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THE EFFECT OF CHROMIUM PICOLINATE SUPPLEMENTATION ON IMMUNE RESPONSE AND HEAT SHOCK PROTEINS OF LAYING HENS UNDER HEAT STRESS CONDITIONS.

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ABSTRACT: The present study aimed to investigate the effects of heat shock programs during the growth period without or with chromium picolinate (CrPic) on productive and physiological performance, blood biochemical traits as well as, immune response, heat shock proteins of Mandarah laying hens during first 90 days of egg production (EP) reared under Egyptian summer condition. Seventy hundred-one day old of unsexes Mandarah chicks were randomly divided into seven equal groups (100 chicks each). The 1st treatment was served as a control group and fed a control basal diet and reared under natural conditions. While, the chicks in 2nd, 3rd, 4th, 5 th, 6 th and 7 th treatments were exposed to early heat shock (40±1 °C for 4 hours from 12.00 to 16.00 p.m. for 3-5 consecutive days). The 2nd treatment was exposed to early heat shock at 3 days of age (HSE1). Whereas, the 3rd treatment was exposed to heat shock at 3 days of + 800 µg/ CrPic/kg diet (HSE2). The 4th treatment was exposed to heat shock at 3 days and at 8 weeks of age (HSE3). The 5th treatment was exposed to heat shock at 3 days and at 8 weeks of age + 800 µg/ CrPic /kg diet (HSE4). The 6th treatment was exposed to heat shock at 3 days and at 8 and 16 weeks of age (HSE5). The 7th treatment was exposed to heat shock at 3 days and at 8, 16, weeks of age + 800 µg/ CrPic /kg diet (HSE6).

The obtained results revealed that the egg production (EP) %, egg mass (EM) in hens and some semen characteristics in cocks exposed to heat shock were significantly (P≤0.05) improved in HSE6. Rectal temperature (RT), respiration rate (RR), heterophils % and Heterophils/ lymphocyte (H/L) ratio were significantly (P≤0.05) decreased. Birds in the treatment groups exposed to heat shock in HSE5 or HSE6 were significantly (P≤0.01) increased globulin, HSP70 of Liver, superoxide dismutase (SOD) and glutathione peroxidase (GPX). While, Triiodothyronine (T3) and total antioxidant capacity (TAOC) were significantly (P≤0.01) decreased compared to the control group.

Keywords: Heat shock programs –Chromium -Productive and physiological performance.

INTRODUCTION

In Egypt, the climate is characterized by a long hot period (from May to October) and short mild one (from December to March). One of the problems in the poultry industry is the high ambient temperature, which stay around 6 months of the year (May to October) in Egypt, as it is can be trade off the ability of birds to look after homeostasis (Kadim et al., 2008). In summer, high environmental temperatures can be dangerous to laying hens, on account of high mortality, as well as due to the lessening in the number and quality of the eggs produced (Daghir, 1995). Additionally, Mack et al. (2013) showed that birds subjected to heat-stress condition invests less time feeding, more time drinking and panting and additional time with their wings elevated, less time moving or walking, and additional time resting. Lin et al. (2008) reported that heat stress caused decreased production performance, lessened eggshell thickness, and increased egg breakage. Moreover, heat stress has been appeared to cause a significantly reduction in the EW (−3.24%), egg shell thickness (−1.2%), eggshell weight (−9.93%), and eggshell percent (−0.66%) according to Ebeid et al. (2012). Heat stress can influence on the reproductive function of poultry in various ways. In females, heat stress can upset the typical status of reproductive hormones at the hypothalamus, and at the ovary, leading to reduced systemic levels and functions (Elnagar et al., 2010). Likewise, negative impacts were caused by heat stress in males have been appeared in changed investigations.

Chromium (Cr) is one of the basic minerals, which is required for enhancing beneficial production in poultry because of its important functions in digestion, growth and decrease of lipid and protein peroxidation (Farag et al., 2017). Chromium supplementation was helped in reestablishing the decrease in performance, productivity, nutrient digestibility, immune status and antioxidant profile as a result exposure to heat stress. Chromium was proposed to be a proactive piece of a biomolecule called chromodulin, which is a piece of the insulin flagging pathway and seems to influence sugar and lipid digestion (Vincent, 2000). The essential part of Cr in digestion is to potentiate the activity of insulin through its quality in an organometallic particle called glucose resistance factor (GTF) (Kegley and Spears, 1995). Immunological capacity upgrades by trivalent Cr and its belongings appear to be more articulated amid the seasons of stress (Borgs and Mallard, 1998). Abdallah et al. (2013) reported that supplementing the chicken diet with 800 µg chromium picolinate improved most of the previous mentioned productive traits, egg quality, semen quality, fertility and hatchability carcass parameters, blood parameters as well as the immune response. Therefore, the present study aimed to define work the effects of heat shock programs during the growth period without or with chromium picolinate (CrPic) on productive and physiological responses on

Mandaraha laying hens under condition of Egypt.

MATERIALS AND METHODS

Birds, diet and treatments:

The experimental work of this study was carried out at the Inshas Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt, from February to October, 2016). Seventy hundred-one day old of unsexes Mandarah chicks during the growth period were randomly divided into seven equal treatment groups (100 chicks each). The 1st treatment was served as a control group (non-exposure to heat shock during growth period) and fed a control basal diet and reared under natural conditions. While, the chicks in 2nd, 3rd, 4th, 5 th, 6 th and 7 th treatments were exposed to early heat shock (40±1 °C for 4 hours from 12.00 to 16.00 p.m. for 3-5 consecutive days, using gas heaters). The 2nd treatment was exposed to early heat shock at 3 days of age (HSE1). Whereas, the 3rd treatment was exposed to heat shock at 3 days of age + 800 µg/ chromium picolinate (CrPic) /kg diet (HSE2). The 4th treatment was exposed to heat shock at 3 days and at 8 weeks of age (HSE3). The 5th treatment was exposed to heat shock at 3 days and at 8 weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet (HSE4). The 6th treatment was exposed to heat shock at 3 days and at 8 and 16 weeks of age (HSE5). The 7th treatment was exposed to heat shock at 3 days and at 8, 16, weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet (HSE6). During first 12 weeks of EP, treatments (HSE1, HSE2, HSE3, HSE4, HSE5 and HSE6) were exposed to heat challenge (38±1 °C for 4 hours from 12.00 to 16.00 P.M.) at 24, 30 and 34 weeks of age in a completely randomized design. Each group was housed on the floor pens until they reached to 18 weeks old. Then, birds were transferred and housed individually in single cages in an open system house. Birds were fed ad libitum and fresh water was provided continuously. The basal experimental diet was formulated according to NRC (1994) to meet the nutrition requirements of chickens during the experimental period (from one-day old to 36 weeks of age) as shown in Table (1). Birds were submitted to the same managerial condition in a window house with light cycle regimen (16 hours light: 8 hours darkness). Birds were examined against diseases and treated with antibiotics and vaccines to keep them healthy.

The average minimum and maximum of ambient temperature during the experimental period ranged between 21.71 and 31.80 ^{0}C, relative humidity from 25.67 to 77.23% and temperature-humidity index (THI) from 20.07 to 30.55 under Inshas, Sharkia Governorate, Egypt as shown in Table 2. THI was estimated according to the formula as follows: THI=db ^{o}C-{(0.31-0.31 RH) (db ^{o}C -14.4)}, where db ^{o}C = bulb temperature in Celsius and RH= RH%/100. The values obtained indicate the following: <22.2 = absence of heat stress; 22.2 to <23.3 = moderate heat stress: 23.3 to <25.6 = severe heat stress and 25.6 and more = extreme severe heat stress (Marai et al., 2000).

Measurements:-

Laying performance traits:

Body weight (BW) of laying hens at sexual maturity was recorded at 5 % EP (22 Weeks) and at the end of the experiment (36 weeks). While, the egg number (EN) and egg weight (EW) were recorded daily. Feed consumption (FC) was calculated weekly. The EP rate was calculated during the experimental period. Where: EP rate = EN / hen/ x 100. EM was calculated by multiplying EN by average EW. Feed conversion (g feed/g egg) (FCR) was also calculated. The mortality rate was recorded daily for each treatment from one day to the end of the experiment.

Physiological parameters:

Rectal temperature (RT) and respiration rate (RR) were measured at 24, 30 and 34 weeks of age after exposure to heat challenge. Rectal temperature (°C) was randomly measured in 10 birds in each group for each age by inserting clinical thermometer (2-3 cm) into the cloaca for one minute. RR (breaths/minute) was recorded at random for 10 birds in each group for each age by counting the body wall movements per one minute.

Egg quality: At the end of the experimental period ten eggs, were randomly taken from each treatment to study egg quality measurements. Egg quality (egg shape index %, yolk index %, shell thickness and Haugh unit) and percentage of egg components (yolk, albumin and shell) was determined. The Haugh unit score for each egg was calculated according to Haugh (1937).

Immunological analysis: Blood samples were withdrawn from 3 hens per treatment (5 ml/ hen) from the brachial vein into tubes containing EDTA as anticoagulant at 24, 30 and 34 weeks of age after exposure to heat challenge to examine immediately hematological parameters. Fresh blood samples were taken to determine hemoglobin (Hb), total count of red blood cells (RBCs), total count of white blood cells (WBCs) and their differentiations (Heterophils%, lymphocyte%, and H/L ratio) according to Clark et al. (2011). The Infectious Newcastle virus (NDV), Influenza viruses (H5N1) and (H9N2), detection were performed using a commercial ELISA kit (Shenzhen Lvshiyuan Biotechnology Co., Shenzhen, Guangdong, China), according to the manufacturer's instructions. The assay was performed to obtain a qualitative evaluation of the infections, NDV, H5N1, and H9N2, disease virus production. The immunoglobulin IgG and IgM in blood plasma were determined using a commercial ELISA kit from Bethyl Laboratories (Montgomery, AL, USA), as the method described by Gao et al. (2008).

Blood samples: Blood was collected in tubes with EDTA centrifuged at 1000 g for 20 minutes. Blood plasma samples were collected and stored at -20°C until analysis of total protein, albumin, glucose, cholesterol, LDL, HDL, total lipids and triglyceride concentrations. Antioxidant components and antioxidant enzymes (SOD, GPX and TAOC) were determined using commercial Kits produced by Bio-diagnostic, Egypt. Triiodothyronine (T3), progesterone and estradiol-17B concentration were determined in blood plasma using radioimmunoassay Kit. Heat

shock protein 70 level (HSP 70) of liver was determined after exposure to heat challenge by ELISA method using kits of Uscn Life Science Inc. Wuhan, Chain.

Semen quality:

After production of the primary egg, all females were artificially inseminated with a blend of semen collected from the same group of cocks twice every week. Semen was collected at three times amid the trial time frame in 24, 30 and 34 weeks of age from 5 cocks in each treatment. Cocks were randomly chosen using the massage method. Immediately after semen collection, semen-ejaculate volume (ml) was measured utilizing graduate collecting tubes and hydrogen-ion concentration (pH) was measured by Universal Indicator Paper and Standard Commercial Stain. A drop of semen with the guide of a micro-pipette was set on a pre warmed microscope slide, which was then covered with a glass cover slip and inspected at a magnification of ×400. Motility of semen samples was tested as the percentage of motile spermatozoa having moderate to rapid progressive movement and cells that are motile under their own power (Ommati et al., 2013). At least 10 microscopic fields were inspected for every semen test. Eosin-Nigrosine stain was utilized to decide the percent of morphologically sperm abnormalities and dead spermatozoa. For sperm cell concentration (X 10^9/ml) a droplet of diluted semen (1:200 in distilled water) semen was tenderly put on both councils of a Neubauer hemocytometer and the number of spermatozoa was determined microscopically (Ommati et al., 2013). Acrosomal damage (%) of spermatozoa was determined according to Waston (1975). No less than 10 minuscule fields were inspected for every semen test.

Statistical analysis:

Data were analyzed by the least square analysis of variance according to Snedecor and Cochran (1982) using the General Linear Model Procedure (SAS, 2004) at the 5% level of significance as the following model:

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

Where: Y_{ij} = Any observation, μ = Overall mean, N_i = Effect of treatment (i = 1….7)., e_{ij} = Experimental random error. All percentages, data were transferred to percentage angle using arcsine equation before subject to statistical analysis. Significant differences among means were tested using Duncan Multiple New Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Productive performance:

Results in Table 3 revealed that, BW at sexual maturity, final body weight (FBW), FC and FCR were not significantly affected by HSE programs in laying hens fed diets supplemented without or with CrPic as compared with the control group during all the experimental periods. Whereas, the EP (%) and EM (g/hen) were significantly ($P<0.05$) increased in hens exposed to HSE6 as compared to control group. Similarly, EW was significantly ($P<0.05$) increased in hens exposed to HSE1 as compared to the control group. The increase of EW, EP % and EM with CrPic supplementation are in agreement with those of Abdel-Mageed et al. (2012) who observed that feeding Japanese quail diets supplemented with CrPic was enhanced

EP; EW and EM ratio under hot climate. It is understood that Cr is incorporated into protein mix and there is a relationship of Cr with DNA formats that realized a basic impelling of RNA mix. The oligopeptide was low-nuclear weight Cr-limiting protein (chromoduline) solidly ties four chromic particles before the oligopeptide gets an adjustment required for authority to the tyrosine kinase dynamic site of the insulin receptor (Vincent, 2000). In this way, chromodulin appears to accept a section in an auto upgrade framework in insulin hailing (Sahin et al. 2002). Also, Ezzat et al. (2016) reported that CrPic supplementation, diet significantly ($P\leq0.05$) increased EW, EP rate and EM as compared with control treated group. The results in Table (3) show that the mortality rate was significantly ($P<0.05$) decreased in hens exposed to HSE4 and HSE6 compared to with the control group. This exhibited heat shock program prompted to decrease mortality and this may be because of an improve of thermo- tolerance these hens. Increasing mortality rates might be credited to the inability to control body temperature under heat stress conditions (Nagwa et al., 2012 and Morsy, 2013). Similar trend Ezzat et al. (2017) who reported that broiler diets supplemented with 1200 µg CrPic /kg diet during heat stress resulted in a significant ($P\leq0.05$) decrease mortality rate about 11.67% from 0-42 days of age. This showed that heat shock programs prompted to decreased mortality and this might be because of improving thermo- tolerance and immunity.

Physiological parameters:

Figures (1) and (2) shows that rectal temperature (RT) and respiration rate (RR) was significantly ($P\leq0.05$) decreased by HSE programs in laying hens fed diets supplemented with CrPic as compared with the control group at the end of the experimental period. On the other hand, HSE6 decreased RT (40.73) and RR (90.33) of Mandarah than all other groups and untreated groups. These results might be of useful effect of heat acclimation in lessening the metabolic heat production through their effects on thyroid hormone secretion rates or by means of the effect of heat acclimation on adrenal gland function. Under compromised hot conditions in summer (31.6-38.1°C). Tanizawa et al. (2014) found that the RT experienced chicks was lower than that in control while there was no difference in RR between the different groups. Besides, hen diets supplemented with 800 µg CrPic /kg diet lead decreased RT and RR compared to those untreated and HSE programs. These results are in concurrence with those reported by Norain et al. (2013) who detailed that chromium supplementation as feed additives resulted in a slightly lower RT, and significantly ($P\leq0.05$) lower RR of the broiler chickens received a diet supplemented with chromium compared to the control.

Egg quality and egg components (%):

Table (4) shows that egg quality and egg components were not significantly affected by HSE programs in laying hens fed diets supplemented without or with CrPic. While, yolk index % and shell thickness were significantly ($P<0.05$) higher for all

treated layers as compared with the control group. These improvements in egg shell thickness in the treated with HSE programs and fed diets supplemented with CrPic may be because of the lessening of respiratory rate of hens (Figure 2). Nagwa et al. (2012) found that significantly ($P \leq 0.05$) differences among treatments were observed in egg shell thickness exposed to early heat shock at 3 days and at 8 weeks of age or at 3 days, at 8 weeks and 16 weeks of age of White Leghorn hens. On the other hand, Haugh units was significantly ($P<0.05$) increased in Mandarah laying hens exposed to HSE1 compared to with the control groups or other treated. These results are in agreement with those of Kirunda et al. (2001) who revealed that Haugh units of eggs of heat-stressed birds were significantly ($P<0.05$) decreased after heat exposure. In addition, Sahin et al. (2002) found that supplemental chromium was significantly ($P<0.05$) increased egg shell thickness, egg specific gravity, Haugh unit, yolk index and yolk weight percent of laying Japanese quail.

Some blood hematological parameters and immune response:

Table 5 result shown that HSE programs in laying hens fed diets supplemented without or with CrPic were a significant ($P \leq 0.01$) increased of hemoglobin concentration (Hb), red blood cells (RBC's), white blood cells (WBC's) and lymphocyte (L%). Well, there was a decrease of heterophils (H %) and H/L ratio as compared with the thermoneutral control layer hens. These results may be due to improve in the thermoregulation responses (decrease RT and RR) in these groups (Figures 1 and 2) which reflected in increased Hb concentration, RBC's, WBC's counts and Hb concentration. Additionally, the increase of Hb, RBC's, WBCs and L% concentrations were obtained in the present examination might be credited either to its direct stimulatory effect on bone marrow, which was already revealed by Anwar et al. (1998). These results are in agreement with Nagwa et al. (2012) and Morsy (2013) they announced that hematological concentrations increased under HSE programs in laying hens and cocks compared with control birds. Morsy (2013) found also that Heterophil/Lymphocytes ratio (H/L ratio) was improved ($P<0.05$) in cocks exposed to heat shock (two or three times) at 3 days, at 8 and 16 weeks of age contrasted with the control group. On the other hand, in the present examination Hb (%), total count of RBCs, WBCs, L (%), H (%) and H/L ratio were significantly enhanced by Cr supplementation. These results might be due to make of chromium in balancing out the red blood cells against cellular changes caused by peroxidation (Linder, 1991). These results are in agreement with those of El-Samra et al. (2014) who found that laying hens fed the Cr (1200 and 1400 μg/kg diet) - supplemented diets achieved significantly higher total counts of RBCs, WBCs, and lymphocytes, hemoglobin as compared to their control groups. Toghyani et al. (2007) specified that Hb was increased significantly and the H:L ratios, reduced in broilers fed 1,000 and 1,500 ppb of chromium picolinate under heat-stress condition.

Also, the results show that HSE programs in laying hens fed diets supplemented without or with CrPic were significantly (P≤0.01) increased of antibody titers against infectious Newcastle disease virus (NDV), Influenza viruses (H5N1), Influenza viruses (H9N2), immunoglobulin G (IgG) and immunoglobulin M (IgM) concentrations as compared with the thermoneutral control laying hens (Table 5). These results are in agreement with those of Mashaly et al. (2004) where antibody production increased in commercial laying hens, which pre-conditioning and then exposed to heat stress at a later age. Also, in cocks, Morsy (2013) found that HSE, particularly at 3 days, at 8 and 16 weeks of age prompted increase in HSP70 which thus act to restrain the arrival of cytokines, oxygen free radicals and nitric oxide, consequently increased immunity responses (Polla and Cossarizza, 1996).

On the other hand, NDV, H5N1, H9N2, IgG, IgM were significantly enhanced by Cr supplementation. These results are in agreement with the findings of Mirfendereski and Jahanian (2015) who detailed that dietary consideration of CrMet was enhanced NDV antibody response at day 14 post vaccination (P<0.001) and caused a numerical (P=0.142) increase in NDV titers at day 7 post vaccination. Bhagat et al. (2008) exhibited that the supplementation of chromium at appropriate dose might be helpful to enhance the IFN-gamma mRNA expression in response to NDV in broiler chickens. So also, Farhad Hajializadeh et al. (2017) found that antibody titers against avian influenza and infectious of bronchitis of 21 to 42 days of age in broilers fed supplemented with chromium and nano-chromium were higher than broiler chickens fed the control diet (P≤0.05). Toghyani et al. (2007) uncovered that immune response titers against Newcastle, influenza viruses and serum IgG increased in broiler chickens fed 1,000 and 1,500 ppb of chromium picolinate at 30 days of age under heat-stress condition. Ezzat et al. (2017) found that IgG and IgM titers had a tendency to be higher in broiler chicks fed Cr supplementation. Farag et al. (2017) showed that dietary expansion of Cr has promising impacts on the immune system through increasing relative weights of lymphoid organ, for example, thymus, spleen and bursa of Fabricius, declined heterophil/ lymphocyte (H/L) ratio, upgrading the Cell Mediated Immune (CMI) reaction and enhancing the antibody response versus the infectious diseases.

Blood constituents:

Data presented in Table 6 shows that HSE programs in laying hens fed diets supplemented without or with CrPic were no significant differences among the 7 treatments in total proteins, albumin, glucose, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total lipids, triglycerides, progesterone and estradiol-17B hormone. The birds treatment exposed to heat shock at 3 days and at 8 and 16 weeks of age without or with Cr was significantly (P≤0.01) increased globulin, HSP70 of Liver, superoxide dismutase (SOD) and glutathione peroxidase (GPX). While, Triiodothyronine (T3) and total antioxidant

capacity (TAOC) were significantly ($P \leq 0.01$) decreased than the control group. The antioxidant enzyme system, including SOD, CAT, and GPX, works in concert with free radical scavengers to quench ROS and to protect cells from oxidative damage (Weiss, 1986). The balance between the production of free radicals and antioxidant systems could be disturbed by heat stress in chickens (Lin et al., 2008). The same author added that plasma SOD activity was increased in heat-stressed laying hens. Acute heat stress, increased serum activities of SOD, CAT, and GPX, (Yang et al., 2010). The increase of globulin might be a change of the immune responses of birds that exposed to HSE programs in laying hens fed diets supplemented without or with CrPic compared with control groups. In addition, the significant increase of globulin concentration utilized as a pointer of immune responses and source of antibody production (El-Kaiaty and Hassan, 2004). These results are in agreements with those of Mashaly et al. (2004) and Nagwa, et al. (2012). They detailed that HSE prompted increased levels of HSP70. The upgraded of Hsp70 expression might be a reaction to stressful environments, and may enhance cell survival by protecting proteins from corruption and encouraging their refolding (Pratt, 1993). The over expression of heat shock protein may be the purpose of the extended essential ATP after warmth push (Koelkebeck and Odom, 1995). The more prominent HSP70 expression may suggest that the proteins are involved in the stress caused by HSE in chickens. The mechanism to combat heat stress includes decreasing hyperthermia, which might be halfway because of the security of tissues to hyperthermia because of the prior condition (Rajkumar et al., 2015). Triiodothyronine hormone (T3) assumes an important role in regulating metabolism and thermogenesis in chickens (Tao et al., 2006). Accordingly, heat tolerance enhances, as thyroid function is lessened (Bowen and Washburn, 1985).

On the other hand, some blood constituents were significantly enhanced by CrPic supplementation (Table 6). Khan et al. (2014) found that supplementation of Cr has promising effects on the immune system by method for the relative increase in lymphoid organ weight (bursa of Fabricius, spleen and thymus), was decreased heterophil:lymphocyte ratio, improved antibody response against infectious diseases and increased Cell-Mediated Immune (CMI) response. Sahin et al. (2003) declared that Cr supplementation in broiler chickens caused an increase in serum T3 and T4. These results affirm the information that Cr demonstrations synergistically with antioxidant enzymes, including superoxide dismutase, glutathione peroxidase and total antioxidant capacity. In the meantime, HSP70, layer hens exposed to HSE programs caused a significantly increased in HSP70 expression of liver compared with treatment group Cr had a lower HSP70 level than those fed the basal diet control. Kulkarni et al. (2012) found that the addition of chromium picolinate significantly down regulated relative expression of HSP70 gene in jejunum on the 28^{th} day (40 mg/kg) and 42^{nd} day (20

mg/kg) of age. Literature regarding the effect of dietary chromium supplementation on HSP70 expression is very scarce. HSP 70 is a pressure actuated protein, which is an important part of the cell's apparatus for protein collapsing. High levels can be delivered by cells in light of hyperthermia. The protein goes about as a sub-atomic chaperone by official to other cell proteins, helping intracellular transport and collapsing into the correct optional structures, in this manner counteracting conglomeration of protein during stress (Hartl, 1996). Farag et al. (2017) demonstrated that chromium is an antioxidant and influences lipid peroxidation by battling free radical harm in the body. Cheng et al. (2016) found that chromium trichloride (CrCl3) in drinking water was significantly ($P < 0.05$) decreased of the total antioxidant capacity compared with the controls.

Semen characteristics:

Table 7 shows that semen ejaculate volume and hydrogen-ion concentration (pH) of cocks were not significantly affected by among the seven treatments. Nevertheless, sperm motility (%), sperm-cell concentration **(**X 10^9/ml) and acrosomal damage (%) were significantly ($P<0.05$) improved, while dead spermatozoa (%) and sperm abnormalities (%) were significantly ($P< 0.05$) decreased in cocks exposed to early age exposed to heat shock at 3 days and at 8 and 16 weeks of age without or with 800 µg CrPic/kg diet compared with the control group or other treatments. Heat stress influences on all periods of semen production in raiser cocks as reported by King`ori (2011). The improvement in semen quality characteristics may be explained by heat shock at 3 days and at 8 and 16 weeks of age without or with 800 µg CrPic/kg diet led to increase expression of HSP70 which stimulates testicular growth in the early phase and promotes increased sperm motility and sperm-cell concentration. In addition, HSP70 is one of the most boundless atomic chaperone particles and is fundamental to the upkeep of cellular homeostasis in response to stressful cellular condition (Duagaard et al. 2007). As a rule, intracellular restriction of HSP70 within the cytosol and in organelles, for example, the nucleus, mitochondria, and endoplasmic reticulum have been accounted for as this protein lacks an N-terminal signal sequence (Duagaard et al. 2007). HSP70 ensure the somniferous epithelial cell differentiation against heat stress damage, which is shown in increase semen characteristics, as well as, keep up to homeostasis under the stress condition (Obidi et al., 2008).

On the other hand, semen quality was significantly ($P\leq0.05$) enhanced by CrPic supplementation. The improvement in semen quality might be attributed to the antioxidant activity of chromium which kept up the respectability of cell layer and decreased the oxidants damage. These are results agreement with those of Abdallah et al. (2013) who reported that Cr supplementation from Cr picolinate (800 ppb) was significantly ($P\leq0.05$) detailed semen-ejaculate volume, advanced motility (%) and a live sperm (%) compared to the control group in the cocks of Golden Montazah local strain. Likewise, Ezzat et al. (2016) who found that addition of

chromium at levels of 1200 or 1800 µg Cr/kg to cock diets was significantly (P≤0.05) increased sperm motility (%) and sperm-cell concentration and significantly (P≤0.05) decreased dead spermatozoa (%) and sperm abnormalities (%) than the control group.

CONCLUSIVELY,

based on the pervious data, it could be concluded that exposures of female and male Mandarah to heat shock {three times during the growth period of 3 days and at 8 and 16 weeks of age without or with chromium picolinate (HSE5and HSE6)} improve most of the productive traits, blood parameters, egg production, as well as, improved immune responses and semen quality.

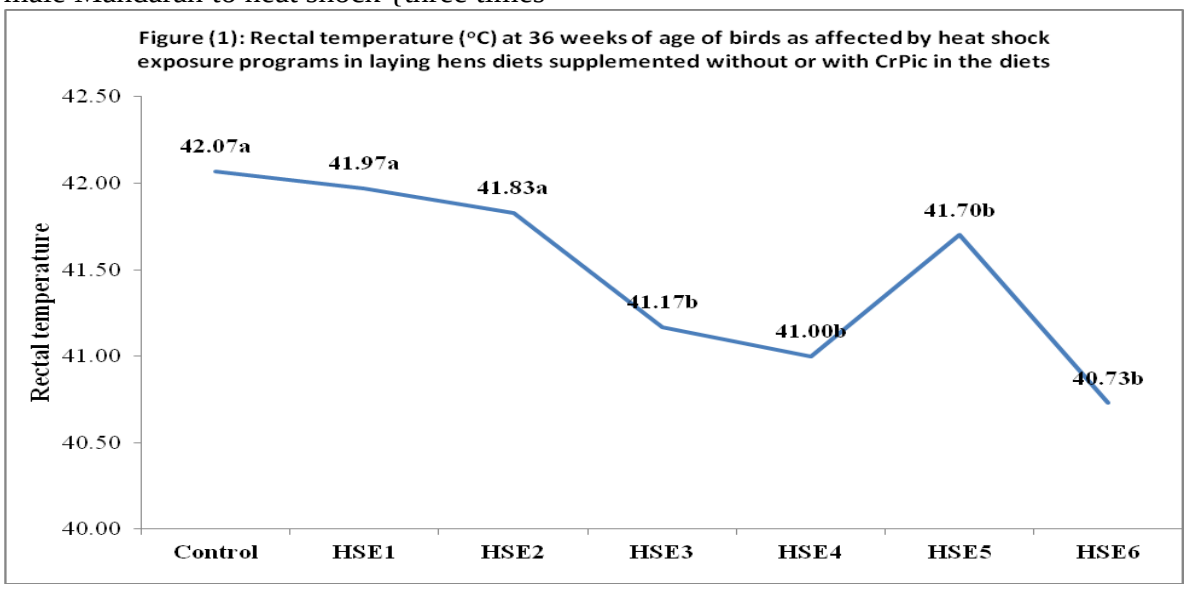

Figure (1): Control, non heat exposure during growth period; HSE1, heat exposure at 3 days of age; HSE2, heat shock at 3 days of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE3, heat shock at 3 days and at 8 weeks of age; HSE4, heat shock at 3 days and at 8 weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE5, heat shock at 3 days and at 8 and 16 weeks of age; HSE6, heat shock at 3 days and at 8, 16, weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet.

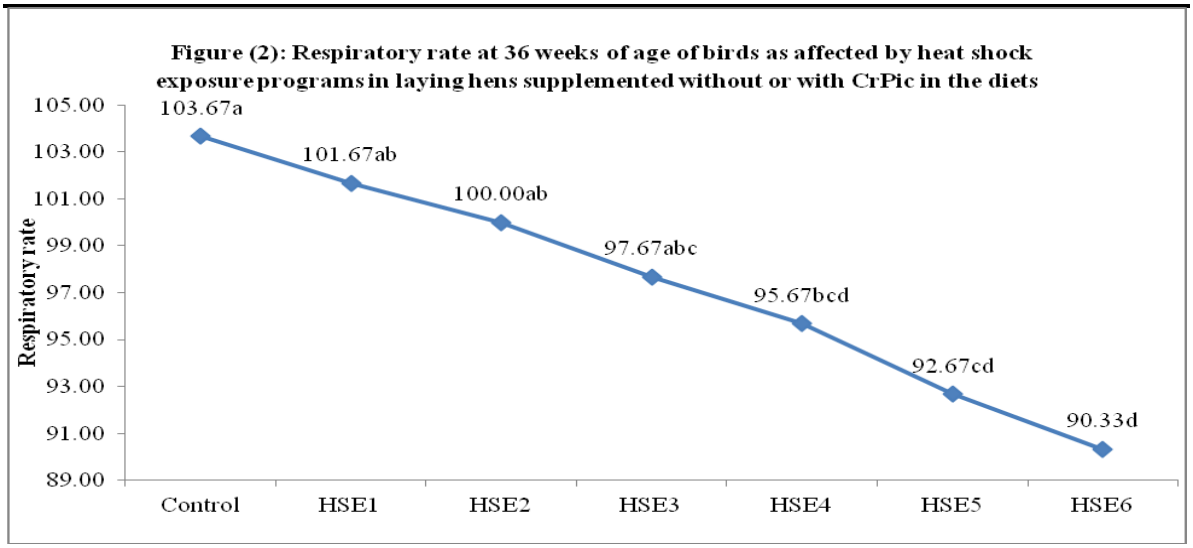

Figure (2): Control, non heat exposure during growth period; HSE1, heat exposure at 3 days of age; HSE2, heat shock at 3 days of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE3, heat shock at 3 days and at 8 weeks of age; HSE4, heat shock at 3 days and at 8 weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE5, heat shock at 3 days and at 8 and 16 weeks of age; HSE6, heat shock at 3 days and at 8, 16, weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet.

Table (1): Composition and calculated analysis of basal diets.

Composition (per 100 Kg)	Starter (1-10Weeks)	Grower (10-20 Weeks)	Layer (20-36 Weeks)
Yellow corn	62.00	63.51	61.80
Soybean meal (44% CP)	23.25	15.50	15.10
Wheat bran	7.22	17.50	8.28
Corn gluten meal (60% CP)	3.90	0.00	4.75
Dicalcium phosphate	1.50	1.25	1.35
Vegetable oil	0.00	0.00	0.00
Salt	0.30	0.35	0.30
Limestone	1.50	1.55	8.10
Vit + Min. premix*	0.30	0.30	0.30
DL-Methionine	0.03	0.04	0.02
Total	100	100	100
Calculated analysis :(NRC, 1994)			
Crude protein (CP); %	19.00	15.01	16.07
ME; kcal/kg	2834	2702	2691
Ether extract	3.019	3.171	2.942
Crude fiber	3.906	4.454	3.434
Calcium	1.018	0.970	3.468
Av. Phosphorus	0.348	0.309	0.304
Lysine	0.857	0.676	0.653
Methionine	0.360	0.290	0.314
Methionine + cystine	0.699	0.527	0.608

*Vitamin and mineral premix: added to the 1 kg of diet including Vit. A 10000 I.U; Vit. D3 2000 I.U; Vit. E 15 mg; Vit. K3 1 mg; Vit. B1 1mg; Vit. B2 5 mg; Vit. B12 10 µg; Vit. B6 1.5mg; Niacin 30mg; Pantothenic acid 10mg; folic acid 1mg; Biotin 50 µg; choline 300 mg; zinc 50mg; copper 4mg; iodine 0.3 mg; iron 30mg; selenium 0.1mg; manganese 60mg; cobalt 0.1mg and carrier CaCo3 up to 1kg.

Table (2): Means of air temperature, relative humidity and temperature-humidity index (THI) during experimental period according to Egyptian Meteorological Authority

Months	Air temperature (ºC)		Relative humidity (%) (RH)		Temperature-humidity index (THI)	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
February	13.18±0.51	23.59±0.92	22.76±1.63	78.07±2.70	13.47	22.97
March	16.04±0.40	26.33±0.68	17.78±1.49	73.65±2.33	15.62	25.36
April	19.07±0.49	32.70±0.78	13.47±1.25	74.47±3.67	17.82	31.25
May	20.88±0.51	32.84±0.77	15.33±0.99	70.49±2.44	19.18	31.15
June	26.17±0.54	38.70±0.57	17.87±1.28	75.00±3.36	23.17	36.82
July	26.52±0.29	32.94±0.10	42.59±0.99	77.13±1.21	24.36	31.63
August	26.42±0.38	33.3±0.16	42.39±1.04	77.42±1.59	24.27	31.98
September	24.54±0.26	34.3±0.44	26.07±1.01	81.30±1.33	22.22	33.15
October	22.20±0.20	31.30±0.42	32.00±1.45	87.55±1.10	20.56	30.64
Averages	21.71±0.31	31.80±0.32	25.67±0.76	77.23±0.83	20.07	30.55

Table (3): Productive and reproductive performance as affected by heat shock exposure programs in laying hens fed diets supplemented without or with CrPic, from 22 to 36 weeks of age of age.

Items	Control	HSE1	HSE2	HSE3	HSE4	HSE5	HSE6	SEM	Sig.
Body weight at sexual maturity (gm)	1217	1197	1277	1208	1276	1253	1287	40	NS
Final body weight (gm)	1487	1437	1487	1470	1492	1490	1553	31	NS
Feed consumption (gm / hen/ day)	99.14	96.91	100.11	100.14	102.18	101.83	103.31	1.77	NS
Feed conversion (g. feed/ g. egg mass)	3.55	3.42	3.59	3.50	3.57	3.52	3.44	0.07	NS
Egg production %	64.74^{cd}	63.55^{d}	63.30^{d}	67.32^{ab}	66.47^{bc}	68.01^{ab}	69.6^{a}	0.72	**
Egg weight (gm)	43.18^{bc}	44.63^{a}	44.12^{ab}	42.53^{c}	43.10^{bc}	42.60^{c}	43.28^{bc}	0.30	**
Egg mass (gm/hen)	27.95^{b}	28.35^{b}	27.93^{b}	28.63^{b}	28.65^{b}	28.97^{b}	30.12^{a}	0.30	**
Mortality rate (from 1 day to 36 weeks)	18.00^{a}	14.00^{ab}	9.00^{cd}	12.00^{bc}	7.00^{d}	10.00^{bcd}	7.00^{d}		*

Control, non heat exposure during growth period; HSE1, heat exposure at 3 days of age; HSE2, heat shock at 3 days of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE3, heat shock at 3 days and at 8 weeks of age; HSE4, heat shock at 3 days and at 8 weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE5, heat shock at 3 days and at 8 and 16 weeks of age; HSE6, heat shock at 3 days and at 8, 16, weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet.

Means having different letters in the same row, differ significantly. * = ($P<0.05$); ** = ($P<0.01$); NS= Not significant.

Table (4): Egg quality and egg components (%) as affected by heat shock programs in laying hens fed diets supplemented without or with CrPic, from 24 to 36 weeks of age.

Items	Control	HSE1	HSE2	HSE3	HSE4	HSE5	HSE6	SEM	Sig.
Egg quality									
Egg shape index %	76.10	77.29	75.61	74.90	78.00	75.41	71.65	1.29	NS
Yolk index %	33.50^{b}	42.38^{a}	42.99^{a}	41.16^{a}	43.09^{a}	45.84^{a}	44.36^{a}	1.69	**
Shell thickness (mm)	0.322^{b}	0.361^{a}	0.359^{a}	0.363^{a}	0.350^{ab}	0.352^{a}	0.382^{a}	0.01	*
Haugh unit	84.83^{bc}	96.33^{a}	86.39^{bc}	85.79^{bc}	88.40^{bc}	81.60^{c}	88.85^{bc}	2.09	**
Egg components (%)									
Albumin %	57.94	57.69	58.53	56.73	56.51	57.05	55.37	0.92	NS
Yolk %	30.39	30.49	29.7	31.79	32.65	30.36	32.95	0.93	NS
Shell %	11.68	11.83	11.79	11.5	10.85	12.61	11.69	0.36	NS

Control, non heat exposure during growth period; HSE1, heat exposure at 3 days of age; HSE2, heat shock at 3 days of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE3, heat shock at 3 days and at 8 weeks of age; HSE4, heat shock at 3 days and at 8 weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE5, heat shock at 3 days and at 8 and 16 weeks of age; HSE6, heat shock at 3 days and at 8, 16, weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet.
Means having different letters in the same row, differ significantly. * = ($P<0.05$); ** = ($P<0.01$); NS= Not significant.

Table (5): Some hematological parameters and immune response $\left(\overline{X} \pm SE\right)$ as affected by heat shock programs in laying hens fed diets supplemented without or with CrPic, at 36 weeks of age.

Items	**Control**	**HSE1**	**HSE2**	**HSE3**	**HSE4**	**HSE5**	**HSE6**	**SEM**	**Sig.**
Some hematological parameters									
Hemoglobin (Hb) mg/dl	10.61^{e}	11.43^{d}	11.78^{c}	12.2^{b}	12.5^{ab}	12.68^{a}	12.82^{a}	0.07	**
Red blood cells (RBC's) $\times 10^{6}$	2.68^{d}	2.73^{d}	2.77^{d}	3.25^{c}	3.56^{b}	3.78^{b}	4.05^{a}	0.06	**
White blood cells (WBC's) $\times 10^{3}$	3.18^{f}	3.43^{e}	3.64^{d}	3.80^{d}	4.07^{c}	4.28^{b}	4.48^{a}	0.04	**
Heterophils (H %)	31.46^{a}	30.17^{b}	29.36^{b}	27.81^{c}	25.96^{d}	24.33^{e}	23.49^{e}	0.25	**
lymphocytes% (L%)	60.29^{f}	61.39^{e}	62.70^{d}	64.18^{c}	65.15^{c}	66.22^{b}	67.36^{a}	0.23	**
H/L	0.53^{a}	0.50^{b}	0.47^{c}	0.44^{d}	0.40^{e}	0.37^{f}	0.35^{f}	0.01	**
Immune response									
Newcastle disease virus (NDV)	6.39^{e}	7.47^{d}	8.13^{c}	8.56^{bc}	8.87^{ab}	9.11^{a}	9.30^{a}	0.10	**
Influenza viruses (H5N1)	0.63^{d}	1.24^{c}	2.14^{b}	2.83^{a}	2.97^{a}	3.16^{a}	3.36^{a}	0.11	**
Influenza viruses (H9N2)	2.31^{f}	3.26^{e}	4.15^{d}	5.07^{c}	5.57^{bc}	5.97^{ab}	6.21^{a}	0.12	**
IgG	1.17^{e}	1.34^{de}	1.62^{cde}	2.01^{bcd}	2.29^{bc}	2.57^{ab}	3.18^{a}	0.10	**
IgM	$0.73\pm^{f}$	0.94^{e}	1.24^{d}	1.36^{d}	1.55^{c}	1.85^{b}	2.25^{a}	0.04	**

Control, non heat exposure during growth period; HSE1, heat exposure at 3 days of age; HSE2, heat shock at 3 days of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE3, heat shock at 3 days and at 8 weeks of age; HSE4, heat shock at 3 days and at 8 weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE5, heat shock at 3 days and at 8 and 16 weeks of age; HSE6, heat shock at 3 days and at 8, 16, weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet. Means having different letters in the same row, differ significantly. ** = (P<0.01); NS= Not significant.

Table (6): Blood plasma components $\left(\overline{X} \pm SE\right)$ as affected by heat shock programs in laying hens fed diets supplemented without or with CrPic, at 36 weeks of age.

Items	Control	HSE1	HSE2	HSE3	HSE4	HSE5	HSE6	SEM	Sig.
Total protein (g/dl)	5.13	5.23	5.55	5.85	6.01	6.26	6.45	0.34	NS
Albumin (g/dl)	3.16	3.11	3.30	3.47	3.50	3.66	3.84	0.24	NS
Globulin (g/dl)	1.98^{d}	2.12^{cd}	2.25^{bcd}	2.38^{abc}	2.51^{ab}	2.60^{a}	2.61^{a}	0.10	**
Glucose (mg/dl)	210.12	196.49	202.09	187.86	200.84	194.71	185.14	11.29	NS
Cholesterol (mg/dl)	190.72	180.00	167.86	174.05	187.67	176.81	162.43	11.95	NS
LDL (mg/dl)	111.8	109.28	97.37	106.13	97.32	92.33	89.61	8.53	NS
HDL (mg/dl)	59.4	60.81	60.68	65.03	67.31	67.85	69.05	8.98	NS
Total lipids (mg/dl)	643.54	612.04	651.7	601.07	636.2	601.42	573.85	22.46	NS
Triglycerides (mg/dl)	153.46	144.10	147.52	136.32	134.63	130.41	123.83	12.83	NS
Triiodothyronine (T3) (ng\ml)	2.76^{a}	2.15^{b}	2.10^{bc}	2.01^{bcd}	1.71^{cd}	1.89^{bcd}	1.65^{d}	0.08	**
Progesterone (ng\ml)	0.83	0.91	0.84	0.96	0.97	1.00	1.05	0.06	NS
Estradiol-17B (pg\ml)	292.00	297.67	283.67	287.67	319.00	323.34	322.00	21.39	NS
HSP70 of Liver	6.18^{d}	7.34^{bc}	7.00^{c}	7.75^{ab}	6.83^{c}	8.22^{a}	7.08^{c}	0.10	**
Superoxide dismutase (SOD)	2.14^{c}	2.11^{c}	2.20^{bc}	2.27^{bc}	2.35^{bc}	2.71^{a}	2.55^{ab}	0.10	*
Glutathioneperoxidase (GPX)	6.47^{c}	6.35^{c}	6.77^{bc}	7.24^{bc}	7.66^{abc}	8.51^{ab}	9.35^{a}	0.53	*
Total antioxidant capacity (TAOC)	0.93^{a}	0.91^{a}	0.87^{ab}	0.82^{abc}	0.78^{bcd}	0.74^{cd}	0.66^{d}	0.03	**

Control, non heat exposure during growth period; HSE1, heat exposure at 3 days of age; HSE2, heat shock at 3 days of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE3, heat shock at 3 days and at 8 weeks of age; HSE4, heat shock at 3 days and at 8 weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE5, heat shock at 3 days and at 8 and 16 weeks of age; HSE6, heat shock at 3 days and at 8, 16, weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet.

Means having different letters in the same row, differ significantly. * = (P<0.05); ** = (P<0.01); NS= Not significant.

Table (7): Semen characteristics $\left(\overline{X} \pm SE\right)$ as affected by heat shock programs in laying hens fed diets supplemented without or with CrPic, at 36 weeks of age.

Items	Control	HSE1	HSE2	HSE3	HSE4	HSE5	HSE6	SEM	Sig.
Ejaculate volume (ml)	0.47	0.40	0.24	0.34	0.24	0.14	0.17	0.08	NS
Hydrogen-ion concentration (pH)	7.17	7.10	7.20	7.24	7.24	7.17	7.30	0.04	NS
Sperm motility (%)	80.00^{c}	83.34^{bc}	88.33^{abc}	86.67^{abc}	91.67^{ab}	95.00^{a}	95.00^{a}	2.25	*
Dead spermatozoa (%)	18.34^{a}	19.00^{a}	18.67^{a}	16.67^{ab}	13.67^{bc}	12.34^{c}	12.67^{c}	1.05	**
Sperm abnormalities (%)	12.00^{ab}	13.34^{a}	11.67^{ab}	8.34^{bc}	6.67^{cd}	5.34^{d}	5.00^{d}	1.07	**
Sperm cell concentration (X 10^{9}/ml)	2.64^{c}	3.47^{b}	4.10^{ab}	4.04^{ab}	4.09^{ab}	4.08^{ab}	4.43^{a}	0.11	**
Acrosomal damage (%)	6.67^{ab}	7.34^{a}	6.67^{ab}	4.67^{abc}	3.34^{bc}	3.67^{bc}	2.34^{c}	0.90	*

Control, non heat exposure during growth period; HSE1, heat exposure at 3 days of age; HSE2, heat shock at 3 days of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE3, heat shock at 3 days and at 8 weeks of age; HSE4, heat shock at 3 days and at 8 weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet; HSE5, heat shock at 3 days and at 8 and 16 weeks of age; HSE6 , heat shock at 3 days and at 8, 16, weeks of age + 800 µg/ chromium picolinate (CrPic) /kg diet.

Means having different letters in the same row, differ significantly. * = ($P<0.05$); ** = ($P<0.01$); NS= Not significant.

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Heat shock programs –Chromium -Productive and physiological performance.

الملخص العربي

تأثير اضافة بيكلونات الكروميوم علي الاستجابة المناعية وبروتينات الصدمة الحرارية للدجاج البياض تحت ظروف الاجهاد الحراري

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تهدف هذه الدراسة إلى التعرف على تأثير برامج الصدمة الحرارية خلال فترة النمو بدون أو مع الكروم علي الأداء الإنتاجي والفسيولوجي والصفات الكيميائية الحيوية في البلازما وكذلك الاستجابة المناعية وبروتينات الصدمة الحرارية لدجاج المندرة البياض خلال أول 90 يوم من إنتاج البيض التي تربى في ظل الظروف الطبيعية في مصر
تم تقسيم سبعه مائة كتكوت غير مجنس عمر يوم من دجاج المندرة المقسم عشوائيا إلى سبع مجموعات متساوية (100 كتكوت لكل معاملة). المعاملة الأولى كنترول، وتغذي علي عليقه أساسيه وتربي في ظل الظروف الطبيعية. بينما تعرضت كتاكيت المعاملات الثانية والثالثة والرابعة والخامسة والسادسة والسابعة إلى صدمة حرارية (40 ± 1 درجة مئوية لمدة 4 ساعات من 12,00 إلى 16,00 لمدة 3 أيام متتالية، تعرضت المعاملة الثانية للصدمة الحرارية في وقت مبكر عند 3 أيام من العمر، وتعرضت المعاملة الثالثة للصدمة الحرارية عند 3 أيام من العمر و 800 ميكروجرام بيكولنات الكروميوم /كجم عليقة ، تعرضت المعاملة الرابعة للصدمة الحرارية عند 3 أيام و 8 أسابيع من العمر ، تعرضت المعاملة الخامسة للصدمة الحرارية عند 3 أيام و 8 أسابيع من العمر و 800 ميكروجرام بيكولنات الكروميوم /كجم عليقة ، تعرضت المعاملة السادسة للصدمة الحرارية عند 3 أيام و 8 و 16 أسبوعا من العمر، وتعرضت المعاملة السابعة للصدمة الحرارية عند 3 أيام و 8 و 16 أسبوعا من العمر و800 ميكروجرام بيكولنات الكروميوم /كجم عليقة.
وأظهرت النتائج المتحصل عليها الي تحسن معنويا للنسبة المئوية لإنتاج البيض وكتلة البيض في الدجاج وبعض صفات السائل المنوى للذكور التي تعرضت للصدمة الحرارية عند 3 أيام و 8 و 16 أسبوع من العمر+ 800 ميكروجرام كروم بيكولينات/كجم عليقه (HSE6). وقد انخفض معنويا درجة حرارة المستقيم ومعدل التنفس و% هيتيروفيلس، ونسبة الخلايا الهيتيروفيلس الي الليمفاوية. وكانت المعاملة التي تعرضت للصدمة الحرارية عند 3 أيام و 8 و 16 أسبوع من العمر بدون او مع بيكولنات الكروميوم زاد فيها معنويا الجلوبيولين، وبروتينات الصدمة الحرارية في الكبد، ومضادات الأكسدة SOD، GPX بينما انخفض معنويا وهرمون تراي ايدوثيرونين ، مضاد الأكسدة TAOC عند المقارنة بمجموعة الكنترول.
التوصية: إن استخدام التعرض الحراري ثلاث مرات خلال فترة النمو عند عمرثلاثة أيام وعمر 8أسابيع وعمر16 أسبوع بدون أو مع الكروم (HSE5، HSE6) حسن الاستجابات الفسيولوجية لإناث وذكور دجاج المندره المعرض للإجهاد الحراري في مرحلة التناسل .وقد تم تحقيق التأثير الإيجابي من خلال الاستجابات الحرارية التنفسية، ومعدل الوفيات، وأداء الدجاج البياض، وتعزيز الاستجابات المناعية، وجودة السائل المنوى.