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ENHANCING BROILER GROWTH, CARCASS QUALITY, BLOOD PARAMETERS AND INTESTINAL MICROBIAL LOAD: THE ROLE OF DIETARY GARLIC POWDER AS A NATURAL GROWTH PROMOTER

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Abstract

This study evaluated the effects of garlic powder (GP) as a natural growth promoter in broiler diets, replacing traditional antibiotics. Four experimental groups of 264 day-old broiler chicks (Ross 308) were established in a fully randomized design experiment. Each group had six replicates, each with eleven unsexed chicks. The treatments were used as follows: The 1st group was the basal diet without any additive (control group). 2nd, 3rd and 4th groups (GP1, GP2 and GP3) receiving basal diet + 1, 2, and 3 g GP/kg feed, respectively. Results demonstrated that 2 and 3 g GP/kg diets significantly improved feed conversion ratio (FCR), while 1 g GP/kg feed led to the greatest gains in body weight, weight gain, and feed intake. Blood analysis revealed elevated total protein, albumin, and A/G ratios across all GP groups, alongside reduced creatinine and ALT levels. The 3 g GP/kg diet notably increased HDL and reduced total cholesterol (TC) and LDL concentrations. Immunoglobulin Y (IgY) levels were highest in birds fed 1 and 3 g GP/kg, with IgM levels peaking in the 1 g GP group. Additionally, the 3 g GP/kg diet enhanced superoxide dismutase (SOD) activity and reduced malondialdehyde (MDA) levels, a marker of oxidative stress. In contrast, the control group exhibited the highest MDA levels. Additionally, the highest lactic acid bacteria (LAB) and total bacterial count (TBC) were found in the GP1 group. However, the control group had the highest levels of *E. coli.* In conclusion, incorporating garlic powder into broiler diets enhanced immune response, blood parameters, overall growth, and gut bacteria counts with optimal effects observed at 1 and 3 g GP/kg.

Key words: garlic powder, broilers, growth, blood, oxidative stress, microbiota

The demand for chicken products has increased due to the significant increase in the world's population, and these goods must be provided while maintaining their quality and safety standards (Bist et al., 2024). It is commonly known that conventional antimicrobials, also called antibiotics, can treat and prevent contagious bacterial illnesses in chicken production (Kaur et al., 2024). Unfortunately, antibiotic resistance is a serious public health concern brought about by the overuse and abuse of these drugs (Ahmed et al., 2024; Abd El-Hack et al., 2024). Yet, when antibiotics were removed from the chicken diet, the growth was decreased (Yang et al., 2024). As a result, natural substances such as probiotics (Cufadar et al., 2024), prebiotics (Youssef et al., 2023 a, b, c; 2024 a), synbiotics (Youssef et al., 2024 b), essential oils, herbal extracts, bioactive medicinal plants, and phytogenic mixes (Dosoky et al., 2024) gained popularity as alternatives to pathogen resistance and growth stimulants. Due to their high profitability and low cost, medicinal and herbal plants have been used extensively as natural antioxidants in recent years (Odubo et al., 2024).

Garlic (*Allium sativum*) is one of the most significant spices in medicine. Because of its many uses, it has been dubbed the "wonder drug" of the herbal world. Bioactive substances found in garlic include molecules that contain sulfur (alliin, diallyl sulfides, and allicin), which have antibacterial, antifungal, anti-parasite, antiviral, antioxidant, antithrombotic, anti-cancer, and vasodilator properties (Sjofjan et al., 2019; Batiha et al., 2020; Abd El-Ghany, 2024). Studies on garlic have shown that it lowers blood and liver cholesterol, reduces oxidative stress, and inhibits the growth of bacteria, platelets, and other microorganisms (Abdulkareem, 2024; Prabayanti et al., 2024). Furthermore, garlic has demonstrated strong antibacterial properties against bacterial infections linked to poultry (Ariza et al., 2024). According to earlier research, garlic has a major positive impact on poultry development performances

and other biochemical traits. Garlic has been shown to enhance broiler growth and FCR and lower the mortality rate when added to feed naturally (Tollba and Hassan, 2003; Williams et al., 2024). Adding garlic powder (GP) to diets can improve broiler performance and carcass attributes (Demir et al., 2003; Malik et al., 2024).

Nonetheless, the extant literature regarding the effects of garlic on poultry nutrition has yielded inconclusive findings, potentially attributable to discrepancies in garlic preparation techniques, species choice, doses, study duration, and additional variables. For example, feeding garlic to broilers increased their growth performance, according to Elbaz et al. (2021) and Sangilimadan et al. (2019). However, Elsagheer et al. (2020) observed no discernible impact on growth. Comparably, garlic-treated broilers showed improved immune responses (Elbaz et al., 2021; Noruzi and Aziz-Aliabadi, 2024), but Toghyani et al. (2011) found no discernible effect on immune function. This discrepancy suggests that more research and precise recommendations regarding garlic's place in poultry nutrition are required.

The novelty of this study lies in numerous specific and original contributions that distinguish it from comparable reports on garlic powder as a natural growth promoter in broilers. With dual-focused approach on growth and gut health, this study incorporates an investigation of gut microbiota, demonstrating how supplementing with garlic powder affects lactic acid bacteria, total bacterial count, and *E. coli* levels. This is in contrast to many studies that only concentrate on growth performance. Additionally, three different GP levels – 1, 2, and 3 g/kg – are evaluated in this trial, offering a more nuanced knowledge of dose-specific advantages. Poultry farmers can easily implement the levels in practice.

Therefore, the current study aimed to determine how different doses of GP in the feed affected the growth performance, blood parameters, carcass features, immunology, antioxidant activity, and intestinal bacterial count of broiler chickens.

Material and methods

Design, birds and diets

The Poultry Experimental Farm, Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt, was the site of this study. The Institutional Animal Care and Use Committee (ZU-IACUC/2/F/313/2023) and the Ethics Committee of the Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt, approved before any studies could begin.

In a fully randomized design experiment, 264 one-day-old, unsexed broiler chicks (Ross 308) with roughly similar initial weights were divided into four experimental groups. Six replicates, each with eleven unsexed chicks, made up each group. The treatments were as follows: a base diet devoid of any additives was given to the control group. GP1, GP2, and GP3 treatments comprised a basal diet plus 1, 2, or 3 g of garlic powder/kg of feed, respectively. Basal diets were created to meet dietary requirements according to NRC (1994). All chicks were fed pellet diets from the first to the 38th day of their lives, as shown in Table 1. The two phases of the diets were starter (1–21 days) and finisher (22–38 days). The NRC lists the general nutritional needs of broilers and other poultry species. It is a well-accepted standard that works with many genetic strains and production processes.

A commercial company (2 M Group, 10th of Ramadan in Sharqia, Egypt) provided the garlic powder. Aqueous garlic solution was made by soaking 300 g of crushed garlic bulbs in a large extraction solvent (water + ethanol); the mixture was then allowed to stand at room temperature for at least 24 hours before being filtered through Whatman No. 42 filter sheets (produced by Hawach Scientific Co., Ltd., Xi'an, China). One liter of the garlic filtrate was then combined with two kilograms of aluminum silicate powder (in a 1:2 ratio to obtain a final powder ratio of 1:6) and baked at 60–70°C for a full day.

The chicks were raised in a room with regulated environmental conditions on the floor. The birds were reared in standard cages (1.1 × 1.1 × 50 cm^3) and kept at 33°C throughout the first week of the trial, which was then decreased gradually by 2–3°C every week. Birds had feed and fresh water provided *ad libitum*. Throughout the experiment they were kept on a 23-hour light schedule with one hour of darkness. Throughout the trial, the chicks were treated in compliance with the protocols for the care of experimental animals, and all procedures complied with applicable laws and guidelines.

Plant materials

A commercial company called 2 M Group, located in Sharqia, Egypt, supplied powdered garlic (*Allium sativum*) (A.S.). For chemical analysis, Merck (Merck KGaA, Darmstadt, Germany) provided folin and Ciocalteu's phenol reagent (DPPH) 2,2-diphenyl-1-picrylhydrazyl, gallic acid, quercetin, methanol, ethanol, and aluminum chloride.

Sample preparation

Ten grams of powdered garlic (*Allium sativum*) were combined with 200 milliliters of 70% methanol, agitated for three hours, and then filtered using Whatman No. 2 filter paper. A BuCHI water bath B-480 evaporator was used to remove methanol from an extract under vacuum at 45°C. A freeze-dryer (Thermoelectron Corporation Heto PowerDry LL 300 Freeze Dryer) was then used to lyophilize the extract. Before being employed for the subsequent analyses, the resultant extract was stored at 20°C (Abd El-Hack et al., 2017 a; Ashour et al., 2020).

Estimation of total phenolic compounds (TPCs)

The Folin–Ciocalteu assay examined the TPCs of the methanolic extract (1000 mg/mL) obtained from each sample. The standard curve [y ¼ 0.001x þ 0.0563 (R2 ¼ 0.9792)] was prepared using gallic acid as a standard

phenolic chemical at various doses (10–1000 mg/mL), where y and x represent the gallic acid absorbance and condensation in mg/ml, respectively. Three milliliters of diluted Folin–Ciocalteu reagent, two milliliters of sodium carbonate 7.5%, and one milliliter of each sample or standard gallic acid were combined and left in the dark for half an hour at 25°C. Lastly, a spectrophotometer (Jenway 6405 UV/Vis, U.K.) was used to record the blend absorbance at 760 nm (Abdel-Shafi et al., 2019).

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
Chemical 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
Calculated analysis***		
moisture (%)	13.33	12.35
dry matter (%)	86.69	87.67
crude protein (%)	22.73	20.57
metabolizable energy (kcal/kg diet)	2932.5	3128.80
crude fiber (%)	2.98	3.64
ether extract (%)	4.65	5.91
crude ash (%)	6.87	5.81

Each 1 kg of vitamin mixture contained 120,000 IU vit. A, 35,000 IU vit. D_3, 400 mg vit. E, 30 mg vit. K_3, 20 mg vit. B_1, 60 mg vit. B_2, 50 mg vit. B_6, 200 mic vit. B_{12}, 1 mg cobalt, 750 mic 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%), its chemical analysis: crude protein: 45%, ME: 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).

***Chemical determined analysis according to AOAC (2006).

Total flavonoids estimation

As advised by Oritz et al. (2006), the extract's total flavonoids (TFs) should be 1000 mg/mL. The standard curve [y ¼ 0.0012x þ 0.008 (R2 ¼ 0.944)] was prepared using quercetin as a standard phenolic chemical at various concentrations (10–1000 mg/mL), where x is the concentration of quercetin in mg/mL and y is the absorbance. One milliliter of quercetin extract or solution was combined with one milliliter of an aliquot of 20 g/L $AlCl_3$ ethanol. The color absorbance at 420 nm was measured by the spectrophotometer (Abdel-Shafi et al., 2019).

Antioxidant activity estimation

The ability of the garlic (*Allium sativum*) powder to scavenge DPPH-assay was used to assess the antioxidant performance of the methanolic extractor (Ramadan et al., 2008; Osman et al., 2014). Three milliliters of methanolic-DPPH solution were mixed with one milliliter of each sample separately, and a spectrophotometer was used to measure the absorbance at 520 nm (DPPH-assay) following a 30-minute incubation period. According to Abdel-Hamid et al. (2017), the extract condensation that scavenges 50% of the DPPH and ABTS radicals (SC50) was calculated.

Data collection

Each replicate was weighed at 1, 15, 30, and 38 days to determine its live body weight (LBW) and daily body weight gain (DBWG) by dividing by the number of chickens of that replicate. Also, the feed conversion ratio (FCR) and average daily feed intake (FI) were calculated. Twelve birds from each group (2 birds/replicate) were randomly selected for carcass evaluations at 38 days of age, weighed, and manually slaughtered at the end of the experiment. The ratio of abdominal fat, giblets, and carcass to live body weight was calculated.

Carcass weight % = eviscerated weight / live weight × 100.

Dressing weight % = weight of carcasses plus giblets / live weight × 100.

Giblets (%) = Giblets weight (empty gizzard + liver + heart weight) / Live weight of bird × 100.

Liver, gizzard and heart (%) were expressed as a percentage of the live weight of the bird.

Blood biochemical analysis

The same 12 slaughtering birds from each group had their blood drawn into non-heparinized tubes after they had fasted for the whole night. The serum was then centrifuged at 5000 rpm for 15 minutes at 4ºC and stored at –20 ºC until biochemical analysis. The levels of total protein (TP), albumin (ALB), globulin (GLOB), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and high-density lipoprotein (HDL) were measured. Also, the serum samples were used to measure the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Moreover, creatinine and immunological response of immunoglobulins Y and M (IgY, IgM) was assessed following the directions provided by the manufacturer of commercially available kits.

Also, the activities of oxidative status parameters such as malondialdehyde (MDA) and superoxide dismutase (SOD) were ascertained (Abd El-Hack et al., 2017 b; Seidavi et al., 2022).

Microbiological analysis

The same 12 birds from each group were used to get their ceca. To guarantee consistent extraction and lower variability, the cecal tissue from each group was combined and homogenized. After that, 10 grams of the homogenate and 90 milliliters of sterile water peptone were combined, and the mixture was stimulated for half an hour. A 1:10 dilution (10^{-1} dilution) was obtained by collecting the liquid component. Up to a dilution of 10^{-7}, additional dilutions were carried out in tenfold increments. The total bacterial count (TBC), lactic acid bacteria (LAB), *Escherichia coli*, and coliform counts were made. Finally, the bacterial data (log CFU/g) were converted to logarithmic units.

Statistical examination

Utilizing a complete randomization design and the GLM procedures of SAS, data was provided for the ANOVA procedure (SAS Institute Inc., 2001). 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

Chemical characterization of *Allium sativum*

Table 2 shows the methanolic extract obtained from garlic (*Allium sativum*) (A.S) powder's total phenolic (TP; mg GAE g^{-1} extract) and total flavonoid (TF; mg QE g^{-1} extract) contents as well as its DPPH activity (SC50; mg mL^{-1}). For A.S., the overall amount of flavonoids was 2.7 mg QE g^{-1} extract, and the total amount of phenol was 6.9 GAE g^{-1} extract. As measured by the extract's SC50 (mg mL^{-1}), the antioxidant activity was 195 mg mL^{-1}.

Live body weight and daily body weight gain

The effects of different doses of GP supplementation in the diet on LBW and DBWG features in broiler chickens were shown by the data in Table 3. When broilers were fed 1 g GP/kg feed at 15 days of age, their LBW was higher ($P<0.05$) than the broiler control group and other treatments. Furthermore, during the research, broilers which received 1 g GP/kg feed from 1 to 15 days of age exhibited increased DBWG. However, there were no appreciable variations during the other experimental periods at LBW and DBWG.

Table 2. Total phenolic (TP), and total flavonoid contents (TF), and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) activity of the methanolic extract acquired from garlic (*Allium sativum*)

Items	*Allium sativum* content
TP (mg GAE g^{-1} extract)	6.9
TF (mg QE g^{-1} extract)	2.7
DPPH activity (SC50; mg mL^{-1})	195

Feed intake and feed conversion ratio

The results shown in Table 4 demonstrated how different levels of GP supplementation affected broiler FI and FCR. The results indicated that the 1 g GP/kg and control groups had the highest levels of FI during the experiment's duration (31–38 and 1–38 days of age), whereas the 3 and 2 g GP/kg groups had the lowest levels of FI.

Furthermore, the results showed that during the 31–38 and 1–38-day periods, the control and 1 g GP/kg groups had the highest (worst) FCR. During the same experimentation periods, the diet groups with 3 and 2 g GP/kg had the lowest (best) FCR rates compared to the control group.

Carcass characteristics

Table 5 presents an overview of the effects of adding GP to the birds' feed on the characteristics of their carcasses at the end of the experiment. The carcass, heart, liver, gizzard, spleen, dressing, and abdominal fat did not significantly differ throughout the various GP treatments as a percentage of the body's total weight. Nonetheless, the examination of the data revealed that the GP treatments had a significant effect on the giblets ($P<0.05$). In particular, the largest percentage of giblets was seen in broilers fed a diet containing 3 g of GP /kg f feed.

Blood biochemical parameters

Kidney, liver functions and lipid profile

The effects of various GP treatments on liver and kidney function are displayed in Table 6. TP, ALB, A/G ratio, ALT enzyme, and creatinine blood serum levels differed significantly among GP-treated birds and the control group. Compared to the control group, treatments containing all levels of GP had the highest TP, ALB, and A/G ratio levels. Additionally, the birds fed with a 1 g GP/kg diet showed a substantial decrease in ALT concentration, whereas those fed a 3 g GP/kg diet showed the highest level of ALT. Furthermore, compared to the control group, the creatinine levels of birds fed by all treatment groups decreased dramatically. Nonetheless, there was no significant variation in globulin or AST levels across the experimental treatments.

Table 3. Effects of using different levels of garlic powder (GP) on live body weight and daily body weight gain of broiler chickens

Items	Treatments (g garlic powder/kg diet)				SEM	P value
	control	GP1	GP2	GP3		
Live body weight (g)						
1 day	45.27	45.09	44.82	45.00	0.23	0.947
15 days	438.86 b	468.64 a	447.85 b	446.14 b	0.88	0.010
30 days	1079.55	1096.82	1075.02	1070.91	1.22	0.893
38 days	1656.91	1726.00	1730.00	1789.55	1.57	0.079
Daily body weight gain (g/day/bird)						
1–15 days	26.24 b	28.24 a	26.87 b	26.74 b	0.26	0.012
16–30 days	42.71	41.88	41.81	41.65	0.72	0.969
31–38 days	72.17	78.65	81.87	89.83	1.09	0.253
1–38 days	43.56	45.43	45.55	47.15	0.50	0.077

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

Table 4. Effects of using different levels of garlic powder (GP) on broiler chickens' feed intake and feed conversion ratio

Items	Treatments (g garlic powder/kg diet)				SEM	P value
	control	GP1	GP2	GP3		
Feed intake (g/day/bird)						
1–15 days	32.80	32.80	33.81	32.80	0.25	0.441
16–30 days	70.09	65.92	60.91	64.98	0.59	0.630
31–38 days	149.15 b	159.75 a	139.92 c	130.40 d	1.42	0.000
1–38 days	84.01 a	86.16 a	78.21 b	76.06 b	1.36	0.002
Feed conversion ratio (g feed/g gain)						
1–15 days	1.25	1.16	1.26	1.23	0.01	0.127
16–30 days	1.65	1.58	1.46	1.55	0.05	0.757
31–38 days	2.08 a	2.03 a	1.72 b	1.48 b	0.08	0.005
1–38 days	1.93 a	1.90 a	1.72 b	1.61 b	0.04	0.009

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

Table 5. Effects of using different levels of garlic powder (GP) on carcass characteristics of broiler chickens

Items (%, relative to slaughter weight)	Treatments (g garlic powder/kg diet)				SEM	P value
	control	GP1	GP2	GP3		
Dressing	76.65	77.67	74.07	75.24	0.59	0.132
Carcass	72.55	73.80	70.51	70.93	0.55	0.119
Heart	0.43	0.40	0.37	0.42	0.10	0.189
Gizzard	1.35	1.33	1.20	1.58	0.60	0.162
Liver	2.32	2.14	2.33	2.31	0.56	0.115
Giblets	4.11 a	3.86 ab	3.56 b	4.31 a	0.11	0.028
Spleen	0.09	0.07	0.13	0.12	0.13	0.361
Abdominal fat	12.33	16.33	10.00	12.00	0.70	0.056

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

Table 6 illustrates the changes in the birds' lipid profiles caused by varying dosages of GP treatment. Compared to the control group, the birds treated with a 3 g GP/kg diet had the lowest levels of TC, TG, LDL and VLDL. The birds who received zero GP, however, had the highest levels of TG and VLDL. Additionally, the birds fed with a 3 g GP/kg diet exhibited the highest levels of HDL when compared to the control and other treatment groups.

Table 6. Effects of using different levels of garlic powder (GP) on blood biochemical parameters of broiler chickens

Items	Treatments (g garlic powder/kg diet)				SEM	P value
	control	GP1	GP2	GP3		
Liver and kidney functions						
TP (g/dL)	3.52 b	4.69 a	4.34 a	4.07 ab	0.15	0.015
ALB (g/dL)	1.41 b	2.40 a	2.27 a	2.11 a	0.12	0.003
GLOB (g/dL)	2.12	2.30	2.07	1.96	0.57	0.221
A/G (%)	0.67 b	1.04 a	1.10 a	1.10 a	0.06	0.006
ALT (IU/L)	29.14 a	7.87 c	18.83 b	15.34 bc	2.50	0.004
AST (IU/L)	199.61	151.02	143.75	147.20	1.90	0.056
creatinine (mg/dL)	0.88 a	0.73 b	0.72 b	0.76 b	0.02	0.023
Lipid profile						
TC (mg/dL)	273.30 a	193.37 bc	202.30 b	170.00 c	1.16	0.000
TG (mg/dL)	153.90 a	138.30 ab	148.39 a	117.22 b	1.82	0.036
HDL (mg/dL)	43.34 b	45.29 b	45.69 b	49.40 a	0.79	0.018
LDL (mg/dL)	205.66 a	120.42 b	129.29 b	89.57 c	1.16	0.000
VLDL (mg/dL)	30.78 a	27.68 b	29.68 a	21.44 c	1.36	0.036

a, b, c – means within the same row with different letters are significantly different (P≤0.05).
TP: total protein, ALB: albumin, GLOB: globulin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TC: total cholesterol, TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein, and VLDL: very low-density lipoprotein.

Table 7. Effects of using different levels of garlic powder (GP) on broiler chickens' immunoglobulins and blood antioxidant parameters

Items	Treatments (g garlic powder/kg diet)				SEM	P value
	control	GP1	GP2	GP3		
Immunoglobulins						
IgY (ng/ml)	319.47 c	571.97 a	431.41 b	464.84 ab	1.30	0.004
IgM (ng/ml)	209.99 c	383.64 a	343.34 ab	275.57 bc	1.22	0.004
Antioxidant parameters						
SOD (U/ml)	77.91 b	119.86 a	103.86 a	132.04 a	1.90	0.005
MDA (nmol/ml)	8.74 a	3.62 c	4.36 b	2.09 d	0.78	0.000

a, b, c – means within the same row with different letters are significantly different (P≤0.01).
IgY: immunoglobulins Y, IgM: immunoglobulins M, SOD: superoxide dismutase activity, MDA: malondialdehyde.

Immune and antioxidant parameters

Table 7 shows that different GP treatments dramatically affected the blood antioxidant parameters and immunoglobulin levels of broiler chickens. Immunoglobulin Y (IgY) levels were lowest in birds from the control group but higher in the birds fed 1 and 3 g GP/kg diet, respectively. Comparatively to the control group, the birds fed a diet containing 1 g GP/kg had the highest levels of IgM.

By supplementing with GP, all antioxidant markers, including SOD and MDA, showed significant improvements. The birds given a feed containing 3 g GP/kg had the greatest levels of SOD, whereas the birds fed a diet containing 0 g GP/kg had the lowest levels. Notably, the birds fed a control diet had the highest levels of MDA, while the birds provided a diet with 3 g of GP/kg had the lowest levels.

Microbial analysis of gut microbiota

The treatments had a significant effect on all bacterial populations (P>0.05), with the exception of coliform bacteria (Figure 1). The highest amounts of TBC and LAB were found in the GP1 group. In contrast to the other treatment groups, the control group exhibited the highest concentrations of harmful bacteria (*E. coli*).

Discussion

High antioxidant activity is indicated by low SC50 values (Osman et al., 2019). The antioxidant activity (SC50) for A.S. in the DPPH-radical scavenging activity experiment was 195 mg mL^{-1}. Polyphenol aggregation supports the antioxidant capacity of various foods, including coffee (Acidri et al., 2020). Garlic compounds may raise cellular GSH levels and encourage the production of antioxidant enzymes like SOD or catalase (Borlinghaus et al., 2014). Taking 80–4000 mg of garlic daily for 2–24 weeks raises blood SOD levels, which lowers malondialdehyde and boosts total antioxidant capacity (Askari et al., 2021).

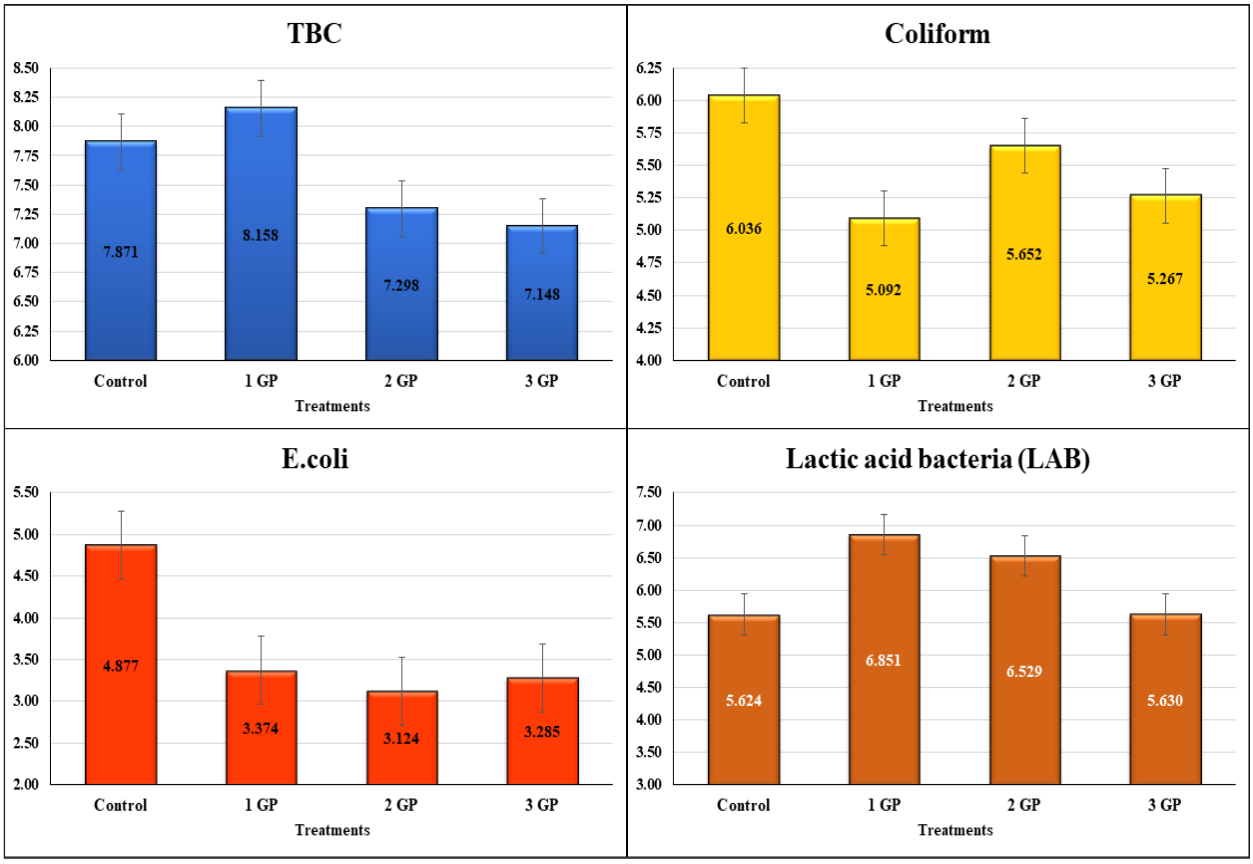


Figure 1. Effects of using garlic powder (GP) on cecal microbial count (Log CFU/g) of broiler chickens

Garlic's constituents include polysaccharides, organosulfur compounds, phenolic compounds, and saponins (Szychowski et al., 2018). Its most significant biological and pharmacological characteristics are attributed to its organosulfur compounds, which are identified and extracted for medicinal uses (Locatelli et al., 2017; El-Saber Batiha et al., 2020). Garlic has compounds with anti-inflammatory, antioxidant, and antibacterial properties that have been shown to help prevent cancer, heart disease, and immune system illnesses (Lu et al., 2017). Garlic's phenolic components, organosulfur compounds, flavonoids, and saponins are primarily responsible for its antioxidant capacity. The existence of water-soluble organosulfur compounds with antioxidant capacity (like SAC and SAMC) is probably the reason why this potential is larger in aqueous extracts (like AGE) than in fresh garlic or other preparations. Additionally, flavonoids, saponins, and certain micro- and macronutrients found in aqueous garlic extracts have been shown to have a synergistic antioxidant impact with these substances (Moutia et al., 2018; Rodrigues and Percival, 2019).

As per the results, significant improvements in LBW and DBWG were observed in the group supplemented with 1 g GP/kg diet. These findings are consistent with earlier research conducted by Khalil et al. (2007), Premavalli and Omprakash (2020), Olayinka et al. (2022), and Jalal et al. (2024), which also reported increased body weight gain in poultry supplemented with GP at various levels (0.5, 1, 1.5, 2, and 4%). Allicin, a strong antibiotic found in garlic that has been demonstrated to suppress dangerous bacteria and fungi that produce aflatoxin, is probably the cause of this improvement (Sivam, 2001; Nazareth et al., 2024). Furthermore, according to Abd El-Ghany (2024), allicin may benefit the intestinal epithelium, enhancing food absorption. It may also improve gastric and salivary secretions, further improving body weight (Abdelkader et al., 2024). Our findings align with those of Habibi and Ghahtan (2019), who found that adding dietary GP at levels between 1% and 3% considerably increased FI compared to the control.

Raya et al. (2014), Olayinka et al. (2022), and Jalal et al. (2024) found no significant variations in FI between the groups, which is in contradiction to these findings. The differences in poultry species, environmental factors (such as temperature, humidity, and lighting schedules), garlic powder content and supplementation, and the basal diet's design may all be responsible for these differences in FI responses to GP supplementation among studies. The results of this investigation showed a significant difference in FCR between the groups between the ages of 31–38 and 1–38 days, which is in line with findings from other research (Karangiya et al., 2016; Elsagheer et al.,

2020; Elbaz et al., 2021). Garlic has antibacterial qualities that aid nutrient absorption in the gastrointestinal system, which is probably why there has been an improvement in FCR. However, Olayinka et al. (2022) found that GP supplementation did not affect broiler chickens' FCR. The inclusion of various organosulfur components, such as diallyl sulfide, S-allyl cysteine, ajoene, dithiin, and allicin, may be the strategy by which the garlic powder improves these parameters (Rais et al., 2023).

Likewise, the research conducted by Ross et al. (2001) revealed that garlic's diallyl polysulfide, an antibacterial component, is a key factor in raising broiler BWG. In the ileum of broiler hens, a mixed meal containing 10 g/kg of each of garlic and turmeric decreased the pH of the digestive system. It increased apparent and digestible metabolized energy (Olukosi and Dono, 2014). Garlic may also improve pancreatic enzyme function, which improves the environment for food absorption and digestion (Amerian et al., 2024). In addition, allicin can heighten the villus and deepen the crypt, improving the physiological structure of the intestinal epithelial layer and ultimately enhancing the capacity to absorb and assimilate nutrients. Longer villi with deep crypts are thought to be a sign of a robust intestinal architecture, indicating a healthy digestive system and high pancreatic enzyme activity. Moreover, the antioxidant properties of GP can promote the consumption of nitrogen energy and enhance overall gut function (Abd El-Ghany, 2024).

The current study's findings on broiler carcass yield agreed with those of earlier investigations (Issa and Omar, 2012; Raya et al., 2014). They concluded that the dressing percentage values of both broilers were not considerably affected by GP supplementation. Saghi and Zarghi (2022) discovered that broilers which were given a diet supplemented with GP displayed a greater dressing percentage than the control group. This implies that GP may have a dose-dependent effect on carcass features, with greater concentrations possibly having more noticeable impacts (Sheoran et al., 2017).

Additionally, comparable findings about the percentage of edible offal and meat slices were published by Lukanov et al. (2015). Although our study did not find any significant impacts on the belly fat, other researchers have found that taking supplements containing garlic can lower the amount of fat deposited. For example, Issa and Omar (2012) found that broilers supplemented with GP had lower belly fat; they attributed this to garlic's ability to both inhibit and enhance lipolysis.

Garlic may prevent liver damage by increasing the liver's antioxidant enzyme activity, reducing hepatic apoptosis, and inhibiting the metabolism of 1,3-dichloro-2-propanol (Dorrigiv et al., 2020). Furthermore, garlic's organosulfur compounds may repair liver damage by reducing the production of pro-inflammatory cytokines in the liver and boosting antioxidant activity by reducing the expression of cytochrome P450 2E1 (McAlpine et al., 2024). Allicin, flavonoids, and sulfur-containing chemicals are among the powerful antioxidant-containing substances found in garlic. These substances can counteract dangerous free radicals, which can destroy kidney cells and cause renal failure. Garlic may help shield the kidneys from inflammatory-related injury by lowering oxidative stress (Bedouhene et al., 2024).

Results indicated that the birds treated with a 3 g GP/kg diet had the lowest levels of TC, TG, LDL and VLDL and the highest levels of HDL. Issa and Omar (2012) and Sewatkar et al. (2024) noticed reduced serum total cholesterol in the groups that received 0.4% and 2% of garlic powder, respectively. In addition, Aziz-Aliabadi et al. (2024) found that their serum cholesterol levels were lower when broilers were treated with 1.5% garlic powder compared to the control group. Moreover, Prasad et al. (2009) obtained comparable results. They explained the mechanism of the hypocholesterolemic and hypolipidemic effects of GP, which reduce the hepatic activities of lipogenic and cholesterolemia enzymes. Additionally, it was noted that treatment groups had a significantly higher serum HDL cholesterol level than control groups.

Nonetheless, a noteworthy distinction was also noted within the groups that received garlic supplements (Sewatkar et al., 2024). This decrease could be explained by the organic tellurium compounds present in high concentrations in garlic buds and may block enzymes involved in the cholesterol synthesis pathway (Moses et al., 2024). Garlic's allicin can prevent the liver from synthesizing fat and cholesterol and lower broiler fatty acid levels (Abd El-Ghany, 2024). Enzymes found in garlic may have a part in controlling lipid metabolism, boosting biliary cholesterol production, and reducing the fraction of dietary cholesterol absorbed (Corzo-Martínez et al., 2007). Additionally, the blood cholesterol level can be lowered by inhibiting the enzymes 3-hydroxyl-3-methylglutaryl-CoA reductase and acetyl CoA synthetase, which are necessary for cholesterogenesis and the manufacture of fatty acids. Similarly, the mechanism of hypocholesterol and hypolipid may be related to the potential effect of garlic on lipid metabolism in layers by reducing the lipogenic and cholesterogenic-depressing impacts of some hepatic enzymes, like fatty acid synthase, glucose 6 phosphatase dehydrogenase, and malic enzyme (Mahmoud et al., 2010).

Results indicated a significant increase in IgY and IgM levels in the birds fed 1 g GP/kg feed. Our findings concur with Ismail et al. (2021), who found IgM and IgG increased when broiler chickens were fed GP. The physiologically active molecules in essential oils, which have antibacterial, antioxidant, and anti-inflammatory qualities and supply vital nutrients for the growth of immune cells, may be responsible for an enhancement in the immune response (Chircov et al., 2021). Additionally, enhancing intestinal integrity and encouraging lymphocyte proliferation in the primary immune organs may increase the synthesis of immunoglobulin (Ig), including IgG, IgM, and IgA, which is linked to raising the immune organs' relative weight (Abd El-Ghany, 2024). Garlic contains various immunostimulant chemicals, such as members

of the lectin family, which have been shown to interact with immune cell surfaces' pathogen recognition receptors (Percival, 2016). One of the body's most remarkable immune system conductors, garlic increases the activity of killer cells, or macrophages, promoting immune function. Furthermore, by increasing cytokine production and/or the phagocytic capacity of antigen-presenting cells, garlic can enhance the actions of humoral immune cells (Hanieh et al., 2010). Garlic polysaccharides regulate the expression of interleukin (IL)-6, IL-10, tumor necrotizing factor-α, and interferon-gamma (INF-γ) in RAW 264.7 macrophages, demonstrating an immune potentiation mechanism.

Furthermore, concanavalin A (ConA)-induced splenocytes, thymocyte proliferation, and the *in vitro* expression of IL-2 and INF-γ genes may all be enhanced by garlic extract (Hanieh et al., 2012). Furthermore, in activated splenocytes, adding garlic extracts to a culture increased the induction of IL-2 and IL-12, INF-γ, and tumor necrosis factor α (Kyo et al., 2001). Venkatesh (2018) reported that aged garlic extract improved phagocytosis and cytokine release and boosted T-cell and natural killer cell proliferation and activity.

Results indicated that the birds fed 3 g GP/kg had the greatest levels of SOD and the lowest levels of MDA. In birds, garlic showed high antioxidant properties. Garlic contains phenols and saponins, both of which have potent antioxidant properties. A class of chemical compounds called saponins, which are present in many different types of plants, may be able to guard against hydrogen peroxide's (H_2O_2) ability to damage DNA and impede growth (Stoev, 2024). Oxidative stress results from the host cells being significantly destroyed due to an imbalance between oxidation and reduction. Nevertheless, Pawłowska et al. (2023) state that antioxidant enzymes can stop free radicals from damaging cell membranes.

Many natural antioxidants can be found in essential oils, which are found in various aromatic plants (Ismail et al., 2021). By lowering the generation of free radicals, garlic and/or garlic tocopherol produced a considerably stronger antioxidant effect, particularly in birds under heat stress (Elbaz et al., 2022). Garlic powder polar fractions (garlic equivalent to 1, 2, 4, 6, and 8% fresh garlic paste) have been shown to decrease the activities of hydroxymethylglutaryl coenzyme A reductase, cholesterol 7 α-hydroxylase and fatty acid synthetase (Qureshi et al., 1983). An aged garlic extract's diallyl polysulfides may shield cell membranes from lipid peroxidation (Sahidur et al., 2023). Furthermore, by lowering malondialdehyde (MDA) and raising glutathione peroxidase (GPx) and superoxide dismutase (SOD) levels, essential oils found in garlic and other plants help eliminate oxygen free radicals (Huang et al., 2023).

The health and development of broiler chickens are significantly influenced by their gut microbiota (Amer et al., 2023). Numerous harmful bacteria that cause enteritis have been proven to be effectively combatted by garlic and garlic-derived products (Kumar et al., 2021). Propyl-propane thiosulfonate, a derivative of garlic, has shown antibacterial action against *Salmonella* species, *E. coli*, *Campylobacter jejuni*, and Enterobacteria. Propyl-propane thiosulfonate has been demonstrated to alter the composition of the intestinal microbiota and enhance the growing broiler's ability to digest nutrients (Adjei-Mensah et al., 2023). Additionally, after feeding on 11.3% propyl-propane thiosulfonate, a notable increase in bacteroides and total bacterial contents was noted in the ileum (Abd El-Ghany, 2024). One important antibacterial ingredient that disrupts the structure and metabolism of bacterial cells is garlic oil. Garlic has been shown to have potent antibacterial properties (Liu et al., 2024). Also, Rahimi et al. (2011) showed that adding a mixture of garlic, thyme, and coneflower to the digesta of broiler chickens' ileo-cecum dramatically decreased the amount of *E. coli*.

Elbaz et al. (2021) recently discovered that garlic treatment might raise the *Lactobacillus* count while decreasing the ileal enumeration of *E. coli* and total coliform bacteria. Furthermore, it has been documented that a combination of garlic and eugenol improves the intestinal health and performance of broilers with necrotic enteritis (Kumar et al., 2022). By preventing pathogenic development and encouraging beneficial microbial growth, microencapsulated eugenol and garlic tincture altered the microbiota balance and lessened the severity of intestinal lesions in broilers suffering from necrotic enteritis (Kumar et al., 2021). An allicin-active component found in garlic extract is linked to its bacteriostatic and antibacterial qualities (Chamseddine and Abiad, 2024). Certain vancomycin-resistant enterococci were bacteriostatically affected by allicin.

Furthermore, certain thiol-containing enzymes in pathogens are deactivated and suppressed by allicin's SH group reactivity on cysteine residues (Elmahallawy et al., 2022). Certain thiol-containing enzymes in pathogens were deactivated and suppressed as a result of this process (Bhatwalkar et al., 2021). Garlic is a nucleophilic agent that can reverse the effects of electrophilic compounds on microorganisms (Abd El-Ghany, 2024).

Conclusion

Our results demonstrated that a dietary supplement containing GP at all dosages, particularly 3 g GP/kg feed, enhanced the broiler chicks' performance, blood parameters, immunity, antioxidant levels, and intestinal bacterial counts as they grew. This entails their blood's fat composition being more balanced. Growing broiler hens seem to benefit from this supplement in terms of overall health.

Conflicts of interest statement

The authors declare no conflict of interest.

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