

## Full-Length Article

# Consequences of dietary olive leaf powder supplementation on growth performance, carcass traits, blood biochemical parameters and gut microbiota in broilers

Mohamed H. Negm<sup>a</sup>, Ahmed K. Aldhalmi<sup>b</sup>, Elwy A. Ashour<sup>a</sup>, Laila A. Mohamed<sup>a</sup>, Islam M. Youssef<sup>c</sup>, Mahmoud Kamal<sup>d</sup>, Ahmed A. Elolimy<sup>e,\*</sup>, Samir A. Mahgoub<sup>f</sup>, Mohamed E. Abd El-Hack<sup>a,g</sup>, Ayman A. Swelum<sup>h,\*</sup>

<sup>a</sup> Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

<sup>b</sup> College of Pharmacy, Al-Mustaqbal University, 51001 Babylon, Iraq

<sup>c</sup> Animal Production Systems Research Department, Animal Production Research Institute, Agricultural Research Center, Dokki, Giza 12618, Egypt

<sup>d</sup> Laboratory of Gastrointestinal Microbiology, National Center for International Research on Animal Gut Nutrition, Nanjing Agricultural University, Nanjing 210095, China

<sup>e</sup> Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, Al Ain P.O. Box 15551, Abu Dhabi, United Arab Emirates

<sup>f</sup> Agricultural Microbiology Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

<sup>g</sup> Department of Industrial Pharmacy, College of Pharmaceutical Sciences and Drug Manufacturing, Misr University for Science and Technology (MUST), P.O. Box 77, Giza, Egypt

<sup>h</sup> Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh 11451, Saudi Arabia

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## ABSTRACT

This experiment examined the potential of olive leaf powder (OLP) as a natural growth enhancer in broiler feed and its influences on growth performance, carcass characteristics, blood parameters, and intestinal bacterial count. A total of 210 one-day-old Arbor Acres chicks were randomly allocated into three groups. The control group was given a basal diet only, the 2<sup>nd</sup> and 3<sup>rd</sup> groups were given basal diet supplemented with 1 g OLP and 2 g OLP/kg diet, respectively. Each group consisted of seven replicates with 10 unsexed chicks each. The experimental trial lasted for thirty-one days. Results showed that, the OLP2 group exhibited a significant increase in live body weight (LBW) at days 14 and 21 of age, whereas the OLP1 group experienced a considerable rise in body weight gain (BWG) between days 29 and 31 of age. Average daily feed intake (ADFI) considerably decreased during the first 8–14 days of the trial, but increased during the next 29–31 days throughout the entire duration (1–31 days). While, Feed conversion ratio (FCR) was improved in OLP groups during the 15–31 days of trial. Carcass and breast yields improved significantly with OLP supplementation, while abdominal fat content was reduced. Blood analysis revealed considerable enhances in total protein, albumin, and globulin levels in both OLP groups, while alanine transaminase (ALT), creatinine, total cholesterol (TC), triglycerides (TG), and very low-density lipoprotein (VLDL) levels were considerably reduced. Notably, immunity and antioxidant markers showed significant improvement with 2 g OLP supplementation. Due to OLP supplementation, the number of beneficial bacteria such as *Lactobacillus* rose while the number of all harmful bacteria (*E. coli* and *Clostridium*) in caecal samples declined. In conclusion, OLP supplementation at 1 g and 2 g per kg of feed demonstrated a significant positive impact on broiler growth performance, carcass quality, lipid profile, immunity, antioxidant status, and raised the number of beneficial bacteria in the caecal contents of the broiler chickens, making it a promising natural growth promoter in poultry production.

\* Corresponding authors.

E-mail addresses: [elolimy@uaeu.ac.ae](mailto:elolimy@uaeu.ac.ae) (A.A. Elolimy), [aswelum@ksu.edu.sa](mailto:aswelum@ksu.edu.sa) (A.A. Swelum).

## Introduction

Recently, it has become illegal worldwide to utilize antibiotics as feed additives in the production of chickens (Youssef et al., 2024a; Abd El-Hack et al., 2024). Consequently, scientists have been searching far and wide for substitutes that exhibit comparable mechanisms of action to antibiotic growth promoters (AGP) in an effort to enhance chicken health, poultry product safety, and production competence. Artificial growth stimulants and antibiotics have recently been replaced with organic feed and supplements including medicinal plants (El-Abasy et al., 2025; Deeb et al., 2024). Youssef et al. (2024b) and Dosoky et al. (2024) assert that these supplements enhance immunity and boost productivity. In order to prevent and treat infections from viruses, bacteria, parasites, and immunopotentiators, a great deal of research has been done on alternatives to antibiotic supplements for chicken production (Youssef et al., 2023a, b, c; D'Alessandro et al., 2024).

According to Sola-ojo et al. (2019), antioxidants play a critical role in mitigating these harmful effects. While artificial antioxidants such as propyl gallate, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) used in animal breeding may be effective, their safety for both humans and animals is questionable given the higher incidence of malignant tumors in animals. Conversely, natural antioxidants that employed in birds are secure, beneficial, trustworthy and known to be wide-sourced. In order to lessen oxidative stress and enhance the quality of meat and chicken, look for naturally occurring, safe antioxidants from agriculture residues (Felter et al., 2021).

Olive leaves (OL) (*Olea Europea L.*) are obtained by beating the olive trees (agricultural leftovers) for fruit harvest. Oleuropein, tyrosol, and hydroxytyrosol are among the many useful compounds located in OL (Silva et al., 2006; Batçioğlu et al., 2023). Between 8 % and 14 % of the olive leaf was made up of oleuropein (Erragued et al., 2024). Olive leaves also include particular phenols (vanillin, caffeic acid, tyrosol, hydroxytyrosol, etc.) and flavonoids (luteolin, apigenin, and rutin, etc.) (Blasi et al., 2024).

Numerous studies on the safe and efficient addition of natural plant (extracts) to poultry feed to improve meat quality have increased (Koné et al., 2019; de Paiva et al., 2021). Dried OL is a powerful supplement that can be utilized to improve chicken *in vivo* industry growth and antioxidant capacity. Additionally, by-products of olives like olive cake were also widely used in poultry for a variety of phenolic components to enhance growth, quality of meat, and overall health (Selim et al., 2021; Saleh and Alzawqari, 2021). According to Elhrech et al. (2024), olive leaves include carbohydrates, trans fats, vitamins, minerals, and antioxidants compounds that the body needs the most of. When combined, OL is a rich and moderate supplier of phenolic components that are now utilized in functional foods and animal husbandry. Therefore, this research aims to study the effect of adding olive leaf powder to broiler chicken feed on growth, carcass characteristics, blood characteristics, and intestinal bacterial count, in order to provide nutritious food.

## Materials and methods

### Design, birds and diets

This search was carried out at the Poultry Research Farm, Department of Poultry, College of Agriculture, Zagazig University, Zagazig, Egypt. All experimental protocols were authorized by the Institutional Animal Care and Use Committee (IACUC) (ZU-IACUC/2/F/313/2023) and the Ethics of the Institutional Agency of the Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

In a completely randomized design experiment, 210 one-day-old broiler chicks (Arbor Acres) with about similar initial body weights ( $44.66 \pm 0.17$ ), were divided into three groups. Each was divided into seven replicates, each containing ten chicks. The trial lasted for thirty-one days. To meet the dietary requirements, the diets were created by guidelines for Arbor Acres broiler chicks, and amino acid (AA)

management guidelines were taken into consideration when formulating the diet. As indicated by Table 1, all birds were given pellets for the first thirty-one days of their lives. There were two feeding stages: starter (one to 21 days) and finisher (22 to 31 days). Every bird was raised in the same environment. These were the treatments that were administered: control group (basal diet) not including additive. OLP1 and OLP2 are the basal diet plus 1 and 2 g of olive leaf powder per kg of diet, respectively. The chicks were housed in semi-closed house and were kept in standard cages measuring  $100 \times 100 \times 50$  cm. The birds had *ad libitum* supply of water and feed. The water temperature for broiler chickens was maintained between 20°C to 25°C. The experiment's ambient temperature was maintained at 33°C for the first week, 30°C for the second, and 26°C till the end of the experiment. The experimental room's relative humidity was maintained at 55–65 %. Throughout the experiment period, the chicks were also kept alive for 23 hours under light and 1 hour without. Using LED lights with white light, with 20–40 lux during day 1–7, and 5–20 lux after day 7.

### Plant materials

The olive leaves were supplied by a commercial enterprise. Quercetin, gallic acid, methanol, ethanol, aluminum chloride, folin, and Ciocalteu's phenol reagent (DPPH) 2, 2-Diphenyl-1-picrylhydrazyl and Merck were procured for chemical analysis (Merck KGaA, Darmstadt, Germany).

### Sample preparation

Ten grams of powdered olive leaves were combined with 200 milliliters of 70 % methanol, stirred for three hours, and then filtered through Whatman No. 2 filter paper. After extracting methanol from an

**Table 1**

Basal diet composition and chemical analysis.

Items	Starter (1–21 days)	Finisher (22–31 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:		
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
*** Calculated analysis:		
Calcium %	0.99	0.98
Phosphorous (Available) %	0.45	0.44
Lysine %	1.3	1.1
Methionine + Cysteine %	0.9	0.75

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 Mangan, 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, 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 + cysteine: 2.19 % and fiber: 2.18 %.

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

\*\*\* Calculated according to NRC (1994).

extract using a vacuum in a BuCHI water bath-B-480 evaporator at 45°C, the extract was lyophilized using a Thermoelectron Corporation Heto power dry LL 300 freeze drier. The resulting extract was kept at 20°C until it was used for the subsequent analyses (Abd El-Hack et al., 2017; Ashour et al., 2020). The methanolic extract (1000 mg/mL) extracted from each sample was used as the standard phenolic compound at different concentrations (10–1000 mg/mL) in order to estimate total phenolic compounds (TPCs) using the Folin-Ciocalteu method (Sánchez-Rangel et al., 2013). Consequently, a standard curve was created, which is shown by the equation [ $y = 0.001x + 0.0563$  ( $R^2 = 0.9792$ )], where  $y$  and  $x$  stand for gallic acid absorbance and condensation in milligrams per milliliter, respectively. Following the millilitration of each sample or standard gallic acid, two milliliters of 7.5% sodium carbonate and three milliliters of diluted Folin-Ciocalteu were mixed. After that, the mixture was kept at 25°C in the dark for 0.5 hours. A spectrophotometer (JENWAY, 6405 UV/Vis, U.K.) was used to quantify the blend absorbance at 760 nm (Abdel-Shafi et al., 2019).

#### Total flavonoids (TFs) estimation

To determine the TFs content, the Ordóñez et al. (2006) process was used. The normal curve for quercetin (10–1000 mg/mL),  $y = 0.0012x + 0.008$  ( $R^2 = 0.944$ ), was used. One milliliter of extract or solution quercetin was combined with one milliliter of 20 g/L AlCl<sub>3</sub> ethanol. The color intensity at 420 nm was measured using a spectrophotometer.

#### Antioxidant activity assessment

The antioxidant efficacy of the olive leaves methanolic extractor was assessed by its ability to scavenge the DPPH-assay (Osman et al., 2014). One milliliter of each sample was mixed with three milliliters of methanolic-DPPH solution using a spectrophotometer. After 30 minutes of incubation, the absorbance at 520 nm (DPPH-assay) was determined. The extract condensation that scavenges 50 % of the DPPH and ABTS radicals (SC<sub>50</sub>) was determined.

#### Data collection

Each bird was weighed using an electronic scale once a week to determine its live body weight (LBW) and body weight gain (BWG). Also, average daily feed intake (ADFI), and feed conversion ratio (FCR) were computed cumulatively and by time. Every day, feed loss was noted, and feed intake was calculated using this information. When the experiment is over, seven chickens were taken at random from separately group (1 bird / replicate), weighed (total 21 birds), and manually slaughtered, following Islamic tradition, which calls for cutting the jugular vein with a sharp knife and allowing it to bleed for three to five minutes in accordance with ethical committee guidelines, when they were 31 days old in order to evaluate the carcasses. We calculated the weight of the carcass and the total amount of edible portions (breast, thigh, liver, heart, and gizzard). Carcass (%) = (Weight of edible carcass / Total weight of carcass) \* 100. Dressing (%) = (Weight of carcass / Live weight of bird) \* 100.

#### Blood biochemical analysis

Following an overnight fast, seven birds in each group had their blood samples drawn at random and put into tubes devoid of heparin. Serum was extracted from the samples by centrifuging them for 15 minutes at 4°C at 5000 rpm. It was then kept at -20°C until biochemical analysis. The levels of total protein (TP), albumin (ALB), globulin (Glob), alanine transaminase (ALT), and aspartate transaminase (AST), and total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL). In addition, creatinine and immunological response (IgG, IgM) were measured concurring to the commercial kits' manufacturer's

guidelines (Youssef et al., 2023a). According to the previous work (Abd El-Hack et al., 2017), the activities of oxidative status parameters such as malondialdehyde (MDA) and superoxide dismutase (SOD) were ascertained.

#### Microbiological analysis

Following the removal of the cecum from seven birds in each group, ten grams of the cecum sample were homogenized in ninety milliliters of sterile peptone water and agitated for thirty minutes. Serial dilutions (10<sup>-1</sup> to 10<sup>-7</sup>) of the supernatant were made (Youssef et al., 2024b). According to Oxoid (2006), the total bacterial count (TBC), Coliform, *Escherichia coli*, *Salmonella* spp., *Clostridium*, *Lactobacilli*, *Lactococci*, and *Bacillus* spp., and were counted on certain medium. Log CFU/g was used to convert the bacterial counts.

#### Statistical analysis

GLM procedure of SAS was utilized to apply an ANOVA approach with a complete randomized design on the data (SAS Institute Inc., 2001). The post-hoc Tukey's assay was utilized to ascertain the variation amongst means. Unless otherwise indicated, claims of statistical significance were predicated on  $P < 0.05$ . The statistical model utilized was as follows:

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

where  $T_i$  is the treatment effect,  $Y_{ij}$  is the value that was observed of the treatment under consideration,  $\mu$  is the observed mean for the treatment under consideration, and  $e_{ij}$  is the error associated with a single observation.

#### Results

##### Chemical characterization of *thymus vulgaris*

The total phenolic (TP; mg GAE g<sup>-1</sup> extract), flavonoid (TF; mg QE g<sup>-1</sup> extract), and DPPH activity (SC<sub>50</sub>; mg mL<sup>-1</sup>) contents of the methanolic extract made from olive leaves are displayed in Table 2. Total flavonoid content was 40.66 mg QE g<sup>-1</sup> extract, but total phenol content was 148.33 GAE g<sup>-1</sup> extract. Furthermore, 61.66 mg mL<sup>-1</sup> was the extract's DPPH activity (SC<sub>50</sub>; mg mL<sup>-1</sup>).

##### Live body weight (LBW) and body weight gain (BWG)

Results of Table 3 and 4 illustrated the influence of dried OLP on LBW and BWG of broiler chicken. It was found that the highest LBW when birds fed of 2 g of OLP at 14 day and 21 days in comparison with other level and control group. While BWG was significantly ( $P < 0.05$ ) increasing by using 1 g of OLP at the period of 29 – 31 days of age comparing to other level and untreated group.

##### Average daily feed intake (ADFI) and feed conversion ratio (FCR)

As evidence in Table 5 and 6, FI was significantly ( $P < 0.05$ ) reduced by feeding 1 g and 2 g of OLP at 8 – 14 days of age in comparison to the control birds. On contrary, a significantly increasing in ADFI as a result

**Table 2**

Total phenolic (TP), and total flavonoid contents (TF), and 2,2-diphenyl-picryl-hydrazyl-hydrate (DPPH) activity of the methanolic extract acquired from olive leaves.

Items	Olive leaves content
TP (mg GAE g <sup>-1</sup> extract)	148.33
TF (mg QE g <sup>-1</sup> extract)	40.66
DPPH activity (SC <sub>50</sub> ; mg mL <sup>-1</sup> )	61.66

**Table 3**

Live body weight (LBW) of broilers fed different levels of dietary OLP supplementation.

Items	LBW (g)					
	Initial	7 D	14 D	21 D	28 D	31 D
OLP (g/kg diet)						
0.0	44.81	170.09	388.94 <sup>c</sup>	901.35 <sup>c</sup>	1574.71	1895.29
1.0	45.12	163.75	394.81 <sup>b</sup>	918.94 <sup>b</sup>	1595.10	1921.16
2.0	45.29	169.81	412.50 <sup>a</sup>	923.17 <sup>a</sup>	1597.98	1900.96
SEM	0.17	2.11	4.33	4.09	7.08	7.94
P value	0.580	0.440	0.037	0.036	0.393	0.432

- OLP: olive leaf powder.

- D: days

- Different letters within one column are significantly different ( $P < 0.05$ ).

**Table 4**

Body weight gain (BWG) of broilers fed different levels of dietary OLP supplementation.

Items	BWG (g/day)					
	1-7 D	8-14 D	15-21 D	22-28 D	29-31 D	1-31 D
OLP (g/kg diet)						
0.0	17.79	34.67 <sup>a</sup>	72.95	96.40	100.99 <sup>c</sup>	59.86
1.0	16.95	33.01 <sup>b</sup>	74.88	96.59	108.69 <sup>a</sup>	60.52
2.0	17.90	31.26 <sup>c</sup>	73.20	96.20	106.86 <sup>b</sup>	59.69
SEM	0.29	0.51	0.67	1.02	1.20	0.25
P value	0.392	0.000	0.509	0.991	0.000	0.425

- OLP: olive leaf powder.

- D: days

- Different letters within one column are significantly different ( $P < 0.05$ ).

**Table 5**

Average daily feed intake (ADFI) of broilers fed different levels of dietary OLP supplementation.

Items	ADFI (g/bird/day)					
	1-7 D	8-14 D	15-21 D	22-28 D	29-31 D	1-31 D
OLP (g/kg diet)						
0.0	28.04	52.97 <sup>a</sup>	86.04	126.36	129.01 <sup>b</sup>	84.48 <sup>b</sup>
1.0	30.73	50.03 <sup>b</sup>	85.69	122.61	149.81 <sup>a</sup>	87.77 <sup>a</sup>
2.0	30.25	48.96 <sup>c</sup>	85.16	122.28	150.06 <sup>a</sup>	87.34 <sup>a</sup>
SEM	0.53	0.66	0.47	1.62	3.75	0.60
P value	0.064	0.006	0.797	0.586	0.002	0.020

- OLP: olive leaf powder.

- D: days

- Different letters within one column are significantly different ( $P < 0.05$ ).

**Table 6**

Feed conversion ratio (FCR) of broilers fed different levels of dietary OLP supplementation.

Items	FCR (g feed/g gain)					
	1-7 D	8-14 D	15-21 D	22-28 D	29-31 D	1-31 D
OLP (g/kg diet)						
0.0	1.58	1.53	1.18 <sup>a</sup>	1.31	1.28 <sup>c</sup>	1.41 <sup>c</sup>
1.0	1.81	1.52	1.14 <sup>c</sup>	1.27	1.38 <sup>b</sup>	1.45 <sup>b</sup>
2.0	1.69	1.57	1.16 <sup>b</sup>	1.27	1.40 <sup>a</sup>	1.46 <sup>a</sup>
SEM	0.05	0.01	0.01	0.01	0.02	0.01
P value	0.122	0.056	0.019	0.102	0.029	0.021

- OLP: olive leaf powder.

- D: days

- Different letters within one column are significantly different ( $P < 0.05$ ).

of feeding diet supplemented with 1 g and 2 g of OLP at 29–31 day and 1–31 days of age respectively comparing to untreated group. FCR was significantly reduced by using 1 g and 2 g OLP at 15–21 days of age comparable to the untreated treatment. Further, FCR was significantly

increasing when feeding on 2 g OLP at 29–31 days and 1–31 days of age compared to the other level and control group.

#### Carcass features

**Table 7** inferred a significant increasing on dressing percentage and breast percentage due to feeding 2 g OLP compared to other level of OLP and control group. Carcass percentage was significantly increment due to feeding 1 g and 2 g of OLP comparing with untreated group. On the other hand, abdominal fat was reduced significantly when birds feeding of OLP concentrations in comparison with untreated group, but no considerable effects on thigh, liver, heart, gizzard and giblets percentages by OLP supplement.

#### Blood parameters

**Table 8** illustrated the serum TP, ALB, Glob, A/G ratio, ALT, AST, creatinine, TC, LDL, TG and VLDL of broilers in the different experimental categories. Serum TP, ALB and Glob were significantly increased when birds feeding of 2 g OLP and (1 g and 2 g OLP), respectively compared to the control group. On contrary, ALT, Creat, TC, TG and VLDL were significantly reduced due to OLP levels comparing with the control group. However, no considerable effects were observed on A/G ratio, AST, HDL and LDL due to OLP supplementation.

#### Immunity and antioxidant parameters

Outcomes in **Table 9** exhibit that IgG, IgM and SOD were improved significantly ( $P < 0.05$ ) because of feeding of 2 g OLP comparison to 1 g group and control group. Nevertheless, MDA was significantly reduced by all levels of OLP supplementation comparing to control group.

#### Microbial analysis of the broiler cecum

The cecal microbial count in response to various OLP treatments is shown in **Fig. 1**. Variations in the total bacterial count (TBC) were significant ( $P < 0.05$ ). The OLP groups showed the maximum TBC levels. The higher levels of *Lactobacilli*, *Lactococci*, and *Bacillus* spp. were seen in the OLP groups supplemented with 1 or 2 g of OLP, suggesting positive effects on gut health. Interestingly, in comparison with other treatments, the control group exhibited the highest levels of *E. coli*, *Salmonella*, *Clostridium*, and *Coliform*.

#### Discussion

Low scavenging capacity 50 % (SC50) values are indicative of high antioxidant activity (Osman et al., 2019). The antioxidant potential of a variety of meals is supported by polyphenol aggregation (Acidri et al., 2020). Oleuropein, hydroxytyrosol, and flavonoids are the main bioactive substances found in olive leaves; they prevent food from oxidizing on its own (Adel et al., 2024). All of the derivatives of the olive tree (*Olea europaea* L.) are rich in polyphenols. Compared to olive fruit and olive oil, the quantities of total phenols in olive leaves and leaf extract are even higher (Finicelli et al., 2021). Furthermore, the olive leaf has long been utilized as a basic material in phytotherapy. Numerous experimental, clinical, and epidemiological studies have identified polyphenols from virgin olive oil, olive leaves, and leaf extract, as well as other polyphenols derived from the olive tree, as substances that can have a variety of positive effects on the body, particularly when consumed over an extended period of time (Bucciantini et al., 2021). Oleuropein, oleocanthal, oleacein, hydroxytyrosol, and tyrosol are the polyphenols that have been studied the most.

Numerous studies have demonstrated the antioxidant qualities of OLP polyphenols and their potential to combat oxidative stress in brain tissues via a variety of methods. For instance, hydroxytyrosol and oleocanthal are strong cyclooxygenase (COX) inhibitors, but oleuropein

**Table 7**

Carcass characteristics of broilers fed different levels of dietary OLP supplementation.

Items	Relative to pre-slaughter weight, %								
	Dressing	Carcass	Breast	Thigh	Liver	Heart	Gizzard	Giblets	Abdominal fat
OLP (g/kg diet)									
0.0	76.45 <sup>c</sup>	71.60 <sup>b</sup>	30.61 <sup>c</sup>	28.05	2.44	0.47	1.94	4.85	0.71 <sup>a</sup>
1.0	79.45 <sup>b</sup>	74.81 <sup>a</sup>	31.58 <sup>b</sup>	28.38	2.51	0.43	1.70	4.64	0.66 <sup>b</sup>
2.0	80.44 <sup>a</sup>	75.95 <sup>a</sup>	33.43 <sup>a</sup>	29.29	2.27	0.44	1.78	4.49	0.51 <sup>c</sup>
SEM	0.61	0.65	0.52	0.35	0.06	0.01	0.07	0.08	0.03
P value	0.000	0.000	0.047	0.364	0.264	0.139	0.491	0.144	0.000

- OLP: olive leaf powder.

- Different letters within one column are significantly different ( $P < 0.05$ ).

and hydroxytyrosol scavenge free radicals. Furthermore, oleuropein prevents LDL from oxidizing (Beauchamp et al., 2005). Due to its ability to donate electrons, oleuropein primarily scavenges free radicals to demonstrate its strong antioxidant activity. Actually, the hydroxyl groups in oleuropein's molecular structure work as hydrogen donors, preventing oxidation. Its benefits include limiting neuroinflammation by lowering the release of pro-inflammatory cytokines and chemokines and boosting the brain's antioxidant capacity (Butt et al., 2021).

Olive leaves are an excellent way to boost the growth of livestock and poultry (Adel et al., 2024). Our outcomes were likeness with Almu-hayawi et al. (2023) who found that final body weight (FBW) had a significant influence in all groups fed diverse additives of OLP comparing to untreated group. Amini et al. (2019) noticed that birds feeding the diet provided of 0.25 % OL had the best BWG than the control birds in the starter period, but dietary supplemented levels 0.25, 0.50 and 0.75 % of OL lead to a significant increment in BWG through 22-42 days and 1-42 days. Also, Agah et al. (2019) indicated that birds having OL dietary supplementation at 200 mg/kg diet had the higher BW comparison to other groups. Further, Nassar et al. (2019) recorded the best body weight gain in the treats that feeding on diet contain of 2.0 % olive leaves powder, but the control group had the lowest values. Similar to this, studies by Bahsi et al. (2016), Ait-Kaki et al. (2018), and Nafea and Hussein (2018) showed that adding OLP to broiler feed at a rate of 20 or 25 g/kg diet greatly boosted the amount of BWG.

The powdered olive leaves include phenolic components that function similarly to steroid hormones. Hormone steroids and phenolic compounds increase basal metabolic rate and improve feed absorption, which increases diet nutrient consumption (Esenbuga and Ekinci, 2023). Olive leaf powder has demonstrated encouraging benefits on broiler body weight. It is rich in bioactive components including as oleuropein, hydroxytyrosol, and flavonoids. Compounds found in olive leaves can aid in reducing inflammation, which has a detrimental effect on feed efficiency and growth. Furthermore, olive leaf powder can help maintain a healthy intestinal environment, which is necessary for nutrient absorption, by lowering inflammation (Adel et al., 2024).

Also, parallel findings were noticed by Nassar et al. (2019) who pointed that the best FCR was found in the group that having diet supplemented with 2.0 % OLP, while fewer values were noticed in the control birds. Also, Nafea and Hussein (2018) notified that, the OLP supplementation at level 20 or 25 g/kg diet to broiler diets was enhanced FCR. Also, Erener et al. (2020) recorded that birds that feeding of 600 mg/kg of OLE had the highest FI comparing to the control group. Furthermore, there was a considerably improving in FCR by 300 and 600 mg OLE of broilers in comparison with control, OLE 75 mg, and OLE150 mg group.

The primary chemicals in dietary olive leaves are oleuropein and polyphenols, which may provide certain health benefits (Ronca et al., 2024). There are numerous phenolic components found in olive leaves (Agah et al., 2019). The primary phenolic component in olive oils is oleuropein (Debbou-Iouknane et al., 2021). Oke et al. (2017) demonstrated that the biological effects of the phenolic components found in olive leaves in previous studies include analgesic, anti-inflammatory,

antioxidant, antibacterial, antitumor, and anticancer characteristics.

Moreover, our outcomes were similarity with Erener et al. (2020) who found that carcass and dressing percentage of chickens feeding of 600 mg olive leaf extract (OLE) was higher than those in the other groups, and control birds. While, the abdominal fat was lower in the control birds than in all OLE birds. Also, no significant influenced were noticed in weight of liver, spleen, and heart in all treatment categories feeding of 50, 75, 100, 125, and 150 mg/g of OLP comparing with control birds, while the significant difference on carcass weight showed in 100 mg/g OLP in contrast with control. But the abdominal fat was significantly lowered in 75, 100, 125 and 150 mg/g OLP in comparison to control group (Almu-hayawi et al., 2023). On contrary, El-Damarawy et al. (2013) showed that, birds having 2 % dietary olive leaves reduced significantly weight of liver comparing to the untreated birds. Conversely, Shafey et al. (2013) notified that eviscerated carcass weight and its compounds of birds that slaughtered at 21 and 36 days of age did not affect significantly when feeding of diet supplemented with OLE. Additionally, Jabri et al. (2017) found that the supplementation of two doses of OLE (10ml/L and 20ml/L) to the water didn't impact on most of carcass attributes.

Furthermore, our finding was parallel with Nassar et al. (2019) who recorded that TP and Glob were increased significantly, whereas A/G ratio were reduced significantly in birds that fed diet with 2 % OLP in comparison to control birds. These findings are aligned with Nafea and Hussein (2018) who found a significantly increasing in serum TP and Glob levels of birds fed 2 % olive leaves supplementation. While, serum TC and TG levels of chicks were significantly reduced when consumed a diet complemented with OLP when compared to untreated birds. Also, Ait-Kaki et al. (2018) declared that no considerable influence on TG levels by adding several of olive leaves levels to broiler feed. Also, Xie et al. (2022) reported that, olive leaf extract didn't make any considerable influence on serum concentrations of TC, LDL, HDL, and creatinine in the broilers. However, Sarica and Toptas (2014) noticed that decreasing on serum TC and LDL concentrations by dietary supplementation with 200 mg kg<sup>-1</sup> oleuropein in quail. Almu-hayawi, et al. (2023) indicated that lipid profiles and ALT or AST levels were improved by feeding of OLP compared to untreated group. Further, Agah et al. (2019) found that reduction of TC, TG, ALT, and AST of birds that feeding diets include of OLP. Also, Parsaei et al. (2014) obtained that a significantly decreased in blood cholesterol, HDL, LDL and VLDL by dietary supplementation of olive leaf at level 0.5 and 0.75 % comparable to control group.

Compounds found in olive leaves have the capability to prevent the release of inflammatory moderators and cytokines, which lessens inflammation in the kidneys and liver (Sahnoun et al., 2024). Also, the antioxidants in olive leaf powder can shield these organs from oxidative stress-related harm. Additionally, the normal function of kidney and liver cells can be preserved by using olive leaf powder, which reduces oxidative stress and inflammation. Furthermore, chemicals found in olive leaves may improve the liver's capacity to metabolize harmful compounds, lessening the load on the kidneys (de Oliveira et al., 2024). Among of the polyphenols in OL is oleuropein, is a powerful

**Table 8**  
Blood indices of broilers fed different levels of dietary OLP supplementation.

Items	TP (g/dl)	ALB (g/dl)	GLOB (g/dl)	A/G ratio	ALT (U/L)	AST (U/L)	CREAT (mg/dl)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
OLP (g/kg diet)	4.15 <sup>c</sup>	2.35 <sup>b</sup>	1.80 <sup>c</sup>	1.34	23.16 <sup>a</sup>	137.26	0.80 <sup>a</sup>	167.39 <sup>a</sup>	133.92 <sup>a</sup>	44.19	89.87	26.78 <sup>a</sup>
0.0	4.63 <sup>b</sup>	2.66 <sup>a</sup>	1.96 <sup>b</sup>	1.36	17.43 <sup>b</sup>	110.69	0.77 <sup>b</sup>	152.20 <sup>b</sup>	117.71 <sup>b</sup>	47.01	81.65	23.54 <sup>b</sup>
1.0	5.21 <sup>a</sup>	2.62 <sup>a</sup>	2.59 <sup>a</sup>	1.02	15.01 <sup>c</sup>	74.64	0.34 <sup>c</sup>	108.70 <sup>c</sup>	82.98 <sup>c</sup>	50.73	47.91	16.59 <sup>c</sup>
2.0	0.18	0.06	0.15	0.08	2.84	11.74	0.08	10.52	9.13	1.30	8.14	1.83
SEM					0.044	0.001		0.065	0.027		0.106	
P value	0.019	0.022	0.044									0.034

- OLP: olive leaf powder.

- Different letters within one column are significantly different ( $P < 0.05$ ).

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

**Table 9**

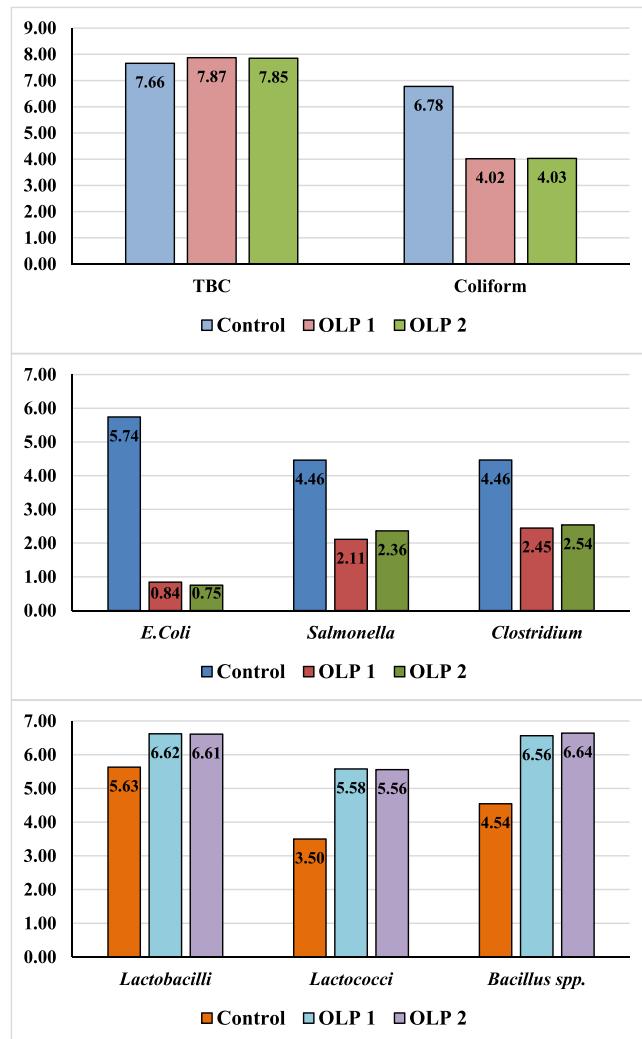
Immunity and antioxidative parameters of broilers fed different levels of dietary OLP supplementation.

Items	IgG (ng/ml)	IgM (ng/ml)	SOD (U/ml)	MDA (nmol/ml)
OLP (g/kg diet)				
0.0	212.40 <sup>c</sup>	254.41 <sup>c</sup>	94.49 <sup>c</sup>	7.71 <sup>a</sup>
1.0	349.10 <sup>b</sup>	318.89 <sup>b</sup>	144.81 <sup>b</sup>	3.08 <sup>b</sup>
2.0	589.27 <sup>a</sup>	405.28 <sup>a</sup>	233.36 <sup>a</sup>	1.43 <sup>c</sup>
SEM	55.44	23.45	20.47	0.95
P value	>0.001	0.002	>0.001	>0.001

- OLP: olive leaf powder

- Different letters within one column are significantly different ( $P < 0.05$ ).

- IgG: Immunoglobulin G, IgM: Immunoglobulin A, SOD: Superoxide dismutase, MDA: Malondialdehyde.



**Fig. 1.** Cecal microbial count (Log CFU/g) of broilers fed different levels of dietary OLP supplementation.

antioxidant. As lipid peroxidation destroys cell membranes and can result in raised cholesterol levels, they can counteract dangerous free radicals that contribute to this process (Gonçalves et al., 2024). Also, olive leaf chemicals could inhibit with the assimilation of dietary cholesterol in the guts. This could be achieved through processes like attaching to cholesterol or affecting bile acid metabolism. According to Shahidi and Danielski (2024), some polyphenols found in olive leaves may encourage the liver to create more bile acids, which may aid in the body's removal of cholesterol. Hepatic cholesterol production is

decreased by oleuropein's inhibition of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the enzyme that limits the rate of cholesterol biosynthesis (Gnoni et al., 2021). Additionally, polyphenols inhibit the synthesis of fatty acids and triglycerides by suppressing the expression of genes such as fatty acid synthase (FAS) and sterol regulatory element-binding protein 1c (SREBP-1c) (Qin et al., 2023). According to Tahri-Joutey et al. (2021), OLP increases lipid breakdown for energy by activating peroxisome proliferator-activated receptor-alpha (PPAR- $\alpha$ ), a nuclear receptor that stimulates fatty acid  $\beta$ -oxidation in mitochondria and peroxisomes. Furthermore, AMP-activated protein kinase (AMPK) is triggered by hydroxytyrosol, which causes metabolism to shift from lipid storage to fatty acid oxidation (Munteanu et al., 2025).

Additionally, our results were similarity with Oke et al. (2017) and Ahmed et al. (2017) who noticed a significantly decreased of MDA concentration because of adding various levels of OLP to broiler diets. Hayes et al. (2011) suggested that the antioxidant advantages of OL correspond to phenolic compounds which constitute free radical scavenger by preventing the free radical chain reaction. Furthermore, a number of chemicals found in OL and OLE have been reported by Silva et al. (2006) and Jemai et al. (2008) to potentially function as sources of antioxidants. Additionally, hens fed OLE exhibited a considerable rise in SOD plasma levels and a significant drop in MDA concentrations (Oke et al., 2017).

Agah et al. (2019) noticed that OLE can provide antioxidant protection and reduced lipid peroxidation and this can enhance broilers' health and redox status. Furthermore, El-Damarawy et al. (2013) realized that an improvement of most of immunity and biochemical traits of Mandarah chick when feeding of OLP at the level of 2.0 %. Christaki et al. (2004) observed that olive leaf contains flavonoids, these components are able improve the immunity status of chicken. The body's natural defense against oxidative stress may be strengthened by the utilization of OLP, which might increase the synthesis of antioxidant enzymes like SOD. OLP may also assist in stabilizing cell membranes, which would lessen the likelihood of lipid peroxidation and, in turn, lower MDA levels (Al-Shammari and Zamil, 2024). In order to avoid oxidative damage to lipids, proteins, and DNA, oleuropein and hydroxytyrosol give electrons to neutralize reactive oxygen species (ROS), such as hydrogen peroxide ( $H_2O_2$ ), hydroxyl radicals ( $OH^-$ ), and superoxide anions ( $O_2^-$ ) (Gonçalves et al., 2024). Furthermore, polyphenols suppress Fenton processes that produce dangerous free radicals by binding to iron ( $Fe^{2+}$ ) and copper ( $Cu^{2+}$ ) (Ohiagu et al., 2024).

In the present study, OLP drastically reduced the quantity of caeca pathogens and raised the number of beneficial bacteria. These outcomes were consistent with those of Abdel-Kader et al. (2024), who discovered that the most effective activity against intestinal pathogenic bacteria was exhibited by an OLP at a concentration of 300, and 600 ppm /kg diet for growing Japanese quail. Additionally, it greatly accelerates *Lactobacilli* growth. Bioactive substances with potent antibacterial qualities, including as hydroxytyrosol, flavonoids, and oleuropein, are found in OLP. These substances have the ability to damage bacterial cell membranes, which can result in bacterial mortality and the leaking of cellular contents (Alowaiesh et al., 2023). Certain phenolic substances inhibit bacterial growth and metabolism by interfering with their enzymatic systems (Nemzer et al., 2025). Furthermore, several bioactive substances found in olive leaves have the ability to prevent bacterial communication, or quorum sensing, which lessens the capacity of pathogens to create biofilms and spread illnesses (Arrigoni et al., 2024). As prebiotics, the fiber and polyphenols in OLP promote the growth of good bacteria, such as *Lactobacillus* and *Bifidobacterium* (Plamada and Vodnar, 2021). Additionally, probiotic-induced improved fermentation raises short-chain fatty acids (SCFA), such as butyrate, which support intestinal cells and prevent the formation of pathogens (Al-Smadi et al., 2023).

## Conclusions

Based on the findings of this study, supplementing broiler chicken diets with OLP at concentrations of 1 g and 2 g per kilogram of feed significantly enhanced overall growth performance, involving LBW and FCR. Furthermore, the addition of OLP positively influenced the birds' lipid profiles, evidenced by a decrease in TC, TG, and VLDL concentrations, contributing to improved cardiovascular health. The supplementation also bolstered immune function and increased antioxidant capacity, suggesting enhanced disease resistance and better protection against oxidative stress. Also, the OLP supplementation improved the number of beneficial bacteria and reduced the number of pathogens bacteria, suggesting boosted disease resistance and improved the overall health of birds. These results strongly support the potential of OLP as a natural, effective alternative to antibiotics, promoting both growth and health in broiler production systems. The use of OLP could serve as a sustainable strategy in poultry nutrition, fostering healthier birds while addressing consumer demand for antibiotic-free meat products.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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