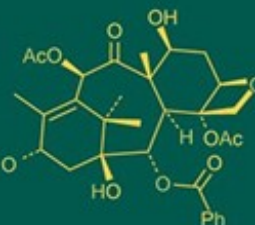


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Effect of plant based herbal vitamins on carcass traits and meat attributes of broiler chicken

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ABSTRACT

An experiment was conducted with one hundred- and seventy-five-day old broiler chicks (Ven Cobb 430y strain) to assess the effect of dietary supplementation of herbal vitamins on meat quality of broiler chicken. The chicks were divided into control, positive control, T_1 , T_2 and T_3 having 5 replicates of seven chicks in each group. Chicks in control were given basal diet (BIS, 2007) without vitamins supplementation and positive control, T_1 , T_2 and T_3 were supplied with synthetic vitamin E (0.01 %) + synthetic vitamin C (0.02 %), herbal vitamin E (0.01 %), herbal vitamin C (0.02 %), herbal vitamin E (0.01 %) + herbal vitamin C (0.02 %) respectively. Non-significant effects in performance observed in carcass traits with dietary supplementation of herbal vitamins. The proximate composition was non-significantly ($P \geq 0.05$) different among experimental groups. Incorporation of vitamin E/selenium, vitamin C and combination results increase in pH, TBARS, tyrosine value, total plate count and psychrophilic count with increase in storage period from 0 to 8 days. Coliform count and *Salmonella* counts were not detected during storage period in both treatment and control group. Fresh meat from each group was packed aerobically in LDPE pouches and was acceptable up to 6th day of storage at refrigerated ($4 \pm 1^\circ\text{C}$) temperature compared to control group. Based on findings, it is concluded that supplementation of herbal vitamin E/Selenium (0.01%) + herbal vitamin C (0.02%) is beneficial in terms of carcass traits, proximate composition and keeping quality of meat.

Keywords: Herbal vitamins, Proximate composition, Carcass traits, TBARS, Tyrosine value, Total plate count, Psychrophilic count, Coliform count, Salmonellacount

Introduction

Chicken meat accounted for roughly 39% of world meat output (FAO, 2019) [1]. Total poultry has reached to 16.81% and total poultry production is 851.81 million. The total commercial poultry production is 534.74 million which has increased by 4.5% in 2019. India's agricultural industry is currently one of the fastest expanding sectors, with broiler production rising at an annual rate of 8 to 10% (APEDA, Annual report, 2020-21) [2]. Indeed, poultry breast meat, fits the modern consumer demand for low fat, sodium and cholesterol (Cavani *et al.*, 2009) [3]. Proteins, lipids and minerals make up the bulk of raw poultry flesh in proportions ranging from 18.4 to 23.4, 1.3 to 6.0, and 0.8 to 1.2%, respectively (Culioli *et al.*, 2003) [4].

Vitamin E is the primary lipid-soluble antioxidant present, performs a crucial role as a chain-breaking lipid antioxidant and free radical scavenger in the membranes of cells and subcellular organs (Young *et al.*, 2003) [5]. Natural vitamin E is physiologically and chemically distinct from manufactured vitamin E and it has the highest bioavailability in animals (Wilburn *et al.*, 2008) [6]. Due to the action of free radical sequestration, selenium and vitamin E can be used alone or in combination in broiler diets to reduce the effects of stress brought on by high temperatures and thereby enhance the performance of the birds' growth (Habibian *et al.*, 2013). The combination of vitamin E and selenium is known to influence biological functions, particularly as an antioxidant that can improve the flavour of meat (Dalia *et al.*, 2018). Vitamin E and selenium (Se) significantly contribute to the defence of cells against reactive oxygen species by lowering free radicals and limiting the peroxidation of lipids (Ghazi *et al.*, 2012) [7]. Selenium is a vital antioxidant mineral for animals and is known to have an impact on feather development and tissue cellular integrity in avian species (Surai, 2002) [8].

Vitamins C and vitamin E worked together to reduce the harmful effects of persistent heat stress in chickens (Sahin *et al.*, 2002) [9]. Tocopheroxy radicals converting back to their active form of vitamin E and improved antioxidant activity in the presence of vitamin C (Doba *et al.*, 1985) [10]. Vitamin E, Vitamin C and Vitamin E+C supplementation increased the number of lymphocytes and white blood cells (WBCs) in birds and lowered the number of heterophils and the heterophil/lymphocyte ratio (Ipek *et al.*, 2007) [11]. The properties of vitamin C are used to protect cells from oxidative damage and coupled with alpha-tocopherol are responsible for many of its physiological functions (Whitehead *et al.*, 2003) [12]. The holistic approach (from farm to

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fork) is crucial for the manufacture of meat and meat products that contain low initial quantities of bacteria because meat does not naturally possess intrinsic barriers that are sufficient to restrict the growth of microorganisms. Considering the above facts and in order to increase health concern needs of consumers the experiment was carried out to study the efficacy of supplementation of herbal vitamins on meat quality of broiler chicken.

Material and Methods

The experiment was conducted at experimental broiler shed of College of Veterinary and Animal Sciences, Udgir (Maharashtra).

Dietary formulation

The experimental diet was designated and mentioned as per the table 1 as control, positive control, T₁, T₂ and T₃ in that control served as (with no vitamin supplementation) while positive control (synthetic vitamin E (0.01 %) + synthetic vitamin C (0.02 %), T₁ (Herbal vitamin E/ selenium (0.01 %), T₂ (Herbal vitamin C (0.02 %) and T₃ (Herbal vitamin E/ selenium (0.01 %) + Herbal vitamin C (0.02 %). Herbal vitamin E, selenium and vitamin C was collected from Indian Herbs Specialities Private Limited, C215, 2nd floor, Elante offices, Industrial and business park, Chandigarh and Synthetic vitamin E and Vitamin C was procured from Motera Biotech Udgir, (Maharashtra).

Table 1. Dietary treatments allotted for various experimental groups

Sr. No	Ingredients	Experimental groups (%)				
		Control	Positive Control	T ₁	T ₂	T ₃
1	Synthetic vitamin E	-	0.01	-	-	-
2	Synthetic vitamin C	-	0.02	-	-	-
3	Herbal vitamin E/Se	-	-	0.01	-	0.01
4	Herbal vitamin C	-	-	-	0.02	0.02

Experimental design

Day old one hundred and seventy-five broiler chicks were randomly divided into five equal groups. The experiment was conducted for the period of six weeks (42 days) by adopting standard managerial practices. The 1 sq. ft. space was allotted per chick and remaining space was used for drinkers and feeders. Brooder area was stabilized for 95 °F temperature before arrival of chicks. Biosecurity measures were followed in order to avoid infection to arriving chicks. Pre-starter, starter and finisher broiler feeds were prepared at farm feed mill before arrival of chicks as per BIS (2007). The recommended vaccination schedule was followed for all the experimental chicks to prevent them from various diseases. Feed formulation was done according to BIS, (2007) and mentioned as per the table 2. Procured feed ingredients were grinded in feed mill situated at college farm and diets were prepared as pre-starter (1st week), starter (2-3 weeks) and finisher (4-6 weeks) and formulation of the same is given to feed. At the end of the experiment four birds from each treatment group were randomly selected and sacrificed. All activities were carried out in Poultry Processing Unit of Department of Livestock Products

Technology, College of Veterinary and Animal Sciences, Udgir (Maharashtra).

Experimental procedure

A. Determination of Proximate composition

The meat samples were analysed for moisture, total fat, crude protein and ash by following the method of AOAC (1995) [14].

B. Thiobarbituric acid reactive substances (TBARS) evaluation of broiler chicken

Thiobarbituric acid (TBA) reagents were prepared by dissolving 0.283 g of thiobarbituric acid in sufficient quantity of 90% acetic acid and by slight warming it to dissolve the contents. Then the volume is made up to 100 ml with 90% glacial acetic acid.

Thiobarbituric acid number (TBA) was measured as per the method described by Witte *et al.*, (1970) [6] with slight modification in the technique. Five ml of TCA extract was mixed with 5 ml of TBA reagent in a test tube. The test tubes containing sample were kept in a water bath at 100 °C for 30 min along with control (a blank with 5 ml of 10% TCA and 5 ml TBA reagent). After cooling the tube in running tap water for 10 minutes the developed colour was measured as absorbance value at 530 nm using spectrophotometer with medium sensitivity and reported as TBA number.

C. Tyrosine value

Tyrosine value of fresh meat samples was estimated by the extraction method of Strange *et al.*, (1977) [15]. TCA extract (2.5 ml) was added with equal amount of distilled water. To this freshly prepared 10 ml of 0.5 N NaOH solution was added. The mixture was kept for 10 min and then mixed with 3 ml of diluted Folin and Ciocalteu reagent (1:2 with distilled water). After mixing, it was kept in dark for 15 minutes at room temperature the developed colour was measured as absorbance at 660 nm using spectrophotometer at low sensitivity using blank containing 5 ml 10% trichloroacetic acid (TCA) with 10 ml of 0.5 N sodium hydroxide and 3 ml of diluted Folin and Ciocalteu reagent. With reference to standard graph the tyrosine value was calculated as mg tyrosine per gm of sample.

D. Microbiological analysis of chicken meat samples

A)

Carcass traits

At the end of the sixth week, four birds from each group were slaughtered at random for carcass examination. The jugular vein of the birds was severed, and the butcher used the Halal procedure to perform the sacrifice. After being severed, it was placed in a tank that was set to scald at (60 °C for 30 seconds). A defeathered machine was used to perform the defeathering. The atlanto-occipital joint and the hock joint were used to remove the heads and shanks of dead birds, respectively. The carcass traits were examined through manual evisceration. All activities were carried out in Poultry Processing Unit of Department of Livestock Products Technology. Carcass traits such as dressing percentage, giblet yield, cut-up parts yield and eviscerated yield was evaluated based on (Varun *et al.*, 2017) [13]

Table 2. Composition of experimental diet of pre-starter, starter and finisher phase

Ingredients g/kg	Pre starter	Starter	Finisher
Maize	518.1	536.2	586.1
Soyabean DOC	418.1	392.9	333.2
Vegetable Oil	27.2	36.2	45.3

(continued on next page)

Ingredients g/kg	Pre starter	Starter	Finisher
Di-calcium phosphate	15.4	16.3	17.2
Limestone powder	9.1	7.2	7.6
Salt	2.7	2.7	2.7
DL-Methionine	1.5	1.7	1.9
Lysine	1.4	1.2	1.3
Trace -mineral mixture	0.9	0.9	0.9
Coccidiostat	0.4	0.4	0.4
Vitamin B complex	0.1	0.4	0.4
Toxin binder	0.9	0.9	0.9
Acidifier	1.3	1.3	1.3
Choline chloride	0.5	0.5	0.5

i) Dressed percentage

After slaughter of birds of respective treatment groups visceral organs such as head, neck, shanks and feet were removed and carcass weight

of particular bird was weighed. The carcass weight was expressed in terms of its dressing percentage.

$$\text{Dressing percentage (\%)} = \frac{\text{Carcass weight}}{\text{Live weight}} \times 100$$

ii) Giblet yield

Liver, heart and gizzard was collected from selected birds and weighed. This giblet weight was calculated with live body weight as

giblet yield.

$$\text{Giblet yield (\%)} = \frac{\text{Giblet weight}}{\text{Live weight}} \times 100$$

iii) Cut-up parts yield

Cut up parts were collected from selected birds and weighed. This weighed cut-up parts was calculated with live body weight as cut-up

parts yield.

$$\text{Cut-up parts yield (\%)} = \frac{\text{Weight of the individual cut-up part}}{\text{Carcass weight}} \times 100$$

iv) Eviscerated yield

Eviscerated parts were collected from selected birds and weighed. This weighed eviscerated parts was calculated with live weight basis

yield.

$$\text{Evisceration yield (\%)} = \frac{\text{Eviscerated weight without giblet}}{\text{Live weight}} \times 100$$

The microbiological quality of chicken meat was assessed on the basis of total plate count (TPC), psychrophilic count, coliform count and salmonella count as per the method of APHA (1992) [16].

The samples were prepared according to APHA (1992) [16]. 10 gm of sample was aseptically weighed and transferred to a presterilized mortar containing 90 ml of 0.1 % sterile peptone water. The sample was homogenized using a sterile pestle for 2 minutes for uniform dispersion and to get a 10^{-1} dilution of the sample. To prepare 10^{-2} dilution, 1 ml of this diluted solution was quantitatively transferred and then mixed uniformly in a test tube containing 9 ml of 0.1 % sterile peptone water. Again 1 ml of 10^{-2} dilution was added to 9 ml of 0.1 % sterile peptone water and mixed to obtain 10^{-3} dilution and so on. Appropriate dilution of 1 ml were inoculated into the different culture media (Nutrient agar for total plate count, Eosin methylene blue agar (EMB) for coliform count, Xylose-Lysine-Deoxycholate agar (XLD Agar) for salmonella count, Plate count agar for psychrophilic count) using the pour plate technique. The cultures were then incubated at 37 °C for 24-48 hours inside incubator and for psychrophilic count incubated at 4 ± 1 °C for 5 to 7 days. The inoculated plates were observed for growth after period of incubation and readings of the count

were recorded for each sample. The average number of colonies was counted and count was then expressed as $\log_{10}$ CFU/g of sample.

F) Statistical Analysis

The data obtained in the study on various parameters were statistically analysed on 'SPSS-20.0' software package as per standard methods of (Snedecor and Cochran (1995)). The experiments were conducted in four replications each and the collected data were subjected to One Way ANOVA (analysis of variance) and Duncan's Multiple Range Test (DMRT) for comparing the means to find the effects between samples at 5 % level.

Results

1. Effect of dietary herbal vitamins on carcass traits

The carcass characteristics dressing percentage, giblet percentage, cut-up parts yield and eviscerated percentage did not differ significantly ($P \geq 0.05$). From the table 3 it is observed that group T₃ showed the highest carcass traits values at the end of six weeks than T₁, T₂, positive control and control group.

Table 3. Mean $\pm$ SE values of Carcass traits of broilers fed with herbal vitamins

Treatment	Carcass Traits						
	Dressing (%)	Eviscerated wt. (%)	Giblet wt. (%)	Breast wt. (%)	Leg wt. (%)	Back wt. (%)	Wing wt. (%)
Control	76.24 $\pm$ 1.17	62.81 $\pm$ 1.10	4.58 $\pm$ 0.46	36.19 $\pm$ 1.20	30.11 $\pm$ 1.48	20.50 $\pm$ 1.32	11.30 $\pm$ 1.06
Positive Control	76.40 $\pm$ 1.19	63.13 $\pm$ 1.25	4.60 $\pm$ 0.45	36.31 $\pm$ 1.35	30.15 $\pm$ 1.28	20.55 $\pm$ 1.27	11.33 $\pm$ 1.00
T ₁	76.75 $\pm$ 0.99	63.42 $\pm$ 1.25	4.65 $\pm$ 0.44	36.25 $\pm$ 1.05	30.20 $\pm$ 1.44	20.50 $\pm$ 1.48	11.34 $\pm$ 0.97
T ₂	75.75 $\pm$ 0.68	64.22 $\pm$ 1.30	4.67 $\pm$ 0.42	36.60 $\pm$ 1.27	30.32 $\pm$ 1.56	20.60 $\pm$ 1.31	11.38 $\pm$ 0.91
T ₃	76.80 $\pm$ 0.88	64.23 $\pm$ 1.32	4.68 $\pm$ 0.44	36.79 $\pm$ 1.30	30.38 $\pm$ 1.30	20.65 $\pm$ 1.32	11.40 $\pm$ 1.02
SE Mean	76.39 $\pm$ 0.40	63.56 $\pm$ 1.32	4.63 $\pm$ 0.17	36.42 $\pm$ 0.49	30.23 $\pm$ 0.56	20.56 $\pm$ 0.53	11.35 $\pm$ 0.39
F Value	0.179 ^{NS}	0.264 ^{NS}	0.010 ^{NS}	0.042 ^{NS}	0.006 ^{NS}	0.002 ^{NS}	0.001 ^{NS}

Control: Basal diet (BIS, 2007), Positive control: Synthetic vitamin E (Alpha-Tocopherol) (222.22 IU) and Synthetic vitamin C (L-Ascorbic acid) (4000 IU), T₁: Herbal vitamin E (Alpha-Tocopherol)/Se (149.25 IU), T₂: Herbal vitamin C (L-Ascorbic acid) (4000 IU), T₃: Herbal vitamin E (Alpha-Tocopherol)/Se (149.25 IU), + Herbal vitamin C (L-Ascorbic acid) (4000 IU)

NS- $P \geq 0.05$; *- $P \leq 0.05$; **- $P \leq 0.01$

2. Effects of herbal vitamin on proximate composition of broiler chicken meat

The proximate composition of broiler chicken meat as affected by vitamins supplemented diet. The data reveal non-significant ($P \geq 0.05$) difference between the control and treatment group. The group T₃ showed higher moisture, protein, fat and ash content than T₁, T₂, positive control and control.

Table 4. Mean $\pm$ SE values of proximate composition of broiler chicken meat fed

Treatments	Proximate composition (%)			
	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Control	72.80 $\pm$ 1.19	18.18 $\pm$ 1.09	4.76 $\pm$ 0.42	1.18 $\pm$ 0.04
Positive control	73.18 $\pm$ 0.89	18.22 $\pm$ 1.10	4.77 $\pm$ 0.41	1.20 $\pm$ 0.04
T ₁	73.85 $\pm$ 0.91	19.10 $\pm$ 1.11	4.81 $\pm$ 0.41	1.20 $\pm$ 0.04
T ₂	73.58 $\pm$ 0.92	18.31 $\pm$ 0.90	4.79 $\pm$ 0.40	1.19 $\pm$ 0.04
T ₃	73.87 $\pm$ 0.91	19.44 $\pm$ 1.07	4.84 $\pm$ 0.40	1.21 $\pm$ 0.04
SE Mean	73.46 $\pm$ 0.39	18.65 $\pm$ 0.43	4.79 $\pm$ 0.16	1.19 $\pm$ 0.01
F Value	0.223 ^{NS}	0.298 ^{NS}	0.006 ^{NS}	0.080 ^{NS}

with herbal vitamins

Control: Basal diet (BIS, 2007), Positive control: Synthetic vitamin E (Alpha-Tocopherol) (222.22 IU) and Synthetic vitamin C (L-Ascorbic acid) (4000 IU), T₁: Herbal vitamin E (Alpha-Tocopherol)/Se (149.25 IU), T₂: Herbal vitamin C (L-Ascorbic acid) (4000 IU), T₃: Herbal vitamin E (Alpha-Tocopherol)/Se (149.25 IU), + Herbal vitamin C (L-Ascorbic acid) (4000 IU)

NS- $P \geq 0.05$; *- $P \leq 0.05$; **- $P \leq 0.01$

3. Effect of herbal vitamins on pH of broiler chicken meat

The pH of broiler chicken meat at refrigerated (4 ± 1 °C) storage as affected by vitamins supplemented diet. During storage pH values differ highly significantly between entire periods of refrigerated storage. Data revealed highly significant ($P \leq 0.01$) difference between entire storage period (0, 2, 4, 6, 8 days) for control, positive control, T₁, T₂ and T₃.

Table 5. Mean $\pm$ SE values of pH of broiler chicken meat fed with herbal vitamins during refrigerated (4 ± 1 °C) storage

Treatment	pH						F value
	0 th day	2 th day	4 th day	6 th day	8 th day	SE Mean	
Control	6.12 ^B $\pm$ 0.13	6.46 ^{AB} $\pm$ 0.18	6.55 ^{AB} $\pm$ 0.23	6.66 ^{AB} $\pm$ 0.14	6.78 ^A $\pm$ 0.11	6.51 $\pm$ 0.08	2.176*
Positive Control	6.14 ^B $\pm$ 0.14	6.48 ^{AB} $\pm$ 0.19	6.57 ^{AB} $\pm$ 0.24	6.69 ^{AB} $\pm$ 0.11	6.80 ^A $\pm$ 0.12	6.53 $\pm$ 0.08	2.182*
T ₁	6.25 ^B $\pm$ 0.16	6.54 ^{AB} $\pm$ 0.18	6.62 ^{AB} $\pm$ 0.17	6.76 ^A $\pm$ 0.12	6.86 ^A $\pm$ 0.09	6.61 $\pm$ 0.07	2.333*
T ₂	6.18 ^B $\pm$ 0.15	6.45 ^{AB} $\pm$ 0.22	6.54 ^{AB} $\pm$ 0.16	6.70 ^{AB} $\pm$ 0.15	6.78 ^A $\pm$ 0.10	6.53 $\pm$ 0.08	2.051*
T ₃	6.22 ^B $\pm$ 0.15	6.50 ^{AB} $\pm$ 0.25	6.60 ^{AB} $\pm$ 0.19	6.73 ^{AB} $\pm$ 0.14	6.84 ^A $\pm$ 0.13	6.58 $\pm$ 0.08	1.666*
SE Mean	6.18 $\pm$ 0.06	6.49 $\pm$ 0.08	6.57 $\pm$ 0.08	6.71 $\pm$ 0.05	6.81 $\pm$ 0.04		
F Value	0.126 ^{NS}	0.032 ^{NS}	0.029 ^{NS}	0.076 ^{NS}	0.091 ^{NS}		

Control: Basal diet (BIS, 2007), Positive control: Synthetic vitamin E (Alpha-Tocopherol) (222.22 IU) and Synthetic vitamin C (L-Ascorbic acid) (4000 IU), T₁: Herbal vitamin E (Alpha-Tocopherol)/Se (149.25 IU), T₂: Herbal vitamin C (L-Ascorbic acid) (4000 IU), T₃: Herbal vitamin E (Alpha-Tocopherol)/Se (149.25 IU), + Herbal vitamin C (L-Ascorbic acid) (4000 IU)

NS- $P \geq 0.05$; *- $P \leq 0.05$; **- $P \leq 0.01$

4. Effects of herbal vitamins on TBARS Value during refrigerated (4 ± 1 °C) storage vitamins

The thiobarbituric acid reactive substances (TBARS) of broiler chicken meat at refrigerated (4 ± 1 °C) storage as affected by vitamins supplemented diet. Enhanced lipid oxidation in meat contributes to deterioration of meat quality. The TBARS values measures the malondialdehyde (MDA) that is degradation product of lipid oxidation. During storage TBA values differ significantly ($P \leq$

0.05) between entire period (0, 2, 4, 6, 8 days) of refrigerated storage. Results were within limit of 0.2 mg MDA/kg that showed acceptabil-

ity for consumers and indicate freshness of meat (Brewer *et al.*, 1992) [17]

Table 6. Mean $\pm$ SE values of TBA of broiler chicken meat fed with herbal vitamins during refrigerated ($4 \pm 1^\circ\text{C}$) storage vitamins

Treatment	TBA (mg MDA/kg)						F value
	0 th day	2 nd day	4 th day	6 th day	8 th day	SE Mean	
Control	0.22 ^E $\pm$ 0.02	0.42 ^{aD} $\pm$ 0.02	0.58 ^{aC} $\pm$ 0.03	0.67 ^{aC} $\pm$ 0.02	0.83 ^{aA} $\pm$ 0.02	0.54 $\pm$ 0.04	74.863**
Positive Control	0.18 ^D $\pm$ 0.02	0.37 ^{abC} $\pm$ 0.02	0.53 ^{abB} $\pm$ 0.02	0.58 ^{bB} $\pm$ 0.02	0.78 ^{abA} $\pm$ 0.02	0.48 $\pm$ 0.04	71.371**
T ₁	0.14 ^D $\pm$ 0.02	0.33 ^{bC} $\pm$ 0.02	0.48 ^{bB} $\pm$ 0.02	0.52 ^{bB} $\pm$ 0.03	0.71 ^{bA} $\pm$ 0.02	0.43 $\pm$ 0.04	59.661**
T ₂	0.16 ^D $\pm$ 0.02	0.35 ^{abC} $\pm$ 0.02	0.51 ^{abB} $\pm$ 0.02	0.55 ^{bB} $\pm$ 0.02	0.75 ^{abA} $\pm$ 0.03	0.46 $\pm$ 0.04	61.161**
T ₃	0.15 ^D $\pm$ 0.02	0.34 ^{bC} $\pm$ 0.02	0.48 ^{bB} $\pm$ 0.02	0.53 ^{bB} $\pm$ 0.02	0.73 ^{abA} $\pm$ 0.02	0.45 $\pm$ 0.04	63.164**
SE Mean	0.17 $\pm$ 0.01	0.36 $\pm$ 0.01	0.51 $\pm$ 0.01	0.57 $\pm$ 0.01	0.76 $\pm$ 0.01		
F Value	1.348 ^{NS}	2.168*	2.187*	4.036*	2.465*		

Control: Basal diet (BIS, 2007), Positive control: Synthetic vitamin E (Alpha-Tocopherol) (222.22 IU) and Synthetic vitamin C (L-Ascorbic acid) (4000 IU), T₁: Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), T₂: Herbal vitamin C (L-Ascorbic acid) (4000 IU), T₃: Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), + Herbal vitamin C (L-Ascorbic acid) (4000 IU)

NS- $P \geq 0.05$; *- $P \leq 0.05$; **- $P \leq 0.01$

5. Effects of herbal vitamins on Tyrosine value during refrigerated ($4 \pm 1^\circ\text{C}$) storage

The Tyrosine of broiler chicken meat at refrigerated ($4 \pm 1^\circ\text{C}$) storage as affected by vitamins supplemented diet. The degree of autolysis and bacterial proteolysis in meat could be measured tyrosine value that actually determined tyrosine and tryptophan present in extract of meat. Data revealed significant ($P \leq 0.05$) difference between storage period (0, 2, 4, 6, 8 days) for control, positive control, T₁, T₂ and T₃

Table 7. Mean $\pm$ SE values of Tyrosine of broiler chicken meat fed with herbal vitamins during refrigerated ($4 \pm 1^\circ\text{C}$) storage

Treatment	Tyrosine (mg/g)						F value
	0 th day	2 th day	4 th day	6 th day	8 th day	SE Mean	
Control	0.210 ^{aD} $\pm$ 0.00	0.235 ^{aD} $\pm$ 0.00	0.385 ^{aC} $\pm$ 0.00	0.440 ^{aB} $\pm$ 0.00	0.565 ^{aA} $\pm$ 0.00	0.367 $\pm$ 0.03	3410.675**
Positive Control	0.206 ^{abE} $\pm$ 0.00	0.231 ^{abD} $\pm$ 0.00	0.381 ^{abC} $\pm$ 0.00	0.436 ^{abB} $\pm$ 0.00	0.561 ^{abA} $\pm$ 0.00	0.363 $\pm$ 0.03	3767.202**
T ₁	0.199 ^{bE} $\pm$ 0.00	0.225 ^{bD} $\pm$ 0.00	0.375 ^{bC} $\pm$ 0.00	0.430 ^{abB} $\pm$ 0.00	0.555 ^{bA} $\pm$ 0.00	0.357 $\pm$ 0.03	3997.778**
T ₂	0.204 ^{abE} $\pm$ 0.00	0.229 ^{abD} $\pm$ 0.00	0.379 ^{abC} $\pm$ 0.00	0.434 ^{abB} $\pm$ 0.00	0.559 ^{abA} $\pm$ 0.00	0.361 $\pm$ 0.03	3937.665**
T ₃	0.202 ^{Be} $\pm$ 0.00	0.227 ^{abD} $\pm$ 0.00	0.377 ^{abC} $\pm$ 0.00	0.432 ^{bB} $\pm$ 0.00	0.557 ^{bA} $\pm$ 0.00	0.359 $\pm$ 0.03	3441.222**
SE Mean	0.204 $\pm$ 0.00	0.229 $\pm$ 0.00	0.379 $\pm$ 0.00	0.434 $\pm$ 0.00	0.559 $\pm$ 0.00		
F Value	2.807*	2.255*	2.492*	2.453*	2.638*		

Control: Basal diet (BIS, 2007), Positive control: Synthetic vitamin E (Alpha-Tocopherol) (222.22 IU) and Synthetic vitamin C (L-Ascorbic acid) (4000 IU), T₁: Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), T₂: Herbal vitamin C (L-Ascorbic acid) (4000 IU), T₃: Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), + Herbal vitamin C (L-Ascorbic acid) (4000 IU)

NS- $P \geq 0.05$; *- $P \leq 0.05$; **- $P \leq 0.01$

6. Effect of herbal vitamin on storage of broiler chicken meat during refrigerated ($4 \pm 1^\circ\text{C}$) storage

The total plate count of broiler chicken meat at refrigerated ($4 \pm 1^\circ\text{C}$) storage as affected by vitamins supplemented diet. Data revealed significant ($P \leq 0.05$) difference between storage period (0, 2, 4, 6, 8 days) for control, positive control, T₁, T₂ and T₃. The total plate count was considerably below the incipient spoiling criterion of less than 5 log₁₀ CFU/gm at the conclusion of storage indicating that meat was acceptable at about 6th day of refrigerated storage as compare to control (El barbri *et al.*, 2008) [18] (Jay, 1996).

Table 8. Mean $\pm$ SE values of Total plate count of broiler chicken meat fed with herbal vitamins during refrigerated ($4 \pm 1^\circ\text{C}$) storage

Treatment	Total plate count (log CFU/g)						F value
	0 th day	2 th day	4 th day	6 th day	8 th day	SE Mean	
Control	2.35 ^{aE} $\pm$ 0.17	3.80 ^{aD} $\pm$ 0.10	4.58 ^{aC} $\pm$ 0.24	5.40 ^{aB} $\pm$ 0.26	6.75 ^{aA} $\pm$ 0.11	4.57 $\pm$ 0.34	741.139**
Positive Control	1.30 ^{bE} $\pm$ 0.17	2.29 ^{bD} $\pm$ 0.21	3.72 ^{bC} $\pm$ 0.13	4.50 ^{bB} $\pm$ 0.18	5.35 ^{bA} $\pm$ 0.19	3.43 $\pm$ 0.34	79.860**
T ₁	1.20 ^{bE} $\pm$ 0.14	2.20 ^{bD} $\pm$ 0.19	3.63 ^{bC} $\pm$ 0.16	4.41 ^{bB} $\pm$ 0.18	5.27 ^{bA} $\pm$ 0.18	3.34 $\pm$ 0.34	88.193**
T ₂	1.26 ^{bE} $\pm$ 0.17	2.25 ^{bD} $\pm$ 0.17	3.69 ^{bC} $\pm$ 0.17	4.46 ^{bB} $\pm$ 0.24	5.31 ^{bA} $\pm$ 0.18	3.39 $\pm$ 0.34	70.923**
T ₃	1.26 ^{bE} $\pm$ 0.19	2.22 ^{bD} $\pm$ 0.19	3.65 ^{bC} $\pm$ 0.17	4.43 ^{bB} $\pm$ 0.23	5.29 ^{bA} $\pm$ 0.16	3.37 $\pm$ 0.34	68.993**
SE Mean	1.47 $\pm$ 0.12	2.55 $\pm$ 0.16	3.85 $\pm$ 0.11	4.64 $\pm$ 0.12	5.59 $\pm$ 0.14		
F Value	7.949*	14.832*	4.995*	3.545*	13.888*		

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Control: Basal diet (BIS, 2007), Positive control: Synthetic vitamin E (Alpha-Tocopherol) (222.22 IU) and Synthetic vitamin C (L-Ascorbic acid) (4000 IU), T_1 : Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), T_2 : Herbal vitamin C (L-Ascorbic acid) (4000 IU), T_3 : Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), + Herbal vitamin C (L-Ascorbic acid) (4000 IU)

NS- $P \geq 0.05$; *- $P \leq 0.05$; **- $P \leq 0.01$

a. Effect of herbal vitamin on psychrophilic count of broiler chicken meat

The psychrophilic count was considerably below the incipient spoiling criterion of less than $4.6 \log_{10}$ CFU/gm at the conclusion of stor-

age indicating that meat was acceptable at about 6th day of refrigerated storage as compare to control (Cremer *et al.*, 1977) [19] and (Biswas *et al.*, 2017) [20]. The total psychrophilic count of broiler chicken meat at refrigerated ($4 \pm 1^\circ\text{C}$) storage as affected by vitamins supplemented diet. During storage total psychrophilic count values differ significantly ($P \leq 0.05$) between entire period of refrigerated storage. Data revealed significant ($P \leq 0.05$) difference between storage period (0, 2, 4, 6, 8 days) for control, positive control, T_1 , T_2 and T_3 . (Attia *et al.*, 2017) [21].

Table 9. Mean $\pm$ SE values of Total Psychrophilic count of broiler chicken meat fed with herbal vitamins during refrigerated ($4 \pm 1^\circ\text{C}$) storage

Treatment	Total psychrophilic count (log CFU/g)						F value
	0 th day	2 th day	4 th day	6 th day	8 th day	SE Mean	
Control	1.89 ^{aD} $\pm$ 0.13	2.30 ^{aD} $\pm$ 0.14	3.20 ^{aC} $\pm$ 0.11	4.50 ^{aB} $\pm$ 0.18	5.23 ^{aA} $\pm$ 0.16	3.42 $\pm$ 0.29	89.409**
Positive Control	1.29 ^{bD} $\pm$ 0.21	1.81 ^{abCD} $\pm$ 0.21	2.20 ^{bC} $\pm$ 0.12	3.54 ^{bB} $\pm$ 0.20	4.69 ^{bA} $\pm$ 0.18	2.71 $\pm$ 0.29	52.770**
T_1	1.21 ^{bD} $\pm$ 0.13	1.72 ^{bC} $\pm$ 0.16	2.12 ^{bC} $\pm$ 0.13	3.46 ^{bB} $\pm$ 0.21	4.62 ^{bA} $\pm$ 0.13	2.63 $\pm$ 0.29	75.609**
T_2	1.27 ^{bD} $\pm$ 0.17	1.78 ^{abC} $\pm$ 0.20	2.17 ^{bC} $\pm$ 0.18	3.50 ^{bB} $\pm$ 0.12	4.66 ^{bA} $\pm$ 0.12	2.68 $\pm$ 0.29	69.965**
T_3	1.24 ^{bD} $\pm$ 0.18	1.75 ^{abC} $\pm$ 0.10	2.14 ^{bC} $\pm$ 0.11	3.48 ^{bB} $\pm$ 0.15	4.64 ^{bA} $\pm$ 0.15	2.65 $\pm$ 0.29	90.350**
SE Mean	1.38 $\pm$ 0.08	1.87 $\pm$ 0.08	2.36 $\pm$ 0.11	3.70 $\pm$ 0.11	4.77 $\pm$ 0.08		
F Value	2.785*	2.005*	11.013*	6.387*	2.857*		

Control: Basal diet (BIS, 2007), Positive control: Synthetic vitamin E (Alpha-Tocopherol) (222.22 IU) and Synthetic vitamin C (L-Ascorbic acid) (4000 IU), T_1 : Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), T_2 : Herbal vitamin C (L-Ascorbic acid) (4000 IU), T_3 : Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), + Herbal vitamin C (L-Ascorbic acid) (4000 IU)

NS- $P \geq 0.05$; *- $P \leq 0.05$; **- $P \leq 0.01$

b. Effect of herbal vitamin on coliform and salmonella count of broiler chicken meat

Non-detection of coliform population throughout the refrigerated ($4 \pm 1^\circ\text{C}$) storage in both control and treatment groups might be due to destruction of count during hygienic practices followed during handling and packaging of fresh meat. It is indicated that meat was microbiologically safe throughout the storage period.

Table 10. Mean $\pm$ SE values of Total coliform count of broiler chicken meat fed with herbal vitamins during refrigerated ($4 \pm 1^\circ\text{C}$) storage

Treatment	Total coliform count (log CFU/g)						F value
	0 th day	2 th day	4 th day	6 th day	8 th day	SE Mean	
Control	ND	ND	ND	ND	ND		
Positive Control	ND	ND	ND	ND	ND		
T_1	ND	ND	ND	ND	ND		
T_2	ND	ND	ND	ND	ND		
T_3	ND	ND	ND	ND	ND		
SE Mean							
F Value							

Control: Basal diet (BIS, 2007), Positive control: Synthetic vitamin E (Alpha-Tocopherol) (222.22 IU) and Synthetic vitamin C (L-Ascorbic acid) (4000 IU), T_1 : Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), T_2 : Herbal vitamin C (L-Ascorbic acid) (4000 IU), T_3 : Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), + Herbal vitamin C (L-Ascorbic acid) (4000 IU)

NS- $P \geq 0.05$; *- $P \leq 0.05$; **- $P \leq 0.01$

c. Effect of herbal vitamin on salmonella count of broiler chicken meat

Non-detection of salmonella population throughout the refrigerated ($4 \pm 1^\circ\text{C}$) storage in both control and treatment groups might be due to destruction of count during hygienic practices followed during handling and packaging of fresh meat. It is indicated that meat was microbiologically safe throughout the storage period.

Table 11. Mean $\pm$ SE values of Total *Salmonella* Count of broiler chicken meat fed with herbal vitamins during refrigerated ($4 \pm 1^\circ\text{C}$) storage

Treatment	Total salmonella count (log CFU/g)						F value
	0 th day	2 th day	4 th day	6 th day	8 th day	SE Mean	
Control	ND	ND	ND	ND	ND		
Positive Control	ND	ND	ND	ND	ND		
T_1	ND	ND	ND	ND	ND		
T_2	ND	ND	ND	ND	ND		

(continued on next page)

Treatment	Total salmonella count (log CFU/g)					F value
	0 th day	2 th day	4 th day	6 th day	8 th day	
T ₃	ND	ND	ND	ND	ND	
F Value						

Control: Basal diet (BIS, 2007), Positive control: Synthetic vitamin E (Alpha-Tocopherol) (222.22 IU) and Synthetic vitamin C (L-Ascorbic acid) (4000 IU), T₁: Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), T₂: Herbal vitamin C (L-Ascorbic acid) (4000 IU), T₃: Herbal vitamin E (Alpha-Tocopherol)/Se(149.25 IU), + Herbal vitamin C (L-Ascorbic acid) (4000 IU)

NS- $P \geq 0.05$; *- $P \leq 0.05$; **- $P \leq 0.01$

Discussion

The primary goal of the study was to assess the effects of dietary supplementation with herbal vitamins on broiler chicken growth performance and meat quality. From the data it is observed that group T₃ showed the highest carcass traits values at the end of six weeks than T₁, T₂, positive control and control group. Similar results were observed by Singh *et al.*, (2021) that stated carcass characteristics such as dressing percentage, giblet weight and lymphoid organ weight did not differ significantly ($P \geq 0.05$) due to supplementation of antioxidants. Bobade *et al.*, (2009) and Niu *et al.*, (2009) [22] found that dressing percentage was not influenced by the dietary supplementation of vitamin E and Se in broilers. Similar line of findings was observed by Wu *et al.*, (2012) [23] that showed increase in breast yield by vitamin E supplementation (60 or 120 mg/kg feed) whereas the eviscerated carcass yield was not affected. Similarly Rao *et al.*, (2012) showed that no significant ($P \leq 0.05$) difference found in giblet weight on selenium supplementation in broiler diet. Sahin *et al.*, (2003) [24] reported slight increase in giblet weight with supplementation of vitamin C in broiler diet.

Similar results were observed by Pecjak *et al.*, (2022) [25] that showed carcass yield, breast, legs, wings, back yields not affected by the dietary supplementation of vitamin E, Vitamin C and selenium. Zeferino *et al.*, (2016) [26] and Habibian *et al.*, (2016) showed that dietary supplementation with vitamins E and C and combination of vitamin E and selenium had no effect on carcass, breast and thigh yields. Leskovec *et al.*, (2019) [27] reported that dietary supplementation with vitamins E, C, and selenium or their combination did not cause differences in the carcass dressing and traits in broilers under oxidative stress induced by high PUFA (Poly-unsaturated fatty acids) intake.

The results were observed by (Santosa *et al.*, 2021) [28] that showed no effect of vitamin E supplementation on moisture and fat content of breast muscles. Similarly dietary supplementation with red ginseng marc and alpha tocopherol did not significantly influence moisture level in chicken muscle (Kim *et al.*, 2013). Similar results were observed by (Mazur-Kuśnerek *et al.*, (2019) [29] that showed supplementation of antioxidants had no effect on the crude protein and ash content of breast muscles. The results reported by (Adediran *et al.*, 2022) [30] that showed supplementation of vitamin E (200 mg/kg) had non-significant ($P \geq 0.05$) effect on crude protein and fat composition of breast muscle meat.

The increase in pH may be associated with fractional proteolysis leading to produce more free alkaline groups depending on the state of variable Hassanien *et al.*, (2016) [31]. A non-significant ($P \geq 0.05$) difference was observed between control, positive control, T₁, T₂ and T₃. Similar results observed by Coetzee *et al.* (2001) [32] that showed dietary supplementation of 40, 80, 120, 160 mg vitamin E did not affect pH value of broiler meat during storage. There was slight increase in pH that was related to deamination reactions with liberation of ammonia results in increase in pH Gill (1986) [33].

The pH of meat was non significantly ($P \geq 0.05$) increased with incorporation of vitamin E/selenium, vitamin C and combination in diet. Comparable results were observed by Pečjak *et al.* (2022) [25] and Mazur-kusnierk *et al.* (2019) [29] revealed that the pH values of the breast muscle tested 15 minutes and 24 hours after post mortem were

unaffected by the various vitamin E levels. A significant ($P \leq 0.05$) difference was observed between control, positive control, T₁, T₂ and T₃ group. T₁ group showed lower TBA value as compare to T₂, T₃, positive control and control. Bartov *et al.* (1992) [34] showed that oxidative stability was best in tissues from broilers that received vitamin E and had higher tocopherol content in the meat. Similar results were observed by Coetzee *et al.* (2001) [32] that stated in case of refrigerated carcass the storage time can be extended by one day by supplementing 40 mg vitamin E/Kg that means antioxidants have certain chance to trap radical according to its chemical characteristics or specific distribution pattern. Similar results were observed by Sheldon *et al.*, (1996) that showed inverse relationship between TBA values and dietary tocopherol concentrations and indicates birds fed with 250 to 300 IU vitamin E/Kg had significantly lower breast TBA values and thus greater oxidative stability than samples from birds that received NRC recommendation diet. A significant ($P \leq 0.05$) difference was observed between control, positive control, T₁, T₂ and T₃. The increment in tyrosine value might be due to enhanced microbial load, enhanced production of proteolytic enzymes in the late logarithmic phase of microbial growth that responsible for autolysis and bacterial proteolysis Dainty *et al.*, (1975) [35]. Similar results were observed by Das *et al.*, (2014) [36] and Pearson (1968) [37]. A significant ($P \leq 0.05$) difference was observed between control, positive control, T₁, T₂ and T₃ group. T₁ group showed lower total plate count value as compare to T₂, T₃, positive control and control might be due to vitamin E decreased aspartate amino transferase (AST), serum albumin, cholesterol that leads to improvement in liver functions and increase the immune status and causes antimicrobial activity Attia *et al.*, (2017) [21]. A significant ($P \leq 0.05$) difference was observed between control, positive control, T₁, T₂ and T₃ group. T₁ group showed lower psychrophilic count value as compare to T₂, T₃, positive control and control might be due to higher phagocytic activity when supplemented with vitamin E compare to vitamin C and E combination (Attia *et al.*, 2017) [21]. Non-detection of coliform and salmonella population throughout the refrigerated (4 ± 1 °C) storage in both control and treatment groups might be due to destruction of count during hygienic practices followed during handling and packaging of fresh meat. It is indicated that meat was microbiologically safe throughout the storage period. The psychrophilic count was considerably below the incipient spoiling criterion of less than 4.6 log₁₀ CFU/gm at the conclusion of storage indicating that meat was acceptable at about 6th day of refrigerated storage as compare to control (Cremer *et al.*, 1977) [19] and (Biswas *et al.*, 2017) [20]. A significant ($P \leq 0.05$) difference was observed between control, positive control, T₁, T₂ and T₃ group. T₁ group showed lower psychrophilic count value as compare to T₂, T₃, positive control and control might be due to higher phagocytic activity when supplemented with vitamin E compare to vitamin C and E combination. Non-detection of coliform population throughout the refrigeration storage in both control and treatment groups might be due to destruction of count during hygienic practices followed during handling and packaging of fresh meat. It was observed that salmonella count was not detected in the control and treatment groups throughout the refrigeration storage. It was possible that these organisms might have not encountered during handling, hygienic practices and clean packaging at storage. It is indicated that meat was microbiologically safe throughout the storage period.

Conclusion

The result of the present experiment revealed that there was significantly better carcass traits in broilers due to supplementation of herbal vitamin E, C and selenium in different combinations that showed better carcass traits and better keeping meat quality parameters and it was concluded that incorporation of herbal vitamin E/Selenium (0.01

%) + herbal vitamin C (0.02 %) could improve keeping meat quality parameters of broiler meat. The result of present experiment revealed that there was better meat quality parameters due to supplementation of vitamins E, C and selenium in different combinations and observed that incorporation of herbal vitamin E/Selenium (0.01 %) + herbal vitamin C (0.02%) could improve quality parameters of broiler meat. Fresh meat obtained by incorporation of herbal vitamin E/Selenium (0.01 %) + herbal vitamin C (0.02 %) in diet and packed aerobically in LDPE pouches were acceptable up to 8th days at refrigeration temperature ($4 \pm 1^\circ \text{C}$) and cost-effective broiler production could be made with incorporation of herbal vitamin E (0.01 %) and herbal vitamin C (0.02 %) with combination.

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Declaration of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Authors' contribution

All authors contributed equally to this study.

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