ECRONICON OPEN ACCESS

EC VETERINARY SCIENCE
Research Article

Effect of Mitigation of Heat Stress by Early Heat Acclimation and Glutamine Injection on Some Physiological, Productive and Reproductive Traits of Sinia Chickens

YS Rizk[1], Ehab A Abdallah[1], Doaa MM Yassein[1], Fatma TF Abd-ElGhany[2], Hassan abde-lkarim[1], Nahla A El Razik[1], MH Abdelfattah[1], HA Abdellatif[1] and Osama A El-Sayed[1]*

[1]Poultry Breeding Research, Department Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt

[2]Agricultural. By-product Utilization Research, Department Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt

***Corresponding Author:** Yaser Saddek Rizk, Poultry Breeding Research Department, Animal Production Research Stations, Agriculture Research Centre, Minimum of Agriculture., Giza Egypt.

Received: November 17, 2020; **Published:** December 30, 2020

Abstract

This work aimed to investigate the resistance of female Sinia chickens against hot climate by using both early heat shock exposures (HSE) and glutamine injections (GI). four hundred and fifty one-day old of female Sinia chicks were randomly divided into five groups with three equal replicates to enhance the effect of (GI) and (HSE) on some productive and physiological performance in laying hens during reproductive period under heat stress conditions. The 1st group of chicks was reared under natural conditions and served as a control. The 2nd group of chicks was managed thermally like the control group during growth period, while through reproductive period the female were exposed to heat challenge (38 ±1ºC for four hours from 12 pm till 4pm for one day) at 24, 30 and 34 weeks of age. The 3rd group (HSE1) was injected with glutamine GI (0.75 mg/kg weight intra peritoneal injection (at age of two days) then, exposed to early heat shock (41 ±1ºC for four hours from 12pm till 4pm for 3 consecutive days) at age of 3 days, the 4th group (HSE2) was twice injected with GI at 0.75 mg/kg weight (intra peritoneal injection) at 2 and 34 days of age, then exposed to heat shock (41 ±1ºC for four hours from 12pm till 4pm for 3 consecutive days) at age of 3 days and 35 days. The 5th group (HSE3) was injected three times with GI at 0.75 mg/kg weight (intra peritoneal injection) at 2,3 and 111 days of age, and then, exposed to three times of heat shock (41 ±1ºC for four hours from 12 pm till 4 pm for 3 consecutive days) at ages of 3, 35 and 112 days. During reproductive period, HSE1, HSE2, and HSE3 groups were reared under natural conditions and they were exposed to heat challenge at 24, 30 and 34 weeks of age (similar to that applied for the second group). Results revealed that there was a significant decrease in the values of respiratory rate and rectal temperature for the groups treated with injection and temperature, as well as the T5 (HSE3) group females had a significant increase in both heat shock protein 70, final body weight, total protein, albumin, globulin and hormonal level Progesterone and estrogen, and final body weight. The 5 (HSE3) group showed that females showed a significant increase in egg number.

Keywords: *Early Heat Shock Exposures; Glutamine; Female And Heat Shock Protein70*

Introduction

The climate of Egypt is characterized by a very long hot period of year that extends from May up to October. Chronic heat stress causes disturbance of homeostatic ability of birds [1]. So poultry production suffers from huge economic losses every year resulting from extended period of heat stress [2]. Hyperthermia is associated with biochemical and physiological events disturbing avian homeostasis through excessive formation of reactive oxygen species that cause. Lipid peroxidation in cell membranes and oxidative injury of biological

molecules including proteins and DNA [3]. Drawbacks of heat stress on broilers and laying hens ranges from reduced growth and egg production up to reduction of egg quality and poultry safety due to challenge of productivity and immune response of birds [4]. Moreover Ebeid *et al.* [5] reported that heat stress reduced egg weight and egg shell thickness. They attributed that to reduced calcium level in blood of laying hens under hot conditions as well as increased panting that elevates carbon-dioxide subsequent alkalosis that hampers blood bicarbonate availability for egg shell mineralization. The target of poultry industry in any tropical country is to resist the damaging effects of heat stress by several methods including early heat exposure especially at growth period. It is well established that early short –term sub lethal (acute heat stress) induces heat shock response causing rapid synthesis of heat shock protein (HSE) and dramatic change in gene expression [6]. The heat shock proteins (HSP) are a set of proteins synthesized in response to physical, chemical or biological stresses including heat exposure [7]. Heat shock protein70 (HSP70) is the most conserved and important protein in the family of heat shock proteins, as it plays an important role in protection and repair of injured cells and tissues exposed to heat stress [8]. Dietary additions are the most economically means to resist heat stresses, one of these additives is glutamine that is essential amino-acid used as a potent enhancer of HSP70 synthesis both *in vivo* and *in vitro* [9]. Moreover glutamine improves absorption in digestive system and hence enhancing performance [10]. Diet supplemented with L-glutamine increased significantly levels of follicle stimulating hormone (FSH) and Luteinizing hormone (LH) as well as enhanced egg mass, egg production, egg weight, egg shell thickness, hatchability and one-day chick weight [11]. So, this work was done to investigate enhancement of heat shock protein 70 (HSP70) gene expression by using only heat shock exposures in some groups that were compared with other groups exposed to both heat shocks during growth period and glutamine injection of female Sinia chicken. Also, their effects on productive and physiological performance in laying hens at reproductive period were evaluated.

Materials and Methods

Experimental design and bird's management

This experiment was carried out at EL- Serw Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. The experiment lasted for 8 month from December up to August. A total number of 450 one day old of female Sinia chicks were taken, weighted and randomly divided into equal five experimental groups (90 chicks each), each of three equal replicates. The 1st group was reared under natural conditions without either early heat exposure or glutamine injection and served as a control. The 2nd group of birds was reared under natural conditions during growth period, while through reproductive period (hot summer months Jun to Augusts) the chicks were exposed to heat challenge (38 ± 1°C for four hours from 12 pm till 4pm for one day at 24, 30 and 34 weeks of age. The 3rd group (HSE1) was GI (0.75 mg/kg weight intra peritoneal injection) at age of two days and exposed to early heat shock (41 ± 1ºC for four hours from 12:00 till 16:00 for 3 consecutive days) at age of 3 days, the 4th group (HSE2) was two GI (0.75 mg/kg weight intra peritoneal injection) at 2 and 34 days of age, then exposed to heat shock (41 ±1ºC for four hours from 12:00 till 16:00 for 3 consecutive days) at age of 3 days and 35 days. The 5th group (HSE3) was three GI (0.75 mg/kg weight intra peritoneal injection) at 2,3 and 111 days of age, and exposed to three times of heat shock (41 ±1ºC for four hours from 12:00 till 16:00 for 3 consecutive days) at age of 3, 35and 112 days). During reproductive period, HSE1, HSE2, and HSE3 groups were reared under natural conditions and they were exposed to heat challenge at 24, 30 and 34 weeks of age (similar to that applied for the second group. 38 ±1ºC for four hours from 12 pm till 4pm for one day). The chicks of each group were housed in floor pens in semi-open system.Birds were exposed to natural day-light and artificial light to increase the day light length until reaching 14 hours at 18 weeks of age. Then, the day light length period was increased 30 minutes every week until fixed at 17 hours daily to the end of experiment. Birds were provided with clean fresh water and fed ad-libitum according to recommended standard diets for each age as show in table 1. according to NRC, (1994) [12]. Birds were kept under the same hygienic conditions to protect birds against diseases. At 22 wks of age, females were weighted and adjusted number of birds 30 females for each treatments were continued in the same house for all treatments then fed on the same layer diet to evaluate the subsequent effect of treatments during rearing period on sexual maturity and productive performance till 34 wks of age. In door climate conditions were shown in (Table 2) ambient temperature AT ^{0}C and relative humidity RH% were recorded during the experimental period using electronic digital thermo-hygrometer. The relationship between relative humidity and ambient temperature was determined as temperature-humidity index (THI) according to Maria., *et al.* [13]. THI= db^{0}C - {(0.31-0.31xRH) x (db ^{0}C -14.4)}.

Ingredients %	Starter 0-8 wks	Grower 9-18 wks	Pre-Layer 19-20 wks	Layer 21-40 wks
Yellow Corn	64.00	71.25	69.00	68.00
Soybean meal (44 %)	32.10	18.50	22.45	22.45
Wheat bran	0.00	6.00	1.7	0.0
Di-calcium phosphate	1.80	1.35	1.5	1.5
Limestone	1.40	2.00	4.7	7.4
Vit. and Min. premix[1]	0.30	0.30	0.3	0.3
NaCl	0.30	0.30	0.3	0.3
DL. Methionine	0.10	0.30	0.05	0.05
Total	100	100	100	100
Calculated Analysis [2]				
Crude protein %	19.11	14.57	15.47	15.14
ME (Kcal / kg)	2863	2750	2836	2781
Crude fat%	2.91	3.00	3.4	3.2
Crude fiber %	3.82	3.65	3.03	2.92
Calcium (%)	1.06	1.14	2.18	3.2
Av. phosphorus (%)	0.47	0.40	0.405	0.398
Lysine %	1.10	0.82	0.80	0.82
Methionine %	0.43	0.33	0.336	0.33
Methio + Cyst %	0.75	0.58	0.600	0.587

Table 1: *Ingredients composition and chemical analysis of the basal diet**s.***

Each 3 kg of the Vit and Min. premix manufactured by Agri-Vit Company, Egypt contains: Vitamin A 10 MIU, Vit. D 2 MIU, Vit E 10 g, Vit. K 2 g, Thiamin 1 g, Riboflavin 5 g, Pyridoxine 1.5 g, Niacin 30 g, Vit. B12 10 mg, Pantothenic acid 10 g, Folic acid 1.5 g, Biotin 50 mg, Choline chloride 250 g, Manganese 60 g, Zinc 50 g, Iron 30 g, Copper 10 g, Iodine 1g, Selenium 0. 10 g, Cobalt 0.10 g. and carrier CaCO3 to 3000 g. According to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001).

Items	AT (^{0}C)		RH (%)		THI	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
December	10.6 ± 0.62	23.6 ± 0.66	33.6 ± 1.49	59.2 ± 1.27	11.0 ± 0.51	21.7 ± 0.50
January	6.2 ± 0.67	18.4 ± 0.71	35.0 ± 1.60	60.3± 1.37	7.2 ± 0.55	17.6 ± 0.53
February	7.1 ± 0.91	18.7 ± 0.97	32.0 ± 2.19	55.8 ± 1.87	8.1 ± 0.76	17.7 ± 0.73
March	10.5 ± 0.58	19.6 ± 0.62	35.5 ± 1.40	52.5 ± 1.20	11.1 ± 0.48	18.5 ± 0.47
April	12.1 ± 1.40	24.3 ± 1.48	30.3 ± 3.34	45.6 ± 2.86	12.5 ± 1.16	22.2 ± 1.12
May	17.1 ± 0.58	29.5 ± 0.62	25.4 ± 1.40	47.5 ± 1.20	16.7 ± 0.48	26.0 ± 0.47
June	22.6 ± 0.22	32.8 ± 0.72	26.4 ± 1.16	39.4 ± 0.99	21.0 ± 0.40	28.5 ± 0.38
July	22.2 ± 0.91	34.7 ± 0.97	25.1 ± 2.19	41.1 ± 1.08	20.8 ± 0.76	29.9 ± 0.73
August	23.9 ± 0.91	36.2 ± 0.97	31.1 ± 2.19	47.1 ± 1.87	22.3 ± 0.76	31.6 ± 0.73

Table 2: *Indoor Ambient Temperature (AT), Relative Humidity (RH) and Temperature –Humidity Index (THI) During Experimental Period.*

Where: db^{0}C = dry bulb temperature in centigrade, the values of THI were classified as absence of heat stress (< 27.8), moderate heat stress (27.8 - 28.8), sever heat stress (28.9 - 29.9) and very sever heat stress (> 30.0). Rectal temperature (RT) and Respiratory rate (RR) were measured at 24, 30 and 34 weeks of age after exposure to heat challenge.

Data collection and estimated parameters

Productive performance traits

Live body weight (LBW) and feed consumption (FC) were recorded for each replicate per each treatment and expressed grams per chick throughout the overall experimental period (22 - 34wks of age). feed conversion ratio (FCR) were calculated during the same period. Age of the sexual maturity was recorded at the 1st egg laid. Subsequent laying traits such as egg number, egg weight, egg mass, feed consumption were recorded during studied laying period (22 - 34 wks of age).

Egg quality parameters

At 34 weeks of age (after exposure to heat challenge), a total number of 45 eggs (9 from each treatment) were randomly taken to determine some egg quality parameters.Laying rate, feed conversion ratio and body weight change were calculated. Rectal temperature and respiration rate were measured at 24, 30 and 34 weeks of age after exposure to heat challenge. It was randomly measured in 15 birds in each group for each age by inserting clinical thermometer (2 - 3 cm) into the cloaca for one minute. Respiration rate (breaths/minute) was recorded at random for 15 birds in each group for each age by counting the body wall movements per one minute.

Blood constituents

At the age of 34 weeks after exposure to heat challenge, blood samples were withdrawn from the wing vein into heparinized test tubes tube and centrifuged at 3000 rpm to get plasma, blood Hormones levels (progesterone and estrogen) were determined by ELISA method using commercial kits. Also, blood total protein, albumin, glucose and cholesterol) were determined by using commercial kits, globulin was calculated by subtract albumin from total proteins values, A/G ratio was calculated by dividing albumin /globulin values. Levels of heat shock protein 70 were estimated in liver tissues also at 24, 30 and 34 weeks of age immediately after heat challenge by ELISA method using kits of USCN Life Science Inc. Wuhan, Chain. Specificity of this assay has high sensitivity and excellent specificity for detection of gallinaceous HSP70.

Statistical Analysis

Data obtained were statistically analyzed using the General Liner Model of SPSS, (2008) [14]. The following model was used: Yij = μ + Ti + eij where: Yij = an observation, μ = overall mean, Ti = effect of treatment (I = 1,2,3 and 4) and eij = experimental random error. Significant differences between means were tested by Duncan's Multiple Range Test Duncan (1955) [15] at 5% level of significance.

Results and Discussion

Productive performance

Table 3 shows the age of sexual maturity, weight of the first egg, rectal temperature and respiration rate. The age of Sexual maturity (based on first egg laid) and weight of the first egg were significantly affected due to early heat exposure and glutamine injection under hot condition (Table 3). Pullets at T2, T3 and T4 reached the sexual maturity earlier with heavier weight of first egg than other treatments (133.3,135.6 and 138.6 days respectively) while, pullets at T5 reached at sexual maturity later with lighter weight of first egg than control group. These results were confirmed by the obtained by Nagwa., *et al*. [16] who mentioned that age at sexual maturity of laying hens showed unobserved increase by multiple heat acclimation cycles during growth period. Table (3) demonstrated that rectal temperature

(RT) and respiration rate (RR) were significantly affected by treatments. The negative effect of hot summer conditions could be clearly observed in the control and 2nd group. However the 2nd group (T2) exposed to additional heat challenge showed significantly ($p < 0.05$) higher RT and RR than of control and the other experimental groups. Hover, the highest values of RT and RR (42.51 and 107.68) were recorded for the 2nd while the lowest values were recorded for the 4th. These results were consistent with that obtained by Rizk., *et al.* [17] who showed that RT and RR were significantly higher for Sinia hens reared under heat stress than those reared in thermo neutral conditions at 24 and 34 weeks of age. T3 and T4 groups showed insignificant decrease of RT from that of control groups. As the time elapsed between the 1st exposure (at 3 days of age) and the 2nd exposure (at 35 days of age) seemed was too short to enhance thermo tolerance of birds that exposed to heat stress during reproduction period [16,18]. The T5 HSE3 group had significant ($p < 0.05$) reduction in RT by - 0.88,- 4.31 C and RR by 11.32% and 20.82% in comparison with both control group and the 2nd group. The multiple early heat exposure and glutamine injections might stimulate production of HSP70 which having vital role in cellular homeostasis during development of thermo-tolerance [19]. The present results could be confirmed by that obtained by Ezzat., *et al.* [20] who mentioned that laying hens acclimatization receiving multiple early heat programs had significant reduction of RR and RT as compared with control group. This could be due to the effect of heat acclimation on thyroid hormones that reduce the metabolic heat production. The results of table 4 showed that there were a significant differences among treatments in all studied parameters except of yolk index, and Haugh units. However, the highest values for egg shell thickness was recorded for egg of T5 (HSE3) group compared to other treatments, meanwhile, the highest values of shell and yolk relative weights as well as yolk color were recorded for eggs of T5,T3 and T4 respectively. Final live body weight (FBW), total feed intake and feed conversion of Sinai hens at 34-wks of age during the period of (22 to34 wks of age) were significantly ($P \leq 0.05$) differ among treatment groups (Table 5). The 2nd group followed by control group exposed the worst results regarding FI, FC and FBW. FBW was affected significantly among tasted groups, the highest value recorded for 5th group (HSE3) which was1570.07 comparing with the control group value which was1395.52. These results are consistent well known facts that stress conditions affect FI, absorption of food constituents and hence FC. Also this could be seen in the values of FBW of 2nd group which is the lowest due to reduced FI and FC [21]. HSE1 and HSE2 exposed insignificant increase in FBW from each other and their values of FBW control group. However HSE3 expressed the highest FBW ($p < 0.05$) compared to all other groups. It is proved that early heat acclimation improves growth performance of chickens due to advancement of both FI and FC. Ever if not significant [22]. Hence repeated early thermal cycles could produce better growth performance as shown in FBW of HSE3 group whose results are consistent e that obtained by [16,23].

Treatments						
T1	**T 2**	**T3**	**T4**	**T5**	**Pooled SEM**	**Sig.**
Age of sexual maturity (days) (SM)						
144^{a}	133.3^{c}	135.6^{bc}	138.6^{b}	145.3^{a}	1.36	**
The weight of the first egg (g)						
31.53^{b}	32.36^{b}	31.83^{b}	34.23^{a}	31.03^{b}	0.34	
Rectal temperature (c^{o})						
41.11^{bc}	42.51^{a}	41.41^{b}	41.36^{b}	40.75^{c}	0.16	**
Respiration rate/ min						
99.21^{b}	107.68^{a}	96.84^{c}	94.64^{d}	89.12^{e}	1.60	**

Table 3: *Growth performance and sexual maturity age of Sinai pullets as affected by glutamine injection and early heat exposure under hot condition.(1-20 wks)*

a,b,c,..: means in the same row bearing different superscripts are significantly different ($p \leq 0.05$).

NS= non-significant.

Treatments						
T1	T2	T3	T4	T5	Pooled SEM	Sig.
Egg index						
0.776^{bc}	0.764^{c}	0.765^{c}	0.800^{a}	0.792^{ab}	0.003	**
Yolk index						
48.03	44.86	44.92	45.91	47.17	0.56	N.S
Yolk weight %						
27.61^{b}	29.95^{ab}	31.46^{a}	29.03^{ab}	28.61^{b}	0.41	**
Albumen weight %						
60.14^{a}	58.11^{ab}	56.72^{b}	58.72^{ab}	57.20^{b}	0.38	**
Shell weight %						
12.26^{b}	11.91^{b}	11.81^{b}	12.23^{b}	14.17^{a}	0.23	**
Haugh unit						
96.55	96.78	99.22	99.89	96.89	0.82	N.S
Shell thickness						
0.29^{bc}	0.28^{c}	0.30^{ab}	0.31^{abc}	032^{a}	0.003	**
Yolk colour						
6.44^{bc}	6.00^{c}	6.22^{c}	7.11^{a}	6.89^{ab}	0.11	**

Table (4): *Egg quality traits of Sinai hens as affected by glutamine injection and early heat exposure under hot condition at 34wks of age.*

a,b,c,..: means in the same row bearing different superscripts are significantly different (p ≤ 0.05). NS= non-significant.

Treatments						
T1	T2	T3	T4	T5	Pooled SEM	Sig.
Initial body weight at 22 wks of age, g						
1156.67	1139.67	1185.33	1164.0	1156.6	7.43	N.S
Final body weight at 36 wks , g						
1395.52^{d}	1358.31^{e}	1486.07^{c}	1540.1^{b}	1570.07^{a}	21.81	**
Total feed intake/hen (22-34)weeks						
8324.68	8252.16	8414.56	8535.52	8575.28	50.7	N.S
Feed conversion						
3.9^{ab}	4.00^{a}	3.54^{bc}	3.46^{c}	3.28^{c}	0.084	**
Egg weight g						
41.62^{c}	41.14^{c}	42.59^{bc}	43.5^{ab}	44.6^{a}	0.39	**
Egg number/hen						
51.20^{b}	50.11^{b}	55.86^{b}	56.6^{a}	58.51^{a}	0.92	**
Egg mass						
2131.43^{c}	2061.48^{c}	2381.26^{b}	2461.85^{ab}	2608.78^{a}	57.59	**

Table 5: *Performance of Sinia laying hens as affected by glutamine injection and early heat exposure under hot conditions (22-34) weeks of age .. *

a,b,c,..: means in the same row bearing different superscripts are significantly different (p ≤ 0.05). NS= non-significant.

Moreover glutamine injection caused more HSP70 synthesis which increased activity of digestive enzymes [24] of chicken under heat stress. Also glutamine improves FC through increasing height of villi of intestine subsequent increase of nutrient absorption [25], hence triple injection of glutamine in HSE3 produced the highest FBW. Also, Jazideh., *et al.* [26] noticed the improvement in the intestinal morphology and body weight gain of broilers supplemented with 0.5 glutamine in diet under heat stress comparing with control diet and there was increasing in the villus. Results of table 5 represented the egg number, egg weight and egg mass of the tested groups. The data showed that 5th group (HSE3) had the significantly ($p < 0.05$) largest egg weight (44, 60 gm) and highest numbers 58.54 of egg production. So its egg mass (2608.78 gm/hen) was the highest among all other groups. These results could be explained by the gradual increase of body weight at sexual maturity reaching its maximum value in 5th group (HSE3). These results were consistent with that obtained by Nagwa., *et al*. [16] who reported that laying hens receiving multiple early heat acclimation cycles had highest number of egg, largest egg weight and egg mass. Also the increase of egg weight, egg number and egg mass of HSE3 group might be attributed to triple injection of glutamine. This was confirmed by Vahid Gholipour., *et al*. [11] who conducted that diet supplemented by L- glutamine significantly increased egg weight, egg production and egg mass.

Blood constituents

Table 6 revealed that there were significant differences in all blood parameter and it could be noticed that the highest superiority values of studied parameters (heat shock protein, total protein, albumin, globulin, glucose, calcium, progesterone and estrogen) were exhibited by hens of T5 (HSE3). On the other hand, the same previous treatment (T5 HSE3) had recorded the least plasma cholesterol and phosphorus concentrations, The lower level of HSP70 in the 2nd group may be due to additional heat challenge at 24, 30 and 34 weeks of age without early thermal acclimation during the growth period. This could be confirmed by significant (< 0.05) increase of HSP70 concentration in T3 (HSE1) group from that of control and 2nd groups. As HSE1 had dual mechanisms enhance HSP70 synthesis which early heat shock exposure together with glutamine injection. It is well established that early thermal acclimation increased synthesis of heat shock protein HSP70 [6,16,18,24,27] had reported that GI stimulates synthesis of HSP70.There was no significant difference in the level HSP70 between of T3(HSE1) group and T4(HSE2) group that which received double of both early heat stress and GI. However T5 (HSE3) showed significant increase level of HSP70 ($p < 0.05$) from that of both HSE1 and HSE2 groups. These results are in agreement with that obtained by Ezzat., *et al.* [20] who found that triple exposure to early heat shock during growth period caused significant increase of HSP70 level in laying hens. Hence the present results showed that three times of GI and triple exposure of early heat shock had synergistic effect on the level of HSP70 in T5(HSE3) group.

Treatments						
T1	**T2**	**T3**	**T4**	**T5**	**Pooled SEM**	**Sig.**
	Heat shock protein 70					
7.31^{c}	6.21^{d}	8.14^{bc}	8.34^{ab}	9.11^{a}	0.28	**
Total protein (g/dl)						
4.90^{d}	4.48^{e}	5.51^{c}	6.30^{b}	7.09^{a}	0.25	**
Albumin (g/dl)						
2.74^{d}	2.45^{e}	2.95^{c}	3.41^{b}	3.78^{a}	0.12	**
Globulin (g/dl)						
2.16^{c}	2.03^{d}	2.56^{bc}	2.89^{ab}	3.31^{a}	0.13	**
A/G ratio						
1.27	1.22	1.17	1.18	1.41	0.039	N.S
Glucose(mg/dl)						
187.14^{d}	186.12^{e}	189.85^{c}	192.28^{b}	195.4^{a}	0.90	**

Cholesterol (mg/dl)						
176.78^{b}	185.99^{a}	167.1^{c}	157.00^{d}	139.22^{e}	4.30	**
Phosphorus(mg/dl)						
8.02^{b}	9.30^{a}	7.92^{b}	7.58^{c}	6.92^{d}	0.20	**
Calcium (mg/dl)						
8.55^{c}	7.32^{d}	9.19^{b}	9.14^{b}	10.22^{a}	0.25	**
Progesterone(ng/ml)						
0.288^{c}	0.21^{d}	0.33^{b}	0.37^{b}	0.43^{a}	0.02	**
Estrogen(ng/nl)						
265.5^{d}	295.8^{c}	342.8^{b}	344.1^{b}	400.0^{a}	12.34	**
T3((ng\ml)						
3.82^{a}	3.27^{b}	2.62^{c}	1.98^{d}	1.53^{e}	0.22	**

Table 6: *Blood constituents of Sinia hens as affected by glutamine injection and early heat exposure under hot condition. a,b,c,..: means in the same row bearing different superscripts are significantly different ($p \leq 0.05$).*
NS= non-significant.

The 3rd,4th and 5th groups (HSE1, HSE2 and HSE3) expressed significant increases ($p < 0.05$) of total protein, albumin and globulin comparing with control group (Table 6). The highest values were for 5th (HSE3) group which recorded 7.095, 3.78 and 3.315 and respectively, whereas the control group recorded 4.906, 2.749 and 2.157 respectively. But they showed significant ($p < 0.05$) reduction of cholesterol level from the 2nd group. However A/G ratio level had insignificant change in between tested groups. These results are in agreement with those obtained by Selim (2011) [28] who observed a significant decrease in plasma total protein, albumin in the heat stressed chickens (38^{0}C for 6 hours and 70 ± 5% Rh). Gursu., *et al.* [29] discovered an increase in the concentration of cholesterol in heat-stressed broilers**.** Moeini., *et al.* [30] noticed that positive effect on serum lipid by decrease both cholesterol, and LDL) and increase HDL,) concentrations in broiler chickens exposed to heat stress. Moreover the best improvement of total protein, albumin, G/A ratio in HSE3 group might be explained by reduction of corticosterone level by multiple early heat exposures. Also the observed increase of these parameters in 5th group could be attributed to multiple glutamine injection which is an abundant amino acid involved the nucleic acid synthesis in the body [31,32]. Plasma Phosphorus level was decreased significantly in the 5th (HSE3) group as compared to control and experimental other groups (Table 6), On the contrary, calcium levels were increased ($p < 0.05$) in the 5th group compared with other experimental groups. These results may be due to the reverse relationship between calcium and phosphorus in which increase blood calcium level resulted in an increase of parathyroid hormone secretion which inhibits the renal tubules reabsorption of phosphorus [33,34]. These results are in agreement with those of Rahimi [35] and Nagwa., *et al*. [16] who reported that calcium level in broilers was increased significantly in treatments exposed to heat shock compared to control group. Table (6) showed that the 2nd group had significantly ($p < 0.05$) lower level of progesterone hormones than other experimental treatments. As the heat stress applied on females can upset the typical status of reproductive hormones at both the hypothalamus and the ovarian levels. Also these results are confirmed by the obtained by Amen and Doraji [36] who reported that heat stress decreased LH and FSH hormones.Hens of T3 (HSE1),T4 (HSE2) and T5 (HSE3) groups showed significant increase in sex-hormones over than of control and the 2nd groups. These results are very realistic as it is well established that early heat acclimation at the growth period of bird can adapt the thermoregulatory system of immature hypothalamus where LH and FSH are released to increase secretion of progesterone and estrogen. However T5 (HSE3) showed significantly higher level of progesterone and estrogen compared to that of T3 (HSE1) and (HSE2) groups. Triiodothyronine (T3) decreased significantly ($P < 0.05$) compared with control group, where, the lowest value was expressed by HSE3 group. Moraes., *et al.* [37] reported that birds are able to reduce plasma (T3) concentration especially during thermal challenge. Also, Yalcin., *et al.* [38] mentioned that the concentration of (T3) was reduced in

heat acclimatization treated birds. Our results are in agreement with Youssef., *et al*. [6] who reported that glutamine injection alleviate the negative effects of heat stress, as (T3) hormone is responsible for thermogenesis in chickens [39]. Some researches indicated that the multiple early heat shock during growth period produces heat acclimation of hypothalamus of the bird e more subsequent more release of LH and FSH that stimulate production of reproductive hormones at the ovarian. Also it is well established that the enhancement of reproductive hormone synthesis in the hens is dependent on the dose of the glutamine that is added to the diet [11,40,41]. Hence in this study triple glutamine injections produces the highest levels of progesterone and estrogen hormones.

Conclusion

The previous results of this experiment indicated that the 5th group (HSE3) that received three times early heat shock and triple glutamine injection, showed significantly ($P < 0.05$) increase level of HSP70 compared to other groups. Also the level of T3, progesterone hormone, estrogen hormone, egg number, egg weight, egg mass group. while significant ($P < 0.05$) reduction of RT and RR.Moreover all the physiological parameters were significantly improved in HSE3 group.

Acknowledgements

I would also like to extend my thanks to all staff members at EL- Serw Poultry Research Station.

Conflict of Interest

No any financial interest or any conflict of interest exists.

Bibliography

1. Kadim IT., *et al.* "Effect of seasonal temperature and ascorbic acid supplementation on performance of broiler chickens maintained in closed and open-sided houses". *International Journal of Poultry Science* 7 (2008): 655-660.

2. Tawfeek SS., *et al.* "The effect of dietary supplementation of some antioxidants on performance, oxidative stress and blood parameters in broilers under natural summer condition". *The Journal of World's Poultry Research* 4.1 (2014): 10-14.

3. Aslam F., *et al.* "Toxico pathological changes induced by cypermethrin in broiler chicks: their attention with Vitamin E and selenium". *Experimental Toxicology* 62 (2010): 441-450.

4. Lucas J Lara and Marcos H Rostagno. "Impact of heat stress on poultry production". *Animals* 3 (2013): 356- 369.

5. Ebeid TA., *et al.* "High temperature influences eggshell quality and calbindin-D28k localization of eggshell gland and all intestinal segments of laying hens". *Poultry Science* 91 (2012): 2282-2287.

6. Youssef SF., *et al.* "Increasing the activity of antioxidant system in broiler chicks during summer seasons :1- Effect of some anti-heat stress agents on broiler performance, immune response and heat shock protein 70". *Egyptian Poultry Science Journal* 36.1 (2016): 355-374.

7. Staib JC., *et al.* "Increased temperature, not cardiac load, activates heat shock transcription factor 1 and heat shock protein 72 expression in the heart". *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* 292 (2007): 432-439.

8. Ming J., *et al.* "Molecular cloning and expression of two HSP70 genes in the Wuchang bream (Megalobrama amblycephala Yih)". *Fish and Shellfish Immunology* 28 (2010): 407-418.

9. Kojima R., *et al.* "Expression of heat shock proteins induced by L-(1)-glutamine injection and survival of hypothermic ally stored heart grafts". *Transplantation Proceedings* 30 (1998): 3746-3747.

10. Wu G. Functional amino acids in growth, reproduction and health". *Journal of Biological Chemistry* 1 (2010): 31-37.

11. Vahid Gholipour., *et al.* "Effect of dietary L-glutamine supplementation on performance, egg quality, Fertility and some blood biochemical parameters in Guinea Fowls (Numida meleagris)". *Kafkas Universitesi Veteriner Fakultesi Dergisi* 23.6 (2017): 903-910.

12. NRC. National research council. Nutrient Requirements of chickens. 9th Editions. National Academy of Science Washington D.C (1994): 19-26.

13. Marai IFM., *et al.* "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". *Tropical Animal Health and Production* 33 (2001): 1-12.

14. SPSS. SPSS User´s Guide Statistics. Ver. 17. Copyright SPSS Inc., USA (2008).

15. Duncan DB. "Multiple range and multiple F tests". *Biometrics* 11 (1955): 1-42.

16. Nagwa A Ahmed., *et al.* "Effect of using heat shock programs on thermoregulation responses and performance of laying hens under desert conditions". *Egyptian Poultry Science Journal* 32 (2012):777-790.

17. Rizk YS., *et al.* "Effect of dietary source of selenium on productive and reproductive performance of Sinia laying hens under heat stress conditions". *Egyptian Poultry Science Journal* 37.2 (2017): 461-489.

18. Morsy AS. "Effect of heat shock exposure on the physiological responses and semen quality of male chickens under heat stress conditions". *Egyptian Poultry Science Journal* 33 (2013): 143-161.

19. Hahn GM and Li GC. "Thermo tolerance, thermo resistance and thermos sensitization. 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 (1990): 79-100.

20. Ezzat W., *et al.* "The effect of chromium picolinate supplementation on immune response and heat shock proteins of laying hens under heat stress conditions". *Egyptian Poultry Science Journal* 38.1 (2018): 65-89.

21. Lagana C., *et al.* "Effect of the reduction of dietary heat increment on the performance, carcass yield and diet digestability of broilers submitted to heat stress". *Brazilian Journal of Poultry Science* 9 (2007): 45-51.

22. Franco-menez DJ., *et al.* "Differential effect of heat stress in three strains of laying hens". *The Journal of Applied Poultry Research* 16.4 (2007): 628-634.

23. Yahav S., *et al.* "The effect of thermal manipulation during embryogenesis of broiler chicks (Gallus domesticus) on hatchability, body weight and thermoregulation after hatch". *The Journal of Theoretical Biology* 29 (2004): 245-250.

24. Hao Y., *et al.* "Over expression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers:1. Intestinal structure and digestive function". *Poultry Science* 91 (2012): 781-789.

25. Bartell SM and Batal AB. "The effect of supplemental glutamine on growth performance, development of the gastrointestinal tract and hormonal immune response of broilers". *Poultry Science* 86 (2007): 1940-1947.

26. Jazideh F., *et al.* "The effects of dietary glutamine supplementation on growth performance and intestinal morphology of broiler chickens reared under hot conditions". *Turkish Journal of Veterinary and Animal Sciences* 38.3 (2014): 264-270.

27. Mashaly MM., *et al.* "Effect of heat stress on production parameters and immune response of commercial laying hens". *Poultry Science* 83 (2004): 889-894.

28. Seliem MME. "Effect of harmal seeds on heat stress chickens". *The Journal of American Science* 7.12 (2011): 606-612.

29. Gursu MF., *et al.* "Effects of vitamin C and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress *In vivo*". *Nutrition Research* 24 (2004): 157-164.a

30. Moeini MM., *et al.* "The effect of different levels of organic and inorganic chromium supplementation on production performance, carcass traits and some blood parameters of broiler chicken under heat stress condition". *Biological Trace Element Research* 144 (2011): 715-724.

31. Newsholme P., *et al.* "Glutamine and glutamine-their central role in cell metabolism and function". *Cell Biochemistry and Function* 21 (2003): 1-9.

32. Olubodun JO., *et al.* "Glutamine and glutamic acid supplementation enhances performance of broiler chickens under the hot and humid tropical condition". *The Italian Journal of Animal Science* 14 (2015): 25-29.

33. Tyler DD. "Water and minerals metabolism". In: physiological chemistry. 17th Edition. Long medical publication, California, USA (1979).

34. Pavlik A., *et al.* "Blood plasma mineral profile and qualitative indicators of the eggshell in laying hens in different housing systems". *Acta Veterinaria Brno* 78 (2009): 419-429.

35. Rahimi G. "Effect of heat shock at early phase on glucose and calcium regulation axis in broiler chickens". *International Journal of Poultry Science* 4.10 (2005): 790-794.

36. Amen MH and HJ Al-Daraji. "Effect of dietary supplementation with different level of zinc on Sperm egg penetration and fertility traits of broiler breeder chicken". *Pakistan Journal of Nutrition* 10.11 (2011): 1083-1088.

37. Moraes VMB., *et al.* "Effect of thermal conditioning during embryonic development on aspects of physiological responses of broilers to heat stress". *Journal of Thermal Biology* 28.2 (2003): 133-140.

38. Yalcin S., *et al.* "Response to heat stress in commercial and local broiler stocks. 1. Performance traits". *British Poultry Science* 42 (2001): 149-152.

39. Tao X., *et al.* "Responses of thyroid hormones of market-size broilers to thermoneutral constant and warm cyclic temperatures". *Poultry Science* 85 (2006): 1520-1528.

40. Dong Xiao-Ying., *et al.* "Effect and mechanism of glutamine on productive performance and egg quality of laying hens". *Asian-Australasian Journal of Animal Sciences* 23.8 (2010): 1049-1056.

41. Feed Composition Tables For Animal and Poultry Feedstuffs Used In Egypt, 2001. Technical builetin No, 1, Center Lab Feed and Food; Ministry of Agriculture, Egypt (2001).

Volume 6 Issue 1 January 2021