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EFFECT OF MITIGATION OF HEAT STRESS BY EARLY HEATE ACCLIMATION AND GLUTAMINE INJECTION ON SOME PHYSIOLOGICAL MEASUREMENTS, IMMUNE RESPONSES AND SEMEN QUALITY OF SINIA MALE CHICKENS.

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ABSTRACT: This work aimed to enhance the resistance of cocks against hot climate by using both early heat shock exposures (HSE) and glutamine injections (GI). Eight hundred one-day old of male Sinia chicks were randomly divided into eight groups to investigate the effect of (HSE) and (GI) on some physiological parameters, immune responses and semen quality during reproductive period under heat stress conditions. The 1st group of chicks served as a control that reared under natural conditions. The 2nd group of chicks was managed thermally like the control group during growth period, while through reproductive period the cocks were exposed to heat challenge (38 ±1ºC for four hours from 12:00 till 16:00 for one day) at 24, 30 and 34 weeks of age. The 3rd group (HSE_1) was 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 similarly, the 4th group (HSE_2) received the same early heat stress, in addition to GI (0.75 mg/kg weight intra peritoneal injection) at age of two days. The 5th group (HSE_3) was 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. Also, 6th group of chicks (HSE_4) received heat shock two times (at age of 3 and 35 days) and two GI (0.75 mg/kg weight intra peritoneal injection) at 2 and 34 days of age. Three times of heat shock (41 ±1ºC for four hours from 12:00 till 16:00 for 3 consecutive days) were applied to chicks of 7th group (HSE_5) at 3, 35 and 112 days of age. Similar schedule of heat shock was applied to 8th group (HSE_6) that received also, three times of GI (0.75 mg/kg weight intra peritoneal injection) at 2, 34 and 111 days of age. During reproductive period, HSE_1, HSE_2, HSE_3, HSE_4, HSE_5 and HSE_6 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 12:00 till 16:00 for one day). Cocks of 8th group (HSE6) expressed the best level ($P<0.05$) of heat shock protien70 (HSP_{70}) as well as all other measured parameters compared to control and other groups.

Keywords: Early Heat shock exposures - Glutamine - Cocksand Heat shock protein70.

INTRODUCTION

Stress can be defined as responses of the body to abnormal conditions that potentially interrupt homeostasis or normal physiological equilibrium (Lara and Rostagno, 2013).

The global climate has changed, so tropical and subtropical areas are affected more frequently by high ambient temperature, especially traditional farms that depended on natural ventilation (Fouad et al., 2016), In Egypt, summer stress is severe due to elevated temperature that may go up to 40°C.

High ambient temperature is one of the most important factors exerting a negative influence on the performance of poultry and causes huge losses regarding reduced feed intake, body weight gain and feed efficiency (Habibian, et al., 2016) as well as decrease of absorption of nutrients and secretion of digestive enzymes (Liu et al., 2016).

The heat stress leads to production of excess quantities of reactive oxygen species that damage cell phospholipid membrane and other macromolecules (Rahman, 2003). Exposure of young cockerels to hot stress stimulates mitochondrial superoxide production (Mujahid, et al., 2007).

Also, heat stress affects reproductive efficiency of poultry that has shown reduction of relative weights of reproductive organs (testis, ovary and oviduct) and impaired semen quality (Chen et al., 2015; Turk et al., 2015 and 2016). Moreover, immune competence is affected due to reduced proportions of immune organs and synthesis of antibodies (Jahanian and Rasouli, 2015; Tang and Chen, 2015).

Many researches have been done to increase thermotolerance of chicks and hence minimizing heat stress related mortality as well as maintenance of productivity. Exposure to short and daily heat shocks at early growth phases acclimate birds to heat stress and enhance their physiological responses (Yahav, 2009 and El-Moniary et al., 2010). However, the under lying mechanism of this thermal acclimation is still vague. Some researchers contribute thermal adaptation of birds to exploiting the incompletely mature hypothalamic thermoregulatory system (Yossifoff et al., 2008). While others suggested that early heat stress causes increasing in synthesis of heat shock proteins, also known as stress proteins (HSPs). The increased levels of HSPs defend cells against damage and apoptosis (Khan et al., 2012). Heat shock protein 70 is the most common and conservative in the HSPs family, as its synthesis is abundant in most organisms after cell stress (Hao et al., 2012).

The amino-acids are the constituents of protein and peptides. Also, some amino acids are involved in regulation of metabolic pathways to affect growth and immunity. Glutamine is a free, neutral, non-essential amino acid that is found in higher levels in muscles and plasma. Moreover, glutamine represents about 50-80% of total free amino acids in the body (Sakamoto et al., 2006). Glutamine could have several roles in metabolism and tissue homeostasis (Menconi et al., 2013). Moreover, dietary supplementation of glutamine improves growth performance and humoral immune response in poultry (Dai, et al., 2009 a and b). Glutamine is considered as a conditionally essential nutrient under heat stress conditions (Olubodun, et al., 2015).

The assessment of semen quality of cocks gives an excellent indicator of their reproductive potential, fertility and subsequent hatchability of eggs (Peters, et al., 2004). So, this work was done to investigate enhancement of heat shock protein 70 (HSP_{70}) 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 male Sinia chicken. Also, their effects on physiological performance, immune response and semen quality at reproductive period were evaluated.

MATERIALS AND METHODS

This study was conducted at El-Serw Poultry Research Station, Animal production Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. The experiment started in December 2015 up to August 2016. Eight hundred one-day old of male Sinia chicks were randomly divided into eight equal groups (100 chicks each). The 1st group served as a control that reared under natural conditions without either early heat exposure or glutamine injection. The 2nd group of birds was reared under natural conditions during growth period, while through reproductive period (that was already present in June, July and August it means hot summer months) the chicks were exposed to heat challenge (38 ±1°C for four hours from 12:00 till 16:00) for one day at 24, 30 and 34 weeks of age.

The 3rd group (HSE_1) was exposed to early heat shock (41 ±1°C for four hours from 12:00 till 16:00 for 3 consecutive days) at age of 3 days. The 4th group (HSE_2) received the same early heat shock in addition to GI (0.75 mg/kg weight intra peritoneal injection) at age of 2 days.

The 5th group (HSE_3) was exposed to heat shock (41 ±1°C for four hours from 12:00 till 16:00 for 3 consecutive days) two times at age of 3 days and 35 days of age. Moreover, the 6th group (HSE_4) exposed to the same two times of heat shock, but with two times of GI (0.75 mg/kg weight intra peritoneal injection at 2 days and 34 days of age).

The 7th group (HSE_5) chicks were exposed to three times of heat shock (41 ±1°C for four hours from 12:00 till 16:00 for 3 consecutive days) at 3 days, 35 days and 112 days of age. The same schedule of heat shock was applied to 8th group chicks (HSE_6) that were injected with glutamine GI (0.75 mg/kg weight intra peritoneal injection) three times at 2, 34 and 111 days of age.

HSE_1, HSE_2, HSE_3, HSE_4, HSE_5 and HSE_6 groups were reared under natural conditions during reproductive period up to the age of 34 weeks. Moreover, they exposed to heat challenge at 24, 30 and 34 weeks of age (38 ±1°C for four hours from 12:00 till 16:00 for one day). The chicks of each group were housed on floor pens in semi-open system up to age of 18 weeks old. Then the birds were transferred to wire cages. 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 other week until fixed at 17 hours daily from 30 weeks of age to the end of experiment. Birds were provided with clean fresh water and fed ad-libitum following recommended standard diets for each age (from one day to 10 weeks of age, 18% crude protein (CP) and 2600 k cal/kg metabolizable energy (ME): from 11 to 20 weeks of age, 15% CP and 2600

k cal/kg ME and from 21 weeks of age up to 34 weeks (end of the experiment), they fed diet with 17% CP and 2800 kcal/ kg (ME) according to NRC, (1994).

Birds were kept under the same hygienic conditions to protect birds against diseases and treated with antibiotics and vaccines to keep birds healthy.

Indoor climatic conditions were shown in (Table, 1) ambient temperature AT°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) and calculated according to Maria 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).

Rectal temperature and respiration rate were measured at 24, 30 and 34 weeks of age after exposure to heat challenge. Rectal temperature (°C) 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.

At the age of 24, 30 and 34 weeks after exposure to heat challenge, blood samples were withdrawn from the wing vein. Each blood sample was divided into three parts; the first part was placed into tube containing EDTA to get fresh blood, while the 2nd part was placed into tube containing heparin to get plasma. The 3rd part of blood sample was put in non-heparinized tubes to obtain serum.

The following parameters were determined in:

Fresh blood

Hematological parameters including count of Red Blood cells (RBC's) that was determined under microscope using hemocytometer according to Jaime, (2000). Packed cell volume (PCV) was estimated using microhematocrit tubes by Wintrobe methods. Mean corpuscular volume (MCV), mean corpuscular Hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by the following equations:

MCH (In picogram, pg) = (Hb content g/dl ×10)/ RBCs in million.

MCHC (%) = (Hb content × 100)/Ht%.

MCV in femto-liter, FL) = (Ht % × 10)/RBCs In million.

Heterophil/Lymphocyte ratio (H/L ratio) was estimated according to Gross and Siegel, (1983) where one hundred different white blood cells were counted and differentiated into lymphocytes and heterophils to calculate the ratio between them.

Plasma sampling

The tube containing heparin was centrifuged at 3000 rpm for 15 minutes to get plasma that was stored at -70°C to determine hormones, blood metabolites and antioxidant enzymes. Hormones levels including tri-iodothyronine (T_3), corticosterone and testosterone were determined by ELISA method using commercial kits.

Also, blood metabolites (total protein, globulin, albumin, albumin/globulin ratio (A/G ratio), glucose, cholesterol) were

determined in plasma by using commercial kits, globulin was calculated by subtract albumin from total proteins values, A/G ratio calculated by divided albumin about globulin values. Also, antioxidant enzymes were superoxide dismutase (SOD), glutathione peroxidase (GPX) and total antioxidant capacity (TAOC) were evaluated using commercial kits produced Bio-diagnostic, Egypt

Serum samples

The blood samples were allowed to clot at 37°C and centrifugation was done to obtain serum in which antibody titers against Newcastle disease virus (NDV), Infections bronchitis virus (IBV) and Avian Influenza virus (AIV) were detected by hemagglutination inhibition tests and expressed as log2 of the reciprocal of the highest serial dilution in which there was hemagglutination. Immunoglobulin concentrations of (IgG and IgM) were determined by enzyme-linked Immunosorbent assays using commercial ELISA kits according to manufacturer's Instructions (Sun Biomedical Technology Co., BeiJing, 10039).

Moreover, ten cocks of each treatment at 24 and 28 weeks of age were injected intravenously with 1 ml of 10% suspension of sheep red blood cells (SRBC's). Then blood samples were collected at 25 and 29 weeks of age and centrifuged to get serum and frozen until the measurements of anti SRBC's antibody levels for primary and secondary immune responses were determined using micro-hemagglutination procedure (Witlin, 1967). The highest dilution exhibited by hemagglutination was recorded as titer of serum sample. The titer of each sample was converted to $\log_2$ value (Thaxton and Siegel, 1972).

Individual body weight was recorded at 1st day and 34 weeks of age. At 24, 30 and 34 weeks of age, five cocks of each group were weighed and slaughtered, the weights of spleen, Liver, heart, thyroid, adrenal and testis glands were determined to the nearest 0.1 gm.

Levels of heat shock protein 70 of liver were recorded also at 24, 30 and 34 weeks of age 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 HSP_{70}.

Semen was collected three times during the experiment (at 24, 30 and 34 weeks of age after exposure to heat challenge). 15 cocks from each group were randomly chosen, abdominal massage at the level between the pelvic bones was done to get semen. The ejaculate volume was determined to the nearest 0.01 ml using 1.00 ml tuberculin syringe. Sperm concentration was evaluated by using Thomes -Zeis haemocytometer (Kalamah et al., 2000).

Total sperm output = ejaculate volume × spermatozoa concentration.

Percentage of live and abnormal sperms were determined by staining with Eosine and Nigrosine (Blom, 1950), then calculated as percentage out of randomly chosen 100 sperm counted.The percentage of motile sperm was estimated by visual examination under low- power magnification (10x) using a phase-contrast microscope according to Melrose and Laing (1970).

TMS = MS% × TSO

$$SQF = :V \times \frac{LS}{100}$$

Where: TMS: total number of motile sperm, MS%: percentage of motile sperm, TSO: total sperm output, SQF:

semen quality factor, SC: sperm concentration, EV: ejaculate volume, LS: live spermatozoa. Hydrogen ion concentration (pH) of semen was determined immediately after collection using pH paper.

Mortality rate were recorded daily for each group from one day to end of the experiment.

Statistical Analysis

All statistical analyses were performed using IBM SPSS 22.0 Software Package (IBM corp., NY, USA, 2013). One-way ANOVA was used to determine statistical differences between treatment groups for the performance traits, the physiological) and the immunological measurements. Results are expressed as LSM ±SEM and the significance level was set at $P<0.05$, and model used was as follows:

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

Where: Y_{ij}= The observations of j^{th} bird within i^{th} group, μ = Overall mean , T_i = Effect of i^{th} group or treatments, (i: 1-8) , e_{ij} = Experimental error.

Significant differences between treatments means were tested using Duncan multiple range test (Duncan, 1955).

RESULTS OF DISCUSSIONS

Levels of Heat shock protein 70 (HSP_{70})

Heat shock protein 70 (HSP_{70}) showed significant ($P<0.05$) reduction from the control group to the 2nd group (Figure, 1). Both groups were reared during reproductive period in June, July and August (the peak of summer season in Egypt), but the lower value of 2nd group may be due to the additional heat challenge at 24, 30 and 34 weeks of age without thermal acclimation during growth period.

On this base, significant increase of HSP_{70} level in HSE_1 group compared to the 2nd group could be attributed to early heat shock exposure at growth period. This assumption could be confirmed by insignificant increase of HSP_{70} from HSE_1 group through HSE_5. It is worthy to mention that all treated groups had better levels of HSP_{70} than that of the control group. However, HSE_6 expressed the highest significant level ($P<0.05$) of HSP_{70} that has increased in HSE_6 by 20.88% and 41.09% from that of control and 2nd groups, respectively. These results are in agreement with that obtained by Nagwa et al., (2012) and Morsy (2013) who reported that level of HSP_{70} increased by multiple heat shock exposures. Moreover, the observed rise of HSP_{70} in HSE_6 group could be due to triple glutamine injection that enhanced synthesis of HSP_{70} (Hao et al., 2012 and Youssef et al., 2016).

Rectal temperature (RT) and respiratory rate (RR)

Adverse effects of heat stress could be observed in RT and RR of both the control and 2nd groups. However, RT and RR of the 2nd group expressed significantly ($P<0.05$) higher values than those of the control. As 2nd group was exposed to heat challenge during reproductive period without development of thermo-tolerance at growth period (Figures 2a and 2b). There were no observable differences in RT and RR of birds belonging to HSE_1, HSE_2, HSE_3 and HSE_4. As, the long time elapsed between 1st exposure (at 3 days of age) and 2nd exposure (35 days of age) wasn't enough to enhance thermo-tolerance of birds exposed to heat stress during reproduction (Nagwa et al., 2012 and Morsy, 2013).

The HSE_6 group showed significant ($P<0.05$) reduction of RT by (-2.2ºC and -3.8ºC) and RR by (14.26% and 24.52%) in relation to both control group and the 2nd groups. This was due to both increasing number of early heat

exposures together with glutamine injections .As both could enhance synthesis of HSP_{70} that play vital role in cellular homeostasis during development of thermo-tolerance (Hahn and Li, 1990).

Final body weight and organs weights:

Table (4) has shown that the 2nd group expressed the lowest final weight at 34 weeks of age. It was a realistic result, as this group exposed to continuous heat challenge as well as high ambient temperature that affect feed intake and feed conversion (Lagana et al., 2007).

The improvement in body weight was insignificant from that of the control group. However, HSE_6 recorded the highest significant ($P<0.05$) body weight by 22.09% and 24.03% from that of control and 2nd groups on sequence. This result may be due to repeated, short and daily heat shocks especially at early phases of growth with subsequent thermal conditioning of poultry (Narongsak, 2004 and Yahav et al., 2004). Thermo-tolerances improve growth and feed efficiency during reproductive phase (Franco-Jimenez et al., 2007).

Moreover, repeated injections of glutamine improved growth performance by increasing height of villi of intestine which resulted in more surface area for absorption of nutrients (Bartell and Batal, 2007). Also Hao et al., (2012) found strong positive correlation between over expression of HSP_{70} and increased activity of digestive enzymes of chicken under heat stress. Moreover, Table (4) reveals that the weight of thyroid gland was reduced significantly ($P<0.05$) in HSE_6 by 27.67% and 30.30% successively compared to the control 2nd groups. This result was confirmed by reduced secretion of (T_3) in the same group. This result agrees with that obtained by Melesse et al., (2011).

Spleen weight recorded insignificant increases from that expressed by control group that showed the least weight of spleen. This result is confirmed by that obtained by Jahanian and Rasouli (2015) who reported significant reductions in the relative weights of immune organs (including spleen) due to heat stress. However no significant differences were observed between groups regarding adrenal weight, testis weight and heart weight. These results were on line with that obtained by Morsy, (2013).

Hematological parameters:

Results of Tables (2a and 2b) has clarified that the 2nd group showed significant reduction of number of RBC's count, hemoglobin (Hb) and packed cell volume (PCV) in relation to the control group. This reflected impairment of synthesis of red blood cells resulting from nutritional stress during hot summer months (Oladele et al., 2001).

RBC's count, Hb concentration and PCV expressed slight elevation from each group to the next one. But all treatment groups had significant ($P<0.05$) improvement from the 2nd group HSE_6 group increased ($P<0.05$) regarding RBC's count (36.01% and 63.32%), Hb concentration (19.89% and 24.30%) and PCV (5.37% and18.22%) as compared with control and 2nd groups. These results are in line with that obtained by Nagwa et al., (2012) and Morsy, (2013) who demonstrated that hematological parameters decreased under heat stress and increased in acclimated chickens compared with unacclimated ones. Also, Youssef et al., (2016) have mentioned that exposure of chickens to early heat shock together with glutamine injection resulted in significant increase of RBC's count, Hb concentration and hematocrit value. Both heat shock exposure and

glutamine injection produced insignificant effects on MCV, MCH and MCHC.

Heterophil/Lymphocyte (H/L) ratio was significantly (P<0.05) reduced compared with the 2nd group that had the greatest numbers of heterophils and lowest number of lymphocytes. Physiologically, this result was expected because of surrounding hot weather and heat challenge that resulted in release of gluco-corticoids bringing about disintegration of lymphocytes in lymphoid tissues as well as an increase in heterophils discharged by bone marrow. Hence, H/L ratio is considered to be a reliable index of stress response in birds (Rajalekshmi et al., 2014).

The observed reduction of H/L ratio represented enhancement of thermo-regulation responses in the remaining groups of experiment. The small H/L ratio suggests that the birds tolerate and adapt to the heat stress (Al-Aqil and Zukifli, 2009). Moreover, the improved H/L ratio could be observed with glutamine injection that is essential for lymphocyte proliferation (Frauwirth, 2015), so the lowest result was that of HSE_6 that had 26.66% and 29.78% reduction (P<0.05) from that of control and 2nd groups, respectively.

Hormonal profile

As shown in Figure (3a), the 2nd group showed the highest level of corticosterone followed by the control one. Sahin et al., (2009) showed that introduction of chickens to high natural temperature causes an increase in plasma corticosterone. The observable improvement (P<0.05) of corticosterone level in the next groups in relation to 2nd group indicated that multiple heat exposures improve thermotolerance of birds (Nagwa, et al., 2012 and Morsy, 2013). It was observed that groups injected with glutamine produced better levels of corticosterone, as glutamine improves the stress response (Wischmeyer, 2002).

Triiodothyronine (T_3) decreased significantly (P<0.05) compared with 2nd group, where, the lowest value was expressed by HSE_6 group (Figure, 3b). Moraes et al., (2003) reported that birds are able to reduce plasma (T_3) concentration especially during thermal challenge. Also, Yalcin et al., (2001) mentioned that the concentration of (T_3) was reduced in heat acclimatization treated birds. Our results are in agreement with Youssef et al., (2016) who reported that glutamine injection alleviate the negative effects of heat stress, as (T_3) hormone is responsible for thermogenesis in chickens (Tao et al., 2006).

The 2nd group expressed the lowest level of testosterone (T_2) hormone followed by the control one (Figure, 3c). As the reduction in (T_2) level might be due to reduced ability of Leydig and Sertoli cells to respond to Luteinizing hormone leading to decrease of biosynthesis of testosterone hormone (Gomes et al., 1971). Moreover, other groups expressed significant increase (P<0.05) of (T_2) hormone in relation to 2nd group. Morsy, (2013) mentioned that triple heat shock exposures improve greatly (T_2) level and they attributed their results to over expression of HSP_{70} that protect Sertoli and Leydig cells against heat stress (Parminder and Bansal, 2003). Also, in our study injection of glutamine enhanced production of HSP_{70} (Hao et al., 2012) and hence the best level of (T_2) was obtained in HSE_6 having triple injection of glutamine with triple heat shock exposures that increased (P<0.05) (T_2)

level by 16.73% and 64.11% from that of control and 2nd groups, respectively.

Blood constituents

There were significant increases (P<0.05) of levels of total protein, albumin, globulin and albumin/globulin ratio relative to 2nd group. On the other hand, cholesterol level showed significant (P<0.05) reduction from 2nd group. However, glucose level expressed insignificant change (Table 3).

These results are confirmed with that obtained by Seliem (2011) who reported a significant decrease of total protein and albumin in the heat stress groups. This might be attributed to high corticosterone level under heat stress, where it stimulates gluconeogenesis using non-carbohydrate sources such as fat and protein to obtain their needs. These sources are characterized by lower heat increment leading to reduction of heat production under heat stress (Deaton et al., 1984).

Gursu et al., (2004) discovered increased concentrations of cholesterol and triglycerides in heat stress birds. So, the obtained results were consistent with reduction of corticosterone level by multiple heat shock exposures. Moreover, increased levels of total protein, albumin, globulin and albumin/globulin ratio could be attributed to multiple glutamine injection which is an abundant amino acid in the body and it is involved in nucleic acid synthesis (Newsholme et al., 2003 and Olubodun et al., 2015). This is confirmed by our results of HSE_6 group that showed increased (P<0.05) level of total protein by (44.50% and 58.21%), albumin level (45.68% and 59.99%) and globulin level (43.06% and 55.81%) from that of control and 2nd groups, respectively.

Immune parameters

Figures (4a, 4b and 4c) demonstrated that IgM, IgG and antibodies against (Newcastle disease virus (NDV), infection bronchitis virus (IBV) and influenza virus (AIV) as well as primary antibody titer against sheep red blood cells (SRBC's). The HSE_6 group showed significant (P<0.05) increase from that of control and 2nd groups sequentially regarding primary Ab against (SRBC's) by (44.50% and 73.40%), Ab against (NDV) (20.51% and 27.42), (AIV) Ab (44.25% and 65.10%) and (IBV) Ab (33.7% and 40.6%). As multiple heat shock exposures led to overexpression of HSP_{70} that inhibit release of cytokines, oxygen free radicals, nitric oxide and hence increased immune responses (Polla and Cassarrza, 1996 and Morsy, 2013). The groups that received glutamine injection in addition to heat shock exposures showed best titers of antibodies, as glutamine causes excessive production of HSP_{70} (Youssef et al., 2016). It is worthy to mention that in our study, the immunological performance of Sinia cocks was improved as 2ry immune response against SRBC's showed significant increase not only in relation to 2nd group but also on comparing any treatment group with the preceding one and also HSE_6 had the highest 2ry Ab titer against SRBC's. Moreover, the significant increase of antibody titers in this study was in parallel with the significant of globulin concentration that is used as an indicator of immune responses and source of antibodies production (El-kaiaty and Hassan, 2004)

Additionally, heat shock proteins are widely involved in immunotherapy of cancer; this is due to their nature as biological adjuvants preventing bacterial and viral disease in poultry to support

immune system against stress (Kapakin et al., 2013)

On the other hand, the lowest significant antibody titers of the 2nd group were in agreement with that obtained by Tang and Chen (2016) who revealed that heat stress significantly reduce the plasma levels of IgA, IgG and IgM in chickens. Moreover, Hosseini-Vashan et al., (2016) mentioned that heat stress decreased titers of total and IgG antibodies of the 2ry response to SRBC's and antibody production against NDV. This reduction of antibody titer with heat stress could be attributed to the release of a sustained high level of corticosterone resulting in reduced IgM and IgG synthesis (Habibian et al., 2013).

Also, heat stress causes oxidative damage to the cell membranes of immune organs leading to significant reductions of B- and T-lymphocytes with subsequent decrease in antibody production, changes in cytokines secretion and lower numbers of macrophages with reduced phagocytic ability (Ohtsu et al., 2015 and Varasteh et al., 2015).

Antioxidant enzymes

Glutathione peroxidase (GPX), superoxide dismutase (SOD) and total antioxidant capacity (TAOC) recorded significant (P<0.05) reduced levels in the 2nd group compared with that of the control one (Figure, 5), Spurlock and Savage (1993) reported that high ambient temperature increased the free radicals and other reactive oxygen species (ROS) in the body fluids and tissues. They added that accumulation of (ROS) results from decreased antioxidant defense.

Moreover, Assimako Poulos et al., (2006) mentioned that GPX, SOD and TAOC were reduced high oxidative stress associated with heat stress. However, these antioxidant enzymes showed significant (P<0.05) improvement from HSE_1 group, through HSE_6 group that expressed the best results of antioxidant enzymes (Figure, 5). These results could be associated with over expression of HSP70 by multiple heat shock and glutamine injection, as Gu et al., (2012) demonstrated that GSX, SOD and TAOC had positive correlation with HSP70 expression.

In the jejuenal mucosa of chicken at 3 hours of heat stress. Also, glutamine is a component of glutamine peptide and hence it is a precursor for the important antioxidant glutathione synthesis improvement of GPX in group injected with glutamine than that exposed only to heat shock. The possible antioxidant effects of glutamine occur also through the formation of nitric oxide (Fathi et al., 2014). As nitric oxide released from glutamine either scavenges or prevents the formation of reactive oxygen species derived from hydrogen peroxide or superoxide (Wink et al., 1993).

Semen Quality

Table (5) demonstrated that the drawback of heat stress on semen quality was clear in the 2nd group followed by the control one, as the highest value (P<0.05) of sperm abnormality and dead sperm were observed in the 2nd group. Heat stress induces testicular injury by elevating testicular lipid peroxidation, as lipids are essential component of sperm cell membrane and plasma of birds (Zaniboni et al., 2006). Histopathological studies that were done by Kapakin et al., (2013) have revealed that heat stress in broilers induced loss of spermatogenic cells with arrest of spermatogenesis and degenerative alterations in seminiferous tubules of testis. So, heat stress affected negatively the weight of testis, semen volume sperm concentration, live and normal sperms count, spermatogenia,

spermatocytes and spermatids (Turk et al., 2015 and 2016).

On contrary, the ejaculate volume, sperm concentration, sperm motility and live sperm were increased gradually from HSE_1 up to HSE_5 and HSE_6 that expressed the highest significant ($P<0.05$) values compared with control and the 2nd groups. These results could be explained by over expression of HSP_{70} in cocks receiving multiple heat shock and three times injections of glutamine. Generally, HSP_{70} defends cells against apoptosis and keeps homeostasis of cells (Khan et al., 2012). So, HSP_{70} protects the seminiferous epithelial cell differentiation against damage of heat stress. Also, over expression of HSP_{70} stimulates testicular growth in early phase to increase semen volume and sperm concentration (Obidi et al., 2008 and Morsy, 2013). The HSP_{70} is important for spermatogenesis in testis of poultry (Kapakin et al., 2013), additionally, early heat shock could activate nitric oxide synthesis which is beneficial to sperm motility (Garcia-Cardena et al., 1998). It is worthy to mention that nitric oxide is released also from glutamine.

Mortality rate

The 2nd group expressed the highest significant ($P<0.05$) mortality rate compared with control (Figure, 6), as both groups were reared under hot climate in addition to heat challenge during reproductive period of the 2nd group of Then mortality was reduced significantly ($P<0.05$) in HSE_6 by 45.98% and 60.46% from that control and 2nd groups respectively. As HSE_6 received three times heat shock exposures and three times injections of glutamine enhanced not only thermotolerance but also immunity of cocks. these results agree with that obtained by morsy, (2013) and yousef et al., (2016).

CONCLUSION

According to these results, three times of early heat shock exposures and triple times injection of glutamine are recommended during growth period of Sinia cocks that were reared under hot summer months and exposed to heat stress (38 ±1°C for four hours from 12:00 till 16:00 for one day at 24, 30 and 34 weeks of age) during reproductive period to enhance over expression of HSP_{70} that may alleviate the negative effects of heat stress on thermoregulation responses, hematological parameters, semen quality, mortality rate and may improve immunity responses by increasing antibody titer and globulin concentration and reducing H/L ratio.

Table (1): Indoor ambient temperature (AT), relative humidity (RH) and temperature-humidity index (THI) during experimental period.

Items	AT (°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-a): Hematological parameters of Sinia cocks as affected by early heat exposure and glutamine injection under hot condition.

Treatments		Hematological parameters			
		PCV%	MCV (fl)	MCH (pg)	MCHC%
Control group (gp_1)		45.19^{b} ±1.13	117.00 ±7.61	29.27 ±2.85	28.47 ±2.98
Second group (gp_2)		40.28^{c} ±1.81	122.22 ±7.23	28.47 ±2.76	25.53 ±2.76
Treatment groups	HSE_1 (gp_3)	45.75^{b} ±1.62	116.33 ±6.73	29.94 ±2.29	28.90 ±2.81
	HSE_2 (gp_4)	46.13^{b} ±1.83	114.67 ±7.21	30.47 ±2.95	28.13 ±2.59
	HSE_3 (gp_5)	46.52^{b} ±1.57	115.33 ±6.91	32.87 ±2.81	28.93 ±2.68
	HSE_4 (gp_6)	47.28^{b} ±1.92	114.33 ±7.21	32.53 ±2.51	29.03 ±2.93
	HSE_5 (gp_7)	47.36^{a} ±2.10	113.67 ±6.90	33.03 ±2.85	30.33 ±2.87
	HSE_6 (gp_8)	47.62^{a} ±1.72	111.00 ±7.40	33.36 ±2.90	30.40 ±2.79

a, b, c, ... Means with different superscript within columns are significant differences ($P<0.05$).
PCV: Mean packed cell volume, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin and MCHC: Mean Corpuscular Hemoglobin Concentration

Table (2-b): Hematological parameters of Sinia cocks as affected by early heat exposure and glutamine injection under hot condition.

Treatments		Hematological Parameters				
		Hb (g/dl)	RBC ($\times 10^6$)	H%	L%	H/L ratio
Control group (gp_1)		11.86^{b} ±0.06	3.11^{c} ±0.06	28.51^{a} ±0.21	62.70^{f} ±0.40	0.45^{b} ±0.01
Second group (gp_2)		11.44^{c} ±0.13	2.59^{d} ±0.08	29.13^{a} ±0.14	61.39^{g} ±0.12	0.47^{a} ±0.00
Treatment groups	HSE_1 (gp_3)	12.25^{b} ±0.06	3.21^{c} ±0.06	26.74^{b} ±0.30	64.18^{e} ±0.21	0.42^{c} ±0.01
	HSE_2 (gp_4)	12.48^{b} ±0.02	3.45^{c} ±0.04	25.45^{c} ±0.1	65.15^{d} ±0.24	0.39^{d} ±0.00
	HSE_3 (gp_5)	12.75^{b} ±0.08	3.69^{b}c ±0.05	24.48^{d} ±0.76	66.08^{c} ±0.12	0.37^{e} ±0.01
	HSE_4 (gp_6)	12.94^{b} ±0.02	3.74^{bc} ±0.03	23.54^{de} ±0.12	67.16^{b} ±0.26	0.35^{f} ±0.01
	HSE_5 (gp_7)	13.03^{a} ±0.04	3.86^{a} ±0.02	23.02^{ef} ±0.09	67.98^{a} ±0.07	0.34^{f} ±0.00
	HSE_6 (gp_8)	14.22^{a} ±0.06	4.23^{a} ±0.03	22.54^{f} ±0.14	68.46^{a} ±0.12	0.33^{g} ±0.00

a, b, c, ... Means with different superscript within columns are significant differences ($P<0.05$).
Hb: hemoglobin, RBC's: red blood cells, H: heterophils, L:lymphocytes, H/L ratio: heterophils/Lymphocytes ratio

Table (3): Blood metabolites of Sinia cocks as affected by early heat exposure and glutamine injection under hot condition.

Treatments		Blood metabolites					
		Glucose (mg/dl)	**Cholest. (mg/dl)**	**Total protein (g/dl)**	**Albumin (g/dl)**	**Globulin (g/dl)**	**Alb/Glu ratio**
Control group (gp_1)		186.46 ±2.062	190.16^{b} ±2.696	4.956^{g} ±0.050	2.830^{g} ±0.064	2.127^{f} ±0.015	1.33^{c} 0.041
Second group (gp_2)		185.12 ±5.578	205.52^{a} ±3.654	4.530^{h} ±0.042	2.577^{h} ±0.038	1.953^{g} ±0.023	1.32^{e} 0.025
Treatment groups	HSE_1 (gp_3)	187.33 ±1.695	177.76^{c} ±2.445	5.333^{f} ±0.054	3.113^{f} ±0.027	2.220^{ef} ±0.026	1.40^{ab} 0.006
	HSE_2 (gp_4)	188.16 ±4.000	170.64^{c} ±2.382	5.570^{e} ±0.042	3.270^{e} ±0.031	2.300^{e} ±0.012	1.42^{a} 0.007
	HSE_3 (gp_5)	188.87 ±2.464	162.19^{d} ±2.466	5.910^{d} ±0.096	3.477^{d} ±0.043	2.433^{d} ±0.055	1.43^{a} 0.015
	HSE_4 (gp_6)	190.57 ±2.986	153.31^{e} ±1.330	6.370^{c} ±0.078	3.657^{c} ±0.032	2.713^{c} ±0.047	1.35^{bc} 0.010
	HSE_5 (gp_7)	191.57 ±3.475	144.04^{f} ±1.528	6.740^{b} ±0.046	3.873^{b} ±0.029	2.867^{b} ±0.018	1.35^{bc} 0.003
	HSE_6 (gp_8)	193.66 ±5.315	127.73^{g} ±2.963	7.167^{a} ±0.105	4.123^{a} ±0.047	3.043^{a} ±0.062	1.36^{b} 0.018

a, b, c, ... Means with different superscript within columns are significant differences (P<0.05).
A/G ratio = Albumin / globulin ratio.

Table (4): Body weights and some carcass traits of Sinia cocks as affected by early heat exposure and glutamine injection under hot condition.

Treatments		Carcass traits (gm)							
		Initial body weight	Final body weight	Adrenal weight	Thyroid weight	Testis weight	Spleen weight	Liver weight	Heart weight
Control group (gp_1)		35.7 ±2.1	1569.9^{b} ±14.6	0.205 ±0.01	0.159^{a} ±0.01	19.00 ±0.61	1.81 ±0.06	22.31 ±1.10	10.29 ±0.32
Second group (gp_2)		39.9 ±2.9	1545.2^{b} ±43.9	0.186 ±0.01	0.165^{a} ±0.01	18.11 ±1.69	1.79 ±0.09	25.10 ±1.16	10.13 0.39
Treatment groups	HSE_1 (gp_3)	38.9 ±1.1	1608.2^{b} ±27.2	0.201 0.01	0.148^{ab} ±0.01	19.34 ±1.75	1.82 ±0.13	21.20 ±1.19	11.21 ±0.41
	HSE_2 (gp_4)	36.9 ±1.9	1617.4^{b} ±69.4	0.195 0.01	0.143^{ab} ±0.02	19.99 ±1.91	1.84 ±0.14	23.10 ±1.30	11.34 ±0.46
	HSE_3 (gp_5)	38.3 ±0.8	1666.6^{b} ±102.9	0.198 ±0.01	0.130^{b} ±0.01	21.12 ±2.01	1.86 ±0.16	24.60 ±1.45	10.89 ±0.49
	HSE_4 (gp_6)	39.2 ±2.2	1718.7^{b} ±61.8	0.179 ±0.01	0.127^{b} ±0.01	21.89 ±2.02	1.92 ±0.11	23.21 ±1.61	10.99 ±0.51
	HSE_5 (gp_7)	40.0 ±1.5	1722.5^{b} ±56.3	0.188 ±0.01	0.119^{b} ±0.01	22.17 ±2.41	1.95 ±0.17	24.50 ±1.71	11.21 ±0.62
	HSE_6 (gp_8)	38.1 ±1.0	1916.7^{a} ±53.5	0.202 ±0.01	0.115^{b} ±0.02	23.99 ±2.56	1.97 ±0.21	22.14 ±1.85	11.30 ±0.72

a, b, ... Means with different superscript within columns are significant differences ($P<0.05$).

Table (5): Semen quality of Sinia cocks as affected by early heat exposure and glutamine injection under hot condition.

Treatments		Traits				
		EV (ml)	**SC (× 10^6 ml)**	**TSO**	**SM (%)**	**TMS (× 10^6 ml)**
Control group (gp_1)		0.22 ^{b} ±0.01	620.35 ^{b} ±0.90	136.48 ^{b} ± 24.12	62.10 ^{b} ±4.23	84.75 ^{b} ±15.12
Second group (gp_2)		0.21 ^{b} ±0.02	610.00 ^{b} ±0.99	128.10 ^{b} ±32.11	59.81 ^{b} ±5.17	76.61 ^{b} ±23.10
Treatment groups	HSE_1 (gp_3)	0.23 ^{b} ±0.01	710.40 ^{b} ±109.3	163.34 ^{b} ±34.15	67.10 ^{ab} ±5.92	109.63 ^{b} ±34.14
	HSE_2 (gp_4)	0.24 ^{ab} ±0.02	740.19 ^{b} ±123.14	177.64 ^{b} ±37.16	69.21 ^{ab} ±5.17	122.94 ^{b} ±39.15
	HSE_3 (gp_5)	0.25 ^{ab} ±0.02	815.65 ^{ab} ±162.24	203.91 ^{ab} ±41.12	72.44 ^{ab} ±6.42	147.71 ^{ab} ±47.16
	HSE_4 (gp_6)	0.27 ^{ab} ±0.03	880.91 ^{ab} ±130.24	237.84 ^{ab} ±42.33	78.15 ^{ab} ±7.21	185.87 ^{a} ±49.15
	HSE_5 (gp_7)	0.28 ^{a} ±0.02	1000.68 ^{a} ±13.14	280.19^{a} ±46.48	80.62 ^{a} ±8.12	225.88 ^{a} ±52.10
	HSE_6 (gp_8)	0.29 ^{a} ±0.02	1180.24 ^{a} ±147.24	342.26^{a} ±52.11	84.17 ^{a} ±9.12	288.08 ^{a} ±63.41
		LS (%)	**DS (%)**	**SA (%)**	**SQF**	**pH**
Control group (gp_1)		62.1^{c} ±3.9	37.9^{a} ±3.9	19.6^{a} ±0.9	84.8^{b} ±17.3	7.9 ±0.0
Second group (gp_2)		61.3^{c} ±3.2	38.7^{a} ±3.2	18.2^{a} ±1.0	78.6^{b} ±19.4	77.7 ±0.0
Treatment groups	HSE_1 (gp_3)	64.1^{c} ±2.5	35.9^{b} ±3.5	15.1^{b} ±1.3	104.7^{b} ±29.2	7.8 ±0.1
	HSE_2 (gp_4)	67.2^{b} ±2.1	32.8^{b} ±2.1	14.0^{b} ±1.6	119.4^{b} ±30.6	7.7 ±0.1
	HSE_3 (gp_5)	70.3^{b} ±1.9	29.7^{b} ±1.9	12.1^{b} ±2.0	143.4^{b} ±24.2	7.8 ±0.1
	HSE_4 (gp_6)	73.2^{b} ±1.8	26.9^{b} ±1.8	11.2^{b} ±2.2	174.0^{a} ±26.2	7.7 ±0.1
	HSE_5 (gp_7)	75.2^{a} ±1.7	24.8^{b} ±1.7	10.1^{b} ±1.6	210.7^{a} ±42.2	7.8 ±0.1
	HSE_6 (gp_8)	78.3^{a} ±1.6	21.7^{c} ±1.4	9.9^{c} ±1.6	268.1^{a} ±45.5	7.7 ±0.1

a, b, c, ... Means with different superscript within columns are significant differences (P<0.05). EV, ejaculate volume; SC, sperm concentration; TSO, total sperm output; SM, sperm motility; TMS, total motile sperm; LS, live spermatozoa; DS, dead spermatozoa; SA, sperm abnormalities; SQF, semen quality factor; pH, hydrogen ion.

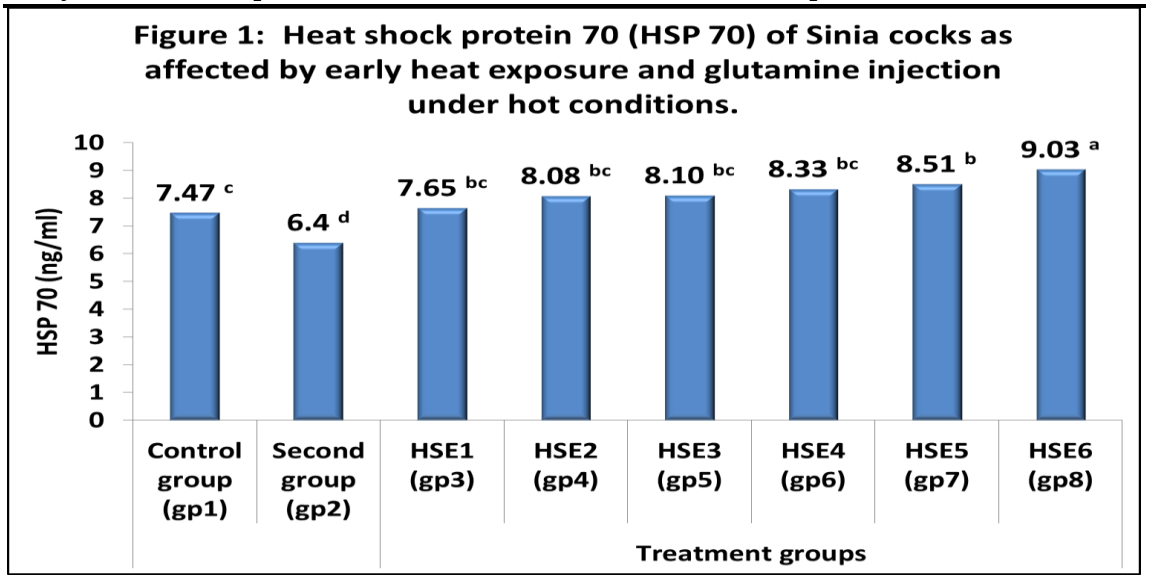


Figure 1: Heat shock protein 70 (HSP 70) of Sinia cocks as affected by early heat exposure and glutamine injection under hot conditions.
HSP 70 (ng/ml)
10
9
8
7
6
5
4
3
2
1
0
7.47 c
6.4 d
7.65 bc
8.08 bc
8.10 bc
8.33 bc
8.51 b
9.03 a
Control group (gp1)
Second group (gp2)
HSE1 (gp3)
HSE2 (gp4)
HSE3 (gp5)
HSE4 (gp6)
HSE5 (gp7)
HSE6 (gp8)
Treatment groups

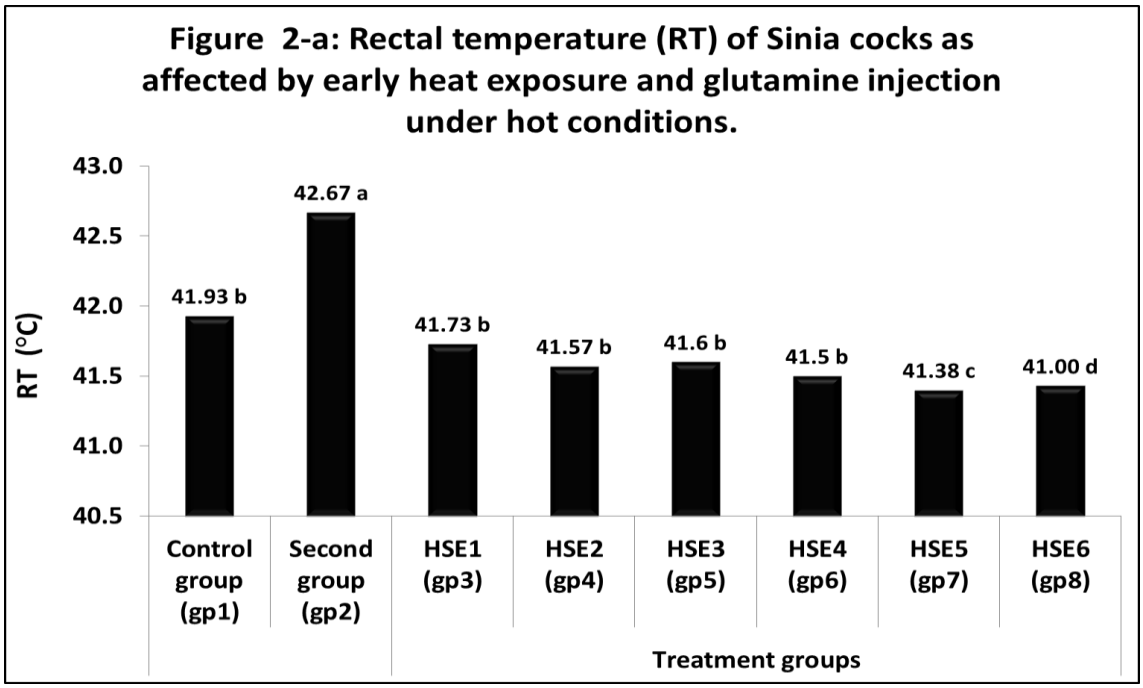


Figure 2-a: Rectal temperature (RT) of Sinia cocks as affected by early heat exposure and glutamine injection under hot conditions.
RT (°C)
43.0
42.5
42.0
41.5
41.0
40.5
41.93 b
42.67 a
41.73 b
41.57 b
41.6 b
41.5 b
41.38 c
41.00 d
Control group (gp1)
Second group (gp2)
HSE1 (gp3)
HSE2 (gp4)
HSE3 (gp5)
HSE4 (gp6)
HSE5 (gp7)
HSE6 (gp8)
Treatment groups

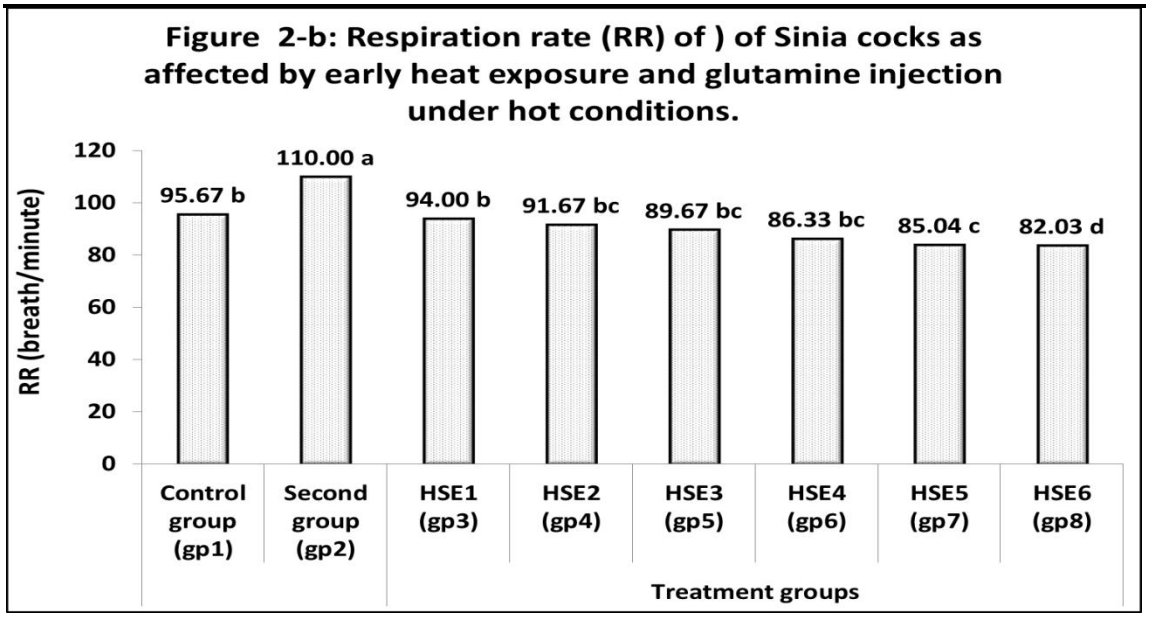


Figure 2-b: Respiration rate (RR) of) of Sinia cocks as affected by early heat exposure and glutamine injection under hot conditions.
RR (breath/minute)
120
100
80
60
40
20
0
95.67 b
110.00 a
94.00 b
91.67 bc
89.67 bc
86.33 bc
85.04 c
82.03 d
Control group (gp1)
Second group (gp2)
HSE1 (gp3)
HSE2 (gp4)
HSE3 (gp5)
HSE4 (gp6)
HSE5 (gp7)
HSE6 (gp8)
Treatment groups

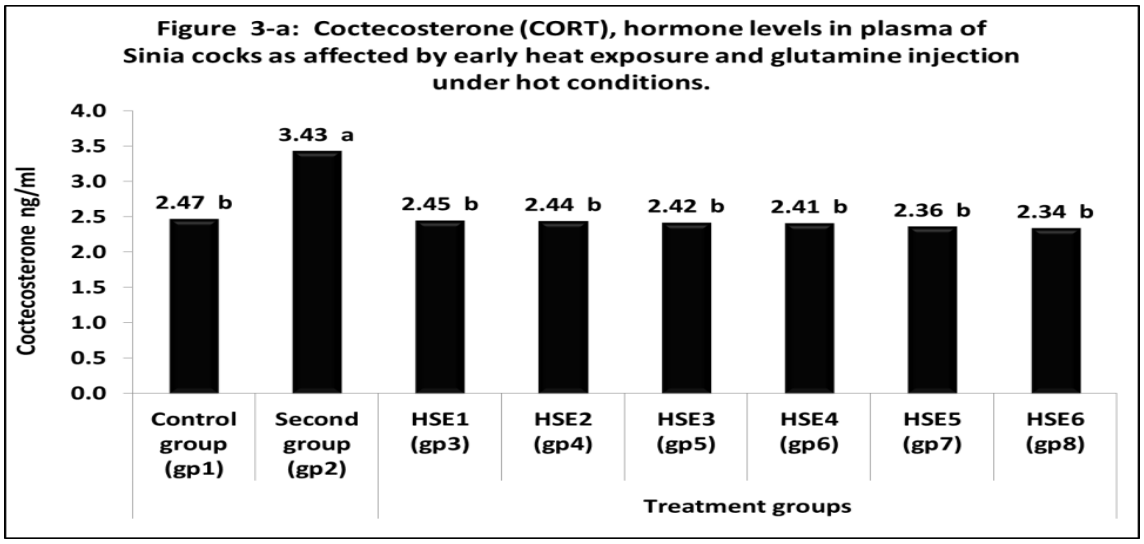
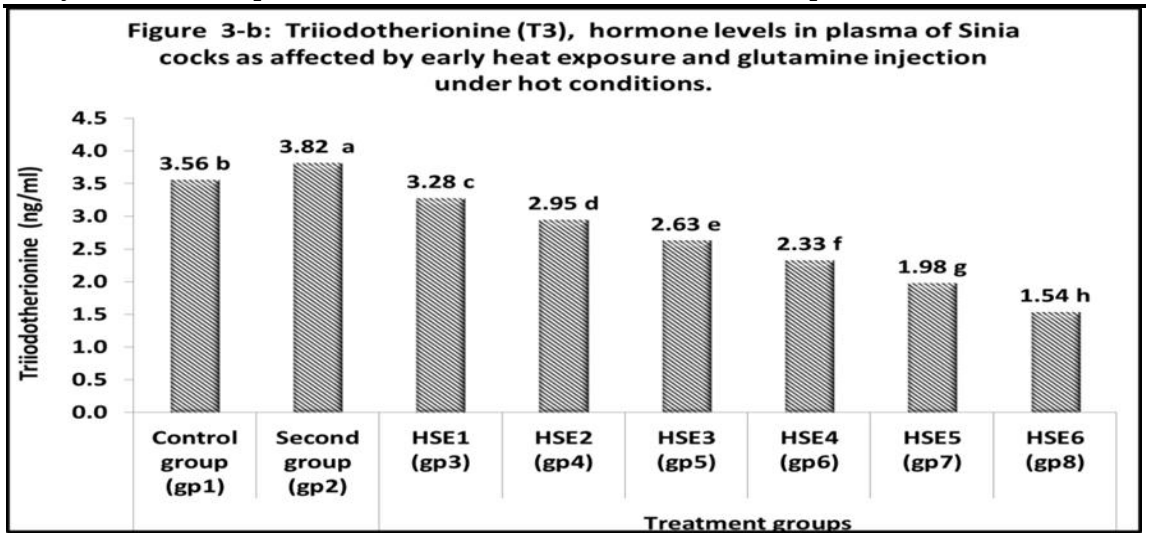


Figure 3-a: Coctecosterone (CORT), hormone levels in plasma of Sinia cocks as affected by early heat exposure and glutamine injection under hot conditions.
Coctecosterone ng/ml
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2.47 b
3.43 a
2.45 b
2.44 b
2.42 b
2.41 b
2.36 b
2.34 b
Control group (gp1)
Second group (gp2)
HSE1 (gp3)
HSE2 (gp4)
HSE3 (gp5)
HSE4 (gp6)
HSE5 (gp7)
HSE6 (gp8)
Treatment groups

Early Heat shock exposures - Glutamine - Cocks and Heat shock protein70.



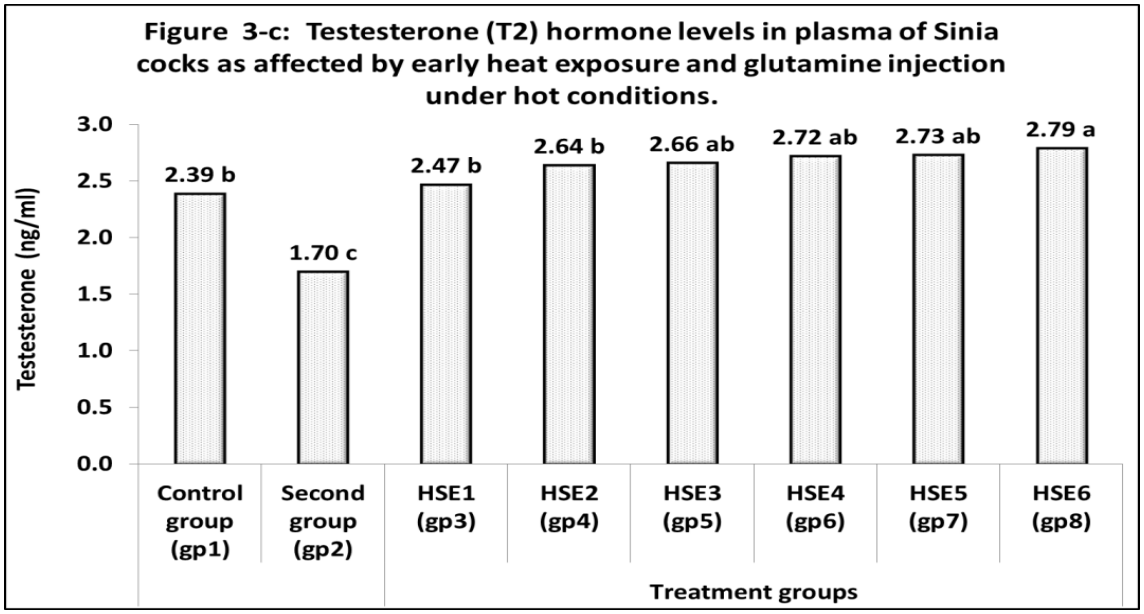


Figure 4-a: Primary Sheep Red Blood Cells (SRBC,s) and secondary Sheep red Blood Cells (SRBC,s) of Sinia cocks as affected by early heat exposure and glutamine injection under hot conditions.

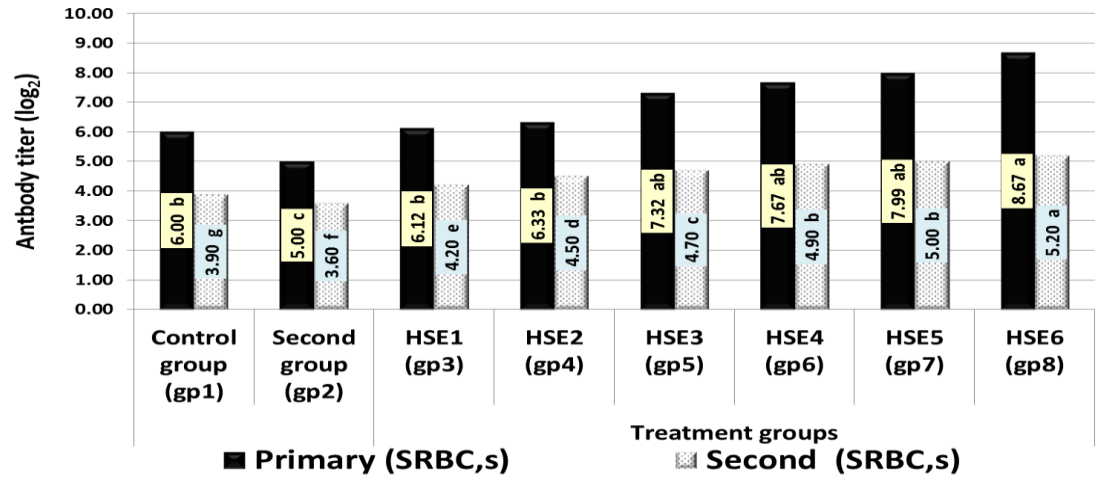


Figure 4-b: IgG and IgM antibodies titer (log 2) of Sinia cocks as affected by early heat exposure and glutamine injection under hot conditions.

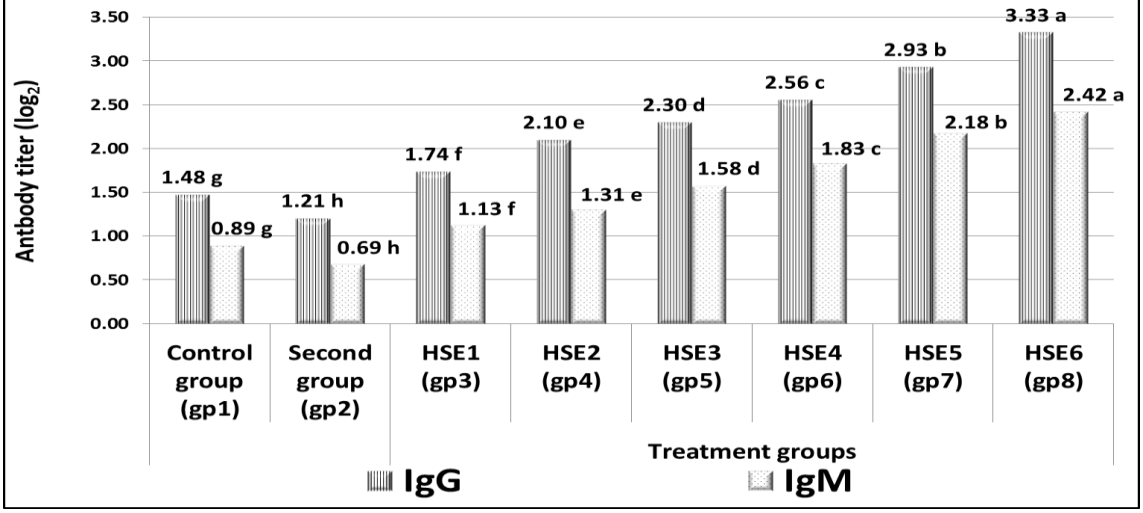


Figure 4-c: Newcastle disease virus (NDV), Infectious bronchitis virus (IBV) and Avian Influenza (AI) antibodies titer (log_2) of Sinia cocks as affected by early heat exposure and glutamine injection under hot conditions.

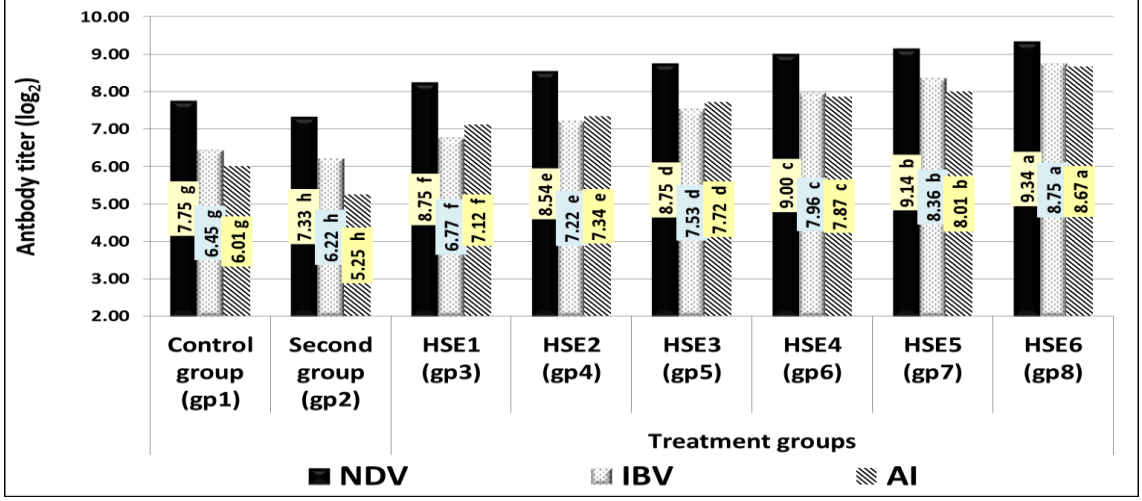
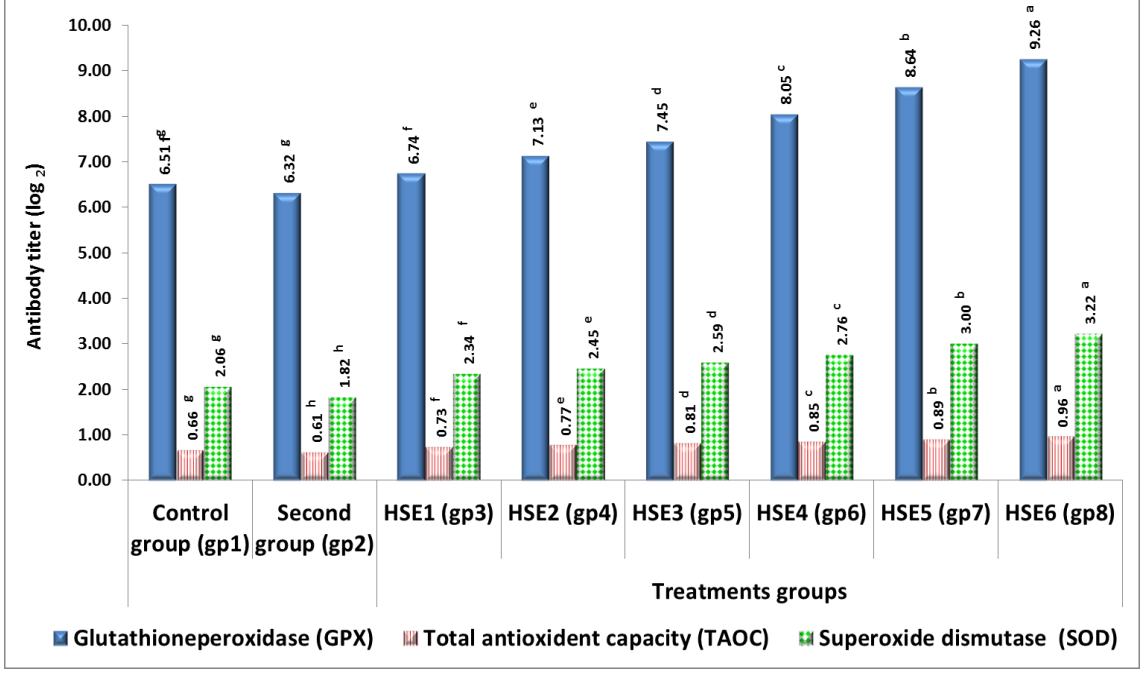


Figure 5: Some antibodies titer of male Sina chickens as affected by heat acclimation and glutamine injection treatments.



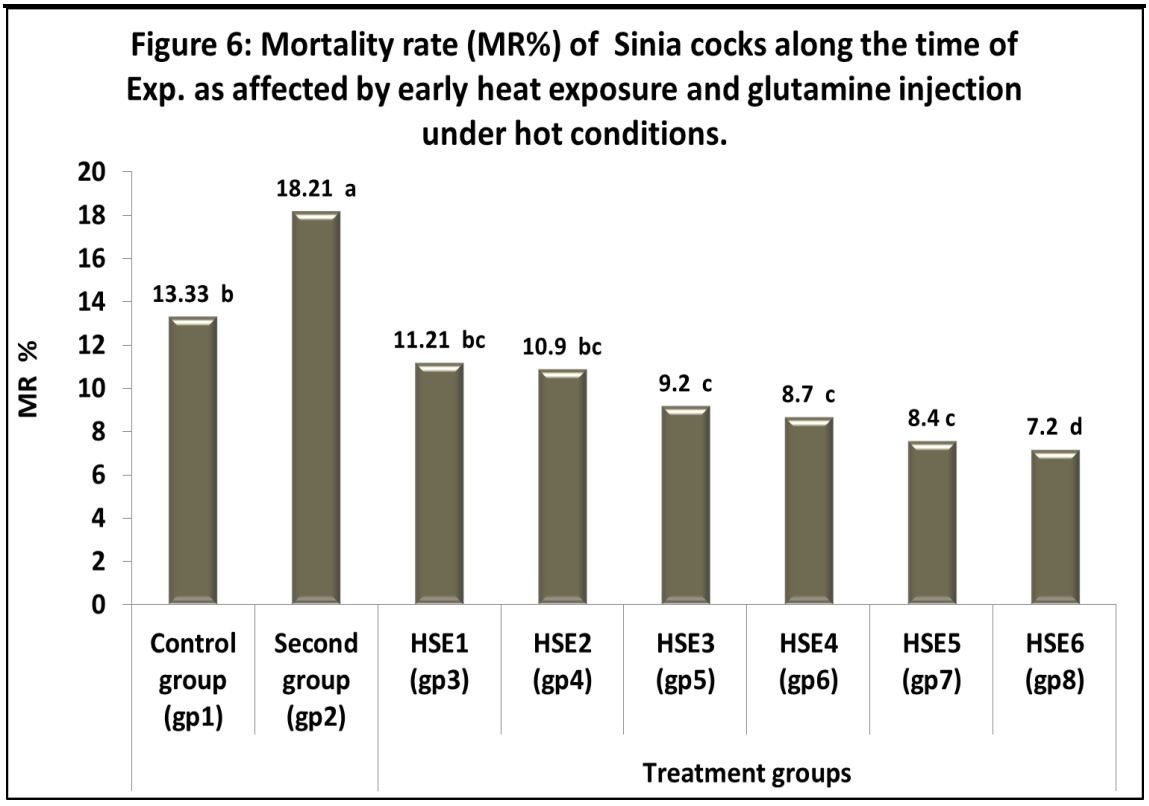


Figure 6: Mortality rate (MR%) of Sinia cocks along the time of Exp. as affected by early heat exposure and glutamine injection under hot conditions.
MR %
20
18
16
14
12
10
8
6
4
2
0
13.33 b
18.21 a
11.21 bc
10.9 bc
9.2 c
8.7 c
8.4 c
7.2 d
Control group (gp1)
Second group (gp2)
HSE1 (gp3)
HSE2 (gp4)
HSE3 (gp5)
HSE4 (gp6)
HSE5 (gp7)
HSE6 (gp8)
Treatment groups

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الملخص العربى

تأثير تخفيف الاجهاد الحرارى بالتأقلم المبكر للحرارة وحقن الجلوتامين على بعض القياسات الفسيولوجية والأداء المناعى وجودة السائل المنوى لذكور دجاج سينا.

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يهدف هذا البحث إلى زيادة أو تحفيز التعبير الجينى لبروتينات الصدمة الحرارية 70 عن طريق حقن الجلوتامين والتعريض لصدمات حرارية خلال مرحلة النمو وبحث تأثيرات ذلك على الاستجابات الفسيولوجية والمناعية وصفات جودة السائل المنوى خلال مرحلة التناسل لذكور دجاج سينا المحلى التى تربى تحت ظروف الأجواء المصرية الحارة خاصة فى أشهر الانتاج الحارة (يونيو، يوليو، أغسطس). استخدم عدد ثمانمائة كتكوت عمر يوم من ذكور دجاج سينا المحلى قسمت عشوائيا إلى ثمان مجموعات متساوية كما يلى:
المجموعة الاولي (كنترول) تم التربية تحت ظروف البيئة الطبيعية دون التعرض لاى صدمة حرارية او الحقن بالجلوتامين خلال مرحلةالنمواوالانتاج. المجموعة الثانية: لم تتعرض للحقن للجلوتامين او لصدمة حرارية خلال مرحلة النمو، بينما خلال مرحلة النمو تعرضت كتاكيت المجموعة (الثالثة والرابعة والخامسة والسادسة والسابعة والثامنة) الى:
1- صدمات حرارية (41°م ±1) لمدة 4 ساعات من الساعة 12: الي الساعة 16: لمدة ثلاثة ايام متتالية. حيث تعرضت كل من المجموعة (الثالثة والرابعة) لصدمة حرارية مبكرة عند عمر ثلاثة ايام فقط . بينما تعرضت المجموعة المجموعة (الخامسة والسادسة) لصدمتين حراريتين عند عمر ثلاثة ايام وعمر 8 اسابيع .في حين ان المجموعة (السابعة والثامنة) تعرضت لثلاث صدمات حرارية عند عمر ثلاثة ايام وعمر 8 اسابيع وعمر 16 اسبوع .
2- تم الحقن بالجلوتامين داخل الغشاء البريتوني (0.75 مللجم /1 كجم وزن حي): تم للمجموعة (الرابعة والسادسة والثامنة فقط) كالتالى:
تم حقن المجموعة الرابعة مرة واحدة فقط عند عمر يومين .وتم حقن المجموعة السادسة مرتبن عند عمر(يومين و34 يوم من العمر).
بينما تم حقن المجموعة الثامنة ثلاث مرات عند عمر (يومين ، 34 ، 111 يوم من العمر).
اثناء مرحلة النمو عادت كل المجموعات بعد الحقن بالجلوتامين وفترة التعريض للصدمة الحراية الى الرعاية تحت الظروف الطبيعية.
اثناء مرحلة التناسل تعرضت كل المجموعات الى الظروف الجوية الحارة وخلال تلك الفترة تم تعريض كل المجموعات (الثانية والثالثة والرابعة والخامسة والسادسة والسابعة والثامنة) فيما عدا الكنترول الى اختبار حرارى (38°م ±1) (لمدة 4 ساعات من الساعة 12: الى الساعة 16: لمدة يوم واحد عند اعمار (24 ، 30 ، 34) اسبوع من العمر.
أوضحت النتائج أن: ديوك المجموعة الثامنة حدث لها زيادة معنوية فى كل من (بروتين الصدمة الحرارية 70 ووزن الجسم النهائي وعدد كرات الدم الحمراء وتركيز الهيموجلوبين ونسبة المكونات الخلوية والبروتين الكلى والالبيومين والجلوبيولين (ونسبة الالبيومين الى الجلوبيولين) والانزيمات المضادة للاكسدة وكذلك القياسات المناعية وهرمون التستيسترون ونسبة الحيوانات المنوية الحية وحجم القذفة وقدرة الحيوانات المنوية الحية على الحركة وتركيز الحيوانات المنوية) بينما انخفض معنويا (درجة حرارة المستقيم ومعدل التنفس وعدد كرات الدم البيضاء (المتعادلة الى الليمفاوية) وهرمون الكورتيكوستيرون وهرمون التراي ايودوثيرونين ونسبة الحيوانات المنوية الميتة والمشوهة ومعدل النفوق. مقارنة بالمجموعة الكنترول وباقى المجموعات.
تخلص هذه الدراسة إلى أنه ربما يؤدى استخدام التعريض الحرارى والحقن بالجلوتامين خلال مرحلة النمو (ثلاثة مرات حقن بالجلوتامين عند عمر يومين وعمر 34 يوم وعمر 111 يوم ، وثلاثة مرات صدمة حرارية عند عمر ثلاثة أيام وعمر 35 يوم وعمر112يوم) تحسن الاستجابات الفسيولوجية لذكور دجاج سينا المحلى التى تعرضت لإجهاد حرارى فى مرحلة التناسل من خلال زيادة التعبير الجينى لبروتين الصدمة الحرارية 70 وتحسنت الاستجابات المناعية عن طريق زيادة انتاج الاجسام المضادة وتركيز الجلوبيولين وانخفاض نسبة كرات الدم المتعادلة لنسبة كرات الدم الليمفاوية وبالتالى أدى ذلك إلى تخفيف التأثيرات السلبية للإجهاد الحرارى على استجابات التنظيم الحرارى وصفات الدم وجودة السائل المنوى ومعدل النفوق.