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Effects of Artemisia and Nano-Artemisia on Growth, Health Indicators, and *Pseudomonas aeruginosa* Resistance in Japanese quail

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Abstract

A PRELIMINARY field investigation conducted in Kafrelsheikh Governorate, Egypt, revealed a 9.2% prevalence of *Pseudomonas aeruginosa* among quails raised in local farms. Antimicrobial susceptibility tests showed complete sensitivity to Gentamicin, intermediate sensitivity to Ciprofloxacin, and full resistance to Enrofloxacin and Erythromycin. Molecular identification via 16S rDNA sequencing confirmed the isolates, while virulence gene analysis detected the presence of *oprL* and *toxA* genes. In the experimental phase, A total of 220 Japanese quail chicks, each one day old, seven mutually exclusive groups were formed through random grouping of chicks, with 30 birds in each group to evaluate the effects of Artemisia, Nano-Artemisia, or Ciprofloxacin on physiological and immune responses following *P. aeruginosa* challenge over a 35-day feeding trial. Results demonstrated that birds supplemented with 1% Artemisia, 0.5% Nano-Artemisia, or treated with Ciprofloxacin showed improved growth performance, elevated antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)) with fewer clinical symptoms and pathological lesions. Moreover, supplementation with 1% Artemisia or 0.5% Nano-Artemisia reduced ether extract content in breast muscles, enhanced tissue antioxidant capacity, and improved intestinal histology. Overall, the findings suggest that incorporating Artemisia or Nano-Artemisia at the tested levels in quail diets supports immune function, enhances health, and promotes better growth under bacterial challenge.

Keywords: Artemisia, Nano-Artemisia, Growth performance, Health status, Japanese quail and *Pseudomonas aeruginosa.*

Introduction

The Japanese quail (*Coturnix japonica*) is considered a favourable species for commercial farming due to its fast growth rate, early reproductive maturity, consistent and high egg yield, frequent generational turnover (up to four per year), and short hatching period [1].

Pseudomonas aeruginosa is recognised as a prominent opportunistic pathogen in poultry, with particular relevance in Japanese quail *(Coturnix japonica*). It is frequently implicated in a range of clinical manifestations, including septicaemia, respiratory distress, and enteric disorders such as diarrhoea. The pathogen's high infectivity, marked ability to spread rapidly within flocks, and its capacity to cause severe morbidity and mortality result in substantial economic losses. Moreover, its zoonotic potential raises concerns regarding public health, particularly in settings with close human-animal interaction or inadequate biosecurity measures [1].

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Antimicrobial resistance represents a growing threat to animal health, public health, and the sustainability of poultry production systems. Its consequences extend beyond therapeutic failures to include significant economic losses due to prolonged illness, increased mortality, and reduced productivity. Moreover, resistant pathogens may act as reservoirs for antimicrobial resistance genes, contributing to their dissemination across microbial populations and potentially to humans. In light of these risks, the careful selection of effective antimicrobial agents, guided by susceptibility testing, becomes imperative. Equally important is the adoption of rational and responsible antimicrobial usage practices to curb the development and spread of resistance. Within this framework, the accurate detection and identification of etiological agents in commercial quail farms is a critical step in designing targeted intervention strategies and ensuring optimal flock health [2].

The World Health Organization (WHO) has identified *Pseudomonas aeruginosa* as one of the three most critical antibiotic-resistant pathogens of global concern [3]. In light of escalating concerns about antimicrobial resistance and increasing efforts to minimize antibiotic usage in poultry farming, there is an urgent demand for safe, cost-effective, and efficient alternatives to conventional antibiotics for controlling bacterial diseases in quail production systems.

Aim of the Work

This study aimed to investigate the potential effects of dietary supplementation with Artemisia and Nano-Artemisia on growth, immune response, and resistance to *P. aeruginosa* infection in Japanese quails.

Material and Methods

Bacteriological Examinations (P. aeruginosa Isolation and Identification)

A total of 87 Japanese quail chicks comprising both healthy and symptomatic individuals were obtained from a range of geographically distinct farms located within Kafrelsheikh Governorate, Egypt. On reaching the laboratory, tracheal and cloacal swabs were obtained from birds showing clinical signs of illness, while internal organ samples comprising the liver, heart, and lungs, kidneys, spleen, and yolk sacs were collected from freshly deceased chicks. All samples taken under aseptic conditions to prevent contamination and immediately transferred to Tryptic Soy Broth (TSB), followed by aerobic incubation at 37 °C for 24 hours.

Following incubation, a sterile loop used to transfer aliquots from each broth culture onto MacConkey agar, *Pseudomonas* agar, and Cetrimide agar plates. These incubated again at 37 °C for an-another 24 hours. Colonies displaying morphological traits consistent with *Pseudomonas aeruginosa* were isolated and subsequently cultured on nutrient agar to evaluate pigment production. The identification process for the presumptive isolates was conducted based on the guidelines described by [4], which included the oxidase test, pigment analysis, detection of the organism's distinctive earthy or musty odor, as well as detailed assessment of colony appearance, cellular morphology, and a range of conventional biochemical tests.

Antibiotic Susceptibility Testing

The antibiotic susceptibility of the bacterial isolates assessed using the Kirby-Bauer disk diffusion method. Nine antibiotics tested, including Tetracycline (TE) 30 µg, Fosfomycin (FO) 200 µg, Erythromycin (E)15 µg, Doxycycline (DO) 30 µg, Streptomycin (S) 10 µg,Ciprofloxacin (CIP) 5 µg, Florfenicol (FL) 30 µg, Enrofloxacin (ENR) 5 µg, and Gentamicin (GEN) 10 µg. After incubation, measurements were taken for the inhibition zone diameters around each antibiotic disc and evaluated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [5]. Isolates that showed resistance to at least one antimicrobial drug in no fewer than three antibiotic classes classified as multidrug-resistant (MDR), following the criteria established by [6].

Detection of **P. aeruginosa** *16S rDNA and Virulence Genes Using Polymerase Chain Reaction (PCR)*

DNA Extraction

Genomic DNA was isolated from *Pseudomonas aeruginosa* strains using the QIAamp DNA Mini Kit (Qiagen, Germany), with slight adjustments made to the manufacturer's standard procedure. Briefly, 200 µl of the bacterial suspension was incubated at 56 °C for 10 minutes with 200 µl of lysis buffer and 20 µl of proteinase K. After incubation, 200 µl of absolute ethanol was added and thoroughly mixed to ensure complete homogenization. The mixture was then processed through purification and centrifugation steps following the kit instructions. Finally, the extracted DNA was eluted in 100 µl of the provided elution buffer.

Oligonucleotide Primers

Primers used for PCR amplification were obtained from Metabion (Germany), and their sequences along with related details are listed in Table 1.

PCR Amplification

The PCR mixture had a total volume of 25 µl and consisted of 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol), 5.5 µl of nuclease-free water, and 5 µl of the extracted DNA template. The amplification process was carried out using an Applied Biosystems 2720 Thermal Cycler. Detailed information on the target

genes, expected amplicon sizes, and thermal cycling conditions is provided in Table 1.

PCR Product Analysis

The PCR products were analysed by electrophoresis on a 1.5% agarose gel (Applichem, Germany) prepared with 1× TBE buffer. Electrophoresis was undertaken at room temperature at a constant voltage of 5 V/cm for 10 minutes. A volume of 20 µl from each PCR sample was loaded into individual gel wells. DNA fragment sizes were determined by comparing the migration patterns to a 100 bp DNA ladder (Generuler, Fermentas, Thermo-Scientific, Germany). Visualization of the gel was performed using a gel documentation system (Alpha Innotech, Biometra), followed by analysis of the resulting band patterns through specialized imaging software.

Experimental Design

A total of 220 one-day-old Japanese quail chicks were used in this study. At the beginning of the experiment, ten randomly selected chicks were humanely sacrificed for bacteriological examination to confirm the absence of *Pseudomonas aeruginosa* infection. The remaining 210 chicks were randomly distributed into seven experimental groups, each consisting of 30 birds. Group 1 served as the uninfected negative control. Group 2 was orally challenged on day 10 of age with 0.5 ml of a previously isolated virulent *P. aeruginosa* strain at a concentration of 1.5×10^8 CFU/ml following the method described by [7]. And designated as the infected positive control. Group 3 received a basal diet supplemented with 1% Artemisia without infection. Group 4 was infected and received the same Artemisia-supplemented diet. Group 5 was uninfected and fed a basal diet supplemented with 0.5% Nano-Artemisia. Group 6 was infected and received a Nano-Artemisia-supplemented diet at the same concentration. Group 7 was infected and treated with Ciprofloxacin (1 g/L) starting 48 hours post-infection for five consecutive days. The grouping design and treatments are summarized in table 2.

All birds were fed the balanced formulated diet carefully designed to full fill the nutritional requirements of Japanese quail chicks for growth, In line with the recommendations of the [8]. as shown in table 3.

All birds were housed in a clean, well-ventilated facility that had been thoroughly disinfected using a mixture of formalin and potassium permanganate. A 4-centimeter layer of fresh wheat straw was used as bedding. The ambient temperature within the facility regulated according to the age of the chicks to maintain optimal comfort. Feeders and drinkers were positioned to provide the birds with uninterrupted access to feed and water for the entire duration of the study.

Throughout the experimental period, a daily clinical assessment was carried out to monitor the birds for any signs of illness, and cloacal swabs were collected periodically over two weeks post-infection to assess bacterial shedding. Any birds exhibiting severe illness or that died during the study were submitted for necropsy and bacterial re-isolation from organs. Mortality was recorded on a daily basis. On day 25 after infection, all surviving quails were humanely euthanized and underwent thorough post-mortem examination.

Preparation of Artemisia and Nano-Artemisia

Artemisia leaves procured from a local market in Kafrelsheikh, Egypt. The leaves thoroughly cleaned with double-distilled water, filtered, & then air-dried at room temperature. Once completely dried, the leaves were ground using a household grinder and passed through a fine silk sieve with an approximate pore size of 1 µm to produce Nano-Artemisia powder. The grinding process carried out using a Braun mixer grinder set to the third speed for 10 minutes. Any large particles remaining were reground and sieved under the same conditions. The resulting fine powder was stored in airtight glass containers until further use [9].

Characterizations of Nanoparticles

The physical features of Nano-Artemisia powder, Encompassing particle size, morphology, and grain boundaries, were analyzed using Field Emission Scanning Electron Microscopy (FESEM, Quattro S, Thermo-Scientific). Elemental composition was analyzed through Energy Dispersive X-ray (EDX) spectroscopy. For structural and functional group identification, the sample was freeze-dried and mixed with potassium bromide (KBr) in a 1:100 (w/w) ratio. The mixture compressed into 2 mm discs, and Fourier Transform Infrared (FTIR) spectroscopy conducted using a JASCO spectrophotometer across a spectral range of 4000 to 400 cm^{-1}.

Growth Assessment

The initial body weight of each bird was measured at the start of the experiment, at one week of age, and subsequently on a weekly basis until it reached five weeks old. The following parameters calculated throughout the study duration according to [10].

Total Weight Gain (TWG): Determined by subtracting the body weight recorded at one week of age from the final body weight measured at five weeks.

Total Feed Intake (TFI): The cumulative amount of feed consumed by each bird from one to five weeks of age.

Average Feed Conversion Ratio (AFCR): Calculated by dividing the total feed intake by the total weight gained, providing a measure of feed utilization efficiency.

Average Protein Efficiency Ratio (APER): The weight gain achieved per unit of protein consumed, which reflects the effectiveness of protein utilization.

Average Efficiency of Energy Utilization (AEEU): The ratio of total weight gain to the total energy intake, representing how efficiently dietary energy convert into body mass.

These parameters used to evaluate the birds' growth rate, feed efficiency, and nutrient utilization during the course of the study.

Assessment of some blood serum parameters

Blood samples were collected from six birds per group (two birds from each replicate) on days 14, 21, and 35 of age without the use of anticoagulants. Samples were centrifuged at 4000 rpm for 5 minutes to separate the serum, which was carefully transferred into Eppendorf tubes and stored at –20 °C until analysis. The serum samples were subjected to biochemical evaluation using spectrophotometric methods and commercial diagnostic kits (LABOMED Co., Lab. American Inc., USA). Total protein (TP) and albumin (Alb) concentrations were measured following the protocols described by [11] and [12], respectively. Serum globulin (Glob) levels were calculated by subtracting albumin from total protein values (expressed in g/dL). Kidney function parameters, including urea and creatinine (mg/dL), were determined based on the methods outlined in [13] and [14]. Liver function was assessed by measuring the activities of aspartate transaminase (AST) and alanine transaminase (ALT) in IU/L, according to [15]. The antioxidant status was evaluated through the enzymatic activities of superoxide dismutase (SOD) and catalase (CAT), determined using the methodologies described in [16] and [17], respectively and measured in units per millilitre (U/ml).

Meat quality

Upon completion of the five-week growth period, six birds were drawn through a randomization process from each treatment group (two birds from every replicate) and humanely euthanized. Samples of breast muscle were then collected and promptly frozen for subsequent analysis.

Chemical analysis

The crude protein levels in Artemisia, feed, and breast meat samples analyzed using the Kjeldahl method. Ether extract was determined through the Soxhlet extraction procedure. Samples were dried at 105 °C for 8 hours to determine dry matter (DM), and ash content was assessed by incinerating the samples overnight at 550 °C. All analyses conducted in accordance with the methods described by the [18].

Measurement of Meat Oxidative Status

Breast muscle samples were homogenized in an ice-cold 0.86% sodium chloride solution at a 1:9 weight-to-volume ratio and kept at 4 °C. The homogenates were then centrifuged at 3500 × g for 10 minutes at 4 °C. The supernatant obtained was carefully collected for the measurement of thiobarbituric acid reactive substances (TBARS). TBARS concentrations were determined spectrophotometrically using commercial assay kits from Roche Diagnostica (Basel, Switzerland), following the manufacturer's protocol. Results were expressed as nanomoles of malondialdehyde (MDA) per milligram of protein (nmol/mg protein) according to [19].

Intestinal histopathology

At the end of the experiment, jejunal tissue samples were chosen from six birds in each tested group (2 birds per replicate) and immediately fixed in 10% neutral buffered formalin. Samples were dehydrated using a graded ethanol series, cleared in xylene, and subsequently embedded in paraffin wax. Following the protocol described by [20], 5 μm-thick tissue sections were cut and stained with hematoxylin and eosin (H&E). For each sample, three sections were examined under a Leica DM 5000 light microscope equipped with a Leica EC3 digital imaging system. Morphometric measurements were conducted, and images were captured. Villus height and width were quantified in micrometres using ImageJ software (National Institutes of Health, MD, USA).

Statistical Analysis

Analytical procedures were conducted using two-way ANOVA, followed by Tukey's post hoc test to assess the main effects and interactions between the independent variables. All statistical procedures were conducted with SPSS software (version 22). Results are expressed as means ± standard error of the mean (SEM) to reflect variability within the data. A significance level of $P < 0.05$ was used to determine statistical differences.

Results

Incidence of Pseudomonas aeruginosa Infection in Quail Samples

Out of 87 quail samples examined, *Pseudomonas aeruginosa* was isolated from eight birds, resulting in an overall infection prevalence of 9.2%.

Clinical Signs and Post-Mortem Finding

Eighty-seven quails, including both symptomatic and apparently healthy individuals, collected from farms in Kafrelsheikh. Affected birds showed signs of respiratory distress and gastrointestinal disturbances. Post-mortem examination frequently revealed signs of septicemia and enteritis. Characteristic findings included focal necrosis in the intestinal mucosa and, in some cases, the presence of cheesy cores within the ceca. The spleen and liver commonly enlarged and congested.

Antibacterial Susceptibility

According to the data presented in Table 4, *P. aeruginosa* isolates exhibited complete sensitivity to Gentamicin. The isolates exhibited intermediate susceptibility to Ciprofloxacin, while showing complete resistance to both Enrofloxacin and Erythromycin.

Prevalence of P. aeruginosa 16S rDNA and some virulence genes (oprL and toxA) in isolates

As shown in table 5 and figure 1 PCR amplification of the 16S rDNA gene was observed in all eight isolates. Moreover, the *oprL* and *toxA* virulence genes were also detected in all isolates, with a 100% detection rate

Characterization of Synthesized Nano-Artemisia

Scanning Electron Microscopy (SEM)

Field Emission Scanning Electron Microscopy (FE-SEM) employed to observe the surface morphology of the Nano-Artemisia powder (Fig. 2A). The micrographs revealed the presence of both larger, well-separated particles approximately 450 nm in diameter and smaller, uniformly distributed semi-spherical particles measuring around 70 nm.

Energy-Dispersive X-ray (EDX) Analysis

The elemental composition of the Nano-Artemisia powder assessed using EDX spectroscopy (Fig. 2B). The EDX spectrum confirmed the presence of several elements, including carbon (40.63%), nitrogen (13.69%), oxygen (39.81%), sodium (2.14%), magnesium (0.57%), sulfur (0.85%), chlorine (0.64%), potassium (1.07%), and calcium (0.61%).

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR analysis was conducted to identify the functional groups present in the Nano-Artemisia extract. A broad, intense peak observed at 3413 cm^{-1}, indicative of O–H stretching vibrations. Two distinct fork-shaped peaks appeared at 2924 cm^{-1} and 2845 cm^{-1}, corresponding to C–H stretching. Peaks at 1440 cm^{-1} and 1324 cm^{-1} were associated with C=C and C=O bonds in aromatic rings and carboxyl groups, respectively. Additionally, prominent peaks at 1309 cm^{-1} and 502 cm^{-1} suggested the presence of aromatic N–H and ester-related C–O–C stretching. Bands at 1104 cm^{-1} and 1065 cm^{-1} were attributed to C–O and secondary O–H bonds, indicating the presence of polyphenolic compounds.

Clinical Signs, Mortality, and P. M Lesions In Experimentally Infected birds

Infected birds exhibited several clinical symptoms, including depression, reduced feed intake, weight loss, whitish watery diarrhea, and occasional lameness. These symptoms were most severe in Group 2, which infected but received no treatment. In contrast, symptom severity noticeably reduced in Groups 4, 6, and 7. These observations illustrated in Figure 3.

No mortality recorded in Groups 1, 3, and 5. However, mortality rates of 10%, 3.3%, and 3.3% were observed in Groups 2, 6, and 7, respectively. Post-mortem examinations of the infected birds revealed several pathological changes, including air sacculitis, marked congestion of the liver and spleen, gas accumulation within the intestines, and congested kidneys with visible urate deposits in the ureters. These findings illustrated in Figure 4.

Fecal Shedding and Re-isolation from Internal Organs of Experimentally Infected birds

Fecal swabs collected one day post-inoculation tested positive for *P. aeruginosa* in four infected groups (G2, G4, G6, and G7), confirming active fecal shedding of the pathogen. Moreover, bacteriological analysis of homogenized tissue samples from deceased birds specifically from the liver, heart, lungs, kidneys, spleen, and yolk sac confirmed the presence of the inoculated *P. aeruginosa*. Remarkably, the inclusion of 1% Artemisia powder (AP) or 0.5% Nano-Artemisia powder (NAP) in the diet, as well as antibiotic treatment, led to a noticeable reduction in both fecal shedding and the re-isolation of *P. aeruginosa* from internal organs compared to the positive control group.

Body Weight Development

As presented in Table 6, dietary supplementation with 1% Artemisia powder (AP) or 0.5% Nano-Artemisia powder (NAP) did not significantly influence body weight during the initial three weeks of the experiment. However, by day 35, both treatments resulted in increased final body weights approximately 3.6% and 4.6% higher, respectively compared to the control group. In contrast, quail chicks challenged with *Pseudomonas aeruginosa* without any treatment exhibited a reduction in final body weight by approximately 4.2% relative to the control. Notably, the group receiving 0.5% NAP along with the bacterial challenge maintained a final body weight comparable to that of the unchallenged control group.

Feed Efficiency Parameters

As illustrated in Table 7, dietary supplementation with 1% Artemisia powder (AP) or 0.5% Nano-Artemisia powder (NAP) led to a non-significant increase in total weight gain by approximately 4.2% and 4.9%, respectively, compared to the control group. Conversely, quail chicks infected with *Pseudomonas aeruginosa* without treatment experienced a reduction in total weight gain of about 4.7%. Notably, birds receiving a 0.5% NAP-supplemented diet along with the bacterial challenge achieved a final body weight comparable to that of the control group.

Furthermore, the inclusion of 1% AP or 0.5% NAP, regardless of infection status, significantly ($P < 0.05$) reduced total feed intake compared to the

control. These dietary additives also significantly improved feed conversion ratio (FCR), protein efficiency ratio (PER), and efficiency of energy utilization (EEU) over the course of the experiment.

In contrast, birds challenged with *P. aeruginosa* alone showed marked deterioration in FCR, PER, and EEU. However, quail fed with diets containing 1% AP or 0.5% NAP alongside bacterial challenge exhibited similar or enhanced feed efficiency values relative to the control group. Overall, no significant differences ($P > 0.05$) observed in performance metrics between birds receiving Artemisia in its regular or Nano form and those treated with the antibiotic.

Serum Biochemical Parameters

Tables 8, 9, and 10 illustrate the impact of incorporating dietary Artemisia or Nano-Artemisia on the serum protein profile, as well as on kidney and liver function indicators, in Japanese quail chicks exposed to *Pseudomonas aeruginosa*. The findings demonstrate that supplementing the diet with 1% Artemisia powder (AP) or 0.5% Nano-Artemisia powder (NAP), whether in the presence or absence of *P. aeruginosa* infection, did not significantly alter serum levels of total protein, albumin, globulin, urea, creatinine, alanine aminotransferase (ALT), or aspartate aminotransferase (AST) throughout the study period compared to the control group.

Antioxidant Status

Table 11 demonstrates that dietary supplementation with 1% Artemisia powder (AP) or 0.5% Nano-Artemisia powder (NAP) significantly ($P < 0.05$) enhanced serum activities of superoxide dismutase (SOD) and catalase (CAT) across all experimental periods compared to the control group. In contrast, Japanese quail chicks infected with *Pseudomonas aeruginosa* without dietary supplementation showed reduced levels of SOD and CAT in their serum. Meanwhile, quails that received either 1% AP or 0.5% NAP in combination with Ciprofloxacin treatment did not show a statistically significant enhancement in serum SOD and CAT activities relative to the control.

Breast Meat Chemical Composition and Oxidative Index

When compared to the control group, adding 1% Artemisia powder (AP) or 0.5% Nano-Artemisia powder (NAP) to the diet had no significant impact on the moisture content (%) or crude protein (CP%) of the breast meat. However, both supplements caused a significant decrease ($P < 0.05$) in ether extract (EE %) by approximately 7.6% and 13.7%, respectively. Japanese quail chicks challenged solely with *Pseudomonas aeruginosa* showed an increase in EE% of breast meat by around 16.6% compared to the control. Interestingly, the group receiving 1% AP along with the bacterial challenge displayed EE% levels comparable to the control, while those given 0.5% NAP or treated with antibiotics showed a numerical increase, though not statistically significant.

As shown in Table 12, dietary inclusion of AP, NAP, or antibiotics resulted in a non-significant reduction in thiobarbituric acid reactive substances (TBARS), indicating slightly improved oxidative stability of breast meat compared to the control. Both conventional and Nano forms of Artemisia significantly ($P < 0.05$) reduced EE% and enhanced the antioxidant properties of breast meat in comparison to antibiotic treatment.

Intestinal Morphology

Based on the findings in Table 13 and Figure 5, supplementing the diet with 1% Artemisia powder (AP) caused a slight, non-significant increase in villus height by day 35. In contrast, the addition of 0.5% Nano-Artemisia powder (NAP) significantly ($P < 0.05$) enhanced villus height compared to the control group. Both AP and NAP treatments were linked to a non-significant reduction in villus width and a significant rise ($P < 0.05$) in the number of goblet cells when compared to the control. In contrast, quail chicks infected with *Pseudomonas aeruginosa* without any dietary additives showed a marked reduction in villus height and goblet cell numbers, accompanied by an increase in villus width. Notably, the group fed 0.5% Nano-Artemisia powder (NAP) alongside the bacterial challenge showed enhanced ileal morphology relative to the control group. Throughout the duration of the study, no significant differences ($P > 0.05$) were observed in intestinal structure or goblet cell numbers between birds receiving either form of Artemisia supplementation and those administered antibiotics.

Discussion

P.aeruginosa is widely distributed in the environment and originally considered a saprophytic organism [21]. However, it has since been recognized as an important opportunistic pathogen capable of causing infections in various animal species, including poultry [2]. The current study identified a 9.2% prevalence of *Pseudomonas aeruginosa* in quail samples, which is comparable to the findings of [22], who reported a 6.25% incidence among 224 samples collected from diseased chickens. A slightly lower prevalence of 4.9% was documented by [23], who isolated the pathogen from 20 deceased chickens. Conversely, higher prevalence rates have been reported in previous studies, such as [24], who observed a 20.5% incidence in infected chickens, and [25] who detected *P. aeruginosa* in 31% of poultry respiratory tract samples. Notably, [7] reported a substantially higher prevalence of 60% (30 out of 50 samples) in naturally infected quails. These variations in prevalence rates could be

associated with a range of factors, such as, regional socio-economic conditions, climatic variations, differences in bird health status, and disparities in feed and water quality across different geographical areas.

The results of the antibacterial susceptibility testing (Table 4) demonstrated that *Pseudomonas aeruginosa* isolates exhibited complete sensitivity to Gentamicin (100%) and intermediate susceptibility to Ciprofloxacin (100%). In contrast, the isolates demonstrated high resistance rates to Enrofloxacin and Erythromycin (100% each), as well as to Tetracycline and Doxycycline (87.5% each). These findings are in line with those of [7], who reported that *P. aeruginosa* isolates from quails were sensitive to Gentamicin but resistant to Doxycycline & Erythromycin. Similarly, [26] documented resistance to Tetracycline and Enrofloxacin. However, a discrepancy was noted with [7], who previously reported sensitivity to Enrofloxacin.

Such variations in antibacterial resistance patterns may be attributed to the indiscriminate use of antibiotics in field settings and to structural characteristics of the bacterial cell wall, rather than solely to the action of antibiotic-inactivating enzymes [27]. Furthermore, our study confirmed the presence of multidrug resistance among the *P. aeruginosa* isolates, likely resulting from the frequent and unregulated use of antibiotics for both treatment and prevention in quail farms. Additionally, the presence of the *oprL* gene, encoding an outer membrane lipoprotein, contributes to efflux system function and regulates cell permeability, may contribute to the resistance mechanisms observed [28].

The 16S rDNA gene, specific to *Pseudomonas aeruginosa*, serves as a reliable molecular marker for early identification, even in uncommon strains [29]. In the current study, PCR amplification of the 16S rDNA gene yielded distinct bands at 956 bp in all eight isolates, confirming their identity as *P. aeruginosa* (Table 5 & Figure 1a). Comparable findings were reported by [30], who observed identical amplicon sizes in isolates from *Pangasianodon hypophthalmus* fish. Likewise, [31]. verified *P. aeruginosa* presence in all seven isolates using the same genetic marker and reported matching amplicon lengths.

Further confirmation of virulence was achieved through the detection of the *oprL* gene, which encodes an outer membrane lipoprotein. This gene was identified in all isolates, producing amplicons at 504 bp (Table 5 and Figure 1b). The *oprL* gene is known to contribute to antibiotic resistance and is considered a dependable genetic target for the rapid detection of *P. aeruginosa* [32]. The results are in agreement with the findings of [33], who observed 100% prevalence of *oprL* in isolates from broiler chickens.

Moreover, all isolates tested positive for the *toxA* gene, generating amplicons at 396 bp (Table 5 & Figure 1c). The *toxA* gene encodes exotoxin A, a key virulence factor implicated in respiratory diseases in poultry, primarily by disrupting host protein synthesis [34]. This 100% detection rate of *toxA* in isolates is consistent with [35], although [34] reported a slightly lower prevalence of 80% in broiler chicken isolates.

Field Emission Scanning Electron Microscopy (FE-SEM) utilized to examine the morphological features of the Nano-Artemisia powder. The micrographs displayed the presence of both larger, isolated particles measuring around 450 nm and smaller, uniform semi-spherical particles approximately 70 nm in diameter. Elemental composition analysis using Energy Dispersive X-ray (EDX) spectroscopy identified peaks corresponding to carbon (40.63%), nitrogen (13.69%), oxygen (39.81%), sodium (2.14%), magnesium (0.57%), sulfur (0.85%), chlorine (0.64%), potassium (1.07%), and calcium (0.61%). These results align with the known elemental makeup of Artemisia species as described by [36].

Fourier-transform infrared spectroscopy (FTIR) analysis further verified the existence of distinct functional groups associated with Artemisia compounds. A broad, intense absorption band at 3413 cm^{-1} attributed to O–H stretching vibrations, indicative of phenolic groups [37]. Two distinct peaks at 2924 cm^{-1} and 2845 cm^{-1} corresponded to C–H stretching vibrations in methylene groups [38]. Peaks observed at 1630 cm^{-1} and 1724 cm^{-1} were assigned to C=C and C=O stretching in aromatic rings and carboxylic groups, respectively. Additional bands at 1440 cm^{-1} and 1324 cm^{-1} linked to O–H bending in phenolic structures. Strong absorptions at 1309 cm^{-1} and 502 cm^{-1} indicated N–H stretching in aromatic amines and C–O–C bonds from ester groups [39]. Furthermore, bands at 1104 cm^{-1} and 1065 cm^{-1} corresponded to C–O and secondary O–H bonds, reflecting the polyphenolic content of the extract [40]. These FTIR findings suggest that the nanosizing process maintained the chemical integrity of the Artemisia plant, with spectral peaks largely consistent with those of bulk material but varying mainly in intensity.

The clinical signs noted in the infected birds throughout this investigation closely resembled those reported by [7], including lethargy, decreased feed consumption, weight loss, watery white diarrhea, and occasional lameness. Post-mortem examinations revealed lesions comparable to those described by [7, 41]. Affected birds of all ages exhibited severe respiratory distress, difficulty breathing, and profuse diarrhea. Necropsy findings included inflammation

of the air sacs, pronounced congestion of the liver and spleen, gas buildup in the intestines, and renal congestion with visible urate deposits in the ureters. However, mortality rates in this study lower than those documented [28], who reported mortality as high as 78.26%. This difference may be attributed to variations in the infection method, as Badr et al. [26] used subcutaneous inoculation, which could have increased the virulence and mortality rates.

The observed increase in final body weight of quails fed with 1% Artemisia powder (AP) or 0.5% Nano-Artemisia powder (NAP) can be attributed to the plant's bitter compounds, which are known to enhance gastric motility, promote blood circulation to the stomach lining, and stimulate acid secretion [42, 43]. Moreover, Artemisia contains bioactive substances with anti-inflammatory, liver-protective, and bile-promoting effects, all of which may contribute to better growth performance and more efficient feed utilization [44, 43]. The obtained data support the findings of [45], who documented improved feed conversion ratios and decreased feed intake in birds supplemented with varying levels of Artemisia annua. Likewise, [46] reported that supplementation with Artemisia dracunculus extract enhanced weight gain, final body weight, and specific growth rate in treated birds. Supporting this, [47] found that broilers receiving Artemisia annua water extract showed improved growth performance and nutrient absorption, underscoring Artemisia's positive role in poultry health and productivity.

Blood biochemical indicators are essential markers of an animal's metabolic condition and overall health status, directly influencing poultry performance [48]. Serum total protein (TP) and albumin (ALB) are particularly important as they reflect protein synthesis efficiency and nutrient assimilation. Variations in TP and ALB levels often indicate liver function and the bird's nutritional state. Higher TP levels typically correlate with enhanced protein metabolism, better feed efficiency, and improved growth [49].

Given the liver's vital role in metabolism, liver enzymes serve as key indicators of physiological health in poultry. This study evaluated the effects of dietary AP and NAP, both with and without *P. aeruginosa* infection, on liver enzyme activities. Contrasting our findings, [50] observed a significant reduction in liver enzymes in broilers fed 3–5% Boswellia serrata, although the enzyme levels remained within normal ranges. The hepatoprotective properties in that case attributed to the plant's rich polyphenol and flavonoid content [51]. Similarly, [46] reported significant decreases in serum ALT and AST in fish supplemented with Artemisia dracunculus extract. These discrepancies may arise due to differences in species, dosages, or environmental conditions.

Maintaining a balance between the production and detoxification of reactive oxygen species (ROS) is crucial for cellular homeostasis. Under normal physiological conditions, antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) play essential roles by neutralizing ROS, converting them into harmless molecules like water and oxygen [52]. In this study, supplementation with AP increased serum activities of SOD and CAT, likely due to the high levels of phenolic compounds in Artemisia that support antioxidant defenses in quails [53]. Previous research by [54], also confirmed Artemisia's antioxidative and immunomodulatory benefits in broilers. Additional studies by [55, 56] further corroborate these findings, noting enhanced antioxidant enzyme activity in birds fed diets enriched with medicinal plants, including Artemisia leaf powder.

Regarding meat composition, results revealed a reduction in intramuscular fat and preservation of crude protein content in breast muscle, aligning with findings by [57], who observed similar effects in Arbor Acres chickens fed Artemisia leaves. Comparable results reported by [58] in broilers and [34] in Japanese quails. Lipid oxidation critically influences meat quality and shelf life, particularly in poultry due to its high polyunsaturated fatty acid content [59]. Oxidative stress negatively affects meat characteristics [51], with thiobarbituric acid reactive substances (TBARS) serving as reliable markers of lipid peroxidation and quality [60]. In this study, dietary AP and NAP significantly lowered TBARS levels in breast muscle, indicating improved oxidative stability likely resulting from Artemisia's antioxidant properties, consistent with observations by [61].

Enhancements in intestinal morphology also observed with AP and NAP supplementation. Damage to the intestinal lining typically impairs nutrient absorption, increases fluid and electrolyte loss, and reduces overall performance [62], Artemisia supplementation improved key histological parameters, such as villus height and goblet cell density, likely by promoting epithelial cell regeneration [63]. Similar improvements in gut structure reported by [64, 61] in birds fed diets containing medicinal herbs, including Artemisia.

Conclusion

This research confirmed that the 16S rDNA gene serves as a dependable molecular marker for identifying *Pseudomonas aeruginosa*. The detection of the *toxA* gene correlated with respiratory infections, while the *oprL* gene was associated with increased antibiotic resistance. Despite some observed resistance to frequently used antibiotics, Gentamicin and Ciprofloxacin showed strong effectiveness in vitro.

Notably, this study offers novel insights into the use of herbal nanoparticles within poultry production. Supplementation with Artemisia powder (AP) and its nano-formulation (NAP) significantly improved growth performance, intestinal health, overall well-being, and disease resistance to *pseudomonas aeruginosa* in Japanese quail. These findings indicate that AP and NAP could be promising natural alternatives to conventional antibiotics in poultry feeding programs. In particular, incorporating 10 g/kg of AP or 5 g/kg of NAP into the diet may promote a more sustainable and health-conscious poultry industry. Nevertheless, additional research is necessary to thoroughly evaluate their safety, effectiveness, and long-term impacts.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval and Animal Welfare Statement

The experimental procedures, protocols, and animal care practices used in this study strictly adhered to the guidelines established by the Animal Health Research Institute, Agriculture Research Center, Giza, Egypt (ARC AHRI). Additionally, informed consent for the use of Japanese quail was obtained via email from the owner of the quail farm. All efforts were made to minimize animal discomfort and ensure humane treatment throughout the infection and treatment processes.

TABLE 1. Primer Sequences, Target Genes, Expected Amplicon Sizes, and Thermal Cycling Conditions Used in Conventional PCR

Target gene	Primers sequences	Amplified segment (bp)	Amplification (35 cycles)				Final extension	Reference
			Primary denaturation	Secondary denaturation	Annealing	Extension		
toxA	F-ACAACGCCCTCAGCATCACCAGC R-CGCTGGCCCATTCGCTCCAGCGCT	396 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	[65]
oprL	F-ATGGAAATGCTGAAATTCGGC R-CTT CTT CAG CTC GAC GCG ACG	504 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[66]
P. aeruginosa 16S rDNA	F-GGGGGATCTTCGGACCTCA R-TCCTTAGAGTGCCCACCCG	956 bp	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 50 sec.	72˚C 10 min.	[67]

TABLE 2. the experimental design

Groups	Treatment Description
1	Negative control group (neither infected nor treated).
2	Positive control group (infected* but not treated).
3	Uninfected group receiving a basal diet enhanced with 1% Artemisia**.
4	Infected group receiving a basal feed enriched with 1% Artemisia.
5	Uninfected group fed a basal diet supplemented with 0.5% Nano-Artemisia.
6	Infected group fed a basal feed enriched with 0.5% Nano-Artemisia.
7	Infected and treated with Ciprofloxacin*** (1 g/L) starting 48 hours post-infection for five consecutive days.

*Infection was administered orally on day 10 of age with 0.5 ml of a previously isolated virulent *P. aeruginosa* strain at a concentration of 1.5×10^8 CFU/ml following the method described by [7].

**Artemisia was sourced from the local market in Kafrelsheikh, Egypt. The 1% Artemisia supplementation was based on the dosage used by [68], while the 0.5% Nano-Artemisia dose followed the recommendations of [69].

***Ciprofloxacin used for treatment was prepared by medical professionals according to the manufacturer's instructions provided by Pharco Pharmaceuticals.

TABLE 3. The specific ingredients and nutrient composition of the experimental diets based on their dry matter content

	Artemisia type and its inclusion%		
Items	**0**	**1.0% Artemisia**	**0.5% Nano Artemisia**
Ingredients%			
Corn	52	51	51.5
Soybean meal (44%)	34.5	34.5	34.5
Artemisia[1]	0	1	0
Nano Artemisia	0	0	0.5
Corn gluten meal (60%)	8	8	8
Soybean oil	1.6	1.6	1.6
Di-calcium phosphate (DCP)[2]	1.8	1.8	1.8
Calcium carbonate[3]	1.3	1.3	1.3
Lysine[4]	0.05	0.05	0.05
DL-Methionine[5]	0.1	0.1	0.1
Choline chloride (50%)	0.05	0.05	0.05
Sodium chloride	0.25	0.25	0.25
Mineral & vitamin premix[6]	0.3	0.3	0.3
Mycotoxin binder[7]	0.05	0.05	0.05
Total	100	100	100
Nutrient profile:			
Moisture%	11.15	10.98	11.06
Crude protein%	24.06	23.95	23.89
Ether extract%	4.08	4.05	3.98
Crude fiber%	2.56	2.98	2.79
Calcium%[7]	1.06	1.09	1.07
Available phosphorus%[8]	0.39	0.39	0.39
Lysine%[8]	1.16	1.09	1.05
Methionine%[8]	0.53	0.51	0.49
ME (kcal/kg)[9]	2928	2921	2920

Artemisia[1] = Artemisia chemical composition (DM 89.1%, Moisture 10.9%, CP 3.7%, EE 4.3%, Ash 1.24% and CF 43.4%). Dicalcium phosphate[2]= contain 25% calcium & 16% phosphorus. Calcium carbonate[3]= locally produced lime stone contain 37% calcium. Lysine[4]= lysine hydrochloride (contain 98.5% Lysine). DL-methionine[5]= produced by Evonik Company (99.5% DL-methionine). Premix[6]= [3]Vitamin and mineral mix each 3kg contains: Vit A (12000000IU), vit D (2000000IU), vit E(10gr), vit K_3 (2gr), vit B_1 (1gr), vit B_2 (5gr), vitB_6 (1.5gr), vit B_{12} (10gr), nicotinic acid (30gr), pantothenic acid (10gr), folic acid (1gr), biotin (50mg), choline chloride50% (250gr), iron (30gr), copper (10gr), zinc (50gr), manganese (60gr), iodine (1gr), selenium (0.1gr), cobalt (0.1gr) and carrier up to 3kg. mycotoxin binder[7]= Mycotoxin adsorbent: Beta Mos plus (Zoomaria Sri company). 8= calcium, phosphorus, lysine and methionine content calculated according to [8]. ME[9] = Metabolizable energy was calculated according to [8].

TABLE 4. Antibacterial susceptibility test for *P. aeruginosa* isolates (no=8)

Antimicrobial agent	**Sensitive**		**Intermediate**		**Resistant**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Enrofloxacin (ENR)	-	-	-	-	8	100
Erythromycin (E)	-	-	-	-	8	100
Tetracycline (TE)	-	-	1	12.5	7	87.5
Doxycycline (DO)	1	12.5	-	-	7	87.5
Fosfomycin (FO)	3	37.5	-	-	5	62.5
Floramphenicol (FL)	3	37.5	3	37.5	2	25
Streptomycin (S)	6	75	-	-	2	25
Ciprofloxacin (CIP)	-	-	8	100	-	-
Gentamicin (GEN)	8	100	-	-	-	-

TABLE 5. Prevalence of *P. aeruginosa* 16S rDNA and some virulence genes (*oprL and toxA*) in isolates

Sample	***P. aeruginosa* 16S rDNA**	***oprL***	***toxA***
1	+	+	+
2	+	+	+
3	+	+	+
4	+	+	+
5	+	+	+
6	+	+	+
7	+	+	+
8	+	+	+
Totat %	8(100%)	8(100%)	8(100%)

TABLE 6. Influence of Dietary Artemisia and Nano-Artemisia Supplementation on Body Weight Gain (g per Chick) in Japanese Quail Challenged with *Pseudomonas aeruginosa*

Treatments	Week 1	Week 2	Week 3	Week 4	Week 5
G1	18.66±05 [a]	52.08±1.27[a]	107.13±2.65	158.52±2.62[a]	188.56±3.83[ab]
G2	18.61±0.49[a]	49.16±1.71[a]	103.71±3.25[a]	151±3.61 [ab]	180.72±4.39[b]
G3	18.24±0.54[a]	52.58±1.63[a]	107.38±3.11[a]	145.28±3.47[b]	195.36±3.97[a]
G4	18.35±0.57[a]	50.03±1.99[a]	107.57±4.13[a]	155.24±4.72[ab]	182.64±4.99[b]
G5	18.81±0.62[a]	52.31±1.75[a]	108.30±3.91[a]	154±4.96 [ab]	197.28±4.25[a]
G6	18.37±0.6 [a]	50.59±1.72[a]	108.14±2.47[a]	150.63±3.12[ab]	188.25±3.83[ab]
G7	18.22±0.48[a]	54.12±1.80[a]	111.15±2.67[a]	152.72±3.13[ab]	198±3.07 [a]
P-values					
Artemisia	0.913	0.83	0.516	0.592	0.734
Infection	0.846	0.88	0.908	0.042	0.345
Interaction	0.897	0.215	0.866	0.513	0.008

Results expressed as means ± standard error. Values in the same column followed by different letters indicate significant differences at ($P < 0.05$).

TABLE 7. Impact of Dietary Artemisia and Nano-Artemisia Supplementation on Feed Efficiency Parameters in Japanese Quail Chicks Challenged with *Pseudomonas aeruginosa*

Treatment	Parameters				
	Total gain (g/chick)	Total feed intake (g/chick)	Average FCR	Average PER	Average EEU
G1	169.43±3.37[ab]	630.99±6.78[a]	3.76±0.08[a]	1.12±0.02[c]	11.23±0.23[a]
G2	161.49±3.94[b]	611.69±9.55[b]	3.85±0.11[a]	1.1±0.03 [c]	11.49±0.33[a]
G3	176.49±3.47[a]	602.79±8.65[g]	3.44±0.07[b]	1.22±0.02[ab]	10.29±0.19[b]
G4	163.63±4.51[b]	604.78±7.99[d]	3.77±0.11[a]	1.13±0.03c	11.26±0.34[a]
G5	177.8±3.73 [a]	602.68±9.08[f]	3.43±0.08[b]	1.23±0.03[ab]	10.24±0.23[b]
G6	169.19±3.32[ab]	608.54±8.94[c]	3.63±0.08[ab]	1.16±0.02[bc]	10.85±0.24[ab]
G7	179.39±2.62[a]	604.34±5.85[e]	3.39±0.05[b]	1.24±0.02[a]	10.12±0.16[b]
P-values					
Artemisia	0.740	0.001	0.308	0.238	0.308
Infection	0.248	0.001	0.040	0.035	0.040
Interaction	0.003	0.001	0.003	0.001	0.003

Data presented as means ± standard error. Different letters within the same column indicate statistically significant differences at ($P < 0.05$).

TABLE 8. Influence of Dietary Artemisia and Nano-Artemisia Supplementation on Serum Protein Profile in Japanese Quail Chicks Exposed to *Pseudomonas aeruginosa*

Treatment	14th day			21st day			35th day		
	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
G1	6.65 ±0.25	5.39 ±0.55	1.26 ±0.35	6.82 ±0.23	4.76 ±1.59	2.07 ±0.12	6.59 ±0.18	4.97 ±0.09	1.62 ±0.13
G2	6.42 ±0.46	4.95 ±1.65	1.35 ±0.41	7.26 ±0.16	5.55 ±1.85	1.84 ±0.22	7.19 ±0.28	5.27 ±0.31	1.92 ±0.56
G3	7.14 ±0.46	5.32 ±0.11	1.83 ±0.55	6.32 ±2.08	5.22 ±0.17	1.46 ±0.56	7.32 ±0.63	5.28 ±0.22	2.03 ±0.65
G4	6.86 ±0.35	5.22 ±0.14	1.64 ±0.46	6.56 ±2.21	4.66 ±1.55	2.04 ±0.32	7.12 ±3.04	5.07 ±0.01	1.64 ±0.42
G5	7.06 ±2.35	5.16 ±0.33	1.91 ±0.5	6.97 ±2.32	4.86 ±0.21	1.9±0.34	7.22 ±0.7	5.07 ±1.69	2.24 ±0.86
G6	7.16 ±2.39	5.55 ±1.85	1.94 ±0.38	6.25 ±2.09	4.95 ±0.19	1.41 ±0.35	7.58 ±0.35	5.25 ±0.17	2.33 ±0.45
G7	7.34 ±0.89	4.85 ±1.62	2.37 ±0.78	7.06 ±0.23	5.08 ±0.29	1.98 ±0.49	7.09 ±2.39	5.59 ±1.87	2.39 ±0.39
P-values									
Artemisia	0.282	0.978	0.363	0.117	0.783	0.979	0.711	0.32	0.259
Infection	0.698	0.584	0.636	0.726	0.292	0.23	0.558	0.42	0.688
Interaction	0.645	0.286	0.89	0.409	0.812	0.986	0.894	0.332	0.19

Values presented as means ± standard error. Means in the same column with different letters indicate significant differences at ($P < 0.05$).

TABLE 9. Impact of Dietary Artemisia and Nano-Artemisia Supplementation on Serum Kidney Function Parameters in Japanese Quail Chicks Challenged with *Pseudomonas aeruginosa*

Treatment	Urea 1 (mg/dl)	Urea 2 (mg/dl)	Urea 3 (mg/dl)	Creatinine 1 (mg/dl)	Creatinine 2 (mg/dl)	Creatinine 3 (mg/dl)
G1	23.6±0.53	22.85±0.53	21.74±0.14	2.2±0.13	2.31±0.13	2.28±0.14
G2	23.19±1.28	22.19±1.11	24.84±0.18	2.27±0.09	2.57±0.08	2.09±0.31
G3	16.14±4.16	22.86±0.29	23.25±0.3	2.42±0.22	2.15±0.25	2.25±0.26
G4	24.05±0.24	21.55±0.52	22.85±6.63	2.42±0.07	2.15±0.3	2.07±0.22
G5	24.05±0.23	21.13±0.49	20.52±1.49	1.97±0.27	2.44±0.28	1.71±0.05
G6	15.17±5.86	22.04±0.34	22.71±0.34	2.19±0.03	2.35±0.16	2.09±0.26
G7	24.04±0.55	14.86±7.43	21.17±1.29	2.00±0.18	2.22±0.33	2.28±0.26
P-values						
Artemisia	0.939	0.053	0.067	0.54	0.848	0.197
Infection	0.197	0.44	0.112	0.407	0.387	0.392
Interaction	0.99	0.92	0.356	0.251	0.665	0.875

Values expressed as means ± standard error. Means within the same column bearing different letters differ significantly at ($P < 0.05$).

TABLE 10. Influence of Dietary Artemisia and Nano-Artemisia Supplementation on Serum Liver Function Parameters in Japanese Quail Chicks Challenged with *Pseudomonas aeruginosa*

Treatment	ALT 1 (IU/L)	ALT 2 (IU/L)	ALT 3 (IU/L)	AST 1 (IU/L)	AST 2 (IU/L)	AST 3 (IU/L)
G1	9.21±0.51	9.72±1.16	9.35±0.54	35.67±1.67	38.67±2.6	42.00±3.00
G2	9.37±0.45	8.47±0.51	7.73±0.72	39.00±0.01	37.00±3.01	40.33±1.33
G3	8.03±0.39	8.73±1.61	8.25±0.51	37.00±3.01	41.67±1.33	37.33±1.67
G4	8.74±1.56	7.53±0.28	10.84±2.45	37.33±1.67	37.00±3.00	51.33±10.39
G5	8.6±0.85	7.29±0.5	10.11±1.25	38.67±2.60	37.33±1.67	38.33±4.66
G6	7.86±0.87	9.04±0.87	8.39±0.29	39.00±0.01	40.33±1.33	41.67±1.33
G7	9.71±1.67	8.41±0.54	8.68±0.68	38.67±2.6	39.00±0.01	41.67±4.09
P-values						
Artemisia	0.524	0.102	0.198	0.484	0.641	0.352
Infection	0.419	0.396	0.359	0.478	0.239	0.273
Interaction	0.945	0.212	0.945	0.85	0.984	0.536

Data presented as means ± standard error. Means in the same column with different letters indicate significant differences at ($P < 0.05$).

TABLE 11. Impact of Dietary Artemisia and Nano-Artemisia Supplementation on Serum Antioxidant Enzyme Activities in Japanese Quail Chicks Challenged with *Pseudomonas aeruginosa*

Treatment	SOD 1 (u/ml)	SOD 2 (u/ml)	SOD 3 (u/ml)	CAT 1 (u/ml)	CAT 2 (u/ml)	CAT 3 (u/ml)
G1	347.31 ± 13.47^{cd}	402.29 ± 3.63^{c}	456.19 ± 11.49^{bc}	3.15 ± 0.19^{b}	3.38 ± 0.19^{bc}	3.91 ± 0.79^{b}
G2	314.53 ± 7.33^{d}	363.55 ± 13.34^{d}	417.47 ± 11.67^{c}	2.85 ± 015^{b}	2.93 ± 0.14^{c}	3.41 ± 0.11^{c}
G3	395.45 ± 12.94^{ab}	458.36 ± 17.82^{ab}	491.69 ± 11.57^{ab}	3.85 ± 0.11^{a}	4.3 ± 0.17^{a}	4.99 ± 0.11^{a}
G4	368.62 ± 12.72^{bc}	405.75 ± 3.46^{c}	451.92 ± 4.46^{bc}	3.16 ± 0.04^{b}	3.56 ± 0.17^{b}	4.09 ± 0.08^{b}
G5	414.72 ± 6.89^{a}	473.35 ± 16.96^{a}	507.53 ± 10.63^{a}	3.87 ± 0.13^{a}	4.32 ± 0.19^{a}	5.06 ± 0.17^{a}
G6	386.03 ± 10.33^{abc}	425.81 ± 7.52^{bc}	481.1 ± 3.92^{ab}	3.13 ± 0.11^{b}	3.87 ± 0.09^{ab}	4.22 ± 0.15^{b}
G7	358.72 ± 17.25^{bc}	411.05 ± 6.64^{c}	449.72 ± 16.08^{bc}	2.96 ± 0.07^{b}	3.63 ± 0.21^{b}	4.16 ± 0.05^{b}
P-values						
Artemisia	0.001	0.018	0.008	0.37	0.017	0.014
Infection	0.026	0.025	0.015	0.072	0.007	0.001
Interaction	0.001	0.001	0.001	0.001	0.001	0.001

Values expressed as means ± standard error. Within each column, means followed by different letters indicate significant differences at ($P < 0.05$).

TABLE 12. Influence of Dietary Artemisia and Nano-Artemisia Supplementation on the Breast Meat Quality of Japanese Quail Chicks Challenged with *Pseudomonas aeruginosa*

Treatment	Parameters				
	Moisture%	Protein%	Ether extract (EE)%	Ash%	TBARS (nmol of MDA/mg meat)
G1	73.62±0.28	21.16±0.31	1.45±0.18	1.95±0.05	0.81±0.02
G2	73.52±0.48	21.94±0.37	1.69±0.26	2±0.11	0.79±0.05
G3	73.77±0.44	21.99±0.37	1.34±0.15	2.11±0.05	0.67±0.01
G4	74.01±0.85	22.38±0.59	1.47±0.3	2.08±0.09	0.7±0.04
G5	74.48±0.29	22.86±0.19	1.45±0.09	2.12±0.05	0.67±0.05
G6	73.72±0.6	22.79±0.58	1.53±0.37	2.08±0.02	0.75±0.09
G7	73.28±0.35	22.61±0.93	1.71±0.22	1.93±0.08	0.79±0.03
P-values					
Artemisia	0.851	0.111	0.956	0.205	0.272
Infection	0.87	0.354	0.84	0.502	0.677
Interaction	0.22	0.267	0.689	0.414	0.13

Data expressed as mean ± standard error. Within each column, means labeled with different letters are significantly different at (*P <0.05*).

TABLE 13. Impact of Dietary Inclusion of Artemisia and Nano-Artemisia on the Intestinal Morphology of Japanese Quail Chicks Challenged with *Pseudomonas aeruginosa*

Treatments	Villi length (µm)	Villi width (µm)	goblet cells no/mm2
G1	881.97±18.73^{cd}	95.11±7.96^{abc}	351.96±5.29^{d}
G2	445.17±48.19^{f}	112.74±11.91^{ab}	159.96±10.46^{f}
G3	972.89±29.36^{bc}	83.58±5.11^{c}	489.77±6.39^{b}
G4	763.26±8.11^{de}	94.5±4.04 ^{abc}	272.62±6.39^{e}
G5	1221.42±82.26^{a}	83.09±9.68^{c}	667.33±12.42^{a}
G6	1013.99±12.35^{b}	87.45±6.65^{bc}	400.84±14.29^{c}
G7	736.44±22.22^{e}	117.25±11.8 ^{a}	256.83±9.71^{e}
P-values			
Artemisia	0.001	0.23	0.001
Infection	0.002	0.308	0.001
Interaction	0.001	0.118	0.001

Values presented as means ± standard error. Means within the same column that are marked with different letters indicate statistically significant differences at (*P <0.05*).

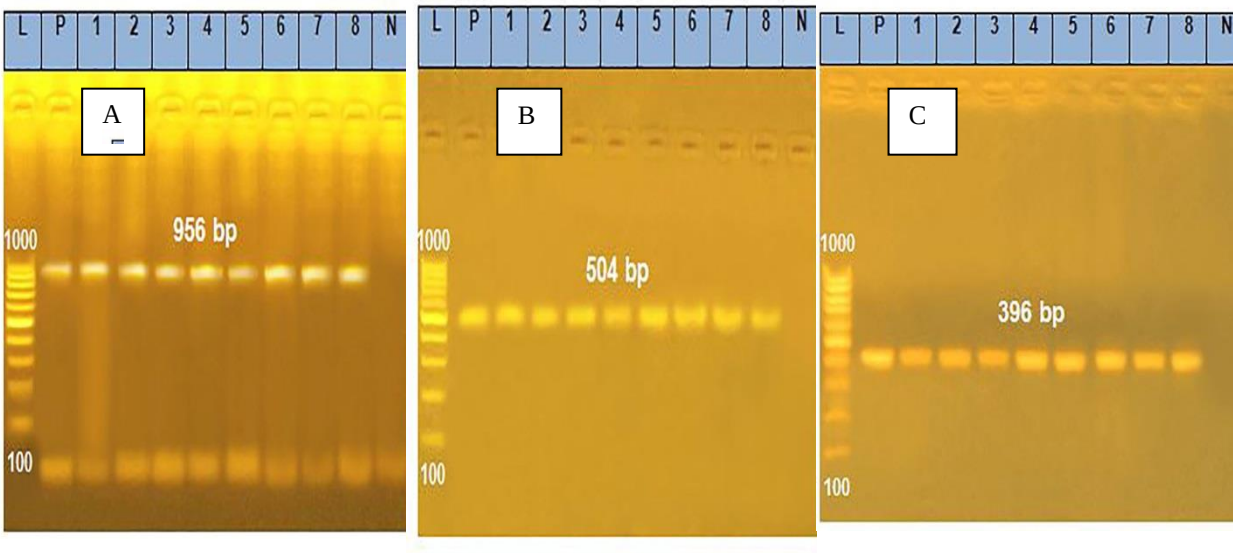

Fig.1. (A) Agarose gel electrophoresis showing PCR products of *Pseudomonas aeruginosa* 16S rDNA. Lane L: DNA ladder (100–1000 bp). Lane Pos: Positive control for *P. aeruginosa* 16S rDNA at 956 bp. Lanes 1 to 8: Samples positive for *P. aeruginosa* 16S rDNA. (B) Agarose gel electrophoresis of PCR products targeting the *oprL* virulence gene for *P. aeruginosa* identification. Lane L: DNA ladder (100–1000 bp). Lane Pos: Positive control for *oprL* gene at 504 bp. Lanes 1 to 8: Samples positive for the *oprL* gene. (C) Agarose gel electrophoresis of PCR products for the *toxA* virulence gene characterization of *P. aeruginosa*. Lane L: DNA ladder (100–1000 bp). Lane Pos: Positive control for *toxA* gene at 396 bp. Lanes 1, 2, 7, and 8: Samples positive for the *toxA* gene.

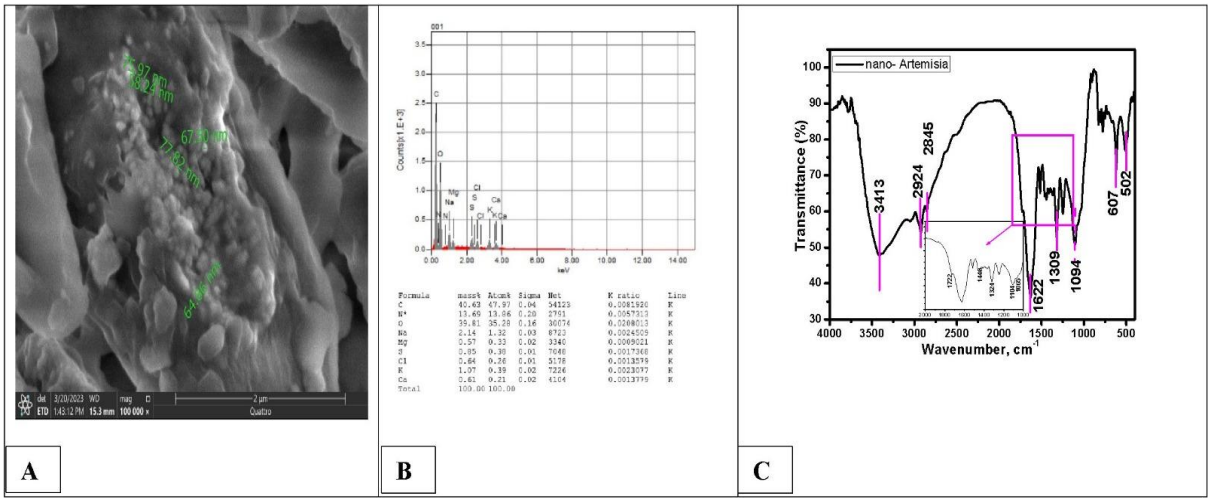

Fig.2. A) The SEM image of the prepared Nano-Artemisia.
B) The EDX pattern of Nano-Artemisia.
C) The FTIR spectrum of Nano Artemisia.

Fig.3. Depression, decreased appetite, loss of body weight (a& b), white watery diarrhea appeared (c) in quails challenged with *pseudomonas aeruginosa*

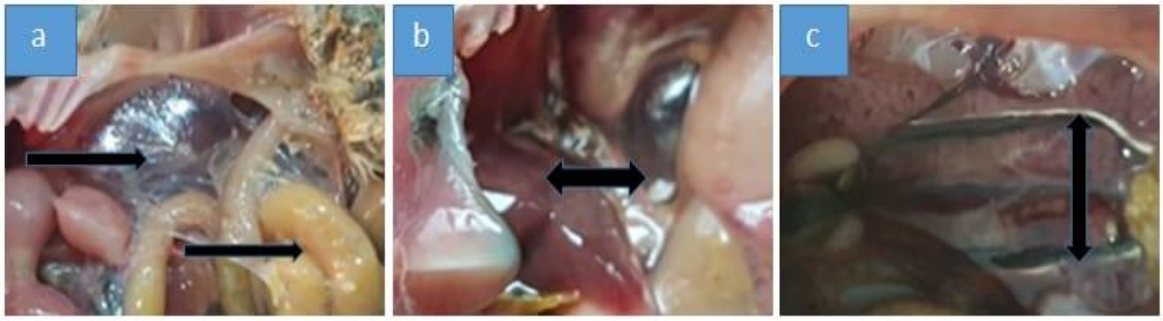

Fig.4. Airsaculitis and gas accumulation (a), congested liver and spleen (b), congested kidney and deposition of urates (c).

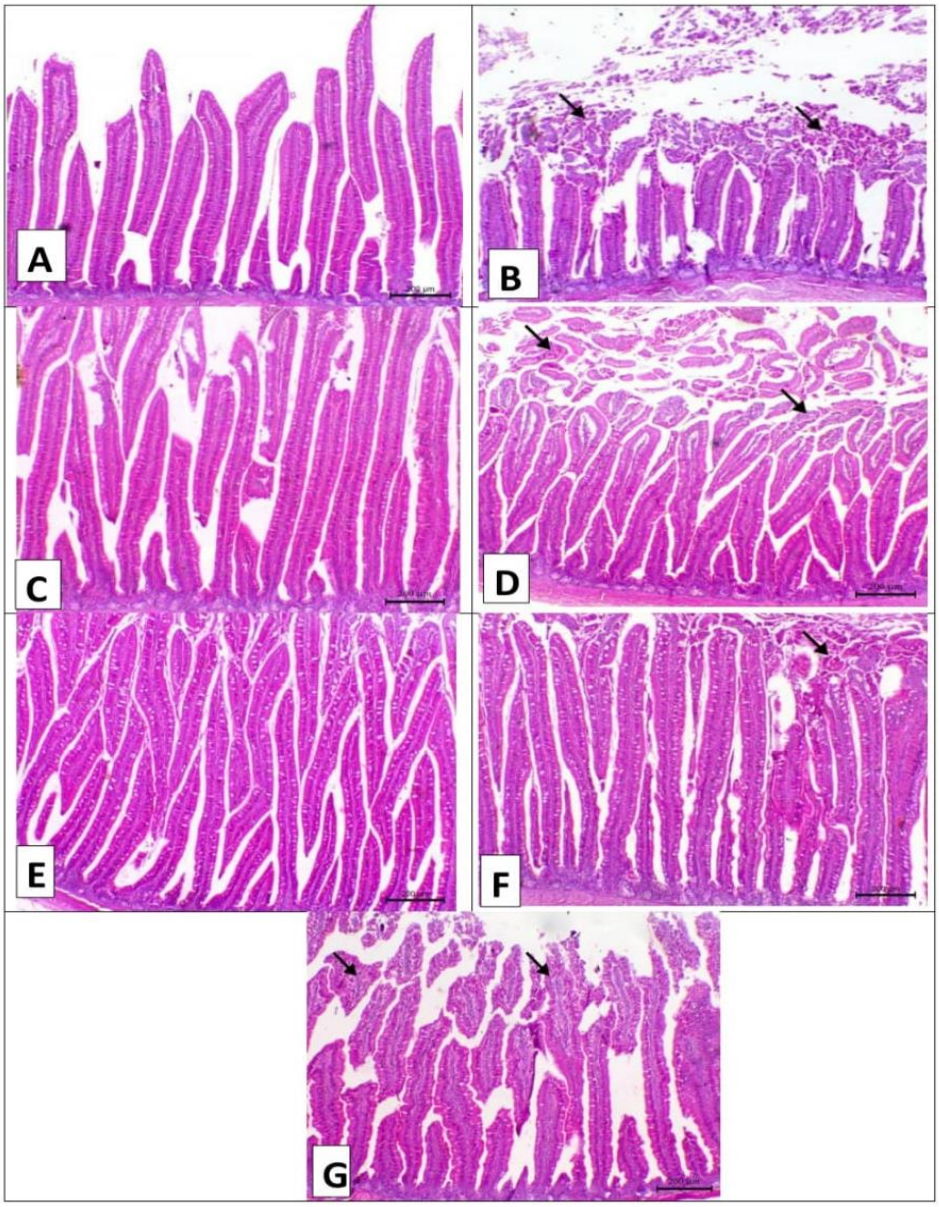

Fig. 5. Jejunal morphology of quail chicks from different experimental groups (H&E staining, X50 magnification, scale bar = 200 µm): (A) Group 1 (G1): Quail chicks displaying normal villus architecture. (B) Group 2 (G2): Presence of necrotic enteritis characterized by shortened villi, extensive mucosal necrosis, and epithelial sloughing (indicated by arrows). (C) Group 3 (G3): Noticeable elongation of the intestinal villi. (D) Group 4 (G4): Necrotic alterations in the upper mucosal layer (arrows) alongside increased villus height. (E) Group 5 (G5): Prominent elongation of villi with an elevated number of goblet cells. (F) Group 6 (G6): Mild and localized necrosis within the mucosal tissue (arrows) with a marked increase in villus height. (G) Group 7 (G7): Reduced signs of necrosis and sloughing (arrows), accompanied by increased villus length.

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تأثيرات الشيح والنانو شيح على النمو، مؤشرات الصحة، والمقاومة لبكتيريا السيدوموناس اريجينوزا في السمان الياباني

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[4] وحدة صحة أغذية معمل كفر الشيخ الاقليمى، معهد بحوث الصحة الحيوانية، مركز البحوث الزراعية، الجيزة، مصر.

[5] معهد علوم و تكنولوجيا النانو، جامعة كفر الشيخ، مصر.

الملخص

كشفت دراسة ميدانية اولية اجريت في محافظة كفر الشيخ ، مصر،عن وجود بكتريا السيدوموناس اريجينوزا بنسبة انتشار بلغت 9.2% بين طيور السمان المرباه فى المزارع المحلية،اظهرت اختبارات الحساسية للمضادات الحيوية حساسية كاملة تجاة الجنتاميسين، وحساسية متوسطة تجاه السبروفلوكساسين ومقاومة كاملة تجاه كلا من انروفلوكساسين و الاريثرومايسين. وقد اكدت عملية التعرف الجزيئى باستخدام تسلسل الحمض النووى الريبوزى هوية المعزولات،بينما أظهر تحليل جينات الضرواة وجود جينى *oprL* و*toxA*

وفى المرحلة التجريبية،تم أستخدام 220 فرخ سمان ياباني عمر يوم وقسمت الطيور عشوائيا الى سبع مجموعات منفصلة ،تضم كل مجموعة 30 طائرا ،لتقييم تأثير نبات الشيح ،والنانو شيح أو السبروفلوكساسين على الأستجابات الفسيولوجية والمناعية بعد تعرض الطيور للعدوى ببكتريا السيدوموناس اريجينوزا. أظهرت النتائج ان الطيور التى تلقت مكملات غذائية تحتوى على 1% شيح أو 0.5% نانوشيح،أو عولجت بالسيبروفلوكساسين قد سجلت تحسنا فى اداء النمو و وأرتفاعا فى مستويات إنزيمات مضادات الاكسدة، بالإضافة إلى انخفاض الاعراض السريرية والافات الباثولوجية.

علاوة على ذالك أدى استخدام 1% شيح او 0.5% نانو شيح فى العلف الى تقليل محتوى الدهن فى عضلات الصدر ، وتعزيز قدرة الأنسجة المضادة للاكسدة، و تحسين تركيب الامعاء النسيجى. وبشكل عام تشير النتائج الى ان ادراج الشيح او النانو شيح بالمستويات المختبرة فى علائق السمان يسهم فى دعم الوظائف المناعية، وتحسين الصحة العامة، وتعزيز النمو فى ظل التحديات البكتيرية.