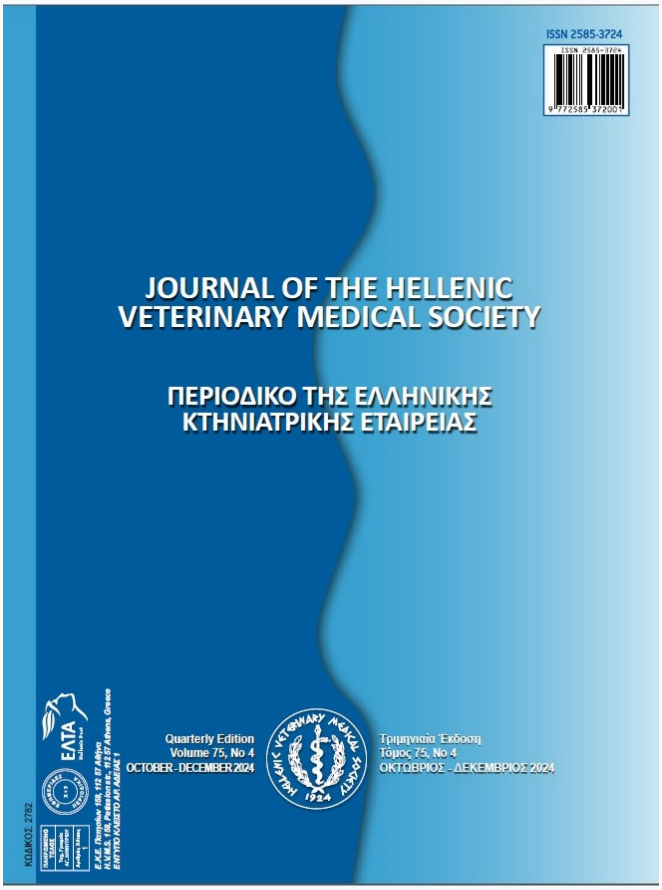
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 4 (2024)



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doi: 10.12681/jhvms.36511

J HELLENIC VET MED SOC 2024, 75 (4): 8241-8252
ΠΕΚΕ 2024, 75 (4): 8241-8252

Research article
Ερευνητικό άρθρο

The effect of green tea leaf on broiler's performance, blood profiles, and immunity

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ABSTRACT: Green tea leaf powder (GT) is used as a therapeutic supplement in humans and animals worldwide. The aim of this study was to evaluate the effects of GT on growth performance and carcass characteristics, including meat color, breast pH, cooking loss, immune response, and blood profile. The study, which included 120 local broiler chickens, was conducted using a completely randomized design with five treatments with different levels of GT in diet (0; 0.1; 0.2; 0.3; 0.4%). The results showed that GT (0.4 %) markedly improved body weight and body weight gain, while reducing feed intake and the feed conversion ratio. The effectiveness of GT in the diets was evident in improving carcass characteristics, including breast and thigh weights, compared to the control treatment ($P \leq 0.05$). The treatment with 0.4% GT resulted in the highest immune organ indices. GT increased the antibody titers of birds at 47 d and 85 d ($P \leq 0.05$). Although GT slightly enhanced the index of serum protein and decreased cholesterol levels, this study did not find any changes in the blood profiles ($P > 0.05$). Thus, the application of 0.4% GT to the diet can improve the growth performance and health of broiler chickens.

Key words: blood profile; Camellia sinensis; growth performance; immune response; local chicken.

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Date of initial submission: 11-1-2024
Date of acceptance: 21-7-2024

INTRODUCTION

Vietnam's poultry sector has developed in recent years. Poultry farming encounters many difficulties concomitant with progress, particularly disease challenges. Newcastle disease is among the most prevalent chicken diseases worldwide. It has numerous detrimental effects on productivity and economic efficiency and can lead to high mortality. Vietnamese crossbred chickens play a significant role in local chicken production in Vietnam and help to meet the country's growing demand for poultry products (Vo et al., 2019).

Legislations that limit the use of antibiotics in livestock partially affects the efficiency of chicken production. In addition, the immune system of chickens is affected by intensive breeding, medication misuse, and improper vaccinations. Therefore, it is critical to explore other options for promoting chicken growth while boosting their immune system. According to a study by Seidavi et al. (2017), medicinal herbs such as green tea (GT) have been used for 4000 years as anti-aging agents in humans and confer many benefits upon humans and animals. Recent studies have further demonstrated the beneficial effects of medicinal herbs or GT in improving growth performance and carcass quality, and strengthening the immune system in chickens (Farahat et al., 2016). The growth-promoting effects of GT (*Camellia Sinensis*) may also be due to the epigallocatechin-3-gallate (EGCG) content (Singh et al., 2011), which damages membrane proteins, cytoplasmic enzymes, bacterial cytoplasmic lipids and thus exerting antibacterial effects (Mahlake et al., 2022). In addition, the active compounds in herbal products are responsible for boosting the immune system, lowering the number of harmful bacteria, and increasing the number of beneficial bacteria in the digestive tract (Aziz-Aliabadi et al., 2023), thereby improving gut health and growth performance. Furthermore, polyphenols in green tea leaves have antibacterial and antioxidant effects and are considered to be of therapeutic value in enhancing immunity against avian influenza and Newcastle disease (Santini and Novellino, 2017). Additionally, the L-theanine component of GT leaves has been recognized as a powerful immunostimulant in broiler (Saeed et al., 2018). According to Mahlake et al. (2022), GT contains phenolic acids, catechins, and flavanols with antioxidant, anti-inflammatory and antimicrobial effects as well as synthetic epigallocatechin gallate, which helps improve poultry production and health status (Song and Seong, 2007).

In a study by (Seidavi et al., 2020), the supplement of GT leaf powder at different levels (0-1%) to the diet did not have any negative effects on the immune system of experimental chickens. The effects of GT have also been shown in a study by (Mahlake et al., 2022); growth performance and carcass performance were increased, but hematological results showed no marked responses. Similar results by Xu et al. (2020) showed a non-statistically significant increase in cholesterol levels. Whether supplemented in any form, powder, or extract, GT has a positive effect on growth by improving growth performance and increasing immunity, and can be used as a stimulant. As a natural growth promoter, GT can be used as an alternative to antibiotics in livestock (Seidavi et al., 2020). In a study by Jelveh et al. (2018), the use of GT leaf powder resulted in a higher growth efficiency than the extract; 10 g/kg GT leaf powder had an equivalent effect to 40 g/kg GT leaf extract.

To date, only a few studies have been conducted on the effects of feed and medicinal herbs, such as GT leaf powder, on growth and immunity against Newcastle disease in poultry, particularly Vietnamese backyard chickens. Given the recent increase in antibiotic residues, the use of natural feed sources to reduce medication use and improper vaccination is crucial. This study aimed to determine the effects of green tea leaf powder on the growth performance, carcass quality, blood profile, and immune response of Vietnamese crossbred chickens to the Newcastle disease vaccine.

MATERIALS AND METHODS

Location

The experiment was conducted on the experimental farm in Tra Vinh Province, Vietnam (9°55'05.8" N, 106°21'00.3" E) from July, 2022 to December, 2022. Research on animals was conducted according to the Committee of Education and Science and Vietnam regulation (No. 137/2022/HĐ.HĐKH&ĐT-ĐHTV and TCVN 8400-4:2010).

GT powder preparation

GT leaves were separated from the petiole and cut horizontally to a size of 2-3 cm. After shredding, the GT leaves were washed with water. The GT leaves were then removed, drained, and dried. GT leaves were ground into powder and spread thinly on a cool aluminum tray for an hour. The GT was further dried for 4 h at 60-65 °C at a thickness of 1.5-2 cm on the tray, with turning once every 20 min. GT was mixed according to the levels of GT in the treatments and stored under cool conditions before feeding the chickens. The chemical composition of GT was 972.6 g/kg dry matter, 295.8 g/kg DM (dry matter) crude protein, 924.8 g/kg organic matter, 145.7 g/kg DM acid detergent fiber, and 200.5 g/kg DM neutral detergent fiber. The polyphenolic components of GT include 0.7% catechin, 1.6% epicatechin, 1.5% gallocatechin, and 3.5% epigallocatechin as similar to (Farahat et al., 2016).

Experimental design and house management

This study was conducted on 120 local Noi broiler chickens with an average initial live weight of 30.17 g/bird, from one day-old to 103 days of age. The experiment was performed using a completely randomized design with five treatments and three replicates. A total of 24 chickens were allocated to each treatment and were balanced in terms of sex (1:1 for males and females). Once per week, the bird house was cleaned and disinfected, and the birds were closely monitored for abnormalities. No deaths were documented during the study period (100% survival rate). The experiment was conducted under natural conditions (12 hours of daylight) with temperatures ranging from 29 °C to 32 °C and indoor humidity averaging 60%.

The treatments were fed the same diets in terms of nutritional value and differed only in the amount of GT leaf powder supplementation. The Control (T1) had no GT addition; in T2, T3, T4, and T5, GT was added at concentrations of dietary DM of 0.1%, 0.2%, 0.3%, and 0.4%, respectively.

Vaccination schedule

This study focused on Newcastle disease vaccination. However, other vaccines were administered to protect the chickens throughout the experiment. The first and second doses of the Newcastle disease vaccination were scheduled when the chicks were 5 and 19 days old, respectively. The first and second doses of the Gumboro vaccination were scheduled on the 3rd and 14th days, respectively. Chicken pox and highly pathogenic avian influenza vaccinations were administered at 7 and 14 d of age, respectively.

Feed formulation

All ingredients were bought from a feed store in Tra Vinh Province, and the chemical composition was checked prior to feed formulation (Table 1). The chemical composition of feed, including crude protein (CP), dry matter (DM), organic matter (OM), total minerals (Ash), calcium (Ca), phosphate (P), and neutral detergent fiber (NDF) was analyzed according to (AOAC, 1990), and the ME value of feed ingredients was estimated according to the method of the (NRC, 1994)

Five treatments (Table 2) were formulated to meet the nutritional requirements of local crossbred broiler chickens. Chickens were fed *ad libitum* with the same basal feed but different levels of GT leaf powder. The basic ingredients (Table 2) were analyzed as described above, following the methods described by (AOAC, 1990).

Growth performance measurement

At the onset of the trial, one-day-old chicks were weighed. The average weekly body weight gain (BWG) per bird was determined by weighing all birds in each pen until the age of 14 weeks to record their average live body weight (BW). Calculating the average feed intake (FI) per bird required first deducting the weight of feed refusals from the weight of feed delivered (this was done daily starting at the beginning of week one and continuing until the end

Table 1. Ingredient composition used in the feed formulation (%)

Ingredients	DM	OM	CP	EE	NFE	CF	Ca	P	ME
Corn	87.2	98.4	7.15	1.8	88.2	1.24	0.004	0.14	3690
Broken rice	86.2	99.7	7.98	0.91	90.7	0.10	0.020	0.10	3480
Rice bran	88.7	92.6	13.2	8.25	63.6	7.60	0.030	2.03	2610
Soybean	87.2	94.2	45.5	1.73	43.3	3.7	0.25	0.64	2660
Fish meal	91.6	85.8	50	10	25.4	0.40	3.30	2.43	3220
DCP	100	14.8	-	-	-	-	22.0	17.0	-
Stone meal	100	-	-	-	-	-	37.9	0.01	-
Lysine	97.4	-	-	-	-	-	-	-	-
Methionine	99.3	-	-	-	-	-	-	-	-
Mineral-premix	100	-	-	-	-	-	-	-	-

Table 2. Composition of feed ingredients in the treatments (% air-dry)

Ingredients	Growing period		Chemical composition		
	1-4 weeks	5-14 weeks		1-4 weeks	5-14 weeks
Corn	24.0	30.2	DM	88.0	88.0
Broken rice	13.0	12.5	CP	21.0	19.0
Rice bran	29.0	28.3	ME (Kcal/kg)	2957	2957
Soybean	22.8	18.0	Ca	1.28	1.34
Fish meal	8.00	7.60	P	1.04	1.00
DCP	0.30	0.30			
Stole meal	2.00	2.20			
Lysin	0.20	0.20			
Methionine	0.10	0.10			
Vitamin-Mineral premix*	0.30	0.30			
Salt	0.30	0.30			
Total	100	100			

*: The vitamin and mineral - premix was formulated according to the requirements of broiler chickens including Vitamin A: 2,500,000 UI, Co: 0.28 g, Se: 0.04 g, Vitamin D_3: 600,000 UI, Vitamin K_3: 400 mg, Choline: 100,000 mg, Cu: 48 g, Mn: 14 g, Zn: 40 g, Iodine: 0.5 g, Fe: 32 g;

of week 14), and then dividing the resulting difference by the total number of birds in each pen. The amount of body weight gain over time was compared to the total amount of feed that was ingested in order to calculate the average feed conversion ratio (FCR).

Immunity analysis

Serum analysis: chickens were randomly chosen. Blood samples were collected three times. The first time was 28 d after the second vaccination dose when chickens were 47-d of age, and then at 75 and 103 d (28-d intervals). Two milliliters of blood were collected from the wing vein, using 5 mL disposable syringes with 23 G needles tilted at a 45 °C. The aim of this study was to obtain blood serum samples. Each syringe was assigned a code to differentiate between treatments. The samples were placed in cooler bags and moved to the animal analysis center. During transportation, the samples were placed in cooler bags at temperatures between 2 °C and 8 °C for 12-48 h. Blood serum was used to analyze antibody titers between treatments. Serum samples were checked for antibody titers using the hemagglutination inhibition (HI) technique, according to the instructions in the TCVN 8400-4:2010 standard regulations of Vietnam.

Blood chemistry indicators

At the end of the experiment, using 5 mL disposable syringes equipped with 23 G needles, approximately 2 mL of blood was drawn from two randomly selected broilers from each replication. For hematological analysis, blood samples were promptly transferred to ETDA. Blood tubes were stored in cooler containers, and blood samples were analyzed within 48 h of collection. The samples were transported to an animal hospital for blood biochemical analysis, including total protein (mg/dL), albumin (mg/dL), globulin (mg/dL), glucose (mg/dL), high-density lipoprotein cholesterol (HDL-c) (mg/dl), total cholesterol (mg/dL), triglycerides (mg/dL), low-density lipoprotein cholesterol (LDL-c) (mg/dl).

Carcass characteristics

Thirty birds were randomly selected and euthanized at the end of the experiment. One female and one male bird were chosen from each replicate. On the day of slaughter, the carcasses were dissected and eviscerated to determine the size of internal organs, carcass features, and meat quality. Carcass weight, breast and thigh weight, internal organs (liver and gizzard), and immunological organs (spleen, thymus, and bursa of Fabricius) were measured using a digital weighing scale. The immune organ indices were computed using Equation 1:

$$\text{Immune organ index} = (\text{immune organ weight [g]} / \text{BW [g]}) \times 1{,}000 \qquad (1)$$

After slaughter, the pH of breast meat was measured using a digital pH meter (pH/ORP/Temperature Laboratory Bench Meter Mi 151, USA) equipped with a spear-type electrode. The pH meter was cleaned and calibrated using standard solutions provided by the manufacturer. A color analyzer (Lutron RGB-1002, China) was used to determine the color rating (and a^* = redness, L^* = lightness, b^* = yellowness) of the breast muscle surface. The color analyzer was calibrated as prescribed by the manufacturer before and after measuring each experimental unit.

Cooking loss assessment

After slaughter, pieces of breast and thigh meat from each experimental unit were cut to record the initial weight of each sample. The samples were boiled in fresh water for 5 min. The samples were then removed and weighed. Cooking loss was calculated by subtracting the final weight (after cooking) from the initial weight (before cooking).

Data analysis

All the information and data collected during the research were primary data and were processed for preliminary calculations using Microsoft 365 software. In order to mitigate the impact of inadequate replication, the experimental unit for growth performance was defined as the pen, while for all other characteristics, it was the individual bird. Growth performance, hematology, antibody titers, and meat trait data were analyzed using the General Linear Model (GLM) procedure in Minitab 16.0 and GraphPad Prism v9.0. All tested parameters were considered statistically significant at $P \leq 0.05$ using the model:

$$Y_{i,j} = \mu + T_i + \text{random error}, \qquad (2)$$

where $Y_{i,j}$ is any observation for which $X_1 = i$ (i and j denote the level of the factor and replicates within the level of GT leaf powder, respectively); μ is the mean parameter; and T_i is the effect of GT leaf powder (level i).

RESULTS

Effects of GT on growth performance

The initial weights did not differ between treatments ($P > 0.05$) (Table 3). This implies that the final weight and data from the entire experiment were not affected by the differences in the initial weights. The body weights of the chickens were different among the treatments ($P \leq 0.05$). The 0.4% GT treatment showed the greatest difference at week 4 (318 g/bird), week 8 (683.1 g/bird), and week 14 (1552 g/bird) ($P \leq 0.05$). Similar to BW, BWG showed the highest performance in the 0.4% GT treatment ($P \leq 0.05$). From the first to the fourteenth week, the BWG in the treatment with 0.4% GT (15.5 g/bird/day) was better than the BWG in the treatment without GT supplementation (14.14 g/bird/day). The 0.4% GT group had the best feed consumption ($P \leq 0.05$), and good results

Table 3. Effects of GT on broiler growth performance

Criteria	Treatments					SEM	P
	T1	T2	T3	T4	T5		
BW, g							
Initial weight, g	31.1	30.2	29.8	29.6	30.0	0.49	0.30
At week 1, g	45.3	44.7	46.3	46.2	46.8	0.59	0.17
At week 4, g	278.2[b]	282.9[ab]	293.1[ab]	303.6[ab]	318.0[a]	7.59	0.024
At week 8, g	635.2[b]	636.8[b]	646.4[ab]	652.8[ab]	683.1[a]	9.81	0.037
At week 14, g	1414[b]	1442[b]	1488[ab]	1506[ab]	1552[a]	22.8	0.014
FI, g/bird							
From 1-4-week-old	13.6	14.8	14.5	14.4	14.7	0.26	0.078
From 4-8-week-old	37.4	37.4	36.8	36.6	37.9	0.44	0.30
From 8-14-week-old	64.4[a]	61.3[b]	62.0[b]	60.7[b]	62.5[ab]	0.41	0.001
From 1-14-week-old	42.2[a]	41.2[ab]	41.1[ab]	40.6[b]	41.8[ab]	0.29	0.024
BWG, g/bird/day							
From 1-4-week-old	8.82[b]	9.02[ab]	9.40[ab]	9.78[ab]	10.2[a]	0.27	0.023
From 4-8-week-old	12.7[ab]	12.6[b]	12.6[b]	12.5[b]	13.1[a]	0.060	0.002
From 8-14-week-old	18.6[b]	19.2[ab]	20.0[ab]	20.3[a]	20.7[a]	0.33	0.008
From 1-14-week-old	14.1[b]	14.4[b]	14.8[ab]	15.1[ab]	15.5[a]	0.23	0.013
FCR							
From 1-4-week-old	1.54	1.64	1.53	1.47	1.43	0.050	0.15
From 4-8-week-old	2.93	2.96	2.91	2.93	2.90	0.030	0.78
From 8-14-week-old	3.46[a]	3.19[ab]	3.09[b]	2.99[b]	3.02[b]	0.070	0.007
From 1-14-week-old	2.98[a]	2.86[ab]	2.76[ab]	2.69[b]	2.694[b]	0.050	0.010

T1: control; T2: 0.1% of GT; T3: 0.2% of GT; T4: 0.3% of GT; T5: 0.4% of GT; Means with different superscript differ significantly ($P \leq 0.05$).

Table 4. Effect of GT on broiler carcass characteristics

Criteria	Treatments					SEM	P
	T1	T2	T3	T4	T5		
Live weight, g	1426^{c}	1429^{c}	1442^{bc}	1508^{ab}	1526^{a}	16.8	0.01
Carcass weight, g	997^{b}	994^{b}	1000^{b}	1053^{ab}	1075^{a}	14.1	0.01
Carcass percentage, %	69.9	69.6	69.4	69.8	70.4	1.15	0.97
Breast weight, g	207.1^{b}	207.1^{b}	211.6^{ab}	220.8^{ab}	225.6^{a}	3.50	0.01
Breast percentage, %	20.77	20.82	21.18	20.98	20.99	0.48	0.97
Thigh weight, g	215.2^{b}	216.2^{b}	224.5^{ab}	231.9^{ab}	240.8^{a}	5.16	0.02
Thigh percentage, %	21.5	21.7	22.4	22.1	22.4	0.63	0.81
Liver weight, g	22.6	24.7	25.6	26.1	25.4	1.70	0.64
Heart weight, g	6.73	6.53	6.60	6.70	7.03	0.20	0.48
Gizzard weight, g	30.5	32.1	31.7	31.8	33.3	2.71	0.96
Small intestine length, cm	135.3	135.3	135.5	133.3	133.2	9.09	0.99
Large intestine length, cm	11.7	11.7	11.2	12.1	12.1	0.63	0.79
Cecal length, cm	17.0	17.0	18.8	16.8	17.17	0.77	0.39

T1: control; T2: 0.1% of GT; T3: 0.2% of GT; T4: 0.3% of GT; T5: 0.4% of GT; Means with different superscript differ significantly ($P \leq 0.05$).

Table 5. Effect of GT on broiler carcass quality

Criteria	Treatments					SEM	P
	T1	T2	T3	T4	T5		
pH (breast)	6.35	6.23	6.44	6.15	6.49	0.14	0.48
Meat color							
*L**	52.8	54.3	54.2	55.6	56.0	1.09	0.31
*a**	5.53	5.80	6.10	5.76	6.03	0.27	0.61
*b**	12.4	13.1	12.0	13.3	12.1	0.87	0.75
Breast meat cooking loss							
Before boiling, g	5.43	5.00	5.40	5.70	5.80	0.30	0.41
After boiling, g	4.26	4.00	4.30	4.53	4.60	0.24	0.46
Cooking loss, %	21.4	20.0	19.9	20.4	20.6	2.59	0.99
Thigh meat cooking loss							
Before boiling, g	5.56	5.46	5.83	5.63	5.06	0.28	0.44
After boiling, g	4.43	4.33	4.53	4.43	4.06	0.19	0.55
Cooking loss, %	20.3	20.6	22.2	21.1	19.7	1.61	0.85

T1: control; T2: 0.1% of GT; T3: 0.2% of GT; T4: 0.3% of GT; T5: 0.4% of GT; Means with different superscript differ significantly ($P \leq 0.05$).

Table 6. Effect of GT on blood parameters of broilers

Criteria	Treatments					SEM	P
	T1	T2	T3	T4	T5		
Total protein, mg/dL	4400	4183	4796	4160	4046	249.5	0.29
Albumin, mg/dL	1822	1876	1848	1759	1681	55.3	0.17
Globulin, mg/dL	2580	2310	2916	2400	2366	214.4	0.32
Glucose, mg/dL	245.7	258.4	256.6	250.0	254.5	7.29	0.73
Total cholesterol, mg/dL	59.5	69.2	67.1	69.3	61.3	5.75	0.65
Triglycerides, mg/dL	25.2	21.4	29.3	26.9	27.7	4.55	0.77
LDL-cholesterol, mg/dL	18.3	21.2	21.9	19.9	14.5	4.52	0.78
HDL-cholesterol, mg/dL	38.6	40.6	33.5	37.1	34.2	3.31	0.54

T1: control; T2: 0.1% of GT; T3: 0.2% of GT; T4: 0.3% of GT; T5: 0.4% of GT; Means with different superscript differ significantly ($P \leq 0.05$).

were also recorded for diets with different concentrations of GT. With low feed intake and high body weight gain, the feed conversion ratio was greatest at 0.4% GT from 8-14 w (3.027) and 1-14 w (2.694).

Effects of GT on carcass parameters

Effects of GT were evident between treatments (Table 4) ($P \leq 0.05$). The highest carcass weight was 1,075 g in the 0.4% GT treatment, followed by the treatments with 0.3% GT, 0.2% GT, and 0.1% GT; the lowest carcass weight was recorded in the treatment without GT supplementation. Furthermore, breast and thigh weights were different among the five levels of GT supplementation ($P \leq 0.05$). The highest weights were recorded in the 0.4% GT treatment (breast and thigh weight of 225.6 g and 240.8 g, respectively). However, the breast and thigh percentages were similar ($P > 0.05$). Differences were not recorded in the internal organs (liver, heart, and gizzard weight) or intestinal length (small intestine, large intestine, and cecum) ($P > 0.05$).

Effects of GT on carcass quality

This study did not observe any effects of GT consumption on meat quality (Table 5). The pH of breast meat did not differ between treatments. Additionally, no difference was observed in the breast meat color ($P \leq 0.05$). However, breast meat was lightest in the 0.4% GT treatment and darker in the treatments without GT. Furthermore, there were no substantial differences in redness or yellowness between the groups. Breast and thigh meat cooking loss percentage indicated no differences between treatments, pre- or post-cooking ($P > 0.05$) (Table 5).

Effect of GT on the immune organ indices

GT positively affected the immune organ index (Figure 1). Differences were observed in spleen and thymus indices ($P \leq 0.05$). In particular, the spleen and thymus indices were highest in the 0.4% GT treatment (2.073 and 6.118, respectively) and lowest in the treatment without GT (1.708 and 5.635, respectively). The bursa of Fabricius index (2.147-1.504) was similar among the five treatments ($P > 0.05$).

Effect of GT on the immune response to Newcastle disease

GT positively impacted the immune response to the Newcastle disease vaccine. The antibody titers decreased over time (Figure 2). For female broilers, the administration of GT increased the antibody titer in response to the Newcastle disease vaccine in birds at 75 days of age in the 0.4% GT (4.67) treatment compared to the control treatment without GT (3.00). In contrast, there was no statistical effect of green tea on antibody titers (Figure 2A and 2C). In male birds, the administration of GT increased antibody titers at both 47 and 85 days of age. The highest values were recorded in the 0.4% GT treatment at 47 days (6.33) and 85 days (6.00) of age. The lowest performance was observed in the treatment without GT (4.67 at 103 d).

Effect of GT on blood profiles

GT had no effect on blood profiles, including total protein, albumin, globulin, glucose, total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol ($P > 0.05$) (Table 6). However, marginally better results were recorded in the 0.4% GT treatment, especially for globulin.

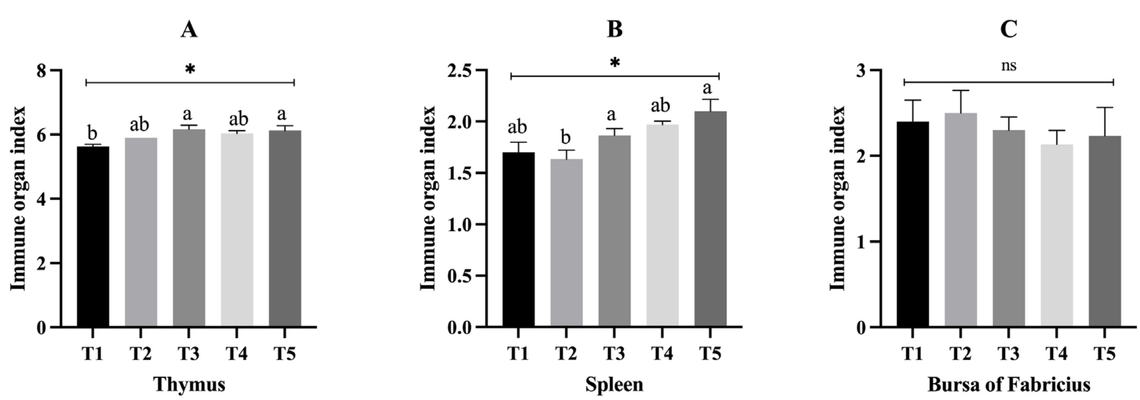

Figure 1. Effects of GT on immune organ indices. A: Effects of GT on thymus index; B: Effects of GT on spleen index; C: Effects of GT on bursa of Fabricius index; [a, b, c] - means within a column with different letters are significantly different at $P \leq 0.05$.

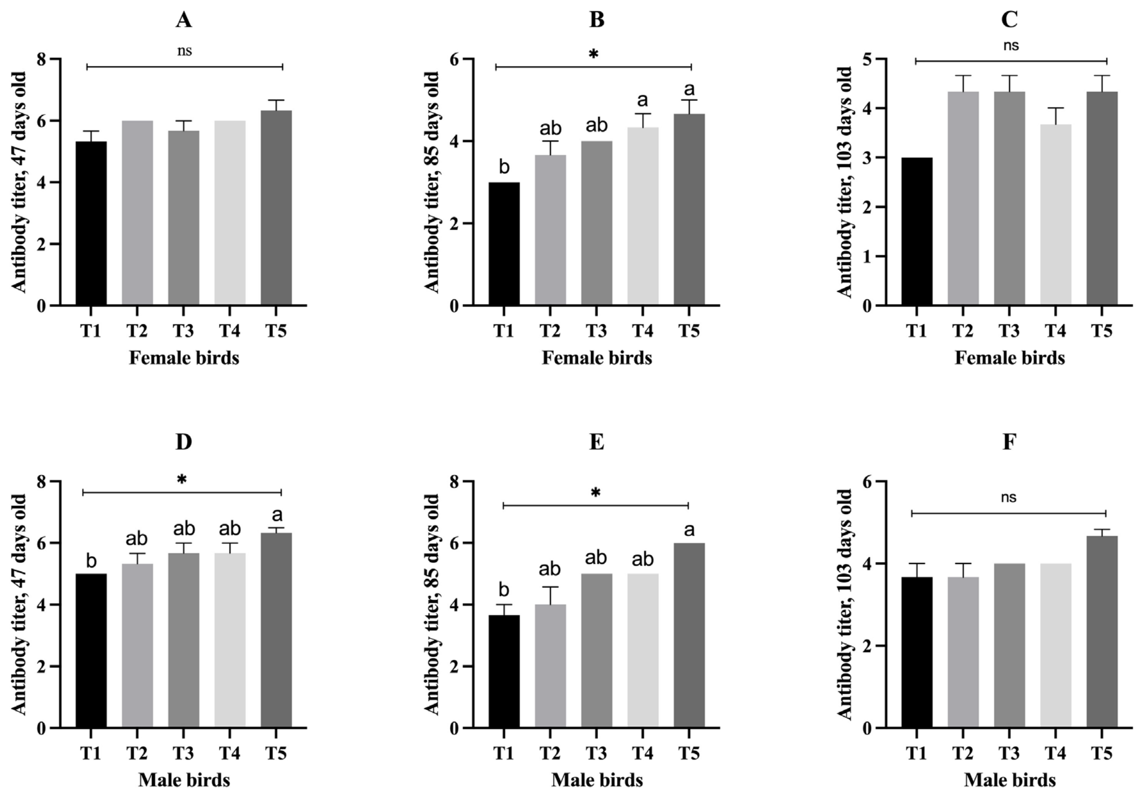

Figure 2. Effects of GT on immune response to Newcastle disease vaccine. A, B, C: Effects of GT on female bird antibody titer at 47 d, at 75 d, and at 103 days of age. D, E, F: Effects of GT on male bird antibody titer at 47 d at 75 d, and at 103 days of age; [a, b, c] - means within a column with different letters are significantly different at $P \leq 0.05$.

DISCUSSION

Tea is one of the most popular beverages globally. It is rich in phenolic compounds and catechins; GT has high polyphenol and catechin contents (Chen et al., 2019). The effects of GT leaf powder were clearly demonstrated in the current study. This is in line with the findings of (Aziz-Aliabadi et al., 2023; Mahlake et al., 2022; Saeed et al., 2018; Seidavi et al., 2017; Xu et al., 2020). At the beginning of the growing period, often before week four, intestinal function is not yet well established and the microbiota are still developing, which may explain why GT did not affect the growth performance of young broilers, as seen in this study. This has also been reported by (Chen et al., 2019). Despite this, the incorporation of GT resulted in improved weight gain at four weeks of age, indicating that the ability of birds to utilize the diets differs depending on their age. In the current study, GT supplementation improved body weight, weight gain, and feed conversion ratio, while reducing feed intake. These results are similar to those reported by (Mahlake et al., 2021). This result can be further explained by the properties of green tea, which includes a high concentration of polyphenols (Wu et al., 2016), including catechins, as antibacterial agents (Santini and Novellino, 2017), which may suppress the proliferation of harmful bacteria and improve animal intestinal health and performance. (Mahlake et al., 2021) reported that GT can improve the performance of quails by exploiting protein, amino acid, vitamin, calcium, phosphorus, selenium, and zinc absorption for sustainable poultry production (Saeed et al., 2018). L-theanine, is a non-protein, also known as gamma-glutamylethylamide, water-soluble amino acid and is the most abundant amino acid in GT (approximately 50%). According to Mahlake et al. (2022), it has pharmacological features that make it useful for poultry production. These properties include the ability to act as antioxidants and growth promoters. Fat accumulation typically requires two to three times as much feed as other tissues, and the increased abdominal fat deposition causes the broilers to consume more feed (Missio et al., 2015). However, GT powder for chickens could decrease their abdominal fat (Missio et al., 2015). This could help birds improve their growth performance.

The growth-promoting benefits of GT can be attributed to the presence of catechins, flavanols, and phenolic acids, which have antibacterial, anti-inflammatory, and antioxidant properties (Santini and Novellino, 2017). These chemicals can specifically alter the gut microbiota through their antimicrobial actions. This is achieved by changing the ability of the bacteria to survive by increasing their hydrophobicity. Consequently, the properties of cell membranes are altered, resulting in ion leakage. This makes the bacteria less virulent, which, in turn, leads to higher feed conversion, increased nutrient utilization, and stimulation of the immune system. Enhanced nutrient utilization is also a result of protection against oxidative degradation of lipids and improved nutrient absorption in the gut (Mahlake et al., 2022).

In this study, there were substantial effects of GT on carcass, breast, and thigh weights. No other indicators showed marked effects. This result is similar to that reported by Mahlake et al. (2021); there were no differences between the treatments with GT supplementation. The differences in thigh and breast weights in the current study could be due to the improved body weight gain at the end of the experiment; caffeine in GT powder could stimulate brain neurons and generate excitement in chickens, resulting in more frequent movement and a larger proportion of leg muscle mass (Chen et al., 2019), and breast muscle. Missio et al. (2015) showed that the dietary addition of Japanese GT powder to broilers reduced their abdominal fat content. This can improve breast and thigh weights. The significant increase in breast and thigh weights could also be attributed to the presence of biologically active compounds in GT, including epigallocatechins and flavanols (Khalesi et al., 2014). Synthetic epigallocatechin gallate increases carcass weight and dressing percentage under stressful conditions (Mahlake et al., 2021). Moreover, herbal antioxidants improve the properties of the carcass and its performance by blocking the production of reactive oxygen species or cell injury induced by free radicals and oxidative stress (Mateos et al., 2012; Surai et al., 2019). Mateos et al. (2012) stated that birds fed fibrous diets had enlarged gastrointestinal organs as an anatomical adaptation to efficiently utilize high dietary fiber levels. However, we did not observe any changes in the gastrointestinal organ weights. Hrnčár and Bujko (2017) demonstrated that the cecal and small intestine weights of broilers fed diets containing 1% or 1.5% GT were substantially lower than those of broilers in the control group. However, neck, crop, heart, and gizzard weights and intestinal length were higher. This can be explained by the fact that local crossbred broilers may adapt to diets with low fiber content, such as GT; therefore, there was no difference in cecal and small intestine length.

The pH of chicken meat in this study indicated that pale, soft, exudative meat did not occur. According to Fletcher (1999), there is a correlation between meat color and pH: acidic meat pH frequently results in darker meat color, and vice versa. Petracci et al. (2004) reported equivalent CIE brightness values of $L* < 50$, $50 < L* < 56$, and $L* > 56$. Based on these findings, the values of $a*$, $b*$ and $L*$ for all treatments in the current study were within the normal range and were not considered too pale. Besides, GT gets its color from chlorophyll and polyphenols found in tea, and catechins, such as epigallocatechin gallates found in GT extract, are among the most powerful natural antioxidants. The color and antioxidant capabilities of GT can result in a lighter color but are unrelated to its pH value (Erener et al., 2011). The current study showed that cooking loss was not affected by the different GT inclusions, either before or after boiling, indicating that the use of GT does not compromise the quality of broiler meat during storage or processing. As Samuel et al. (2011) reported, a lower $L*$ value is associated with lower water content in meat. The $L*$ value was not markedly different between the different inclusion levels and consequently led to no differences in the cooking loss of each treatment group.

The mammalian immune system is comprised of immunological organs, immunocytes, and immune chemicals. Immune organs, which are the key locations for the immune response, execute protective reactions to ensure the removal of detrimental stimuli. The spleen and the thymus are vital immune organs. Thus, the thymus and spleen indices are regarded as the most significant indicators of immune function (Li et al., 2017; Sun et al., 2019). According to Tong et al. (2022), the thymus plays a crucial role in the growth and maturation of T-cells. The bursa of Fabricius is crucial for B lymphocyte maturation and growth (Ribatti, 2015). As a peripheral immunological organ, the spleen is essential for mature cells to settle, present antigens, and stimulate the immune response (Song et al., 2021). In the current study, the thymus and spleen indices were increased by the GT treatment, indicating that GT had immunostimulatory activity. The antioxidant, antifungal, anti-inflammatory, and antiviral effects of flavonoids and phenolic compounds are well known, and they are abundant in GT. The high concentration of secondary plant components in GT, such as phenolics and condensed tannins, which call for detoxification by immunological organs, could have been the cause of diets (Mahlake et al., 2021). This is the reason for the improved immune organ indices with the addition of GT to the diet. Additionally, these compounds are effective in the immune response to the Newcastle disease virus (Farahat et al., 2016). GT enhances the antibody response to Newcastle virus vaccinations and might therefore be used to enhance vaccination efficacy and immunological responses (Farahat et al., 2016). In the current study, an increase in GT supplementation led to an increase in the antibody titer. The antioxidant components of GT (polyphenolic catechins and their derivatives) contributed to its humoral immunostimulatory activity. With the increased proliferation and differentiation of B lymphocytes into antibody-producing plasma cells, the body preserves immune cells and guards them against oxidative stress and environmental harm when there is an adequate supply of antioxidants (Farahat et al., 2016). Polyphenols in green tea can trigger the expression of Foxp3 and interleukin-10, leading to an increase in the number of regulatory T cells in the spleen and lymph nodes, thereby improving the immune response (Farahat et al., 2016).

There were no marked differences among the groups in the following serum parameters: total protein, glubolin, albumin, glucose, total cholesterol, triglycerides, HDL-c, and LDL-c. These results are consistent with those of Farahat et al. (2016) and Chen et al. (2019). GT is expected to increase the globulin index, an immune index in mammals. Similarly, globulin levels were increased by GT addition, but there were no statistically significant differences among the GT inclusions. There was no marked effect of GT on the cholesterol indices in this study. This study recorded lower levels of cholesterol, HDL-c and VDL-c in treatments with GT addition. As mentioned previously, GT can reduce total cholesterol, LDL-c, and HDL-c. The mechanism underlying the favorable effect of GT on lipid regulation may be linked to its high catechin content (Xu et al., 2020). Inactivation of 3-hydroxy-3-methylglutaryl coenzyme inhibits cholesterol synthesis in the liver. Additionally, this reduction may be due to a reductase and the activation of adenosine monophosphate kinase which could be due to the antioxidant actions of catechins that give a defense against lipid peroxidation for the cell walls (Mahlake et al., 2022).

CONCLUSION

The growth performance and health of Vietnamese crossbreed chickens were boosted by GT dietary supplementation. The diet with 0.4% GT increased BW, DWG, and FCR. Carcass weights, including thigh and breast weights, were also improved. GT tends to lighten breast meat, but does not affect its pH, which is critical for meat preservation. Dietary inclusion of GT resulted in increased immune organ indices. The effects of GT were also reflected by a notable increase in antibody titers in response to the Newcastle disease vaccine. However, in this study, improvements in blood profiles were not noted.

ACKNOWLEDGMENT

We acknowledge the support of time and facilities from Tra Vinh University (TVU) for this study.

CONFLICT OF INTEREST

None declared

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