B-5261
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RESEARCH ARTICLE Indian Journal of Animal Research

Effect of Vitamin E and Selenium Supplementation on Growth Performance and Heat Shock Protein 70 Levels in Broiler Chickens Exposed to Summer Heat Stress

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10.18805/IJAR.B-5261

ABSTRACT

Background: The effect of vitamin E and Selenium supplementation in diet of broiler chicken on growth performance and Heat shock protein70 (HSP70) level under summer heat stress was studied.

Methods: The current study was conducted on 240 day-old commercial broiler chicks, which were divided randomly into four treatment groups of 60 numbers, with three replicates of 20 in each group. The control (T_0) group was allotted with basal diet, group T_1 (basal diet + Vitamin E @ 100 mg/kg and Selenium @ 0.2 mg/kg), T_2 (basal diet + Vitamin E @ 125 mg/kg and Selenium @ 0.25 mg/kg) and T_3 (basal diet + Vitamin E @ 150 mg/kg and Selenium @ 0.3 mg/kg).

Result: In the present study, it has been found that, vitamin E and selenium supplementation in the diet of heat stressed broilers influenced the body weight (P=0.002), Body weight gain (P=0.001), Feed Intake (P=0.311) and BPEI (P=0.013) but not the Feed conversion ratio. Dietary vitamin E and Selenium supplementation increased the dressing percentage (P<0.001), Breast meat yield percentage (P=0.004), Thigh yield percentage (P=0.018), Drum stick yield percentage (P=0.013) and decreased abdominal fat percentage (P=0.004) under heat stress. The Heat shock protein level was improved (P<0.001) by dietary supplementation of vitamin E and Selenium under heat stress. The study indicated that growth performance, carcass characteristics and HSP70 level could be improved by dietary vitamin E and selenium supplementation under heat stress.

Key words: Antioxidant, Broiler chicken, Heat stress, HSP70, Selenium, Vitamin E.

INTRODUCTION

Poultry are usually sensitive to temperature-associated environmental challenges, especially heat stress. It is reported that, modern poultry genotypes produce more body heat, due to their faster growth rate and greater metabolic activity (Mack, 2013). Poultry which are raised in warm areas are susceptible to heat stress (Zulkifli *et al*., 2009; Ajakaiye *et al*., 2010) due to their bio-physiological characteristics. Heat stress leads to oxidative stress associated with a reduced antioxidant status in birds, as reflected by increased oxidative damage and lowered plasma concentrations of antioxidant vitamins and minerals (Sahin *et al*., 2009). In chronic heat stress condition, increased levels of 'Reactive Oxygen Species' (ROS) occurs and the body enters into a stage of oxidative stress resulting in the production and release of Heat Shock Proteins (HSP) in order to try and protect itself from the deleterious cellular effects of ROS (Droge, 2002). Amongst all members of the 70 kDa family, Heat Shock Protein 70 (HSP70) has attracted most attention to researchers as it directly responds to heat stress (Al-Aqil and Zulkifili, 2009) and higher concentrations were found in broilers and laying hens when exposed to heat stress (Gu *et al*., 2012).

Vitamin E (α-tocopherol) is a biological antioxidant that contributes to improved growth, physiological and immunological performance in broiler chickens because of its ability to neutralize free radicals and reduce lipid peroxidation in both the plasma and skeletal muscle (Gao *et al*. 2010). Selenium enhances the actions of vitamin E in reducing peroxide radicals.

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How to cite this article: Bora, S., Sonowal, M., Baishya, P., Mahanta, J.D., Saikia, A.K., Sarma, M., Deka, P. and Borah, P. (2024). Effect of Vitamin E and Selenium Supplementation on Growth Performance and Heat Shock Protein 70 Levels in Broiler Chickens Exposed to Summer Heat Stress. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5261.

Submitted: 06-11-2023 **Accepted:** 18-03-2024 **Online:** 29-03-2024

Hence, keeping in view the above facts, the present study was planned to investigate the effects of supplementing Vitamin E and Selenium on broiler chicken performance; exposed to summer heat stress.

MATERIALS AND METHODS

Birds, diets and experimental design

A total of 240-day-old commercial broiler chicks (cob 400) from a single hatch were obtained, weighed individually and wing banded for identification. The birds were maintained following standard feeding and uniform management practices under deep litter system of rearing with strict bio-security measures.

The chicks were divided randomly into four treatment groups of 60 numbers with three replicates (n=20) in each group. Four experimental diets with different levels of vitamin E and Se were formulated (Table 1) for three phases *i.e.* Pre starter, Starter and Finisher as per BIS (2007). The control (T_0) group comprised of basal diet (Standard broiler ration), group T_1 (basal diet + Vitamin E @ 100 mg/kg and Se @ 0.2 mg/kg), T_2 (basal diet + Vitamin E @ 125 mg/kg and Se @ 0.25 mg/kg) and T_3 (basal diet + Vitamin E @ 150 mg/kg and Se @ 0.3 mg/kg). The average maximum temperature inside the house was 36.5°C with maximum relative humidity 92.5%. The experimental design employed were completely randomized design (CRD).

Total body weight (BW), Body weight gain (BWG), Total feed intake (FI), feed conversion ratio (FCR) and Broiler Performance efficiency Index (BPEI) were determined during the experiment. The Broiler Performance Efficiency Index (BPEI) was calculated out as per Narahari and Kumararaja (2008).

Carcass characteristics

At the end of the experiment the birds were slaughtered as per improved Koshers method to determine the dressing percentage, prime cuts yield, giblet percentage and lymphoid organ weight percentage.

Serum Heat shock protein *(HSP)* concentration was measured using Chicken Heat Shock protein 70 (HSP70) ELISA kit (Catalog No: E 1383025, Type II), 96T/48T, Sincere Biotech Co. Ltd., Shunyi, Beijing) by standard method.

Statistical analysis was done as per the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Effect on growth performance

In the present study, it has been found that, vitamin E and Se supplementation in the diet of heat stressed broilers influenced BW (P=0.002), BWG (P=0.001), FI (P=0.311) and BPFI (P=0.013) but not the FCR (Table 2). Heat stress influences the peripheral thermal receptors which transmit nerve impulses that suppresses the activity of the appetite center in the hypothalamus causing reduction in feed intake (Marai *et al*., 2007) and fewer nutrients are available for enzymatic activities, hormone production and heat generation. Geraert *et al*. (1996) reported that high ambient temperature reduces body weight and they found that when birds were exposed to 32°C there was 14% reduction in body weight from 2 to 4 weeks of age and a 24% reduction from 4 to 6 weeks of age. Various researchers (Mansoub *et al*., 2010 and Tawfeek *et al*., 2014) also reported that the hot weather conditions (35°C) significantly reduced body weight (P<0.01) and feed intake (P<0.05) in broilers and birds kept in heat stress condition. The decreased body weight was not only due to the lower feed intake, but also due to a direct effect of environmental temperature on broiler physiology and metabolism (Ain-Baziz *et al*., 1996 and Geraert *et al*., 1996).

The antioxidants vitamin E and Se synergistically destroys the cell damaging free radicals (Biswas *et al*. 2011) leading to better utilization, digestion, absorption and metabolism of feed nutrient essential for health and body weight gain. Se has a protective role on pancreatic tissue against oxidative damage that might have helped the pancreas to function properly including secretions of digestive enzymes, thus improving digestibility of nutrients and consequently, performance (Sahin *et al*., 2009). Further, Vitamin E and Se supplementation reduced stress by a reduction in lipid peroxidation that might have helped the

Table 1: Ingredient composition (%) and calculated nutritive value of broiler prestarter, starter and finisher diet.

Ingredients	Prestarter (0-7 days)	Starter (8-21 days)	Finisher (22-42 days)
Yellow Maize	44.05	41.79	52
Rice Polish	8	12	5.5
Vegetable oil	3.5	5	5.5
Soybean meal	15	10	16.62
GNC	25.95	27.99	16.62
Limestone	1.3	1.3	2.5
Mineral mixture	1	1	-
Trace mineral	-	-	0.1
Salt	0.5	0.5	0.4
Lysine	0.45	0.21	0.35
Methionine	0.2	0.16	0.2
Multi enzyme	-	-	0.55
Vitamin mixture	0.01	0.01	0.01
Liver tonic	-	-	0.1
Toxin binder	-	-	0.05
Coccidiostat	0.02	0.02	-
Growth promoter (commercial)	0.02	0.02	-
Nutritive value			
CP (%)	23	22	20
Metabolizable energy (Kcal/kg)**	3004.51	3084.25	3194.98
Vitamin E mg/kg)**	19.4	23.2	17.5
Selenium (mg/kg)**	0.08	0.07	0.09

**In the calculated value.

birds in improving their performance by reducing the metabolic requirement of nutrients and diverting these nutrients for muscle growth (Rama Rao *et al*., 2013).

Effect on dressing percentage

Vitamin E and Se supplementation increased the dressing percentage (Table 3) with ($P<0.001$) and without giblet ($P<0.001$) in the present study, which might be due to reduced release of cortisol resulting prevention of body catabolic activity as well as increased feed intake and consequently more nutrients were available to improve growth rate and body weight.

At high ambient temperatures, birds reduced their feed intake and consequently less nutrients were provided to the internal organs, which compromise their developments (Ghazi *et al*., 2012b). High ambient temperature induced production and release of corticosteroids, which exert catabolic effects (mobilization of proteins and lipids) through muscle wasting and hence reduce growth rate (Hayashi *et al*., 1994). Carcass and liver yields were adversely affected by high temperature where they represented 96% of that of normal control group (Tawfeek *et al*., 2014). A strong relationship reported between live body weight and carcass yield of broiler chickens (Fanatico *et al*., 2005). The effect of selenium on meat yield could be due to changes in thyroid hormone metabolism or a result of changes in broiler feathering (El-Sheikh *et al*., 2006) and cellular integrity (Peric *et al*., 2009). Heat stress decreased ($P<0.05$) dressing percentage (-2.9%) and Vitamin E (100 mg/kg diet) supplementation increased dressing percentage in broilers under heat stress (Attia *et al*., 2017).

Effect on mean cut of parts (per cent of carcass weight)

Increased percentage of breast (p=0.004), Thigh (p=0.018) and Drum stick (p=0.013) in vitamin E and Se supplemented groups were observed in the present study (Table 3). It might be due to increased dressing percentage and also effective mechanism of vitamin E in its reactions with active radicals to break chains, to exert pressure, to renovate, to increase endogen defence and better protein accretion to improve breast meat characteristics (Rokade, 2014). Carcass characteristics especially breast yield improved with an increase of both vitamin E and Selenium (Sahin and Kucuk, 2001). Vitamin E supplementation in diet helps in fatty acid composition and oxidative stability of thigh muscle in broilers under heat stress which might have contributed to increase

Table 2: Growth performance of broiler chicken under different treatment groups.

Variable	T_0 (Control)	T_1	T_2	T_3	*P*-value
Total body weight (g)	$2120.0^{a}\pm33.5$	$2292.9^{c}\pm14.7$	$2219.0^{b}\pm16.4$	$2179.9^{ab}\pm9.08$	0.002
Total body weight gain (g)	$2077.7^{a}\pm14.5$	$2250.6^{c}\pm14.7$	$2176.6^{b}\pm14.3$	$2137.6^{b}\pm9.08$	<0.001
Total feed intake (g)	$4062.75^{a}\pm0.08$	$4156.53^{d}\pm0.77$	$4151.91^{b}\pm0.35$	$4117.85^{c}\pm11.67$	0.001
Total FCR	1.92±0.04	1.81±0.01	1.88±0.04	1.87±0.04	0.311
BPEI	$110.67^{a}\pm4.67$	$126.67^{b}\pm1.764$	$118.00^{a}\pm0.58$	$116.33^{a}\pm0.33$	0.013

Means with different superscripts between groups differed significantly ($P<0.05$).

Table 3: Carcass characteristics of broiler chicken under different treatment groups.

Parameter (%)	T_0 (Control)	T_1	T_2	T_3	*P*-value
Dressing percentage with giblet	$77.42^{a}\pm0.21$	$80.13^{c}\pm0.59$	$78.64^{b}\pm0.09$	$78.54^{b}\pm0.21$	<0.001
Dressing percentage without giblet	$71.86^{a}\pm0.25$	$74.79^{c}\pm0.53$	$73.12^{b}\pm0.05$	$72.87^{b}\pm0.24$	<0.001
Thigh	$13.61^{a}\pm0.34$	$15.07^{b}\pm0.19$	$14.27^{a}\pm0.17$	$14.16^{a}\pm0.72$	0.018
Neck	$5.86^{b}\pm0.32$	$4.64^{a}\pm0.43$	$5.38^{b}\pm0.38$	$5.54^{b}\pm0.18$	0.029
Drum stick	$11.65^{a}\pm0.39$	$13.05^{b}\pm0.49$	$12.59^{b}\pm0.62$	$12.56^{b}\pm0.15$	0.013
Wings	10.76±0.21	9.94±0.66	10.27±0.57	10.58±0.43	0.271
Breast	$29.64^{a}\pm1.08$	$32.46^{c}\pm0.32$	$31.25^{b}\pm0.53$	$31.03^{b}\pm0.14$	0.004
Back	16.60±0.67	16.08±0.33	16.33±0.47	16.18±0.54	0.647
Abdominal fat	$3.49^{c}\pm0.16$	$2.09^{a}\pm0.50$	$2.89^{b}\pm0.29$	$2.72^{b}\pm0.12$	0.004
Giblet yield	$5.34^{a}\pm0.07$	$5.56^{b}\pm0.11$	$5.52^{b}\pm0.04$	$5.68^{c}\pm0.04$	0.002
Heart	0.77±0.02	0.81±0.06	0.81±0.03	0.85±0.05	0.196
Gizzard	$2.15^{a}\pm0.04$	$2.35^{b}\pm0.07$	$2.26^{b}\pm0.03$	$2.30^{b}\pm0.05$	0.007
Liver	3.75±0.09	4.02±0.23	3.95±0.10	4.08±0.06	0.082
Thymus	0.47±0.00	0.53±0.02	0.46±0.06	0.48±0.03	0.211
Bursa	0.14±0.03	0.17±0.01	0.17±0.00	0.17±0.00	0.146
Spleen	$0.10^{a}\pm0.01$	$0.12^{b}\pm0.01$	$0.11^{ab}\pm0.00$	$0.11^{ab}\pm0.00$	0.026

Means with different superscripts between groups differed significantly ($P<0.05$).

Table 4: Serum HSP 70 (ng/ml) of broiler chicken under different treatment groups.

Variable	T_0 (Control)	T_1	T_2	T_3	SEM[a]	*P*-value
HSP 70	$6.20^d \pm 0.08$	$2.28^a \pm 0.05$	$2.81^c \pm 0.07$	$2.65^b \pm 0.09$	1.65	<0.001

SEM- Standard error of Mean; Means with different superscripts between groups differed significantly (P<0.05).

in the thigh yield as was found in the present experiment (Vakili and Rashidi, 2011). The reduced breast (%) in heat stressed broilers due to abundant of white fast-contracting glycolytic fibers (McKee, 2003), which are richer in ATP and rely on glycogen supply for its metabolism and hypertrophy. Therefore, as feed intake was limited under heat stress, glycogen supply decreased leading to decreased protein synthesis in the breast muscle (Temim *et al*., 2000). Heat stress decreasing the proportion of breast muscle in the carcass were observed in several studies (Zhang *et al*., 2012; Lara and Rostagno, 2013; Zeferino *et al*., 2016).

The abdominal fat percentage decreased significantly (p=0.004) in all the vitamin E and Se supplemented groups. Vitamin E strongly influences the lipid metabolism (Albuquerque *et al*., 2017) and Se supplementation declined the activity of cytosolic malic enzyme leading to decline in abdominal fat deposition (Vadhanavikit and Ganther, 1994). Konca *et al*. (2009) reported that supplementation of Se alone decreased the abdominal fat pad. Fat deposition in the abdominal area of broilers is regarded as waste in the poultry industry and represents a loss in the market and consumer acceptability (Toghyani *et al*., 2011). The increase in carcass fat of broilers raised under hot ambient temperature is a major concern since the fat content of meat products has become increasingly important to consumer perceptions of the healthfulness of meat and abdominal fat pad yield increased from 1.8 in normal control to 2.78 % by more than 50 % in heat stressed control group (Tawfeek *et al*., 2014). Retention of more abdominal fat in chickens exposed to heat stress was credited to decreased capacity of protein synthesis and of peripheral lipolysis (Ain-Baziz *et al*., 1996 and Zhang *et al*., 2012).

Giblet yield (percentage of dressed weight)

The supplementation of vitamin E and Se in the diet of heat stressed broilers in the present study (Table 3) increased the giblet yield (p=0.002). At high ambient temperatures, birds reduced their feed intake and subsequently less nutrients were supplied to the internal organs leading to compromised developments (Park *et al*., 2013) and resulted decrease in the giblets yield (Attia *et al*., 2011). Supplementations of anti-oxidants in the diet ameliorate this effect.

Lymphoid organ weight (percentage of dressed weight)

The relative weight of spleen showed significant (p=0.026) increase in T_1 group as compared to control. No variations were observed in thymus and bursa amongst experimental groups. The reason for enhanced relative weight of spleen in the present study might be due to proliferation of lymphocyte in this organ (Akbari *et al*., 2018). Swain *et al*. (2000) reported non-significant effect of vitamin E and Se (150, 300 mg/kg vitamin E + 0.1, 0.5, 1 mg/kg Se) supplementation in broiler chicken on relative weight of thymus, while Shaik *et al.* (2005) in their experiment on supplementation of 150, 300 IU Vitamin E/kg+0.15 mg Selenium / kg di*et al*so could not find any effect on the weight of bursa in broiler chicken.

Effect on serum HSP70 (ng/ml)

Supplementation of Vitamin E and Se significantly reduced the HSP70 level (Table 4) in all the supplemented groups (T_1, T_2 and T_3). Dietary antioxidant supplementation suppressed the expression of HSP70 by eliminating ROS and stabilizing antioxidant status of birds in summer season (Jang *et al*., 2014). It is likely that Se or Vitamin E diet supplementation may restrict the expression of HSPs by increasing the activities of superoxide dismutase and subsequent removal of ROS (Dokladny *et al*., 2006). Dietary vitamin E significantly decreased the mRNA expression of HSP70 (Jang *et al*., 2014). Recently, it has been proposed that the lipid composition and the architecture of membranes act as membrane censors and modulate HSPs response through the HSF-1. Therefore, the up regulation of heat shock proteins in stressed birds may be due to the damages of oxidative stress in the muscle cells. Furthermore, changes in HSP70 may be an indication of cellular damage within the intestines (Dokladny *et al*., 2006). Moreover, dietary antioxidants vitamin E and Se might alleviate heat stress in birds during summer conditions by down-regulation of HSP expression which might be associated with the modulation of pro-inflammatory cytokine expression (Rettenbacher and Palme, 2009 and Chang *et al*., 2010) and by eliminating ROS *via* stabilizing antioxidant status in birds under summer conditions (Jang *et al*., 2014). Combined action of Se and Vitamin E increased the antioxidant capacity and enhanced the GPx1 and GPx4 mRNA levels, which might successfully eliminate most of the ROS as the Se plus Vitamin E supplementation in broiler feed could be able to enhance the endogenous antioxidant defense system by suppressing the lipid oxidation and by regulating the heat shock proteins and thus ameliorate the negative effects of summer heat stress conditions (Kumbhar *et al*., 2018).

CONCLUSION

On the basis of the present findings, it can be concluded that supplementation of antioxidants vitamin E and selenium to the diets of broilers during heat stress period at the supplementation rate of 100 g vitamin E and 0.2 mg selenium per kg of diet is beneficial in terms of growth performance, carcass characteristics and stabilizing the antioxidant status of birds in summer season.

Conflict of interest

All authors declared that there is no conflict of interest.

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