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# THERAPEUTIC POTENTIAL OF POMEGRANATE-COATED NANO ZINC IN BROILER CHICKS INFECTED WITH *E. COLI* O78: A HISTOMORPHOLOGICAL AND MOLECULAR APPROACH

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## ABSTRACT

Avian Pathogenic *E. coli* (APEC) colibacillosis is a highly fatal, multisystemic bacterial infection of broilers with high mortality rates, reduced productivity, and inferior growth rates. The current study aimed to evaluate the effectiveness of pomegranate-coated zinc oxide nanoparticles (PEE-ZnONPs) at different concentrations against *E. coli* infection (Colibacillosis) in broiler chicks, considering the increasing *E. coli* antibiotic resistance. One hundred and fifty chicks were divided into five groups: G1: a negative control; G2: a positive control with *E. coli* O78 infection; and three treatment groups with 40 mg/L as G3, 80 mg/L as G4, and 120 mg/L as G5 of PEE-ZnONPs in their drinking water for 35 days. Bird performance and deaths were monitored throughout the duration of the study, and intestinal tissue samples were prepared for molecular and pathologic analysis. The findings showed that the supplementation of PEE-ZnONPs significantly improved the body weight, feed consumption, and feed conversion ratio relative to the infected control ($P<0.05$). Furthermore, PEE-ZnONPs raised the mRNA levels of occludin (OCLN) and mucin (MUC) in the jejunum ($P<0.05$), while inhibiting TNF mRNA levels, which represent reduced inflammation. These findings revealed that PEE-ZnONPs supplementation attenuates *E. coli* O87 infection-induced impairment of intestinal mucosal barrier function, perhaps via increased occludin and mucin expression. Microscopically, the infected group showed a severe inflammatory response in the mucosa and submucosa. The intestinal villi showed shedding and desquamation, with an accumulation of desquamated cells, debris, and bacterial colonies in the lumen. These lesions were improved in groups treated with different doses of PPE-ZNONPs, especially the 80 mg NZ. In conclusion, supplementing PEE-ZnONPs in drinking water considerably increases growth performance, gut histomorphology, immune response, and intestinal integrity in *E. coli O78*-challenged broilers.

***Keywords:*** Pomegranate, zinc nanoparticles, *E. coli* O78.

## INTRODUCTION

The use of antibiotic growth promoters (AGPs) in poultry is raising public health concerns due to the risks of antibiotic resistance, environmental pollution, and antibiotic carry-over into meat and eggs (Anee *et al.,* 2021; Fotouh *et al.,* 2024a). In response, researchers are investigating alternative strategies, including metal oxide nanoparticles (NPs)

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for bacterial pathogen control (Dizaj *et al.,* 2014; Mahmoud *et al.,* 2025). Furthermore, poultry nutritionists are increasingly exploring natural phyto-additives as safe and environmentally friendly replacements for antibiotics (Salah *et al.,* 2025a), with promising research demonstrating their ability to improve poultry health and productivity without adverse side effects (Chavan *et al.,* 2022; Salah *et al.,* 2025b).

NPs, with a size range of 1–100 nm, are employed in various sectors, including health, food, chemical, and cosmetic industries and agriculture (Shoker *et al.,* 2025; Akdaşçi *et al.,* 2025). NPs possess antioxidant and antimicrobial properties are considered a promising new type of antimicrobial therapeutic agent for food preservation and pathogenic micro-organism control (Elsayed *et al.,* 2024). NPs offer a promising solution to antimicrobial resistance. Due to their coordinated contact with the bacterial cell wall without needing to enter the cell, NPs may be less prone to fostering bacterial resistance compared to antibiotics (Huang *et al.,* 2019).

Pomegranate peels, often considered a waste product, are packed with beneficial compounds. Rich in nutrients and bioactive substances like proanthocyanidins and flavonoids, which confer growth-promoting, immune-modulating, antioxi-dant, and anti-inflammatory properties (Tozzi *et al.,* 2022). Recent trials have demonstrated that pomegranate peel powder (PPP) supplementation in broilers can enhance growing parameters and carcass traits (Abdel Baset *et al.,* 2022).

Zinc, an essential trace element for all living organisms, is found in both plant- and animal-based diets, though its absorption can be hindered by phytates, which bind strongly to zinc ions in the digestive tract. To ensure adequate intake, poultry feeds are supplemented with zinc in various forms, including chelated organic and amino acids, both zinc oxide and chloride, and inorganic feed-grade zinc (Zaki *et al.,* 2025a). Zinc deficiency in chickens manifests as frizzed feathers, shortened and thickened legs, enlarged hocks, and a slow growth rate, highlighting the element's critical role in immune function, enzyme activity, cell division, protein and nucleic acid synthesis, and overall protein and carbohydrate metabolism (Abdel-Kareem *et al.,* 2025).

Researchers have been exploring the applications of these tiny particles, and zinc oxide nanoparticles (ZnONPs) have gained important attention. ZnONPs were found to affect the growth performance and physiological rank of poultry and livestock in a dose-dependent way (Shukla *et al.,* 2013; Fotouh *et al.,* 2024b). Their effectiveness in trace mineral supply is key to enhancing mineral bioavailability (Wijnhoven *et al.,* 2009; Soufy *et al.,* 2016), owing to their large proportion of surface area to volume. Low toxicity, biocompatibility, and chemical stability of ZnONPs also make them a research hotspot in biology (Teow *et al.,* 2018; Shosha *et al.,* 2024).

This research aims to estimate the effectiveness of PEE-ZnONPs as a potential alternative to antibiotics for *E. coli*-infected poultry, focusing on bird performance, genetic analysis, and pathological analysis of intestinal tissue across varying concentrations of PEE-ZnONPs.

## MATERIALS AND METHODS

### Materials

Pomegranate peel extract (PPE): Obtained by macerating dried *Punica granatum* peels in ethanol.

**Solvents and reagents:**

**1.** Ethanol (analytical grade), was used as a solvent for PPE extraction and was purchased from Sigma Company.

**2.** Deionized water was used throughout all experimental procedures.

**3.** Zinc acetate dihydrate [$Zn(CH_3COO)_2 \cdot 2H_2O$], was purchased from Sigma Company and used as the zinc source.

**4.** Sodium hydroxide (NaOH) pellets, was used as the precipitating agent in the synthesis of ZnO nanoparticles, and was purchased from Sigma Company.

All chemicals and reagents were of analytical grade and used without further purification.

**Preparation of Nano Zinc Oxide**

Nano zinc oxide was created using chemical precipitation. First, a 0.1 M solution of zinc acetate dihydrate and a 0.2 M solution of sodium hydroxide were prepared. The sodium hydroxide was added to the zinc acetate, with vigorous swirling, resulting in the precipitation of zinc hydroxide [$Zn(OH)_2$] as a white substance. The mixture was stirred for approximately six hours to ensure complete precipitation, after which the precipitate was separated from the solution via centrifugation. The precipitate was washed multiple times with deionized water to remove any unreacted precursors or byproducts, dried at 80-100°C in an oven or vacuum oven, and finally calcined at a temperature above 400°C for approximately six hours to produce micro ZnO particles (Liu *et al.,* 2007).

**Preparation and characterization of pomegranate peel extract**

Fresh peels are collected from ripe pomegranates, thoroughly cleaned with water to remove any impurities, and then air-dried on a sanitized surface or oven-dried at a low temperature (40-50°C). The dried peels, stored in an airtight container until use, are then ground into a coarse powder using a food processor or blender. The powdered peels are mixed with ethanol at a ratio of 1:10 (w/v) in a suitable container, and the mixture is heated to 60°C for 2-4 hours with intermittent stirring to facilitate the extraction of solvent-soluble phytochemicals like tannins, flavonoids, and polyphenols. After extraction, the resulting pomegranate peel extract is stored in an airtight container, preferably in a freezer or refrigerator, until further use (Nasiriboroumand *et al.,* 2018).

**Coating with Pomegranate Peel Extract**

The pomegranate peel extract is used to coat the produced nano ZnO particles by first mixing them, followed by a 60-minute sonication to ensure thorough dispersion and coating. The coated particles are then separated from the liquid via centrifugation, followed by washing with the original extraction solvent to remove any pomegranate extract that didn't bind to the ZnO. Finally, these coated nanoparticles are dried in an oven or vacuum oven at a gentle 60°C, using a 1:9 ratio of ZnO nanoparticles to pomegranate extract (Nasiriboroumand *et al.,* 2018).

**Characterization of POM-ZnON nanoparticles**

The synthesized pomegranate-coated zinc oxide nanoparticles (PEE-ZnONPs) were characterized by different methods. Dynamic light scattering (DLS) was used to analyze the nanoparticles' stability and size. FTIR spectroscopy (Nexus 670 FTIR, USA) in the range of 400 to 4000 $cm^{-1}$ revealed the functional groups of the PEE-ZnONPs. Transmission electron micro-scopy (TEM) was employed to examine the size, shape, and composition of the synthesized PEE-ZnONPs. This was carried out by sonication of the nanoparticle solution for five minutes to facilitate particle dispersal and discourage agglomeration on the copper grid, followed by the addition of a drop of the solution onto carbon-coated copper grids. Finally, high-resolution TEM (HR-TEM; JEOL,

JEM2100, Electron Microscope, Japan) was used to further analyze the particle size and morphology of the synthesized solution (Mohan and Renjanadevi 2016).

**Acute toxicity**

The toxicity of PEE-ZnONPs was evaluated by administering a single gavage dose (0, 50, 100, 250, or 500 mg/kg body weight) of PEE-ZnONPs dissolved in distilled water to different groups of five rats each, after which the solutions were shaken. The animals were continuously monitored for 24 hours for signs of behavioral changes, toxicity, and/or death, including the latency of death. Over a subsequent 14-day period, the birds were supplemented with adequate food and water, and daily records were maintained regarding their food intake, water consumption, body weight, deaths, and any changes in their appearance. The median lethal dosage (LD50), defined as the dose resulting in death in 50% of the experimental animal population, was calculated using the method described by Litchfield and Wilcoxon (1949).

**Bird Management**

During the study, birds were provided with a well-balanced meal, formulated according to NRC (1994) recommend-dations, as detailed in Table 1. Chicks received a starter diet until day 14, followed by a grower diet until day 28, and a finisher diet until the experiment's conclusion on day 35. Broiler feed intake, body weight, and FCR were documented in two phases throughout the duration of the study.

**Table 1:** The ingredient composition of the different basal diets.

| Ingredient composition (kg) | Starter | Grower | Finisher |
|---|---|---|---|
| **Yellow corn** | 56 | 59 | 64 |
| **Soybean meal 44%** | 39.5 | 35 | 29 |
| **Vitamin and mineral premix** | 0.3 | 0.33 | 0.33 |
| **Monocalcium phosphate** | 1.5 | 1.4 | 1.3 |
| **Sodium chloride** | 0.2 | 0.2 | 0.2 |
| **Bicarbonate** | 0.2 | 0.2 | 0.2 |
| **Vegetable oil** | 0.6 | 2.2 | 3.3 |
| **Limestone** | 1.1 | 1.1 | 1.1 |
| **Methionine** | 0.3 | 0.3 | 0.3 |
| **Lysine** | 0.2 | 0.16 | 0.18 |
| **Choline chloride** | 0.18 | 0.15 | 0.15 |
| **Crude protein** | 22.14 | 20.42 | 18.2 |
| **Metabolizable energy** | 2811 | 2955 | 3088 |

**Bacterial agents**

*E. coli* O78 was supplied by the Faculty of Veterinary Medicine's Central Laboratory, Benha University, Egypt. The colonies were grown in nutrient broth at 37°C for 24 hours, as suggested by Macfaddin (1980), and the viable colonies of *E. coli* strain were then diluted to $4 \times 10^8$ CFU/ml (colony forming units/ml) by phosphate-buffered saline (PBS).

**Experimental design and animals**

The study protocol conducted in this study was permitted by the Benha University Faculty of Veterinary Medicine Animal Research Ethics Committee (approved no. BUFVTM 22-09-23) to guarantee ethical conduct of the study.

150 chicks were allocated into five groups (30/group).

- G1 (Negative Control): Uninfected, untreated.

- G2 (Positive Control): Infected with *E. coli* O87, and untreated.
- G3 (Treatment Group 1): Infected and supplemented with 40 mg/L pom-ZnONPs in drinking water.
- G4 (Treatment Group 2): Infectedand supplemented with 80 mg/L pom-ZnONPs in drinking water.
- G5 (Treatment Group 3): Infected and supplemented with 120 mg/L pom-ZnONPs in drinking water.

**Growth performance**

Body weights of broilers were taken on day 21 and day 35 (end of experiment). Feed intake and weight gain were measured for different periods, and the FCR was thereafter measured (Abd-El Hamed *et al.,* 2025).

**Molecular analysis**

For molecular studies, approximately 0.5 g of intestinal tissue was placed in Eppendorf tubes, snap-frozen directly in liquid nitrogen, and stored at -80°C. This tissue was later utilized for RNA extraction to detect the gene expression of Muc2, Occludin, TLR4, IL1B, and TNF alpha in the chicken intestine by real-time quantitative polymerase chain reaction (real-time qPCR). The threshold cycle (Ct) of the target genes was normalized to that of β-actin, a housekeeping gene, using the ΔΔCt method (Yuan *et al.,* 2007). different basal diets.

**Table 2:** Oligonucleotide primers and probes used in real-time PCR

| Gene | Primer sequence (5'-3') | Reference |
|---|---|---|
| ***28S rRNA*** | GGCGAAGCCAGAGGAAACT | **Suzuki *et al*., 2009** |
| | GACGACCGATTTGCACGTC | |
| | (FAM) AGGACCGCTACGGACCTCCACCA (TAMRA) | |
| ***IL1β*** | GCTCTACATGTCGTGTGTGATGAG | **Samy *et al*., 2015** |
| | TGTCGATGTCCCGCATGA | |
| | (FAM) CCACACTGCAGCTGGAGGAAGCC (TAMRA) | |
| ***TNF alpha*** | CCCCTACCCTGTCCCACAA | **Chen *et al*., 2017** |
| | ACTGCGGAGGGTTCATTCC | |
| | (FAM) CTGGCCTCAGACCAG (TAMRA) | |
| ***Muc2*** | GCCTGCCCAGGAAATCAAG | **Chen et al., 2015** |
| | CGACAAGTTTGCTGGCACAT | |
| ***Occludin*** | GAGCCCAGACTACCAAAGCAA | |
| | GCTTGATGTGGAAGAGCTTGTTG | |
| ***TLR4*** | GTTCCTGCTGAAATCCCAAA | **Lu et al., 2014** |
| | TATGGATGTGGCACCTTGAA | |
| ***B. actin*** | CCACCGCAAATGCTTCTAAAC | **Yuan *et al*., 2007** |
| | AAGACTGCTGCTGACACCTTC | |

**Histopathological examination**

For histological investigations, intestines from ten birds per group (two birds per replication) that were slaughtered were selected to assess histopathological lesions. The tissues were sliced to a 3–4 um thickness and fixed in 10% neutral buffered formalin, followed by dehydration, clearing, and embedding in paraffin. The paraffin blocks were stained with hematoxylin and eosin and examined under a microscope (Elbarbary *et al.,* 2024a).

### Statistical analysis

The data are reported as Mean ± Standard Error (S.E.). Statistical analysis was performed using one-way ANOVA and the post-hoc Tukey's multiple comparisons test. The statistical significance was designated as $p \leq 0.05$, and the analyses were carried out using GraphPad Prism 7 (GraphPad Software).

## RESULTS

### Characterization of PEE-ZnO NPs

FT-IR spectroscopy was conducted to discover the functional groups of the produced PEE-ZnoNPs. The IR absorbance spectra of the samples were obtained in the range from 4000 to 400 $cm^{-1}$. Fig. 1 shows the FT-IR spectrum of PEE-ZnO NPs. Different peaks of absorption for biosynthesized PPE-ZNONps at 3309.59, 1905.75, 1638.05, 1085.21, 1045.51, 615.52, and 588.18 $cm^{-1}$ were noticed from Figure 2. The broadband of absorption at 3309.59 $cm^{-1}$ corresponding to free hydroxyl group stretching is due to the phenolic compounds present in pomegranate peel extract. The maximum at 1638.05 $cm^{-1}$ corresponds to (C–N) stretching vibrations of amines, and 1045 $cm^{-1}$ for C-O stretching of the functional molecules' primary alcohol. Again, the band observed at 1085.21 cm corresponds to C-O stretching of alcohols, and 1045 $cm^{-1}$ is for the -C-O stretch of alcohols, carboxylic acid esters, and ether functional groups. These results as a whole demonstrated the existence of functional groups on the synthesized ZnONPs, which is crucial for their functionalities and applications.

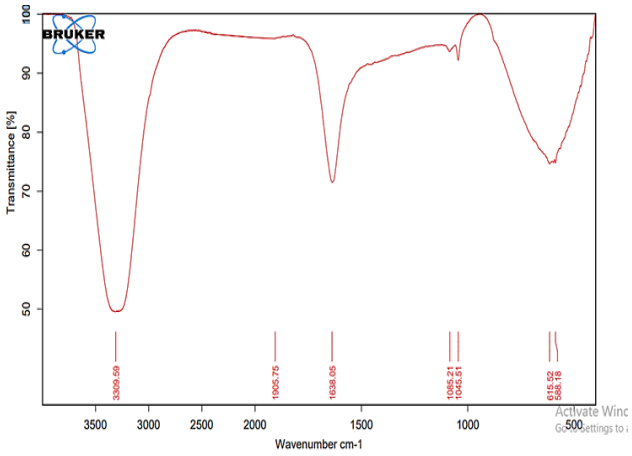


**Fig. 1:** The FT-IR spectrum of PEE-ZnONPs TEM was performed on (HR-TEM; JEOL, JEM2100, Electron Microscope, Japan). The average particle size of PEE-ZnONPs was 30 nm and appeared as spherical. (Fig. 2)

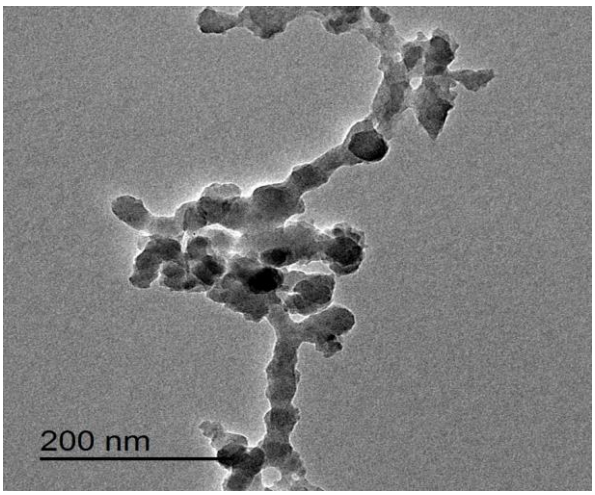


**Fig. 2:** Electron microscopy transmittance graphs of PEE-ZnONPs

### Acute toxicity

Following oral administration of PEE-ZnONPs at doses up to 500 mg/kg body weight, no evidence of toxicity or mortality was detected. The current study revealed minor clinical symptoms of colibacillosis across all infected treatment groups. Chicks in Group 2, the positive control group, exhibited characteristic signs of colibacillosis three days post-infection, including weakness, depression, loss of appetite, dyspnea, coughing, sneezing, gasping, and nasal discharge. In contrast, Group 1, serving as the negative

control, consisted of chicks that remained active throughout the testing period and displayed no signs of illness. Although all infected groups experienced a 100% morbidity rate, no mortalities were observed in any of the groups.

**Growth performance**

The effect of (PEE ZnO NP) added in drinking water on chicken growth performance is shown in Tables 3 and 4.

**Table 3:** Effect of zinc oxide nanoparticles (PEE-ZnO NPs) on broiler production at 21 days of age.

| Item | control (G1) | Infected (G2) | Infected treated with 40 (G3) | Infected treated with 80 (G4) | Infected treated with 120 G5) |
|---|---|---|---|---|---|
| **Total body Weight (BW)** | 911±31.82$^{b}$ | 700±17.32$^{a}$ | 801.1±28.87$^{ab}$ | 845±15.9$^{b}$ | 825±13.02$^{b}$ |
| **Feed intake (FI)** | 1229±45.53$^{b}$ | 1100±28.87$^{a}$ | 1143±8.82$^{a}$ | 1120±16.01$^{b}$ | 1136±20.24$^{b}$ |
| **Feed conversion ratio** | 1.354± 0.02$^{b}$ | 1.568±0.001$^{a}$ | 1.430± 0.05$^{b}$ | 1.317±0.01$^{b}$ | 1.378±0.002$^{b}$ |

a: significant difference compared with the control group, b: significant difference compared with the infected group (P<0.05). TBW: total body weight, FCR: feeding conversion rate

`**Table 4**: Effect of zinc oxide nanoparticles (PEE-ZnO NPs) on broiler production at 35 days of age.

| Item | Control G1 | Infected G2 | Infected treated with 40 G3 | Infected treated with 80 G4 | Infected treated with 120 G5 |
|---|---|---|---|---|---|
| **Total body weight** | 2100±88.19$^{b}$ | 1820±20.28$^{a}$ | 2050±57.45$^{b}$ | 2250±130.2$^{ab}$ | 1950±20.48$^{b}$ |
| **Feed intake** | 3600±104.1$^{b}$ | 3550±88.19$^{a}$ | 3450±60.09$^{ab}$ | 3500±72.5$^{a}$ | 3400±29.36$^{b}$ |
| **Feed conversion ratio** | 1.71±0.05$^{b}$ | 1.85±0.01$^{a}$ | 1.68±0.02$^{b}$ | 1.55±0.08$^{b}$ | 1.74±0.04$^{b}$ |

a: significant difference compared with the control group, b: significant difference compared with the infected group (P<0.05). TBW: total body weight, FCR: feeding conversion rate.

In the starter phase (days 0-21), bird weight gain (BWG) was greatly improved in groups 3, 4, and 5 compared to the infected group (G2). The mean final body weight and weight gain (g/bird) were significantly higher (P<0.05) for group G4 and then group G5 compared to groups G2 and G3 (Table 2). On day 35, the increase was significantly higher (P<0.05) in group G4 (80 mg/L PEE-ZnO NP-treated) compared to the positive control group. Feed intake was lower (P<0.05) on day 35 (Table 3) in the group treated with water containing 80 mg/L of PEE-ZnO NP. In contrast, group G5 (120 mg/L PEE-ZnO NP-treated) had lower TBW and improved feed intake. There was a significant reduction (P<0.05) of BWG in the challenged treatment group versus the challenged treatment group. There was an impressive reduction in the body weight of the E. coli-challenged untreated group in this research.

**Intestinal inflammation, gene expression, and tight junction-related gene expression**

As shown in Table 5. Tight junction (TJ) protein occludin is a significant protein in maintaining gut integrity. Infection with E. coli lowered occludin mRNA expression at

a highly significant level (P<0.05) in the ileum of chickens (Table 4). However, supplementation with different levels of PPE-ZnONPs increased the mRNA expression of occludin in the jejunum. The mucus barrier, composed predominantly of MUC2 secreted by goblet cells, is a key component of the intestinal mucus layer. APEC challenged decreased MUC2 levels (P<0.05). MUC2 levels gradually increased following PPE-ZnONPs supplementation. In chicks receiving PPE-ZnONPs at 40, 80, and 120 mg/L, MUC2 levels were higher than with no supplementation with PPE-ZnONPs (P<0.05).

**Table 5:** gene expression and tight junction-related gene expression

| Parameter /Group | Group1 | Group2 | Group3 | Group4 | Group5 |
|---|---|---|---|---|---|
| Occludin | 1.0± 0.001 $^{b}$ | 0.1140±0.004$^{a}$ | 0.3082±0.015$^{ab}$ | 0.8313± 0.058$^{b}$ | 0.6213± 0.07$^{ab}$ |
| Muc2 | 1.0± 0.001 $^{b}$ | 0.2399± 0.02$^{a}$ | 0.5264± 0.04$^{ab}$ | 0.9910± 0.05$^{b}$ | 0.7168± 0.06$^{ab}$ |
| TLR4 | 1.0± 0.001 $^{b}$ | 10.16± 0.176$^{a}$ | 4.518±0.141 $^{ab}$ | 2.688±0.12$^{ab}$ | 7.683±0.37$^{ab}$ |
| IL1B | 1.0± 0.001 $^{b}$ | 8.758± 0.242 $^{a}$ | 3.586± 0.552 $^{ab}$ | 2.292± 0.10$^{b}$ | 4.014±0.01 $^{ab}$ |
| TNF alpha | 1.0± 0.001 $^{b}$ | 7.112± 0.148 $^{a}$ | 5.333± 0.443 $^{ab}$ | 3.608± 0.337 $^{ab}$ | 5.246± 0.218 $^{ab}$ |

a: significantly difference compared with the control group, b: significant difference compared with the infected group: P≤0.05.

**Histopathological findings**

In the control group, intestinal histoarchitecture appeared normal, characterized by intact mucosal and submucosal layers, well-preserved villi, and an absence of inflammatory infiltrates. In contrast, the infected group exhibited pronounced pathological alterations, including a marked inflammatory response with extensive infiltration of heterophils and mononuclear cells within both the mucosa and submucosa. The intestinal vasculature was notably dilated and congested with erythrocytes. Severe villous damage was evident, including epithelial shedding and desquamation, leading to the accumulation of sloughed epithelial cells, cellular debris, and dense bacterial colonies within the intestinal lumen.

Treatment with varying doses of *Punica granatum* peel extract–zinc oxide nanoparticles (PPE-ZnONPs) resulted in dose-dependent amelioration of these lesions. Notably, the group treated with 80 mg/kg PPE-ZnONPs demonstrated significant histological improvement, with only mild vascular congestion and minimal hemorrhagic changes observed, suggesting a protective and restorative effect of the treatment (Fig. 3, A–E).

## DISCUSSION

Avian pathogenic *Escherichia coli* (APEC) is the causative agent of colibacillosis, a disease that causes high economic losses in the poultry sector globally. Beyond broiler chickens, APEC infection also adversely affects broiler breeders, leading to decreased egg production and increased mortality within these flocks (Fotouh *et al.,* 2020). In recent decades, antimicrobial growth promoters have been extensively used in chicken feed to enhance the growth performance and overall health of the animals (Abu El Hammed *et al.,* 2022).

Antibiotics are commonly used to prevent and control colibacillosis (Diab *et al.,* 2021). However, the prolonged use of antibiotics can lead to antibiotic residues in poultry goods and the emergence of multidrug-resistant bacteria, confusing colibacillosis inhibiting efforts (Panth 2019; Elmeligy *et al.,* 2024). Therefore, there is a need to develop new strategies. One promising approach involves the combined use of nanoparticles (NPs) with natural phyto-additive compounds to control bacterial pathogens (Morsy *et al.,* 2023).

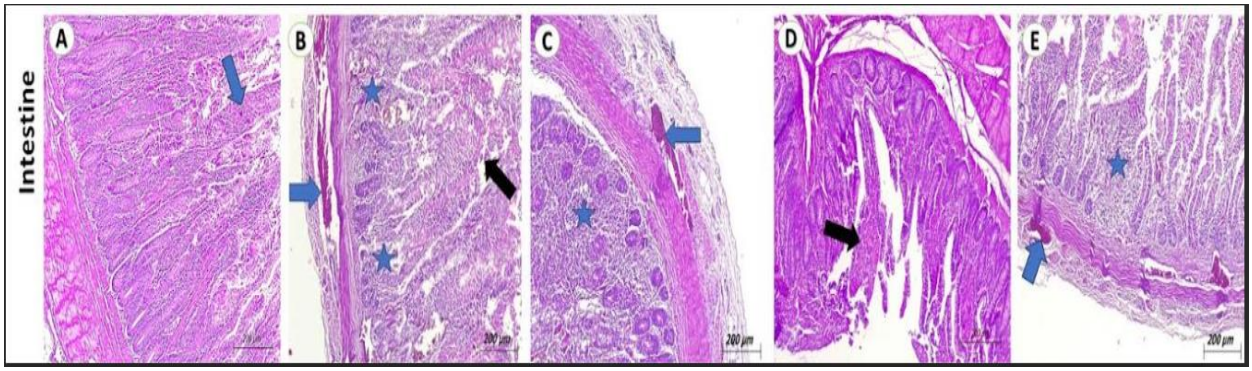


**Fig. 3:** Histopathological sections of intestinal tissue from broiler chickens experimentally infected with Escherichia coli O87 (H&E staining, scale bar 200um). A) Control group displaying normal intestinal architecture with intact mucosal layers and well-preserved villi (arrow). B) Infected group demonstrating severe pathological changes, including marked congestion of the muscularis layer (blue arrow), extensive infiltration of inflammatory cells within the mucosa (stars), and significant villous destruction (black arrow). C) Broilers treated with 40 mg/kg PPE-ZnONPs (NZ) showing persistent vascular congestion in the muscular layer (arrow) and diffuse inflammatory infiltration in the mucosa (stars), indicating partial amelioration. D) 80 mg/kg NZ-treated group exhibiting only mild villous damage, with largely preserved intestinal structure. E) 120 mg/kg NZ-treated group showing mild congestion of the muscularis layer and minimal inflammatory cell infiltration within the mucosa (star), indicating improved histological restoration.

In this study, the *E. coli*-challenged and untreated group exhibited a significant reduction in body weight, which aligns with the findings of da Rosa *et al.* (2020)**.** They reported that *E. coli* infection can negatively impact broiler development and performance, leading to symptoms such as sadness, reduced appetite, and decreased feed intake. The decrease in weight was attributed to oxidative stress caused by the *E. coli* infection (Abu El Hammed *et al.,* 2022).In this study, supplementing chicks' drinking water with PEE-ZnONPs enhanced both body weight gain (BWG) and feed conversion ratio (FCR) across all treatment groups compared to the positive control group. The most significant improvements in FCR and BWG were observed at a concentration of 80 mg PEE-ZnONPs per kg of diet. The superior growth performance (BWG and FCR) of the G4 group, which received 80 mg PEE-ZnONPs, suggests that the combination of zinc nanoparticles (ZnNPs) and pomegranate extract results in improved growth, likely due to their contributions and potential synergistic interactions.

In his study on zinc nano's effects on broilers, Alian *et al.* (2023) reported that supplementing the diet with 40 mg/kg of nano ZnO led to a notable increase in both body weight gain and ultimate weight ($P<0.001$), in contrast to the control group. Also, zinc nanoparticles (ZnNPs) enhance weight gain and feed efficiency in broilers, largely due to their positive effects on gastrointestinal absorption, specifically increasing mucosal efficacy. This is supported by studies such as Fazilati *et al. (2013) and* Zhao *et al. (2014).* Where birds supplemented with nano-ZnO exhibited significantly higher weight gains and improved feed conversion ratios. Furthermore, Radi *et al.* (2021) documented improved body weight in broiler chicks fed ZONPs, highlighting PEE-ZnONPs' efficacy in promoting growth. And this improved performance is likely attributed to the unique properties of ZnONPs, which expand the intestinal absorptive capacity. This involves lengthening and extending the villi, increasing the mucosal surface area, and deepening the crypts (Hafez *et al.,* 2017; Abedini *et al.,* 2018)

Regarding the impact of pomegranate peel on growth performance, Xu *et al.* (2024) explored its potential to enhance growth due to its high tannin content. Xie *et al.* (2021) demonstrated that pomegranate peel polyphenols, when incorporated into chitosan

nanoparticles, exhibited inhibitory effects against *E. coli* O157:H7. This aligns with findings from Hanafy *et al.* (2021), who reported that pomegranate peel extracts, rich in tannins, enhance growth performance and reduce the prevalence of E. coli in broilers, effectively suppressing several bacterial species, including *E. coli, Staphylococcus aureus*, and *Listeria monocytogenes*. These improvements may stem from the polyphenol compounds in pomegranate peel, which possess antimicrobial activity that inhibits bacterial growth, improves digestion, and enhances energy utilization. Additionally, the presence of proantho-cyanidins in pomegranate peel boosts the activities of pancreatic and small intestinal digestive enzymes, leading to improved nutrient absorption (Thema *et al.,* 2019; Reddy *et al.,* 2014).

The observed decrease in body weight with a PEE-ZnONPs dosage of 120 mg/L in our study may stem from the broilers' overall absorption of zinc and phenolic compounds. Similarly, Mahmoud *et al.* (2013) showed that the inclusion of 1% guava leaves in broiler diets resulted in reduced feed consumption. This effect is likely because phenolic compounds, particularly tannins, are recognized as antinutrients. It is hypothesized that high concentrations of polyphenols, as present in pomegranates, can reduce the digestibility of proteins and amino acids by interacting with endogenous proteins (protein carbonyl groups) within the intestinal lumen (Pascariu *et al.,* 2017).

Intestinal integrity plays a significant role in maintaining health, preventing tissue damage and disease, and facilitating the sufficient delivery of food nutrients into the body. In light of its highest importance. Intestinal mucosal barriers are primarily composed of epithelial cell membranes and mucus gel covering the epithelium (Turner, 2006; Abdel-Maguid *et al.,* 2019). The most significant mucin gene in the intestine is MUC2. Mucin secretion and expression patterns could affect the efficacy of the protective barrier (Al-Sadi *et al.,* 2009). *The E. coli* challenge in this research decreased MUC2 expression. Another crucial intestinal mucosal barrier constituent was mainly controlled by an effective system of the epithelial junctional complex, termed tight junctions (TJs), which act as a barrier that blocks macromolecular transmission (Elbarbary *et al.,* 2024b).

Occludin is the most important constituent in regulating intestinal epithelial barrier function. According to this study, intestinal physical barrier damage occurred when mRNA expression of OCLN decreased in the jejunum following an APEC challenge. This is consistent with Yang *et al.* (2014)'s result. Our findings indicated that birds ingested water containing PEE ZnONP (40, 80, and 120 mg/L) greatly elevated OCLN, which significantly relieved the physical barrier injury. Zinc has also been shown to enhance intestinal mucosal repair and gastrohepatic protection (Sturniolo *et al.,* 2002; Bucker *et al.,* 2020). This aligned with Zhang *et al.* (2012), who discovered that Zn enhanced the expression of OCLN in Salmonella-challenged broiler chicken ileum. Also, pomegranate can alleviate the reduction of occludin in the mouse intestine caused by alcoholic liver disease or hyperlipidemia (Xuan *et al.,* 2017). This study confirmed that E. *E.coli* challenge results in injury of the jejunal barrier of broilers and increases the permeability of jejunal mucosa with the passage of antigenic chemicals (e.g., LPS) to the blood environment, thus initiating jejunal inflammation and oxidative stress, with systemic inflammation consequently impairing the capacity for intestinal digestion and absorption, ultimately resulting in stunted growth.

Toll-like receptors (TLRs) are significant components of pattern recognition receptors; TLR-4 can detect lipopoly-saccharides (LPS), which are specific to Gram-negative bacteria (Pasare and Medzhito 2005; Liu *et al.,* 2017). Toll-like receptors (TLRs) can trigger subsequent inflammatory responses, ultimately culminating in the production of pro-inflammatory mediators, such as cytokines, *TNF-α* and *IL-1β* (Mahmoud *et al.,* 2020). Cytokines are small molecular-weight immune regulatory peptides that contributs to both innate and adaptive host immunological

responses in the context of interleukin expression (Abo-Aziza *et al.,* 2022).

In this study, the E. coli challenge increased the mRNA expression level of TLR-4 as in the non-infected. It is consistent with Huang *et al.'s* (2019) findings for the ileum and jejunum mRNA expression level of TLR4 in E. coli-challenged piglets. Activation of TLR4 ultimately results in the production of IL-1, IL-6, and TNF (Liu *et al.,* 2017). The expression of TNF-α and IL-1β was significantly greater in the APEC-challenged group, compared to the unchallenged group, consistent with findings in broiler chickens (Ateya *et al.,* 2019). For such negative impacts in this study, the incorporation of PEE-ZNOP supplements tended to suppress jejunum TLR4 mRNA expression. The findings are consistent with earlier research (Hu *et al.,* 2017), which demonstrated that supple-menting with CS-Zn suppressed TLR4 protein expression and decreased IL-2 and TNF. Zn supplementation has also been found to suppress the generation of IL-1β, IL-6, and TNF-α when duck intestinal epithelial cells are damaged by LPS (Xie et al., 2021). Previous research demonstrates that zinc plays a key role as an immunological mediator against inflame-mation and infection, and zinc deficiency increases myeloid cell secretion of TNF-α and IL-1 through epigenetic changes (Wessels *et al.,* 2013; Zaki *et al.,* 2025b).

The observation that PEE ZnONP reduces intestinal inflammation by modulating the level of inflammatory cytokines. These findings suggest that PEE ZnONP protective effects against intestinal inflammation were largely contributed by inhibiting the production of pro-inflammatory cytokines IL-1β and TNF-α.

Economic feed efficiency

Total cost of feed reduced marginally in treatment group diets due to a reduction in average consumption per unit relative to the control positive unit, most notably for the G4 group that received 80 mg of the PEE-ZnoNPs, which was equally effective in reducing cost of production (Mahmoud *et al.,* 2025).

## CONCLUSION

In conclusion, in accordance with findings, PPE ZnO NP at 40, 80, and 120 ppm/l may be supplemented in broiler chickens' drinking water to prevent an intestinal mucosal barrier function loss caused by an E. coli challenge. A partial mechanism is likely involved with the enhanced occludin and MUC2 expression and reduced inflammatory cytokine levels in broiler chickens. These findings are further confirmation of the significant role of PPEZnO NP in inhibiting E. coli infection.

**Conflict of interest**

The authors affirm that they have no conflicts of interest.

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# الإمكانات العلاجية للزنك النانوي المغلف بالرمان في كتاكيت التسمين المصابة ببكتيريا الإشريشية القولونية O78: نهج هيستومورفولوجي وجزيئي

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داء العصيات القولونية، الذي تسببه البكتيريا الإشريشية القولونية الممرضة للطيور، هو عدوى بكتيرية في الدجاج اللاحم، مما يؤدي إلى ارتفاع معدل الوفيات وانخفاض الإنتاجية وضعف أداء النمو. هدفت هذه الدراسة الى تقييم فعالية جسيمات أكسيد الزنك النانوية المغلفة بالرمان (PEE-ZnONPs) بتركيزات مختلفة في السيطرة على عدوى الإشريشية القولونية في دجاج التسمين، مع الأخذ في الاعتبار زيادة مقاومة المضادات الحيوية من الإشريشية القولونية. تم تقسيم ١٥٠ كتكوت إلى خمس مجموعات: G1: مجموعة ضابطة سلبية، G2: مجموعة ضابطة إيجابية مصابة بـالإشريشية القولونية O78، وثلاث مجموعات علاجية مجموعة ٣ (G3) تلقت ٤٠ مجم/لتر ، مجموعة ٤ (G4)تلقت ٨٠ مجم/لتر، ومجموعة ٥ (G5) تلقت ١٢٠ مجم/لتر من PEE-ZnONPs في مياه الشرب لمدة ٣٥ يومًا. خلال مدة الدراسة، رُصد أداء الطيور ونفوقها، وأُعدّت عينات من أنسجة الأمعاء للتحليل الجزيئي والمرضي. تأظهرت النتائج أن إضافة PEE-ZnONPs حسّنت بشكل ملحوظ وزن الجسم، واستهلاك العلف، ونسبة تحويل العلف مقارنةً بالمجموعة الضابطة المصابة ( P <0.05). علاوة على ذلك، زادت PEE-ZnONPs من التعبير عن mRNA للأوكلودين والميوسين في الصائم (P<0.05)، مع تثبيط التعبير عن mRNA لعامل نخر الورم TNFa، مما يُشير إلى انخفاض الالتهاب. تُشير هذه النتائج إلى أن إضافة PEE-ZnONPs تُقلل من فقدان وظيفة الحاجز المخاطي المعوي الناتج عن عدوى الإشريشية القولونية O78، ربما عن طريق زيادة التعبير عن الأوكلودين والميوسين. وبالتالي، خلصت الدراسة إلى أن إضافة PEE-ZnONPs إلى مياه الشرب يُحسّن بشكل ملحوظ أداء النمو، ونسيج الأمعاء، والوظيفة المناعية، وصحة الأمعاء لدى دجاج التسمين المُصاب بالإشريكية القولونية O78.