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Effect of Vitamin E and Zinc supplementation on the growth performance, immunity, serum biochemistry and antioxidant profile of broiler chicken under heat stress

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ABSTRACT

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A six-week feeding trial was carried out to evaluate the effects of dietary vitamin E and zinc on growth performance, immune response, and serum biochemical profile in broiler chickens. A total of 360 mixed-sex day-old chicks were randomly assigned to nine dietary treatments in a 3×3 factorial design, with three levels of vitamin E (50, 100, and 150 IU/kg diet) and three levels of zinc (40, 80, and 120 mg Zn/kg diet). Each treatment had five replicates of eight birds each. Birds were reared under *ad libitum* feeding and watering conditions, and body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded across starter (0–3 weeks) and finisher (4–6 weeks) phases. Results indicated that birds supplemented with 100 IU/kg vitamin E and 80 mg Zn/kg diet exhibited significantly higher BWG and better feed efficiency. FI was higher at 50 IU/kg vitamin E with 40 mg Zn/kg diet, whereas FCR improved with 150 IU/kg vitamin E and 120 mg Zn/kg diet. Significantly ($p \leq 0.05$) higher serum total protein was observed at 150 IU/kg vitamin E with 40 mg Zn/kg diet compared to 150 IU/kg vitamin E with 120 mg Zn/kg diet and 50 IU/kg vitamin E with 40 mg Zn/kg diet. Serum triglycerides was significantly higher at 50 IU/kg vitamin E than those recorded at other levels. Immune responses were significantly enhanced at 100 IU/kg vitamin E with 80 mg Zn/kg diet. Spleen weight was significantly affected by dietary treatments, but thymus and bursa weights remained unaffected. The study concluded that supplementation of 100 IU/kg vitamin E with 80 mg Zn/kg diet is optimal for improving growth performance, immune response, and feed efficiency in broiler chickens under heat-stressed conditions.

Keywords: Broiler chickens, Vitamin E, Zinc, Growth performance, Immune response, Feed efficiency

INTRODUCTION

Reducing heat stress in broiler chickens remains a major concern for both researchers and poultry producers. Heat stress often leads to high mortality rates, reduced feed intake, lower body weight gain, and poor feed efficiency, which negatively impact meat-type poultry flocks (Yegani, 2008). Each year, poultry farmers face substantial economic losses due to heat stress, as temperatures exceeding 30°C create stressful conditions for birds and significantly affect production. The optimal temperature range for broiler chickens to achieve maximum body weight is 10-22°C, while the range of 15-27°C is considered ideal for improved feed efficiency (Rama Rao *et al.*, 2011).

Various strategies have been explored to mitigate the harmful effects of high environmental temperatures on broiler performance, with dietary modifications being one of the most effective approaches (Dukare *et al.*, 2021). Among these, supplementing broiler diets with vitamin E and zinc has proven beneficial in enhancing diet stability, strengthening the immune system, and improving overall performance (Bou *et al.*, 2004). Additionally, Bou *et al.* (2005) reported that zinc supplementation significantly increased selenium levels in chicken. Moreover, studies by Sahin and Kucuk (2003) demonstrated that dietary zinc supplementation led to higher serum concentrations of vitamin C, vitamin E, and zinc in poultry, further supporting its role in improving bird health and productivity.

Adaptive immunity is based on activity of B- and T-lymphocytes which produce antigen specific antibodies or directly attached the pathogens to be expelled from the cell, respectively. Feeding selenium and vitamin E at increased levels than the recommended dose increase the antibody titre against sheep red blood cell antigen (Yamuna and Thangavel, 2011; Nageswara *et al.*, 2003). Dietary Zn is essential for normal immune function (Dardenne and Bach, 1993) and substantial evidences are available which show that adding Zn above the dietary requirement enhances disease resistance in chicken. Zhang *et al.* (2006) reported that optimum supplemental levels of Zn should range between 80-120 mg/kg diet for broiler chicken to get better immune competence. Kumar (2007) and Kumar *et al.* (2009) reported that immune response of broiler chicks was better at higher levels of Zn (60 and 80 mg/kg diet) compared to 40 mg Zn/kg diet, irrespective of Zn sources. However, Mohanna and Nys (1999) reported that the antibody titres

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in response to sheep red blood cells (SRBC) injection and plasma alkaline phosphatase activity remained unaffected by dietary Zinc. During heat stress, an increased respiratory rate serves as a mechanism for heat dissipation. However, this elevated respiration leads to respiratory alkalosis and a rise in blood pH, which in turn reduces blood calcium levels. As a result, bone mineralization and strength are compromised (Raza *et al.*, 2021). Therefore, this study was undertaken to assess the impact of vitamin E and zinc supplementation on the growth performance, immune response, serum biochemistry, and antioxidant profile of broiler chickens.

MATERIALS AND METHODS

A six-week feeding trial was conducted to evaluate the performance of broiler chickens in terms of growth, immune response, and serum biochemical profile in broiler birds. A total of 360 mixed-sex day-old chicks were obtained from the experimental hatchery of the Central Avian Research Institute, Izatnagar, for this study. The experiment followed a 3×3 factorial design, incorporating nine dietary treatments with three levels of vitamin E (50, 100, and 150 IU/kg diet) and three levels of zinc (40, 80, and 120 mg Zn/kg diet). Each treatment was assigned five replicates of birds with 8 birds in each making a total of 40 birds per treatment. The birds were reared for 6 weeks – starter (0-3 weeks) and finisher (4-6 weeks) phase. There was the provision of *ad lib* feeding and watering to the birds during the whole experimental period. The ingredients and nutrient composition of broiler starter and finisher ration are presented in Table 1. The experiment was conducted during May to June, 2017. The temperature humidity index (THI) of 85.18 of the shed was obtained from the recorded temperature (minimum 31.54±0.08 and maximum 35.00±0.36) and humidity (minimum 59.25±1.21 and maximum 69.25±0.89) during the whole experimental period. All experimental procedures involving the birds were reviewed and approved by the Animal Ethics Committee of the Indian Veterinary Research Institute, Izatnagar, India.

Weekly body weight and feed intake were recorded, and overall body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were calculated for different periods (0–3 weeks, 4–6 weeks, and 0–6 weeks). The immune response of the birds to varying dietary levels of vitamin E and selenium was assessed through humoral immunity (haemagglutination-HA titer against sheep red blood corpuscles - SRBC) and cell-mediated immunity (CMI) measured by the foot web index response to phytohaemagglutinin (PHA-P, a lectin derived from *Phaseolus vulgaris*) at three weeks of age. For this 90 birds (10 birds per treatment) were selected randomly for each SRBC and CMI test. At the end of the trial immune organs such as thymus, spleen and bursa taken from 90 randomly sacrificed birds (10 birds per treatment) were weighed and presented as % of pre slaughter live weight.

Table 1: Ingredients and nutrients composition of basal diets for broiler chicks used during starter (0-3wks) and finisher (4-6wks) of age

Ingredients %	Starter (0 – 21 days)	Finisher (22-42 days)
Maize	53.570	61.650
Soya bean meal	42.000	34.000
Oil	1.000	1.300
Lime stone	0.920	1.100
Di-calcium phosphate	1.730	1.285
Salt	0.300	0.300
DL-Methionine	0.165	0.050
TM-Premix*	0.100	0.100
Vit-Premix	0.150	0.150
B complex	0.015	0.015
Ch. Chloride	0.050	0.050
Total	100.000	100.000
Analyzed values %DM		
Crude protein, %	23.22	20.40
Calcium, %	1.02	0.95
Total P %	0.72	0.66
Selenium (mg/kg)	0.15	0.13
Calculated values		
ME, Kcal/kg	2905	3006
Available phosphorus%	0.45	0.36
Lysine, %	1.25	1.06
Methionine%	0.53	0.38
Threonine%	0.99	0.87

Trace mineral premix supplied mg/kg diet: Mg300, Mn 60, I0.4, Fe 80, Cu 8, Zn* 40, Se-variable

[2]Vitamin mixture provided mg/kg diet: Choline chloride 500, Niacin12, Pyridoxine hydrochloride 1.6, vitamin A 82500IU, Vitamin $D_3$2000IU, Vitamin B_1 0.8, Vitamin B_2 6, Vitamin B_{12}8, Vitamin K 1, Vitamin E*-variable

*Values variable in test die

During the time of slaughter, blood samples were collected and serum was separated for the assay of serum biochemistry and antioxidant enzyme assay. The diagnostic kits were used for the estimation of serum cholesterol (Wybenga *et al.*, 1970), triglycerides (Bucolo and David, 1973), glucose (Barham and Trinder, 1972), total protein (Daumas, 1976), alkaline phosphatase (Kind and King, 1954), serum glutamic-pyruvic transaminase (Reitman and Frankel 1957), and serum glutamic-oxaloacetic transaminase (Reitman and Frankel, 1957). The Cayman diagnostic kits were utilized to assess serum antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (Wheeler *et al.,* 1990). The experimental data were analyzed using a two-way analysis of variance (ANOVA) following a completely randomized design, employing the GLM procedure in SPSS 20. Significant differences among means were determined using Duncan's multiple range test, with a significance level set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Growth performance

Temperatures exceeding 30°C create heat stress conditions for poultry, significantly impacting production parameters. Heat stress is particularly critical in intensive poultry farming, especially in broiler lines, due to their enhanced production performance and feed conversion efficiency, making modern broilers more vulnerable to heat stress than before (Lin *et al.*, 2004). Common negative effects of heat stress in meat-type poultry include increased mortality, reduced feed intake, lower body weight gain, and poor feed efficiency (Yegani, 2008). Vitamin E plays a crucial role in protecting lipid components of biological membranes, acting as a key chain-breaking antioxidant (Halliwell and Gutteridge, 1999). The present study was conducted from May to June under recorded environmental conditions, with temperatures ranging from a minimum of 31.54±0.08°C to a maximum of 35.00±0.36°C, and humidity levels varying between 59.25±1.21% and 69.25±0.895% throughout the experimental period.

During 0-3 week of age, significantly ($p \leq 0.01$) higher BWG was recorded at 100 IU/kg vitamin E with 80 and 40 mg Zn/kg diet and 50 IU/kg vitamin E with 120 mg Zn/kg diet than those observed in other dietary combinations (Table 2). During 4-6 weeks of age, significantly higher BWG was recorded at 100 IU/kg vitamin E with 40, 80 and 120 mg Zn/kg diet and 150 IU/kg vitamin E with 40 mg Zn/kg diet than those recorded in other dietary combinations. Throughout the 0-6 weeks period, the highest cumulative body weight gain (BWG) was observed in broilers fed a diet supplemented with 100 IU/kg vitamin E and 80 mg Zn/kg, surpassing the BWG recorded in other dietary combinations. The cumulative BWG during the 0-3, 4-6, and overall, 0-6 week periods varied significantly depending on the different levels of vitamin E included in the diets. During the 0-3- and 0-6-week periods, broilers fed a diet with 100 IU/kg vitamin E exhibited significantly higher body weight gain (BWG) compared to those receiving other levels of vitamin E supplementation. During 4-6 weeks of age, significantly higher BWG was recorded at 50 and 100 IU/kg vitamin E than that recorded at 150 IU/kg vitamin E in the diets. The cumulative body

Table 2: Effect of different levels of vitamin E and zinc (Zn) on growth performance of broiler chicken

Vitamin E	Zn	Body weight gain (g)			Feed intake (g)			Feed conversion ratio		
(IU/kg)	(mg/kg)	0-3 week	4-6 week	0-6 week	0-3 week	4-6 week	0-6 week	0-3 week	4-6 week	0-6 week
50	40	692^{a}	1253^{bc}	1945^{bc}	733	2690^{d}	3423	1.06^{c}	2.15^{c}	1.76^{c}
	80	718^{bc}	1260^{bc}	1978^{cd}	717	2610^{c}	3327	1.00^{abc}	2.07^{ab}	1.68^{abc}
	120	747^{d}	1258^{bc}	2005de	695	2531^{b}	3226	0.93^{a}	2.01^{ab}	1.61^{a}
100	40	745^{d}	1269^{c}	2014de	713	2591^{c}	3304	0.96^{ab}	2.04^{ab}	1.64^{ab}
	80	749^{d}	1284^{c}	2033^{e}	727	2538^{b}	3265	0.97^{ab}	1.98^{a}	1.61^{a}
	120	736^{cd}	1269^{c}	2005de	750	2453^{a}	3203	1.02^{abc}	1.93^{a}	1.60^{a}
150	40	704^{ab}	1265^{c}	1969^{bcd}	718	2438^{a}	3156	1.02^{bc}	1.93^{a}	1.60^{a}
	80	713^{b}	1214^{ab}	1927^{ab}	712	2480^{ab}	3192	1.00^{abc}	2.04^{ab}	1.66^{a}
	120	701^{ab}	1192^{a}	1893^{a}	629	2483^{ab}	3112	0.90^{a}	2.08^{b}	1.64^{bc}
Pooled SEM	3.9	3.39	7.61	7.94	24.67	26.31	0.018	0.026	0.019	
Main effect										
Vitamin E										
50	719^{n}	1257^{n}	1976^{n}	715^{mn}	2604^{n}	3319^{n}	0.99	2.07	1.68	
100	743^{o}	1274^{n}	2017^{o}	730^{n}	2527^{mn}	3257^{mn}	0.98	1.98	1.61	
150	706^{m}	1223^{m}	1929^{m}	686^{m}	2467^{m}	3153^{m}	0.97	2.02	1.63	
Zinc										
40	714^{x}	1262	1976	721	2573	3294	1.01^{y}	2.04	1.67	
80	727^{y}	1252	1979	719	2482	3201	0.99^{xy}	1.98	1.62	
120	728^{y}	1240	1968	691	2543	3234	0.95^{x}	2.05	1.64	
Probability										
Vitamin E	P<0.01	P<0.01	P<0.01	P<0.05	P<0.05	P<0.05	NS	NS	NS	
Zinc	P<0.05	NS	NS	NS	NS	NS	P<0.05	NS	NS	
Interaction	P<0.01	P<0.01	P<0.01	NS	P<0.01	NS	P<0.05	P<0.01	P<0.01	

Value bearing different superscripts within a column differ significantly, NS-Non-significant weight gain (BWG) during the 4-6- and 0-6-week periods remained unaffected by varying zinc levels in the diet. However, during the 0–3-week period, birds fed diets containing 80 and 120 mg Zn/kg exhibited significantly higher BWG compared to those receiving 40 mg Zn/kg.

The cumulative feed intake (FI) during the 4–6-week period varied significantly due to the interaction between different dietary levels of vitamin E and zinc. Significantly higher cumulative FI was observed at 50 IU/kg vitamin E with 40 mg Zn/kg diet than those recorded in other dietary combinations. Cumulative FI during 0-3 and 0-6 wk of age, however, did not differ significantly due to interaction between varied levels of vitamin E and zinc in the diets. Significantly higher cumulative FI was observed at 100 IU/kg vitamin E than that recorded at 150 IU/kg vitamin E during 0-3 weeks of age. However, cumulative FI recorded at 50 IU/kg vitamin E was found intermediary. The cumulative FI during 4-6 and 0-6 wk of age was significantly higher at 50 IU /kg vitamin E than that recorded at 150 IU/kg vitamin E. However, cumulative FI recorded at 100 IU/kg vitamin E was found intermediary. Non-significant differences in cumulative feed intake (FI) were observed across different zinc levels in the diets.

During 0-3 weeks of age, significantly lower and better FCR was recorded at 150 IU/kg vitamin E with 120 mg Zn/kg diet followed by 50 IU/kg vitamin E with 120 mg Zn/kg diet than those recorded in other dietary combinations. The cumulative FCR recorded at 100 IU/kg vitamin E with 40, 80 and 120 mg Zn/kg diet, 150 IU/kg vitamin E with 80 mg Zn/kg diet and 50 IU/kg vitamin E with 80 mg Zn/kg diet was found comparable to that fed diet having 150 IU/kg vitamin E with 120 mg Zn/kg diet and 50 IU/kg vitamin E with 120 mg Zn/kg diet. During 4-6 wk of age, significantly lower and better FCR was recorded at 150 IU/kg vitamin E with 40 mg Zn/kg diet, 100 IU/kg vitamin E with 80 and 120 mg Zn/kg diet than those recorded in other dietary combinations. However, cumulative FCR recorded at 50 IU/kg vitamin E with 80 and 120 mg Zn/kg diet, 100 IU/kg vitamin E with 40 mg Zn/kg diet and 150 IU/kg vitamin E with 80 mg Zn/kg diet was found comparable to that fed diet having 150 IU/kg vitamin E with 40 mg Zn/kg diet, 100 IU/kg vitamin E with 80 and 120 mg Zn/kg diet. The cumulative FCR during 0-6 wk of age was recorded significantly lower and better at 150 IU/kg vitamin E with either 40 and 80 mg Zn/kg diet, 100 IU/kg vitamin E with either 80 and 120 mg Zn/kg diet, and 50 IU/kg vitamin E with 120 mg Zn/kg diet than those recorded in other dietary combinations. However, cumulative FCR recorded at 50 IU/kg vitamin E with 80 Zn/kg diet and 100 IU/kg vitamin E with 40 mg Zn/kg diet was found comparable to that fed diet containing 150 IU/kg vitamin E with 40 and 80 mg Zn/kg diet, 100 IU/kg vitamin E with 80 and 120 mg Zn/kg diet and 50 IU/kg vitamin E with 120 mg Zn/kg diet. The cumulative feed conversion ratio (FCR) of broiler chickens showed no significant differences across varying levels of vitamin E in the diets. Likewise, during the 4-6 and 0-6-week periods, cumulative FCR remained unaffected by different zinc levels. However, during the 0-3 weeks period, a significantly lower and improved cumulative FCR was observed at 120 mg Zn/kg diet compared to 40 mg Zn/kg diet, while the FCR recorded at 80 mg Zn/kg diet was intermediate.

Based on the findings of this study, it is recommended that broiler diets be supplemented with at least 100 IU/kg vitamin E along with 80 mg Zn/kg to achieve optimal body weight gain. These results align with previous research by Khattak *et al.* (2012), who reported improved performance in heat-stressed broilers when supplemented with 300 mg/kg vitamin E compared to a control group receiving 35 mg/kg. Similarly, Lin *et al.* (2004) found that pullets fed 160 mg/kg vitamin E exhibited significantly higher ($p \leq 0.01$) body weight gain between 26 and 35 weeks of age than those receiving 0 or 40 mg/kg. However, findings by Niu *et al.* (2009) contrast with the present study. Their research on birds raised in either a thermoneutral environment (constant 23.9°C) or under heat stress (cycling between 23.9–38°C) showed that dietary supplementation with 0, 100, or 200 mg/kg vitamin E did not significantly affect body weight or feed intake, although feed conversion improved at 100 mg/kg. Regarding zinc supplementation, the present findings are supported by Burrell *et al.* (2004), who observed that optimal body weight gain in broilers was achieved at 80 mg Zn/kg, exceeding the NRC recommendation of 40 mg/kg. The BIS (2007) also suggested 80 mg Zn/kg for optimal growth performance. Furthermore, Zhang *et al.* (2006) recommended a zinc supplementation range of 80-120 mg/kg for improved performance and immune function in broilers. Conversely, Bartlett and Smith (2003) found that dietary zinc levels (32, 40, and 100 mg/kg) did not significantly impact the performance of broilers raised under cyclic heat stress (23.9–35°C). Similarly, Akbari *et al.* (2016) conducted an experiment evaluating three dietary zinc levels (0, 60, and 120 mg/kg) in combination with three vitamin E levels (0, 150, and 300 mg/kg). Their results indicated no significant effects on average daily feed intake, average daily gain, feed conversion ratio, mortality rate, or the European production efficiency factor.

Regarding feed intake, present results are in accordance with Raza *et al*. (1997) who found that supplementing vitamin E @ 300 IU/kg diet increased the feed intake of broiler chicken as compared to control. Bou *et al.* (2004, 2005) reported that growth performance of broiler chicken was not affected by variable dietary zinc levels. Lagana *et al.* (2007) reported no differences in feed intake of broiler chicks fed control diet supplemented with 60/30 IU vitamin E and 80 mg/kg inorganic zinc. Similarly, Daniel *et al.* (2017) observed that the levels of selenium and vitamin E did not affect feed intake of broiler chicken. However, the supplementation of 100 IU/kg vitamin E and 80 mg/kg Zn was found optimum for better efficiency of feed utilization. The improvement in FCR of broiler chicken in the present results has been related to increased body weight gain which might be due to positive effects of Vitamin E supplementation. Vitamin E functions as a biological antioxidant that can enhance broiler performance by neutralizing free radicals and minimizing lipid peroxidation in both plasma and skeletal muscle (Gao *et al.*, 2010; Selim *et al.*, 2013b). Niu *et al.* (2009) reported that dietary vitamin E supplementation did not have a significant impact on body weight or feed intake in broilers, although feed conversion showed significant improvement at a supplementation level of 100 mg/kg. Conversely, Akbari *et al.* (2016) found that supplementing broiler diets with vitamin E and zinc had no notable effect on the feed conversion ratio.

Immune response and immune organs

Results pertaining to immune response and immune organ weights in response to dietary vitamin E and Zn supplementation are presented in Table 3. The interaction effect revealed significantly ($P \leq 0.05$) higher cellular immune response at 150 IU/kg vitamin E with 40 mg Zn/kg diet than those observed in other dietary combinations. However, cellular immune response recorded at 100 IU/kg vitamin E with 80 mg Zn/kg diet and 50 IU /kg vitamin E with 120 mg Zn/kg diet was found intermediary. Non-significant differences were observed on cellular immune response of broiler chicks due to different levels of vitamin E as well as different supplementary levels of zinc in the diets. On similar lines, significantly higher ($P \leq 0.01$) humoral immune response was observed at 100 IU/kg vitamin E with 80 mg Zn/kg diet than those recorded in other dietary combinations. However, humoral immune response recorded in birds at 50 IU/kg vitamin E with 120 mg Zn/kg diet, 100 IU/kg vitamin E with 40 mg Zn/kg diet and 150 IU/kg vitamin E with 120 mg Zn/kg diet was found comparable to those fed diet containing 100 IU/kg vitamin E with 80 mg Zn/kg diet. A significantly ($P \leq 0.05$) higher humoral immune response was observed at a dietary level of 100 IU/kg vitamin E compared to other levels. Additionally, a

Table 3: Effect of different levels of vitamin E and zinc (Zn) on immune response and immune organs weight of broiler chicken

Vitamin E	Zinc	Immunity status		Immune Organ weight (%)		
(IU/kg)	(mg/kg)	CMI (mm)	HA titre (log_2)	Thymus	Spleen	Bursa
50	40	0.41^{a}	1.95^{a}	0.29	0.16^{ab}	0.10
	80	0.58^{a}	2.47^{bc}	0.34	0.24^{c}	0.09
	120	0.66^{ab}	2.58^{cd}	0.32	0.21^{bc}	0.10
100	40	0.57^{a}	2.62^{cd}	0.30	0.23^{c}	0.10
	80	0.78^{ab}	2.84^{d}	0.35	0.17^{ab}	0.10
	120	0.52^{a}	2.23^{ab}	0.33	0.15^{a}	0.10
150	40	0.97^{b}	2.25^{ab}	0.34	0.20^{bc}	0.11
	80	0.53^{a}	2.21^{ab}	0.35	0.17^{ab}	0.08
	120	0.53^{a}	2.66^{cd}	0.31	0.16^{ab}	0.07
Pooled SEM	0.043	0.042	0.006	0.006	0.003	
Main Effect						
Vitamin E (IU/kg)						
50	0.55	2.34^{m}	0.31	0.20	0.10	
100	0.63	2.56^{n}	0.33	0.18	0.10	
150	0.68	2.37^{m}	0.34	0.18	0.09	
Zinc (mg/kg)						
40	0.65	2.27^{m}	0.31	0.20	0.10^{y}	
80	0.63	2.51^{n}	0.34	0.19	0.09^{x}	
120	0.57	2.49^{n}	0.32	0.18	0.09^{x}	
Probability						
Vitamin E	NS	P<0.05	NS	NS	NS	
Zinc	NS	P<0.05	NS	NS	P<0.05	
Interaction	P<0.05	P<0.01	NS	P<0.05	NS	

Value bearing different superscripts within a column differ significantly, NS-Non-significant

Table 4: Effect of different levels of vitamin E and zinc (Zn) on serum biochemistry and antioxidant enzymes of broiler chicken

Vitamin E (IU/kg)	Zinc (mg/kg)	Glucose (mg/dl)	TP (g/dl)	TG (mg/dl)	TC (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	SOD (IU/ml)	GSH-Px (nmol/min/ml)
50	40	190.17	4.01^{a}	131.67	146.67	123.25^{c}	32.48	216.11	104.67	6.83
	80	187.83	4.51^{bc}	122.17	146.83	122.78^{c}	28.62	217.13	114.67	9.58
	120	188.50	4.57^{bc}	115.17	122.50	125.45^{c}	27.62	211.02	126.33	11.30
100	40	217.67	4.64^{bc}	102.33	118.33	97.39^{ab}	26.84	230.72	108.00	7.77
	80	218.17	4.78^{bc}	101.50	121.00	80.00^{a}	25.67	231.17	116.00	12.35
	120	203.17	4.54^{bc}	103.50	120.83	92.87^{ab}	30.60	225.17	130.67	12.62
150	40	207.67	4.82^{c}	101.17	125.00	89.97^{a}	29.43	225.85	108.00	8.07
	80	204.83	4.55^{bc}	111.00	128.50	115.83^{bc}	29.41	219.17	124.67	12.40
	120	197.83	4.39^{b}	114.17	122.50	112.93^{bc}	25.95	224.60	130.67	11.87
Pooled SEM		2.37	0.045	2.73	3.44	2.95	0.69	3.67	5.15	0.47
Main Effect										
Vitamin E(IU/kg)										
50		188.83	4.38^{m}	123.00^{n}	138.67	123.83^{o}	29.57	214.75	113.22^{m}	9.24^{m}
100		213.00	4.65^{n}	102.44^{m}	120.06	90.09^{m}	27.70	229.02	120.22^{n}	10.91^{n}
150		203.44	4.59^{n}	108.78^{m}	125.33	106.24^{n}	28.26	223.21	121.11^{n}	10.78^{n}
Zinc (mg/kg)										
40		205.17	4.49	111.72	130.00	103.54	29.58	224.23	106.89^{x}	7.56^{x}
80		203.61	4.63	111.56	132.11	106.20	27.90	222.49	118.45^{y}	11.44^{y}
120		196.50	4.50	110.94	121.94	110.42	28.06	220.26	129.22^{z}	11.93^{y}
Probability										
Vitamin E		P<0.01	P<0.05	P<0.01	NS	P<0.01	NS	NS	(P<0.05)	(P<0.05)
Zinc		NS	NS	NS	NS	NS	NS	NS	(P<0.01)	(P<0.01)
Interaction		NS	P<0.01	NS	NS	P<0.05	NS	NS	NS	NS

Value bearing different superscripts within a column differ significantly, TP: Total protein; TG: Triglycerides; TC: Total cholesterol; Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; ALP: Alkaline phosphatase; SOD; Superoxide dismutase; GPx: Glutathione peroxidase; NS-Non-significant

significantly (P≤0.05) higher humoral immune response was recorded at 80 and 120 mg Zn/kg diet compared to 40 mg Zn/kg diet.

The immune organ weights such as thymus and bursa did not differ significantly due to the interaction between vitamin E and zinc levels in the diets. However, significantly higher (P≤0.05) weight of spleen was recorded at 50 IU/kg vitamin E with 80 mg Zn/kg diet and 100 IU/kg vitamin E with 40 mg Zn/kg diet than those observed in other dietary combinations. However, weight of spleen recorded in birds at 50 IU vitamin with 120 mg Zn/kg diet and 150 IU /kg vitamin E with 40 mg Zn/kg diet was found comparable to those fed diet having 50 IU/kg vitamin E with 80 mg Zn/kg diet and 100 IU/kg vitamin with 40 mg Zn/kg diet. The different levels of vitamin E had no significant effect on any of the recorded immune organ weights. Similarly, thymus and spleen weights remained unaffected by varying levels of dietary zinc. However, bursa weights were significantly higher at 40 mg Zn/kg diet compared to other zinc levels.

It is evident for the results that supplementation of 100IU/kg vitamin with 80mg/kg zinc was found optimum for better immunity in term of cellular and humoral immune response. Present results get strengthen by the work reported by Singh *et al*. (2006) who also found better immunity at supplementary vitamin E levels of 100 and 200 IU/kg diet in broiler chicken. Yamuna and Thangavel (2011) observed that feeding of selenium and vitamin E at higher levels than the recommended dose increased the antibody titre against sheep red blood cell antigen (SRBC). Nageswara *et al.* (2003) also observed an increased antibody titre against SRBC on selenium and vitamin E supplementation at levels higher than the recommended dose. Consistent with the findings of the present study, Zhang *et al*. (2006) reported that the optimal supplemental zinc levels for broiler chickens should range between 80-120 mg/kg diet to enhance immune competence. Kumar *et al*. (2009) reported improved immune response of broiler chicken at higher levels of Zn (60 and 80 mg /kg diet) compared to 40 mg

/kg diet, irrespective of Zn sources. In the present study relative organs weight were not affected by different levels of vitamin E in the diets which was corroborated by the results obtained by Swain and Johri (2000) who suggested that the relative weights of spleen, thymus, and liver were not affected by supplemental vitamin E (0 to 300 IU/kg). Similarly, Konjufca *et al*. (2004) also concluded that vitamin E supplementation (110 and 220 IU/kg diet) did not increase relative organ weight of thymus and bursa. However, Basmacýoglu *et al*. (2009) reported significantly increased relative spleen weight in broiler chicken supplemented with 200 IU/kg vitamin E. Consistent with the findings of the present study, Kulkarni *et al.* (2017) reported that supplementing the diet with ZnO at 60 or 120 mg/kg had no significant effect on the relative thymus weight in broiler chickens at 42 days of age. In contrary, Bartlett and Smith (2003) and Sunder *et al*. (2008) observed that weight of immune organs, cellular, and humoral immune response were increased significantly by supplementing zinc up to 40 ppm. Further they reported that increase in dietary zinc up to 80 ppm had no disadvantage on immunity.

Serum biochemistry and antioxidant profile

Results of serum biochemistry and antioxidant profile of broiler chicken under the influence of dietary vitamin E and Zn supplementation are given in Table 4. Among the serum biochemical and antioxidant parameters only total protein and SGOT levels differed significantly due to interaction between different levels of vitamin E and Zn in the diets. Significantly ($p \leq 0.05$) higher serum total protein was observed at 150 IU/kg vitamin E with 40 mg Zn/kg diet compared to 150 IU/kg vitamin E with 120 mg Zn/kg diet and 50 IU/kg vitamin E with 40 mg Zn/kg diet. All the dietary combinations resulted in intermediate total protein values which were statistically similar to birds fed 150 IU/kg vitamin E with 40 mg Zn/kg diet. Significantly lower serum SGOT was observed at 100 IU/kg vitamin E with 80 mg Zn/kg diet and 150 IU/kg vitamin E with 40 mg Zn/kg diet compared to birds fed diet containing 50 IU/kg vitamin E with 40,80, and 120 mg Zn/kg diet. The other dietary combinations yielded intermediate results. The other serum biochemistry and antioxidant parameters were not influenced by the interaction between vitamin E and Zn supplementation.

Significantly higher serum total protein was observed at 100 and 150 IU/kg vitamin E than that recorded at 50 IU/kg vitamin E in the diets. Serum triglycerides was significantly higher at 50 IU/kg vitamin E than those recorded at other two levels. The serum SGOT level of birds was found to be significantly lower at 100 IU/kg vitamin E followed by 150 IU/kg vitamin E compared to 50 IU/kg level. Significantly lower SOD and GSH-Px levels were observed in birds fed with diets containing 50 IU/kg vitamin E compared to other two levels which did not differ significantly from each other. Likewise, serum SOD levels showed a linear increase with the dietary zinc concentration ranging from 40 to 120 mg Zn/kg diet. However, serum GSH-Px levels were significantly lower at 40 mg Zn/kg diet compared to the other two levels, which did not exhibit any significant difference from each other.

In concurrence with the findings of present study, Sahin *et al*. (2002) and Speranda *et al*. (2008) reported that dietary vitamin E levels had no significant effects on the glucose level in quail serum. Mobaraki *et al*. (2013) reported that total protein concentration increased significantly in quail fed diet containing 80 and 160 mg/kg vitamin E and 0.20 and 0.40 mg/kg Se compared to control groups. However, significantly higher plasma total protein level was also reported with dietary Zn supplementation in broiler chicken (Feng *et al*., 2010). The addition of vitamin E to diets of broiler chicken had no significant effect on serum triglyceride levels (Speranda *et al*., 2008; Zaghari and Mohiti Asli, 2010; El-Mallah *et al*., 2011). Contrary to the findings of the present study, Herzig *et al.* (2009) observed a significant reduction in plasma cholesterol levels in broiler chickens fed high levels of dietary zinc. Zinc has been reported to exhibit anti-atherogenic properties in hypercholesterolemic rabbits (Rashtchizadeh *et al.,* 2008). Additionally, Bolkent *et al.* (2006) demonstrated the protective role of zinc supplementation on lipid metabolism indices in laboratory rats. Zinc deficiency has been associated with increased plasma lipid levels in LDL recipient mice. Aksu and Ozsoy (2010) also noted a decrease in total and LDL cholesterol, along with an increase in HDL cholesterol, in the blood plasma of chickens when their diets were supplemented with organic complexes of zinc, copper, and manganese. However, in agreement with the present study, Kucuk *et al*. (2008) reported no significant alterations in total cholesterol, triglycerides, and glucose levels as a result of zinc supplementation. Furthermore, no changes were observed in turkey plasma ALP levels due to vitamin E supplementation (Franchini *et al*., 1990).

The findings on the antioxidant profile in this study align with previous research, which established the role of Cu/Zn-SOD in safeguarding cells by minimizing lipid peroxidation and superoxide anion production (Fawzy *et al*., 2016). Similarly, Ozkan *et al*. (2007) reported that supplementation of inorganic Selenium with vitamin E significantly increased glutathione peroxidase and total glutathione concentration in the liver of broiler chicken. Supplemented diets with antioxidants alleviated these negative impacts of the heat stress by a significant improvement in GPx activity in all the supplemented dietary groups (Samar Sayed Tawfeek *et al*., 2014). Contrary to present finding, Tawfeek *et al*. (2010) found no significant changes in liver SOD in Zn supplemented chicks.

CONCLUSIONS

The present study concludes that broiler chicken raised under heat stress perform better in terms of growth, immunity, and serum biochemical profile on dietary supplementation of 100 IU vitamin E /kg diet along with 80 mg Zn /kg diet.

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