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Effect of different vitamin E sources on growth performance, nutrient utilization, anti-oxidant status and carcass characteristics in broiler chicken

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ABSTRACT

This study aimed to compare the efficacy of supplementation with a synthetic (DL-α-tocopherol acetate) or herbal source of vitamin E on performance of broiler chicken during a 35-days feeding trial. Day-old broiler chicks (180) were randomly assigned to one of four treatment groups, each with three replicates. T_1 (control) received a basal diet, Group T_2 received a basal diet with synthetic vitamin E @ 100 g/quintal of feed, Group T_3 received a basal diet with herbal vitamin E @ 100 g/quintal of feed and Group T_4 received a basal diet with synthetic vitamin E @ 50 g/quintal of feed + herbal vitamin E @ 50 g/quintal of feed. Supplementation of herbal vitamin E enhanced growth performance traits (average weight gain, feed intake, feed conversion ratio and economics of broiler production), retention of nutrients (dry matter, nitrogen, and ether extract), haematological parameters (Hb, PCV, and TEC) and serum total cholesterol in broiler chicks. Antioxidant status revealed significant increase in GSH but not SOD and CAT. A marked increase in the antibody titer against Newcastle Disease vaccine was recorded in the herbal vitamin E supplemented group. The carcass quality was statistically similar among different dietary treatments, however, lowest abdominal fat and meat cholesterol were recorded in the group supplemented with herbal vitamin E. The study depicts that dietary supplementation of synthetic or herbal vitamin E and their combination @ 100 g/quintal of feed improved the growth performance, antioxidant status and carcass quality of broiler chickens; and that herbal vitamin E was economical and more effective as compared to the synthetic vitamin E in improving the performance of broiler chickens.

Keywords: Antioxidant, Broiler, Carcass, Performance, Vitamin E

Vitamin E is a fat-soluble vitamin, which is essential for proper metabolism, growth performance, immunity and quality of animal products. Vitamin E is found in high quantities in vegetable oils. Eight naturally occurring substances have been found to have vitamin E activity: α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols (Voljc *et al*. 2011). Of these, the α-tocopherol is the most biologically active and most widely distributed form (Halliwell and Gutteridge 2000). The naturally occurring molecule is the D-α-tocopherol (RRR-α-tocopherol) configuration that has the highest vitamin activity (McDonald *et al*. 2011). Vitamin E is usually added to poultry feed in the synthetic form of DL-α-tocopherol acetate. An alternate dietary source of vitamin E is from the natural sources. It was reported that herbal vitamin E got quickly absorbed, slowly excreted, and remains more bioactive in animal system as compared to synthetic vitamin E. Herbal vitamin E assimilates far better in the body than synthetic vitamin E as specific binding and transport proteins in the liver have selective affinity for the natural form, and they largely ignore all other forms (Hosomi *et al*. 1997).

The superior bioavailability of natural vitamin E over synthetic vitamin E has been recorded in turkeys resulting in improved meat quality and muscular antioxidant capacity (Rey *et al*. 2015). Kaiser *et al*. (2012) reported that broilers fed natural vitamin E had a significantly lower lipopolysaccharide-induced inflammatory response in comparison to synthetic vitamin E-supplemented birds. The present study aimed to ascertain the effects of partial to complete replacement of synthetic with natural vitamin E on growth performance, antioxidant capacity and carcass characteristics of broiler chickens.

MATERIALS AND METHODS

Experimental design: The efficacy of synthetic and herbal vitamin E were compared during a 35-days feeding trial in terms of growth performance, nutrients utilization, haemato-biochemical profile, antioxidant status, antibody titer and carcass quality in broiler chickens. Day-old broiler chicks (180) were randomly assigned to one of four

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treatment groups, each with three replicates. T_1 (control) received a basal diet, group T_2 received a basal diet with synthetic vitamin E @ 100 g/quintal of feed, group T_3 received a basal diet with herbal vitamin E (AV/HEP/18; M/s Ayurvet Limited, India) @ 100 g/quintal of feed and group T_4 received a basal diet with synthetic vitamin E @ 50 g/quintal of feed + herbal vitamin E @ 50 g/quintal of feed. All the standard managemental practices, including vaccination were followed during the experimental period. Chicks had *ad lib.* access to feed and water throughout the feeding trial. All diets were formulated to meet the requirements for the energy, protein, calcium, and phosphorus that have been established for broiler chicken as per Bureau of Indian Standards (BIS 2007). Feeding was done in three-phased manner, viz. pre-starter (0-7 days), starter (8-21 days), and finisher (22-35 days). The representative samples of feed were analyzed for proximate principles, phosphorus, and calcium (AOAC 2007). The composition of the basal diet and the nutrient composition of the feed ingredients are shown in Table 1.

Table 1. Ingredient and chemical composition (% DM basis) of basal diet of broiler chicken

Ingredient	Pre-starter	Starter	Finisher
Maize	57.90	56.00	59.20
Soybean meal	26.50	29.00	25.50
Groundnut cake	6.00	4.00	3.00
Meat cum bone meal	5.00	4.00	3.70
Vegetable oil	1.82	3.89	5.32
Limestone powder	0.80	0.90	0.90
Dicalcium phosphate	0.70	0.85	1.00
Common salt	0.30	0.30	0.30
Trace mineral premix[1]	0.15	0.15	0.15
Vitamin Premix[2]	0.10	0.10	0.10
L-Lysine	0.25	0.15	0.050
DL-Methionine	0.12	0.10	0.130
Additives [3]	0.06	0.06	0.150
Total	100	100	100
Chemical composition (% DM basis)			
Crude protein (%)	23.09	22.13	20.21
Ether extract (%)	8.11	8.23	8.41
Crude fibre (%)	3.81	3.90	3.98
Total ash (%)	6.26	6.34	6.34
Calcium (%)	1.12	1.08	1.04
Total phosphorus (%)	0.71	0.73	0.73
Available phosphorus (%)*	0.41	0.44	0.44
ME (kcal/kg)*	3012.96	3125.36	3219.87
Lysine (%)*	1.01	1.00	1.00
Methionine (%)*	0.45	0.44	0.44

*Calculated values; [1]Trace mineral premix supplied (per kg diet): Magnesium- 300 mg, Manganese- 55 mg, Iodine- 0.4 mg, Iron- 56 mg; Zinc- 30 mg and Copper- 4 mg. [2]Vitamin premix supplied (per kg diet): vitamin A-8250 IU, vitamin D_3- 1200 ICU; vitamin K- 1 mg; vitamin B_1- 2 mg, vitamin B_2- 4 mg; niacin- 60 mg, pantothenic acid-10 mg, vitamin B_{12}-10 µg and choline- 500 mg. [3]Premix contains: Toxin binder 0.01%, Coccidiostat 0.05% and sodium bicarbonate 0.15%.

Growth performance: Performance parameters such as body weight (BW), feed intake (FI) were recorded at weekly interval upto 5th week. Feed conversion ratio (FCR) was calculated as the feed consumed per kg body weight gain. Feeding economics were calculated in terms of feed cost per body weight gain of the experimental birds. Mortality and birds' health status were observed daily and overall mortality percentage was calculated at the end of experiment.

Metabolic trial: A metabolism trial of 3 days duration was conducted during 5th week for the evaluation of nutrient (crude protein, ether extract, calcium and phosphorus) retention. Data on feed intake, excreta voided were recorded, dry matter content of feed and excreta was estimated. The samples of feed and excreta were analysed for proximate principles, phosphorus, and calcium (AOAC 2007) and retentions were expressed as proportion of the nutrients retained per unit weight of intake.

Haematological and biochemical analyses: At 35th day, six birds per treatment group were slaughtered and blood samples were collected in a sterile heparinzed container for estimation of various haematological profiles. Hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH) were determined in whole blood samples of chicken in different experimental groups following standard procedure as described by Weiss and Wardrop (2011). Serum was analysed for total protein (Valtzidis 1977), albumin (Gustaffson 1978), glucose (Tietz 1976), total cholesterol (Doumas *et al.* 1971), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) and alanine transaminase (ALT) (Reitman and Frankel (1957) using diagnostic kits (Span Diagnostic Ltd., India). The serum albumin content was subtracted from serum total protein content to calculate globulin (Total protein - Albumin). Serum catalase activity was estimated spectrophotometrically as per the method described by Cohen *et al.* (1970). Reduced glutathione (GSH) level was estimated using the method described by Lin *et al.* (1988). Serum superoxide dismutase (SOD) activity was measured using the method given by Madesh and Balasubramanian (1998).

Carcass characteristics: At the end of the experiment on day 35, two representative birds from each replicate were sacrificed for evaluation of carcass characteristics and sensory evaluation. The birds were sacrificed after an overnight fast by decapitation, and processed for carcass characteristics. Birds were scalded, defeathered and eviscerated. Weight of the carcass was recorded as dressed yield by the following formula:

Dressing yield = live weight – (weight loss as blood, feathers, head, shank and viscera)

The weights of different organs (heart, liver, spleen, thymus, Bursa of Fabricius) were recorded by separating them from carcass and expressed as a percentage. The sensory quality of cooked meat samples was evaluated by the standard sensory evaluation method (Keeton and

Foegeding 1984) in terms of appearance, flavour, texture, juiciness, and overall acceptability.

Lipid peroxidation of meat samples was measured by determining the malondialdehyde (MDA) production using thiobarbituric acid (TBA) as per method given by Suleiman *et al.* (1996). Reduced glutathione (GSH) level was estimated using the method described by Lin *et al.* (1988). Serum superoxide dismutase (SOD) activity was measured using the method given by Madesh and Balasubramanian (1998).

Statistical analysis: The experimental data obtained were analyzed statistically (Snedecor and Cochran 1994) as a completely randomized design by analysis of variance (ANOVA) by using general linear model (GLM) procedure of statistical package for the social sciences (SPSS 20.0). Statistical significance was declared at P<0.05.

RESULTS AND DISCUSSION

Growth performance and nutrients utilization: The supplemental vitamin E effect on production traits *viz.* body weight gain, feed intake, feed conversion ratio (FCR) and feed economics of broiler chicken are shown in Table 2.

The final BW at 35 days of age was significantly (P<0.05) highest in T3 (herbal vitamin E) followed by T4 (synthetic + herbal vitamin), T2 (synthetic vitamin E), T1 (basal diet). Feed intake followed the same trend as body weight. It was significantly (P<0.05) highest in T3. The FCR was significantly lower (P<0.05) in T3 followed by comparable values in T4, T2 and greatest (P<0.05) in T1. Best feed economics was observed in T3 as compared to other treatment groups.

Improvement in body weight gain in birds fed vitamin E supplemented diets is in agreement with the reports of Chae *et al*. (2006). Feed intake during the entire rearing period (0-35 days) was maximum in the herbal vitamin E group. These findings were similar to the findings of Shaik *et al*. (2005), Liu *et al*. (2009), and Jhirwal *et al*. (2018) who observed significant improvement in feed consumption. The results of significantly better feed conversion ratio due to incorporation of herbal vitamin E are similar to those of Shaik *et al*. (2005), and Liu *et al*. (2009), all of whom observed significant effect on feed conversion ratio by supplementing control diet with vitamin E. Feeding cost per kg body weight gain was highest (₹ 55.00) in control group and lowest (₹ 53.84) in herbal vitamin E treatment group.

The effect of supplemental vitamin E on nutrient utilization of broiler chicken of 35 days of age (Table 2) revealed that dry matter retention was significantly (P<0.05) highest in T3 followed by T2 and T1 but it was comparable with T4 whereas, crude protein retention in T3 was significantly higher as compared to all other treatment groups. The ether extract retention was significantly (P<0.05) highest in T3 and comparable with T2. Differences in phosphorus and calcium retention were non-significant among all treatment groups. Supplemental vitamin E had significantly (P<0.05) positive impact on dry matter and ether extract retention of broiler birds. Our finding is similar to that of Secrist *et al.* (1997), who reported a positive effect on DMI and its digestibility in crossbred steers due to vitamin E supplementation.

Improvement in growth performance may be attributed to effect of vitamin E on better utilization of nutrients in broilers. Khalifa *et al.* (2021) reported that dietary inclusion of vitamin E upregulate the expression of some genes related to growth performance including growth hormone receptor (GHR) and insulin-like growth factor 1 (IGF1) in the liver tissue of broilers resulting in improved production parameters. GHR play an important role in broilers' production parameters because of its cardinal role in the development (Al-Kelabi *et al.* 2019). The lowest feeding cost in herbal vitamin E group might be due to better FCR, better nutrient utilization and lower price of herbal vitamin E as compared to synthetic vitamin E.

Blood profile: The effect of supplemental vitamin E on hemato-biochemical parameters (Table 3) of broiler chicken at 35 days of age revealed that haemoglobin, packed cell volume, total erythrocyte count, total cholesterol, and GSH

Table 2. Effect of synthetic and herbal vitamin E on growth performance of broiler chicken

Parameter	Group					
	T1	T2	T3	T4	SEM	P-value
Growth performance (0-35 days)						
Weight gain (g)	1523.00[a]±21.68	1558.10[b]±23.61	1608.46[d]±27.62	1575.01[c]±24.81	9.40	0.001
Feed intake (g)	2699.89[a]±39.87	2741.28[b]±34.52	2810.75[c]±44.25	2764.72[b]±32.91	12.47	0.001
FCR	1.77[c]±0.02	1.76[b]±0.05	1.75[a]±0.03	1.76[ab]±0.02	0.01	0.002
Mortality (%)	3.44	3.21	2.22	3.12	-	----
Feed cost (₹ / kg weight gain)	55.00	54.70	53.84	54.44	-	---
Nutrients utilization						
Dry matter (%)	70.78[a]±0.70	72.59[b]±0.32	75.11[c]±0.33	73.71[bc]±0.29	0.515	0.001
CP (%)	62.43[a]±0.55	64.57[b]±0.42	67.09[c]±0.47	65.30[b]±0.41	0.542	0.001
EE (%)	73.78[a]±0.43	75.12[b]±0.20	77.32[c]±0.63	76.75[c]±0.39	0.460	0.002
Calcium (%)	45.02±0.88	45.52±0.27	46.91±1.20	46.72±0.68	0.422	0.329
Phosphorus (%)	72.62±1.41	74.21±1.19	76.11±1.07	76.03±0.62	0.599	0.114

[abc] Means with different superscripts in a row differ significantly (P≤0.05).

Table 3. Effect of synthetic and herbal vitamin E on hemato-biochemical parameters of broiler chicken at 35 days of age

Parameters	T1	T2	T3	T4	SEM	P-value
Haemoglobin (g/dL)	8.46[a]±0.09	8.75[ab]±0.08	9.53[c]±0.21	9.11[bc]±0.25	0.112	0.001
PCV (%)	26.95[a]±0.31	28.30[b]±0.43	30.11[c]±0.50	28.93[bc]±0.39	0.360	0.001
TEC (million/ mm^3)	2.27[a]±0.02	2.35[b]±0.03	2.59[c]±0.02	2.42[b]±0.01	0.027	0.001
MCV	115±1.07	120.57±2.73	116.25±1.25	119.57±1.63	0.961	0.127
MCH	37.30±0.37	37.27±0.68	36.79±0.66	37.67±0.81	0.313	0.826
Glucose (mg/dL)	258.43±1.36	250.43±3.18	251.46±3.40	249.10±3.95	1.642	0.294
Total protein (g/dL)	4.73±0.11	4.82±0.18	5.08±0.15	5.00±0.10	0.073	0.324
Albumin (g/dL)	3.12±0.15	3.01±0.04	3.13±0.05	3.18±0.04	0.052	0.599
Globulin (g/dL)	1.61±0.21	1.80±0.18	1.95±0.17	1.82±0.09	0.079	0.547
Total cholesterol (mg/dL)	158.02[b]±3.58	136.50[a]±3.51	128.26[a]±6.20	133.73[a]±4.15	3.155	0.001
ALT (IU/L)	27.63±0.85	28.05±0.75	29.52±0.64	26.85±0.87	0.419	0.144
AST (IU/L)	170.31±2.41	168.01±2.73	167.87±1.16	171.89±2.77	1.156	0.581
ALP (IU/L)	214.66±3.15	208.97±3.10	216.38±5.73	218.12±5.84	2.285	0.547
GSH (U/mL)	6.31[a]±0.20	8.89[b]±0.08	9.96[c]±0.14	9.21[b]±0.07	0.293	0.001
CAT (U/mL)	4.96±0.06	5.12±0.05	5.05±0.16	5.16±0.04	0.046	0.467
SOD (U/mL)	152.08±2.28	156.76±2.94	162.81±3.99	159.31±2.16	1.594	0.102

[abc] Means with different superscripts in a row differ significantly ($P \leq 0.05$).

content differed significantly (P<0.05) among different groups; the remaining parameters were found to differ non-significantly among all treatment groups.

Haemoglobin, PCV and TEC of group T3 was significantly higher than T1 and T2 but comparable to T4. Total cholesterol in T3 was significantly (P<0.05) lowest with respect to T1 but comparable with T2 and T4. El-Sheikh *et al*. (2006) and Kant *et al*. (2014) also reported increase in haemoglobin due to vitamin E supplementation. This could be attributed to the role of vitamin E as fat soluble antioxidant which protects the biological membranes from oxidative damage and decreases osmotic fragility of erythrocytes (Dlouha *et al*. 2008). Total erythrocyte count (TEC) was significantly (P<0.05) higher in herbal vitamin E supplemented group (2.59 million/mm^3). Kant *et al*. (2014) also observed the same finding. The values of mean serum glucose, total protein, albumin or globulin among the treatment groups did not differ significantly (P>0.05). Similar results were reported in case of glucose (Behera *et al*. 2019) and total protein (Kant *et al*. 2014). There was significant change (P<0.05) in the serum cholesterol level after supplementing vitamin E. Kant *et al*. (2014) showed that total cholesterol levels were lower (P<0.05) in treatment groups than the control. In the present study, no significant (P>0.05) change was observed in the AST, ALT and ALP levels.

Carcass traits: Carcass characteristics were not influenced by the dietary treatments (Table 4). Our results regarding carcass characteristics are in agreement with the findings of Daniel *et al*. (2017), who reported that feeding vitamin E had no significant effects on visceral organs of broilers. The addition of vitamin E resulted in decrease of abdominal fat in birds from the experimental group. The abdominal fat content of T3 was significantly lower than T1 and T2 but comparable to T4. Castellini *et al*. (2002) and Skrivan *et al*. (2010) observed a positive effect of vitamin E on the fat content of breast muscles at high supplementation of vitamin E. There was no significant effect of dietary treatment on lymphoid organ weight.

The effect of supplemental vitamin E on sensory attributes of broiler chicken at 35 days of age (Table 4) revealed that the juiciness, tenderness and overall acceptability was significantly (P<0.05) better in T3 as compared to the other treatment groups. The results of sensory evaluation are in agreement with the findings of Cheng *et al*. (2016) and Sasiadek *et al*. (2016). In the current study, meat from the experimental group had better consumer scores. By inhibiting oxidative processes, vitamin E reduces the quantity of generated oxidation products that may deteriorate the quality of meat by negative effects on taste, aroma, colour and texture (Kennedy *et al.* 2005). Dietary supplementation of vitamin E significantly (P<0.05) affected the breast meat cholesterol. Lowest meat cholesterol was recorded in T3 followed by T4, T2, and T1. Decrease in meat cholesterol level due to vitamin E supplementation was also reported by Sasiadek *et al.* (2016), where meat from chicken fed with higher dose of vitamin E was characterized by a low cholesterol level. There was no significant change in GSH-Px and SOD level in the breast muscle of different groups. There was significant change (P<0.05) in the MDA accumulation in the breast muscle among the different groups. The lowest MDA was accumulated in the group supplemented with herbal vitamin E. Maximum MDA accumulation occurred in the control group T1, whereas groups T2 and T4 had intermediate values. Chicken breast meat contains high levels of unsaturated fatty acids, especially PUFA, and is therefore more susceptible to lipid oxidation, leading to a higher risk of oxidative rancidity in the muscle tissue of live birds, and persisting in the fresh carcass and in stored and processed meat products (Salami *et al.* 2015). In our study, the lowest MDA content, as a secondary by-product

Table 4. Effect of synthetic and herbal vitamin E on carcass characteristics, sensory attributes and antioxidant status of breast meat of broiler chicken

Parameter	T1	T2	T3	T4	SEM	P-value
Carcass characteristics (% of body weight)						
Dressing (%)	68.39±0.55	69.12±0.93	71.48±0.89	70.44±0.38	0.42	1.420
Eviscerated yield (%)	63.68±0.49	63.96±0.39	65.34±0.52	65.01±0.32	0.25	0.734
Giblets (%)	5.25±0.07	5.27±0.13	5.35±0.12	5.28±0.05	0.05	1.151
Abdominal Fat (%)	1.64[c]±0.02	1.49[b]±0.01	1.41[a] ±0.02	1.45[ab] ±0.01	0.196	0.001
Heart (%)	0.62±0.03	0.64±0.03	0.71±0.02	0.66±0.02	0.01	1.473
Liver (%)	2.34±0.08	2.36±0.04	2.51±0.13	2.39±0.08	0.04	0.663
Spleen (%)	0.11±0.005	0.11±0.007	0.11±0.007	0.11±0.005	0.003	0.637
Thymus (%)	0.35±0.001	0.36±0.017	0.35±0.004	0.36±0.002	0.002	0.096
Bursa of Fabricius (%)	0.07±0.005	0.08±0.005	0.07±0.005	0.08±0.003	0.002	0.264
Meat:bone	2.12±0.04	2.25±0.08	2.31±0.16	2.28±0.11	0.05	0.851
Sensory attributes and antioxidant status of breast meat						
Appearance	6.33±0.21	6.50±0.22	6.33±0.33	6.66±1.42	0.147	0.849
Flavour	6.00±0.25	6.33±0.33	6.16±0.30	6.83±0.16	0.143	0.193
Juiciness	5.33[a]±0.21	6.33[b]±0.21	6.83[b]±0.30	6.66[b]±0.42	0.185	0.010
Tenderness	5.33[a]±0.21	6.16[ab]±0.40	6.83[b]±0.30	6.50[b]±0.22	0.180	0.012
Overall acceptability	5.50[a]±0.22	6.16[ab]±0.30	7.00[b]±0.36	6.16[ab]±0.30	0.182	0.021
Breast meat cholesterol (mg%)	48.53[b]±1.31	42.66[a]±1.27	39.23[a]±1.63	40.52[a]±2.29	1.082	0.004
GSH-Px (nmol/mg)of protein)	14.83±0.18	14.98±0.27	15.46±0.13	15.06±0.12	0.100	0.145
SOD (U/mg of protein)	40.59±0.57	41.23±0.10	40.87±0.15	41.02±0.10	0.151	0.532
MDA (nmol/mg protein)	0.48[d]±0.001	0.47[c]±0.001	0.45[a]±0.001	0.46[b]±0.001	0.002	0.001

[abc]Means with different superscripts in a row differ significantly (P≤0.05).

of the lipid peroxidation, was observed in breast meat of vitamin E supplemented fed broiler chickens. Coetzee and Hoffman (2001) demonstrated a significantly lower level of MDA during storage of meat when the animals were fed on diets supplemented with vitamin E. Our result is consistent with a theory of Mitsumoto *et al.* (1993), who suggested that dietary vitamin E incorporates itself into the bilayer phospholipid structure. Hence, in order to perform the function of an antioxidant, vitamin E should be administered for a longer period of time because only then it may be accumulated in cells and effectively protect the meat against oxidation (Flachowsky *et al.* 2002).

This study demonstrated that dietary supplementation with synthetic or herbal vitamin E alone or their combination @ 100 g/quintal of feed improved the growth performance, antioxidant status and carcass quality of broiler chickens. Herbal source of vitamin E was economical and more efficacious as compared to the synthetic vitamin E at improving the performance, carcass and meat characteristics of broiler chicken.

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