

Comparison Effect of Adding Green Tea and Vitamin E, In Performance and Blood Parameters in Broiler under Dexamthason Stress

Ali Azizipouri¹, Mahdi Hedayati^{2*}, Milad Manafi³ and Saeed Khalaji⁴

¹Ali Azizipouri, Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran.

^{2*}MahdiHedayati, Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran.

*Email: mahvet77@gmail.com or hedayati@malayeru.ac.ir

³MiladManafi, Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran.

⁴Saeed Khalaji, Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran.

Abstract

This research aims at studying the effects of adding green tea and vitamin E on the performance, blood parameters, immune system, visceral traits, and blood cells in broilers under dexamethasone stress. In this experiment, 210 male broiler chicks were studied by completely randomized block design in 7 treatments, and each group with three replications (each replication with 10 male chicks). Treatment groups, include group 1: control (basic diet, without adding green tea and vitamin E, and no dexamethasone injection), group 2(with green tea and dexamethasone injections, without adding vitamin E), group 3(with addition of 300 mg/kg of vitamin E, without dexamethasone injection and consumption of green tea), group 4(a basic diet containing 1.5% green tea, without vitamin E and dexamethasone injections), group 5(a basic diet containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea), group 6(basic diet with 1.5% green tea with dexamethasone injection, without adding vitamin E.), and group 7(Basic diet taking 1.5% of green tea and 300 mg/kg of vitamin E with dexamethasone injection). Dexamethasone was injected twice in two stages during the 31th and 33th days for the first time and at 37th and 40th days for the second time of breeding. The minimum feed consumption was observed by green tea, and the maximum feed consumption was observed in the control group with the dexamethasone and vitamin E taking group ($P<0.05$). The maximum weight gain in the initial growth, growth, and final growth stages were in the control group and the minimum weight gain was in a group of 1.5% added green tea and dexamethasone groups ($P<0.05$). In this study, consumption of green tea reduced the performance through its effect on reducing feed consumption. Therefore, broilers' weight was reduced. Using dexamethasone as a stressful factor harmed the performance of broilers. Adding the green tea to the blood biochemical parameter reduced blood cholesterol and LDL ($P<0.05$). Blood cholesterol reduction was observed by the impact of adding green tea to the diet.

Keywords: Green Tea, Vitamin E, Blood Parameters, Flock Health

Introduction

The new generation of feed additives to livestock and poultry feed includes medicinal plants and essential oils (Brenes and Roura, 2010). Stress is a situation in which it creates biological stress mechanisms (Selye, 1963). Dexamethasone is a synthetic glucocorticoid that suppresses plasma corticosterone by inhibiting pituitary gland corticosterone secretion (Cole et al., 2000). Green tea has active medicinal components such as catechins, flavonoids, flavadiols, flavonoids, and phenolic acids. Catechin has a wide range of biological effects (Lin et al 1996), antioxidant effects. It prevents cholesterol, anti-hyperglycemia, cardiovascular disease. It has an anti-cancer effect, antibacterial activity, and antihypertensive, anti-inflammatory activity, and antimicrobial, fat-reducing, anti-viral effect. Many pieces of evidence show stress has many harmful effects on the health and performance of broilers. For example, it reduces the feed intake, growth rate, feed efficiency, carcass nutritional value, and meat quality (Temim et al., 2000; Virden et. al., 2007). Yin et al. (2008) stated that green tea can reduce the stress effects on mice. In addition, they concluded that the green tea can prevent stress as a chelating and antioxidants (Yin et al., 2008). Vitamin E is a natural antioxidant because of its ability to neutralize free radicals and reduce fat peroxidation in plasma and skeletal muscle. It can improve meat quality and prolong storage (Smet et al., 2008; Gao et al., 2010). Adding high levels of vitamin E in the diet of broilers under stress leads to a positive effect on their growth performance (Bou et al., 2004), improves body quality and weight of various body parts, quality of meat production, reduces lipid peroxidation membrane, and improves immune system function (Hashizawa et al., 2013). The polyphenolic compounds of green tea improved body weight and conversion ratio in pigs (Hossain et al., 2012), cattle (Sarker et al., 2010b), and broilers (Biswas and Wakita, 2001a). Green tea leaves, by product, and tea polyphenols are used as a supplement in broiler feed to reduce mortality in sick birds (Cao et al., 2005) to improve the performance of laying hens (Uganbayar et al., 2005; 2006) and reduce the cholesterol content of eggs (Uganbayar et al., 2005; 2006). The compounds in green tea are proved to have antiparasitic activities in inhibiting egg hatching and larval development and inactivate the infected larvae of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Green tea polyphenols reduce bacteriophage and increase staphylococci in the intestinal tract. Polyphenols extracted from green tea have inhibitory effects on gram-positive as well as gram-negative bacteria (Gadang et al., 2008). It was indicated that adding a green tea to the broilers has positive effects on the growth performance and production of the broilers' meat. Supplementing green tea powder reduces food intake, increases body weight, and improves the feed conversion ratio.

Materials and Methods

This study was conducted for 42 days in the agricultural faculty research farm of Malayer University. 210 One day chicks, strain of Ross 308 divided into 7 treatment groups in a completely randomized design and with 3 replications in each group (each replication with 10 broilers). Treatment groups, include group 1: control (basic diet, without adding green tea and vitamin E, and dexamethasone injection), group 2(Diet with green tea and dexamethasone injections, without adding vitamin E), group 3(diet with the addition of 300 mg/kg of vitamin E, without dexamethasone injection and consumption of green tea), group 4(a basic diet containing 1.5% green tea, without vitamin E and dexamethasone injections), group 5(a basic diet containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea), group 6(basic diet with 1.5% green tea with dexamethasone injection, without adding vitamin E.), and group 7(Basic diet taking 1.5% of green tea and 300 mg/kg of vitamin E with dexamethasone injection).

Dexamethasone was injected in 4 cc volume in the thigh muscle in 4 times and 2 stages during the 31st and 33rd days for the first stage and at 37th and 40th days for the second stage of breeding.

Performance

The environmental condition was equal for all test groups and birds access freely to water and feed all test period. At the end of each breeding period, feed intake and body weight were measured and the losses were recorded. The number of broilers in the studied periods was presented as the daily criterion of the broiler to eliminate the effect of losses and calculate the actual values of each trait. Therefore, all the measured traits were corrected based on the daily criterion of broiler after measurement (Lemme et al., 2006).

Internal Organs

5 birds were randomly selected from each test group and slaughtered after weighing in 42nd day age. The body was weighed and recorded after plucking features. Carcass performance was calculated by dividing carcass weight into live body weight. The relative weight of internal organs was also obtained by dividing their weight by carcass weight. In addition, the organs were weighed precisely by the laboratory scale and precision of ± 0.01 (Zhao, 2009).

Immunity and Blood Traits

2 broilers from each test unit were randomly selected to study the immune and blood traits in 28th and 42nd day age and 1 cc of blood was taken from the vagina under sterile conditions. Neobar slide was used in the hematology laboratory to count the number of monocytes, lymphocytes, eosinophils, and heterophiles in the blood.

Biochemical traits

1 cc blood is taken from a vein after 42nd day age, poured in the test tubes, and sent to the laboratory. Ordinary tubes were used to measure the concentration of serum metabolites, including cholesterol, triglycerides, calcium, and phosphorus in serum, and tubes containing anticoagulants (EDTA) were used to prepare plasma. After separating their serum, the samples were analyzed using Pars Azmoon kits and autoanalyzer (RATechnicon-1000 USA) (Hedayati et al., 2018).

Analyzing the Statistical Models

The data obtained was analyzed using SAS software version 9.3 of the GLM procedure that was obtained from a completely randomized design. The phrase "Contrast" related to the GLM procedure was used to measure the linear and quadratic nationalities between different levels of vitamin E, green tea use, and the studied variables. $Y_{ij} = \mu + T_i + e_{ij}$

In which, Y_{ij} is the relevant observation of i_{th} replication from test group i , μ = the average trait, T_i = the treatment effect, and $-e_{ij}$ = effect of test error. Duncan's (1995) multivariate test was used to compare the means of experimental groups for the desired traits and a significance level of 0.05 was considered.

Results and Discussion

The obtained results from research showed that supplementing of green tea reduces the feed and body weight increase in higher doses, and it improves FCR. The fed broilers green tea diets consumed less feed

until 28th day age (Table 1). adding the dry green tea to 0.5-1% will reduce the feed of broilers significantly (Saneko et al., 2001; Sarker et al., 2010). In another research by Yang et al. (2003), adding 2% green tea did not show a significant effect on feed intake. Table 2 shows that the maximum weight gain of broilers in the first week of breeding was in the control group (P <0.05), and the maximum weight gain and the minimum weight gain were obtained at the end of the second week in the diet contained vitamin E, and in the same week in the group fed with green tea with dexamethasone injection, respectively (P <0.05). Biswass and Wakita (2001) stated along with the findings of this research that adding higher levels of green tea to the broiler’s diets reduce the feed consumption and weight; although, FCR is improved. Furthermore, Kandeko et al. (2001) reported in research that 1, 2.5, and 5% green tea will reduce the broilers’ boy weights that are in agreement with the findings of this research. Uganbayar (2004) reported that adding 1 and 1.5% green tea supplement will reduce the body weight of broilers. Then test group, using vitamin E will improve the boy weight of broilers. Sohaib et al. (2015) research was conducted with results in agreement with this research about the effect of quercetin and alpha-tocopherol on growth performance, antioxidant potential, fat stability, and fatty acid composition was assessed in broiler breast meat. The results showed that adding dietary alpha-tocopherol had a significant effect on weight gain. Therefore, the maximum weight gain was observed in the groups containing 300 mg of quercetin and 300 and 225 mg of alpha-tocopherol per kg of diet (Sohaib et al., 2015). Despite the third week, no significant difference was observed in FCR in other breeding groups (Table 3). The maximum FCR was with dexamethasone injection at the end of the third week in the control group (P <0.05), and the minimum FCR was obtained in the fed broilers with 1.5% green tea with dexamethasone injection (P <0.05). AbdolAzam (2005) reported that adding 0.25, 0.5, and 0.75% of green ream powder to the Japanese quail diet improves FCR, particularly in 75% level. Abdu et al. (2010) investigated the effect of adding green team levels (1-5%) and liquid extract (0.5 l in 100 kg to 2.5 l in 100 kg) to the diet of laying broilers. The results show that egg production, egg mass, and FCR were improved by adding 1% leaves of green tea than the control group by 5.59, 6.79, and 7.84%, respectively. The relevant level (0.5 l/100 kg diet) of warm green tea extract improved egg production, egg mass, and FCR by 6.78, 7.46, and 8.65%, respectively. The test results are in agreement with this research results about improving the FCR of the fed broilers with the supplemented diets by green tea. Moreover, the responses of broilers to the various levels of green tea were studied by Biswas and Wakita (2001). The results of this research showed that adding high levels of green team to the diet reduces weight and feed consumption. However, this improves FCR which is in agreement with the obtained results from this research.

Table 1. feed consumption of the fed broilers by various first without stress in breeding period (g/bird)

treatment	Day					
	1-7	1-14	1-21	1-28	1-35	1-42
1	172.5 ^a	438.0 ^b	889.6 ^c	1697.3 ^a	2771 ^a	4068.1 ^a
2	157.3 ^b	435.4 ^b	973.5 ^a	1524.1 ^b	2780.8 ^a	4030.7 ^a
3	161.5 ^b	462.2 ^a	908.6 ^b	1446.0 ^c	2654.4 ^b	4107.8 ^a
4	149.8 ^c	370.1 ^d	917.9 ^b	1217.5 ^d	2300.7 ^c	3548.2 ^b
5	163.5 ^b	392.9 ^c	861.0 ^d	1436.2 ^c	2231.1 ^c	3534.7 ^b
6	155.9 ^b	399.0 ^c	815.0 ^e	1191.7 ^d	1777.6 ^e	3059.6 ^d
7	159.9 ^b	442.8 ^b	811.8 ^e	1251 ^d	2095.9 ^d	3265.2 ^c
P-value	0.001	0.000	0.000	0.000	0.000	0.000
SEM	9	14	24	26	45	98

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection, 4: Containing 1.5% of green tea without vitamin E and dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea and dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means ($P \leq 0.05$).

Table 2. weight increase of the fed broilers by various diets without stress during breeding period (g/bird)

treatment	Day					
	1-7	1-14	1-21	1-28	1-35	1-42
1	150 ^a	359 ^b	640 ^d	1088 ^a	1630 ^a	2223 ^a
2	138 ^b	354 ^c	649 ^d	935 ^c	1589 ^a	2067 ^b
3	138 ^b	382 ^a	673 ^c	964 ^b	1599 ^a	2185 ^a
4	128 ^c	311 ^d	685 ^b	801 ^c	1447 ^b	2005 ^b
5	150 ^a	354 ^c	700 ^a	977 ^b	1336 ^c	1841 ^c
6	143 ^b	350 ^c	668 ^c	784 ^c	1118 ^c	1728 ^d
7	139 ^b	360 ^b	615 ^e	840 ^d	1278 ^d	1814 ^c
P-value	0.03	0.000	0.000	0.000	0.000	0.000
SEM	8	11	18	24	42	72

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection, 4: Containing 1.5% of green tea without vitamin E with dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea with dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means ($P \leq 0.05$).

Table 3. FCR of the fed broilers by various diets without stress in breeding period

treatment	Day					
	1-7	1-14	1-21	1-28	1-35	1-42
1	1.15	1.22	1.39 ^b	1.56	1.70	1.83
2	1.14	1.23	1.50 ^a	1.63	1.75	1.95
3	1.17	1.21	1.35 ^b	1.50	1.66	1.88
4	1.17	1.19	1.34 ^b	1.52	1.59	1.77
5	1.09	1.11	1.23 ^c	1.47	1.67	1.92
6	1.09	1.14	1.22 ^c	1.52	1.49	1.77
7	1.15	1.23	1.27 ^c	1.49	1.64	1.80
P-value	0.07	0.58	0.04	0.31	0.20	0.53
SEM	0.09	0.08	0.06	0.13	0.18	0.25

Internal Weights

Weights of the internal organs are shown in table 4 that no significant difference was seen in the organs of heart, heart, proventriculus, gizzard, spleen, and pancreas. However, the weight of the spleen, bursa, and thymus, gizzard, proventriculus, and liver were impressed by different used test groups in this experiment. The percentage of liver weight to body weight in the test group with dexamethasone inserted diets was higher than the other test groups. The maximum liver ratio was observed in the test group with 2.55% dexamethasone and the minimum liver to body weight ratio was observed in the fed broilers by 1.82% green tea contained diet ($P < 0.05$). the maximum percentage of crop in the fed groups with green tea, dexamethasone, with green tea, vitamin E, with dexamethasone, and the green team was higher than other test groups with the percentage of 0.53, 0.52, and 0.49, respectively ($P < 0.05$). Furthermore, the minimum percentage of crop was observed in the control group with 0.32% ($P < 0.05$).

Fabricius bursa weight among groups were significantly different ($P < 0.05$) in a way that it was maximum with 0.2 and 0.22 in control and E vitamin diets, and minimum similarly wit 0.7% in test groups with green tea and dexamethasone, and green tea and E vitamin and dexamethasone injection ($P < 0.05$). In addition, thymus weight in the fed groups by the control and vitamin E diets was significantly higher than other test groups by 0.27 and 0.33, respectively ($P < 0.05$). On the other hand, the fed broilers by the diets containing green tea showed lower thymus weight. The results show that adding vitamin E to the broilers' diet increases the weight of lymphatic organs as well as gizzard, pre-stomach, and liver. Alternatively, added green tea in the diet significantly reduced the weight of the mentioned organs.

Table 4. the ratio of the internal organs of the fed broilers by various test diets with and without stress

Treatment	heart	liver	Crop	gizzard	Pancreas	proventriculus	spleen	bursa	Thymus
1	0.67	2.06 ^{ab}	0.32 ^b	1.59	0.28	0.54	0.09	0.20 ^a	0.27 ^a
2	0.63	2.55 ^a	0.32 ^b	1.69	0.25	0.56	0.07	0.20 ^a	0.14 ^c
3	0.63	2.18 ^b	0.42 ^{ab}	1.84	0.20	0.54	0.12	0.22 ^a	0.33 ^a
4	0.52	1.82 ^c	0.49 ^a	1.56	0.29	0.59	0.08	0.09 ^c	0.14 ^c
5	0.58	2.78 ^a	0.49 ^a	1.69	0.34	0.51	0.10	0.15 ^b	0.19 ^b
6	0.61	2.42 ^a	0.53 ^a	1.73	0.33	0.53	0.07	0.12 ^c	0.10 ^c
7	0.61	2.29 ^{ab}	0.52 ^a	1.69	0.37	0.50	0.07	0.15 ^b	0.13 ^c
P-value	0.11	0.045	0.012	0.26	0.37	0.6	0.31	0.002	0.027
SEM	0.12	0.04	0.012	0.30	0.14	0.12	0.041	0.03	0.03

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection, 4: Containing 1.5% of green tea without vitamin E with dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea with dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means ($P \leq 0.05$).

Length and pH of Gastrointestinal Tract

The length and pH of the gastrointestinal tract of the fed broilers by green tea and vitamin E are shown in Table 5 during breeding. There is so significant difference between test groups about the duodenum

length as a morphological parameter ($P>0.05$). The maximum ileum length was in the fed broilers by vitamin E and the minimum intestine length was in the fed broilers with green tea and dexamethasone injection diet. In broilers fed with green tea, pH was significantly higher than the control group with dexamethasone injection ($P <0.05$). In the ileum, the pH of the control group had the lowest value (4.9) and the fed broilers with vitamin E and green tea with dexamethasone injection had the maximum pH (6.3) among all test groups. In a research, the results of using green tea and biotin on the growth performance and destine development of broilers showed that adding 1% green tea to the diet can solely reduce the period weight, final weight, and consumed feed than other groups. However, the simultaneous use of green tea and biotin had the same effect as the antibiotic group and was higher than the control group (Sarker et al., 2010). The result showed that adding vitamin E to the broilers' diet increases the weight of lymphatic organs as well as gizzard, proventriculus, and liver.

Table 5. the gastrointestinal tract traits of the fed broilers by various test diets with and without stress

treatment	Length (mm)			pH					
	duodenum	jejunum	ileum	crop	stomach	gizzard	Doudenum	jejunum	ileum
1	38.7	82.0	61.3	5.3	3.4	3.6 ^{ab}	5.0	5.6	4.9 ^b
2	35.0	80.3	59.7	5.5	3.2	3.4 ^b	5.0	5.7	6.1 ^a
3	39.3	78.3	68.3	5.4	3.2	3.6 ^{ab}	5.2	5.7	6.1 ^a
4	35.7	89.0	54.3	5.3	3.6	3.9 ^a	5.2	5.7	6.1 ^a
5	34.7	87.0	51.7	5.3	3.3	3.8 ^{ab}	5.1	5.9	6.2 ^a
6	32.7	84.3	44.7	5.4	3.2	3.6 ^{ab}	5.3	5.8	5.7 ^{ab}
7	31.7	82.7	57.7	5.4	3.1	3.8 ^{ab}	5.2	5.7	6.3 ^a
P-value	0.27	0.36	0.28	0.94	0.185	0.044	0.272	0.748	0.0228
SEM	4.11	12.21	16.09	0.25	0.194	0.077	0.148	0.024	0.104

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection, 4: Containing 1.5% of green tea without vitamin E with dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea with dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means ($P\leq 0.05$).

Blood Cells and Immune Parameters

The related results to the blood cells and immune parameters in the 28th and 42nd day are shown in Tables 6 and 7. The results show no significant difference between the test groups about counting WBC cell bloods, heterophils, eosinophils, lymphocytes, and monocytes. Qureshi et al. (2000) stated that the process of phagocytosis by macrophages is a membrane-dependent phenomenon, which is maintained by the availability of high levels of the needed vitamin E for phagocytic integrity. Vitamin E does this by empowering immunity through its antioxidant properties or by reducing prostaglandin synthesis. The ratio of heterophils to lymphocytes is a reliable index for stress in birds (Mashaly et al., 2004). The ratio of heterophils to blood lymphocytes has been widely used to assess the presence of inflammation and has been well established in various models of avian infection (Leshchinsky and Klasing, 2001). Heterophils are the responsible cells to protect cells against invasive microorganisms. Boa-Amponsen et al. (2000)

reported that increasing the ratio of heterophils to lymphocytes occurs in response to higher doses (300 mg /kg feed) of broilers. Leshchinsky and Klasing (2001) reported a decrease in heterophils for moderate levels (50 international units per kg of diet) of vitamin E supplementation. The number of heterophiles increases during mild to moderate stress conditions, so the ratio of heterophils to lymphocytes can be used to identify physiological stresses that may lead to heterophilia and basophilia in broilers.

Table 6. the effect of various test groups on counting the blood cells in the 28th day of breeding (*10⁹/L)

Treatment	WBC	Heterophile	Lymphocytes	Eosinophils	Monocytes
1	13.48	5.25	5.78 ^{ab}	0.69	0.77
2	12.27	5.31	6.14 ^{ab}	0.71	0.73
3	11.36	5.11	4.87 ^b	0.64	0.74
4	13.18	6.14	4.32 ^b	0.66	0.80
5	10.45	3.78	5.23 ^{ab}	0.65	0.76
6	12.16	4.04	4.08 ^b	0.70	0.71
7	11.99	5.20	6.70 ^a	0.76	0.88
P-value	0.06	0.45	0.03	0.21	0.85
SEM	2.08	1.19	1.14	0.28	0.42

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection, 4: Containing 1.5% of green tea without vitamin E with dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea with dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means (P≤0.05).

Table 7. counting the blood cells in the 42nd day of breeding broilers (*10⁹/L)

Treatment	WBC	Heterophile	Lymphocytes	Eosinophils	Monocytes
1	12.05	4.88	7.87	0.61	0.70
2	11.15	4.42	7.27	0.67	0.79
3	11.85	4.76	7.67	0.65	0.76
4	13.18	4.72	5.1	0.64	0.72
5	11.27	4.14	7.73	0.65	0.76
6	11.30	4.72	5.17	0.65	0.76
7	11.30	4.19	5.70	0.64	0.77
P-value	0.61	0.97	0.55	0.28	0.22
SEM	1.44	1.12	1.19	0.03	0.041

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection, 4: Containing 1.5% of green tea without vitamin E with dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea with dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means ($P \leq 0.05$).

Blood Biochemical Parameters

Blood biochemical parameters for 28th and 42nd days are shown in Table 8, 9, 10, and 11. There is a significant difference in the blood parameters concentration (mg/dl) of cholesterol, triglyceride, and LDL in different test groups ($P < 0.05$). In addition, no significant difference was reported in the reported levels of total protein and HDL of the tested broilers ($P > 0.05$). The maximum cholesterol and triglyceride levels were in the control group and the minimum cholesterol and triglyceride levels were in the green tea and vitamin E diet as 92.33 and 129.29 mg/dl, respectively. The maximum and minimum LDL levels were observed in the fed groups by vitamin E and by green tea with vitamin E, respectively ($P < 0.05$). No significant difference was reported between the various total protein levels in various test groups ($P > 0.05$). Total protein level was lower in the fed broilers by green tea added diets (the fed broilers by green tea and green tea with vitamin E) than the other test groups. The maximum albumin level of the fed broilers was in the diet containing vitamin E ($P < 0.05$) and the minimum blood albumin level was in the fed broilers by diets containing green tea. No difference was reported between calcium, phosphorus, and AST factors. The maximum cholesterol level (168.91 mg/dl) was in the 42nd day age of broilers in diets containing vitamin E. The minimum cholesterol level was observed in groups with green tea and diet with green tea and dexamethasone by 151.47 and 151.80 mg/dl, respectively. The maximum triglyceride level was in the control diet and triglyceride was reduced using green tea. The maximum LDL level was obtained in the fed group with vitamin E as 41.30 mg/dl, and the minimum LDL level was obtained in the fed broilers by green tea as 31.23. No significant difference was seen in the phosphorus and blood albumin levels of the broilers. The blood cholesterol level in the fed broiler having a green tea diet with and without dexamethasone injection was 6.89 and 6.63. The minimum and maximum AST were reported in the green tea test group and control group. Readerstorff et al. (2003) stated that green tea has antioxidant effects due to its polyphenolic compounds including catechins, flavonols, flavonols, flavonoids, and phenolic acids. In addition, it prevents increasing cholesterol which is in agreement with the obtained results from this research. The leaves of green tea, by-products, and tea phenols can be used as a component or supplementary in the broilers' diets to prevent birds' death (Cao et al., 2005) and to reduce cholesterol content (Uganbayar et al., 2005; 2006). Yang et al. (2003) announced that using green tea reduces the cholesterol level and improves the plasma of fatty acids and meats. Adding green tea to diets reduces the blood and liver cholesterol than other groups. In addition, the use of vitamin E in the broilers diet improves blood fat. The results of Zeweil et al. (2015) on the fed broilers by vitamin E showed on the performance of blood parameters that vitamin E has a significant effect on total protein, glycoline, glucose, total cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL). Adding vitamin E has a positive effect on blood performance and parameters (Zeweil et al., 2015).

Table 8. blood biochemical parameters of 28th day broilers (mg/dl)

Treatment	Cholesterol	Triglyceride	Total protein	LDL	HDL
1	33 / 121.33 ^a	154.02 ^a	3.53	37.04 ^{ab}	57.43
2	120.0 ^a	153.14 ^a	3.49	37.06 ^{ab}	56.16
3	117.67 ^a	136.19 ^{ab}	3.49	40.36 ^a	59.56
4	119.14 ^a	130.19 ^{ab}	3.38	40.26 ^a	58.12
5	110.67 ^b	128.57 ^b	3.25	37.09 ^{ab}	59.47

6	105.31 ^b	126.43 ^b	3.39	36.73 ^{ab}	56.14
7	92.33 ^c	129.29 ^b	3.45	34.29 ^b	48.80
P-value	0.001	0.010	0.598	0.044	0.899
SEM	5.25	7.66	0.33	2.66	3.20

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection, 4: Containing 1.5% of green tea without vitamin E with dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea with dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means ($P \leq 0.05$).

Table 9. blood factors and liver enzymes in the 28th day broilers (U/L)

Treatment	Calcium	phosphorus	Albumin	ALT	AST
1	9.24	6.25	2.68 ^{ab}	25.67 ^b	123.94
2	8.16	7.32	2.52 ^b	23.32 ^b	116.73
3	9.43	6.87	2.82 ^a	24.95 ^b	114.53
4	8.98	7.36	2.93 ^a	24.65 ^b	121.12
5	7.63	7.72	2.52 ^b	29.35 ^a	119.06
6	8.19	7.52	2.47 ^b	29.28 ^a	120.37
7	8.04	7.68	2.69 ^{ab}	28.96 ^a	119.73
P-value	0.19	0.23	0.01	0.05	0.16
SEM	0.540	0.72	0.08	3.02	4.34

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection, 4: Containing 1.5% of green tea without vitamin E with dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea with dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means ($P \leq 0.05$).

Table 10. blood biochemical parameters of 42nd day broilers (mg/dl)

Test group	Cholesterol	Triglyceride	Total protein	LDL	HDL
1	162.39 ^a	166.31 ^a	4.67	38.03	58.07
2	147.97 ^b	153.60 ^b	4.04	39.23	55.79
3	168.91 ^a	158.17 ^{ab}	4.12	41.30	56.96
4	151.47 ^b	139.57 ^c	4.25	32.23	57.84
5	160.47 ^{ab}	149.93 ^b	4.25	41.00	55.45
6	151.80 ^b	141.13 ^c	3.96	33.27	54.59
7	160.81 ^{ab}	147.76 ^{bc}	4.10	35.97	58.80
P-value	0.02	0.04	0.25	0.048	0.26
SEM	10.01	11.04	0.32	3.23	2.22

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection,

4: Containing 1.5% of green tea without vitamin E with dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea with dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means ($P \leq 0.05$).

Table 11. blood factors and liver enzymes in the 42nd day broilers (U/L)

Test group	Calcium	phosphorus	Albumin	ALT	AST
1	8.96 ^a	6.56	2.73	27.07 ^a	214.68 ^a
2	9.73 ^a	7.38	2.80	25.24 ^a	210.20 ^a
3	8.61 ^a	8.15	2.76	26.15 ^a	211.57 ^a
4	6.63 ^b	6.98	2.66	23.58 ^b	188.58 ^b
5	8.68 ^a	7.35	2.60	27.95 ^a	199.49 ^{ab}
6	6.89 ^b	6.49	2.71	22.32 ^b	190.65 ^b
7	8.82 ^a	6.96	2.74	27.70 ^a	202.84 ^{ab}
P-value	0.04	0.14	0.71	0.04	0.02
SEM	1.00	1.55	0.14	2.69	15.77

1: without adding green tea and vitamin E, 2: without adding vitamin E and green tea by dexamethasone injection, 3: without green tea and by adding 300 mg/kg of vitamin E without dexamethasone injection, 4: Containing 1.5% of green tea without vitamin E with dexamethasone injection, 5: Containing 300 mg/kg of vitamin E and dexamethasone injection without adding green tea, 6: Containing 1.5% of green tea with dexamethasone injection without adding vitamin E, 7: Containing 1.5% green tea and 300 mg/kg of vitamin E with dexamethasone injection.

The non-similar Latin letters in each row show a significant difference between the means ($P \leq 0.05$).

Conclusion

Using green tea in broilers’ diet significantly reduces feed consumption, improves feed conversion ratio (FCR), and reduces cholesterol, blood triglyceride, and LDL. However, it doesn’t have a significant effect on the weight of the heart, proventriculus, and stomach organs, gizzard, spleen, pancreas, and duodenum length in terms of morphology, and pH of stomach and ileum. Moreover, using green tea and vitamin E in the diet doesn’t significantly influence the number of white blood cells (WBC), heterophils, eosinophils, lymphocytes, and monocytes. Generally, birds were under less performance stress than other test groups. Therefore, based on the obtained results, using green tea can reduce the cholesterol and fat of the body and blood. In addition, it improves the immune system of the broilers.

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Conflicts of Interests

There are no conflicts in references.

References

- Abdo, Z.M., Hassan, R.A., El-Salam, A.A. and Helmy, S.A., 2010. Effect of adding green tea and its aqueous extract as natural antioxidants to laying hen diet on productive, reproductive performance and egg quality during storage and its content of cholesterol. *Egyptian Poultry Science Journal*, 30(4), pp.1121-1149.
- Biswas, A.H. and Wakita, M., 2001. Effect of dietary Japanese green tea powder supplementation on feed utilization and carcass profiles in broilers. *The Journal of Poultry Science*, 38(1), pp.50-57.
- Bou, R., Guardiola, F., Tres, A., Barroeta, A.C. and Codony, R., 2004. Effect of dietary fish oil, α -tocopheryl acetate, and zinc supplementation on the composition and consumer acceptability of chicken meat. *Poultry Science*, 83(2), pp.282-292.
- Cao, B.H., Karasawa, Y. and Guo, Y.M., 2005. Effects of green tea polyphenols and fructo-oligosaccharides in semi-purified diets on broilers' performance and caecal microflora and their metabolites. *Asian-Aust. J. Anim. Sci*, 18(1), pp.85-89.
- Cole, M.A., Kim, P.J., Kalman, B.A. and Spencer, R.L., 2000. Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. *Psychoneuroendocrinology*, 25(2), pp.151-167.
- Erf, G.F. and Bottje, W.G., 1996. Nutrition and immune function in chickens: Benefits of dietary vitamin E supplementation. In *Proceedings of the Arkansas Nutrition Conference, Fayetteville, AR* (pp. 113-130).
- Erf, G.F., Bottje, W.G., Bersi, T.K., Headrick, M.D. and Fritts, C.A., 1998. Effects of dietary vitamin E on the immune system in broilers: altered proportions of CD4 T cells in the thymus and spleen. *Poultry Science*, 77(4), pp.529-537.
- Gadang, V.P., Hettiarachchy, N.S., Johnson, M.G. and Owens, C., 2008. Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a turkey frankfurter system. *Journal of Food Science*, 73(8), pp.M389-M394.
- Gao, J., Lin, H., Wang, X.J., Song, Z.G. and Jiao, H.C., 2010. Vitamin E supplementation alleviates the oxidative stress induced by dexamethasone treatment and improves meat quality in broiler chickens. *Poultry science*, 89(2), pp.318-327.
- Guo, F.C., Williams, B.A., Kwakkel, R.P., Li, H.S., Li, X.P., Luo, J.Y., Li, W.K. and Verstegen, M.W.A., 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry science*, 83(2), pp.175-182.
- Hashizawa, Y., Kubota, M., Kadowaki, M. and Fujimura, S., 2013. Effect of dietary vitamin E on broiler meat qualities, color, water-holding capacity and shear force value, under heat stress conditions. *Animal Science Journal*, 84(11), pp.732-736.
- Hedayati M, Manafi M. 2018. Evaluation of An herbal Compound, a Commercial Probiotic, and an Antibiotic Growth Promoter on the Performance, Intestinal Bacterial Population, Antibody Titers, and Morphology of the Jejunum and Ileum of Broilers. *Brazilian Journal of Poultry Science*, 20 (suppl 2):

305-16. <https://doi.org/10.1590/1806-9061-2017-0639>.

Hossain, M.A., AL-Raqmi, K.A.S., AL-Mijizy, Z.H., Weli, A.M. and Al-Riyami, Q., 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris*. *Asian Pacific journal of tropical biomedicine*, 3(9), p.705.

Ko, S.Y. and Yang, C.J., 2008. Effect of green tea probiotics on the growth performance, meat quality and immune response in finishing pigs. *Asian-Australasian Journal of Animal Sciences*, 21(9), pp.1339-1347.

Kondo, M., Kita, K. and Yokota, H.O., 2007. Ensiled or oven-dried green tea by-product as protein feedstuffs: Effects of tannin on nutritive value in goats. *Asian Australasian journal of animal sciences*, 20(6), p.880.

Leshchinsky, T.V. and Klasing, K.C., 2001. Relationship between the level of dietary vitamin E and the immune response of broiler chickens. *Poultry Science*, 80(11), pp.1590-1599.

Lin, Y.L., Cheng, C.Y., Lin, Y.P., Lau, Y.W., Juan, I.M. and Lin, J.K., 1998. Hypolipidemic effect of green tea leaves through induction of antioxidant and phase II enzymes including superoxide dismutase, catalase, and glutathione S-transferase in rats. *Journal of Agricultural and food chemistry*, 46(5), pp.1893-1899.

Mashaly, M.M., Hendricks 3rd, G.L., Kalama, M.A., Gehad, A.E., Abbas, A.O. and Patterson, P.H., 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. *Poultry science*, 83(6), pp.889-894.

Miller, E. R., Pastor-Barriuso, R., Dalal, D., Riemersma, R. A., Appel, L. J., &Guallar, E. (2005). Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Annals of internal medicine*, 142(1), pp.37-46.

Molan, A.L., Sivakumaran, S., Spencer, P.A. and Meagher, L.P., 2004. Green tea flavan-3-ols and oligomericproanthocyanidins inhibit the motility of infective larvae of *Teladorsagiacircumcincta* and *Trichostrongyluscolubriformis* in vitro. *Research in veterinary science*, 77(3), pp.239-243.

Noguchi, T., Cantor, A. H., & Scott, M. L. (1973). Mode of action of selenium and vitamin E in prevention of exudative diathesis in chicks. *The Journal of nutrition*, 103(10), 1502-1511.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Qureshi, M.A., 2003. Avian macrophage and immune response: an overview. *Poultry science*, 82(5), pp.691-698.

Sarker, M.S.K., Kim, G.M. and Yang, C.J., 2010. Effect of green tea and biotite on performance, meat quality and organ development in Ross broiler. *Egyptian Poultry Science Journal*, 30(1), pp.77-88.

Smet, K., Raes, K., Huyghebaert, G., Haak, L., Arnouts, S. and De Smet, S., 2008. Lipid and protein oxidation of broiler meat as influenced by dietary natural antioxidant supplementation. *Poultry science*, 87(8), pp.1682-1688.

- Sohaib, M., Butt, M.S., Shabbir, M.A. and Shahid, M., 2015. Lipid stability, antioxidant potential and fatty acid composition of broilers breast meat as influenced by quercetin in combination with α -tocopherol enriched diets. *Lipids in health and disease*, 14(1), p.61.
- Temim, S., Chagneau, A.M., Guillaumin, S., Michel, J., Peresson, R. and Tesseraud, S., 2000. Does excess dietary protein improve growth performance and carcass characteristics in heat-exposed chickens?. *Poultry Science*, 79(3), pp.312-317.
- Uganbayar, D., Bae, I.H., Choi, K.S., Shin, I.S., Firman, J.D. and Yang, C.J., 2005. Effects of green tea powder on laying performance and egg quality in laying hens. *Asian Australasian Journal of Animal Sciences*, 18(12), p.1769.
- Uganbayar, D., Shin, I.S. and Yang, C.J., 2006. Comparative performance of hens fed diets containing Korean, Japanese and Chinese green tea. *ASIAN AUSTRALASIAN JOURNAL OF ANIMAL SCIENCES*, 19(8), p.1190.
- Van Praag, H., Shubert, T., Zhao, C. and Gage, F.H., 2005. Exercise enhances learning and hippocampal neurogenesis in aged mice. *Journal of Neuroscience*, 25(38), pp.8680-8685.
- Virden, W.S., Thaxton, J.P., Corzo, A., Dozier III, W.A. and Kidd, M.T., 2007. Evaluation of Models Using Corticosterone and Adrenocorticotropin to Induce Conditions Mimicking Physiological Stress in Commercial Broilers 1 2. *Poultry science*, 86(12), pp.2485-2491.
- Wang, H., Provan, G.J. and Helliwell, K., 2000. Tea flavonoids: their functions, utilisation and analysis. *Trends in Food Science & Technology*, 11(4-5), pp.152-160.
- Wheeler, D.S. and Wheeler, W.J., 2004. The medicinal chemistry of tea. *Drug development research*, 61(2), pp.45-65.
- Xu, J., Zhu, S., Yang, F., Cheng, L., Hu, Y., Pan, G. and Hu, Q., 2003. The influence of selenium on the antioxidant activity of green tea. *Journal of the Science of Food and Agriculture*, 83(5), pp.451-455.
- Yang, C.J., Yang, I.Y., Oh, D.H., Bae, I.H., Cho, S.G., Kong, I.G., Uganbayar, D., Nou, I.S. and Choi, K.S., 2003. Effect of green tea by-product on performance and body composition in broiler chicks. *Asian australasian journal of animal sciences*, 16(6), pp.867-872.
- Yin, S.T., Tang, M.L., Su, L., Chen, L., Hu, P., Wang, H.L., Wang, M. and Ruan, D.Y., 2008. Effects of epigallocatechin-3-gallate on lead-induced oxidative damage. *Toxicology*, 249(1), pp.45-54.
- Zeweil, H., Ahmed, M., Eid, Y., Dosoky, W.M. and El-Salam, D.A., 2015. EFFECT OF VITAMIN E AND PHYTOGENIC FEED ADDITIVES ON PERFORMACE, BLOOD CONSTITUENTS AND ANTIOXIDATIVE PROPERTIES OF BROILER CHICKS. *Egyptian Poultry Science Journal*, 35(4), pp.55-58.