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Effect of supplementation of certain antioxidants (Vitamin E, vitamin C and selenium) on the growth performance of broiler chicken during heat stress

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Abstract
An experiment was conducted with one hundred eighty numbers of day-old broiler chicks (Vencobb-400) to assess the effect of dietary antioxidants supplementation on broiler performance. The chicks were divided into T_0, T_1, T_2, T_3 and T_4 having 3 replicates of 12 chicks in each group. Chicks in group T_0 were given basal diet without antioxidant supplementation and T_1, T_2, T_3 and T_4 were supplied with antioxidants into drinking water as vitamin E@100mg/l and Selenium@ 0.2 mg/l, vitamin E@200 mg/l and Selenium@ 0.3 mg/l, vitamin E@100 mg/l and vitamin C@100mg/l and vitamin E@200 mg/l and vitamin C @200 mg/l respectively. Better performance was observed in antioxidant supplemented groups in terms of body weight gain, feed intake and feed conversion ratio, livability, gross profit and physico-chemical profile of breast muscle excluding shear force value while the carcass traits and proximate composition of breast muscle except for crude protein percentage were not affected.

Keywords: Broiler, performance, selenium, vitamin C, vitamin E

Introduction
The poultry industry is a rapidly growing sector of Indian agriculture and has an important position in Indian economy. At present, the total poultry population in India is 729.21 million [1]. Despite the faster growth, there are many factors that pull down the growth of poultry industry. Among these high ambient temperatures is one of the most important factors which affect the poultry industry. High ambient temperature has a direct relationship with the profitability of meat and egg production. It causes major economic losses through reducing feed intake and decreasing utilization of nutrients, body weight gain, yield and quality of egg and feed efficiency. Addition of antioxidant vitamins in diet is also a potent way of reducing heat stress in poultry. Antioxidant such as selenium, vitamin E (α tocopherol) and C (ascorbic acid) is used in poultry diets because of their anti-stress effects and also because their synthesis is reduced during heat stress. Supplementing higher doses of these vitamins may be advantageous to control heat stress that causes reduced performance of poultry. To reduce the negative effects of stress and to optimize performance in broilers, vitamin E can be added to broiler diets at 250mg/kg as a protective measure [2]. Supplementation of about 250mg vitamin C/kg feed in broiler diet seems to cause optimum growth, feed efficiency and livability under heat stress. Selenium is an integral part of glutathione peroxidise (GSH-Px) which acts as an antioxidant defence in the cell and its supplementation in broiler diet was found to improve antioxidant status in broiler chicken [2, 3]. Considering the above facts, an experiment was carried out to study the effect of dietary supplementation of vitamin E, vitamin C and selenium (Se) on the performance of broiler chicken raised under heat stress.

Materials and Methods
Experimental procedure
The experiment was carried out in the poultry shed of Department of Animal Nutrition of College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam. One hundred eighty (180) numbers of day-old broiler chicks of commercial Vencobb strain 400 were selected for the study and reared under intensive deep litter system on standard managerial condition. The chicks were kept in brooder with optimum brooding temperature for 3 days. The chicks were vaccinated against Ranikhet disease with Lasota strain (F1) and

Infectious Bursal Disease (IBD) vaccine (dose as per company's recommendation) on 7th and 14th day of experiment, respectively.

Multivitamin preparation, Ambiplex @ 8-10ml/100 broilers and Multistar @ 5ml/100 broilers daily were given by mixing in water and other healthcare measurements were taken, wherever necessary. The feeding trial was conducted for 6 weeks with an average temperature ranging from 21 to 33ºC and relative humidity from 58 to 98%. The chicks were offered *ad libitum* pre-starter ration (crumbles) formulated as per [4] for the first 7 days of age. On 7th day chicks were weighed individually in chick weighing balance and wing banded for identification. On 8th day of age, chicks were randomly distributed into 5 treatment groups viz. T_0, T_1, T_2, T_3 and T_4 on the basis of their body weight having 3 replicates of 12 chicks in each.

Experimental diets were formulated for starter and finisher phases to meet the nutrient requirement as per [4] by using commonly available ingredients (table 1). The experimental diets were designated as diet T_0, T_1, T_2, T_3 and T_4. T_0 served as control (with no antioxidant supplementation) while T_1, T_2, T_3 and T_4 groups were supplied antioxidants into drinking water as vitamin E@100mg/l and Selenium@ 0.2 mg/l, vitamin E@200 mg/l and Selenium@ 0.3 mg/l, vitamin E@100 mg/l and vitamin C@100mg/l and vitamin E@200 mg/l and vitamin C @200 mg/l respectively. Vitamin E and vitamin C were procured from Indoma marine Private ltd, Panbazar, Guwahati-1 and vitamin E and Se mixture powder was procured from Vetcure Remedies, Delhi Road, Saharanpur.

During the feeding trial, feed intake and body weight gain were recorded weekly. The feed conversion ratio was calculated as the amount of feed (g) required to gain 1 g of body weight. Livability was measured by maintaining mortality record during the whole experiment. At the end of the experiment, five birds from each treatment group were randomly selected to determine carcass quality traits. Processing (slaughtering, bleeding, scalding, defeathering) of birds was done using standard procedures. The dressed weight of the carcass was recorded after bleeding. Evisceration was done following standard procedures. The weight of heart, liver and gizzard was recorded as giblet yield and expressed in terms of percentage on the basis of pre-slaughter live weight. The weight of lymphoid organs was also expressed in terms of percentage based on pre-slaughter live weight. After slaughter, the meat sample was preserved in deep freeze (-20 ^{0}C temp) and pH of fresh meat**,** water holding capacity [5], drip loss [6], shear force value (Warner Bratzler meat shear device) was estimated later. The chemical composition of the meat was estimated by [7]. The average cost of production per broiler for various treatment groups was calculated by the formula described by [8].

Table 1: Percent composition of the experimental diet of starter and finisher phase (Dry matter basis)

Ingredients	Starter	Finisher
Maize	45.3	51
Rice polish	10	10
Ground nut cake	18.5	16.4
Soya bean meal	20.5	17
Mineral mixture	1.5	1.5
Salt	0.5	0.5
Vegetable oil	3.5	3.5
Lysine	0.067	-
Methionine	0.133	0.1

Statistical Analysis

The statistical analysis of the experimental data was carried out by using Statistical Package for Social Science [9] version 23.0. The one and two way analysis of variance (ANOVA) was used to compare the means at 5% level of significance according to Duncan's multiple range test [10].

Results and Discussion

Body weight gain, feed intake, feed conversion ratio and carcass traits

The body weight gain (g), feed intake (g), feed conversion ratio (FCR), livability percentage and carcass traits of broiler chicken under different treatment groups have been presented in table 2. In the present study, the total body weight gain during the experimental trial was recorded as 1552.90, 1852.87, 1685.32, 1687.17 and 1783.34 g for T_0, T_1, T_2, T_3 and T_4 group, respectively. The highest (P<0.01) gain in body weight was observed in the T_1 group followed by T_4, T_3, T_2 and T_0 groups. In conformity with the present study increased body weight was noticed by [11] on supplementation Se and vitamin E in the broiler diet. Similar findings were also reported by [12] and [13] in broilers. They stated that there was significant increase in body weight due to the supplementation of vitamin E and Se during heat stress. The improvement in body weight gain of antioxidants supplemented groups might be due to an excellent chain breaking ability of antioxidant that protects cells and tissues from lipoperoxidative damage [14]. Vitamin E interacts with Se containing enzyme glutathione peroxidase to prevent the oxidative breakdown of cell [15].

The total feed intake per broiler during the experimental trial was higher (P<0.05) in antioxidant supplemented groups and recorded to be 3250.56, 3405.28, 3330.56, 3312.17 and 3308.33 g for T_0, T_1, T_2, T_3 and T_4 groups, respectively. Higher feed intake in the antioxidant supplemented group in the present study might be due to vitamin E supplementation. This was confirmed by the finding that supplementation of Se and vitamin E was efficient for augmenting performance in the layer diet [16]. Similar findings were reported by [12] and [11] in broilers. They reported that there was increased feed intake of broiler during heat stress when they were supplemented with vitamin E and Se.

The overall feed conversion ratio (FCR) during the entire experimental period was recorded as 2.03, 1.79, 1.92, 1.90, and 1.80 for T_0, T_1, T_2, T_3 and T_4 groups, respectively. The T_1 and T_4 group showed a better FCR (P<0.01) than T_2, T_3 and T_0 groups. Improved FCR The T_1 and T_4 group might be ascribable to reduced environmental temperature which is well comparable to the result of [17] who reported that alleviated environmental heat stress owing to supplementation of vitamin E and Se showed significantly (P<0.05) better FCR. [18] observed better FCR in the group of Japanese quail supplemented with vitamin E and C during heat stress. [19] reported that the supplementation of vitamin E and C significantly improved the FCR in broiler chicken during heat stress.

The livability percentage of group T_1, T_3 and T_4 was cent percent and in T_2 and T_0 it was recorded as 97.22 and 94.44 percent, respectively. Similar results were obtained in chicken [19] and breeder hen [20] exposed to the heat stress when supplemented with vitamin E and C. Better livability percentage may be related to better immune status. Vitamin E reduces secretion of immunosuppressive factors and inhibits protein kinase C in cells of monocytes and lymphocytes

thereby improve immunological system [21].

The carcass characteristics *viz* dressing percentage, giblet weight and lymphoid organ weight did not differ significantly (P>0.05) due to supplementation of antioxidants. [22] stated that influence of dietary vitamin E supplementation was not observed in lymphoid organ weight of Japanese Quail. Similarly, [11] also stated that supplementation of vitamin E and Se did not affect the lymphoid organ weight in broiler. Similar lymphoid organ weight was also found on dietary supplementation of vitamin E by [23, 13] found that the dressing percentage was not influenced by the dietary supplementation of vitamin E and Se in broilers. Similar results were reported by [24, 25] in broilers. [3] did not find significant difference in giblet weight on Se supplementation in broiler.

Table 2: Body weight gain, feed intake and feed efficiency ratio, livability and carcass traits under different experimental groups

Parameter	Groups					SEM	P value
	T_0	T_1	T_2	T_3	T_4		
Body weight gain (g)	1552.90^a	1852.87^d	1685.32^b	1687.17^b	1783.34^c	27.61	<0.001
Feed intake (g)	3250.56^a	3405.28^b	3330.56^{ab}	3312.17^{ab}	3308.33^{ab}	17.05	0.037
Feed conversion ratio	2.03^c	1.79^a	1.92^b	1.90^b	1.80^a	0.02	<0.001
Livability	94.44	100	97.22	100	100	-	-
Dressing percentage	71.47	74.37	71.47	72.07	73.83	0.48	0.158
Giblet weight (% of pre slaughter weight)	5.01	5.35	5.01	5.04	5.13	5.10	0.705
Lymphoid organ weight (% of pre slaughter weight)							
Bursa	0.20	0.15	0.19	0.17	0.19	0.01	0.620
Spleen	0.21	0.18	0.26	0.24	0.23	0.01	0.480
Thymus	0.13	0.16	0.20	0.17	0.14	0.01	0.172

Means with different superscripts in a row (a, b, c, d) differ significantly at 5% probability level

Physico-chemical profile and proximate composition of breast muscle

The physico-chemical profile and proximate composition of breast muscle have been illustrated in table 3. The water holding capacity (ml/100g), drip loss (%) and TBARS (mg malonaldehyde/kg) had differed significantly (P<0.05) due to supplementation of dietary antioxidants. The water holding capacity (WHC) of different treatment groups were recorded as 58.00, 68.97, 64.11, 60.33 and 66.22 ml/100g for T_0, T_1, T_2, T_3 and T_4 groups, respectively. The T_1 group showed the highest (P<0.01) WHC followed by T_4, T_2, T_3 and T_0 groups. [26, 27, 28, 22] reported similar findings on WHC (65-70%). Improved WHC in supplemented group might be due to the association of vitamin E with membrane antioxidants that are capable to remove the highly reactive free radicals to prevent oxidation of unsaturated fatty acids within the cells [29]. This oxidation leads to fluidity and disruption of cell membranes that may affect semi-permeable barriers.

The values of the drip loss percentage of different treatment groups were recorded as 6.53, 5.12, 7.29, 7.79 and 6.33 for T_0, T_1, T_2, T_3 and T_4 groups, respectively. The T_1 group showed better (P<0.01) results than T_4, T_0, T_2 and T_3 group which might be the result of higher glutathione peroxidase activity in serum and tissue in T_1 group compared to other groups. The improved antioxidant status may aid in maintenance of cell membrane integrity [27], which ultimately leads to reduced drip loss. It has been observed that chicken muscle drip loss can be reduced by supplementing organic Se [30] and nano-Se [31].

The TBARS value under different treatment groups was noted as 2.15, 1.23, 1.38, 1.76 and 1.62 for T_0, T_1, T_2, T_3 and T_4 groups, respectively. Group T_1 shows better value (P<0.05) than the other groups. The TBARS value decreased due to dietary supplementation of antioxidants. [32, 33, 22, 34] reported similar findings that were well comparable with the findings of present investigation. During heat stress the level of malonaldehyde increases due to the lipid peroxidation of the tissue cells. The addition of vitamin E had beneficial effect on the TBARS value [35].

The pH values under different treatment groups were noted as 5.60, 6.00, 5.85, 5.88 and 5.98 for T_0, T_1, T_2, T_3 and T_4 groups, respectively. The pH value (P<0.05) increased in the supplemented group might be due to low production of lactic acid from glycogen break down [36]. Similar results were obtained from chicken [37] and turkey [38].

The shear force value (kg) of breast muscle did not differ (P>0.05) due to supplementation of vitamin E, C and Se. [37] reported that addition of vitamin E in broiler diet did not alter the shear force value. The study by [39] and [40] stated that addition of vitamin E in the diet did not affect the pH of meat in chicken.

The moisture, ether extract (EE) and total ash (TA) percentage of breast meat did not differ (P>0.05) significantly among different experimental groups whereas, the percent crude protein (CP) of breast meat was significantly (P<0.05) higher in the antioxidants supplemented groups. The result is in line with [41] which might be due to higher intake of feed leads to higher energy intake which improves the rate of total protein deposition. [38] observed improved dry matter, crude protein, crude fat and crude ash content of meat on supplementation of Se in turkeys. On the other hand, [42] reported that proximate composition of breast muscle did not differ in turkeys with or without supplementation of ascorbic acid.

Table 3: Physico-chemical profile and proximate composition of breast muscle in different experimental groups

Attributes	Dietary Treatment					SEM	P value
	T_0	T_1	T_2	T_3	T_4		
Physico-chemical profile of breast muscle							
Water holding capacity (ml/100g)	58.00^a	68.97^e	64.11^c	60.33^b	66.22^d	0.76	<0.001
Drip loss (%)	6.53^b	5.12^a	7.29^{bc}	7.79^c	6.33^b	1.25	<0.001
Shear force value (kg)	4.14	3.57	4.03	3.79	3.57	0.60	0.348
P^H	5.60^a	6.00^b	5.85^{ab}	5.88^{ab}	5.98^b	0.38	0.04
TBARS (mg malonaldehyde/kg)	2.15^c	1.23^a	1.38^a	1.76^b	1.62^b	1.02	0.028

Proximate composition of breast muscle							
Moisture (%)	72.29	70.35	71.63	72.50	70.22	0.36	0.128
Crude protein (%)	17.37[a]	19.60[b]	17.65[ab]	17.33[ab]	19.09[b]	0.30	0.028
Ether extract %)	6.78	6.74[a]	6.95[b]	6.73[b]	6.80[ab]	0.21	0.905
Total ash (%)	1.26	1.31	1.32	1.35	1.32	0.04	0.986

Means with different superscripts in a row (a, b, c, d, e) differ significantly at 5% probability level

Economy of feeding

The cost of production and gross profit per broiler for different treatment groups are shown in table 4. Supplementation of vitamin E and Se @ 100mg/l and 0.2mg/l respectively (T_1) and vitamin E and vitamin C @ 200mg/l each (T_4) were found to be the most cost effective than other supplemented groups and basal diet fed group. The cost of production per broiler was found to be highest in T_1 group whereas it was lowest for T_0 group. The gross profit per broiler was found to be Rs.14.45, 39.46, 23.17, 26.18, and 35.39 for T_0, T_1, T_2, T_3 and T_4 groups, respectively. Hence, the gross profit was higher in T_1 and T_4 groups followed by T_3, T_2 and T_0 groups. Improved body weight gain supported the higher gross profit in the antioxidant supplemented groups. [43] observed increased net profit in Japanese quail supplemented with vitamin E and Se. Similar findings were observed by [44].

Table 4: Economics of feeding per broiler under different treatment groups

Parameters	Groups				
	T_0	T_1	T_2	T_3	T_4
I. Expenditure					
1) Chick cost (A) = cost of one day-old chick	42.00	42.00	42.00	42.00	42.00
2) Feed cost (B) = Live weight in kg x FCR x Cost per kg of feed	96.74	100.39	98.06	97.78	97.42
3) Miscellaneous expenditure (C) = Add 12% of (A+B)	17.01	17.45	17.17	17.13	17.09
4) Additional cost of supplement (D)	Nil	1.00	2.00	0.70	1.40
5) Production cost per broiler (A+B+C+D)	155.75	160.84	159.23	157.62	157.91
II. Return					
Sale of one live broiler @ ` 100 per kg	170.20	200.30	182.40	183.80	193.30
III. Gross profit per broiler	14.45	39.46	23.17	26.18	35.39

Conclusion

The result of the present experiment revealed that there was significantly better performance in broilers due to supplementation of vitamin E, C and Se in different combinations, which showed better feed intake, body weight gain, feed conversion ratio and livability without affecting the carcass traits. The physico-chemical profile of breast muscle except for shear force value was also improved in antioxidant supplemented groups as compared to control. The percent moisture, EE and total ash were not affected by supplementation of antioxidants while the CP percent in breast meat was significantly better on supplementation of vitamin E and Se @100mg/l and 0.2mg/l, respectively and vitamin E and vitamin C@200 mg/l each in the starter and finisher ration.

Therefore, vitamin E@100mg/l and Se@ 0.2 mg/l, vitamin E@200 mg/l and Se@ 0.3 mg/l, vitamin E@100 mg/l and vitamin C@100mg/l and vitamin E@200 mg/l and vitamin C @200 mg/l can be incorporated as antioxidant in broiler ration for better production and optimum health status during heat stress period.

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