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RESEARCH ARTICLE

Olive Leaves' Extract as an Antibiotic Alternative Enhances the Growth, Oxidative Stress, Molecular Markers, and Histological Variation in *Salmonella*-Challenged Japanese Quails (*Coturnix japonica*)

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A B S T R A C T

The overuse of antibiotics in poultry farming has led to a significant rise in antimicrobial resistance (AMR), highlighting the urgent need for sustainable and effective alternatives to maintain quail health and productivity. This study explored the potential of Olive leaves' extract (OLE) as a natural antibiotic alternative for quails challenged with *Salmonella*. OLE is rich in beneficial compounds like polyphenols, flavonoids, and oleuropein, known for their antioxidant, antimicrobial, and anti-inflammatory properties. Our research evaluated OLE's effects on growth performance, oxidative stress markers, molecular indicators of immune response, and histological variations in *Salmonella*-challenged quails. The results were highly encouraging: OLE significantly enhanced body weight gain, feed efficiency, and overall growth performance in treated quails compared to Salmonella-challenged controls. Moreover, OLE supplementation effectively reduced oxidative stress by lowering malondialdehyde (MDA) levels and boosting the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx). At a molecular level, OLE upregulated the expression of immune-related genes, including those involved in inflammatory response and pathogen defense, indicating improved immune function. Histological analysis also revealed that OLE mitigated *Salmonella*-induced damage in intestinal tissues, promoting better gut health and integrity. Beyond these benefits, OLE enhanced the count of beneficial lactic acid bacteria (LAB) while decreasing pathogenic bacteria and even improved the meat color and taste quality. These findings collectively highlight the substantial potential of olive leaf extract as a sustainable and eco-friendly alternative to antibiotics in poultry production. By improving growth performance, reducing oxidative stress, modulating immune responses, and enhancing gut health, OLE offers a compelling alternative strategy to combat *Salmonella* infections and reduce reliance on conventional antibiotics.

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INTRODUCTION

Quail farming has grown into a major global agricultural activity, providing a significant source of animal protein for people worldwide (Reda *et al.*, 2024a). Using natural additives like extracts, phytogenics, and essential oils in animal feed is a common practice (Nhara *et al.*, 2025). These additions help reduce feed costs, improve the quality, productivity, and health of livestock products, and enhance various physiological traits (Surai, 2014; Alagawany *et al.*, 2021a,b; Reda *et al.*, 2024b). These natural feed additives and their extracts are packed with active components that act as growth promoters and antimicrobial agents, providing numerous health and nutritional benefits for poultry (El-Hack *et al.*, 2022a; El-Sayed *et al.*, 2024). Olive trees are packed with natural antioxidants like oleuropein, dimethyl oleuropein, ligostroside, and oleoside. They also contain various beneficial phenolic compounds such as caffeic acid, tyrosol, elenolic acid, catechol, rutin, tocopherols, and hydroxytyrosol. Also, flavonoids like apigHenin, kaempferol, and luteolin are found in olive trees. All these compounds have significant medicinal potential (Ghanbari *et al.*, 2012).

Oleuropein, a powerful compound found in olive leaves, is a strong antioxidant with many health benefits (Gikas *et al.,* 2007). Olive trees produce a lot of leaves when pruned-around 25kg per tree (Paiva-Martins *et al.,* 2009). Since the cellulosic material in these leaves can be harmful to the environment, using them as livestock feed offers significant economic and environmental benefits (Abo Omar *et al.,* 2012). These leaves are also rich in oleuropein, a primary polyphenol, and its derivatives like hydroxytyrosol (Lee and Lee, 2010).

Various diseases infest the poultry farm because of the overuse of antibiotics or the transition of infection (Nechitailo *et al.,* 2024; El-Saadony *et al.,* 2025). Among that, *Salmonella* Enteritidis (SE) is a major concern for poultry farmers, posing significant risks to both animal health and food safety (Mkangara, 2023). This particular strain of *Salmonella* is a leading cause of foodborne illness in humans, primarily due to consuming contaminated poultry products like eggs and meat (Galán-Relaño *et al.,* 2023). While adult poultry often show no visible symptoms of SE infection, young chicks can suffer from diarrhea, lethargy, dehydration, and stunted growth. A critical issue with SE is its ability to colonize the reproductive tract of laying hens. This means the bacteria can be passed directly into eggs, leading to contaminated eggs and a substantial risk to human health (Qosimah *et al.,* 2021). In severe cases, SE infections can even lead to mortality, especially in young or immunocompromised birds (Schat and Skinner, 2022).

Olive leaf extract (OLE), which comes from the leaves of the olive tree (*Olea europaea*), is gaining recognition as a natural and sustainable alternative to antibiotics for fighting bacterial infections in poultry, like *Salmonella enteritidis* (SE). OLE is packed with bioactive compounds such as oleuropein, hydroxytyrosol, and various polyphenols. These powerful ingredients work by disrupting the cell membranes of bacteria, preventing them from forming protective biofilms and interfering with their ability to multiply (Aksoy *et al.,* 2023). This makes OLE particularly potent against SE. Because of its strong antimicrobial, anticoccidial, antioxidant, and anti-inflammatory properties (Şen *et al.,* 2023; Saeed *et al.,* 2023), OLE shows great promise for improving poultry health and reducing the risk of SE contamination in both eggs and meat.

Many studies highlight the positive impact of Olive Leaf Extract (OLE) in poultry. For instance, Obied *et al.* (2005) found that *Olea europaea* L. leaf extracts possess a range of beneficial properties, including antimicrobial, anti-inflammatory, antithrombotic, antiatherogenic, and antioxidant activities. Jabri *et al.* (2017) observed that OLE effectively combated harmful intestinal bacteria. Similarly, El-Damrawy *et al.* (2013) reported that supplementing Mandarah chicks' diets with 2% olive leaf powder led to improved immunity, better serum chemical parameters, increased body weight, and enhanced feed conversion. Further supporting these findings, Jabri *et al.* (2017) also noted improved performance in broiler chickens when OLE was added to their drinking water at a rate of 10 mL/L.

Research shows that oleuropein, an active compound in olive leaves, can significantly benefit poultry. For instance, Bahsi *et al.* (2016) found that feeding quails diets with 400ppm of oleuropein improved their productive performance and the lipid quality of their breast muscle. Similarly, Ahmed *et al.* (2017) observed better performance in laying hens when their diets were supplemented with oleuropein at 50, 100, and 150ppm. These results indicate that the beneficial compounds in olive leaves could be a promising way to modulate the intestinal microbial population and enhance growth performance in various poultry.

While oleuropein and olive leaf extract are promising growth stimulants and alternative feed additives in poultry nutrition, further research is needed to fully understand their effectiveness, mechanisms of action, and antimicrobial properties. Although some studies have explored the effects of OLE and oleuropein as feed additives on growth performance, digestive enzymes, antioxidant parameters, antimicrobial activity, and immunity in quails (Bahsi *et al.,* 2016), more research is needed. Therefore, this study aims to investigate the influence of three different dosages of OLE on growth, serum biochemistry, antioxidant status, immunity, intestinal bacterial composition, and digestive enzyme activity in *Salmonella*-challenged Japanese quails during the growing period.

MATERIALS AND METHODS

Preparation of olive leaves' extract: The olive leaves were collected during the winter (February) from a private farm. The collected leaves were thoroughly cleaned to remove impurities and then shade-dried under natural ventilation. Once dried, the leaves were ground into a fine powder using a Moulenix blender (France) equipped with a specialized size setting to ensure uniformity in the powder consistency.

Ten grams of the olive leaves flour were mixed with 200mL of 70% (v/v) aqueous ethanol and stirred for 2 hours at 38°C using a thermo-shaker set to 180 rpm. The extract was centrifuged at 10000rpm for 5min to separate the residues; then, the supernatant was concentrated using a rotary evaporator (BUCHI, Germany), and the solvent was evaporated. The residues were lyophilized at -50°C and 0.028m bar using a heto-power dry lyophilizer (-80°C), and the powdered extract was obtained (Saad *et al.,* 2021a,b). The lyophilized extract was stored in glass bottles and refrigerated until further use, as Aytul, (2010) described. The phenolic compounds of OLE was found in Table 1.

Table 1: Phenolic compounds profile in olive leaves extract (mg/g dry weight).

Phenolic compound	Nature	Concentration mg/g
Rutin	Flavonol	1.2±0.01
Luteolin	Flavone	0.51±0.01
Oleuropein	Secoiridoid	82.11±1.3
Quercetin	Flavonol	0.44±0.01
Protocatechuic acid	Phenolic acids	1.3±0.2
Caffeic acid	"	2.2±0.5
Coumaric acid	"	2.5±0.3
Gallic acid	"	2.45±0.4
Syringic acid	"	0.11±0.02
Ferulic acid	"	0.2±0.04
Tyrosol	Simple phenol	2.1±0.5
Hydroxytyrosol	Simple phenol	0.36±0.01
p value		<0.0001

Biological activities of OLE

Antioxidant activity: The DPPH scavenging activity of OLE was estimated by Alsulami and El-Saadony (2024) with some modifications. After adding 100µL of ethanolic DPPH to 100µL of OLE (50, 100, 200, and 300µg/mL) and incubating for 30 minutes in the dark, the resulting color was measured using a microplate reader (517nm). The absorbance was incorporated into the subsequent equation.

% DPPH scavenging activity=(Abs control-Abs sample)/(Abs control) x100 (1)

Antimicrobial activity: *Salmonella typhi, Salmonella enteritidis, Escherichia coli,* and *Bacillus cereus* were used to measure the antibacterial activity of OLE. The microbial strains were kept at 4 °C by subculturing them on nutrient agar slants. The agar well-disc-diffusion method were used to assess the antibacterial activity of OLE. Following the addition of 50 ml of melted Muller-Hinton agar (MHA) to plates, a loopful of bacterial inoculum was distributed across the surface of each plate (Saad et al., 2021a). Each plate was punched with 8 mm wells and introduced 6 mm discs saturated with 50 µl of OLE levels (50, 100, 200, and 300µg/mL). Negative control wells were discs with water. For 24–48 hours, MHA plates were incubated at 37°C (Thagfan *et al.,* 2025). Diameters of the resulting inhibition zones (mm) indicated antibacterial activity.

Experimental design, birds, and diets: 500 Japanese quail chicks were housed in electrically heated batteries and received the standard diet (24% crude protein, 2900 kcal ME/kg) until 10 days of age, following NRC (1994) guidelines. At 10 days old, 360 unsexed quail chicks weighed 60.46 g and were allocated to six treatments (60 birds per group, six replicates of 10 birds each). The control group received the basal diet, the *Salmonella*-challenged group, while the following three groups received basal diet supported with OLE at 100, 200, and 300 ppm, respectively, and the *Salmonella*-challenged group and treated with OLE 300 ppm. Each quail was wing-banded on day 10, and the birds were then transferred to cages (60 × 40 × 25cm³). Throughout the experimental period, the quails were kept under continuous illumination and had feed and water. The basal diet's ingredients and chemical composition are shown in Table 2. The OLE was manually incorporated into the diets in a two-step mixing process to ensure homogeneity. The mixed diets were stored in sealed, labeled bags to preserve the additives' efficacy (Khubeiz and Shirif, 2020).

The *Salmonella* challenge was conducted as follows: Salmonella enteritis strain is typically grown in Luria Bertani (LB) broth at 37°C for 24h to reach a high concentration of viable bacteria. The bacterial concentration is determined by serial dilution and plating on Xylose Lysine Desoxycholate (XLD) agar medium to count Colony Forming Units (CFU/mL). The bacterial suspension is then diluted to the desired challenge dose (107 CFU/quail) using sterile phosphate-buffered saline (PBS).

Quails were fasted for 10 hours before inoculation. This can temporarily alter gut conditions, potentially increasing susceptibility to infection by reducing gut motility and gastric acid. However, this also causes stress and must be weighed against welfare considerations and research objectives. A specific volume of 0.5mL of the Salmonella inoculum, containing the calculated challenge dose, is administered directly into the crop of each quail using a sterile gavage needle or cannula attached to a syringe (Marcq et al., 2011).

Growth performance: Birds were weighed individually at the beginning of the study, & feed intake (FI, g) per cage was reported through period time of quails (10–38 days of age). The uneaten feed was discarded, and the body weight gain (BWG, g), feed conversion ratio (FCR), growth rate (GR), and performance index (PI) were calculated using the following equations (North, 1972):

$$BWG10-38 = BW38 - BW10 \quad (1)$$

$$FCR = FI\,(g) - BWG(g) \quad (2)$$

$$GR = \frac{(LBW38 - LBW10)}{0.5 \times (LBW38 + LBW10)} \quad (3)$$

$$PI = \frac{BWG\,(g)}{FCR} \quad (4)$$

Table 2: Composition and calculated analysis of basal diet fed to growing Japanese quail.

Ingredients	%
Yellow corn	53.5
Soybean meal (44 %)	30.5
Corn gluten meal (60%)	9.5
Wheat Bran	1.5
Vegetable oil	0.5
DL-methionine	0.20
L-Lysine hydrochloride	0.30
Salt (NaCl)	0.50
Vitamin and mineral premix*	0.50
Limestone	1.00
Di calcium phosphate	2.00
Total	100
Calculated Analysis**	
Metabolizable energy (kcal/kg)	2900
Crude fiber %	3.60
Crude protein %	24.11
Available P%	0.39
Calcium %	1.24
Lysine	1.35
Methionine	0.62
Methionine + Cystine	0.89

Blood incidences: On day 38 of the experimental period, blood samples were collected from three randomly selected quails from each replicate after slaughter. Slaughter was performed by jugular vein severance according to Islamic methods. Forty-eight blood samples were collected into clean, dry centrifuge tubes and centrifuged at 5000rpm for 20min to separate the serum. The serum samples were stored at -20°C in Eppendorf tubes until analysis.

Lipid Profile: Total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were measured using the method described by James (2001). Antioxidant Status: Total antioxidant capacity (TAC), glutathione peroxidase (GPx), and thiobarbituric acid-reactive substances (TBARS) were measured following the method of Paglia and Valentine (1967). Superoxide dismutase (SOD) activity in whole blood hemolysates was determined spectrophotometrically using an automatic biochemical analyzer (RX Daytona, Randox Laboratories) and the Ransod kit (Randox Laboratories), based on the method of McCord and Fridovich (1969).

Liver Enzymes: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were determined using the method described by Friedman and Young (2005). Digestive Enzymes: Amylase and lipase activities were assayed according to Friedman and Young (2005), while trypsin levels were determined using a Bovine Trypsin ELISA Kit (MBS706461). Immunoglobulins: IgG, IgA, and IgM levels were measured using a Sandwich ELISA, with absorbance read at 450 nm on an ELISA plate reader (Erhard *et al.*, 1992).

Gut microbial count: 1 g of the intestinal component was separated and homogenized in 9mL of saline solution to obtain a 10^{-1} dilution. Serial dilutions were done until a concentration of $1x10^4$. 0.1 ml of the diluted solution was distributed in five different culturing media: MacConkey and MRS agar was used to count *E. coli*, and *Lactobacillus*, respectively. *Salmonella* load was counted on Brilliant Green Agar (BGA) plates, and total bacterial count (TBC) & the count of molds and yeasts were evaluated on Plate Count Agar (PCA) and Sabouraud Dextrose Agar (SDA), respectively (Reda *et al.*, 2020; Alagawany *et al.*, 2025). The microbial counts were estimated as colony-forming units per gram (CFU/g) sample.

Histological studies: Intestinal and liver tissues were collected, fixed in 10% formalin for 48 hours, and then processed using an automated tissue processor. Following fixation, tissues were washed in distilled water for 30 min and then dried using different immersions in alcohol with different concentrations (70% for 120 minutes and 90% for 90 minutes). The dehydration was cleared by applying numerous cycles of xylene. Briefly, tissues were submerged in xylene (50%) for 60 min and alcohol (50%), then pure xylene for an additional 90min. The tissues were put with melted paraffin wax, sealed, and then paraffin was cut into sections with a thickness of 4-5µm and then stained with Hematoxylin & Eosin (Suvarna *et al.*, 2020).

Statistical analysis: All data were acquired in triplicate and presented as mean ± SE. Then, they were statistically analyzed by SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) using a one-way ANOVA & LSD test to assess significant differences at $P<0.05$.

RESULTS

Biological activities of olive leaves' extract: The provided figures clearly illustrate the significant biological activities of olive leaf extract (OLE), specifically its antioxidant and antibacterial properties, both of which are dose-dependent. Fig. 1 demonstrates that OLE possesses potent antioxidant activity, measured by its ability to scavenge DPPH free radicals. As the concentration of OLE increases from 50µg/mL to 300 µg/mL, its antioxidant activity progressively rises, with the highest efficacy observed at 300µg/mL (over 90% antioxidant activity), which is significantly higher than the lower concentrations ($P<0.05$ as indicated by different superscripts).

Fig. 2 showcases OLE's strong antibacterial activity against several common pathogenic bacteria: *B. cereus*, *S. enteritidis*, *E. coli*, and *S. typhi*. Across all tested concentrations (50 to 300µg/mL), OLE consistently produced inhibition zones, suppressing bacterial growth. Notably, the antibacterial efficacy generally increased with higher OLE concentrations, reaching its peak at 300µg/mL for most tested bacteria. For instance, at 300µg/mL, OLE showed the largest inhibition zone diameters against *S. enteritidis* (approximately 40 mm) and *B. cereus* (over 30 mm), significantly outperforming lower concentrations. While the effectiveness varied slightly among bacterial species, with *S. enteritidis* often showing the highest susceptibility, the overall trend confirms OLE's broad-spectrum antibacterial properties.

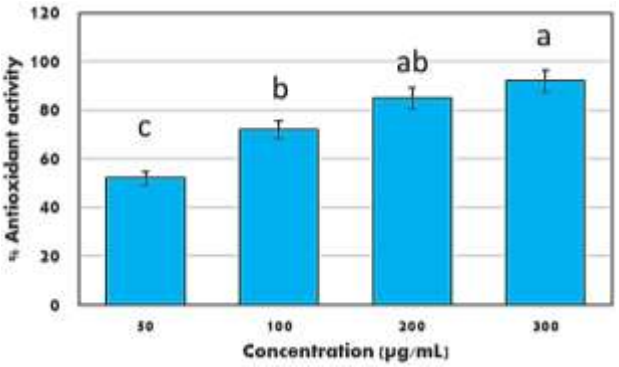

Fig. 1: Antioxidant activity of olive leaves extract against DPPH free radicals.

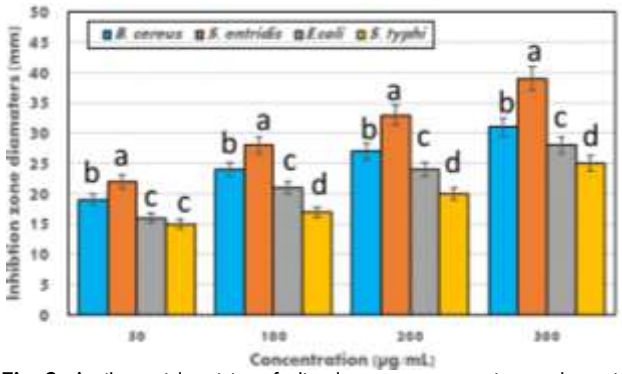

Fig. 2: Antibacterial activity of olive leaves extract against pathogenic bacteria.

Effect of OLE on Growth Parameters: The data in Table 3 demonstrate the detrimental impact of *Salmonella* infection on quail growth performance. Comparing the Control group (healthy quails) with T4 (Salmonella-challenged quails), a significant reduction is observed across most growth parameters. Specifically, T4 quails exhibited a statistically lower final body weight (FBW), body weight gain (BWG), and feed efficiency (FCR), alongside an increased feed intake (FI) compared to the Control, suggesting that the infection impaired nutrient utilization and growth. For instance, the Control group had a BWG of 180±0.2g, while the Salmonella-challenged T4 group had a significantly lower BWG of 175.4±0.9 g ($P<0.0001$). Similarly, the performance index (PI) was notably reduced in the *Salmonella*-challenged group (T4: 138±2.8) compared to the healthy control (Control: 121.3±1.0), further highlighting the negative consequences of the infection on overall productivity. The increase in feed intake (FI) for T4 (341±1.2g) compared to the control (352±2.3g), despite reduced weight gain, indicates poor

feed conversion due to the infection. These findings underscore that Salmonella infection severely compromises the growth and efficiency of quails, likely due to inflammation, nutrient malabsorption, and increased energy expenditure as the immune system combats the pathogen. On the other hand, among the various treatments, T3 (300 ppm OLE) stands out as the most effective in terms of enhancing growth performance in healthy quails. This group consistently achieved the highest values for final body weight (FBW: 239.2±1.0g), body weight gain (BWG: 194±1.0g), and performance index (PI: 149.8±1.5), all with high statistical significance (P<0.0001). While feed intake (FI) was numerically higher in T3 compared to the control

Impact of OLE on digestive enzymes: Table 4 illustrates the significant impact of dietary olive leaf extract (OLE) on the serum digestive enzyme activity in quails. All measured enzymes—Amylase, Lipase, and Trypsin-showed highly significant differences across treatments (P<0.0001). The control group exhibited the lowest enzyme activities. Notably, OLE supplementation in healthy quails (T1, T2, T3) led to a dose-dependent increase in all three digestive enzymes, with the highest activities observed in the T3 group (300ppm OLE), reaching 520±2.0 for Amylase, 28±0.7 for Lipase, and 40±0.7 for Trypsin. Interestingly, while the *Salmonella*-challenged group (T4) showed enzyme levels comparable to or slightly above the control, the group challenged with Salmonella and treated with 300 ppm OLE (T5) demonstrated significantly higher enzyme activities (e.g., Amylase 500±3.2, Lipase 26±0.6, Trypsin 33±0.3), although not always reaching the levels of the healthy T3 group.

Impact of OLE on Blood biochemical parameters: OLE supplementation in healthy quails (T1, T2, T3) progressively *decreased* AST and ALT levels in a dose-dependent manner, with T3 (300ppm OLE) showing the lowest values (175±1.9 U/L for AST and 1.8±0.1 U/L for ALT. Conversely, the *Salmonella*-challenged group (T4) exhibited elevated AST (266±3.2U/L) and ALT (3.5±0.1U/L) compared to the control. However, treatment with OLE 300ppm in *Salmonella*-challenged quails (T5) significantly reduced these elevated levels, bringing them closer to or even below control values (AST 187±1.2U/L, ALT 2.0±0.1U/L. Uric acid levels followed a similar trend, decreasing with OLE in healthy quails and increasing with *Salmonella* but significantly reduced by OLE in the challenged group.

For the Lipid profile, highly significant differences were observed for Total Cholesterol (TC), LDL, HDL, and Abdominal fat. OLE supplementation (T1, T2, T3) consistently led to a dose-dependent reduction in TC, LDL, and Abdominal fat while *increasing* beneficial HDL levels. The T3 group achieved the most favorable lipid profile, with the lowest TC (95±1.8mg/dL), LDL (18±0.2mg/dL), and Abdominal fat (0.71±0.0%) and the highest HDL (98±1.1mg/dL). In contrast, the *Salmonella*-challenged group (T4) showed higher TC, LDL, and Abdominal fat and lower HDL compared to the control, indicating a negative impact on lipid metabolism. Encouragingly, T5 (*Salmonella*-challenged + 300 ppm OLE) effectively mitigated these negative effects, significantly lowering TC, LDL, and Abdominal fat and increasing HDL.

Impact of OLE on immunity: The data for IgG and IgA show highly significant differences across treatments (P=0.001). In healthy quails, olive leaf extract (OLE) supplementation (T1, T2, T3) led to a dose-dependent increase in both IgG and IgA levels. The T3 group (300 ppm OLE) exhibited the highest concentrations of both immunoglobulins (IgG: 1095±3.2mg/dL; IgA: 203.6±1.2mg/dL), suggesting a strong immunostimulatory effect of OLE. Conversely, the Salmonella-challenged group (T4) showed slightly lower IgG (941±3.6 mg/dl) and IgA (171±2.3mg/dL) compared to the control, indicating a potential suppression or dysregulation of the immune response due to infection. Importantly, the T5 group (*Salmonella*-challenged + 300ppm OLE) demonstrated a significant improvement in immunoglobulin levels (IgG: 1088±3.5 mg/dl; IgA: 198.2±1.7mg/dL), bringing them close to or even surpassing the healthy control group.

The role of OLE in regulating the Intestinal microbes: Fig. 3 clearly illustrates OLE's profound impact on gut microbial populations, as evidenced by changes in bacterial counts. For pathogenic bacteria, the *Salmonella* challenge in T4 notably elevated *Salmonella* counts (reaching over 5.0 log CFU/g), but subsequent OLE treatment in T5 resulted in a substantial decrease in *Salmonella* to below 1.0 log CFU/g, representing an approximate 80% reduction. Similarly, *E. coli* counts, while high in the control and T4 (around 5.0 log CFU/g), generally showed a reduction in OLE-treated groups (T1, T2, T3) and a significant decrease in T5. Conversely, beneficial Lactic Acid Bacteria (LAB) counts exhibited a clear increase with OLE supplementation, particularly in T2, T3, and T5,

Table 3: Effect of dietary treatments OLE on growth performance Parameters of *Salmonella*-challenged quails

Parameters	Age (d)	Control	T1	T2	T3	T4	T5	p-value
LBW (g)	10	45±0.1	45.1±0.0	44.8±0.5	45.1±0.2	44.6±0.2	44.5±0.0	0.9
FBW (g)	38	230.0±0.9b	225.0±0.9c	235.0±2.2ab	239.2±1.0a	220±1.5e	231.0±2.1b	<0.0001
BWG (g)	10-38	185±1.1c	180±0.2d	190±1.6b	194±1.0a	175.4±0.9e	186±1.9c	<0.0001
FI (g)	10-38	359.0±2.1c	352.0±2.3d	360.0±1.4b	366.2±2.1a	341±1.2e	355.0±0.9cd	<0.0001
FCR	10-38	1.62±0.1c	1.66±0.2c	1.71±0.5b	1.75±0.1a	1.59±0.6e	1.6±0.2c	0.04
GR	10-38	195.6±1.1b	194.2±1.2b	196.3±1.2b	197.3±1.3a	186±2.1e	192±3.2c	0.035
PI	10-38	132.5±0.6c	121.3±1.0d	142.6±1.0b	149.8±1.5a	138±2.8e	141.3±2.0b	<0.0001

Table 4: Effect of dietary treatments OLE on serum digestive enzymes of quails

Treatments	Amylase	Lipase	Trypsin	p value
Control	320±2.1d	15±0.9d	28±0.2c	<0.0001
T1	410±3.2c	19±0.1c	31±0.5bc	<0.0001
T2	490±2.1bc	25±0.5b	35±0.4b	<0.0001
T3	520±2.0a	28±0.7a	40±0.7a	<0.0001
T4	450±3.0c	16±0.9	29±0.8c	<0.0001
T5	500±3.2b	26±0.6ab	33±0.3b	<0.0001

Table 5: Effect of dietary treatments OLE on serum kidney and liver function, lipid profile and immunity parameters of quails

Serum parameters	Treatments (mg/kg)						*p* value
	Control	T1	T2	T3	T4	T5	
Liver and Kidney functions							
AST (U/L)	252±2.1a	222±3.0b	210±3.0c	175±1.9e	266±3.2	187±1.2d	<0.0001
ALT (U/L)	3.1±0.2a	2.8±0.2b	2.1±0.2c	1.8±0.1d	3.5±0.1	2.0±0.1c	<0.0001
Creat (mg/dl)	0.31±0.01	0.33±0.02	0.34±0.01	0.27±0.00	0.36±0.02	0.33±0.01	0.99
Uric acid (mg/dl)	5.3±0.3a	4.70±0.6b	4.39±0.2c	3.7±0.3d	5.6±0.5	4.4±0.5c	0.001
Lipid profile							
TC (mg/dl)	133±1.1a	126±0.8b	120±1.2b	95±1.8d	139±1.1	105±1.5c	<0.0001
LDL (mg/dl)	45±0.2a	33±0.4b	27±0.8c	18±0.2d	49±0.6	25±0.8c	0.001
HDL (mg/dl)	92±0.6c	94±0.8b	95±0.7b	98±1.1a	75±0.8	94.2±0.5b	0.001
Abdominal fat	1.3±0.1a	1.0±0.2ab	0.88±0.01b	0.71±0.0c	1.41±0.2	0.84±0.01b	0.001
Immunity							
IgG (mg/dl)	953.3±4.2e	1050±5.1d	1071±1.6c	1095±3.2a	941±3.6	1088±3.5b	0.001
IgA (mg/dl)	175.8±0.9e	187.2±1.1d	190.3±1.4c	203.6±1.2a	171±2.3	198.2±1.7b	0.001
T3 (ng/dl)	2.35±0.0	2.32±0.1	2.31±0.2	2.30±0.0	2.33±0.1	2.35±0.1	0.9
T4 (ng/dl)	135.1±1.1	134.3±1.9	134±0.9	136±0.8	133±1.5	131.6±1.1	0.5

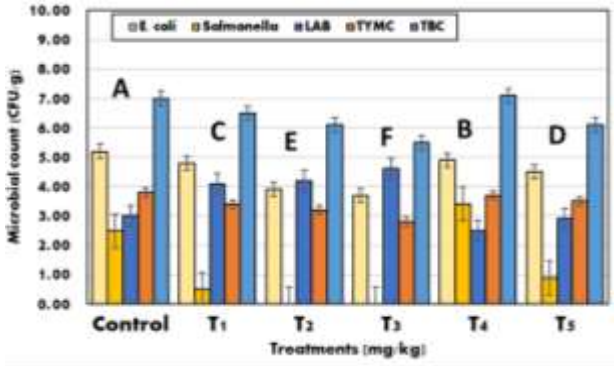

Fig. 3: Effect of dietary OLE on the intestinal microbial count of quails. TBC, total bacterial count; TYMC, total yeast and mold count; *E coli: Escherichia coli* CFU/g: logarithm of colony forming unit per gram of digesta LAB: *Lactobacillus* spp. T1, OLE 100µg/kg; T2 OLE 200µg/kg; T3, OLE 300µg/kg; T4, OLE 300µg/kg+ Salmonella infected groups.

compared to both the control and the challenged T4 group, where LAB was comparatively lower. For instance, in T5, LAB counts were notably higher, showing an approximate 200% increase compared to the diminished levels in T4.

The impact of OLE on the histology of the liver and intestine: Fig. 4 shows different intestinal sections of non-infected, OLE-treated, and *Salmonella*-challenged quails. The histology of the control, T1, T2, and T3 groups revealed typical structures of the intestines. In contrast, the *Salmonella*-infected group (T4) exhibited exfoliation of the gastrointestinal lining and a significant infection of the intestinal tissues (black arrows and star). Fig. 4 F suggests a reduction in *Salmonella* infection within the intestinal lining and the presence of very few counts.

The liver histology of *Salmonella*-challenged quails is presented in Fig. 5. The OLE treatments enhance the structure of liver tissues, which shows normal structure as the control (Fig. 5A, B, C and D). Fig. 5E shows cellular swelling, a reversible increase in cell volume, which can occur due to toxic, infectious, or immune insults. This swelling, characterized by increased cell size and a clearer appearance, is often accompanied by the formation of small vacuoles or clear spaces within the cytoplasm. These changes are a consequence of cellular injury and an increase in water content within the cell; however, the OLE treatment mitigates these impacts and recovers the normal tissues (Fig. 5F).

Impact of OLE on quails' meat quality: Table 6 details the significant effects of dietary olive leaf extract (OLE) on the physicochemical, sensory, and color properties of quail meat. For Chemical composition, most parameters showed significant differences (P≤0.041). OLE supplementation (T1, T2, T3) generally led to a dose-dependent increase in Moisture and Protein content, with T3 (300 ppm OLE) showing the highest values (66.7% moisture, 23.5% protein). Conversely, Fat and Ash content consistently decreased with increasing OLE dosage, with T3 having the lowest fat (7.7%) and ash (0.32%). The *Salmonella*-challenged group (T4) had moisture, protein, and fat levels comparable to or slightly different from the control. However, OLE treatment in *Salmonella*-challenged quails (T5) generally improved these parameters, increasing moisture and protein and reducing fat, although not always reaching the levels of healthy T3 quails. pH generally increased with OLE and in T5, while TVBN (Total Volatile Basic Nitrogen) and TBA (Thiobarbituric Acid Reactive Substances), indicators of spoilage and lipid oxidation, were significantly reduced by OLE (T1-T3, T5). In terms of Sensory properties, all parameters (Juiciness, Tenderness, Aroma, Taste) showed significant differences (P≤0.032). The *Salmonella*-challenged group (T4) generally exhibited the lowest scores for all sensory attributes, suggesting reduced meat quality due to infection. In contrast, OLE supplementation (T1, T2, T3) and, particularly in the T5 group (*Salmonella*-challenged + 300 ppm OLE), generally led to improved sensory scores, indicating enhanced palatability, with T3 often showing the best results, similar to the control for Juiciness and Taste. For Color properties**,** only 'a (redness) and 'b' (yellowness) values showed significant changes (p≤0.046), while 'L' (lightness) remained largely unaffected. OLE generally decreased 'a and 'b' values in a dose-dependent manner (T1, T2, T3), suggesting less redness and yellowness. Interestingly, T5 also showed reduced 'a and 'b' values compared to T4.

DISCUSSION

Salmonella infection, particularly caused by *Salmonella Enteritidis* (SE), is a significant concern in poultry farming, including Japanese quails (*Coturnix japonica*). Conventional antibiotics have been widely used to control *Salmonella* infections, but their overuse has led to the emergence of antimicrobial resistance (AMR), posing risks to both animal and human health (Collignon *et al.*, 2016; El-Hack *et al.*, 2022 b,c). Therefore, finding eco-

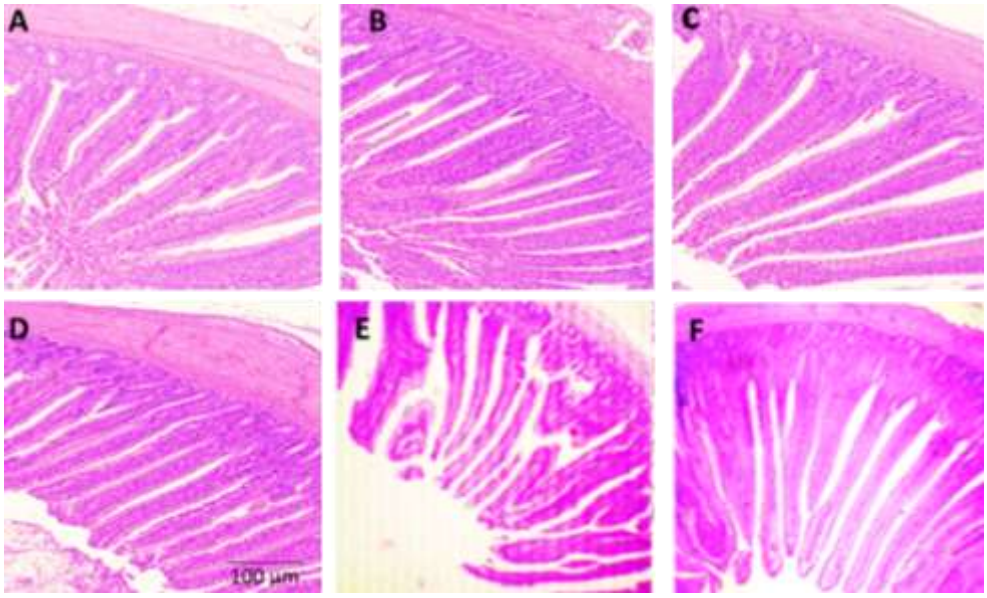

Fig. 4: The intestine epithelium in quails (A) control; (B) dietary OLE 100mg/kg 'quails' group, (C) dietary OLE 200 mg/kg 'quails' group, (D) dietary OLE 300µg/kg 'quails' group; (E) *Salmonella*-challenged groups (F) *Salmonella*-challenged quails and received dietary OLE 300mg/kg. stain was hematoxylin-eosin (H&E); Scale bar 100µm.

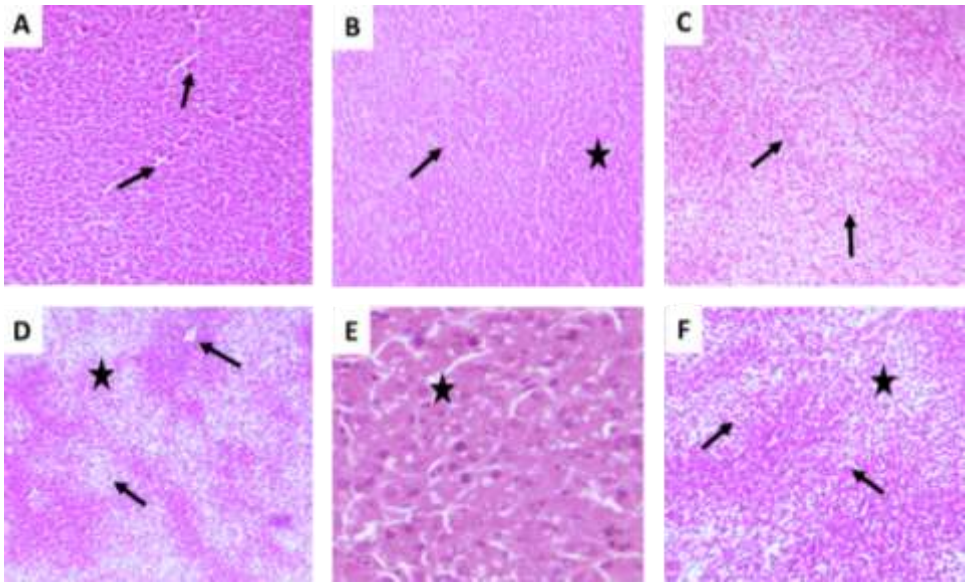

Fig. 5: The liver histology in quails (A) control; (B) dietary OLE 100mg/kg 'quails' group, (C) dietary OLE 200mg/kg 'quails' group, (D) dietary OLE 300mg/kg 'quails' group; (E) *Salmonella*-challenged groups (F) *Salmonella*-challenged quails and received dietary OLE 300mg/kg. stain was hematoxylin-eosin (H&E); Scale bar 100µm.

Table 6: Effect of dietary treatments OLE on physiochemical, sensorial and color properties of 'quails' meat

Parameters	Treatments (mg/kg)						P value
	Control	T1	T2	T3	T4	T5	
Chemical composition							
Moisture	62.5±0.9c	64.9±1.1b	63.6±1.3b	66.7±1.6a	62.9±0.7c	63.1±2.1b	0.001
Protein	20.5±0.2c	21.2±0.2b	20.6±0.8c	23.5±0.7a	21.0±0.5b	20.2±0.5c	0.041
Fat	13.5±0.1a	10.3±0.3b	10.2±0.4b	7.7±0.1c	12.9±0.3ab	9.2±0.2bc	0.001
Ash	0.88±0.01ab	0.45±0.01c	0.78±0.01b	0.32±0.01d	0.91±0.01a	0.33±0.0d	0.001
pH	5.7±0.2c	6.0±0.2c	6.2±0.2b	6.7±0.0a	5.2±0.6d	6.4±0.1b	0.001
TVBN	6.8±0.4ab	5.6±0.9b	5.0±0.2bc	4.4±0.6c	7.1±0.4a	4.8±0.1c	0.001
TBA	0.75±0.0b	0.44±0.01c	0.45±0.06c	0.26±0.02e	0.88±0.03a	0.37±0.02d	0.003
Sensory properties							
Juiciness	9±0.0a	8.7±0.1b	8.4±0.2c	9±0.0a	8.0±0.1d	8.1±0.2d	0.025
Tenderness	8.7±0.1b	8.5±0.0bc	8.5±0.1bc	8.8±0.1a	8.0±0.2c	8.2±0.1c	0.031
Aroma	8.5±0.0a	8.2±0.2b	8.0±0.4b	8.6±0.1a	7.7±0.0c	7.8±0.3c	0.032
Taste	8.6±0.2a	8.3±0.3c	8.6±0.0a	8.7±0.1b	7.7±0.2d	7.9±0.1d	0.012
Color properties							
L	58±0.2	58.2±0.8	58.8±0.9	58.5±1.1	58.1±0.3	58.3±0.5	0.06
a	6.2±0.3a	5.6±0.1b	5.4±0.2b	5.2±0.5c	6.0±0.1	5.4±0.2bc	0.041
b	14.5±0.1b	15.0±0.5a	15.5±0.2a	15.4±0.1a	14.2±0.2	14.6±0.1b	0.046

friendly alternatives that act as antibiotics is critical, where Abdel-Moneim *et al*. (2022) used *Spirulina platensis* to improve the broiler performance. This work examines the promise of olive leaf extract (OLE) as a natural, sustainable substitute for conventional antibiotics in controlling Salmonella infection in Japanese quails. We investigate its influence on growth performance, oxidative stress, molecular markers, and histological changes.

Investigations on olive waste extract reveal distinct outcomes across poultry species. In Japanese quails, researchers have observed consistent improvements in growth performance, body weight, feed intake, and egg production when these supplements are included in their diets (Salem *et al.,* 2022).

For broilers, the results are less uniform. High levels of OLE (7.5% and 15%) have been associated with diminished digestibility and metabolizable energy (Adams *et al.,* 2022). Although suggestions for safe inclusion levels range from 5% in the finisher phase (Papadomichelakis *et al.,* 2019) to 10% overall (Sayehban *et al.,* 2016), an older study by El-Hackemi *et al.* (2007) found no significant impact on broiler weight gain or carcass weight even at 15% OLE inclusion.

In this study, the addition of OLE often enhances growth performance. This improvement is likely due to its active compounds, such as oleuropein and its derivatives (Altiok *et al.,* 2008). Furthermore, olive leaf extract boasts various pharmacological activities, including antioxidant and anti-inflammatory properties (Carluccio *et al*., 2003). Toghyani *et al.* (2011) also suggest that OLE supplementation might boost productive performance by altering caecal microflora and blood metabolites (Zeng *et al.,* 2015). Our findings align with Erener *et al*. (2020), who reported that broilers on diets with 150, 300, and 600 ppm of OLE showed higher daily body weight gain than those on the basal diet.

Bahsi *et al.* (2016) observed improved quail performance with oleuropein supplementation. Similar positive effects were noted in broilers by El-Damrawy *et al.* (2013) and Jabri *et al.* (2017), and in laying hens by Cayan and Erener (2015) and Ahmed *et al.* (2017). Oke *et al.* (2017) also demonstrated that broilers receiving 15mL of OLE had significantly better final live body weight and weight gain compared to lower doses (10mL, 5mL) and control groups. These results suggest OLE is a viable feed additive for growth promotion, partly due to its antimicrobial properties (Ocak *et al.,* 2008; Amad *et al.,* 2011).

Birds fed olive leaves also showed superior European production index values, linked to better livability and feed conversion ratio (FCR) (Varmaghany *et al.,* 2013). However, some studies present contrasting views. Xie *et al.* (2022) found no significant change in broiler average daily gain until OLE supplementation exceeded 0.3% of the basal diet. Similarly, Sarica and Toptas (2014) reported no discernible growth-promoting effects from increasing levels of oleuropein in quail diets. Tarek *et al.* (2013) also noted no substantial performance differences in broilers fed various OLE levels.

Regarding feed intake (FI) and FCR, quails fed diets containing 300 and 600 ppm of OLE had significantly lower feed intake with the best FCR. This FCR improvement might stem from OLE's good flavor and palatability, enhanced digestive enzyme activity, and improved nutrient absorption. It could also be linked to modifications in intestinal bacteria (Toghyani *et al.,* 2011; Zeng *et al.,* 2015) and changes in studied blood metabolites (Jemai *et al.,* 2008) when OLE is included in the diet.

The dietary OLE significantly influenced the total lipid profile, liver functions, and digestive enzymes. Specifically, chicks on a diet supplemented with 300 ppm OLE showed noticeably lower total cholesterol (Chol) and very-low-density lipoprotein (VLDL) levels. Quails receiving the same 300 ppm OLE diet exhibited the lowest levels of triglycerides (TG) and low-density lipoprotein (LDL), alongside the highest high-density lipoprotein (HDL) levels. Furthermore, both 300 and 600ppm OLE diets significantly affected liver enzymes (ALT and AST), but without a notable impact on liver MDA levels. This beneficial effect likely stems from the antioxidant properties of oleuropein, hydroxytyrosol, and oleuropein aglycone, which neutralize free radicals by halting reactive oxygen species activity and capturing radicals before they damage cells (Srinivasan *et al.,* 2007; Liaqat *et al.,* 2023).

Scientific research further supports that many phenolic compounds found in OLE can reduce serum and liver triglycerides (Romani *et al.,* 1999). This is attributed to their ability to alter cholesterol metabolism. This cholesterol-lowering effect is achieved through several mechanisms: reduced intestinal absorption of dietary cholesterol, decreased cholesterol production in the liver, increased biliary cholesterol secretion, and enhanced fecal excretion of cholesterol (Rezar *et al.,* 2015).

Similarly, Erener *et al.* (2009) observed that broilers fed an OLE-supplemented diet had the lowest serum cholesterol levels. Additionally, Sarica and Topbas (2014) reported that a diet enhanced with 200ppm oleuropein reduced serum total cholesterol and LDL levels. This reduction in serum and triglyceride levels is primarily due to oleuropein, a key phenolic component in olive leaf known for its hypocholesterolemic properties. In this context, hydroxytyrosol and oleuropein in olive leaves are known to block the enzyme 3-hydroxy-3-methyglutaryl coenzyme A, which is crucial for cholesterol synthesis, and to prevent LDL oxidation (Cayan and Erener, 2015).

Krzeminski *et al.* (2003) suggested that the reduction in serum cholesterol in OLE-treated chicks might be due to decreased cholesterol absorption from the intestines and reduced liver cholesterol synthesis. Sung *et al.* (2004) also noted that isoflavone contents in OLE inhibit the catalytic domain of 3-hydroxy-3-methyl glutaryl (HMG) CoA reductase, an enzyme vital for cholesterol synthesis. Research into the effects of olive oil and olive leaf products on poultry lipid concentrations shows varied results. While Mahmoud *et al.* (2013) observed a significant reduction in overall lipid concentration with olive oil supplementation, LDL levels remained unaffected. Conversely, Bahrani *et al.* (2012) found that chicks fed a diet with 3% olive oil had lower LDL triglyceride concentrations and notable variations in HDL levels. El-Damrawy *et al.* (2013) similarly reported that olive leaf powder significantly reduced serum cholesterol and triglycerides in birds. Parsaei *et al.* (2014) further supported this, showing that olive leaf powder led to a significant decrease in serum levels of triglycerides, cholesterol, LDL, VLDL, HDL, and hepatic enzymes in chickens.

However, some studies present mixed findings, where Ahmed *et al.* (2017) noted an increase in total cholesterol levels in birds given 150ppm of oleuropein, yet lower LDL and triglyceride levels were recorded at 100 and 150ppm, with HDL levels showing no substantial change across treatments. Erener *et al.* (2020) found that chicks fed diets with OLE at 75, 150, and 600ppm had higher triglycerides and HDL, but lower LDL, with no change in cholesterol levels compared to the control. Additionally, El-Bahra and Ahmed (2012) reported that adding 2% olive oil to broiler diets did not significantly impact cholesterol, triglyceride, or VLDL levels. More recently, Xie *et al.* (2022) found that OLE supplementation did not significantly affect serum cholesterol, LDL, HDL, or the HDL/LDL ratio in chicks.

Studies by Zhang *et al.* (2013, 2021) and Yu *et al.* (2015) indicate that birds fed fermented Ginkgo leaves show higher levels of protease and lipase enzymes, leading to better nutrient absorption in the gut. This improvement is likely due to enhanced villus height and an increased villus height-to-crypt depth ratio. Furthermore, Wenk (2002) suggested that polyphenols can boost intestinal enzyme function by reducing harmful gut microorganisms. Our findings align with Polzonetti *et al.* (2004), who reported that supplementing diets with oleuropein increased pepsin activity. These beneficial effects of olive leaf extract (OLE) may stem from its ability to enhance digestive enzymes, stimulate appetite and food intake, provide antimicrobial and antifungal protection, guard against diseases, and ultimately improve overall animal performance.

Some studies have shown no significant changes in blood parameters, including glucose, cholesterol, triglycerides, and liver enzymes when OLE is in the quail's diet (Elsokkary *et al.*, 2020). However, other studies have observed alterations in blood parameters, such as increased levels of antioxidant enzymes and improved lipid profiles (Ahmed *et al.*, 2019; Salem *et al.*, 2022). All OLE treatments significantly impacted both antioxidant parameters and immunological indices. Diets supplemented with OLE notably increased immune responses, along with glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity. Concurrently, they reduced thiobarbituric acid-reactive substances (TBARS) and plasma malondialdehyde (MDA) compared to the control diet. Specifically, the group treated with 600 ppm of OLE showed the most pronounced positive effects on GPx, TBARS, SOD, plasma MDA, IgG, IgA, and IgM. These findings are consistent with the known biological properties of OLE, which include anti-inflammatory, antioxidant, and anticancer features (Selim *et al.*, 2022; Magyari-Pavel *et al.*, 2024). Likewise, Itumeleng *et al.* (2023) observed no impact on plasma biochemical parameters in jumbo quails fed on OP at levels of 200 and 250g/kg. Furthermore, the data presented indicates higher levels of plasma antioxidant enzymes when olive pomace is included in the diet. This finding aligns with research by Ibrahim *et al.* (2021), who found increased total antioxidant capacity, total superoxide dismutase, and glutathione peroxidase in broilers fed diets containing enzymatically fermented olive pomace at levels of 15% and 30%. The elevated serum antioxidants attributed to olive pomace are due to its phenolic compounds with antioxidant activities. such as oleuropein, oleacein, oleocanthal, tyrosol, and hydroxytyrosol, which can reduce alpha-tocopherol radicals (Rigacci and Stefani, 2016; Constantina *et al.*, 2018).

Our results are consistent with El-Damrawy (2011), who observed that OLE significantly enhanced plasma SOD activity and reduced TBARS. The substantial SOD levels found could reflect a marked improvement in the oxidative status and general health of Mandarah chicks. Oke *et al.* (2017) also found that birds receiving 15mL of OLE showed similar plasma SOD levels to those given 10 mL, both of which were superior to the 5mL group and the untreated control.

Silvan *et al.* (2021) and Pennisi *et al.* (2023) suggest that the antioxidants in OLE neutralize free radicals by halting their chemical reactions. Furthermore, treatments with varying amounts of oleuropein significantly increased SOD levels compared to the control, with the highest SOD observed in groups receiving the largest amount of oleuropein. Younan *et al.* (2018) reported a notable increase ($P<0.05$) in total antioxidant capacity (TAC) and a significant decrease ($P<0.05$) in MDA with increasing OLE in the diet.

Similarly, Ahmed *et al.* (2017) demonstrated that laying hens fed an oleuropein-supplemented diet exhibited considerable improvements ($P<0.01$) in SOD and TAC levels, alongside a substantial reduction in MDA. This underscores that OLE and olive leaf are rich sources of polyphenols, recognized for their antioxidant potential (Jemai *et al.*, 2008). Agah *et al.* (2019) also noted improved blood GPx levels in chicks fed 200 ppm OLE, and a significantly lower blood MDA level in birds given 400 ppm OLE compared to the challenged control group.

Our current study found that adding OLE to quail diets significantly improved their gut health ($p\leq0.001$). This was evident through a notable increase in beneficial intestinal bacteria, specifically *Lactobacillus* (Ibrahim *et al.*, 2024). We also observed increased gut viscosity and a decrease in intestinal pH. Conversely, the populations of harmful bacteria like *Salmonella* and *E. coli* decreased compared to the control group. Notably, quails fed diets supplemented with 300 and 600 ppm of OLE had the lowest counts of *E. coli* and *Salmonella* and the highest *Lactobacilli* numbers. These quails also showed increased intestinal viscosity and pH.

These findings highlight that olive leaf and OLE are rich in phytochemicals, making them excellent sources of antioxidants (Silva *et al.*, 2006; Jemai *et al.*, 2008). In the poultry industry, the use of herbs and plant-derived products is increasingly recognized for their antimicrobial effects, which effectively control and limit the growth of various pathogenic and non-pathogenic bacteria in the gut (Reda *et al.*, 2021; El-Shall *et al.*, 2022; El-Saadony *et al.*, 2022). It's well-documented that olive leaf can inhibit several Gram-negative and Gram-positive bacteria, as well as yeast and parasites (Markin *et al.*, 2003).

Erener *et al.* (2020) further supported this, reporting that most tested OLE doses, excluding the 300ppm group, reduced *E. coli* counts compared to the control. Importantly, these treatments didn't harm beneficial *Lactobacillus* species, while all OLE levels showed significant antibacterial effects against *Clostridium* spp. and *S. aureus* populations compared to the control.

Considering existing in vitro (Markin *et al.,* 2003; Sudjana *et al.,* 2009) and in ovo (Jabri *et al.,* 2017) studies, alongside our current research, OLE shows strong potential as a non-antibiotic growth promoter. Jabri *et al.* (2017) specifically noted that an aqueous olive leaf extract at 10mL/L not only exhibited antimicrobial activity against certain cecal pathogenic bacteria (total germs and coliforms) but also significantly stimulated *Lactobacillus* growth. Early work by Aziz *et al.* (1998) established that oleuropein and other phenolic compounds completely inhibit *Escherichia coli*. Moreover, Sudjana *et al.* (2009) indicated that oleuropein helps regulate gastric flora by selectively reducing *Campylobacter jejuni*, *Helicobacter pylori*, and methicillin-resistant *Staphylococcus aureus* (MRSA).

Xie *et al.* (2022) observed that a 0.3% OLE group in broilers led to a decrease in *Escherichia coli* and a significant increase in *Lactobacillus* and *Bifidobacterium*. These positive shifts in gut microbiota can greatly enhance broiler gut health, contributing significantly to improved meat quality (Chen *et al.,* 2019; Dev *et al.,* 2020). Additionally, reducing *Escherichia* and *Shigella* abundance in broiler ceca (Eeckhaut *et al.,* 2016) is promising for better gut health and could even help reduce issues like woody breast development (Zhang *et al.,* 2021).

Certain cell surface proteins from Lactobacillus are known to prevent *Escherichia coli* from multiplying by immobilizing it (Tsou *et al.* 2017). Similarly, olive leaf extract (OLE) contains compounds such as secoiridoids, flavonoids, and simple phenols that significantly reduce *E. coli* populations by disrupting their cell walls and cytoplasmic membranes (Rodrıguez *et al.* 2009). It's worth noting, however, that the diverse compounds within OLE often work together, making it difficult to isolate the exact contribution of each to its overall effects (Vezza *et al.,* 2019).

Prior studies indicate that OLE offers significantly higher antioxidant activity than vitamins C and E, thanks to the combined action of its flavonoids, oleuropeosides, and substituted phenols (Benavente-Garcı *et al.,* 2000). Within this context, oleuropein and hydroxytyrosol are particularly important for balancing gut microbiota and strengthening gut barrier function (Sarica and Urkmez, 2016).

The mechanism by which polyphenols inhibit *E. coli* growth may involve their binding to bacterial cell membranes, which then impairs membrane functions like permeability and the proton motive force by affecting H+-ATPase (Lin *et al.,* 2005; Cardona *et al.,* 2013). Conversely, beneficial bacteria like *Lactobacillus* and *Bifidobacterium* can actually metabolize these phenols as substrates for their own growth, potentially providing them energy and even improving their uptake of nutrients, especially carbohydrates (Garcıa-Ruiz *et al.,* 2008). Therefore, introducing polyphenols into poultry feed can influence both the diversity and levels of bacteria in the caecum.

Because of the antioxidant and antimicrobial efficiency of OLE, the dietary olive leaf extract (OLE) has demonstrated promising effects on the meat quality of quails, primarily due to its rich content of bioactive compounds like oleuropein and hydroxytyrosol. These powerful antioxidants play a crucial role in mitigating lipid oxidation in meat, which is a major factor in reducing shelf life and developing undesirable off-flavors and odors. Studies indicate that OLE inclusion can lead to lower levels of malondialdehyde (MDA), a common marker of lipid peroxidation, and enhanced antioxidant enzyme activities in quail meat (Xie *et al.,* 2022). This improved oxidative stability contributes to better preservation of color, flavor, and overall sensory attributes during storage, extending the freshness of the meat (Hazaa *et al.,* 2019; Hlatshwayo *et al.,* 2023). Beyond its antioxidant properties, OLE's antimicrobial characteristics may also help reduce microbial spoilage, further contributing to improved meat hygiene and extended shelf life. While the exact optimal inclusion levels and the specific impact on various meat quality parameters continue to be areas of active research, the current evidence strongly supports OLE as a valuable natural additive for enhancing quail meat quality.

Conclusions: *Salmonella* infection poses a significant threat to poultry health and productivity. *Salmonella* can cause a range of issues in quails, including reduced growth rates, decreased feed conversion efficiency, and increased mortality. The bacteria can damage the intestinal lining, impairing nutrient absorption and leading to diarrhea. *Salmonella* infection also triggers an immune response, diverting energy from growth and potentially compromising the bird's overall health. Furthermore, *Salmonella* contamination of poultry products risks human health, making control strategies crucial. Therefore, interventions that can mitigate the negative impacts of *Salmonella* and promote gut health, like the use of OLE in this study, are of considerable importance in quail production. This study suggests that dietary supplementation with olive leaf extract (OLE) at 300 and 600 ppm levels can positively influence various aspects of Japanese quail health and productivity. The results indicate improved productive performance, enhanced antioxidant capacity, beneficial effects on blood biochemical and immunological parameters, and a positive impact on intestinal microbiota composition. Therefore, OLE may be a valuable natural growth stimulant and health promoter for use in Japanese quail rearing.

Authors contribution: Safia M.A. Bahshwan: conceptualization, project administration, funding acquisition, writing the original draft, writing – review, and editing.

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