to the rabbits of T1 group (Figure 3). T_3 hormone plays an important role in regulating metabolism and thermogenesis (Tao et al., 2006). Concentration of T3 hormone is highly correlated to decrease of feed intake and heat stress conditions (Yahav et al. 1995). Hence, exposure of rabbits to heat stress conditions caused decrease of T3 level and decreased heat production and sustain homoeothermic (Uni et al., 2001; Attia et al., 2016). These results were in agreement with finding of Moraes et al. (2003); Nagwa et al. (2012) and Morsy (2018). However, the increase in T3 hormone of the rabbits exposed to heat shock programs may be explained the release of HSP70 which might play a role for maintaining metabolic rate and/or reducing the harmful effects of stress (Yahav and Hurwitz, 1996; Zhou et al., 1996; Yahav and Plavnik, 1999; Yahav and Mc-Murtry, 2001). However, Corticosterone (Cor) hormone decreased (P< 0.05) in the rabbits of T3, T4 T5 and T6 (62.1, 55.3, 52.8 and 60.7%, respectively) when compared to the rabbits of control group (Figure 4). In opposite, corticosteroid secretion increases as a response to heat stress (Morsy, 2018). The Cor hormone considered a more effective biological indicator of sever heat stress response (Siegel, 1995). However, lower Cor hormone level in the group of heat shock exposure programs submitted that the rabbits success to habituate to the heat stress. These results agree with the results of Star et al. (2009); Nagwa et al. (2012) and Morsy (2013). On the other hand, there was no significant (P> 0.05) difference among groups on progesterone hormone, although it insignificantly (P> 0.05) increased by 12.8 and 19.7% in T3 and T6 groups respectively as compared to the rabbits in T1 group (Figure 5).

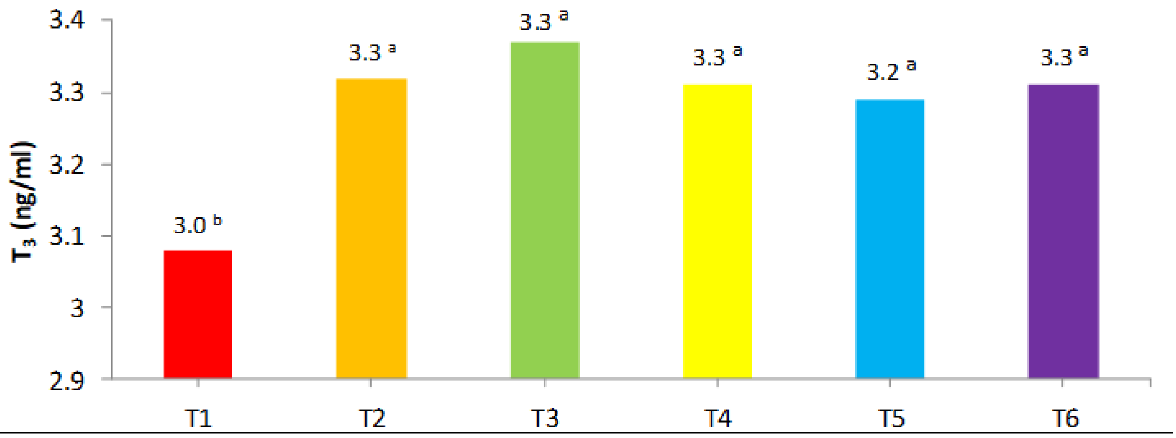

Figure 3. Effect of heat shock programs on tri-iodothyronine hormone in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, T_3 = tri-iodothyronine hormone, [a, b] different superscripts among groups means significant (P< 0.05).

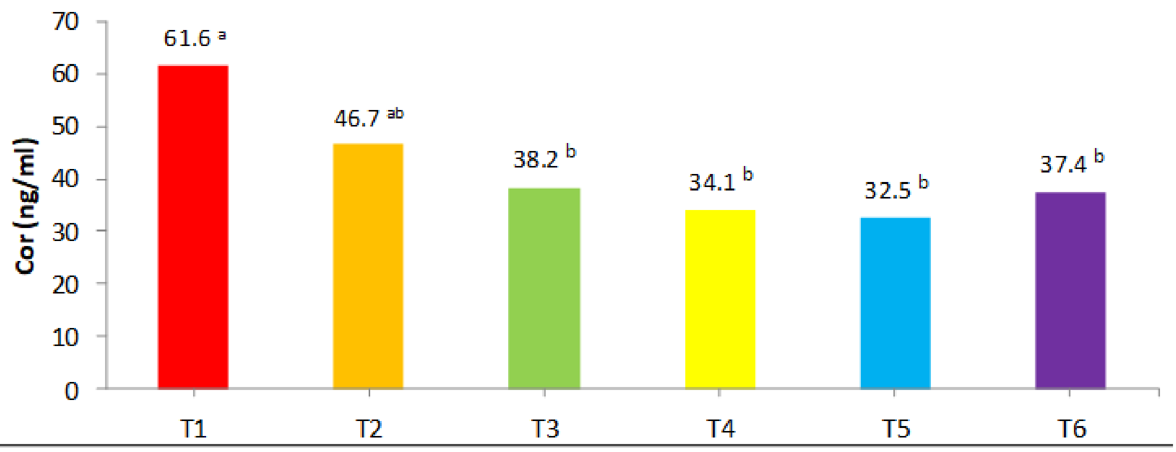

Figure 4. Effect of heat shock programs on corticosterone hormone level in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, Cor = corticosterone hormone, [a, b] different superscripts among groups means significant (P< 0.05).

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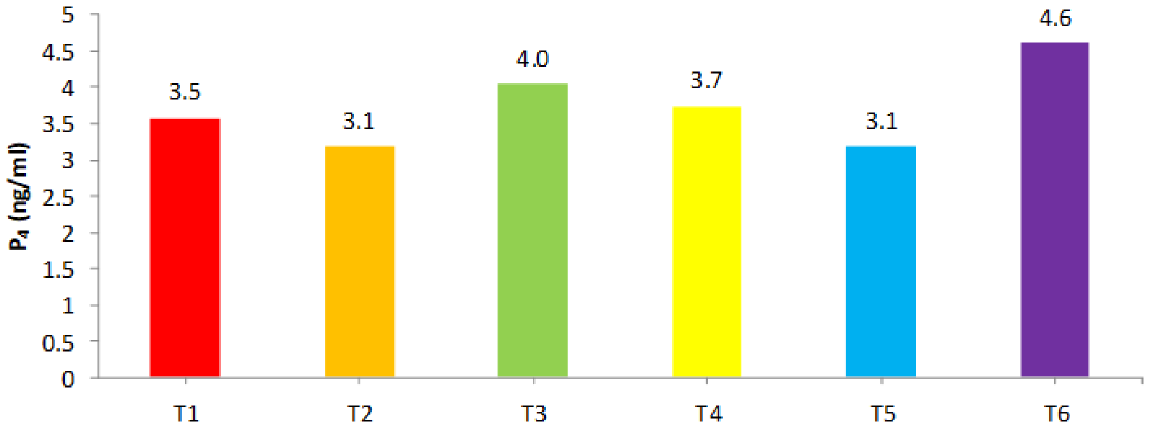

Figure 5. Effect of heat shock programs on progesterone hormone level in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, P_4 = progesterone hormone.

Body weight

Final body weight and body weight changes were significantly (P< 0.05) increased in the does of T2, T3, T4, T5 and T6 groups as compared to the does of control group (Table 5). This increase in final body weight and body weight changes in the does exposed to heat shock exposure during growth period (T2, T3, T4, T5 and T6) might attributed to enhance thermo-tolerance of doe rabbits that would face severe heat stress in advanced or later ages and may be reversed on increased body weight during exposure to heat stress conditions (Faisal et al., 2008; El-Badry et al., 2009; Star et al., 2009; Zulkifli et al., 2009; El-Moniary et al., 2010; Nagwa et al., 2012; Morsy, 2013 and Morsy, 2018).

Table 5. Effect of heat shock programs on doe body weight and body weight changes of Hi-Plus rabbits in South Sinai, Egypt.

Items	T1	T2	T3	T4	T5	T6	±SE
IBW (g)	3104.0	3115.3	3179.3	3194.5	3156.4	3168.1	66.2
FBW (g)	3308.0^{b}	3622.1^{a}	3678.1^{a}	3716.2^{a}	3583.5^{a}	3649.3^{a}	107.3
BWC (g)	204.0^{b}	506.7^{a}	498.7^{a}	521.6^{a}	427.1^{a}	481.2^{a}	67.1

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, IBW = initial body weight, FBW = final body weight, BWC = body weight change. ±SE = standard error. $^{a, b}$ Means bearing different superscripts within the same row are significantly different (P< 0.05).

Reproductive performance

Conception rate was higher (P<0.05) in the does of T5 than that of T3, T4 and T6 groups (Table 6). Furthermore, does in T5 and T1 had insignificant more parities compared to the other treatments. However, number of services/conception was lower (P<0.05) in the does of T5 as compared to the does of T3, T4 and T6 groups. Gestation length significantly (P<0.05) increased in the does of T3 by 2.8% when compared to the does of T1 and insignificantly (P> 0.05) increased in the does of T2, T4, T5 and T6 group by 1.9, 0.63, 1.9 and 1.6%, respectively as compared to the does of T1 (Table 6). These results might attribute to that rabbits exposed to heat shock programs during growth period led to increase gestation period through heat stress conditions. However, release HSP70 which may be suggested to regulate female fertility, enhancing ovulation-inducing and maintaining integrity of the fetal membranes and reducing the frequency of birth defects (Abd El-Kafy, 2006). On the other hand, milk yield significantly (P<0.05) increased in the does of T4 and T6 by 26.4 and 34.5%, respectively as compared to the does of T5 (Figure 6). This increase related to the increasing litter size in both of T4 and T6. These results showed that there is irreversible relationship between milk yield and conception rate. The lower hormonal levels in the suckling rabbits (estrogen hormone) and higher prolactin may reveal poorer ovarian activity, which could result in reducing reproductive efficiency. Our observations confirm the existence of a partial antagonism between lactation and reproduction in rabbits (Marongiu and Dimauro, 2013).

Table 6. Effect of heat shock programs on reproductive performance of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
Number of parity	2.1	1.5	1.7	1.6	2.0	1.6	0.19
CR (%)	51.1^{ab}	52.3^{ab}	45.5^{b}	42.2^{b}	62.3^{a}	45.5^{b}	5.4
NSC	1.9^{ab}	2.1^{ab}	2.4^{a}	2.4^{a}	1.9^{b}	2.3^{a}	0.19
GL (day)	31.4^{b}	32.0^{ab}	32.3^{a}	31.6^{ab}	32.0^{ab}	31.9^{ab}	0.21

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error. GL= gestation length, CR= conception rate, NSC= number of services per conception, $^{a, b}$ Means bearing different superscripts within the same row are significantly different (P< 0.05)

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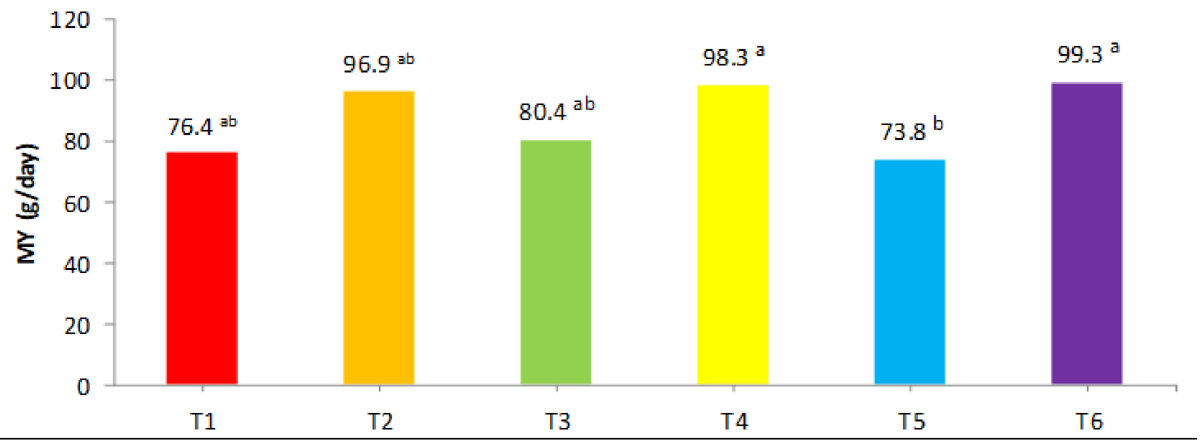

Figure 6. Effect of heat shock programs on milk yield in Hi-Plus doe rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, MY = milk yield, [a, b] different superscripts among groups means significant (P< 0.05).

Litter traits

Viable Litter Size at Birth (VLSB) significantly increased in the does of T6 by about 47.0 and 48.0% as compared to those in T5 and T1, respectively (Table 7). However, there were insignificantly (P>0.05) increased VLSB in the does of T2, T3 and T4 about 32, 25 and 24%, respectively as compared to the does in T1 group. In addition, Litter Size at Weaning (LSW) increased (P< 0.05) in the does of T6, T4, T3 and T2 about 64, 70, 57 and 60%, respectively as compared to the rabbits of T1 group. However, there was insignificantly (P>0.05) increased of LSW in the does of T5 about 25.0% as compared to the does in T1 group. Litter Weight at Birth (LWB) increased (P<0.05) in the does of T2, T4 and T6 groups (43.0, 28.0 and 49.0%, respectively) as compared to the rabbits of control group (T1). Meanwhile, Litter Weight at Weaning (LWW) increased (P<0.05) in the does of T2, T3, T4, T5 and T6 groups as compared to the rabbits in control group (T1). These results indicated that heat shock exposure programs did not have negative effect on litter size and weight at birth which attribute the higher litter size at birth to a role of IGF-1 in protection embryos of mammalian exposed to stress; where the effects of heat shock on total cell number and development to the blastocyst stage (Jousan and Hansern, 2004 and Abd-El Kafy, 2006). In addition, LWW increased (P<0.05) in the does of heat shock programs groups as compared to the does of control group. Exposure of doe rabbits to heat shock exposure at early age led to increase thermo-tolerance and acclimate rabbits at severe heat stress which led to decrease heat-related mortality, improve productive performance and enhance immunity responses. These results agree with the results of Abd El-Kafy (2006); Nagwa et al. (2012) and Morsy (2018).

Table 7. Effect of heat shock programs on litter traits and mortality rate of Hi-Plus rabbits under South Sinai, Egypt conditions

Items	T1	T2	T3	T4	T5	T6	±SE
TLSB	6.4	8.2	7.2	7.8	6.2	8.7	0.80
VLSB	5.6[b]	7.5[ab]	7.1[ab]	7.0[ab]	5.7[b]	8.3[a]	0.76
LSW	3.6[b]	5.8[a]	5.7[a]	6.2[a]	4.5[ab]	6.0[a]	0.62
LWB (g)	268.3[b]	384.3[a]	312.7[ab]	342.2[a]	306.4[ab]	400.8[a]	29.9
LWW (g)	1595.3[c]	2940.0[a]	2831.1[ab]	2795.5[ab]	2248.5[b]	2709.7[ab]	201.7
MRB-W (N)	2.0	1.6	1.3	0.75	1.1	2.3	0.57
MRB-W (%)	35.7[a]	18.3[b]	13.3[b]	9.6[b]	17.6[b]	28.1[ab]	6.7
Stillbirth (n)	0.78	0.75	0.13	0.88	0.57	0.38	0.35
Stillbirth (%)	11.8	7.6	2.5	9.5	5.1	4.3	4.2
Overall MRB-W (%)	47.6[a]	26.0[b]	15.8[b]	19.1[b]	22.8[b]	32.5[ab]	8.8

T1 = control, T2, T3, T4, T5 and T6= rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days+25 days of age and at 3 days+25 days+2 months of age, respectively, ±SE = standard error, TLSB = total litter size at birth, VLSB =viable litter size at birth, LSW = litter size at weaning, LWB= litter weight at birth, LWW= litter weight at weaning, MRB-W = mortality rate from birth to weaning, [a, b, c] Means bearing different superscripts within the same row are significantly different (P< 0.05)

Mortality rate

Results of table 7 declared that Mortality Rate from Birth to Weaning (MRB-W) and overall MRB-W were lower (P<0.05) in does of T2, T3, T4 and T5 groups than control group (T1). These results suggested that release HSP70 synthesis (Figure 1) may protect doe immune function against some forms of stress as reported by Ciavarra and Simone (1990 a and b) and Abd El-Kafy (2006). On the other hand, Yahav et al. (1997 a and b) and Nagwa et al. (2012) reported that early heat shock exposures led to decrease mortality rate and this may be due to enhance thermo- tolerance and immunity. However, Figure 7 demonstrated that heat shock programs led to decrease (P<0.05) mortality in doe Hi-Plus rabbits and this might be due to enhance thermos-tolerance mechanism. Indeed, increasing mortality rate in control group

(T1) may be attributed to inability to regulate body temperature and respiration rate under heat stress conditions as shown in table 2. These results agree with the results of Yahav et al. (1997a); Nagwa et al. (2005) and Abd El-Kafy (2006).

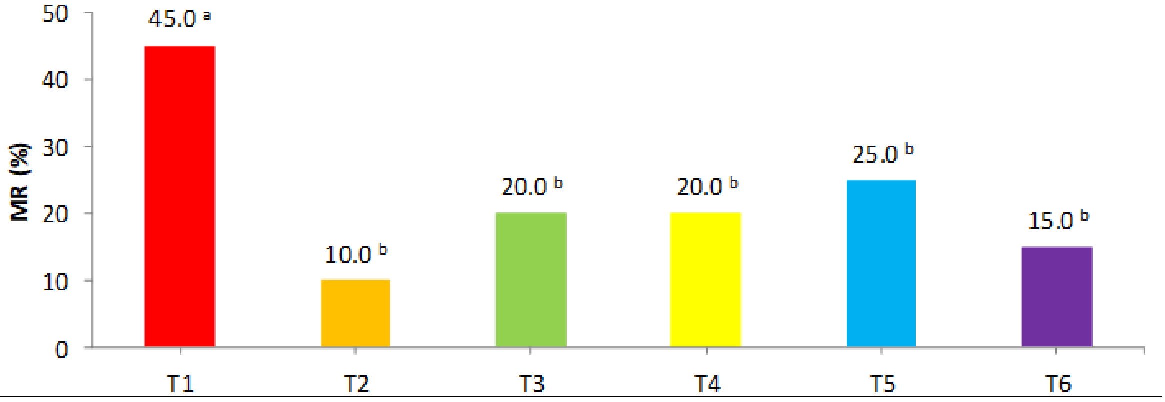

Figure 7. Effect of heat shock programs on doe mortality rate in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, MR = mortality rate, [a, b] different superscripts among groups means significant (P< 0.05).

Offspring's traits

Results in table 8 revealed that early heat shock exposure programs of does had no significant (P>0.05) effect on offspring growth performance, though, does of T2, T3 and T5 had higher bunny weight at weaning and bunny daily gain as compared to does of T1.

Economic indicators

Productive efficiency index and feed conversion were improved (P< 0.05) in the does of T2 (28.8 and 26.1%), T3 (44.3 and 34.3%), T4 (41.5 and 29.5%), T5 (28.8 and 25.6%) and T6 (33.2 and 29.0%), respectively when compared to the does of T1 group (Table 9). However, no significant (P>0.05) differences between treatments in feed intake. Indeed, cost of feeding for producing one kg live weight under desert hot conditions decreased (P<0.05) in the does of T2, T3, T4, T5 and T6 it was 9.2, 12.1, 10.4, 8.9 and 10.2 L.E., respectively as compared to the does of T1 group (Figure 8). This improvement in productive traits might be attributed to application of heat exposure programs (Franco-Jimenez et al., 2007; Emam, 2013 and Morsy, 2018). Heat shock exposure programs at early ages might enhance thermo-tolerance of doe rabbits for facing severe heat stress in later ages (Faisal et al., 2008; El-Badry et al., 2009; Zulkifli et al., 2009; El-Moniary et al., 2010; Nagwa et al., 2012 and Morsy, 2013). So, increase of HSP70 expression might suggesting that the proteins involved in the stress caused by heat shock exposure in the does that play important role in protecting stressed cells and reversing disorders caused by stress (Li and Werb, 1982; Emam, 2013) and hence it reflected on improvement of productive performance of doe rabbits (Abd El-Kafy, 2006).

Table 8. Effect of heat shock programs on offspring's of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
BWB (g)	48.5	52.7	46.9	49.7	56.9	48.5	3.3
BWW (g)	484.1	521.2	545.7	460.1	531.7	490.7	51.4
BDGB-W (g)	15.5	16.7	17.8	14.6	16.9	15.7	1.8
GR (%)	159.8	161.1	166.9	160.4	159.8	162.7	3.4

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error, BWB = bunny weight at birth, BWW = bunny weight at weaning, BDGB-W = bunny daily gain from birth to weaning, GR = growth rate.

Table 9. Effect of heat shock programs on economic indicators of Hi-Plus rabbits in South Sinai, Egypt

Items	T1	T2	T3	T4	T5	T6	±SE
Daily FI (g)	145.3	148.6	148.7	148.0	148.4	146.5	1.4
TFI (kg)	17.8	18.2	18.3	18.2	18.2	18.0	0.17
EI (kg live weight)	3.2^b	4.1^a	4.6^a	4.5^a	4.1^a	4.3^a	0.43
FC	6.4^a	4.7^b	4.2^b	4.5^b	4.7^b	4.5^b	0.64

T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, ±SE = standard error, DFI = daily feed intake, TFI = total feed intake, EI = efficiency index, FC = feed conversion, [a, b] Means bearing different superscripts within the same row are significantly different (P< 0.05)

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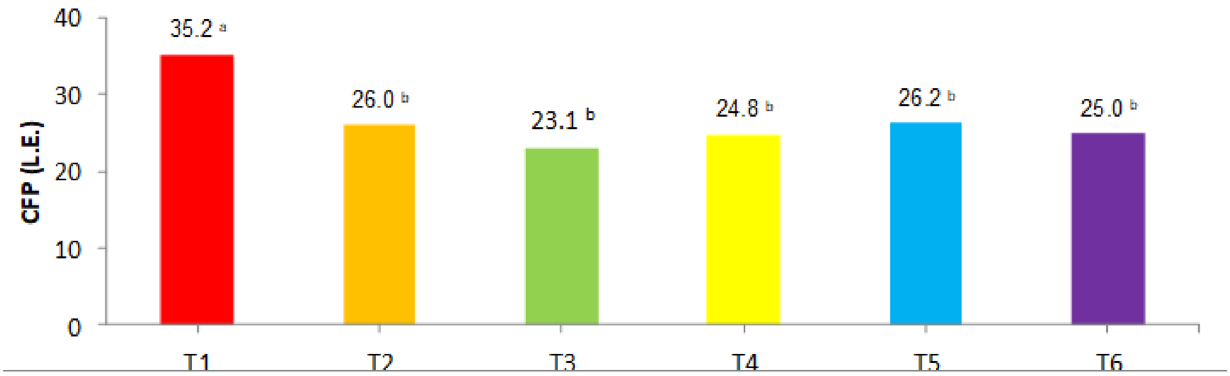

Figure 8. Effect of heat shock programs on cost of feeding for producing one kilogram of live weight in Hi-Plus rabbits under conditions of South Sinai, Egypt. T1 = control, T2, T3, T4, T5 and T6 = rabbits were exposed to heat shock exposure at 3 days, at 25 days of age, at 2 months of age, at 3 days + 25 days of age and at 3 days + 25 days + 2 months of age, respectively, CFP = cost of feeding for producing one kg live weight, [a, b] different superscripts among groups means significant (P< 0.05).

CONCLUSION

In conclusion, applying heat shock exposure programs for doe rabbits especially T3 treatment (rabbits were exposed to heat shock exposure at 25 days of age) might increase HSP70 gene expression and enhance acclimation of doe rabbits, this led to enhance immunity responses, reproductive performance and production under severe heat stress conditions.

DECLARATIONS

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Competing interests

The authors declare that they have no conflict of interests with respect to the research, authorship, and/or publications of this article.

Consent to publish

All the authors approved and agreed to publish the manuscript and declared that this work has not been previously published elsewhere.

Author’s contribution

Dr. Nagwa Abde El-Hady Ahmed designed the experiment, article writing and revision, Dr .Ali Saber Morsy designed the experiment, laboratory analyses, statistical analysis, tabulation of experimental data, manuscript writing, commenting and approval, Dr. Osama Glal Sakr helped in statistical analysis, tabulation of experimental data and article writing; Dr .Khamis Refay Sayed Emam designed the experiment, tabulation of experimental data, manuscript writing, commenting and approval; while, Mr .Baliegh Hamdy Mohammed Mousa helped in field study, collected data, laboratory analyses, manuscript writing. All authors have read and approved the final manuscript.

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