



IMPACT OF CHROMIUM PICOLINATE SUPPLEMENTATION ON PRODUCTIVE PERFORMANCE, IMMUNE RESPONSE AND HEAT SHOCK PROTEINS OF BROILER CHICKENS UNDER HEAT-STRESS CONDITION.

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ABSTRACT: The present study conducted to investigate the effect of heat stress and early age of thermal condition (at 3 days of age) for broiler chickens were fed diets supplemented with or without chromium picolinate (CrPic) to study its effects on growth performance, some physiological parameters, plasma biochemical traits as well as, immune response, heat shock proteins and carcass characteristics. Unsexed 300 one-day-old Hubbard broiler chicks were individually weighed and randomly distributed into 5 experimental treatments with 3 replicates (20 chicks each) per pen from 1–42 days of age. The 1st treatment (T1) served as a control and was fed a control basal diet and reared under natural conditions (25°C). The 2nd treatment (T2) and 3rd treatment (T3) were daily exposed to heat stress (33°C) and 65% relative humidity and 1200 µg/chromium picolinate (CrPic) /kg diet was added for T3 only, the 4th treatment (T4) and 5th treatment (T5) were daily exposed to heat stress (33°C) during the experimental period and at 3 days of age were exposed to early age of thermal condition (early heat shock exposure) and 1200 µg/chromium picolinate (CrPic) /kg diet was added for T5 only. After the end of thermal condition at early age T4 and T5 returned to be reared under daily heat stress (33°C). The obtained results showed that heat stress caused a significantly ($p \leq 0.05$) decrease body weight (BW), weight gain (BWG), feed intake (FI), feed conversion (FC), hemoglobin concentration (Hb), red blood cells (RBCs), white blood cells (WBCs), lymphocytes% (L%), antibody titers against infectious bronchitis virus (IBV), Newcastle virus (NDV), influenza viruses (H9N2), immunoglobulin G (IgG) and immunoglobulin M (IgM) concentrations, total protein, albumin and high density lipoprotein (HDL), superoxide dismutase (SOD), glutathione peroxidase (GPX), Triiodothyronine (T3) and thyroxine (T4) hormones. While, there were significant ($p \leq 0.05$) increases mortality rate, respiration rate, heterophils % (H %), H/L ratio, glucose, cholesterol, LDL, total lipids and triglycerides, corticosterone, total antioxidant capacity (TAOC), HSP70 expression of liver and abdominal fat as compared with the thermoneutral control broiler chicks. However, supplementation of chromium to the broiler chicks diet was able to alleviate many of deleterious effects. The present results indicate that the supplementation of diets with 1200 µg/chromium picolinate (CrPic)/kg diet has been considered to overcome the deleterious effects of heat stress on broiler performance.

Keywords: Broilers Heat Stress-Heat shock-Chromium Growth performance-Blood parameters.

INTRODUCTION

In Egypt, ambient temperature during the summer season can remain reliably high for extended periods of time in addition to sudden, repetitive hot and muggy waves which have more unsafe impacts. So, poultry production suffers significant losses every year due to heat stress (HS), leading to economic losses to the poultry farmers. Temperatures during these periods reaches 40°C most of the time and the humidity reaches 75% (Tawfeek et al., 2014). HS in broiler chickens has been accounted for to specifically stifle the immune system, prompting disappointments in the chickens' response to vaccination and immune organ involvement (Lee et al., 2003). Stressed poultry can be recognized by panting frequency and a decrease in feed consumption, an increase in water utilization and excrement water content (Garriga et al., 2006). An increase in serum corticosterone concentration (Al-Aqil and Zulkifli, 2009) decreased immune competence and expanded investment costs to alleviate the impacts of environmental change (Rajkumar et al., 2015) and heat shock proteins (HSP70) expression (Staib et al., 2007). Heat shock proteins (HSPs) are an arrangement of proteins synthesized in response to physical, substance or natural anxieties, including heat introduction (Staib et al., 2007). Environmental and pathological stresses induce HSPs, particularly the inducible form Hsp-70 and Hsp-72. These programs improved bird's final productive performance and survival ability during exposure to heat stress at later ages. Heat stress was likewise appeared to increase antioxidant enzyme activities, namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidases (GPX). The increased antioxidant enzyme activities in response to the increased Responsive Oxygen Species (ROS) levels aim to maintain the steady state concentrations of

producing free radicals. A few procedures for settling this problem have been proposed to deal with the negative impacts of heat stress, including environmental management, nutritional manipulation, as well as, addition of feed additives in the diet. Several methods are available to alleviate the impact of high environmental temperature on poultry performance. Supplemental dietary chromium, particularly at 1200 ppb, may offer a potential protective management practice in preventing detrimental effects of heat stress on performance of broiler chickens (Sahin et al., 2002a). The essential part of chromium in digestion is to potentiate the activity of insulin through its nearness in an organometallic particle, and glucose resistance figure (Sahin et al., 2001). Chromium insulin cofactor is, consequently, proposed to work as an antioxidant (Preuss et al., 1997). The Cr inadequacy may prompt to decreased insulin affectability infringe tissues and resulting in disrupted carbohydrate and lipid metabolism and consequently impaired growth rate (Vincent, 2000). Lien et al. (2005) found that Cr-Pico particularly upgraded weight gain due to increased feed consumption and supports the immune function by enhancing the cell mediated and humeral immune responses in broilers. Supplementation of broiler diets with natural Cr enhanced final body weight and body weight gain (Mohammed et al., 2014). Therefore, the purpose of the current research was to determine the effects of Cr supplementation to the broiler chickens exposed to heat stress and early age thermal condition (early heat shock exposure) on productive performance and immune response.

MATERIALS AND METHODS

The present study was carried out on a Private Farm near the Inshas Poultry Research Station, Animal Production Research Institute, Agriculture Research Center, Giza, Egypt, from February to March, 2016. Unsexed 300 one-

Broilers - Heat Stress - Heat shock – Chromium - Growth performance - Blood parameters.

day-old Hubbard broiler chicks were housed in a partitioned floor pens, individually weighed and randomly distributed into 5 experimental treatments with 3 replicates (20 chicks each) per pen from 1–42 days of age. The experimental treatments were fed on corn-soybean meal basal diets which met the strain requirements (NRC, 1994). The composition and analytical calculation of the basal diets during the experimental periods (starter and grower) are shown in Table 1. The 1st treatment (T1) served as a control and was fed a control basal diet and reared under natural conditions (25°C). The 2nd treatment (T2) and 3rd treatment (T3) were daily exposed to heat stress (33°C) and 65% relative humidity during the experimental period and 1200 µg/chromium picolinate (CrPic) /kg diet (containing 12.27% Cr) was added for T3 only, the 4th treatment (T4) and 5th treatment (T5) were daily exposed to heat stress (33°C) during the experimental period and at 3 days of age were exposed to early age of thermal condition (early heat shock exposure) (40± 1°C for 4 hours (h) from 12:00 - 16:00 for 3 consecutive days) and 1200 µg/chromium picolinate (CrPic) /kg diet was added for T5 only. After the end of thermal condition at early age T4 and T5 returned to be reared under daily heat stress (33°C). Broiler chicks were fed ad libitum a starter diet until 21 days of age, followed by a grower diet from day 21 to day 42. Feed intake and body weight were recorded at weekly intervals from which weight gain and feed conversion of birds were calculated. Mortality was recorded daily and feed intake was adjusted for mortality. The rectal temperature (RT) of three birds randomly selected out of each replicate was measured with a digital thermometer (0.1°C accuracy) inserted into the rectum (colon) of the birds for one minute as previously described by Yahav and McMurtry (2001). Respiratory rate (RR) of the birds was taken

as the number of breaths per minute. Data on RT and RR were collected two consecutive days in every week.

At the end of the experiment (day 42), three birds from each treatment were randomly chosen, weighed and then slaughtered. Blood samples were collected at the time of slaughtering from each bird. 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). All measurements conducted according to Clark et al. (2009). The Infectious bronchitis virus (IBV), Newcastle virus (NDV), influenza viruses (H9N2), detection was 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, bronchitis virus (IBV), Newcastle and Influenza viruses (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 described by Gao et al. (2008). Blood was collected in tubes with EDTA centrifuged at 4000 rpm 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), thyroxine (T4) and corticosterone were determined in 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 Usen Life Science Inc. Wuhan, China.

After complete bleeding and feather removal, carcass, liver, gizzard, heart, dressing (carcass and giblets) and abdominal fat was weighed and recorded as percentage of body weight.

Data were statistically analyzed by ANOVA, using General Linear Model (GLM) Procedure of SAS software (SAS Institute, version 9.1, 2005). Duncan's multiple range test (Duncan, 1955) was used to detect the differences among means of different groups. Mortality percentages were analyzed using the Chi - square test.

RESULTS AND DISCUSSION

1. Growth traits:

Growth performance as affected by thermo neutral, heat stress and early age thermal condition in broilers fed diets supplemented without or with CrPic during the different experimental periods chicks are shown in Table 2.

Body weight (BW) and body weight gain (BWG) were significantly ($p \leq 0.01$) decreased for broiler exposed to only heat stress and early age thermal condition (early heat shock exposure) compared with the thermo neutral control group. This impact might be because of the diminishing of feed intake and/or the rise in blood levels of corticosterone, which change the energy expenditure in favor of fat deposition and protein catabolism (Siegel, 1995). These results are in agreement with those obtained by Pelicano et al. (2005) who confirmed the effect of high temperature (33°C) on the body weight reduction in 21 day old Ross broilers, when compared to the gain of birds reared under thermo neutral temperature. Laganá et al. (2007) confirmed that the effect of high temperature on body weight was lowered 10 % than the control group in broilers reared under different thermal environments. Marchini et al. (2009) found that 42-day-old broilers submitted to

cyclic high environmental temperature observed a decrease about 7 % in the body weight when compared to birds reared in a warm environment. Moreover, supplementation of broiler diets with chromium at level $1200 \mu\text{g Cr/kg}$ diet significantly ($p \leq 0.05$) increased body weight and weight gain in broiler exposed to either heat stress or early age thermal condition (early heat shock exposure) compared to those without Cr and exposed to early age thermal conditions or with heat stress from 21-42 and 0-42 days of age, respectively. Similarly, Sahin et al. (2002a) reported that increase supplementation Cr at levels of 200, 400, 800 or $1200 \mu\text{g Cr}$ picolinte showed an increase in broilers body weight. Ibrahim (2005) indicated that supplementation of Cr at levels of 10, 20, 30, 40 and 50mg Cr/kg diet significantly increased the final body weight. Mohammed et al. (2014) recommended that dietary organic Cr supplementation improved final BW and BWG in broilers. Our results could be because Cr plays an important role as an integral component of the glucose tolerance factors (GTF), which potentiate the action of insulin and regulate fat metabolism at low insulin level, glucose is changed over into fat and put away in fat cells (Mertz, 1993). The capacity of insulin to control glucose levels in blood and lipid digestion is dependent upon the binding of this pancreatic hormone to particular receptors found in numerous fringe tissues like adipocytes, muscle and liver, expanding the quantity of genuine insulin receptors exhibit in the objective cell. Additionally, chromium has been shown to expand the genuine official of insulin to its receptors. Thus, chromodulin appears to play a role in an auto amplification mechanism in insulin signaling (Sahin et al., 2002b). Steele and Rosebrough (1981) have expressed Cr as a cofactor for insulin movement and that it is vital for ordinary

glucose use and solid creature development. Insulin directs digestion of starch, fat and protein, animating amino corrosive take-up and protein blend, and, glucose usage in tissues (Sahin et al., 2001).

Feed intake (FI) and feed conversion ratio (FCR) were not significantly affected for broiler reared under thermo neutral group (control) and birds exposed to heat stress or with early age thermal condition (early heat shock exposure) from 1-21 days of age. While there were significant ($p \leq 0.05$) negative effects on feed intake and feed conversion of broiler exposed to heat stress and early age thermal condition from 21-42 and 1-42 days of age compared with control group. These results are in agreement with Siegel (1995) who reported that performance and feed intake decreased when the ambient temperature rises above the thermo neutral. The reduced feed intake in heat stress may be due to loss appetite resulting from lesions of appetite center in the lateral nucleus of the hypothalamus. Also, the blood flow and the motility of the intestine decreased, which may result in an increase of food passage time and delay in the thermogenic effect of food intake (Van Handel-Hruska et al., 1997). On the other hand, the deteriorated performance of heat-stressed broilers can be related to a poor appetite and lowered feed intake, which is a defense mechanism designed to reduce heat build-up in the body. El-Tantawy et al. (1998) found that the feed consumption was lower in high environmental temperature by about 36-43%. Laganá et al. (2007) confirmed that the effect of high temperature of feed intake was significantly lower 14% than the control group in broilers reared under different thermal environments. Faria Filho et al. (2006) found that the reduction of feed intake can reach to 36 % in broilers reared at 32°C when compared to birds reared at 22°C. In the present study, FI was not significantly

different between groups, which exposed to heat stress without or with CrPic at different experimental period. These results are in agreement with those reported by Ghazi et al. (2012) who found that supplementation of dietary natural and inorganic Cr has no impact on FI in broilers reared under heat stress conditions and disagreement with Sahin et al. (2002a) who stated that dietary Cr-Pico supplementation increased FI in broilers subjected to heat stress. However, supplementation of broiler diets with chromium at level 1200 µg Cr/kg diet significantly improved FC, which exposed to heat stress and early age thermal condition compared to the ones exposed to only early age thermal condition or heat stress from 21-42 and 0-42 days of age, respectively (Table 2). These results are in agreement with those of Sahin et al. (2002a) who showed that dietary Cr-Pico supplementation improved FC in broilers subjected to heat stress and caused an increase of insulin concentration that increased glucose utilization, consequently feed efficiency improved.

The results obtained for the mortality rate (MR) are shown in Table 3. There were no significant differences in mortality rates among treatments during the starter and finisher period. Moreover, the MR was significantly ($p \leq 0.05$) among the treatments from 0-42 days of age. The MR was increased under heat stress condition and the finisher period of the experiment showed the highest rate. In the group of heat stress, the MR represented about 10.0% from 1-21 days of age and then increased, reaching 15.0% during the period from 21 to 42 days and total nearly 25% in the whole period. While, under thermo neutral condition only four birds died representing about 6.67 % during the whole experimental period. Moreover, broiler diets supplemented with 1200 µg CrPic /kg diet, which was exposed to either early age thermal

condition or heat stressed groups resulted in a significant ($p \leq 0.05$) decrease in MR by about 11.67% compared to those exposed to only heat stress or with heat shock from 0-42 days of age, respectively. These results are in agreement with those reported by Uyanik et al. (2002) who showed that broiler chicks fed 20, 40, or 80 $\mu\text{g Cr/kg}$ diet for 44 days, increased lymphocyte counts, total antibody, IgG, and IgM titers, consequently a decrease of MR. In the same respect, Steele and Rosebrough (1981) found that supplemented Cr in turkey poultry diets increased rate of glucose utilization and immune response which explains the observed decrease in mortality rate.

2. Physiological parameters:

Results in Figures 1 and 2 show that rectal temperature was insignificantly increased, however, respiration rate was significantly ($p \leq 0.05$) increased for broiler exposure to heat stress as compared with those reared under thermo neutral condition. These results are in agreement with those reported by Borges et al. (2004) who found that exposure to heat stress caused an increase in respiratory rate (panting) and resulted in a reduction in blood pCO_2 . At high temperatures, birds increase their respiration rate to regulate heat loss through water evaporation from their lungs (Okela et al., 2003). This panting behavior, increases CO_2 loss from the lungs and partial pressure of CO_2 in blood. The decreased of CO_2 causes a decrease of HCO_3^- concentrations due to the increase in HCO_3^- excretion with a reduction of H^+ excretion by the kidneys to maintain the acid-base balance in the bird. Lowered H^+ concentration raises the level of blood plasma pH, a leading to respiratory alkalosis (Borges et al., 2007). This acid-base imbalance alters Na: Cl ratio, thus reduced feed consumption (Naseem et al., 2005). Moreover, broiler diets supplemented with 1200 $\mu\text{g CrPic /kg}$ diet

lead to a significant ($p \leq 0.05$) decrease in respiration rate compared to those exposed to only early age thermal condition or with heat stress. These results are in agreement with those reported by Norain et al. (2013) who reported that chromium supplementation as feed additives resulted in a slightly lower rectal temperature, and significantly ($p < 0.05$) lower respiration rate for the broiler chickens received a diet supplemented with chromium compared to the control.

3. Blood hematological variables

Results show that heat stress caused a significant ($p \leq 0.01$) decrease of hemoglobin concentration (Hb), red blood cells (RBCs), white blood cells (WBCs) and lymphocyte% (L%). While, there was an increase of heterophils % (H %) and H/L ratio as compared with the thermoneutral control broiler chicks (Table 4). It is known that, heterophils (H) are granulated leukocytes formed from myelocytes in the bone marrow. They are phagocytic cells intended to characterize the living being against disease or foreign bodies, for example, infections, microorganisms and different particles. They are available in plenitude in contamination locales to where they connected by chemotactic compounds from harm cells. Lymphocytes (L) are non-granulated leukocytes framed in lymphoid tissues. They lay an essential physiological effect in immunity, particularly for the generation of antibodies. One of the physiological reactions to exposure to stress is the release of glucocorticoids, bringing about the disintegration of lymphocytes in lymphoid tissues prompting to lymphopenia. Nonetheless, there is an increase in heterophil discharged by the bone marrow, thus increase their numbering dissemination. However, their phagocytes and bactericidal action decreased. These decreases in the total number of RBCs may be due to the hindrance

impact of heat stress on the life expectancy of the present RBCs and also the production of new RBCs from the bone marrow. The H:L ratio has been acknowledged an important record for determining stress in poultry (Siegel, 1995). Our findings are in agreement with those obtained by Nadia (2003) who found that, exposure of Japanese quail to heat stress resulted decrease of lymphocytes. Al-Ghamdi (2008) reported that, Heterophil/Lymphocyte ratio was significantly increased during heat stress. An aftereffect of heterophil increase and lymphocyte reduction, the ratio of heterophil to lymphocyte has been proposed as dependable measures of stress in broiler (Maxwell and Robertson, 1998). Additionally, it has been found that exposure of heat-stressed broiler chickens results in an increase in the heterophil to lymphocyte ratio (Zulkifli et al., 2003). On the other hand, Hb, total count of RBCs, WBCs, L%, H% and H/L ratio were significantly improved by Cr supplementation. Toghyani et al. (2007) found that, Hb was increased significantly in broilers fed 1000 ppb supplemental Cr. Askar et al. (2008) reported that Hb and PCV values were significantly increased ($p \leq 0.01$) due to the effect of dietary Cr-Pic supplementation. These results may be due to the role of chromium in stabilizing the red blood cells against cellular changes caused by peroxidation (Linder, 1991). Toghyani et al. (2007) mentioned that the H:L ratios diminished in broilers fed 1,000 and 1,500 ppb of chromium picolinate under heat-stress conditions. Gross and Siegel (1983) stated that the quantity of heterophils expanded by the blood of chicks fed corticosterone. The increase in lymphocyte counts and decreases in, H: L ratios with natural Cr supplementation in heat-stressed chicks in the present review might be ascribed to a decrease of glucocorticoid.

Results showed that heat stress caused a significantly ($p \leq 0.01$) decrease of antibody titers against infectious bronchitis virus (IBV), Newcastle disease virus (NDV), Influenza viruses (H9N2), immunoglobulin G (IgG) and immunoglobulin M (IgM) concentrations as compared with the thermoneutral control broiler chicks (Table 4). These results are in agreement with Zulkifli et al. (2003) who reported that immune response production in young broiler chicks was decreased under heat-stress conditions. This diminishment could be in a roundabout way because of an expansion in provocative cytokines under anxiety, which invigorates the hypothalamus generation of corticotrophin discharging component (Ogle et al., 1997). Stressors, for example, a high natural temperature actuate a course of neural and hormonal occasions, starting with hypothalamic incitement and the creation of corticotrophin-discharging variable, which fortifies the foremost pituitary to deliver adrenocorticotrophic hormone with incitement of adrenal cortical tissue by adrenocorticotrophic hormone to expand the generation and arrival of corticosteroids, fundamentally corticosterone in flying creatures (Siegel, 1995). Bahrami et al. (2012) found that both the organic and inorganic Cr supplements enhanced the immune response of broilers under heat-stress conditions, and a more positive effect was observed with the addition of 1,200 Cr-l-Met. However, Habibian et al. (2013) revealed that high temperature can reduce the immune function and IgM and IgG concentrations in laying hen; the reason was presumably that high temperature can produce a sustained high level of corticosterone, which induces long luscious reaction in cells, resulting in reduced IgM and IgG synthesis. In chickens, Tang and Chen (2016) showed that HS significantly reduced the plasma levels of IgA, IgG and

IgM. Also, Hosseini-Vashan et al. (2016) reported that heat stress decreased titers of total and IgG antibodies in the secondary response to SRBCs and antibody production against NDV and increased the ratio of H/L ($p < 0.05$). On the other hand, IBV, NDV, IgG and IgM significantly improved by Cr supplementation. These results are in agreement with the finding of Lee et al. (2003) who uncovered that the counter acting agent titer against irresistible bronchitis and immune response titers against NDV were improved in broiler chicks fed 400 ppb of chromium picolinate. Toghyani et al. (2007) revealed that antibody 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 conditions. Similarly, Ebrahimzadeh (2012) found that antibody titers against NDV and IBV at 21 and 42 days of age in broiler fed supplemented Cr were higher than the control diets ($p < 0.05$). Uyanik et al. (2002) found that IgG and IgM titers tended to be higher in broiler chicks fed Cr supplementation.

4. Blood constituents:

The comparison of the blood parameters under thermo neutral, heat stress and early age thermal condition in broilers fed diets supplemented without or with CrPic at the end of the experimental period is shown in Table (5).

The comparison of blood plasma in thermoneutral control and heat stress chicks (control group) revealed total protein, albumin and high-density lipoprotein (HDL) were significantly ($p \leq 0.01$) decreased, while glucose, cholesterol, LDL, total lipids and triglycerides were significantly ($p \leq 0.01$) increased (Table 5). These results are in agreement with those obtained by Seliem (2011) who observed a significant decrease in plasma total protein, albumin in the heat stress

group {exposed to daily heat stress period (38°C for 6 hours and $70 \pm 5\%$ Rh. Body temperature caused a move in tissue fluids and cause a change in the concentration of plasma protein and might be related to elevation of corticosterone which has inspired gluconeogenesis (Malheiros et al., 2003). Gursu et al. (2004) discovered increased concentrations of triglyceride, cholesterol, and HDL in heat-stressed broilers. Moeini et al. (2011) noticed that positive effect on serum lipid (cholesterol, HDL, LDL and triglyceride) concentrations in broiler chickens exposed to heat stress upon the supplementation of the proportion with chromium. Moreover, the Cr supplementation in the present work enhanced blood parameters as it was clearly observed by a significant increase in total protein, albumin and decreased glucose, cholesterol, LDL and triglycerides as compared with heat stress (control) in the broiler. A decrease in glucose level might be inferable from the impact of chromium on serum glucose, cholesterol and triglyceride concentrations were decreased when dietary chromium was added. A reduction in glucose level might be inferable from the impact of chromium on insulin. It is represented that CrPic is able to increase the rate of disguise and take-up of glucose into rodent skeletal muscle cells (Evans, 1992). These results are in agreement with those of Sahin et al. (2002b), who noticed that that total protein and albumin concentrations increased straightly with an increase of Cr supplementation. Ibrahim (2005) found that blood parameters of broiler chicks showed significantly increased in plasma total protein levels and decreased cholesterol concentration with the expanding dietary level of Cr supplementation at levels of 10, 20, 30, 40 and 50 mg Cr/kg diet when contrasted with the control group. Yildiz et al. (2004) detailed that Cr supplementation from Cr picolinate

decreased serum glucose and cholesterol concentrations, while insulin and total protein concentrations increased straightly as the dietary Cr level increased ($p \leq 0.05$). Al-Kotait et al. (2008) demonstrated that a significant ($p \leq 0.05$) decrease in low density lipoprotein cholesterol (LDL) values and increased high density lipoprotein cholesterol (HDL) values in groups received Cr when contrasted to the control. Komorowski et al. (2001) expressed that chromium picolinate significantly raised high-density lipoprotein (HDL) concentrations. Anderson (1995) who found that Cr increased plasma total lipid concentration. Chromium active component in the glucose tolerance factor, which increase of the affectability of tissue receptors to insulin, resulting in increased glucose take-up by cells. Examine recommends Cr contribution in carbohydrate metabolism, including glucose take-up, glucose use for lipogenesis, and glycogen formation (Anderson et al., 1991).

Our results showed that heat stress caused a significant decrease in SOD and GPX and increased of TAOC as compared with the thermoneutral control broiler chicks (Table 6). These results are in agreement with those obtained by Ramnath et al. (2008) who expressed that chickens exposed to heat stress (high temperature–high humidity environmental conditions) for 5 or 10 days perpetually indicated reduced activities of CAT and SOD and GSH levels contrasted with the control. These results might be troublesome to their body system during heat stress because of panting there could be conceivable for oxidative stress, respiratory alkalosis, subsequently an overproduction of free radicals in the body. It is realized that glutathione is considered to be the master antioxidant of the body and is found in every single living cell.

Similar trend was reported by Seven et al. (2009), and Sahin et al. (2011). On the other hand, SOD, GPX and TAOC improved significantly by Cr supplementation.

Data presented in Table 6 show that heat stress (control) decreased significantly ($p \leq 0.01$) Triiodothyronine (T3) and thyroxine (T4) hormones and increase plasma corticosterone concentration compared with the thermoneutral control broiler chicks. These results are in agreement with those obtained by Yahav and McMurty (2001) who revealed that exposure of broiler chickens to high environmental temperature decreased plasma level of T3 yet not T4. Garriga et al. (2006) revealed that heat stress reduced significantly T3 and T4 by 52 and 37%, respectively. It is realized that plasma levels of triiodothyronine (T3) and thyroxine (T4), important growth promoter in broilers reduced with increase of natural temperature, while corticosterone increase (Sahin et al., 2009). El-sayed et al. (2010) demonstrated that T3 concentration of broilers was significantly decreased under a HS condition. Ahmed et al. (2012) detailed that birds able to reduce plasma T3 concentration, especially during a thermal challenge. T3 hormone plays an important role in managing digestion and thermogenesis in chickens (Tao et al., 2006). Yalçin et al. (2009) also revealed that the concentration of T3 was reduced in heat acclimatization treated birds than the control broilers. The decline in T3 in broiler exposed to the heat-stress may be the birds made a rapid adjustment in the secretion rate of thyroid or related to the deiodination of T4 to T3 in the liver and kidney tissues, a response catalyzed 5-deiodinase (Lin et al., 2000). Furthermore, Gross and Siegel (1983) showed that, introduction of chickens to high natural temperature causes an increase in the plasma corticosterone, which in this manner discourages the action of the lymphoid organs

and aggregate leukocyte number. McIntosh and Sapolsky (1996) watched that amid ecological anxiety, higher in the corticosterone level quickens the era of free radicals and stifles the immune function. Moreover, dietary supplementation of Cr increased plasma concentrations of T3 and T4 and decreased plasma corticosterone concentration. The positive effects of chromium, is alleviating the negative effects of heat stress. Sahin et al. (2003) announced that supplemental of Cr in broiler chickens caused an increase in serum T3 and T4. Sahin et al., (2002b) reported that Cr supplementation markedly decreased ($p \leq 0.01$) serum corticosterone concentration. This result was likely because of the more prominent catabolic impact (or concentration) of corticosterone, yielding more glucose in the serum. Chromium is generally accepted to be the active component in the glucose tolerance factor (GTF), which increases the affectability of tissue receptors to insulin, resulting in increased glucose take-up by cells. Examine recommends Cr contribution in carbohydrate metabolism, including glucose take-up, glucose use for lipogenesis, and glycogen formation (Anderson et al., 1991). It was guessed that increased glucose take-up should increase oxidation of glucose, which would be generally changed over to fatty acids and put away as triglycerides in adipose tissues.

With regard to HSP70, broiler chicks exposed to heat stress caused a significant increase in HSP70 expression of liver as compared with supplemented with Cr had a lower HSP70 level than those fed basal diets under heat stress conditions. These results confirm the knowledge that Cr acts synergistically with antioxidant enzymes, including superoxide dismutase, glutathione peroxidase and total antioxidant capacity. Literature regarding the effect of dietary chromium supplementation on HSP70 expression is very scarce.

the thermoneutral control broiler chicks (Table 6). Furthermore, heat stress is known to delay the synthesis of most proteins except for heat shock proteins (Al-Aqil and Zulkifli, 2009). Yu and Bao (2008) showed that heat stress incites an expansion in the levels of HSP70 protein and mRNA in the heart, liver, and kidney of broiler chickens. Guerreiro et al. (2004) examined that both liver and cerebrum HSP70 levels expanded significantly during heat stress in the broiler chicks raised at thermoneutrality, with a higher expression of this peptide in brain tissue. Our discoveries that heat shock protein 70 densities may be used as a biological index of stress is consistent with the report in which heat shock protein 70 expression increased in liver and brain tissues of broiler chicks (Guerreiro et al., 2004). The overexpression of heat shock protein might be the reason behind the expanded prerequisite of ATP after heat stress (Koelkebeck and Odom, 1995). The greater HSP70 expression may suggest that the proteins are involved in the stress caused by heat shock exposure in chickens. The mechanism to combat heat stress includes lessening hyperthermia, which may be halfway because of the security of tissues to hyperthermia because of a prior condition (Rajkumar et al., 2015). Then again, heat shock protein 70 expression of liver in broilers exposed to heat stress was decreased significantly by dietary Cr supplementation. Broilers fed a diet

Carcass characteristics:

The comparison of the carcass characteristics of broilers fed diets with or without CrPic under thermo neutral, heat stress and heat shock conditions at the end of the experimental period is shown in Table 7. Carcass % and Giblets % were not significantly affected among treatments, while abdominal fat significantly ($p \leq 0.05$)

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increased in broilers fed diets supplemented with or without CrPic under thermo neutral, heat stress and heat shock conditions. These results are in good agreement with those obtained by Morsy (2013) who demonstrated that thermal shock during the early raising period had no significant effect on final carcass yield and liver weight. Tawfeek et al.

IN CONCLUSION

The results of the present study suggested that heat stress cause decrease of growth performance, Hb concentration, total count of RBCs, total count WBCs, L%, IBV, NDV, IgG and IgM concentrations, total protein, albumin and HDL, SOD, GPX, T3 , T4 hormones, whereas it increases mortality rate, respiration rate, heterophils % (H %), H/L ratio glucose, cholesterol, LDL, total lipids and triglycerides, corticosterone, TAOC,

(2014) found that high ambient temperature (36°C) increased abdominal fat and mortality rate. However, Cr supplementation significantly ($p \leq 0.05$) decreased abdominal fat percentages (Sahin et al., 2002a; and Ibrahim, 2005).

In addition, Uyanik et al. (2005) reported that

HSP70 expression of liver and abdominal fat as compared with the thermoneutral control broiler chicks. However, supplementation of chromium was able to alleviate many of these effects.

It can be recommended to supplementation of diets with 1200 µg/chromium picolinate (CrPic)/kg diet, which is available to overcome the deleterious effects of heat stress on broiler performance.

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

Composition (per 100 Kg)	Starter (1 to 21 d)	Grower (22 to 42 d)
Yellow corn	52.25	62.8
Soybean meal (44% CP)	34	23.66
Corn gluten meal (60% CP)	5.87	6
Dicalcium phosphate	1.9	1.62
Vegetable oil	3.4	3.75
Salt	0.3	0.3
Limestone	1.4	1.1
Vit. & Min. Premix**	0.3	0.3
DL-Methionine	0.25	0.18
L-Lysine-HCl	0.33	0.29
Total	100	100
Calculated analysis (%):**		
Crude protein (CP)	23.56	19.87
ME; kcal/kg	3037	3190
Ether extract	6.01	6.60
Crude fiber	3.71	3.19
Calcium	1.09	0.89
Av. Phosphorus	0.42	0.35
Lysine	1.42	1.13
Methionine	0.64	0.53
Methionine + cysteine	1.06	0.90

*Vitamin and mineral premix: each 3 Kg of vitamin and mineral premix (Special component from commercial source AGRIVET Co.) contains: Vit. A., 12000000 IU; Vit. D₃, 2000000 IU; Vit. K₃, 2000 mg; Vit. E, 10000 mg; Vit. B₁, 100 mg; Vit. B₂, 5000 mg; Vit. B₆, 1500 mg; Vit. B₁₂, 10 mg; Biotin, 50 mg; Choline chloride, 250000 mg; Pantothenic acid, 10000 mg; Nicotenic acid, 3000 mg; Folic acid, 1000 mg; Manganese, 60000 mg; Iron, 30000 mg; Selenium, 100 mg; Copper, 10000 mg; Iodine, 1000 mg; Cobalt, 100 mg; Carrier (Ca Co₃) add to 3kg.

** Calculated according to NRC (1994) requirements.

Table (2): Growth performance as affected by thermo neutral, heat stress and early age of thermal condition in broilers fed diets supplemented without or with CrPic, during the different experimental periods

Parameters \ Groups	Thermo-neutral (Control)	Heat stress				Sig.
		Heat stress (Control)	1200 µg Cr /kg	Early age of thermal condition	Early age of thermal condition + 1200 µg Cr /kg	
Body weight (gm)						
0 days	40.85±0.69	40.62±0.69	41.02±0.7	40.95±0.66	40.94±0.69	NS
21 days	718.88±11.49 ^a	560.63±10.67 ^c	625.16±10.79 ^b	588.71±12.16 ^c	651.58±12.66 ^b	**
42 days	2115.99±23.11 ^a	1496.74±22.9 ^d	1619.53±20.13 ^c	1521.34±18.65 ^d	1827.4±16.7 ^b	**
Body weight gain (gm)						
0 to 21 days	677.87±11.46 ^a	519.24±10.63 ^c	584.26±10.83 ^b	551.6±11.09 ^c	610.34±12.7 ^b	**
21 to 42 days	1495.63±27.43 ^a	944.69±23.54 ^c	996.04±25.46 ^c	925±21.73 ^c	1168.48±22.89 ^b	**
0 to 42 days	2075.08±23.2 ^a	1456±22.84 ^d	1578.54±20.18 ^c	1480.09±18.53 ^d	1786.2±16.69 ^b	**
Feed intake (gm)						
0 to 21 days	923.71±52.26	824.21±47.77	837±64.94	816.91±40.53	870.24±59.95	NS
21 to 42 days	2947.34±66.79 ^a	2730.71±47.5 ^b	2810.68±72.76 ^b	2739.58±65.18 ^b	2824.17±66.04 ^b	*
0 to 42 days	3871.04±115.81 ^a	3554.92±55.87 ^b	3647.68±10.1 ^b	3556.49±40.51 ^b	3694.41±116.96 ^b	**
Feed conversion (feed/gain; gm/gm)						
0 to 21 days	1.36±0.10	1.59±0.13	1.43±0.11	1.48±0.03	1.43±0.14	NS
21 to 42 days	1.97±0.05 ^c	2.89±0.11 ^a	2.82±0.26 ^a	2.96±0.03 ^a	2.42±0.02 ^b	**
0 to 42 days	1.87±0.06 ^c	2.44±0.07 ^a	2.31±0.12 ^a	2.40±0.07 ^a	2.07±0.04 ^b	**

Means having different letters in the same row are significantly (p<0.05) different.

Table (3): Mortality rate values as affected by thermo neutral, heat stress and early age of thermal condition in broilers fed diets supplemented without or with CrPic, during the different experimental periods

Parameters		Initial number	Total mortality			Mortality rate (%)		
			0 to 21 days	21 to 42 days	0 to 42 days	0 to 21 days	21 to 42 days	0 to 42 days
Thermo-neutral (Control)		60	2	2	4	3.33	3.33	6.67 ^a
Heat stress	Heat stress (control)	60	6	9	15	10.00	15.00	25.00 ^{cd}
	1200 µg Cr /kg	60	6	8	14	10.00	13.33	23.33 ^c
	Early age of thermal condition	60	4	8	12	6.67	13.33	20.00 ^c
	Early age of thermal condition + 1200 µg Cr /kg	60	2	5	7	3.33	8.33	11.67 ^b
Sig.						NS	NS	*

Means having different letters in the same column are significantly ($p < 0.05$) different.

Table (4): Some hematological parameters and immune response as affected by thermo neutral, heat stress and early age of thermal condition in broilers fed diets supplemented without or with CrPic, at the end of experimental periods

Parameters	Thermo-neutral (Control)	Heat stress				Sig.
		Heat stress (Control)	1200 µg Cr /kg	Early age of thermal condition	Early age of thermal condition + 1200 µg Cr /kg	
Some hematological parameters						
Hb mg/dl	11.76±0.14 ^a	8.27±0.47 ^d	9.44±0.24 ^c	10.76±0.12 ^b	10.98±0.04 ^b	**
Red blood cells (RBCs) ×10 ⁶	3.69±0.1 ^a	2.91±0.08 ^c	3.30±0.14 ^b	3.02±0.11 ^c	3.46±0.08 ^b	**
White blood cells (WBCs) ×10 ³	4.27±0.07 ^a	3.86±0.11 ^c	4.13±0.10 ^{ab}	3.60±0.06 ^d	4.04±0.08 ^b	**
Heterophils (H %)	23.42±0.36 ^e	31.79±0.71 ^a	29.84±0.25 ^b	26.55±0.53 ^c	24.70±0.58 ^e	**
lymphocytes% (L%)	66.22±0.42 ^a	61.35±0.47 ^e	62.09±0.2 ^d	63.98±0.38 ^c	65.15±0.42 ^b	**
H/L	0.35±0.01 ^e	0.52±0.01 ^a	0.48±0.01 ^b	0.41±0.02 ^d	0.38±0.02 ^c	**
Immune response						
Infectious bronchitis virus (IBV)	7.53±0.17 ^a	6.23±0.05 ^e	6.45±0.12 ^d	6.78±0.12 ^c	7.23±0.12 ^b	**
Newcastle disease virus (NDV)	8.76±0.1 ^a	7.33±0.09 ^e	7.76±0.13 ^d	8.25±0.07 ^c	8.54±0.1 ^b	**
Influenza viruses (H9N2)	8.67±0.58 ^a	5.34±0.58 ^c	6.00±1.00 ^c	7.34±0.58 ^b	7.67±0.58 ^{ab}	**
IgG	2.31±0.07 ^a	1.21±0.09 ^e	1.48±0.11 ^d	1.74±0.1 ^c	2.11±0.09 ^b	**
IgM	1.58±0.1 ^a	0.69±0.08 ^e	0.90±0.08 ^d	1.13±0.06 ^c	1.31±0.1 ^b	**

Means having different letters in the same row are significantly (p<0.05) different.

Table (5): Blood parameters characteristics as affected by thermo neutral, heat stress and early age of thermal condition in broilers fed diets supplemented without or with CrPic, at the end of the experimental periods

Parameters	Thermo-neutral (Control)	Heat stress				Sig.
		Heat stress (Control)	1200 µg Cr /kg	Early age of thermal condition	Early age of thermal condition + 1200 µg Cr /kg	
Total protein (g/dl)	6.23±0.04 ^a	4.94±0.61 ^c	5.40±0.41 ^{bc}	5.66±0.13 ^{ab}	5.85±0.11 ^{ab}	**
Albumin (g/dl)	3.83±0.08 ^a	2.76±0.15 ^d	3.03±0.22 ^c	3.32±0.17 ^b	3.53±0.07 ^b	**
Glucose (mg/dl)	119.5±2.19 ^d	169.46±7.87 ^a	148.10±6.37 ^b	133.16±2.83 ^c	128.58±3.96 ^{cd}	**
Cholesterol (mg/dl)	162.38±3.60 ^c	203.34±4.07 ^a	186.91±7.19 ^b	170.49±3.63 ^c	162.09±3.61 ^c	**
LDL (mg/dl)	114.04±3.20 ^c	148.29±4.61 ^a	137.33±4.01 ^b	128.47±2.73 ^c	122.62±2.82 ^c	**
HDL (mg/dl)	83.23±3.96 ^a	46.5±1.48 ^d	51.28±1.44 ^d	66.21±5.68 ^c	74.51±3.04 ^b	**
Total lipids (mg/dl)	596.20±21.28 ^b	743.54±22.49 ^a	718.70±6.47 ^a	704.24±57.75 ^a	631.07±5.22 ^b	**
Triglycerides (mg/dl)	153.26±10.81 ^b	168.06±6.08 ^a	150.83±7.31 ^b	151.42±8.78 ^b	136.33±3.07 ^c	**

Means having different letters in the same row are significantly (p<0.05) different.

Table (6): Serum antioxidant components and antioxidant enzymes, T3, T4 hormones and HSP70 of liver as affected by thermo neutral, heat stress and early age of thermal condition in broilers fed diets supplemented without or with CrPic, at the end of the experimental periods

Parameters	Thermo-neutral (Control)	Heat stress				Sig.
		Heat stress (Control)	1200 µg Cr /kg	Early age of thermal condition	Early age of thermal condition + 1200 µg Cr /kg	
Serum antioxidant components and antioxidant enzymes						
Superoxide dismutase (SOD)	2.35±0.06 ^a	2.14±0.05 ^{bc}	2.07±0.15 ^c	2.19±0.04 ^{bc}	2.27±0.07 ^{ab}	**
Glutathioneperoxidase(GPX) (U/L)	7.66±0.14 ^a	6.47±0.17 ^c	6.35±0.32 ^c	6.77±0.58 ^{bc}	7.24±0.23 ^{ab}	**
TAOC	0.71±0.05 ^b	0.93±0.09 ^a	0.73±0.05 ^b	0.82±0.10 ^{ab}	0.77±0.09 ^b	*
T3 (ng\ml)	3.11±0.01 ^a	1.74±0.21 ^d	2.29±0.04 ^c	2.40±0.04 ^{bc}	2.55±0.10 ^b	**
T4 (ng\ml)	18.28±0.90 ^a	13.54±0.82 ^c	13.82±0.50 ^c	14.11±0.29 ^c	15.83±0.60 ^b	**
Corticosterone (mol/L)	1.66±0.18 ^{bc}	2.07±0.12 ^a	1.82±0.16 ^{ab}	1.68±0.14 ^{bc}	1.52±0.11 ^b	**
HSP70 of Liver	3.35±0.20	4.62±0.16 ^a	4.03±0.14 ^b	4.20±0.20 ^b	3.55±0.13 ^c	**

Means having different letters in the same row are significantly ($p < 0.05$) different.

Table (7): Carcass characteristics as affected by thermo neutral, heat stress and early age of thermal condition in broilers fed diets supplemented without or with CrPic, at the end of the experimental periods

Parameters	Thermo-neutral (Control)	Heat stress				Sig.
		Heat stress (Control)	1200 µg Cr /kg	Early age of thermal condition	Early age of thermal condition + 1200 µg Cr /kg	
Carcass %	72.38±2.82	69.84±1.26	70.13±2.48	70.02±2.85	72.15±3.18	NS
Giblets %	4.18±0.76	4.82±0.14	4.66±0.26	4.42±0.53	4.32±0.45	NS
Abdominal fat %	1.82±0.21 ^c	2.90±0.17 ^a	2.53±0.18 ^{ab}	2.31±0.22 ^{abc}	2.18±0.18 ^c	*

Means having different letters in the same row are significantly (p<0.05) different.

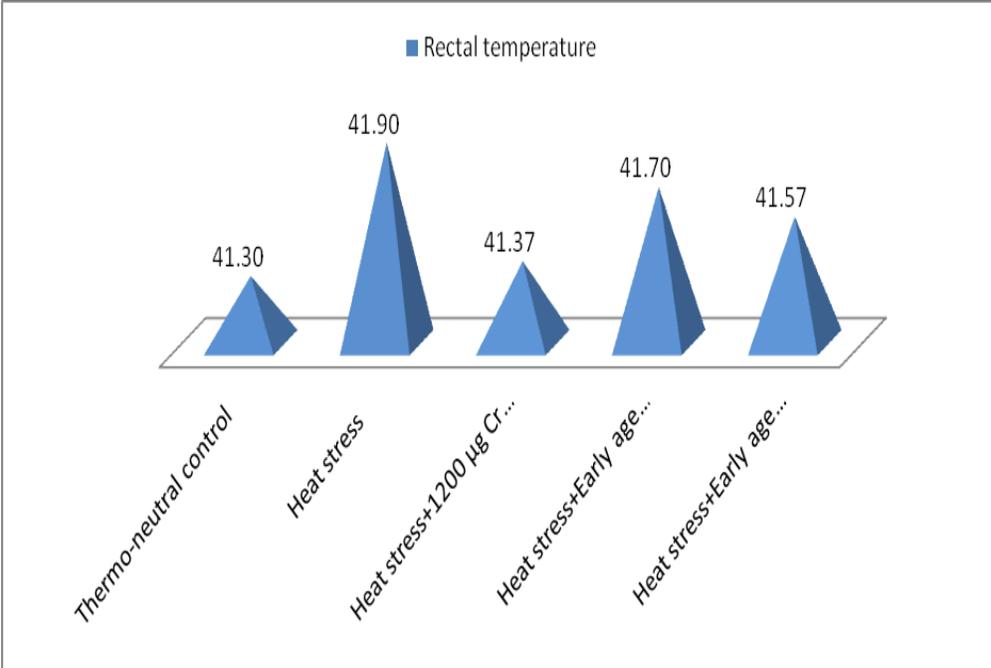


Figure (1): Rectal temperature of broiler chicks as affected by thermo neutral, heat stress and heat shock in broilers fed diets supplemented without or with CrPic, at the end of experimental periods

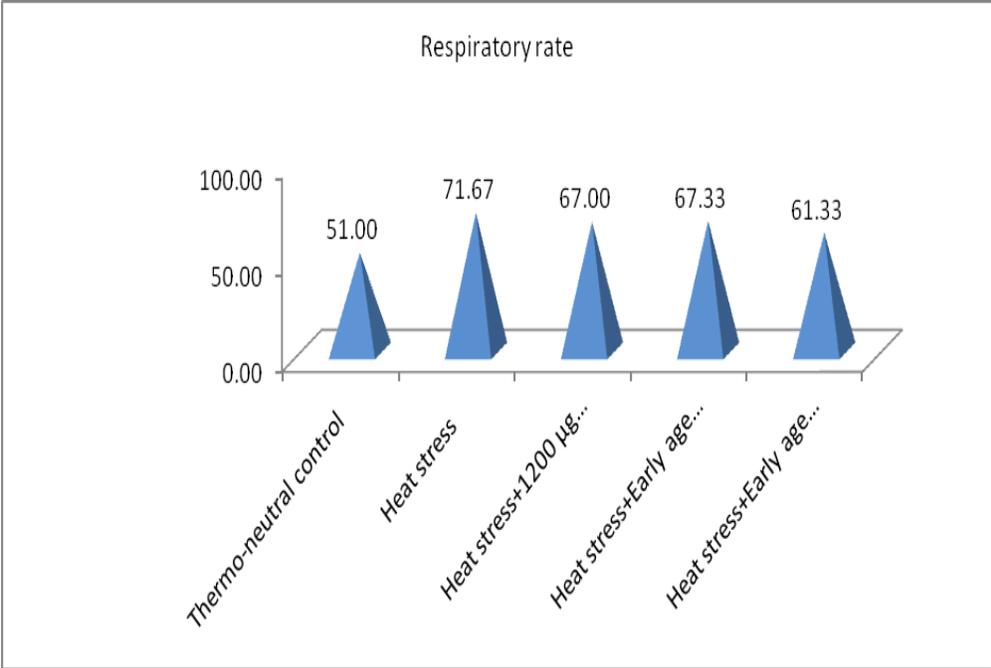


Figure (2): Respiratory rate of broiler chicks as affected by thermo neutral, heat stress and heat shock in broilers fed diets supplemented without or with CrPic, at the end of experimental periods

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الملخص العربي

تأثير إضافة بيكولونات الكروميوم على الإستجابة المناعية وبروتينات الصدمة الحرارية لدجاج التسمين تحت ظروف الإجهاد الحراري
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تهدف هذه الدراسة إلى التعرف على تأثير الإجهاد الحراري، والتكيف الحراري في سن مبكر لدجاج التسمين المغذي علي عليفة مع أو بدون الكروم على أداء النمو وبعض العوامل الفسيولوجية والصفات الكيميائية الحيوية في البلازما وكذلك الإستجابة المناعية وبروتينات الصدمة الحرارية وخصائص الذبيحة في كتاكيت التسمين.

تم وزن 300 كتكوت تسمين من نوع هبارد عمر يوم في حجرات أرضية مقسمة وتم توزيعها عشوائياً إلي 5 مجموعات تجريبية مع 3 مكررات، وكان عدد (20 كتكوت لكل منها) لكل حجرة من 1-42 يوماً من العمر. المعاملة الأولى كمتنول ومربأة في ظروف طبيعية (25م). أما المعاملة الثانية والثالثة فعرضت يوماً للإجهاد الحراري (33م) ورطوبة نسبية 65% خلال فترات التجربة، وتم إضافة 1200 ميكروجرام كروم بيكولينات/كجم عليفة للمعاملة الثالثة فقط. والمعاملة الرابعة والخامسة فعرضت يوماً للإجهاد الحراري (33م) خلال فترات التجربة والتكيف الحراري في سن مبكرة في 3 أيام من العمر، وتم إضافة 1200 ميكروجرام كروم بيكولينات/كجم عليفة للمعاملة الخامسة فقط. بعد نهاية التكيف الحراري في سن مبكر عادت المعاملات الرابعة والخامسة إلي أن تربي تحت ظروف الإجهاد الحراري (33م).

وأظهرت النتائج المتحصل عليها أن الإجهاد الحراري تسبب في إنخفاض معنوي في وزن الجسم والزيادة الوزنية، الغذاء المأكل، ومعامل التحويل الغذائي، وتركيز الهيموجلوبين، خلايا الدم الحمراء، وخلايا الدم البيضاء، والخلايا الليمفاوية (%L)، وتبتر الأجسام المضادة ضد فيروس التهاب الشعب الهوائية المعدية، وفيروس مرض النيوكاسل، وفيروسات الأنفلونزا (H9N2)، وتركيز الجلوبيولين المناعي G و الجلوبيولين المناعي M، والبروتين الكلي والألبومين، والبروتين الدهني العالي الكثافة، ومضادات الأكسدة SOD, GPX، وهرمون تراي أيدوثيرونين و الثيروكسين وزيادة معنوية لمعدل النفوق ومعدل التنفس و% هيتيروفيلس، ونسبة الخلايا الليمفاوية إلي هيتيروفيلس، الجلوكوز، الكوليستيرول، والبروتين الدهني المنخفض الكثافة، والدهون الكلية والدهون الثلاثية، و الكورتيزون، مضاد الأكسدة TAOC، وبروتينات الصدمة الحرارية في الكبد، ودهون البطن بالمقارنة مع ظروف الحرارة العادية لكتاكيت التسمين. ومع ذلك فإن إضافة الكروم لعليفة التسمين كانت قادرة على تخفيف العديد من هذه الآثار الضارة. وتشير النتائج الحالية أن إضافة 1200 ميكروجرام / الكروم بيكولينات / كجم عليفة، هو أحد الطرق المناسبة للتغلب على الآثار الضارة للإجهاد الحراري على أداء دجاج التسمين.