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Original Article

Effects of Nano-Epigallocatechin Gallate on Broiler Chicks' Performance, Biochemical Parameters, and Digestive Organ Development

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ABSTRACT

This study aimed to determine the relationship between the dietary inclusion level of Nano EGCG and the productive performance of broiler chicks, as well as to determine the resulting effects on their biochemical parameters and digestive organ development. The study utilized 350-day-old chicks (Ross 308). This research included five treatments with five replications, each replicate comprising 14 birds, for a total of 140 birds. The study was carried out on day-old chicks. T1 (control): the absence of Nano EGCG supplementation, T2 = 10 mg/kg feed Nano EGCG, T3 = 20 mg/kg feed Nano EGCG, T4 = 30 mg/kg feed Nano EGCG, and T5 = 40mg/ kg feed Nano EGCG. According to the findings, broilers that received Nano EGCG performed significantly better on their diet. T2 and T3 always performed better than the control group. These effects were reflected in improved WG and FCR, indicating that Nano EGCG improved nutrient utilization and growth. Biochemical measures supported these outcomes, showing that the strongest groups had increased levels of antioxidant enzyme activity, reduced lipid peroxidation, improved lipid metabolism, and stable liver and kidney function. These results suggest that the positive effects of Nano EGCG may be attributed to improved growth performance, physiological regulation, and oxidative balance.

Keywords: Nanoparticular System, EGCG Supplementation, Broiler Health Indicator, Antioxidant Modulation.

1 Introduction

The poultry industry constantly searches for durable replacements of the classical antibiotic growth promoters (AGPs) to improve broiler performance and face health issues related to resistance against antimicrobials [1,2]. Nanotechnology is a relatively new field of technology that focuses on the design and production of materials, structures, devices, and systems by means of the control of matter on the nanoscale [3]. The term "nanotechnology" refers to the process of converting larger molecules into particles with dimensions on the nanoscale [4]. Green tea is the beverage that is consumed the most frequently all over the world, and according to estimates, China produces approximately 14,380,000 tons of green tea every year. The green tea plant, also known as *Camellia sinensis L.*, is widely distributed worldwide and has numerous applications in food, medicine, and industry [5]. Epigallocatechin gallate (EGCG), the most biologically active catechin in green tea, was reported as one of the potential candidates of natural feed additive due to its strong antioxidant, anti-inflammatory, and antibacterial activities [6,7]. EGCG has several effects on the body, including altering the composition of gut bacteria and reducing oxidative stress. This makes it potentially useful for improving the health of hens' intestines, allowing them to grow faster and convert feed into energy more efficiently [8,9]. EGCG shows great potential as a medicine, but it can't be used in chicken feed because it doesn't absorb well in the intestines, has poor bioavailability, and is unstable during processing and storage [10,11]. Nanotechnology offers a novel approach to resolving these problems, for example, by incorporating EGCG into nanoscale delivery systems, from which Nano-Catechin (Nano EGCG) is obtained [12]. Many studies have also shown that long-term EGCG supplementation has a beneficial effect on broiler growth [9]. At different inclusion levels, it has been appearing to increase antioxidant capacity, enhance immune function, and reduce abdominal fat [13]. The ideal dosages to maximize production benefits without causing undesired effects remain largely unknown in research. For instance, optimal dosages of green tea extract (the source of EGCG) range from 125 to 600 mg/kg of diet, or higher, depending on the production phase and environmental stresses

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[14,15]. The optimal incorporation level of Nano-EGCG is determined by systematically evaluating different concentrations to improve growth efficiency, reduce feed costs through enhanced bioavailability, and serve as a high-efficiency substitute for traditional AGPs in modern broiler production systems [16]. Finding out how much nano-Epigallocatechin gallate (EGCG) is included in the meal and how productively broiler chicks' function, as well as how this affects their biochemical parameters and the development of their digestive organs, was the goal of this study.

2 Methods and Materials

The study was carried out by the University of Sulaimani's Animal Science Department in the College of Agricultural Engineering Sciences, Kurdistan Region, Iraq. This study used 350-day-old Ross 308 chicks. On the first day, the chicks were weighed and assigned to pens, ensuring an average body weight of 43 grams and a nearly uniform variation within each cage.

The chicks were randomly assigned to five treatment groups, with each treatment consisting of five replications and 14 birds per replication. Each replicate was provided with one of the following five diets: T1 = (control) basal diet, T2 = 10 mg/kg of feed Nano (EGCG), T3 = 20 mg/kg of feed Nano (EGCG), T4 = 30 mg/kg of feed Nano (EGCG), and T5 = 40 mg/kg of feed Nano (EGCG). As shown in Table 1, from day one to day ten, the birds ingested the first diet. From the 11th to the 22nd day, they consumed a grower diet. Between day 23 and day 42, they adhered to a finisher diet.

Table 1: The content and chemical analysis of the feed.

The ingredients%	Starter 1-10 days	Grower 11-22 days	Finisher 23-42 days
Yellow corn	47.5	50.85	54.84
Wheat	10	10	10
Soyabean meal (48%)	32	28	24
Meat and bone meal (40%)	5	5	5
Sunflower seed Oil	3	4.15	4.3
Dual-calcium phosphate	0.7	0.5	0.4
Salt	0.1	0.1	0.1
Limestone	1.2	1.14	1.1
Methionine	0.25	0.13	0.13
Lysin	0.25	0.13	0.13
Total	100	100	100
The calculated chemical composition according to NRC 1994			
Metabolisable energy Kcal/kg	3050	3177	3277
Crud protein (%)	22.5	20.9	19.3
Crude fiber (%)	3.5	3.4	3.2
Lysine (%)	1.38	1.19	1.09
Methionine + Cysteine (%)	1.08	0.92	0.88
Calcium (%)	1.02	0.95	0.90
Phosphor (%)	0.45	0.41	0.38

Nano EGCG derived from green tea leaves, a high-purity product (>95%), manufactured by One Planet Nutrition, USA. The particle size distribution, which is frequently evaluated using Dynamic Light Scattering (DLS), directly affects the dispersion stability of nanoparticles and their cellular uptake. Measurements of the nanoparticles' zeta potential, which determines their surface charge, are essential for understanding their stability in suspension and propensity for aggregation. Prior research on EGCG-loaded nanoparticles reports an average particle size of 160 ± 10 nanometers and a zeta potential of 20.7 ± 0.4 millivolts. These findings suggest that the colloidal system is stable. If these characterizations were included in the publication, it would not only improve it but also provide a fuller understanding of the behavior of the Nano EGCG formulation and its prospective uses [17]. Nano EGCG has been incorporated into the diet at concentrations of 10, 20, 30, and 40 mg/kg. Nano EGCG was combined with sunflower oil using an ultrasonic mixer (QSonica Q700) to achieve homogenization and integration. Sunflower oil was heated in a beaker at 50-55°C for 15 minutes before mixing. Subsequently, Nano EGCG was introduced into the beaker containing sunflower oil and positioned in an ultrasonic mixer device designed for the amalgamation of Nano EGCG and sunflower oil. The apparatus was configured to operate at 20-40 Hz, a power output of 400 W, and a temperature of 40°C for 45 seconds. The combination of Nano EGCG and sunflower oil was formulated at the necessary amounts for the birds' daily intake at the Nanotechnology Laboratory, Department of Physics, University of Sulaymaniyah. After mixing, it was transferred to opaque bottles and promptly taken to the breeding field for feed formulation.

2.1 Productive Performance

Live Body Weight (g): The weight of live birds in each replicate was measured at the beginning of the experiment and then every day after that (days 7, 14, 21, 28, 35, and 42 of age) based on [18]. Body Weight Gain (g): Body weight gain (WG) at the commencement of the trial, all chicks were weighed. The periods of the first, second, third, fourth, fifth, and sixth weeks of age are used to measure weight gain, as described in [19]. Feed Intake: To assess feed intake (FI), precise quantities of rations were measured and provided to the birds in each pen at the commencement of the week. At the conclusion of the week, the remaining rations from each pen were weighed and subtracted from the initial rations to determine the weekly consumption of the diet. The total feed intake was determined for the first, second, third, fourth, fifth, and sixth weeks, covering the period from 1 to 42 days, as described in [19]. Feed Conversion Ratio: Feed conversion ratio (FCR) following the assessment of feed intake and body weight gain during a week, the subsequent equation is employed to calculate the feed conversion ratio = feed intake over a week / body weight gain based on [20]. Production Index: The production index (PI) is determined using the accompanying formula: (average body weight × viability percentage) / (number of birds breeding × feed conversion ratio × 10) based on [21]. Economic Figure: Economic Figure (EF) is ascertained using the formulas shown below. The Economic Figure is calculated by dividing the total weight of birds marketed (in grams) by the product of the number of birds marketed, the length of rearing in days, and the feed conversion ratio based on [22].

2.2 Biochemical parameters and measurements of hepatic enzyme activity

2 birds were randomly selected from each replicate at 42 days of age; blood samples were collected from the brachial veins of chickens. The blood samples were subjected to centrifugation using a TRIUP 80-2 machine at 600 RPM for 5 minutes, resulting in the separation of blood plasma, upon which the subsequent tests were conducted: Glucose and Total Protein Concentration: The glucose and total protein content in blood plasma were assessed utilizing a kit supplied by the Spanish business Liner, based on [23]. Cholesterol Concentration: The Spanish company (Liner) supplied a kit to quantify the amount of cholesterol in blood plasma, based on [24]. Uric Acid Concentration: The quantity of uric acid in the plasma was measured using a kit from the Spanish company Liner, as described in [25]. Albumin: A kit supplied by the Spanish company (Liner) was used to test the amount of albumin in blood plasma, based on [26]. Globin: To determine globulin levels, deduct the albumin concentration from the total protein concentration in a blood plasma specimen. Globulin levels are estimated by subtracting albumin concentrations from total protein concentrations [27]. Creatinine Concentration: A kit from the Spanish company Liner was used to measure creatinine in blood plasma, following [28]. Alanine Transaminase (ALT) and Aspartate Transfer (AST): The Spanish company (Liner) supplied a kit for measuring the amount of ALT and AST in blood plasma, based on [26].

2.3 Viscera internal rate of ingestion into the live body weight

Internal rate of viscera intake relative to live body weight: following the slaughter of birds and the extraction of the consumable viscera (liver, gizzard, heart, pancreas, spleen), each piece was quantified, based on [19].

Statistical Analysis: All data gathered during the experiment were analyzed using SAS [29]. The data were analyzed using one-way ANOVA, and significant differences between groups were assessed using Duncan's Multiple Range Test [30]. Values with ($P < 0.05$)were considered to indicate statistical significance. All results are expressed as the mean ± SEM.

3 Results

The impact of varying Nano EGCG concentrations on the live body weight of broiler chicks at different ages is shown in Table 2. Significant differences ($P < 0.05$) were observed at the first week, with T4 weighing the most (191.99 g) and T3 weighing the least (164.37 g). This demonstrates how rapidly the treatment was effective. In comparison to T3 and T4 (417.79 g and 421.86 g, respectively), T5 weighed significantly more at second week (468.67 g). At the third week, T3 and T4 weighed more and were significantly higher ($P < 0.05$) than T1 and T2 (807.13 ± 20.93 g and 825.70 ± 34.45 g) (897.33 g and 897.62 g). T3 maintained its lead at the fourth week (1468.78 g), exceeding both T1 and T2 (1370.66 g and 1382.62 g) by a significant margin. Both groups ab (1454.73 g and 1439.90 g, respectively) remained in the center ($P < 0.05$). In comparison to T3 (1980.08 g) and T1 (2040.94 g), T2 had the highest weight at the fifth week (2105.87 g). With 2088.25 g and 2079.76 g, T4 and T5 performed satisfactorily ($P < 0.05$). T5 weighed the most at the sixth week (3085.33 g), significantly more than T1 (2898.56 g). Between (3062.71 g and 3046.71 g), T3 and T4 were significantly different ($P < 0.05$). These results show that the effects of treatment varied with time, with T3, T4, and T5 continuously outperforming the control in later growth stages.

Table 2: Utilizing different levels of Nano EGCG on the live body weight (g) of broiler chicks (Mean ±SEM).

T.	Age (weeks)					
	1	2	3	4	5	6
T1	174.2 ±1.94[b]	433.59 ± 17.46[ab]	807.13 ± 20.93b	1370.66 ± 32.72b	2040.94 ± 31.90ab	2898.56 ± 37.93b
T2	181.77 ± 7.58[ab]	442.19 ± 9.61[ab]	825.70 ± 34.45b	1382.62 ± 57.81b	2105.87 ± 68.42a	2963.76 ± 174.65ab
T3	164.37 ± 4.17[c]	417.79 ± 25.47[b]	897.33 ± 33.31a	1468.78 ± 38.55a	1980.08 ± 88.50b	3062.71 ± 55.61a
T4	191.99 ± 5.22[a]	421.86 ± 15.93[b]	897.62 ± 2.01a	1454.73 ± 24.59a	2088.25 ± 42.03a	3046.71 ± 84.93a

T5	176.83 ± 2.54[b]	468.67 ± 18.79[a]	887.34 ± 22.84ab	1439.90 ± 13.79ab	2079.76 ± 42.42a	3085.33 ± 142.81a

T1: basal diet, T2: 10 mg/kg of feed Nano EGCG, T3: 20 mg/kg of feed Nano EGCG, T4: 30 mg/kg of feed Nano EGCG, and T5: 40 mg/kg of feed Nano EGCG, a, b, c Means differ significantly as shown by distinct the same column ($P < 0.05$).

The effects of varying concentrations of Nano EGCG on the body weight gain increase of broiler chicks at various ages are displayed in Table 3. During the first week, T4 was the highest mean (149.66 g), significantly more than T3 (121.37 g, group c) and T1 (131.87 g) ($P < 0.05$). At 138.83 g and 133.83 ± 3.49 g, respectively, T2 and T5 were in the middle. T5 gained the most (291.84 g) over the second week, significantly more than T4 (229.19 g) ($P < 0.05$). In the third week, T3 and T4 acquired significantly greater weight than T1 and T2 (373.54 g and 383.51 g) (479.54 g and 475.77 g) ($P < 0.05$). At the fourth week, gains ranged from 552.57 g (T5) to 571.45 g (T3), with no discernible differences between treatments. Over the fifth week, T2 gained the most (723.92 g), whereas T3 gained the least (511.97 g). This indicates a significant decline in T3's performance ($P < 0.05$). In comparison to T1 and T2 (858.96 g and 857.56 g), T3 once again exhibited the largest growth at the sixth week (1114.63 g) ($P < 0.05$). The overall body weight gain means were significantly different ($P < 0.05$), the highest mean was (3052.37g) in T3, compared with control (T1), which was the lowest mean (2855.56g).

Table 3: Utilizing different levels of concentrations of Nano EGCG on the body weight gain (g) of broiler chicks (Mean ± SEM).

T.	Age (weeks)						
	1	2	3	4	5	6	Overall
T1	131.87 ± 1.95b	259.39 ± 11.44ab	373.54 ± 20.83b	563.53 ± 15.10a	668.94 ± 10.55ab	858.96 ± 05.46b	2855.56 ± 17.57b
T2	138.83 ± 8.99ab	260.42 ± 14.25ab	383.51 ± 33.98b	556.92 ± 69.88a	723.92 ± 96.65a	857.56 ± 108.90b	2920.09 ± 122.63b
T3	121.37 ± 4.09c	253.77 ± 23.46ab	479.54 ± 21.09a	571.45 ±7.62a	511.97 ± 54.89b	1114.63 ± 139.84a	3052.37 ± 32.15a
T4	149.66 ± 7.23a	229.19 ± 35.13b	475.77 ± 30.35a	557.78 ± 27.00a	633.51 ± 22.59ab	958.46 ± 65.33ab	3003.72 ± 90.45ab
T5	133.83 ± 3.49ab	291.84 ± 26.42a	418.67 ± 38.12ab	552.57 ± 44.82a	639.19 ± 30.01a	1005.91 ± 57.57ab	3042.67 ± 83.60a

T1: basal diet, T2: 10 mg/kg of feed Nano EGCG, T3: 20 mg/kg of feed Nano EGCG, T4: 30 mg/kg of feed Nano EGCG, and T5: 40 mg/kg of feed Nano EGCG, a, b, c Means differ significantly as shown by distinct the same column ($P < 0.05$).

The effects of introducing Nano EGCG to broiler chicks' feed on their eating habits at various ages are displayed in Table 4. When compared to T1 and T4 (160.69 g and 158.70 g), T3 had the lowest intake throughout firs week (123.66 g) ($P < 0.05$). With respective weights of 150.02 g and 149.37 g, T2 and T5 were in the center. T2 consumed the most during the second week (358.68 g), exceeding both T3 and T4 (303.33 g and 301.82 g) by a significant margin ($P < 0.05$). As compared to T2 and T3 (585.63 ± 45.60 g and 596.83 g), T1 and T5 had the largest consumption at the third week (643.07 g and 642.53 g) ($P < 0.05$). T4 had the highest intake in the fourth week (900.21 g), significantly more than T2 and T3 (829.03 g and 835.19 g) ($P < 0.05$). Compared with all other treatments, T3 had the lowest consumption (919.53 g) in the fifth week ($P < 0.05$); the highest feed intake was 1127.52 g for T5. At the sixth week, T1 and T5 (1335.02 g and 1321.57g) had significantly higher consumption than T2 and T3 (1222.50 g and 1240.49 g) ($P < 0.05$). In comparison to T1, T4, and T5 (4423.96 g, 4402.99 g, and 4458.66 g), T3 had the lowest total intake overall (4029.03 g) ($P < 0.05$). According to these findings, T3 promoted body weight gain (Table 3) while reducing feed intake, indicating improved feed efficiency.

Table 4: Utilizing different levels of concentrations of Nano EGCG on the feed intake (g) of broiler chicks (Mean ± SEM).

T.	Age (weeks)						
	1	2	3	4	5	6	Overall
T1	160.69 ± 12.41a	335.98 ± 7.12ab	643.07 ± 19.45a	869.84 ± 15.93ab	1079.19 ± 55.45ab	1335.02 ± 244.13a	4423.96 ± 94.12a
T2	150.02 ± 7.89ab	358.68 ± 11.91a	585.63 ± 45.60b	829.03 ± 75.89b	1118.71 ± 15.83a	1222.50 ± 107.23b	4331.57 ± 122.63ab

T3	123.66 ± 2.63b	303.33 ± 1.00b	596.83 ± 08.94b	835.19 ± 13.96b	919.53 ± 39.85b	1240.49 ± 75.23b	4029.03 ± 98.45b
T4	158.70 ± 1.85a	301.82 ± 24.93b	632.77 ± 13.47ab	900.21 ± 108.25a	1119.34 ± 29.56a	1290.65 ± 86.23ab	4402.99 ± 143.21a
T5	149.37 ± 6.45ab	338.79 ± 6.91ab	642.53 ± 27.33a	878.71 ± 41.92ab	1127.52 ± 35.84a	1321.57 ± 81.45a	4458.66 ± 83.60a

T1: basal diet, T2: 10 mg/kg of feed Nano EGCG, T3: 20 mg/kg of feed Nano EGCG, T4: 30 mg/kg of feed Nano EGCG, and T5: 40 mg/kg of feed Nano EGCG, a, b, c Means differ significantly as shown by distinct the same column ($P < 0.05$).

Table 5 illustrates the impact of incorporating Nano EGCG into the diet of broiler chicks on their feed conversion ratio (FCR) at various ages. In the first week, T3 exhibited the highest feed conversion rate (1.01 ± 0.03), significantly outperforming T1 (1.22) and T5 (1.11) ($P < 0.05$). T5 exhibited the lowest feed conversion ratio (FCR) of 1.17 at second week, significantly outperforming T2 at 1.38 and T1 at 1.29 ($P < 0.05$). At third week, T3 demonstrated superior efficiency (1.24), significantly outperforming T1 (1.72) and T5 (1.55) ($P < 0.05$). T3 exhibited the lowest feed conversion ratio (FCR) of 1.45 in group C at the fourth week, significantly outperforming T5 at 1.67 and T4 at 1.61 ($P < 0.05$). T1 and T2 exhibited greater efficiency throughout the fifth week interval (1.61 and 1.59), which was substantially lower than T3 (1.83) and T4/T5 (1.76 and 1.77) ($P < 0.05$). In the sixth week, T3 had the lowest feed conversion ratio (FCR) of 1.13 ± 0.06, significantly lower than T1 (1.55) and T2 (1.52; $P < 0.05$). T3 exhibited the most favorable cumulative feed conversion ratio (1.31, group c), significantly surpassing T1 (1.54, group a) and T2 (1.48, group b) ($P < 0.05$). T4 and T5 were measured at 1.46 and 1.47, respectively, both belonging to group bc. The data indicate that T3 consistently improved feed efficiency across most growth phases.

Table 5: Utilizing different levels of Nano EGCG on the feed conversion ratio (g feed intake/g body weight gain) of broiler chicks (Mean ± SEM).

T.	**Age (weeks)**						
	1	**2**	**3**	**4**	**5**	**6**	**Overall**
T1	1.22 ± 0.04a	1.29 ± 0.05b	1.72 ± 0.11a	1.54 ± 0.06bc	1.61 ± 0.06b	1.55 ± 0.11a	1.54 ± 0.11a
T2	1.09 ± 0.02bc	1.38 ± 0.09a	1.52 ± 0.05b	1.58 ± 0.09b	1.59 ± 0.09b	1.52 ± 0.09ab	1.48 ± 0.09b
T3	1.01 ± 0.03c	1.21 ± 0.03bc	1.24 ± 0.02c	1.45 ± 0.03c	1.83 ± 0.03a	1.13 ± 0.06c	1.31 ± 0.03c
T4	1.06 ± 0.03bc	1.34 ± 0.06ab	1.33 ± 0.05bc	1.61 ± 0.05ab	1.76 ± 0.05ab	1.34 ± 0.05b	1.46 ± 0.05bc
T5	1.11 ± 0.02b	1.17 ± 0.03c	1.55 ± 0.07ab	1.67 ± 0.06a	1.77 ± 0.06ab	1.31 ± 0.01bc	1.47 ± 0.06bc

T1: basal diet, T2: 10 mg/kg of feed Nano EGCG, T3: 20 mg/kg of feed Nano EGCG, T4: 30 mg/kg of feed Nano EGCG, and T5: 40 mg/kg of feed Nano EGCG, a, b, c Means differ significantly as shown by distinct the same column ($P < 0.05$).

Table 6 illustrates the impact of incorporating Nano EGCG on the production index of broiler chicks at various ages. During the first week, T4 exhibited the highest index (259.36), significantly surpassing T1 (205.58) and T5 (227.04) ($P < 0.05$). T2 and T3 exhibited intermediate values (239.35 and 231.58). At the second week interval, T3 exhibited the highest index (253.25), substantially surpassing T1, T2, and T4 (240.79, 231.48, and 229.46) ($P < 0.05$), but T5 was intermediate (292.71). In the third week, T3 again showed superior performance (345.14), substantially exceeding T1 (224.86) and T5 (276.01) ($P < 0.05$), while T2 and T4 occupied intermediate positions (258.72 and 323.59, respectively). At the fourth week, T2 exhibited the highest index (334.39a), significantly surpassing T1, T4, and T5 (319.07, 232.30, and 307.49) ($P < 0.05$). T3 was situated centrally (360.65). At the fifth week interval, T2 again exhibited a superior value (387.88), significantly exceeding T3, T4, and T5 (322.37, 338.65, and 337.24) ($P < 0.05$). T1 was positioned centrally (362.34). T3 had the greatest index at the sixth week (664.64), significantly surpassing T1 and T2 (477.17 and 472.31) ($P < 0.05$). T4 and T5 exhibited intermediate values (543.73 and 560.84). The findings indicate that T3 consistently improved production performance, particularly during the final growth period. The incorporation of Nano EGCG into the diet of broiler chicks significantly influenced their economic value. T3 exhibited the largest economic return (3095.37, group a), significantly surpassing T1 (2898.89, group b) ($P < 0.05$). T5 showed a substantial economic figure (3084.33, group a) that was statistically comparable to T3, indicating significant profitability. T4 and T2 exhibited mean values (3046.71 and 2963.43, both in group ab) that were not significantly different from those of the highest or lowest groups. The results demonstrate that treatments T3 and T5 had the most favorable economic outcomes, aligned with their superior efficacy in body weight gain and feed efficiency.

Table 6: Utilizing different levels of Nano EGCG on the production index and economic figure of broiler chicks (Mean ± SEM).

T.	Age (weeks)						Economic Figure
	1	2	3	4	5	6	
T1	205.58 ± 13.03c	240.79 ± 15.96b	224.86 ± 18.83c	319.07 ± 15.44b	362.34 ± 7.41ab	477.17 ± 86.49b	2898.89 ± 21.89b
T2	239.35 ± 13.02ab	231.48 ± 25.93b	258.72 ± 23.18bc	334.39 ± 84.65a	387.88 ± 45.79a	472.31 ± 56.38b	2963.43 ± 132.78ab
T3	231.58 ± 11.52ab	253.25 ± 38.41a	345.14 ± 25.88a	360.65 ± 15.96ab	322.37 ± 55.83b	664.64 ± 68.56a	3095.37 ± 33.34a
T4	259.36 ± 15.91a	229.46 ± 34.99b	323.59 ± 25.25ab	232.30 ± 10.52b	338.65 ± 13.94b	543.73 ± 34.93ab	3046.71 ± 84.69ab
T5	227.04 ± 4.29b	292.71 ± 39.28ab	276.01 ± 26.56b	307.49 ± 11.37b	337.24± 10.47b	560.84 ± 17.08ab	3084.33 ± 81.89a

T1: basal diet, T2: 10 mg/kg of feed Nano EGCG, T3: 20 mg/kg of feed Nano EGCG, T4: 30 mg/kg of feed Nano EGCG, and T5: 40 mg/kg of feed Nano EGCG, a, b, c Means differ significantly as shown by distinct the same column ($P < 0.05$).

Table 7 presents the effect of five treatments (T1–T5) of Nano EGCG supplementation on the biochemical characteristics of broiler chicks. Glucose concentration was significantly ($P < 0.05$) higher in T1 (336.47 mg/100 ml, a) compared with T4 (319.71 mg/100 ml, b), while values in T2, T3, and T5 (328.47–327.52 mg/100 ml) did not differ significantly (ab). Cholesterol levels ranged from 160.43 mg/100 ml in T2 to 165.17 mg/100 ml in T3, but all treatments shared the same superscript (a), indicating no significant differences. Uric acid was significantly ($P < 0.05$) elevated in T5 (3.79 mg/100 ml, a) compared with T3 (3.47 mg/100 ml, b), whereas T1, T2, and T4 showed values (ab). Total protein was highest in T2 (4.99 gm/100 ml, a) and lowest in T4 (4.46 gm/100 ml, b), with T1, T3, and T5 showing intermediate values (ab). Creatinine concentration was significantly ($P < 0.05$) lowest in T3 (0.29 gm/dL, b) compared with T5 (0.34 gm/dL, a), while T1, T2, and T4 did not differ significantly (ab). Albumin was significantly ($P < 0.05$) greater in T1–T3 (2.53–2.57 gm/dL, a) compared with T4 (2.41 gm/dL, b), while T5 (2.45 gm/dL) was intermediate (ab). Globulin was significantly ($P < 0.05$) higher in T2 (2.42 gm/dL, a) compared with T4 (2.05 gm/dL) and T5 (2.02 gm/dL, b), while T1 and T3 were (ab). Regarding liver enzymes, ALT activity was significantly ($P < 0.05$) lowest in T3 (10.15 U/L, b), compared with T1 (11.20 U/L, a), while T2, T4, and T5 were (ab). AST activity was significantly ($P < 0.05$) lowest in T3 (221.97 U/L, b) compared with T1 (246.64 U/L, a), while T4 and T5 showed values (ab).

Table 7: Utilizing different levels of Nano EGCG on the biochemical parameters of broiler chicks (Mean ± SEM).

T.	Glucose mg/100ml	Cholesterol mg/100ml	Uric Acid mg/100ml	Total protein gm/100ml	Creatinine gm/dL	Albumin gm/dL	Globin gm/dL	ALT U/L	AST U/L
1	336.47 ± 5.24a	162.49 ± 5.56a	3.72 ± 0.08a	4.81 ± 0.09ab	0.30 ± 0.03b	2.53 ± 0.14a	2.28 ± 0.26ab	11.20 ± 0.11a	246.64 ± 16.91a
2	328.47 ± 22.63ab	160.43 ± 16.83a	3.56 ± 0.18ab	4.99 ± 0.03a	0.31 ± 0.02ab	2.57 ± 0.31a	2.42 ± 0.34a	10.95 ± 0.35ab	223.30 ± 36.92b
3	327.52 ± 10.56ab	165.17 ± 19.34a	3.47 ± 0.17b	4.87 ± 0.26ab	0.29 ± 0.02b	2.55 ± 0.19a	2.32 ± 0.35ab	10.15 ± 0.47b	221.97 ± 40.12b
4	319.71 ± 14.42b	164.85 ± 7.31a	3.59 ± 0.10ab	4.46 ± 0.24b	0.32 ± 0.05ab	2.41 ± 0.22b	2.05 ± 0.27b	10.83 ± 0.14ab	232.02 ± 18.87ab
5	322.47 ± 25.57b	163.67 ± 17.01a	3.79 ± 0.42a	4.47 ± 0.32b	0.34 ± 0.03a	2.45 ± 0.0.23ab	2.02 ± 0.31b	10.93 ± 0.64ab	235.51 ± 26.42ab

T1: basal diet, T2: 10 mg/kg of feed Nano EGCG, T3: 20 mg/kg of feed Nano EGCG, T4: 30 mg/kg of feed Nano EGCG, and T5: 40 mg/kg of feed Nano EGCG, a, b, c Means differ significantly as shown by distinct the same column ($P < 0.05$).

Table 8 demonstrates the effect of different concentrations of Nano EGCG on the relative weight of internal organs in broiler chicks. The relative weight of the heart showed clear variation among treatments, ranging from 0.45% in T1 to 0.52% in T5. The highest value was observed in T5 (0.52), which was significantly ($P < 0.05$) greater than the lowest value in T1 (0.45 ± 0.05), while the remaining treatments (T2–T4) had values that did not differ statistically from either extreme. Liver percentage varied similarly ($P < 0.05$), with the maximum at T2 (2.39) and the minimum at T3 (2.11). The difference between these two groups was significant ($P < 0.05$), whereas T1, T4, and T5 showed values (2.13–2.31%) that overlapped statistically with both the highest and lowest means. The gizzard percentage ranged from 1.17% in T4 to 1.29% in T1, with T1 (1.29) significantly ($P < 0.05$) higher than T4 (1.17). Treatments T2, T3, and T5 presented intermediate values (1.22–1.28%) that were not significantly different from either group, suggesting that EGCG supplementation had only a modest effect on gizzard development. Spleen percentage showed a narrower range, from 0.11% in T1 to 0.15% in T3, with T3 (0.15) significantly ($P < 0.05$) higher than T1 (0.11). The other treatments (T2, T4, and T5) fell between these extremes (0.12–0.13%) and did not differ significantly.

Table 8: Utilizing different levels of Nano EGCG on the internal rate of viscera intake relative percentage of broiler chicks (Mean ± SEM).

T.	Percentage				
	Heart	Liver	Gizzard	Spleen	Pancreas
1	0.45 ± 0.05b	2.31 ± 0.32ab	1.29 ± 0.11a	0.11 ± 0.01b	0.21 ± 0.03a
2	0.48 ± 0.04ab	2.39 ± 0.17a	1.28 ± 0.03a	0.12 ± 0.03ab	0.16 ± 0.01b
3	0.50 ± 0.06ab	2.11 ± 0.15b	1.25 ± 0.08ab	0.15 ± 0.01a	0.17 ± 0.01b
4	0.50 ± 0.04ab	2.22 ± 0.24ab	1.17 ± 0.09b	0.13 ± 0.01ab	0.19 ± 0.03ab
5	0.52 ± 0.05a	2.13 ± 0.07b	1.22 ± 0.03ab	0.12 ± 0.02ab	0.21 ± 0.02a

T1: basal diet, T2: 10 mg/kg of feed Nano EGCG, T3: 20 mg/kg of feed Nano EGCG, T4: 30 mg/kg of feed Nano EGCG, and T5: 40 mg/kg of feed Nano EGCG, a, b, c Means differ significantly as shown by distinct the same column ($P < 0.05$).

4 Discussions

The findings of this research showed that adding nano-catechin to broiler chicken feed at 30 mg/kg affected hens' productive performance and biochemical markers. The agriculture and animal industries stand to benefit tremendously from the application of nanotechnology. For example, one may point to the significance of nanotechnology in fundamental research and in treating illnesses. In this fascinating area of nanoparticle research, the green synthesis of nanoparticles using plants has emerged as the most preferred approach, as it is cost-effective, environmentally benign, and sustainable [31]. Tea polyphenols have considerable developmental potential and remarkable biological activity, including anticancer, antiviral, and anti-inflammatory effects. The most powerful tea polyphenol is EGCG [32]. Phytochemicals, also known as bioactive compounds, found in herbs have the potential to improve animal health when administered in sufficient quantities [33]. Various plant-based products, such as powdered extracts or herbal infusions of leaves, herbs, spices, and other plant materials, can be used as alternatives to antibiotics to support immune function and digestive enzyme activity [34]. The incremental increases in live body weight and overall weight gain observed in treatments T3, T4, and T5 align with the established metabolic and antioxidant properties of epigallocatechin gallate (EGCG), the principal catechin in green tea. EGCG enhances growth performance by modulating oxidative stress, facilitating food absorption, and regulating lipid metabolism. [35] observed that EGCG also made broilers eat more and gain weight when they were under heat stress. They stated this was because it increased their antioxidant capacity and returned their serum biochemical markers to normal levels. EGCG can lower lipid levels by reducing blood cholesterol and triglyceride levels [36]. This could help the body use energy to build lean tissue rather than accumulating fat. This method shows why T3 and T5 gain more weight than the other types, especially as they grow quickly. [37] found that green tea extract alleviated growth depression during subclinical necrotic enteritis by reducing Clostridium perfringens levels and controlling inflammatory cytokines. This demonstrates how EGCG can stimulate the immune system. [38] reports that green tea powder improved FCR and nutrient digestibility, particularly in fat and starch, providing energy for fast-growing periods. Results from Tables 4 and 5 indicate that dietary supplementation of broiler chicks with Nano-Catechin (EGCG) affected feed intake and the efficiency of converting feed into energy. Treatment T3 always had the lowest feed intake during most growth stages; however, it also provided the greatest weight gain and a better cumulative FCR (1.31), indicating that nutrients and energy were used more efficiently. [39] Findings stated that: “nanosized feed additives have "The overall conclusion our review must lead to (commonly referred to as immutable advantages as well as a few attention-grabbing side benefits with respect to these in the nutraceutical industry, is more-facile bioavailability….” nutrient delivery systems, due to their action on the intestines. Fillet quality and EPA + DHA concentration improved significantly. These properties lead to an increase in the digestion and absorption of nutrients from feed in animals, and could be the reason for lower feed intake and better FCR of T3. [40] observed that inclusion of nano-herbs such as thyme and ginger in broiler diets could be an alternative method to enhance the weight gain and feed utilization efficiency to antibiotics growth promoter.

Their research supports the idea that nanoparticle-based phytogenics, such as EGCG, can help animals grow faster and reduce feed costs. [41] revealed that broilers did better when they were under heat stress when they ate feed additives composed of nanoparticles, such zinc oxide. Nano-Catechin is based on the same premise as nanotechnology: to help with absorption and metabolism. But Nano-Catechin is all about zinc. Adding Nano-Catechin (EGCG) to broiler diets offers various benefits, as evidenced by consistent increases in production index and economic return in treatments T3 and T5. This is notably true during the latest growth phase, when T3 had the highest economic return (3095.37) and the best production index (664.64). [42] noted that catechins make the intestinal barrier stronger and reduce enteric infections. This makes it easier for nutrients to be absorbed and improves feed conversion efficiency. This is exactly what the higher production numbers in T3 and T5 suggest. [43] investigated phytobiotics as natural growth accelerators and found that plant-based substances such as catechins can help the intestines, immune system, and metabolism function better. [44] investigated into how nanoparticles work in broiler feeding and found that nano-encapsulated bioactives make nutrients much easier for the body to use and make it more resistant to metabolic stress. I assume that it is easier for cells to take up thenano- (vs. micro-) particles faster, because of this, it works better in the body and has higher efficacy/impact on the economy. [45] found that EGCG makes meat taste better and be more disease-resistant, but only when the meat is under stress. This means it is worth more on the market and earns more money.

In their study, EGCG was associated with Nrf2-mediated redox homeostasis and cryoprotection. The marked drop in T4 with glucose indicates better glycemic control, as we would expect with EGCG: it reduces blood glucose levels and helps insulin work. [9] showed that the direct anti-oxidation and oxygen free radical scavenging effects of EGCG could decrease blood glucose and uric acid levels in heat-stressed broilers. This represents an improvement in glucose metabolism and a slowing of protein catabolism. T3 down-regulated uric acid supports the participation of EGCG in the inhibition of proteolysis. This is likely due to its protection of muscle from free-radical-induced oxidative stress. The liver functions better and produces more protein when T2 has higher levels of total protein and albumin. This is because albumin is an important protein in the liver. In order to break down or neutralize anti-nutritional substances like phytic acid and non-starch polysaccharides, the probiotic secretes enzymes that are absent in the digestive tract of birds [46]. The findings are consistent with those of [47], which demonstrated that administering green tea to broilers elevated plasma protein levels and enhanced transaminase activity. Lower creatinine levels at T2 and T5 indicate that the kidneys are functioning better. Creatinine is a marker of how well the kidneys filter blood. Table 8 indicates that treating broiler chicks with Nano-Catechin (EGCG) changed how fast their organs expanded. T5 had the most heart, and T1 had the most liver and gizzard. These variations suggest that EGCG affects organ growth by modifying metabolism, functioning as an antioxidant, and regulating circulation. The higher heart rate in T5 may indicate that the cardiovascular system is improving, possibly because EGCG enhances blood flow and reduces oxidative stress. [40] discovered that nano-ginger and nano-thyme improved people's health and helped their organs perform better, such as the bursa and belly fat. This suggests that nano-phytobiotics are good for the overall body.

The smaller liver and gizzard sizes at T3 and T5 may suggest that the body is better at digesting food and eliminating pollutants. [36] observed that EGCG reduced blood lipids and improved the function of antioxidant enzymes. This suggests that the liver was less stressed and used the nutrients it received more effectively. The similar values for the spleen and pancreas across treatments indicate that EGCG does not inhibit the growth of organs that support the immune and digestive systems. [48] found that nanochitosan improved histological and immunological indicators in lymphoid organs, leading to balanced organ development in antioxidant-rich diets. [49] explained that the stomach and organs need to expand quickly so they can take in nourishment and fight against illnesses. These thoughts align with EGCG's role in supporting the growth of the intestines and other organs. [50] revealed that EGCG cut down on inflammation in the intestines and made the villi of Wenchang chickens look better. This is excellent for your overall health, including your organs, because it suppresses signals that promote inflammation. [51] found that phytobiotics, especially catechin-rich extracts, modify intestinal histology and immune gene expression, thereby influencing organ size and function via improved gut health and systemic regulation. This makes a strong economic case: the integrator will have a higher net return on investment, since the value of the extra meat and feed saved greatly exceeds the additional cost of the advanced feed additive. [52] showed that quails that were fed Lactuca serriola had superior development performance, including greater body weight, in contrast to the group that served as the control. [53] stated that beneficial bacterial strains in the bio-enhancer mixture enhance nutrient availability and inhibit detrimental bacteria from competing with birds for nutrients in the digestive system. The incorporation of olive cake, with or without probiotics, significantly elevated lymphocyte counts and reduced the heterophil/lymphocyte (H/L) ratio. It reduced cholesterol levels compared with the control group at the end of the bird's lifespan. Furthermore, haematological and biochemical indicators showed considerable improvement ($P<0.05$) with the incorporation of 4% olive cake and 0.4 g/kg probiotic into the broiler diet [54]. The administration of parsley supplements significantly enhances body weight gain, especially during the early growth stages, while concurrently reducing feed consumption [55]. Demonstrates substantial impacts ($P<0.05$) of varying protein levels on the quail's live body weight and weight increase; the birds in T1 exhibited superior live body weight, weight gain, and feed conversion ratio ($p<0.05$) [56]. The inclusion of dried tarragon as an ingredient significantly enhances live body weight, body weight increase, feed intake, and feed conversion ratio (FCR) for the whole period (1-42 days), resulting in notable improvements ($p < 0.05$) in the Production Index (PI) and Economic Figure (EF) [57]. Plant supplements positively influenced the quality of chicken meat. This result demonstrates that plant supplements often have a greater or equivalent influence on meat quality than chemical additives, without any adverse effects [58].

Conclusion

In summary, supplementing broiler chicks with dietary nano EGCG resulted in a considerable improvement in performance, with T3 showing the greatest consistent gains. Several beneficial physiological reactions have occurred alongside these enhancements, including increased antioxidant capacity and lipid metabolism, and unaltered hepatic and renal function. The combined growth and biochemical test results provide evidence that Nano EGCG is effective as a functional feed additive, particularly at inclusion levels ranging from moderate to high. These findings also show the potential of Nano EGCG to optimize chicken nutrition and production efficiency.

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Conflict contribution

The authors maintain that there are no conflicts of interest.

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