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Effect of Vitamin E, Vitamin C and Selenium on Blood Haematological, Biochemical, Liver Enzyme, Serum Electrolyte, Antioxidant Profile, Heat Shock Protein (HSP70) and Carcass Quality of Broiler Chickens During Heat Stress

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ABSTRACT

An experiment was conducted in 180 day-old Vencobb 400 strain broilers chicks to determine the effects of Vitamin E, Vitamin C and Selenium in different combinations on blood haematological, biochemical, liver enzyme, serum electrolyte, heat shock protein, antioxidant profile and carcass quality of broiler chickens during heat stress. Chicks were distributed randomly into 5 groups having 36 chicks in each (3 replicates of 12 chicks). Control group (T0) was fed with basal ration and other four groups were offered basal ration and drinking water was supplemented with 100 mg/ltr vitamin E + 0.2 mg/ltr Se (T1), 100 mg/ltr vitamin E + 0.3 mg/ltr Se (T2), 100 mg/ltr vitamin E + 100 mg/ltr vitamin C (T3) and 200 mg/ltr vitamin E + 200 mg/ltr vitamin C (T4). Result showed significant difference ($P<0.05$) in Hb and WBC level. Supplemented groups showed significantly better serum glucose and protein content. Serum chloride, liver enzymes, erythrocytic antioxidants were significantly better ($P<0.05$) in T1 and T4 groups than other groups. Heat shock protein was found to be significant ($P<0.01$), whereas, no significant difference ($P>0.05$) was recorded in carcass characteristics viz. prime cuts percentage and giblet weight. The study revealed that blood haematological, biochemical, liver enzymes, serum chloride, antioxidant profile and heat shock protein were better ($P<0.05$) in the supplemented groups as compared to control. However, due to more pronounced effect of T1 and T4 groups, vitamin E and Selenium @ 100mg/ltr and 0.2 mg/ltr, respectively and vitamin E and vitamin C @ 200 mg/ltr each can be supplemented in broiler rations for better performance and health during heat stress.

KEYWORDS: Broilers, Heat stress, Selenium, Vitamin E, Vitamin C

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INTRODUCTION

Poultry industry is a fast growing segment of Indian economy. Currently the total poultry population in our country is 729.21 million (Census, 2012). Poultry meat production is estimated to 4.2 million tons (carcass weight), translating into volume growth of 7% year-on-year during calendar year 2017 (ICRA, 2018). Broiler contributes about 2193 thousand tonnes of meat to the total meat production in India (B.A.H. Statistics, 2010-2011). Though broiler industry is growing at faster rate, there are many factors which pull down the growth of poultry industry. Among these, high ambient temperature is one of the most important factors, which has a direct relationship on profitability of meat and egg production (Lara and Rostagno, 2013). High environmental temperatures (35-43^{0}C) in tropical countries cause major economic losses by reducing feed intake, nutrient utilization, live weight gain, egg production, egg quality and feed efficiency. High temperature coupled with humidity not only adversely affects production performance but also inhibits immune function (Mashaly et al., 2004; Gicheha, 2021).

Heat stress can be regulated by controlling the shed temperature and by nutritional manipulation, which involves optimizing diet to meet the altered needs of stressed birds. At temperatures above 30^{0}C, consumption decreases by 2.5-4 g per degree rise in temperature. It is advisable to formulate higher density diets in order to maintain daily intake in line with requirements for growth and egg production. It has been observed that birds stressed due to high environmental temperatures have reduced plasma ascorbic acid, á-tocopherol and ascorbic acid concentrations. Heat stress also impairs absorption of vitamins E and C, thereby increasing the dietary requirement of these vitamins. Vitamins E and C and Selenium are used in poultry diets because of their anti-stress effects and also because of their reduced synthesis during heat stress (Horváth and Babinszky, 2018).

Vitamin E is one of the most important natural antioxidants and is an excellent biological chain-breaking antioxidant that protects cells and tissues from lipoperoxidative damage induced by free radicals (Alagaswamy et al., 2021). Vitamin E reduces negative effects of corticosterone induced by stress (Shakeri et al., 2020), provides protection against oxidative damage and enhances proliferation and functions of these cells (Dalólio et al., 2015). Vitamin E can be supplemented in broiler diets at 250 mg/kg to reduce the negative effects of stress and to result in optimum performance in broilers (Calik et al., 2022). Vitamin C enhances the antioxidant activity of vitamin E by reducing the tocopheroxy radicals back to their active form of vitamin E (Shakeri et al., 2020). Vitamin C requirement is higher during stress and several reports have documented a beneficial effect of supplementing poultry feed with ascorbic acid. Supplemental vitamin C limits and alleviates the metabolic sign of stress and improves performance, immunological status and behaviour of birds (Abidin and Khatoon, 2013). Optimum response in growth, feed efficiency and/or livability in broilers under heat stress seems to occur with supplements of about 250 mg vitamin C/kg feed. Selenium (Se) has a special place among the feed-derived natural antioxidants, being an integral part of glutathione peroxidise (GSH-Px) which acts as an antioxidant defence in the cell (Surai, 2002). Se increases antioxidant status in broiler chicken (Rao et al., 2013). Vitamin E and Se supplementation significantly improved broiler growth performance and carcass composition, and reduced heat-related mortality and core body temperature (on d 30) without influencing the mRNA abundance of intestinal nutrient transporters (Calik et al., 2022). Addition of higher levels of vitamin E, vitamin C and Se to broiler diets had no adverse effects on carcass traits, oxidative stability and meat quality parameters when supplemented either alone or in combination (Peèjak et al., 2022).

Considering the above facts, an experiment was carried out to study the effect of supplementation of Vitamin E, Vitamin C and Selenium on blood haematological, biochemical, liver enzyme, serum electrolyte, heat shock protein, antioxidant profile and carcass quality in broiler chicken raised under heat stress.

MATERIAL AND METHODS

Design of experiment and birds

Animal trial was approved by the Ethical committee of Assam Agricultural University, Khanapara, Guwahati, Assam, India. The experiment was carried out in the Poultry shed of Department Animal Nutrition, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam - 781022, during the period of September and November with an average temperature ranging from 21 to 33^{o}C and relative humidity from 58 to 98% (Table 1). One hundred eighty (n=180) day old commercial Ven Cobb strain 400 broiler chicks were procured from the local hatchery. The chicks were kept in brooder with optimum brooding temperature for 3 days. The chicks were offered *ad libitum* pre-starter ration (crumbles) for the first 7 days of age. On 7th day chicks were weighed individually and wing banded. At 8th day, chicks were randomly distributed into 5 treatment groups having 3 replicates of 12 chicks in each (Randomized Block Design).

Table 1. Meteorological data during the experimental period

Week	Average Temperature (^{O}C)	Relative Humidity (%)
1st week	35.25	87.64
2nd week	34.14	87.81
3rd week	35.12	86.34
4th week	32.15	88.22
5th week	34.13	85.57
6th week	31.22	87.61

Experimental diets

Experimental diets were formulated for starter and finisher phases to meet the nutrient requirement as per ICAR (2013). T_0 served as control while groups T1, T2, T3 and T4 were supplied with vitamin E @100 mg/ltr + Se @ 0.2 mg/ltr, vitamin E @ 200 mg/ltr + Se @ 0.3 mg/ltr, vitamin E @ 100 mg/ltr + vitamin C @ 100mg/ltr and vitamin E @ 200 mg/ltr + vitamin C @ 200 mg/ltr, respectively into drinking water.

Procurement of vitamin E, vitamin C and Se powders

Vitamin E and vitamin C were procured from Indoma Marine Private Ltd, Panbazar, Guwahati - 1 and vitamin E and Se mixture powder from Vet Cure Remedies, Delhi Road, Saharanpur, Uttar Pradesh - 247001.

A total of 18 birds (N=90) from each group were selected for collection of blood samples on 21st (starter phase) and 42nd (finisher phase) days of the experiment by puncturing the wing vein. About 2 ml of blood was collected aseptically with anticoagulant from 9 birds (N=45) from each group to estimate haemoglobin (Hb), Packed Cell Volume (PCV) and total White Blood Corpuscles (WBC) with Automatic Haematolyzer in Teaching Veterinary Clinical Complex, College of Veterinary Science, AAU, Khanapara, Guwahati.

Blood samples from other 9 birds (N=45) were brought to the laboratory without disturbing the clots and centrifuged at 1500 rpm for 15 minutes to collect serum and stored at – 20^{o}C in small plastic eppendrof tubes (2ml) for further analysis. Serum samples were analyzed for glucose immediately (Frank et al., 2012) and for total protein, albumin and globulin within 48 hours of collection (Alberghina et al., 2013) using commercial kits manufactured by Aspen Laboratories as per methods. Blood AST and ALT was estimated by modified International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method described in the assay protocol of diagnostic reagent kit manufactured by Aspen Laboratories Maharastra-401303, India.

Blood chloride was estimated by Mercuric Thiocyanate Method described in the assay protocol of diagnostic reagent kit manufactured by Medsource Ozone Biochemical Pvt. Ltd. Sector-31, Faridabad-121003, Haryana, India. Blood potassium and sodium was estimated by colorimetric method described in the assay protocol of diagnostic reagent kit manufactured by Beacon Diagnostics Private Limited, 424 new GIDC, Kabilpore, Navsari - 396424, India.

Blood collected from 9 birds (N=45) from each group using anticoagulant were evaluated for erythrocytic antioxidant activity such as, Catalase, Superoxide Dismutase (SOD) and Glutathione Reductase (GSSG) by methods described by Aebi et al. (1974), Kakkar et al. (1984) and Sedlak and Lindsay (1968) respectively with slight modification.

Quantification of HSP 70 gene expression

Quantification of HSP70 gene expression in the experimental broiler were done by estimating the product of gene i.e. HSP70 concentration using ELISA Kit by Sicere Biotech, Chicken Heat Shock Protein 70 (Hsp-70) ELISA Kit.

Carcass quality

At the end of the experiment, 6 birds from each treatment groups were randomly selected to determine carcass quality viz. prime cut-up parts (neck, wing, back, drumstick, thigh and breast). Processing (slaughtering, bleeding, scalding, defeathering) of birds was done using following standard procedures. The weight of heart, liver and gizzard was recorded as giblet weight and expressed in terms of percentage on the basis of pre-slaughter live weight.

Statistical analysis

The statistical analysis was carried out by using Statistical Package for Social Science (SPSS) version 23.0. ANOVA was used to compare the means at 5% level of significance according to Duncan's multiple range test.

RESULTS AND DISCUSSION

Blood haematological profile

Significant differences were observed in haemoglobin values (Table 2) among the groups due to treatment, period but not due to the treatment x period interaction. Where, T1 and T4 group showed highest (P<0.05) Hb concentration than other groups; whereas T0 group showed lowest Hb concentration. Significant differences (P<0.05) were observed in total WBC count (thousand/mm3) among the groups due to effect of treatment but not due to the period and treatment x period interaction. Group T0 had significantly (P<0.05) higher count of WBC than T1 and T4 groups. There was no significant differences (P>0.05) in PCV values among the groups due to treatment, period and treatment x period interaction.

Hb and WBC level was significantly (P<0.05) better in vitamin E @ 100 mg + Se @ 0.2 mg and vitamin E + vitamin C @ 200 mg each supplemented groups. EL-HACK et al. (2017) treated laying hens with vitamin E and Se found similar result than that of present investigation while EL-SEBAI (2000) found that vitamin E caused a significant rise in WBC count by 4.65%. OSO et al. (2013) reported that supplementation of vitamin C improves Hb and PCV which was well comparable than that of present observation. Altan et al. (2000) reported that broiler selected to heat stress had reduced PCV. Hence it can be explained that administration of vitamin E @ 100 mg + Se @ 0.2 mg and vitamin E @ 200 mg + vitamin C @ 200 mg reduced the effect of heat stress on the birds.

Blood biochemical profile

Significant differences were observed in serum glucose content (mg/dL) (Table 2) among the groups due to treatment, period but not due to the treatment x period interaction. Where, T1 and T4 group showed highly significantly (P<0.05) lower serum glucose value than T0, T2 and T3 groups. Significant differences (P<0.05) were observed in protein content (g/dL) among the groups due to treatment but not due to the period and treatment x period interaction. Group T4 showed significantly higher (P<0.05) serum protein than T0, T1 and T2 groups. There was no significant difference (P>0.05) in serum albumin and serum globulin level (g/dL) among the groups due to treatment, period and treatment x period interaction.

The supplementation of vitamin E and Se @ 100 mg and 0.2 mg respectively and vitamin E and vitamin C @ 200 mg each had significantly (P<0.05) higher effect as compared to control. The present findings of the glucose concentration were in accordance to findings reported by Sahin and Kucuk (2001), Harsini et al. (2012). Whereas, Navid et al. (2010) reported that there was higher glucose concentration in broilers which were supplemented with vitamin E. The increase of glucose level at high ambient temperature may be correlated with the increase in corticosterone hormone secretion to supply the body with energy (Siegel, 1995). El-Sheikh and Salama (2010) found that the treatment with Se caused a significant increase in total protein and albumin. Sheikh et al. (2017) also reported that supplementation of vitamin E increased the level of protein and albumin.

Blood liver enzyme profile

Significant differences were observed in ALT (U/L) and AST (U/L) value (Table 3) among the groups due to treatment, period but not due to the treatment x period interaction. All the supplemented groups showed significantly better (P<0.05) ALT (U/L) and AST (U/L) value than T0 group; whereas group T1 and T4 showed significantly better (P<0.05) AST (U/L) value than T2 and T3 groups.

All the supplemented groups had significantly (P<0.05) better effect as compared to control. Similar findings were reported by Peric et al. (2009) and Biswas et al. (2011). They reported that there was decrease in ALT and AST activities of chicks when supplemented with Se. On contrary to the present findings, Gruzauskas et al. (2013) reported that the supplementation of vitamin E led to increase in serum ALT and AST. These blood enzymes, ALT and AST are used as indicator of liver oxidative damage. Reduction of liver enzymes level means increased protection against oxidative damage of the liver cells.

Serum electrolyte profile

Significant differences were observed in chloride (mMol/L) value (Table 3) among the groups due to treatment, period but not due to the treatment x period interaction. All the supplemented groups were found to be significantly better (P<0.05) than T0 group in chloride value. There was no significant differences (P>0.05) among the groups due to treatment, period and treatment x period interaction in potassium and sodium (mEq/L) values. Supplementation of vitamin E, vitamin C and Se had significantly (P<0.05) better effect as compared to control. Some researcher also reported similar result (Deyhim et al. 1990, Belay and Teeter, 1993). During heat stress the body temperature rises and haemo-dilution occurs which causes a lowering of Na concentration and due to increased permeability K comes into blood stream and excreted. Hooge (1995) reported increase in broiler serum K concentration during acute heat stress but decrease upto 3.5 mEq/L during chronic heat stress in the blood to overcome the respiratory alkalosis caused by the loss of the bicarbonate ion (Ruiz-Lopez and Austic, 1993).

Erythrocytic antioxidant profile

Significant (P<0.05) differences in level of catalase (U/g protein) (Table 3) were observed among the groups due to treatment, whereas there was no significant difference was found due to period and treatment x period interaction. T1 and T4 groups had significantly (P<0.05) higher catalase effect than T0, T2 and T3 groups, however T3 group showed significantly (P<0.05) better catalase value than T0 and T2 groups. Significant (P<0.05) differences in level of SOD (U/g protein) were observed among the groups due to treatment, period and treatment x period interaction. Where, T1 and T4 had significantly higher SOD effect than T0, T2 and T3 groups. In the level of reduced glutathione (nMol/mg protein) significant (P<0.05) differences were observed among the groups due to treatment; whereas, no significant difference was found due to the period and treatment x period interaction. Where, T1 showed significantly (P<0.05) higher effect in terms of reduced glutathione than other groups; however all the supplemented groups had significantly (P<0.05) higher reduced glutathione than T0 group.

Supplementation of vitamin E and Se @ 100 mg and 0.2 mg respectively and vitamin E and vitamin C @ 200 mg each had significantly (P<0.05) higher effect as compared to control. Jena et al. (2013), Zdunczyk et al. (2013), Gultekin et al. (2001) and Bharat et al. (2013) also found comparable results similar to that of present investigation whereas Mikulski et al. (2009) and Jankowski et al. (2011) reported that inclusion of supplemental Se in turkey did not changed antioxidant capacity. Jena et al. (2013), Malayoglu et al. (2009), Gultekin et al. (2001), Zdunczyk et al. (2013) and Bharat et al. (2013) reported that supplementation of vitamin E and C during heat stress enhances the activity of SOD, Catalase and reduced Glutathione to minimize oxidative stress by inhibiting the oxygen free radical production.

Table 2. Blood haematological and biochemical, profile of broiler in different experimental groups

Attributes	Period		Overall Mean±SE	Significant		
	21 d	42 d		T	P	T*P
Blood haematological profile						
Hb (g/dl)						
T0	8.80±0.42	8.40±0.81	8.60^{a}±0.63			
T1	11.7±0.53	13.2±1.31	12.4^{c}±1.22			
T2	10.2±0.13	10.8±0.51	10.5^{b}±0.46	<0.001	0.017	0.116
T3	10.0±0.25	10.2±0.13	10.1^{b}±0.21			
T4	11.4±1.24	12.4±0.62	11.9^{c}±1.06			
WBC (x103/µl)						
T0	30.2±7.85	34.5±3.11	32.3^{c}±5.97			
T1	25.7±1.71	24.7±2.63	25.2^{ab}±2.12			
T2	29.7±6.08	29.0±2.94	29.3^{bc}±4.44	<0.001	0.97	0.52
T3	29.0±3.16	28.5±2.89	28.7^{bc}±2.82			
T4	25.2±2.50	23.0±0.82	24.1^{a}±2.10			
PCV (%)						
T0	29.0±3.65	27.7±3.59	28.3±3.42			
T1	32.5±4.20	31.7±4.43	32.1±4.02			
T2	30.0±2.94	30.5±2.65	30.2±2.60	0.082	0.803	0.974
T3	30.7±1.26	31.2±1.71	31.0±1.41			
T4	32.7±2.75	32.5±2.65	32.6±2.50			
Blood biochemical profile						
Glucose (mg/dl)						
T0	196.1±17.89	204.9±16.88	200.5^{b}±17.21			
T1	175.2±17.16	185.3±1.58	179.8a±13.28			
T2	193.4±9.94	202.9±14.28	198.1^{b}±12.74	0.001	0.002	0.984
T3	187.0±18.23	200.7±8.57	193.8^{b}±15.35			
T4	174.8±12.56	188.7±4.03	181.8^{a}±11.48			
Total Protein (g/dl)						
T0	3.06±0.69	2.84±0.55	2.95^{a}±0.61			
T1	3.90±0.46	3.69±0.67	3.80^{b}±0.54			
T2	3.21±0.80	3.20±0.80	3.20^{ab}±0.76	0.021	0.775	0.325
T3	3.33±0.55	3.25±0.60	3.29^{abc}±0.55			
T4	3.23±0.25	3.99±0.78	3.61^{c}±0.68			
Albumin (g/dl)						
T0	1.76±0.19	1.74±0.25	1.75±0.21			
T1	2.10±0.25	2.01±0.38	2.06±0.30			
T2	1.86±0.32	1.80±0.48	1.83±0.39	0.137	0.594	0.591
T3	1.92±0.49	1.64±0.44	1.78±0.46			
T4	1.90±0.19	2.10±0.25	2.00±0.23			
Globulin (g/dl)						
T0	1.30±0.61	1.10±0.48	1.20±0.53			
T1	1.80±0.61	1.68±0.51	1.74±0.54			
T2	1.35±0.80	1.39±0.34	1.37±0.58	0.053	0.549	0.576
T3	1.42±0.76	1.61±0.75	1.51±0.73			
T4	1.33±0.37	1.89±0.64	1.61±0.58			

*T0 (Control), T1 (Vit E-100mg/ltr + Se-0.2mg/ltr), T2 (Vit E-200mg/ltr + Se-0.3mg/ltr), T3 (Vit E-100mg/ltr + Vit C 100mg/ltr), T4 (Vit E-200mg/ltr + Vit C-200mg/ltr).

^{abc}Means bearing different superscripts differ significantly $P \leq 0.05$

Table 3. Liver enzyme, electrolyte and erythrocytic antioxidant profile of broiler in different experimental groups

Attributes	Period		Overall Mean±SE	Significant		
	21 d	42 d		T	P	T*P
Liver enzyme profile separate						
ALT (U/L)						
T0	7.61±2.44	5.90±1.22	6.76^{b}±2.05			
T1	4.59±0.91	3.69±1.48	4.18^{a}±1.23			
T2	6.07±1.99	4.22±0.70	5.14^{a}±1.72	<0.001	0.001	0.756
T3	4.97±0.93	4.37±1.10	4.67^{a}±1.02			
T4	5.34±0.43	3.94±0.69	4.64^{a}±0.91			
AST (U/L)						
T0	214.6±8.02	221.6±8.08	218.1^{c}±10.27			
T1	180.1±16.59	170.8±11.29	175.9^{a}±14.56			
T2	197.0±10.95	185.4±9.74	191.2^{b}±11.59	<0.001	<0.001	0.902
T3	199.1±8.13	181.3±14.04	190.2^{b}±14.34			
T4	189.5±17.51	180.3±10.46	184.9^{ab}±14.55			
Serum electrolyte profile						
Chloride (mMol/L)						
T0	117.8±3.76	120.0±7.71	118.9^{b}±5.73			
T1	110.8±4.80	104.7±5.72	107.8^{a}±5.87			
T2	115.5±6.99	107.8±3.68	111.7^{a}±6.60	0.005	0.008	0.379
T3	115.6±5.69	109.3±6.09	112.4^{a}±6.40			
T4	113.9±2.38	107.5±4.63	110.7^{a}±4.85			
Potassium (mEq/L)						
T0	4.78±1.06	4.25±0.51	4.51±0.82			
T1	5.00±0.98	6.02±1.49	5.51±1.29			
T2	4.94±0.87	5.05±1.27	4.99±1.01	0.475	0.419	0.668
T3	4.94±1.20	5.04±0.87	4.99±0.97			
T4	4.91±1.75	5.66±0.55	5.29±1.27			
Sodium (mEq/L)						
T0	130.7±8.50	125.4±7.52	128.1±7.96			
T1	139.7±8.16	148.2±16.25	143.9±12.74			
T2	137.4±13.41	141.8±14.05	139.6±12.93	0.156	0.394	0.849
T3	136.6±17.86	144.8±9.71	140.7±14.00			
T4	142.0±17.81	144.8±16.78	143.4±16.09			
Erythrocytic antioxidant profile						
Catalase (U/g protein)						
T0	0.55±0.15	0.51±0.11	0.52^{a}±0.14			
T1	1.43±0.16	1.57±0.18	1.50^{c}±0.17			
T2	0.61±0.13	0.65±0.11	0.62^{a}±0.11	<0.001	0.116	0.692
T3	0.78±0.12	0.90±0.11	0.94^{b}±0.11			
T4	1.91±0.12	1.98±0.30	1.95^{c}±0.10			
SOD (U/mg protein)						
T0	2.03±0.06	1.88±0.83	1.96^{a}±0.10			
T1	2.95±0.41	3.56±0.24	3.26^{d}±0.45			
T2	2.02±0.12	2.18±0.11	2.10^{ab}±0.14	<0.001	0.009	0.011
T3	2.13±0.23	2.39±0.20	2.26^{b}±0.24			
T4	2.96±0.15	2.97±0.19	2.96^{c}±0.16			
Reduced glutathione (nMol/mg protein)						
T0	0.80±0.28	0.70±0.16	0.75^{a}±0.22			
T1	1.26±0.32	1.49±0.23	1.38^{c}±0.28			
T2	0.99±0.25	1.11±0.24	1.01^{b}±0.23	<0.001	0.294	0.810
T3	0.94±0.26	1.12±0.10	1.03^{b}±0.20			
T4	1.16±0.14	1.11±0.11	1.14^{b}±0.12			

[*]T0 (Control), T1 (Vit E-100mg/ltr + Se-0.2mg/ltr), T2 (Vit E-200mg/ltr + Se-0.3mg/ltr), T3 (Vit E-100mg/ltr + Vit C 100mg/ltr), T4 (Vit E-200mg/ltr + Vit C-200mg/ltr).

[abc]Means bearing different superscripts differ significantly $P \leq 0.05$

Heat shock protein (HSP70)

Significant ($p<0.05$) differences were observed in the level of reduced HSP70 (Table 4) among the groups due to treatment, period and treatment x period interaction. All the supplemented groups were found to be have significantly lower ($P<0.05$) HSP 70 value than T0 group; whereas, T1 and T4 groups showed significantly better HSP70 value than T2 and T3 groups.

T1 and T4 showed significantly better HSP70 value than other groups. Similar results were recorded by Kaushal and Bansal (2009) and Ushakova et al. (1996). They revealed that dietary supplementation of Vitamin E can decrease HSP70. HSPs protect the organism from stress through their anti-stress response (Chen et al., 2018). HSP70 mediates the protection against toxic effects (i.e. cell lysis) of reactive oxygen created through oxygen derived free radical action (Zhang et al., 2022). Heat stress generates oxidative stress, which has been recognized as a key factor in the mediation of HSPs induction. Results are in lined with previous study which reported that broilers supplemented with Se and Vitamin E showed lower expression of mRNA levels of HSPs in the breast meat (Kumbhar et al., 2018). Combination of vitamin C and E supplemented diet decreased the mRNA levels of HSPs in brain and ovary of Japanese quail under heat stress conditions (Sahin et al., 2009). Dietary vitamin E significantly decreased the mRNA expression of HSP70 in birds in summer season (Jang et al., 2014).

Table 4. Heat sock protein profile of broiler chickens in different groups during experimental periods

Attributes	Period		Overall Mean±SE	Significant		
	21 d	42 d		T	P	T*P
HSP 70(ng/ml)						
T0	5.78±1.06	6.25±0.51	6.02[c]±0.82			
T1	1.95±0.68	2.02±0.49	1.99[a]±0.29			
T2	2.94±0.87	3.05±1.27	3.00[b]±1.01	0.005	0.001	0.032
T3	2.94±0.20	3.04±0.87	2.99[b]±0.97			
T4	1.98±0.75	2.16±0.55	2.07[a]±0.27			

*T0 (Control), T1 (Vit E-100mg/ltr and Se-0.2mg/ltr), T2 (Vit E-200mg/ltr and Se-0.3mg/ltr), T3 (Vit E-100mg/ltr and Vit C 100mg/ltr), T4 (Vit E-200mg/ltr and Vit C-200mg/ltr),
[abc]Means bearing different superscripts differ significant ($P \leq 0.05$)

Carcass quality

There was no significant ($P>0.05$) difference among the treatment groups in terms of prime cuts percentage (neck, wing, back, drumstick, thigh and breast) and giblet (heart, liver and gizzard) weights on pre-slaughter live weight basis (Table 5). The carcass characteristics *viz.* prime cuts and giblet weight did not differ significantly ($P>0.05$) due to supplementation of vitamin E, vitamin C and Se. Similar results were also reported by Niu et al. (2009), Chitra et al. (2014), Dalia et al. (2018) and Senobar Kalati et al. (2012).

Table 5. Prime cuts and giblet weight (% of PSW) of broiler in different experimental groups

Attributes	Dietary Treatment					SEM	P value
	T0	T1	T2	T3	T4		
Prime cuts (% of pre slaughter weight)							
Neck	5.24±0.12	5.27±0.31	5.07±0.29	5.28±0.21	5.23±0.13	0.12	0.124
Wing	8.03±0.29	8.36±0.35	8.24±0.31	8.23±0.07	8.21±0.35	0.12	0.957
Back	13.5±0.75	15.61±0.79	13.9±0.31	14.2±0.41	13.68±0.55	0.28	0.136
Drumstick	10.0±0.29	9.50±0.71	9.89±0.44	10.0±0.26	9.31±0.27	0.19	0.628
Thigh	10.1±0.13	9.59±0.32	9.60±0.33	9.17±0.43	11.6±0.69	0.24	0.203
Breast	18.0±0.70	19.7±0.88	18.1±1.43	18.6±0.79	20.5±1.55	0.50	0.436
Giblet weight (% of pre slaughter weight)							
Heart	0.44±0.03	0.53±0.02	0.51±0.04	0.50±0.04	0.53±0.03	0.50	0.266
Liver	2.36±0.11	2.37±0.12	2.15±0.03	2.29±0.11	2.32±0.16	2.29	0.586
Gizzard	2.21±0.12	2.45±0.04	2.35±0.12	2.25±0.07	2.28±0.13	2.31	0.496

*T0 (Control), T1 (Vit E-100mg/ltr and Se-0.2mg/ltr), T2 (Vit E-200mg/ltr and Se-0.3mg/ltr), T3 (Vit E-100mg/ltr and Vit C 100mg/ltr), T4 (Vit E-200mg/ltr and Vit C-200mg/ltr).

CONCLUSION

The result of the experiment revealed that the blood haematology, blood biochemical, blood liver enzymes, serum chloride, heat shock protein and antioxidant profile were improved ($P<0.05$) in the supplemented groups as compared to control. However, the effect was more pronounced upon supplementation of vitamin E and Se @ 100 mg/ltr and 0.2 mg/ltr, respectively and vitamin E and vitamin C @ 200 mg/ltr each. Therefore, dietary supplementation of vitamin E @ 100 mg/ltr + Se @ 0.2 mg/ltr and vitamin E @ 200 mg/ltr + vitamin C @ 200 mg/ltr in broiler may be beneficial for production and improvement of health status of broiler chickens during heat stress period.

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