



EFFECTS OF DIETARY LEVELS OF BAY LAUREL LEAF EXTRACT CONJUGATED WITH ZINC NANOPARTICLES ON GROWTH PERFORMANCE, CARCASS TRAITS, AND BLOOD PARAMETERS IN BROILERS

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Abstract

This study aimed to evaluate the effects of bay laurel leaf extract conjugated with zinc nanoparticles (Zn-Nano-BLLE) as a growth enhancer and antibiotic alternative in broiler diets on growth performance, carcass characteristics, and blood markers. In a completely randomized design, 264 one-day-old Ross 308 broiler chicks were divided into four experimental groups to assess the impact of Zn-Nano-BLLE supplementation. Each group consisted of six replicates, each containing 11 unsexed chicks. The treatments were as follows: a control group (basal diet) without any additives, and three experimental groups receiving Zn-Nano-BLLE at 1, 2, and 3 cm³ Zn-Nano-BLLE/kg feed, respectively. Results indicated that Zn-Nano-BLLE supplementation at 1 or 3 cm³/kg feed led to significant improvements in live body weight and weight gain. The lowest feed intake and feed conversion ratio were observed in broilers supplemented with 1 cm³ Zn-Nano-BLLE/kg feed (30.94 and 1.14, respectively). However, the highest dressing, carcass, and heart percentages were found in the control group. Additionally, broilers fed 3 cm³ Zn-Nano-BLLE/kg feed showed the highest levels of total protein, albumin, and globulin, while also exhibiting the lowest levels of total cholesterol and low-density lipoprotein (161.52 and 88.35, respectively). The immunoglobulin G (IgG) levels were elevated in the supplemented groups, although no significant effects were noted on immunoglobulin M (IgM). Birds given 2 cm³ Zn-Nano-BLLE/kg feed demonstrated the highest superoxide dismutase activity (164 U/ml), while the control group had the lowest levels. In conclusion, dietary inclusion of Zn-Nano-BLLE, particularly at 1 and 3 cm³/kg, enhanced broiler immunity, productivity, and antioxidant status, indicating its effectiveness as a natural growth enhancer in broiler production.

Key words: bay laurel leaf liquid, broilers, growth, carcass, blood, oxidative stress

About 60–80% of modern countries' health-conscious population is increasingly seeking natural products and traditional medicine for various illnesses, replacing synthetic products in stores (Shehata et al., 2022 a, b). Consequently, there is growing interest in herbs, medicinal plants, and their oil as potential sources of improved health (Surve et al., 2024). In 2006, the European Union banned the use of antibiotic growth promoters in chicken diets due to antimicrobial resistance, increased foodborne illnesses, and increased consumer infection risks (Youssef et al., 2024 a). Consequently, in order to improve chicken health, poultry product safety, and production competence, researchers have been working hard to find alternatives that show mechanisms of action similar to antibiotic growth promoters (Yang et al., 2024). In poultry farming, organic feed and supplements made from medicinal plants may present a viable substitute for artificial growth boosters

and antibiotics (Youssef et al., 2023 a, b, c; Siregar et al., 2024). These supplements are thought to strengthen the immune system, increase carcass quality, and improve growth performance in birds (Abd El-Hack et al., 2024; Dosoky et al., 2024; Youssef et al., 2024 b; Ibrahim et al., 2024).

The leaf of bay laurel (*Laurus nobilis* L.), which has antifungal and antibacterial properties, can feed chickens by increasing stomach fluids and preventing digestive diseases (Duru and Gölcü, 2023). Apigenin, luteolin, kaempferol, myricetin, cinole, eugenol, and terpinol were among the several potent compounds found in the leaves (Yasar et al., 2012; Fik-Jaskółka et al., 2024). Adding bay leaf to the diets of broiler chickens has been the subject of a few researches (Osman et al., 2020; Ozek, 2012). According to research by Ali and Al-Shuhaib (2021), when *Laurus nobilis* leaves are included in a diet, they contain extremely potent chemicals that can speed up

some growth features and make a notable advancement in the broiler chickens' productivity traits.

Creating nanoparticles (NPs) chemically, biologically, or physically is possible (Salem et al., 2023). Biological synthesis is a safer, cleaner, and more ecologically friendly method than chemical and physical processes, which can require large amounts of energy or hazardous substances, at ambient temperature; it can quickly convert metal ions into nanoparticles (Abdelnour et al., 2021). Advantageous characteristics of NPs include their huge surface area, strong adsorption capacity, and enhanced catalytic activity. Additionally, there are indications that NPs may improve nutrient absorption (Wei et al., 2024), and also have some side effects (Aziz et al., 2022).

Zinc (Zn) is a crucial microelement that has an impact on a variety of biological processes in birds, including immunity, the synthesis of hormones, the metabolism of proteins and carbohydrates, DNA and protein synthesis, and antioxidant capabilities (Hassan et al., 2021; Fatima et al., 2024). The National Research Council (NRC, 1994) states that broilers require 40 parts per million zinc daily. To achieve quick development in the growth of chicks, feed industrialists add 100–120 ppm of zinc to their feed on a commercial scale (Feng et al., 2010). Zinc excretion in feces increases because of this increase in zinc, which raises feed production costs and pollutes the environment. It lowers vitamins and throws off the equilibrium of other microelements. Deficiencies may result from zinc's interference with vitamin A's absorption and use. Additionally, excessive zinc levels might hinder the body's production of vitamin D, which can impact the metabolism of calcium and phosphorus (Bleizgys, 2024). Nevertheless, improving zinc's bioavailability, which rises when the metal is in nanoform, might solve these issues. Because nano-zinc has positive benefits on birds' health and metabolism, it has recently been employed as a feed supplement. Its antimicrobial action strengthens immunity (Akhavan-Salamat and Ghasemi, 2019). The development and reproduction of bacteria can be directly disrupted by zinc ions. They have the ability to interfere with DNA replication, decrease enzyme activity, and damage the bacterial cell membrane (Hamza et al., 2024). Zinc nanoparticles (ZnNPs) have been proven to enhance body weight, feed conversion ratio, meat quality, egg quantity, and immune system function in various trials (Zhao et al., 2014; Attia et al., 2023; Fatima et al., 2024).

We hypothesized that the unique blend of powdered bay laurel leaf extract and biologically generated nanoparticles would have positive and complementary effects on most broiler performance characteristics, because bay laurel leaves may serve as organic stabilizers, preventing clumping and enhancing the stability and dispersion of zinc nanoparticles, thereby ensuring optimal performance. To provide end consumers with wholesome food, this study examined the effects of bay leaf extract conjugated with zinc nanoparticles as a growth promoter and

antibiotic substitute in the broiler diet on carcass features, blood indices, and growth performance.

Material and methods

Preparation of bay laurel leaves extract

The leaves of bay laurel were purchased from a commercial company (2M Group, 10th of Ramadan in Sharqia, Egypt). Whatman No. 42 filter sheets (Hawach Scientific Co., Ltd., Xi'an, China) were used to filter the aqueous bay laurel solutions, which were made by soaking 40 g of powdered bay laurel leaves in a liter of extraction solvent (water-ethanol) for at least 24 hours, at room temperature, which is around 25°C (Mohammed et al., 2021).

Biosynthesis of ZnO-NPs using bay laurel leaves extract

ZnNPs were created by combining 2 mm zinc acetate with 100 ml of extract from bay laurel leaves, stirring continuously, and shaking the combination for 12 hours at 50°C. A white tint appeared at the end of the incubation period, indicating the synthesis of ZnO-NP. A mixture of ethanol and distilled water was centrifuged to gather the ZnO particles. Additionally, the material was dried at 120°C for an entire night. One liter of concentrated solution contained 40 millimoles of Zn-NPs infused with bay laurel leaves extract's active constituents. The concentrated solution was diluted in a 1:3 ratio with distilled water, resulting in 4 liters containing 10 millimoles of Zn-NPs, abundant in the active constituents of the extract utilized (bay laurel leaves powder extract) (Huq et al., 2023). The prepared samples were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM).

Experimental design, birds and diets

The Poultry Research Farm, part of Zagazig University's Department of Poultry in Egypt, served as the site of this study. The University's ethics committee approved all experimental techniques (IACUC-2024) following the Institutional Animal Care and Usage Committee's guidelines. Throughout the trial, the chicks were treated in compliance with the protocols for the care of experimental animals, and all procedures complied with the applicable laws and guidelines, as described in the previous section.

With comparable initial weights, 264-day-old unsexed Ross 308 broiler chicks were split into four experimental groups using a randomized method. Six replicates, each comprising eleven unsexed chicks, were created from each group. The 38-day experiment was conducted. The diets were designed following the NRC (1994) to fulfill the nutritional requirements of Ross 308 broilers. Throughout the experiment, all chicks were fed pelleted feed. The usual temperature range is between 63°C and 85°C. Conditioning moisture: to increase bind-

ing and soften the feed particles, the ideal moisture content is often between 17 and 18 percent. Pellet moisture: To avoid mold growth and deterioration during storage, the pellets' final moisture level should be between 10 and 12%. In order to provide the heat and moisture enough time to permeate the feed particles and activate natural binders, the conditioning period is usually 10 to 20 minutes, as indicated in Table 1. The two phases of the diets were a starter diet for the first twenty-one days and a finisher diet for the final seventeen days. The chicks were reared in a temperature-controlled environment. They were housed in a temperature-controlled facility at 33°C for the first four days. Subsequently, the temperature dropped to 29°C, and 70% humidity was sustained until the experiment concluded at 38 days. A regular diet devoid of additions and three diets containing different concentrations of zinc nanoparticles conjugated with bay leaf liquid were studied. These were designated Zn-Nano-BLLE 1, 2, and 3, which, in turn, stood for 1, 2, and 3 cm³ of liquid per kilogram of feed, respectively. The birds were kept in standard cages measuring 1.2 × 1.2 × 50 cm³, supplied with unlimited freshwater and food. Throughout the experiment, the birds were kept on a 23-hour light schedule with one hour without light.

Table 1. Basal diet composition and chemical analysis

Items	Starter (1–21 days)	Finisher (22–38 days)
Ingredients (%)		
Yellow corn	58.8	60.2
Soybean meal 46%	29.07	24.77
Concentrate 45%*	10.0	10.0
Dicalcium phosphate	0.5	0.5
Limestone	0.43	0.43
DL-Methionine	0.1	0
L-Lysine HCl	0.1	0
Soybean oil	1.00	4.1
Total	100.0	100.0
Calculated analysis**		
Crude protein (%)	23.0	21.0
Metabolizable energy (kcal/kg diet)	2954.1	3152.2
Calcium (%)	1.0	0.98
Phosphorous (available) (%)	0.45	0.44
Lysine (%)	1.3	1.1
Methionine + Cysteine (%)	0.9	0.75
Crude fiber (%)	3.51	3.3

Each 1 kg of vitamin mixture contained: 120,000 IU vit. A, 35,000 IU vit. D₃, 400 mg vit. E, 30 mg vit. K₃, 20 mg vit. B₁, 60 mg vit. B₂, 50 mg vit. B₆, 200 mic vit. B₁₂, 1 mg cobalt, 750 mics. biotin, 1,000 mg manganese, 300 mg iron, 600 mg zinc, 100 mg copper, 20 mg folic acid, 450 mg niacin, 2 mg selenium, 10 mg iodine, 120 mg pantothenic acid, 2,600 mg choline chloride (added per kg concentrate).

*Protein concentrate (45%) chemical analysis: crude protein: 45%, metabolizable energy: 2470 kcal/kg diet, calcium: 6.13%, phosphorus: 2.32%, lysine: 2.67%, methionine + cystine: 2.19% and fiber: 2.18%.

**Calculated according to NRC (1994).

Data collection

Each chick was weighed separately at 1, 15, 30, and 38 days to determine its live body weight (LBW) and daily body weight gain (DBWG). The feed intake (FI) was recorded and feed conversion ratio (FCR) was computed. At the experiment's conclusion, 12 birds from each treatment were randomly selected for carcass evaluations at 38 days of age, weighed and slaughtered according to Halal Islamic method. We calculated the weight of the carcass and the total amount of edible portions.

Blood biochemical analysis

The blood was extracted from the identical 12 birds from each treatment by centrifuging the blood for 15 minutes at 4°C and 5000 rpm in non-heparinized tubes. Before being subjected to a biochemical examination, the serum was kept at –20°C. The levels of total protein (TP), albumin (ALB), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL) and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured in the serum samples. Furthermore, the manufacturer's instructions for commercial kits were followed when measuring creatinine and immunological response (IgG, IgM). The activities of oxidative status markers, including superoxide dismutase (SOD) and malondialdehyde (MDA), were determined according to an earlier publication (Abd El-Hack et al., 2017).

Statistical examination

Utilizing a complete randomization design and the GLM procedures of SAS, data was provided to the ANOVA procedure (SAS Institute Inc., 2001). The pen served as the growth performance experimental unit. Certain broilers were used as test subjects for carcass traits and blood measurements. The Zn-Nano-BLLE effect was the only factor included for analysis. Prior to performing any statistical analysis, the Shapiro-Wilk test was utilized to check for normality. Post-hoc Tukey's test was employed to determine the dissimilarities in means. Statistically significant claims were based on $P < 0.05$ unless otherwise specified. The following statistical approach was applied:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where: Y_{ij} = assessed worth of the relevant treatment intervention, μ = determined mean for the relevant therapy, T_i = treatment consequence and e_{ij} = the error of a single observation.

Results

Live body weight (LBW) and daily body weight gain (DBWG)

Table 2 illustrates the impact of including varying levels of Zn-Nano-BLLE into the diet on broiler's LBW

and DBWG throughout the experiment stages. The initial body weights of one-day-old broiler were statistically comparable across all treatment groups, indicating random assignment to the experimental groups. Compared with the control group, LBW was higher in broilers given 1 and 3 cm³ Zn-Nano-BLLE/kg feed at 38 days of age. Additionally, broilers fed 1 and 3 cm³ Zn-Nano-BLLE/kg feed at 31–38 days of age, and the total duration of the study (1–38 days of age) had higher DBWG.

Feed intake (FI) and feed conversion ratio (FCR)

The findings displayed in Table 3 demonstrated the effects of various Zn-Nano-BLLE supplementation levels on broiler FI and FCR. The results showed that during the period 1–15 days of age, birds fed 3 cm³ Zn-Nano-BLLE/kg feed and the control group had the highest rates of FI, while birds fed 1 cm³ Zn-Nano-BLLE/kg feed had the lowest rates of FI. Additionally, the findings demon-

strated that the control group and 3 cm³ Zn-Nano-BLLE/kg feed group had the highest (worst) FCR during the period 1–15 days of age, while the 1 cm³ Zn-Nano-BLLE/kg feed group had the lowest (best) rate of FCR during the same period of experimentation.

Carcass features

The effects of adding Zn-Nano-BLLE to the birds' feed on the carcass features are displayed in Table 4 at the end of the experiment. Analysis of data demonstrated that treatments showed considerable influences on the dressing (%), carcass (%), and heart (%) ($P < 0.05$, 0.01). The highest percentages of these features were when broilers were fed the control. However, gizzard (%), liver (%), spleen (%), giblets (%), and abdominal fat (%) show no discernible variations between the experimental treatments as a proportion of the total weight of the body.

Table 2. Influences of using different levels of bay laurel leaf liquid conjugated with zinc nanoparticles (Zn-Nano-BLLE) on live body weight and daily body weight gain of broiler chickens

Items	Treatments (cm ³ Zn-Nano-BLLE/kg diet)				SEM	P value
	control	Zn-Nano-BLLE 1	Zn-Nano-BLLE 2	Zn-Nano-BLLE 3		
Live body weight (g)						
1 day	45.27	45.00	45.09	45.82	0.121	0.665
15 days	438	452	439	435	0.215	0.054
30 days	1169	1145	1109	1114	0.375	0.199
38 days	1786 b	1945 a	1872 ab	1893 a	0.970	0.018
Daily body weight gain (g/day)						
1–15 days	26.24 b	27.20 a	26.27 b	25.98 b	0.461	0.045
16–30 days	48.71	46.20	44.67	45.26	0.772	0.208
31–38 days	77.17 b	99.94 a	95.45 a	97.41 a	0.874	0.017
1–38 days	45.83 b	50.01 a	48.10 ab	48.62 a	0.492	0.017

a, b, c – means within the same row with different letters are significantly different ($P \leq 0.05$).

Table 3. Influences of using different levels of bay laurel leaf liquid conjugated with zinc nanoparticles (Zn-Nano-BLLE) on feed intake and feed conversion ratio of broiler chickens

Items	Treatments (cm ³ Zn-Nano-BLLE/kg diet)				SEM	P value
	control	Zn-Nano-BLLE 1	Zn-Nano-BLLE 2	Zn-Nano-BLLE 3		
Feed intake (g/day)						
1–15 days	32.80 a	30.94 b	31.35 b	32.80 a	0.137	0.019
16–30 days	70.09	71.85	65.67	79.52	0.452	0.060
31–38 days	149	147	162	160	0.560	0.485
1–38 days	84.01	83.27	86.42	89.03	0.914	0.487
Feed conversion ratio (g/g)						
1–15 days	1.25 a	1.14 c	1.19 b	1.26 a	0.021	0.002
16–30 days	1.45	1.56	1.47	1.76	0.147	0.097
31–38 days	1.94	1.47	1.70	1.66	0.207	0.089
1–38 days	1.84	1.67	1.80	1.83	0.273	0.353

a, b, c – means within the same row with different letters are significantly different ($P \leq 0.05$).

Table 4. Influences of using different levels of bay laurel leaf liquid conjugated with zinc nanoparticles (Zn-Nano-BLLE) on carcass characteristics of broiler chickens

Items	Treatments (cm ³ Zn-Nano-BLLE/kg diet)				SEM	P value
	control	Zn-Nano-BLLE 1	Zn-Nano-BLLE 2	Zn-Nano-BLLE 3		
Dressing (%)	76.65 a	75.97 a	75.00 ab	73.49b	0.982	0.012
Carcass (%)	72.55 a	72.12 ab	70.83 bc	69.85 c	1.091	0.007
Heart (%)	0.43 a	0.35 b	0.32 b	0.34 b	0.450	0.000
Gizzard (%)	1.35	1.22	1.41	1.40	0.582	0.244
Liver (%)	2.32	2.28	2.44	1.89	0.227	0.139
Spleen (%)	0.09	0.08	0.10	0.10	0.175	0.878
Giblets (%)	4.11	3.86	4.17	3.64	0.229	0.094
Abdominal fat (%)	0.74	1.00	1.17	0.78	0.241	0.114

a, b, c – means within the same row with different letters are significantly different ($P \leq 0.05$).

Table 5. Influences of using different levels of bay laurel leaf liquid conjugated with zinc nanoparticles (Zn-Nano-BLLE) on blood biochemical parameters of broiler chickens

Items	Treatments (cm ³ Zn-Nano-BLLE/kg diet)				SEM	P value
	control	Zn-Nano-BLLE 1	Zn-Nano-BLLE 2	Zn-Nano-BLLE 3		
Liver and kidney functions						
TP (g/dL)	3.52 b	3.94 b	4.00 b	4.90 a	0.194	0.002
ALB (g/dL)	1.41 c	2.17 b	2.11 b	2.52 a	0.170	0.000
GLOB (g/dL)	2.12 ab	1.78 c	1.89 bc	2.38 a	0.112	0.008
A/G (%)	0.67 c	1.22 a	1.13 ab	1.06 b	0.138	0.000
ALT (IU/L)	15.34	9.90	8.75	18.40	0.223	0.112
AST (IU/L)	147	184	164	152	0.215	0.489
creatinine (mg/dL)	0.73	0.60	0.53	0.74	0.359	0.076
Lipid profile						
TC (mg/dL)	273 a	217 b	212 b	161 c	1.041	0.001
TG (mg/dL)	140 a	107 b	122 ab	139 a	1.245	0.048
HDL (mg/dL)	45.69	44.66	44.44	45.20	1.390	0.869
LDL (mg/dL)	205 a	145 b	143 b	88.3 c	1.284	0.000
VLDL (mg/dL)	28.04 a	21.44 b	24.43 ab	27.98 a	1.267	0.048

a, b, c – means within the same row with different letters are significantly different ($P \leq 0.05$).

TP: total protein, ALB: albumin, GLOB: globulin, ALT: alanine aminotransferase, AST: aspartate aminotransferase, TC: total cholesterol, TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein, and VLDL: very low-density lipoprotein.

Table 6. Influences of using different levels of bay laurel leaf liquid conjugated with zinc nanoparticles (Zn-Nano-BLLE) on immunoglobulins and blood antioxidant parameters of broiler chickens

Items	Treatments (cm ³ Zn-Nano-BLLE/kg diet)				SEM	P value
	control	Zn-Nano-BLLE 1	Zn-Nano-BLLE 2	Zn-Nano-BLLE 3		
Immunoglobulins						
IgG (ng/ml)	233 c	571 a	336 b	354 d	0.514	0.002
IgM (ng/ml)	226	277	282	275	0.480	0.814
Antioxidant parameters						
SOD (U/ml)	119 b	136 ab	164 a	142 ab	0.181	0.035
MDA (nmol/ml)	3.25	2.44	2.19	2.04	0.224	0.193
SOD/MDA (%)	36.8 c	55.7 b	75.1 a	70.0 ab	0.040	0.027

a, b, c – means within the same row with different letters are significantly different ($P \leq 0.05$).

IgG: immunoglobulins G, IgM: immunoglobulins M, SOD: superoxide dismutase activity, MDA: malondialdehyde.

Blood biochemical parameters

Kidney, liver functions and lipid profile

Table 5 shows the impacts of several Zn-Nano-BLLE treatments on the markers of the kidneys and liver functions. The blood serum levels of TP, ALB, GLOB, and A/G ratio varied significantly between the Zn-Nano-BLLE-treated birds and the control group. Additionally, treatment with a 3 cm³ Zn-Nano-BLLE/kg diet had the highest TP, ALB, and GLOB levels compared to other treatments and control group. At the same time, treatment with a 1 cm³ Zn-Nano-BLLE/kg diet had the highest levels of A/G ratio compared to the control group. However, the experimental treatments had no discernible impact on ALT, AST, and creatinine levels. Also, Table 5 shows how different Zn-Nano-BLLE treatment dosages altered the lipid profiles of the birds. Compared with the control group, the birds fed 3 cm³ Zn-Nano-BLLE/kg diets had the lowest levels of TC and LDL. Also, the birds fed 0 and 3 cm³ Zn-Nano-BLLE/kg diet had the highest levels of TG and VLDL. However, the experimental therapy had no discernible effect on HDL concentrations.

Immune and antioxidant parameters

Table 6 demonstrates that various Zn-Nano-BLLE treatments significantly impacted broiler chickens' blood antioxidant parameters and immunoglobulin levels. IgG concentrations were lower in the control group, but treatments did not affect IgM levels. Antioxidant markers, including SOD and SOD/MDA ratio, were significantly improved by Zn-Nano-BLLE supplementation. The highest levels of SOD and SOD/MDA (%) were found in the birds fed 2 cm³ Zn-Nano-BLLE/kg diets, while the smallest amounts were seen in the birds provided a control diet. However, the experimental treatments had no discernible effect on MDA concentrations.

Discussion

The present investigation posited that the bioactive chemicals found in Zn-Nano-BLLE might potentially enhance the health status of broilers by improving their performance, lipid oxidation, carcass features, and some blood parameters. Zinc can benefit humans and animals in numerous aspects, including antioxidants, glandular development, protein synthesis, carbohydrate metabolism, nutrition, production performance, and its position as a cofactor in over 300 metalloenzymes (Attia et al., 2019; Abdelnour et al., 2021; Janoš, 2024). It is essential for the growth, formation of bones, and construction of enzymes (Hassan et al., 2021; Stiles et al., 2024). Zinc addition may lessen animal stress and increase surface area, improving the minerals' bioavailability (Manzoor et al., 2024). By promoting lipid breakdown and reducing production, nano-ZnO decreased yolk lipid levels (Abou Ashour et al., 2023).

Our research shows that adding Zn-Nano-BLLE increased broiler chickens' performance. These results are consistent with previous research by Duru and Gölcü (2023) and Fawaz et al. (2019), which found that FI, and LBW tended to rise as the birds' diets contained more laurel leaves powder (LLP) than did the control group. However, there is conflicting evidence regarding how phytogetic agents therapy affects animal performance (Shehata et al., 2022 a, b; Siregar et al., 2024). According to Duru (2016), feed intake, body weight increase, and feed conversion ratio (FCR) in broiler chickens were not significantly affected by dietary LLP (0, 1, 2, and 4 g/kg). According to Karaalp et al. (2011), performance was unaffected by adding 0, 2, and 4 g/kg of bay laurel leaves to the diets of Japanese quails. According to Salim et al. (2021), when dried laurel leaves were added to the diet at 0, 1, 2, or 4 g/kg, rabbits saw increased body weight gain and decreased FCR. Quails' body weights have risen in response to bay laurel supplementation (0%, 1%, 2%, and 3% laurel leaf) (Al Rubaee, 2018). Zinc oxide nanoparticles (ZONPs) at 10 and 20 ppm significantly boosted body weight growth. They decreased the FCR in broilers without influencing the total amount of feed they were fed, according to Fathi et al. (2016). Furthermore, several research studies have found that broiler chicken performance was enhanced by greater dosages of ZONPs, as reported by Pathak et al. (2016) and Hafez et al. (2017). Along with the higher absorption efficiencies of Zn nanoparticles caused by their unique physical characteristics of transport and uptake, which result in improved Zn bioavailability, the performance improvement may be attributed to ZONPs' role in enhancing the intestinal absorptive capacity as it improves mucosal length, villi length and width, and crypt depth (Hafez et al., 2017). More than 300 enzymes involved in the metabolism of proteins, nucleic acids, and energy are thought to require zinc as a necessary component (Jarosz et al., 2017). Furthermore, ZONPs enhanced the broiler's metabolism by boosting insulin-related genes such as growth factor and growth hormone (Ibrahim et al., 2017). However, Aziz et al. (2022) reported side effects which warrants further research for the safety of ZONPs. Based on the findings of Ramiah et al. (2019) it was proposed that the stimulating effect of zinc intake on intestinal metallothionein synthesis could be the reason for the lack of response in performance indicators in treatments given greater doses than 10 ppm ZONPs.

Antioxidants like flavonoids and phenolic substances abound in laurel leaves. By scavenging dangerous free radicals, these antioxidants assist in lessening oxidative stress within the body. Better growth performance can follow from improving animals' general health and well-being (Mrabet et al., 2024). Moreover, laurel leaves might enhance the digestive tract's absorption of nutrients. By doing this, broilers can be guaranteed to receive the most nutritional value from their feed, improving their growth and development (D'Alessandro et al., 2024).

The results show that most carcass characteristics changed considerably between the various experimental treatments. Dosoky et al. (2022) found that broilers' relative intestine and gizzard weights were reduced in response to dietary ZONPs supplementation at dosages of 5, 10, 20, 40, 60, and 80 mg/kg feed, which is consistent with our findings. Furthermore, the relative weights of the kidney, gizzard, and intestine were dramatically decreased, and the liver weight was significantly increased (undesirable effect) in broiler chicks fed diets supplemented with ZONPs at 40 and 60 ppm (Hatab et al., 2022). Since zinc is a cofactor for several metalloenzymes linked to improved digestion enzyme synthesis, the small intestine weight decrease may be explained by increased gut endogenous enzyme excretion and activity under adequate zinc availability (Attia et al., 2019). Zinc benefits the broiler intestine by boosting protein synthesis, barrier architecture, crypt cell activity, and intestinal integrity (Hatab et al., 2022).

Terpenes and flavonoids, two antibacterial substances, are found in laurel leaves. These substances may lessen the likelihood of bacterial infections in the digestive system of broilers. Better development performance and feed utilization can come from a healthy gut, and these factors may eventually raise the dressing percentage (Stefanova et al., 2020; Shehata et al., 2022 a, b). Moreover, laurel leaf has a high antioxidant content that can help shield the broiler's cells from oxidative stress. Reducing oxidative stress may enhance overall performance and dressing percentage because it can harm growth and development (Chatzimitikos et al., 2024). Zn-Nano-BLLE may have synergistic effects that improve the characteristics of the broiler carcass even more. For instance, the zinc in the nanoparticles may strengthen the antioxidant qualities of laurel leaf, improving general health and lowering oxidative stress. Furthermore, zinc nanoparticles may have antibacterial characteristics, which could enhance the laurel leaf's antimicrobial benefits (Movahedi et al., 2024). The observed alterations in carcass features could be attributed to elevated zinc levels in tissue, the impact of zinc on antioxidant status, and oxidative enzymes, particularly those involved in muscle's antioxidant function and water-holding capacity (Abdel-Wareth et al., 2022).

In the current investigation, the broiler feed enriched with Zn-Nano-BLLE at different dosages improved blood biochemical parameters compared to the untreated group. Zn-Nano-BLLE feed supplementation therapy significantly increased TP, ALB, and GLOB compared to control groups. Regarding serum proteins, when ZONPs were added to broiler diets at 30 and 40 ppm (Mahmoud et al., 2020) and 20–100 ppm (Attia et al., 2019), there was a comparable rise in blood proteins and globulins as in the current study. This rise may be related to zinc's critical role in manufacturing enzymes that produce nucleic acids and proteins. Additionally, the hormonal regulation of protein metabolism, such as the growth hormone-induced increase in cellular protein production,

may be responsible for the increased plasma protein content in response to zinc therapies (Al-Daraji and Amen, 2011). Laurel leaf may supply necessary amino acids or increase their bioavailability to boost protein synthesis. Additionally, it may lessen the rate at which proteins break down, raising the total level of serum proteins (Al Chalabi et al., 2020).

By reducing LDL and total cholesterol levels, Zn-Nano-BLLE considerably changed the lipid profile in the current study. Plant-based substances called phytosterols, which resemble cholesterol in structure, may be present in laurel leaf. These phytosterols may lower LDL cholesterol levels because they will compete with cholesterol for intestinal absorption (Evtugun et al., 2023). Laurel leaf may also include high concentrations of phenolic chemicals and flavonoids, two antioxidants. As oxidative stress is linked to several illnesses, including high levels of triglycerides and LDL cholesterol, these antioxidants can aid in shielding cells from its damaging effects (Muscolo et al., 2024). Also, dietary fiber from laurel leaf may be present. This fiber can bind to bile acids and cholesterol in the digestive system and encourage the body to excrete them. As a result, LDL levels may decrease (D'Alessandro et al., 2024). Zinc oxide can scavenge free radicals and aid in neutralizing reactive oxygen species (ROS). These ROS can potentially cause oxidative stress, linked to elevated cholesterol and dyslipidemia. Zinc oxide may also affect the expression of genes linked to lipid metabolism, such as those involved in the production, absorption, and excretion of cholesterol. Fatima et al. (2024) believe this may result in lower cholesterol levels. Additionally, zinc oxide helps support intestinal mucosa integrity, essential for healthy nutrient absorption. According to Yusof et al. (2023), maintaining a healthy gut might enhance the body's ability to absorb vital nutrients and fatty acids, which may impact cholesterol metabolism. Optimizing poultry production requires an understanding of the connection between biochemical alterations and broiler performance attributes. We can learn more about the physiological condition of birds and spot possible health problems that can affect their performance by keeping an eye on blood profiles and liver and kidney enzyme levels (Martinez et al., 2022). Meat quality is influenced by lipid profiles such as triglycerides, cholesterol, and fatty acid profiles. Additionally, increased liver enzyme levels (ALT, AST) may be a sign of liver injury that impacts lipid production and nutrition metabolism (Selim et al., 2021).

The capacity of ZONP supplementation to enhance immune system performance is among its most significant impacts. According to Zarghi et al. (2022), Ross broilers fed zinc supplements showed a significant increase in both the humoral immune response (measured by antibody titer to SRBCs inoculation) and the cellular immune response measured by cutaneous basophil hypersensitivity (CBH) elicited by phytohemagglutinin-P (PHA-P) injection. Additionally, Dosoky et al. (2022) noted that significant differences in the phagocytic activ-

ity, phagocytic index, IgM, and IgG indicated enhanced cellular immunity in chicks fed a diet supplemented with ZONPs. Numerous studies have examined the role of zinc in enhancing immunity and have proposed several pathways for this effect. Zinc is an essential co-factor that affects the thymus gland's ability to produce and release thymulin, a thymic hormone that contains zinc. Thymulin attaches to T lymphocyte surface receptors to drive and regulate T cell maturation and activation. This, in turn, encourages B cell development and differentiation by T-helper cells, which causes the release of macrophages, boosting the immune response (Jarosz et al., 2017).

The considerable rise in SOD and the non-significant decrease in MDA concentration in broiler serum indicate the antioxidant efficacy of Zn-Nano-BLLE in this investigation. Hassan et al. (2023) discovered that antioxidant capacity in chickens was enhanced by nano-Zn generated by various plant extracts at a level of 70 ppm, which supports the current results. Similar findings to this study were also reported by several authors, who found that supplementing broiler diets with ZONPs at 40 to 80 mg/kg greatly increased the antioxidant status of the animals as evidenced by elevated serum SOD, GPx, and TAC, which were correlated with lower MDA concentrations (Zhang et al., 2022). According to Akhavan-Salamat and Ghasemi (2019), at the right concentrations of nano-ZnO, SOD activity will be stimulated. This will boost SOD's ability to eliminate ROS formation and other oxidation-related processes, ultimately leading to a decrease in MDA concentration. SOD is the primary antioxidant enzyme involved in the scavenging of free radicals in cells, and zinc is a crucial and fundamental component of SOD, making up approximately 90% of its structure. Numerous hypotheses have been proposed to explain how zinc exerts its antioxidant effect. Research has shown that the body produces metallothionein, a rich cysteine protein that scavenges free radicals and protects cells from lipid peroxidation and free radicals when zinc is present (Ren et al., 2024). By removing reactive oxygen species, the enzyme SOD plays a crucial role in maintaining the redox equilibrium of the bird's immune system (Abdulrahman et al., 2024). The antioxidant qualities of polyphenols, such as tannins and flavonoids, are abundant in bay laurel leaves. These substances might lessen oxidative stress by directly scavenging free radicals, such as superoxide anions.

Furthermore, polyphenols can activate the body's natural antioxidant defense systems, which may raise SOD activity (Andrés et al., 2024). Lastly, the substances found in bay laurel leaves may function as organic stabilizers, keeping zinc nanoparticles from clumping together and losing their potency. Furthermore, the natural elements found in bay laurel leaves may contribute to the stability and dispersion of zinc nanoparticles, guaranteeing their best possible performance (Özgören Can et al., 2024).

Conclusion

The findings of this study demonstrate that dietary supplementation with Zn-Nano-BLLE effectively enhances growth performance, immune function, antioxidant capacity, carcass quality, and blood biochemical markers in broiler chicks. Specifically, the doses of 1 and 3 cm³/kg feed yielded the most pronounced improvements across these parameters. The notable increases in growth metrics and improvements in immune and antioxidant status suggest that Zn-Nano-BLLE may serve as a promising natural alternative to synthetic growth promoters in poultry farming. Its beneficial effects on carcass yield and quality, combined with positive shifts in blood parameters, underscore its potential to contribute to healthier, more productive poultry farming practices. Further research is encouraged to confirm these effects and assess long-term benefits, establishing Zn-Nano-BLLE as a viable addition to sustainable poultry nutrition strategies.

Conflict of interest

Authors declare no conflict of interests.

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