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EFFECT OF ACUTE HEAT STRESS CONDITIONS ON EGG PRODUCTION, EGGSHELL QUALITY, INTESTINAL CALCIUM TRANSPORT AND CALBINDIN OF THE LAYING HENS

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ABSTRACT: One hundred and fifty Hy-line layer hens, 40 weeks of age were used to study the impact of hot climate on egg production, eggshell quality, intestinal calcium transport and calcium binding protein concentration of the laying hen. Birds were randomly allocated to two experimental groups equally, the 1st group, that served as a control, was reared at room temperature under thermoneutral condition which ambient temperature was 25±1ºC, while the second group was subjected to 40±1ºC for 5 days (4 hrours daily). Egg production, eggshell quality, serum concentrations of calcium, phosphorous, thyroid, aldosterone and estrogen hormones as well as acid phosphatase and alkaline phosphatase activities were estimated. Calcium binding protein concentration and the transport of radioactive calcium (^{45}Ca) across the duodenal were also measured. The results indicated that egg weight, hen-day egg production and eggshell quality were inversely related to high temperature peroids. Serum calcium, calcium binding protein, phosphrous and alkaline phosphatase values were significantly lowered in heat stressed birds. The highest values for serum acid phosphatase and duodenal calcium transport were recorded for heat-treated hens at the 5th days of heat stress period. Thyroid and estrogen hormones unlike aldosterone hormone concentrations declined under hot climates.

In conclusion, the present study recommends avoiding exposure to heat stress conditions because of its negative impact on the level of blood calcium and intestinal calbindin-D28k and the consequent decrease in the egg production rate and the deterioration of the eggshell quality

Keywords: Calcium binding protein. eggshell quality; intestine calcium transport; laying hens.

INTRODUCTION

It is well established that calcium metabolism and eggshell quality are affected by several factors, for instance nutritional status and age of the flock, diseases and heat stress (Roberts, 2004). Unfavorable effects of high temperatures on quality of eggshell and productivity of layers have been previously investigated (Al-Saffar and Rose 2002). High temperature may reduce shell thickness, shell weight and egg specific gravity which increased eggshell breakage (Yahav *et al*., 2000). High environmental temperatures may be affect the feed intake, especially, calcium (Ca) intake of the bird, thus may be resulting in a decrease availability of Ca for shell deposition according to Nidamanuri *et al*. (2017). Regarding birds, heat stress may be also cause several biochemical and physiological changes like shift in acid-base balance, hyperthermia and production of CO_2, increase production of free radical and corticosterone (Deeb *et al*., 2002). These biochemical changes may be coincided with reduction of egg production, egg weight and eggshell quality as reported by Mashaly *et al*. (2004). Calcium carbonate deposition in the eggshell requires Ca concentrations about 4 to 12 times higher in the shell gland fluid than in serum and this required Ca obtained from the gastrointestinal tract, blood, and bones (Dacke, 2001 and Kim *et al*., 2012). About 2-2.5g of Ca are extracted from the blood by the shell gland and transfered to the egg without accumulation over a period of 15 hrs (Lin *et al*., 2004).

Under high ambient temperature, calbindin intensity in intestinal parts was negatively affected and consequently diminish Ca^{+2} absorption along the intestines (Ebeid *et al*., 2012). Under thermoneutral temperature, calcium binding protein (CaBP-D28k) localization in the small intestine segments has been reported to be in the following order: ileum < jejunum < duodenum and also presented in the colon and cecum of laying hens. It was noticed that modification of physiological mechanisms was to mentain birds' survival during hot environment (Ebeid *et al*., 2012). High environmental temperature affect negatively on thyroid hormones (thyroxin, T_4 and triiodothyronine, T_3) which provide a major mechanism of acclimatization on laying hens (Attia *et al*., 2016). Reduction in serum T_4 and T_3 due to heat stress conditions may be lower metabolic rate and feed intake to prevent hyperthermia (Kataria and Kataria, 2005). Moreover the estrogenic hormones play important roles in fowl's reproduction performance so its decrease in serum negatively influences egg production. One more possible cause for the reduction in laying performance might be attributed to the decrease in cholecalciferol-1-hydroxylase production, which has a crucial function in homeostasis of calcium or the hampered uptake of gut calcium (Anjum *et al*., 2014). The alteration in the role of reproductive hormones is attributed to changes in the pathway of neuro hormone linked to stress. High environmental temperature affect negatively on Estrogen (E2) and consequently adversely affects the egg production percentage (Franco and Beck, 2007).

The present study aims to shed more light on the detrioration impacts of heat stress on physiological, productive and reproductive traits of the laying hen.

MATERIALS AND METHODS

The current study was conducted at the Poultry Production Research Unit, Biological Applications Department, Nuclear Research Center, Egyptian Atomic Energy Authority during April month.

One hundred and fifty Hy-line layer hens, 40 weeks of age were numbered and caged (55cm L × 30cm W × 45cm H) individually Hens were provided with 16:8 hrs light:dark cycle, the lights were turned off by a timer. All hens were fed a basel corn-soy mash laying diet formulated to fulfill NRC (1994) requirements of laying hens (Table 1). Along with water, the diet was available *ad libitum*.

Hens were randomly divided equally into two groups with three replication each (25 hens for each replicate). The 1st group reared at room temperature under thermoneutral condition which ambient temperature was 25±1ºC and 53% relative humidity and served as control group. While the 2nd group was exposed to 40±1ºC and 53% relative humidity for 5 days (4 hours daily) from 12:00 to 16:00 and served as heat-stressed group (HS).

Hen-day egg production and quality traits of eggshell were determined. Collected eggs were individually weighed to the nearest 0.1g. Eggshell were individually weighed and proportioned to egg weight. Eggshell breaking strength (kg/cm^2) was determined by subjecting eggs to pressure till the broken of eggshell occures using the machine model 040914 (FHK Co. Ltd., Tokyo, Japan). Eggshell thickness was determined as the mean value of the thickness of air cell, sharp, and equator end locations of the egg after removing the eggshell membranes using a micrometer caliper (Mitutoyo, 0.01 to 20 mm, Tokyo, Japan). Eggshell density (eggshell weight/surface area) was calculated by dividing the weight of eggshell by the surface area. For this purpose, eggshells were washed and dried in an oven at 95°C for 24 hour and weighed. Egg surface area was calculated by the formula of Mueller & Scott (1940) ($S = 4.67 \times W^{2/3}$), where W = egg weight, and S = surface area.

Nine hens from each treated group (three hens per replicate) were slughtered at the end of heat expousre peroid of the first, third and fifth days of heat exposure. While, nine hens from the control group were slughtered at the fifth day end. Blood samples were collected from the slughtered hens then centrifuged at 3000 rpm for 10 min. Serum was separated and stored at −20˚C.

Serum concentrations of calcium (Ca), phosphorous (P) and activities of alkaline phosphatase (Alk-P) and acid phosphatase (Acid-P) enzymes were determined colorimetrically using spectrophotometer Spectronic 1201 (Milton Roy, Ivyland, USA) with commercial kits (Stanbio Laboratory LP, Boerne, USA). Serum thyroid hormones (T_3 and T_4), aldosterone (Aldo) and estrogen (E2) concentrations were estimated by radioimmunoassay (RIA) technique and counted at gama counter model RIA SAR, Pacard, Model 0540501.

Duodenal was collected from slaughtered hens to estimate the concentration of calcium binding protein (CaBp) and the level of calcium transport across the duodenal using the radioactive calcium (^{45}Ca) as described by Bar and Hurwtiz, (1979) and Hurwtiz *et al*. (1973), respectively. Calcium binding protein concentration and the level of ^{45}Ca transport across the duodenal were estimated using Liquid Scintillation Analyser model TRI – CARB 2700 TR (β Counter).

Statistical analysis

Data were statistically analyzed using the General Liner Model Procedure by one-way analysis of variance of the SAS software (SAS Institute, 2000). Mean values were compared using Duncan's Multiple Range Test (Duncan, 1955) at $p < 0.05$.

RESULTS AND DISCUSSION

Egg production is impacted by several factors like nutrition, thermal environment, strain of chicken, age, housing systems, point of lay, peak lay and persistency of lay (Singh *et al*., 2009).

Data in Table (2) demonstrates the effect of heat stress on egg production and eggshell quality characteristics in laying hens. It was found that hen-day egg production percentage, eggshell quality, and egg weight were inversely ($P < 0.05$) related to high temperature. These findings are in agreement with many studies which indicated that heat stress induced a considerable decrease in eggshell weight (Sahin *et al*., 2006), eggshell thickness (Lin *et al*., 2004), and elevation in eggshell breakage (Lin *et al*., 2004). Also, Mashaly *et al*. (2004) noticed that egg production and eggshell quality were significantly lower when White Leghorns hens were reared on high ambience. The reduction in productivity, egg weight and eggshell quality may be due to the reduction in feed intake that deecreasing the amount of nutrients available for egg production (Kirunda *et al*., 2001). High environmental temperature also lessen digestibility of diet ingredients (Dıarra and Tabuacırı, 2014). Moreover, several investigations have been documented that exposure to high ambience reduced plasma protein and Ca concentrations, which are mandatory for egg formation.

In the present study egg and eggshell weights, eggshell percentage, thickness, density and breaking strength were significantly lowered when the laying hens were subjected to high temperatures (Table 2). Eggs weight from the heat-treated groups were remarkably lower than eggs from the thermoneutarl group at the 3rd and 5th days of heat stress. The lowest ($P < 0.05$) values were obtained in the 5th day of heat stress. These results are in agreement with Lin *et al*. (2004) who found that high ambient temperature decreased egg weight and elevated breakage of eggshell. Usayran *et al*. (2001) reported that during summer, heat stressed birds produced smaller eggs with lower shell quality because several of physiological processes such as the reduction in carbonic anhydrase activity which responsible for bicarbonate formation which contributes to the formation of egg shell (Allahverdi *et al*., 2013). In addition, our results are in line with those of Ranjan *et al*. (2019) and Ebeid *et al*. (2012) who revealed that egg weight, eggshell thickness, density and percentage were adversly affected by high temperature.

Table (3) shows heat stress impact on serum calcium, phosphorous (P), calcium binding protein concentrations, alkaline phosphatase (Alk-P), acid phosphatase (Acid-P) and duodenal calcium transport activities. The results showed that serum Ca and P concentrations were lowered ($p < 0.05$) at the 1st and 3rd days of HS exposure and these concentrations returned to the normal values at the 5th day of HS. Alk-P enzyme activity was significantly ($P < 0.05$) increased at the 1st and the 3rd days of HS exposure. While, its activity approximately equal to control at the 5th day of heat exposure. This may be due to acclimation the birds to heat stress condition. Unlike, acid phosphatase activity was significantly ($p < 0.05$) lowered at the 1st day of HS exposure and the reduction was continued to the 3rd day of HS exposure. While its activity was significantly elevated at the 5th day of HS. These findings are agreed with Sogabe *et al*. (2013) who suggested that alkaline phosphatase activity is elevated in cases of improper bone calcification such as when vitamin D3 or dietary calcium is deficient or during high ambient temperature periods. In addition, Kataria *et al*. (2008) noticed that during heat stress, higher secretion of glucocorticoid and alkaline phosphatase enzyme were recorded.

In addition, it could be observed that Ca transport across small intestine was significantly risen in heat stressed hens comparing with control ones. However, it could be observed an extrusive relationship between Ca transport activity and heat exposure duration. Calcium transport was continuously increased with increasing heat exposure days. The increase of duodenal calcium transport across small intestine in heat-stressed hens over the control hens (Table 3) may be occurred to overcome blood calcium deficiency during heat stress periods (Hansen *et al*., 2004). In contrast, CaBp level was significantly ($P < 0.05$) lowered during heat stress periods as compared with the thermoneutral condition. The lowest CaBp concentration was recorded at the 3rd day of heat stress exposure (Table 3).

The deterioration in eggshell quality could be partially attributed to the decrease in serum Ca and calcium binding protein concentrations. Mustaf *et al*. (2009) documented that heat stressed laying hens exhibited lower serum calcium level. Additionally, Mack *et al*. (2013) showed that, under heat stress conditions, Ca use and Ca uptake by duodenal epithelial cells were reduced.

Furthermore, blood flow to the oviduct in heat stressed birds might be affected because blood flow increased to peripheral tissues to transport the excessive inner heat from core of the body to its surface (Bernabucci *et al*., 2010). Furthermore, during heat stress, panting rate of birds increased to lose heat via water evaporation. The elevation in respiration rate leads to increase blood pH which cause respiratory alkalosis and reduce blood partial pressure of HCO^{3-} and CO_2 (Franco and Beck, 2007). The increase in blood pH decreases the blood quantity of ionized Ca^{+2} (Allahverdi *et al*., 2013), which is utilized by the small intestine and eggshell gland. Another main result in this study was the negative impact of heat stress on calbindin intensity in the intestinal segments, which in turn might reduce Ca^{+2} absorption along the intestine. Conversely, Star *et al*. (2008) noticed that at ambient temperature ranged from 42-46°C, the reduction in Ca^{+2} and P could be attributed to the increase in calcitonin level and the decrease in parathyroid hormone. Hypophosphoric and hypocalcemic impacts of calcitonin might be due to the stimulation effect of high temperature to the calcium regulating axis. During hot ambience, low phosphorus could be associated with low calcium metabolism and thyroid activity.

Calbindin exists as two main forms, a protein with high-molecular weight (CaBP-D28k) which found in the mammals brain and kidney, and protein with low-molecular weight (CaBP-D9k) which found in the mammalian intestine (Bar, 2009 a, 2009b). However in avian species, calbindin were found at high concentrations in intestine and eggshell gland and other tissues that are distinguished with their enormous transport of Ca^{+2} (Sugiyama *et al*., 2007). In eggshell gland and intestine tissues, calbindin and Ca^{2+} transportation are strongly associated (Bar, 2009b). Calbindin is presented in birds' intestine prior the beginning of reproduction, and increased at the start of egg production (Bar, 2009a) to accommodate the high demands of Ca^{2+} for eggshell calcification. However, calbindin appears in eggshell gland during first eggshell formation at the commencement of egg production and vanished within three days of its termination (Bar, 2009a). Therefore, calbindin concentration in the small intestine and eggshell gland is relative to Ca^{2+} deposition rate in eggshell (Yosefi *et al.*, 2003). The previous findings obviously explained the decrease in intestinal absorption of Ca^{+2} under high environmental temperature. Due to the significant role of calbindin in absorption capacity of Ca^{2+} which accordingly contributes in calcification of eggshell, it can be presumed that the impairment in eggshell quality of heat-stressed birds in this study could be due to the low concentration of calbindin in the small intestine. Results of Hansen *et al*. (2004) are agreed with this presumption which reported that eggshell calcification was disrupted in heat stressed laying hens and total calcium absorption via intestinal enterocytes was also reduced ($P < 0.05$). Therefore, it seems reasonable to suggest that, heat stress negatively affect calbindin intensity which might in turn adversely affect eggshell calcification process (Ebeid *et al*., 2012).

Thyroid hormones (T_3 and T_4) play important role in maintaining body temperature and regulating metabolic heat production activities in mammals and birds. Our results indicated that thyroid hormones (T_3 and T_4) concentrations were significantly lowered in heat stressed-hens as compared with the control ones. The lowest concentrations of T_3 and T_4 were noticed at the 5^{th} day of heat stress. Moreover, their values are reduced as heat stress days increased (Table 4). In the present study, the reduction in thyroid hormones (T_3 and T_4) due to high environmental temperature may be probably referred to lower metabolic rate in order to thermoregulation and hyperthermia prevention (Ozbey and Ozcelik, 2004). Fewer secretion of T_3 is either due to less conversion of T_4 into T_3 or less fabrication. Servatius *et al*. (2001) documented that exposure of chickens to elevated environmental temperatures decreased feed intake by about 14 % and decreased blood concentration of T_3 but not T_4 level. Anjum *et al*. (2016) reported that heat stress induced an incessant decrease in production of T_3, while T_4 production was also affected either towards decrease, increase or no change. While, Sechman (2013) reported that growing chickens exposed to acute high temperatures (from 35 to 41^oC) did not exhibit a considerable effect on serum T_3 or T_4 concentrations. However, chronic exposure (more than two wks) reduced serum T_3 and T_4 or decreased T_3 but increased T_4 (Kataria and Kataria, 2005).

Results indicating that serum aldosterone concentration was significantly ($P < 0.05$) increased in heat stressed group comparing to the thermoneutral group. The highest aldosterone values were recorded at the 3^{rd} and 5^{th} days of heat stress (Table 4). This results referred to the stimulation of adrenocorticotrophic hormone release which regulates aldosterone secretion which in turn helps in maintaining salt-water balance under heat stress conditions (Kansaku *et al*., 2008). Increased aldosterone level helped in decreasing potassium levels and retaining sodium in birds' body by increasing its absorption from lower segments of the intestine along with water reabsorption. Increased sodium and chloride during hot condition was not only due to haemo-concentration but also be due to increased aldosterone concentration (Khan *et al*., 2002).

Finally, the present results indicating that estrogen hormone concentration was significantly ($P < 0.05$) declined under heat stress conditions due to high temperature at the 1^{st}, 3^{rd} and 5^{th} days of heat stress condition (Table 4). The lowest estrogen value was recorded at the 3^{rd} day of heat stress (Table 4). Estrogen secretion from white nonhierarchical follicles and the theca layer of yellow pre-ovulatory follicles (Ciftci, 2012). Estrogens have direct and indirect relationships with calcium metabolism; the first, they directly elevate blood Ca^{+2} via rising the density of renal receptors of parathyroid hormone and the second, they indirectly elevate the activity of 1-"-hydroxylase in kidneys (Xing *et al*., 2001). The 1-"-hydroxylase is thought to be accountable for the creation of 1,25-di-hydroxy-cholecalciferol which has the ability to mediate absorption of phosphorus and calcium (Bar, 2009a) by a saturable trans-cellular way and augment calcium binding protein concentration in the target cells. As hens aged, egg production reduces accompanied by deterioration in eggshell quality, estrogen receptor, and estrogen levels (Xing *et al*., 2001). The same response was noticed in laying hens subjected to thermal stress. Also, it has been demonstrated that estrogens implantation improves calcium uptake throughout the intestine (Beck and Hansen, 2004).

CONCLUSION

In conclusion, the present study recommends avoiding exposure to heat stress conditions because of its negative impact on the level of blood calcium and intestinal calbindin-D28k and the consequent decrease in the egg production rate and the deterioration of the eggshell quality

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table (1): Composition and calculated analysis of experimental diet.

Contents	Percentage
Ground yellow corn	54
Soybean meal (44%)	25
Concentrate (52%) [1]	10
Fat	1
Di calcium phosphate	3
Limestone	6
Sodium chloride	0.3
Choline chloride	0.25
Mineral premix [2]	0.25
Vitamins premix[3]	0.2
Calculated analysis:	
CrudeProtein	20.5
Kcal ME/Kg diet	2670
Total Calcium	3.7
Available phosphorus	0.13

1-Concentrate contains: 52 % Crude protein, 1.57 % crude fiber, 7 % calcium, 3.5 % available phosphorus, 1.52 % methionine, 2.11 % methionine and cystine, 2.98 % lysine, 2416 K cal / Kg metabolizable energy.
2 – Each kilogram of mineral premix contains Copper 10 mg: Iodine 1 mg: Iron 30 mg: Manganese 55 mg Zinc 55 mg and Selenium 1 mg.
3 – Each kilogram of vitamin premix contains A 12000 I.U.: D_3 2000 I.U: E 10 mg: K 2 mg: B1 1 mg: B_6 1.5 mg: B_{12} 10 Ug : B_2 4 mg: Pantothenic acid 10 mg: Niacin 20 mg: Folic acid 1000 ug: Biotin 50 Ug and Choline chloride 500 Ug .

Table (2): Effect of heat stress on egg production and eggshell quality characteristics in laying hens (means ± SE).

Treatments / Parameters	Thermonetural (25°C)	Heat stress conditions (40°C)		
		1st day	3rd day	5th day
Hen-day egg production (%)	86.85 ± 4.53^a	85.55 ± 4.53^a	81.22 ± 4.53^b	77.95 ± 4.53^c
Egg weight (g)	62.26 ± 0.13^a	62.11 ± 0.13^a	60.29 ± 0.13^b	58.01 ± 0.13^c
Eggshell weight (g)	6.01 ± 0.09^a	5.93 ± 0.09^a	5.72 ± 0.09^b	5.25 ± 0.09^c
Eggshell percent (%)	9.65 ± 0.11^a	9.55 ± 0.11^a	9.39 ± 0.11^b	9.05 ± 0.11^c
Shell thicknes (mm)	0.367 ± 0.002^a	0.341 ± 0.002^b	0.330 ± 0.002^c	0.322 ± 0.002^d
Eggshell density (mg/cm^2)	84.08 ± 1.01^a	83.22 ± 1.01^b	80.01 ± 1.01^c	77.58 ± 1.01^d
Eggshell breaking strenght (Kg/cm^2)	3.92 ± 0.02^a	3.86 ± 0.02^b	3.85 ± 0.02^b	3.79 ± 0.02^c

*Values are expressed as means ± standard error of the mean.
[a, b, c] Means with different superscripts, within row significantly differ ($P < 0.05$).
N = 9 per treatment.

Table (3): Effect of heat stress on some blood biochemicals, duodinal calcium transport and calcium binding protein in laying hens (means ±SE)

Treatments / Parameters	Thermonetural (25°C)	Heat stress conditions (40°C)		
		1st day	3rd day	5th day
Serum Calcium (mg/dl)	*23.08 ± 0.19^{a}	21.17 ± 0.19^{b}	18.22 ± 0.19^{c}	22.71 ± 0.19^{a}
Duodinal Calcium Transport (mmol/L)	319.08 ± 22.41^{d}	334.91 ± 22.41^{c}	351.11 ± 22.41^{b}	371.01± 22.41^{a}
Calcium Binding Protein (CaBp)(mg/dl)	728.17 ± 33.11^{a}	640.22 ± 33.11^{b}	625.81 ± 33.11^{c}	639.21± 33.11^{b}
Serum Phosphorus (mg/dl)	6.95 ± 0.09^{a}	6.31 ± 0.09^{b}	5.85 ± 0.09^{c}	7.3 ± 0.09^{a}
Serum Alkaline –P (Iu/L)	519.01 ± 8.09^{c}	539.22 ± 8.09^{b}	566.19 ± 8.09^{a}	523.19 ± 8.09^{c}
Serum Acid –P (U/L)	362.25 ± 3.17^{b}	321.41 ± 3.17^{c}	301.27 ± 3.17^{d}	387.19 ± 3.17^{a}

*Values are expressed as means ± standard error of the mean.

$^{a, b, c}$ Means with different superscripts, within row significantly differ ($P < 0.05$).

N = 9 per treatment.

Table (4): Effect of heat stress on some blood hormones in laying hens (means ± SE)

Treatments / Parameters	Thermoneutral (25°C)	Heat stress conditions (40°C)		
		1st day	3rd day	5th day
T_3 (ng/ml)	3.82 ± 0.02^{a}	3.61± 0.02^{b}	2.66 ± 0.02^{c}	1.43± 0.02^{d}
T_4 (ng/ml)	19.2 ± 0.4^{a}	17.9 ± 0.4^{b}	12.33 ± 0.4^{c}	9.13± 0.4^{d}
Aldosterone (pg/ml)	116.8 ± 2.38^{c}	135.2 ± 2.38^{b}	141.3 ± 2.38^{a}	144.9 ± 2.38^{a}
Estrogen (pg/ml)	462.9 ± 29.8^{a}	455.7 ± 29.8^{b}	429.2 ± 29.8^{d}	444.5±29.8^{c}

*Values are expressed as means ± standard error of the mean.

$^{a, b, c}$ Means with different superscripts, within row significantly differ ($P < 0.05$).

N = 9 per treatment.

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Calcium binding protein. eggshell quality; intestine calcium transport; laying hens.

الملخص العربي

تاثير الاجهاد الحراري الحاد علي انتاج البيض و جودة قشرة البيض و انتقال الكالسيوم و مستوي الكالسيوم المرتبط بالبروتين بالامعاء في الدجاج البياض

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1- قسم تربية الدواجن – معهد بحوث الانتاج الحيواني- مركز البحوث الزراعية – وزارة الزراعة

2- قسم المعجلات و المصادر الايونية - شعبة العلوم النووية الاساسية - مركز البحوث النووية - هيئة الطاقة الذرية

أجريت التجربة بوحدة بحوث انتاج الدواجن بقسم التطبيقات البيولوجية – مركز البحوث النووية – هيئة الطاقة الذرية خلال شهر ابريل. استخدم في البحث مائة و خمسون طائرا من سلالة الهايلاين عمر اربعون اسبوعا لدراسة تأثير الاجهاد الحرارى علي انتاج البيض و علي جودة قشرة البيض و انتقال الكالسيوم في الامعاء و مستوي الكالسيوم المرتبط بالبروتين. تم تقسيم الطيور عشوائيا الي مجموعتين كل منها ثلاثة مكررات (25 طائر لكل مكرر). تم تربية المجموعة الاولي تحت ظروف درجة حرارة الغرفة (25 ± 1م°) و اعتبرت مجموعة الكونترول. بينما تم تعريض المجموعة الثانية الي درجة حرارة 40 ± 1م° علي مدار خمسة ايام بمعدل 4 ساعات يوميا. تم قياس معدل انتاج البيض اليومي و خصائص جودة قشرة البيض. كما تم قياس تاثير الاجهاد الحرارى علي محتوي سيرم الدم من كل من الكالسيوم و الفوسفور و انزيمات الالكانيز و الاسيد فوسفاتيز و كذلك علي مستوي هرمونات الغدة الدرقية و الالدوستيرون و الاستروجين. كما تم قياس تاثير الاجهاد الحراري علي محتوي ميكوزا الامعاء من الكالسيوم المرتبط بالبروتين و معدل انتقال الكالسيوم خلال الامعاء باستخدام الكالسيوم المشعع. و لوحظ ان الاجهاد الحراري قد تسبب في انخفاض معنوي علي معدل انتاج البيض اليومي و وزن البيض و علي خصائص جودة القشرة. كما تسبب الاجهاد الحراري في الانخفاض المعنوي في محتوي سيرم الدم من كل من الكالسيوم و الفوسفور و الكالسيوم المرتبط بالبروتين و انزيم الالكانيز فوسفاتيز بينما لوحظ ارتفاع مستوي انزيم الاسيد فوسفاتيز و معدل انتقال الكالسيوم خلال الامعاء. كما تسبب الاجهاد الحراري في الانخفاض المعنوي في محتوي سيرم الدم من هرمونات الدرقية و هرمون الاستروجين بينما ارتفع مستوي هرمون الالدوستيرون.

و توصي الدراسة بتجنب التعرض لظروف الاجهاد الحراري لما له من تأثير سلبي علي مستوي كالسيوم الدم و محتوي ميكوزا الامعاء من الكالسيوم المرتبط بالبروتين و ما يتبع ذلك من انخفاض في معدل انتاج البيض و تدهور خصائص جودة القشرة.