International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2025; 10(10): 415-419

www.veterinarypaper.com
Received: 26-08-2025
Accepted: 30-09-2025

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Effect of Vitamin E and selenium supplementation on blood constituents and immune response of broiler chickens during summer

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DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i10f.2670

Abstract
The present experiment evaluates the effects of supplementing vitamin E and Selenium in the diet of broiler chickens during summer season on blood constituents and immune response. Two hundred forty day-old broiler chicks were divided into four equal treatment groups, with three replicates of 20. The control (T_0) group was fed with the Basal diet, group T_1 with Basal diets + Vitamin E @ 100 mg/kg and Selenium @ 0.20 mg/kg, group T_2 with Basal diets + Vitamin E @ 125 mg/kg and Selenium @ 0.25 mg/kg, and group T3, with Basal diets + Vitamin E @ 150 mg/kg and Selenium @ 0.3 mg/kg, respectively, for 6 weeks. The mean values of Hb (%), PCV (%), and WBC (thousand/mm3) of broiler chickens of different treatment groups differed significantly (p<0.001). The mean values of serum glucose, Total protein, Albumin, Globulin, SOD, Glutathione Peroxidase, GSH, AST, ALT, and cortisol were different significantly (p<0.001) among the treatment groups. The overall mean of antibody titre levels against NDV and IBDV in the T_0 group was significantly (p<0.001) lower than the other groups without any significant difference between the T_2 and T_3 groups. It can be concluded from the study that supplementation of vitamin E and selenium @ 100 g and 0.2 mg per kg diet is beneficial for broiler birds during summer heat stress conditions.

Keywords: Vitamin E, selenium, antioxidant, broiler chicken, heat stress, immune response

Introduction
Poultry is very sensitive to temperature-associated environmental challenges, especially heat stress during summer months. The modern poultry genotypes produce more body heat due to their greater metabolic activity [1]. Heat stress leads to oxidative stress associated with a reduced antioxidant status in birds *in vivo*, as reflected by increased oxidative damage and lowered plasma concentrations of antioxidant, vitamins and minerals [2]. It is well established that heat stress can lead to a reduction in the defence mechanisms of birds or to a relative state of immune suppression [3]. Heat exposure results in increased susceptibility of the host to various bacterial, viral, and parasitic infections, which may further be aggravated due to associated non-infectious factors *viz.* humidity, overcrowding, and other stress factors. Immune function in young chicks is impaired due to deficiencies of vitamin E, Se, or both[4]. At the same time, considerable attention is needed for the roles of vitamin E and selenium as antioxidants protecting birds from oxidative damage during heat stress.
Keeping in view to the importance of roles of vitamin E and selenium as antioxidants in protecting birds from oxidative damage during acute heat stress, this study was aimed to investigate the effects of different levels of vitamin E and selenium supplementation on blood constituents and immune response in broiler chickens reared during summer months.

Materials and Methods
Birds, diet and experimental design
A total of two hundred and forty (240) day-old commercial broiler chicks were randomly distributed into four treatment groups, *viz.*

T_0, T_1, T_2 and T_3 containing 60 chicks with 3 replicates of 20 in each group. The birds of the T_0 group (control) were offered standard broiler ration as per BIS, 2007 (Table 1).

Table 1: Ingredient composition (%) and calculated nutritive values of broiler pre-starter, starter, and finisher diets

SL. No.	Ingredients	Pre-starter (0-7 days)	Starter (8-21 days)	Finisher (22-42 days)
1	Yellow Maize	44.05	41.79	52.00
2	Rice Polish	8.00	12.00	5.50
3	Vegetable oil	3.50	5.00	5.50
4	Soybean meal	15.00	10.00	16.62
5	GNC	24.37	26.21	14.62
6	Limestone powder	1.30	1.30	1.50
7	Di-calcium phosphate	1.50	1.70	2.20
8	Mineral mixture	1.00	1.00	1.00
9	Salt	0.50	0.50	0.40
10	Lysine	0.45	0.21	0.35
11	Methionine	0.20	0.16	0.20
12	Vitamin mixture	0.01	0.01	0.01
13	Liver tonic	0.05	0.05	0.05
14	Toxin binder	0.05	0.05	0.05
15	Coccidiostat	0.02	0.02	-
	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

** Calculated value.

The birds under the groups T_1, T_2 and T_3 were fed with standard broiler rations with supplementation of Vitamin E and selenium @ 100 mg/kg and 0.20 mg/kg, 125mg/kg and 0.25 mg/kg, 150mg/kg and 0.30 mg/kg, respectively. The birds were maintained following standard management practices and in similar environmental conditions (temperature 28- 36.5 °C and relative humidity 61.5-92.5%) under a deep litter system of rearing throughout the experimental period (42 days) with strict bio-security measures.

Blood Sample collection

Blood samples were collected from the birds of each of the treatment groups on day 0, 28 and 42. Five numbers of broiler chickens from each treatment group were randomly picked and serum was collected for Haemagglutination Inhibition (HI) Test[5] at day-old and 5th day before immunization and at 7th, 14th, 21st and 28th Day of post vaccination (DPV) of RD vaccine, while was used to for the detection of antibodies to IBD.

The experimental data were subjected to statistical analysis as per standard methods [6].

The investigation was approved by the Institutional Animal Ethics Committee.

Results and Discussion

Effect on Haematological Profile

The blood parameters of broiler chicken under different treatment groups are shown in Table 2. The mean values of Hb (%) and PCV (%) of broiler chickens were lowest in Control (T_0) and highest in the group where Vitamin E and selenium were supplied @ 100 mg/kg and 0.20 mg/kg (group T_1). The mean of white blood cell count (thousand/mm^3) among different treatment groups also differed significantly ($p<0.001$). The highest WBC count was found in the T_0 group, and the lowest values were observed in T_1 group. The mean of WBC (thousand/mm^3) of broiler chicken among different treatment groups differed significantly ($p<0.001$), with the highest and lowest levels observed in T_0 and T_1 groups, respectively.

Table 2: Blood parameters of broiler chicken under different treatment groups

Attributes	Period (day) 0	28	42	Overall mean± SE	SEM	P-Value T	P	T * P
Hb (%)								
T_0	9.10±0.02	10.04±0.18	10.11±0.52	9.75^a±0.50	0.14	<0.001	<0.001	<0.001
T_1	9.15±0.03	12.43±0.09	12.07±0.09	11.22^c±1.56	0.52			
T_2	9.14±0.02	11.06±0.20	11.49±0.43	10.56^b±1.11	0.37			
T_3	9.10±0.03	11.15±0.89	11.24±0.77	10.50^b±1.19	0.35			
PCV (%)								
T_0	26.14±0.02	25.08±0.58	26.10±0.08	25.77^a±0.60	0.19	<0.001	<0.001	<0.001
T_1	26.13±0.17	28.18±0.06	30.16±0.05	28.22^c±1.67	0.56			
T_2	26.18±0.12	26.99±0.32	28.43±0.03	27.20^b±1.01	0.34			
T_3	26.20±0.02	27.31±0.11	28.17±0.21	27.23^b±0.86	0.29			
WBC (thousand/mm^3)								
T_0	24.51±0.31	28.31±0.56	31.26±0.25	28.03^c±2.95	0.98	<0.001	<0.001	<0.001
T_1	24.35±0.14	23.54±0.24	24.50±0.17	24.13^a±0.47	0.16			
T_2	24.38±0.34	24.99±0.19	26.00±0.33	25.13^b±0.75	0.25			
T_3	24.23±0.25	25.09±0.07	26.11±0.26	25.14^b±0.83	0.28			

Means bearing the different superscripts within the same column differ significantly ($p<0.001$)

Vitamin E and Se supplementation at different levels in T_1, T_2 and T_3 groups resulted in significant increase in haemoglobin concentrations at 28th and 42nd day of age as compared to unsupplemented control (T_0) group, which might be due to increased oxygen consumption by the birds of the treated groups [7, 8]. The significant increase in mean value of PCV (p<0.01) in the selenium and vitamin E treated groups of broiler chicken might be due to the role of selenium and vitamin E as antioxidant[9]. Selenium and vitamin E supplementation (T_1, T_2 and T_3) could significantly lower (p<0.001) the WBC or TLC levels in blood, which was significantly higher in T_0 due to heat stress. The increased WBC count observed in (T_0) group could be due to stress related increase in cortisol concentration which is responsible for increased production of leucocytes [10].

Blood Biochemical Profile

The overall mean of serum glucose (mg/dl), total Serum Protein (g/dl), Serum Albumin (g/dl), Serum Globulin (g/dl), Serum AST (U/L) and Serum ALT (U/L) of broiler chickens of different treatment groups are shown in Table 3. The serum glucose level differed significantly (p<0.001) with the lowest and highest levels being recorded in T_1 and T_0 group respectively, which might be due to heat stress, causing greater catabolic effects and increased concentration of adreno-corticotropic hormone yielding more glucose [11].

Table 3: Blood biochemical parameters of broiler chicken under different treatment groups

Parameters	Attribute	0 days	28 days	42 days	Overall Mean ± SE	SEM	P-Value		
Glucose (mg/dl)	T_0	176.1±0.36	232.2±1.13	231.7±0.51	213.3^{d}±27.9	9.31	<0.001	<0.001	<0.001
	T_1	176.3±0.91	190.7±1.06	193.1±1.40	186.7^{a}±7.90	2.63			
	T_2	175.0±0.58	212.9±0.99	221.1±1.36	202.9^{c}±1.36	7.09			
	T_3	174.0±0.60	211.9±0.69	219.6±0.46	201.9^{b}±21.5	7.05			
Total Serum Protein (g/dl)	T_0	2.18± 0.03	2.16±0.02	2.87±0.21	2.40^{a}±0.37	0.12	<0.001	<0.001	<0.001
	T_1	2.19±0.06	3.48±0.55	3.98±0.10	3.21^{c}±0.85	0.28			
	T_2	2.21±0.03	3.08±0.03	3.43±0.01	2.91^{b}±0.54	0.18			
	T_3	2.20±0.02	3.05±0.07	3.57±0.01	2.94^{b}±0.60	0.20			
Serum Albumin (g/dl)	T_0	1.12±0.01	1.02±0.01	1.33±0.07	1.16^{a}±0.14	0.04	<0.001	<0.001	<0.001
	T_1	1.12±0.06	1.52±0.09	1.70±0.17	1.44^{c}±0.27	0.09			
	T_2	1.13±0.05	1.29±0.13	1.38±0.02	1.27^{b}±0.13	0.04			
	T_3	1.13±0.04	1.28±0.13	1.52±0.03	1.31^{b}±0.18	0.06			
Serum Globulin (g/dl)	T_0	1.06±0.03	1.14±0.02	1.54±0.24	1.25^{a}±0.25	0.08	<0.001	<0.001	<0.001
	T_1	1.07±0.02	1.97±0.48	2.28±0.16	1.77^{b}±0.60	0.20			
	T_2	1.08±0.07	1.79±0.14	2.05±0.02	1.64^{b}±0.44	0.15			
	T_3	1.08±0.03	1.77±0.18	2.05±0.02	1.63^{b}±0.44	0.15			
Serum AST (U/L)	T_0	193.1±0.82	186.2±1.30	228.0±0.22	202.4^{d}±19.4	6.48	<0.001	<0.001	<0.001
	T_1	189.9±0.58	170.0±0.64	180.6±0.33	180.2^{a}±8.67	2.89			
	T_2	191.6±1.21	177.7±0.92	214.5±0.89	194.6^{c}±16.1	5.36			
	T_3	190.5±0.81	181.2±1.21	193.4±0.34	188.4^{b}±5.58	1.86			
Serum ALT (U/L)	T_0	5.05±0.67	10.67±0.68	10.47±0.01	8.73^{c}±2.80	0.93	<0.001	<0.001	<0.001
	T_1	5.03±0.32	8.34±0.33	7.79±0.33	7.05^{a}±1.56	0.52			
	T_2	5.23±0.02	9.50±0.33	8.54±0.34	7.76^{b}±1.96	0.65			
	T_3	5.42±0.34	9.12±0.33	9.29±0.24	7.94^{b}±1.92	0.64			

Means bearing the different superscripts within the same column differ significantly (p<0.001)

Total protein and Albumin levels in T_1 group were significantly higher (p<0.001) than the other three groups. The overall mean of Globulin levels differed significantly (p<0.001) with the highest and lowest levels in T_1 and T_0 groups, respectively. The lower serum Total protein observed in T_0 group might be due to increase in glucocorticoids as a result of heat stress, which had primary effects on metabolism, stimulating gluconeogenesis from muscle proteins [11]. The higher serum albumin in group T_1, T_2 and T_3 might be due to reduction of synthesis and secretion of corticoids in birds supplemented with antioxidants [12].

The AST and ALT levels (Table 3) differed significantly (p<0.001) with lower levels in T_1 group than the others. The higher AST and ALT levels in T_0 group during stress might be due to the influence of higher corticosteroid level and keto acids produced by transamination of amino acids provided an important source of substrate for gluconeogenesis [13].

The values of Serum superoxide dismutase (SOD), Blood GPx (microgram/mg protein), Blood GSH (mg/g protein), Serum Cortisol (ng/ml) in different treatment groups are shown in Table 4.

Table 4: Blood SOD, GPx, GSH and Cortisol of broiler chicken under different treatment groups

Parameters	Attribute	28 days	42 days	Overall Mean ± SE	P-Value		
SOD (unit/mg protein)	T_0	1.68±0.02	2.38±0.02	2.03^{a}±0.39	<0.001	<0.001	<0.001
	T_1	2.88±0.01	4.04±0.07	3.46^{d}±0.64			
	T_2	2.32±0.03	3.55±0.01	2.94^{c}±0.68			
	T_3	2.28 ±0.06	3.11± 0.06	2.69^{b}±0.45			
Blood GPx (microgram/mg protein)	T_0	2.26±0.30	2.48±0.23	2.37^{a}±0.27	<0.001	<0.001	<0.001
	T_1	3.23±0.03	3.98±0.16	3.61^{c}±0.18			
	T_2	2.79±0.21	3.17±0.18	2.99^{b}±0.27			
	T_3	2.61±0.23	3.05±0.18	2.83^{b}±0.30			
Blood GSH (mg/g protein)	T_0	0.86±0.02	0.94±0.08	0.90^{a}±0.07	<0.001	<0.001	<0.001
	T_1	0.87±0.02	1.84±0.06	1.36^{c}±0.53			
	T_2	0.86±0.01	1.57±0.21	1.21^{bc}±0.41			
	T_3	0.87±0.01	1.45±0.37	1.17^{b}±0.36			
Serum Cortisol (ng/ml)	T_0	2.39±0.01	2.91±0.02	2.65^{a}±0.28	<0.001	<0.001	<0.001
	T_1	1.26±0.01	1.26±0.05	1.26^{c}±0.01			
	T_2	1.67±0.01	1.75±0.05	1.71^{b}±0.04			
	T_3	1.72±0.02	1.81±0.02	1.77^{b}±0.05			

Means bearing the different superscripts within the same column differ significantly (p<0.001).

The lower SOD in T_0 group might be due to oxidative stress leading to the production of reactive oxygen species (ROS) and decreased in erythrocytic enzymes activity[14]. The lower GPx and GSH level in T_0 group might be due to the effect of heat stress induced changes potentially enhanced the formation of ROS. The lower cortisol level in T_1, T_2 and T_3 groups might be due to suppressed stress responses, which might lead to amelioration of heat stress in treated birds and indicated by reduction in plasma corticosterone levels [15, 16].

Immune profile: Serum immune response against Newcastle Disease Virus (NDV) and Infectious Bursal Disease Virus (IBDV) of broiler chicken under different treatment groups:

The levels of serum humoral immune response against NDVand IBD vaccine control (T_0) group and T_1, T_2 and T_3 groups at 28th and 42nd day of age have been presented in Table 5 and 6. The overall mean antibody titers against NDV in T_0, T_1, T_2 and T_3 groups were 39.98±9.29, 266.51±70.67, 106.61±30.06 and 133.27±35.34, respectively. The overall mean of antibody titer levels against NDV of broiler chickens of different treatment groups differed significantly ($p<0.001$). The antibody titer level against NDV in T_0 group was significantly ($p<0.001$) lower than the rest of the groups.

Table 5: Humoral immune response against NDV of broiler chicken under different treatment groups (Antibody titers)

Attributes	Period		Overall Mean± SE	Significant		
	28 days	42 days		T	P	T*P
T_0	16.00±0.00	63.97±0.00	39.98^a±9.29	<0.001	<0.001	<0.001
T_1	106.62±6.53	426.41±26.11	266.51^c±70.67			
T_2	42.65±6.53	170.58±26.11	106.61^b±30.06			
T_3	53.31±6.53	213.22±26.11	133.27^b±35.34			

Overall means with different superscripts differ significantly ($p<0.001$)

The overall mean antibody titers against IBDV of the broiler chicken in T_0, T_1, T_2 and T_3 groups were 74.94±9.7, 499.91±86.65, 199.98±38.72 and 249.97±43.29, respectively. The lower humoral immune response against NDV and IBDV in T_0 group might be due to decrease in feed intake caused by heat stress, leading to a reduction in the nutrients available to mount an effective immune response.

Table 6: Humoral immune response against IBDV of broiler chicken under different treatment groups (Antibody titers)

Attributes	Period		Overall mean± SE	Significant		
	28 days	42 days		T	P	T*P
T_0	66.59±10.23	83.3±10.23	74.94^a±9.7	<0.001	<0.001	<0.001
T_1	333.29±40.82	666.54±81.63	499.91^c±86.65			
T_2	133.33±20.41	266.64±40.82	199.98^b±38.72			
T_3	166.66±20.41	333.29±40.82	249.97^b±43.29			

Overall means with different superscripts differ significantly ($p<0.001$).

Conclusion

Supplementation of different levels of vitamin E and selenium in the diets of broilers chickens may reduce the oxidative stress and improve humoral immune response against NDV and IBDV during heat stress conditions which was also indicated by improve blood biochemical parameters. Vitamin E @ 100 mg/kg and selenium @ 0.2 mg/kg showed better results than the rest combination levels i.e. @ 125 and 0.25 mg/kg and 150 and 0.3 mg/kg, while all three combinations showed better results in case of immune response. It can be concluded that supplementation of vitamin E and selenium to the diets of broilers during heat stress is beneficial at the rate of 100 g vitamin E and 0.2 mg of selenium per kg of diet for blood constituents and humoral immunity response of broiler birds.

Acknowledgement

The author is thankful to the Department of Animal Nutrition, College of Veterinary Science, Khanapara, Assam Agricultural University, for providing the necessary resources to carry out the research.

Disclaimer

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Conflict of Interest

All authors declared that there is no conflict of interest.

Conflict of Interest

There is no conflict of interest

Financial Support

Not available

Reference

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How to Cite This Article
Bora S, Ahmed HF, Tamuly S, Sonowal M, Dutta D, Saikia AK, *et al*. Effect of Vitamin E and selenium supplementation on blood constituents and immune response of broiler chickens during summer. International Journal of Veterinary Sciences and Animal Husbandry. 2025;10(10):415-419.