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Research Article
Vitamin E Supplementation and Early Age Heat Conditioning to Alleviate the Negative Effects of Heat Stress in Quail Chicks

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Abstract
Background and Objective: Inclusion of vitamin E in poultry diets and manipulation of temperature at early age has been known to help birds cope with heat stress at later age of their life. This study was conducted to investigate the effects of early age heat conditioning (EHC) and vitamin E addition on the performance and blood parameters to alleviate deleterious impact of heat stress in quail chicks. **Material and Methods:** Three hundred one-day-old quail chicks were randomly divided into 4 groups of 5 replicates with 15 birds of each. Treatments were: Control, vitamin E: Chicks were fed the basal diet supplemented with 200 IU kg^{-1} diet vitamin E, EHC: Chicks were exposed to 40$\pm$1°C for 2 h at days 7th and 13th of embryogenesis and EHC+vitamin E: Chicks were exposed to 40$\pm$1°C for 2 h at days 7th and 13th of embryogenesis and fed the basal diet supplemented with 200 IU kg^{-1} vitamin E. The experiment lasted from 1-40 days of age. **Results:** The results indicated that using early age heat conditioning and/or supplementation of vitamin E significantly ($p<0.05$) improved body weight, feed intake and feed conversion ratio at 40 days of age. Significantly ($p<0.05$) improvement was observed in blood pH, H/L ratio and Hb concentration, thyroid hormones (T_3 and T_4), plasma protein fractions and antioxidant status (Total antioxidant capacity, catalase and superoxide dismutase). **Conclusion:** Exposed quail chicks to early age heat conditioning and addition of vitamin E at 200 IU kg^{-1} diet can effectively alleviate the adverse effects of heat stress.

Key words: Quail chicks, plasma protein fractions, vitamin E, heat conditioning, antioxidant

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Heat stress causes significant impairment of productive performance, nutrient utilization, immune and antioxidant statuses, which lead to economic losses in poultry production. Dietary manipulations and heat conditioning are promising procedures to alleviate the negative adverse effects of heat stress. High temperature in poultry houses is one of the most remarkable factors that adversely effects on productive performance of birds through the activation of hypothalamic pituitary adrenal axis and increases corticosterone levels, resulting in decreased body weight gain, feed intake and immunity responses[1]. The effects of heat stress could be overcome by some managerial practices such as increasing ventilation rate, lowering stocking densities, cooling house but the diet manipulation are the simplest method and has been confirmed[2]. Usually dietary manipulation cover altered needs of stressed birds for protein and energy or for supplementing some feed additives such as vitamins and minerals[2]. On the other hand, manipulation of temperature at early age has been known to help birds cope with heat stress at later age of their life. Some studies suggested that acclimatization of birds for heat stress could be induced through exposure to high temperature for short-time at pre-hatch and/or post-hatch[3,4]. So, the time of thermal manipulation has to be related to the development of the hypothalamus-hypophysis-thyroid axis to modification the heat production onset response and to the development of the hypothalamus-hypophysis-adrenal axis to avert increase in stress response[5]. Alkan *et al.*[6] found that thermal manipulation of Japanese quails during the early embryogenesis incubation (at days 6, 7 and 8), temperature was increased from 37.7-41°C for 3 h resulted a higher body weight and rate of maturation at 35 days of age than the control group.

Regarding the dietary manipulation, the vitamin E administration at high levels can be a good management practice in quail nutrition to promote feed intake and growth[7]. Inclusion of vitamin E in poultry diets has been suggested especially during the heat stress not only to reduce oxidative stress but also to improve growth performance[8]. The inclusion of vitamin E to Japanese quail diets is recommended to alleviate heat stress and improve performance[9]. Bollengier-Lee *et al.*[10] revealed that vitamin E can guard the liver from lipid peroxidation and prevents cell membranes from damage. Birds cannot synthesis vitamin E, therefore the level of vitamin E must be increased in the diet during heat stress than at normal conditions. Sahin *et al.*[11] found that supplementation of dietary vitamin E at 250 mg kg^{-1} to Japanese quails diet significantly reduced malondialdehyde (MDA) in serum and tissue. Panda *et al.*[12] added vitamin E at 125 mg kg^{-1} diet and reported a significant lower lipid peroxide and increased glutathione peroxidase level in serum of heat stressed laying hen. Gouda *et al.*[13] reported that adding vitamin E at 200 IU kg^{-1} to the broiler diet showed an improvement in the performance, physiological and immunological measurements of broiler reared under heat stress condition. Thus this study was conducted to investigate the effects of early age heat conditioning and vitamin E supplementation individually and as a combination on performance and some blood parameters in Japanese quails reared under heat stress conditions.

MATERIALS AND METHODS

Experimental animals and feeding: This study was carried out at Nubaria experimental farm affiliated to the Animal Production Department, National research Centre, during the period from July -16 -2018 to August -25- 2018. Three hundred 1 day-old Japanese quail chicks (*Coturnix coturnix japonica*) were divided into 4 groups of 5 replicates with 15 birds of each. Treatments were: Control: (non heat conditioning and without vitamin E supplementation), vitamin E: chicks were fed the basal diet supplemented with 200 IU kg^{-1} diet vitamin E (non heat conditioning), EHC: chicks were exposed to 40±1°C for 2 h at days 7th and 13th of embryogenesis and EHC+vitamin E: chicks were exposed to 40±1°C for 2 h at days 7th and 13th of embryogenesis and fed the basal diet supplemented with 200 IU kg^{-1} vitamin E as α- tocopheryl acetate. The experiment lasted from 1-40 days of age. Chicks of all treatments were kept under similar hygienic and environmental conditions. Feed and water were provided *ad libitum* during the experimental period. Feed composition of the basal diet is shown in Table 1. The basal diet based on corn and soybean meal and was formulated to meet the requirements of quail chicks.

Measurements and estimated parameters: Body weight (BW) and feed intake (FI) were recorded weekly and feed conversion ratio (FCR) was calculated. At the end of the experiment (40 days) blood samples were collected from 2 slaughtered birds for each replicates in heparinized tubes, few drops from the blood were used to determine blood pH by electronic pH meter, blood haemoglobin (Hb) by the colorimetric method using commercial kits. Blood smears were also done, stained with Wright's stain procedure and used to calculate the number of lymphocytes (L) and heterophils (H) in 100 white blood cells then the H/L ratio was

Table 1: Composition of the basal diet

Ingredients	Percentage	Calculated analysis	Percentage
Corn	57.00	Crude protein	23.05
Soybean meal 48 (%)	38.00	Metabolizable energy (kcal kg^{-1})	2970.00
Vegetable oil	1.50	Calcium	0.97
Limestone	1.35	Available phosphorus	0.42
Di-calcium phosphate	1.55	Lysine	1.35
Salt	0.30	Methionine+cysteine	0.76
Vitamin and mineral premix*	0.30		
Total	100.00		

*Vitamin and mineral premix: Each kilogram of diet contains, vitamin A: 12000 IU, vitamin D3: 2500 IU, vitamin E: 10 mg, vitamin B1: 2 mg, vitamin B2: 5 mg, vitamin B6: 4 mg, vitamin B12: 10 µg, niacin 25 mg, pantothenic acid 10 mg, biotin 50 µg, folic acid 1000 µg, vitamin K: 2.0 mg, choline chloride 255 mg, selenium 300 µg, copper 10 mg, iodine 1.0 mg, iron 33 mg, manganese 60, Zinc 60 mg

Table 2: Effect of treatments on performance of quail chicks

	Treatments				
Items	Control	Vitamin E	EHC	EHC+vitamin E	SEM
At 21 days of age					
Body weight (g)	128.1	135.8	138.1	139.7	±5.24
Feed intake (g)	488.9	488.7	488.4	495.7	±11.78
Feed conversion ratio (g g^{-1})	3.820^{a}	3.603^{b}	3.544^{b}	3.552^{b}	±0.070
At 40 days of age					
Body weight (g)	211.9^{c}	233.3^{b}	265.4^{a}	270.2^{a}	±4.48
Feed intake (g)	1188.8^{b}	1199.0^{b}	1250.6^{a}	1290.0^{a}	±15.49
Feed conversion ratio (g g^{-1})	5.610^{a}	5.139^{b}	4.712^{c}	4.774^{c}	±0.067

^{a,b,c}Means within a column with different superscripts are significantly different ($p\leq 0.05$), SEM: Standard Error of mean, EHC: Early heat conditioning, vitamin E supplementation (200 IU kg^{-1})

calculated, the remaining blood were centrifuged at 3000 rpm for 15 min and plasma obtained was stored at -20°C in eppendorf tubes until analysis. Plasma total proteins were determined according to the method described by Henry[14]. The determination of plasma albumin based on a colorimetric method described by Doumas *et al.*[15]. Globulin was calculated by subtraction of plasma albumin from plasma total protein. Plasma triiodothyronine (T_3) and thyroxine (T_4) were determined according to Akiba *et al.*[16]. Total antioxidant capacity (TAC) level was determined according to Koracevic *et al.*[17]. Catalase (CAT) level was determined according to Aebi[18], superoxide dismutase (SOD) level in plasma were determined according to Nishikimi *et al.*[19].

Statistical analysis: Data obtained were statistically analyzed using the general liner model of statistical analysis systems institute[20]. Significant differences among means were tested by Duncan's multiple range test Duncan[21].

The following model was used:

$$Yij = \mu + Ti + eij$$

Where:

Yij = An observation
μ = Overall mean
Ti = Effect of treatment (i = 1, 2, 3 and 4)
eij = Experimental random error

RESULTS

Growth performance: Table 2 shows the effect of early age of heat conditioning and addition of vitamin E to diets at 200 IU kg^{-1} and their combination on growth performance of Japanese quail at 21 and 40 days of age. The results at 21 day of age showed that early age of heat conditioning and addition of vitamin E individually or together significantly ($p<0.05$) improved FCR. However BW and FI showed no significant ($p>0.05$) effect. At 40 days of age BW and FCR were significantly ($p<0.05$) improved with early age of heat conditioning treatment and addition of vitamin E at 200 IU kg^{-1} of diet either individually and/or together compared to the control group. It is obvious that adding vitamin E significantly ($p<0.05$) increased BW however, the best BW were reported for the treatment group that received heat conditioning (with or without vitamin E). The best FCR was recorded with heat conditioning groups (with or without vitamin E). Feed intake was significantly ($p<0.05$) increased with early age heat conditioning and early age heat conditioning with vitamin E supplementation compared with the control group.

Blood parameters: Table 3 explains the effect of early age of heat conditioning and the addition of vitamin E to the diets with 200 IU kg^{-1} diet and their combination on some blood

Table 3: Effect of treatments on blood parameters of quail chicks at 40 days of age

	Treatments				
Items	Control	Vitamin E	EHC	EHC+vitamin E	SEM
Blood pH	7.52^{a}	7.33^{b}	7.35^{b}	7.33^{b}	±0.014
H/L ratio	0.667^{a}	0.560^{b}	0.573^{b}	0.542^{b}	±0.012
Hb concentration	8.45^{b}	9.83^{a}	9.34^{a}	10.06^{a}	±0.315

^{a,b}Means within a column with different superscripts are significantly different ($p \leq 0.05$), SEM: Standard error of mean, EHC: Early heat conditioning, vitamin E supplementation (200 IU kg^{-1})

Table 4: Effect of treatments on thyroid hormones and plasma protein fractions of quail chicks at 40 days of age

	Treatments				
Items	Control	Vitamin E	EHC	EHC+vitamin E	SEM
Triiodothyronine (T_3)	4.07^{c}	4.46^{b}	4.19^{b}	5.05^{a}	±0.103
Thyroxine (T_4)	21.36^{b}	23.95^{a}	24.02^{a}	24.60^{a}	±0.521
Plasma total proteins	3.82^{c}	4.83^{b}	4.26^{b}	5.30^{a}	±0.136
Plasma albumin	1.72^{b}	2.15^{a}	1.55^{b}	2.25^{a}	±0.131
Plasma globulin	2.10^{c}	2.68^{b}	2.71^{b}	3.05^{a}	±0.103

^{a,b,c}Means within a column with different superscripts are significantly different ($p \leq 0.05$), SEM: Standard error of mean, EHC: Early heat conditioning, vitamin E supplementation (200 IU kg^{-1})

Table 5: Effect of treatments on antioxidant status of quail chicks at 40 days of age

	Treatments				
Items	Control	Vitamin E	EHC	EHC+vitamin E	SEM
TAC	10.71^{c}	14.51^{a}	12.51^{b}	15.25^{a}	±0.296
CAT	4.38^{c}	8.51^{a}	6.01^{b}	8.92^{a}	±0.296
SOD	145.76^{c}	164.57^{a}	158.66^{b}	168.64^{a}	±1.621

^{a,b,c}Means within a column with different superscripts are significantly different ($p \leq 0.05$), SEM: Standard error of mean, EHC: Early heat conditioning, vitamin E supplementation (200 IU kg^{-1}), TAC: Total antioxidant capacity, CAT: Catalase enzyme, SOD: Superoxide dismutase

parameters of Japanese quail at 40 days of age, where showed that all the treatments resulted in a significant improvement in all blood measurements studied (pH, H/L ratio and Hb concentration) compared with the control.

Thyroid hormones and plasma protein fractions: Table 4 shows the effect of early age of heat conditioning and the addition of vitamin E to the diets with 200 IU kg^{-1} diet and their combination on thyroid hormones and plasma protein fractions of quail chicks at 40 days of age. Regarding thyroid hormones all the treatments resulted in a significant improvement in thyroid hormones (T_3 and T_4). The EHC+vitamin E treatment showed a significant superiority over the vitamin E and EHC treatments in T_3. As for the plasma protein fractions found that plasma total proteins, plasma albumin and plasma globulin improved significantly with the treatments, where the EHC+vitamin E treatment showed superiority followed by the vitamin E and the EHC treatments and then control on plasma total proteins and plasma globulin as for the plasma albumin the EHC+vitamin E and the vitamin E treatments showed superiority compared with other treatments.

Antioxidant status: Table 5 shows the effect of early age of heat conditioning and the addition of vitamin E to the diets with 200 IU kg^{-1} diet and their combination on antioxidant status of quail chicks at 40 days of age. All treatments resulted in a significant improvement in antioxidant status (TAC, CAT and SOD). The EHC+vitamin E and the vitamin E treatments showed superiority over the rest followed by the EHC treatment and then control.

DISCUSSION

The results of this study showed that Japanese quail chicks that were exposed to heat conditioning had lower body temperature and were more adapted to heat stress than those in control group or treatment group that received vitamin E, birds consumed more feed and gain more weight. On the other hand, vitamin E as an antioxidant have been widely used as feed additives in animal diets to overcome heat stress because of their potential beneficial effects, including performance and health. Heat stress are known to have adverse effects on productive performance[8,22]. Heat stressed birds suffer from decreased vitamins (E and A) and minerals (selenium, iron and zinc) concentrations of tissue[23]. Vitamin E supplementation could be useful in combating some of the heat stress physiological adverse effects responses and improving thermotolerance through antioxidant effects[24]. The data of this study showed that body weight and feed conversion ratio were significantly ($p<0.05$) improved with the addition of vitamin E at 200 IU kg^{-1} of diet compared to control group, which agreed with the findings of Gouda *et al.*[13], who reported improved performance when feeding higher vitamin E to broilers. The results of some blood parameters are consistent with Muller[25], who found that vitamin E improved the hematological parameters where vitamin E works to protect phospholipids of cellular and sub cellular membranes, by preventing the oxidation of fatty acids. In this respect early age heat conditioning was proposed as a management procedure to reinforce the resistance of broiler chicks to acute climate heat condition or sudden heat spells during finishing period[26,27]. The present results lend support to the findings of Hemid *et al.*[27] and El-Moniary *et al.*[28], who found that the hematological responses to acute or chronic heat stress include changes in hemoglobin (Hb) and hematocrit (Ht) levels and leucocytes differential count, especially heterophils and lymphocytes.

Under heat stress conditions, T_3 and T_4 concentration are reduced due to the impair effect of heat stress on thyroid activity. Adding vitamin E to heat-stressed birds resulted in increased concentration of T_3 and T_4. The results of this particular study on T_3 and T_4 concentration on the plasma could be explained by the positive effect of Vit E on alleviating the negative effect of heat stress[11,24]. The results obtained by Sahin *et al.*[11] and Rashidi *et al.*[29] revealed that vitamin E supplementation increased plasma total protein, albumin and globulin concentration, vitamin E is particularly important for the prevention of fatty acid peroxidation[30]. The results of early age heat conditioning of quail chicks are in accordance with the findings of El-Wardany *et al.*[26], who reported that the positive impact of early age heat conditioning on enhancing nutrients utilization and heat tolerance of chicks.

Heat stress disturbs the balance of oxidation as well as antioxidant defense systems and cause oxidative damages to biological molecules including proteins and DNA[31]. Antioxidant system comprising TAC, SOD and CAT acts as a foremost line of antioxidant defense system, the results indicated that early age of heat conditioning and the addition of vitamin E to the diets with 200 IU kg^{-1} diet and their combination brought a significant increase in antioxidant status. These enzymes prevent the generation of free radicals and they protect the cell constituents against oxidative damage[32]. Yardibi and Turkay[33] found that the exposure of chicks to high temperatures at an early age led to an increase in antioxidant status activities when exposed to high temperatures in the late stages of life and this was agreed with the results of this study. Vitamin E is the prim defense against lipid peroxidation, it breaks chain propagation and thus terminates free radical attack at an early stage[34,35]. Antioxidant status was improved by early age of heat conditioning and the addition of vitamin E to the diets with 200 IU kg^{-1} diet.

CONCLUSION

Heat stress causes significant impairment of productive performance, nutrient utilization and immune and antioxidant statuses, which lead to economic losses in poultry production. Dietary manipulations are a promising procedure to alleviate the negative adverse effects of heat stress. From the obtained results it could be concluded that the early age heat conditioning and dietary supplementation of vitamin E at 200 UI kg^{-1} diet of quail chicks resulted in improve of growth performance, antioxidant status and may be a novel tools to alleviate the adverse effects of heat stress.

SIGNIFICANCE STATEMENT

This study discovers the possible synergistic effect of early age heat conditioning and vitamin E at 200 UI kg^{-1} diet of quail chicks to improve the growth performance and antioxidant status. This combination may be a novel tool to alleviate the adverse effects of heat stress.

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