Original Article

Booster Effects of Oregano (*Origanum syriacum*) on Newcastle Disease Vaccination in Chickens

Omar Bassim Al-Tayyar[1], Nawar Ali Jassim[2], Mohammed Ghassan Saeed[1*]

1. Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.
2. Department of Pathology and Poultry Diseases, College of Veterinary Medicine, Tikrit University, Tikrit, Iraq.

Use your device to scan and read the article online

How to Cite This Article Al-Tayyar, O. B., Jassim, N. J. & Saeed, M. Gh. (2026). Booster Effects of Oregano (*Origanum syriacum*) on Newcastle Disease Vaccination in Chickens. *Iranian Journal of Veterinary Medicine, 20*(2), 289-300. http://dx.doi.org/10.32598/ijvm.20.2.1005807

http://dx.doi.org/10.32598/ijvm.20.2.1005807

ABSTRACT

Background: Newcastle disease (ND) is a major threat to poultry health and production worldwide. While vaccination is widely used, adverse vaccine reactions may impair poultry growth and health. Natural feed additives, such as Oregano (*Origanum syriacum*), have shown immunostimulatory and protective properties.

Objectives: This study aimed to evaluate the immunostimulatory, protective, and growth-supporting effects of oregano supplementation in broiler chickens vaccinated against ND.

Methods: A total of 40 one-day-old broiler chicks were divided into four groups (n=10 each): Negative control, positive control (ND vaccine only), and two experimental groups supplemented with Oregano at 1 g/kg or 2 g/kg feed along with ND vaccination. Immune response was assessed by enzyme-linked immunosorbent assay (ELISA). Body weight was recorded at days 1, 21, and 45. Trachea, proventriculus, intestines, and cecum were collected for histopathological evaluation. Data were analyzed using one-way ANOVA followed by Tukey post hoc test, with F values, degrees of freedom, and P values reported.

Results: The oregano-supplemented groups showed enhanced immune response, with the 1 g/kg group exhibiting significantly higher antibody titers ($P \leq 0.05$) than the vaccine-only group. Body weight was highest in the 2 g/kg group ($F_{3,36}=18.54$; $P<0.001$). Histopathological analysis revealed reduced lesion scores in oregano-treated groups, particularly at 2 g/kg. Semi-quantitative scoring confirmed significant protective effects ($P \leq 0.05$)

Conclusion: Oregano supplementation enhances humoral immunity, reduces ND vaccine-induced tissue damage, and improves growth performance. One g/kg dose primarily boosts antibody response, while 2 g/kg confers superior tissue protection and growth benefits.

Keywords: Antibody response, Growth performance, Immunostimulant, Newcastle disease (ND), Oregano

Article info:
Received: 05 Aug 2025
Accepted: 02 Nov 2025
Publish: 01 Mar 2026

*** Corresponding Author:**
Mohammed Ghassan Saeed, Assistant Professor.
Address: *Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.*
Phone: *+964 (770) 1621798*
E-mail: *mgsaeed@uomosul.edu.iq*

Introduction

Newcastle disease (ND) is an infectious viral disease that affects poultry and is caused by ND virus (NDV) of the family Paramyxoviridae (Masoud et al., 2024). It affects many types of birds, including chickens, and poses a significant threat to the export and import trade (Attia et al., 2022). Newcastle virus causes significant losses to the poultry industry. It has different strains that cause a variety of clinical signs, including respiratory, neurological, and digestive symptoms, which depend on the strain and the bird's immune response (Suarez et al., 2020). This diversity in virus strains requires appropriate measures to prevent infections and reduce their pathological effects (Balmer & Tanner, 2011). Vaccination is often effective in protecting against some diseases. However, the vaccines could induce health issues, as ND vaccination techniques can cause strong immune responses and may lead to pathological changes in tissues that resemble clinical signs of the disease itself (Toka & Geinoro, 2023).

ND vaccines include live attenuated (e.g. LaSota, Clone 30), inactivated, and vector-based vaccines (e.g. HVT-ND). Most studies have focused on viral pathogenesis rather than on nutritional modulation of vaccine responses.

In recent years, the focus has been on natural immunostimulants and feed additives as possible answers to these issues. Some of these are *Origanum syriacum*, commonly known as oregano, which is an herbal medicine that contains bioactive compounds such as carvacrol and thymol with antimicrobial and antioxidant activities (Amerah & Ouwehand, 2016). Many studies have reported the antiviral activity of oregano essential oil and aqueous and ethanolic extracts of oregano (Vimalanathan & Hudson, 2012; Deng et al., 2024) against human and animal viruses. Lelešius et al. (2019) studied the antiviral activity of oregano extract on avian infectious bronchitis virus in Vero cells. The mechanisms of action of oregano components consist of up-regulation of cytokines, and inhibition of virus-induced apoptosis in cultured cells (Deng et al., 2024). Besides reducing the viral load (Lelešius et al., 2019; Picoli et al., 2021).

The phytochemicals present in oregano have anti-inflammatory properties, which increase the body's ability to fight diseases, promote gut health, and provide protection against oxidative stress, making oregano a potentially useful source for use in poultry production (Deng et al., 2024). Ruan et al. (2021) reported that oregano supplementation has the ability to improve growth performance, regulate feed microbial conversion profile efficiency, and modulate gut immunity, inflammation, and immune response.

This study aims to analyze how using oregano dried plant as a supplement in the diet can help reduce the effects of the ND vaccine in poultry while also looking into its effects on immune system function and bird growth rates as well as histopathological changes, in birds. The research endeavors to offer a natural option that can boost poultry health and enhance vaccine effectiveness to support sustainable poultry farming practices.

Materials and Methods

Experimental animal

One-day-old Rose chicks *Gallus domesticus* were obtained at the start of the study. Forty chicks were randomly divided into 4 groups of 10 chick. The chickens used were commercial broilers, the purpose was to mimic the chickens in the field.

The sample size was limited to 10 birds per group, which was based on previous poultry immunomodulatory studies and institutional ethical constraints. However, we acknowledge that a larger cohort would enhance statistical power and generalizability.

The chicks were vaccinated intra-ocularly and intranasally at 8 days of age, to pass the remaining maternal immunity, with live ND vaccine (MSD), with a booster dose administered 14 days later, except the negative control group. Oregano was administered in the feed 3 days before and 3 days after each vaccination.

Oregano plant

The oregano plant *O. syriacum* was obtained locally as dried plant, it was grounded and then mixed with the feed at a rate of 1 and 2 g/kg of feed.

The oregano powder was mixed thoroughly with small quantity of feed then it was mixed with larger amount of feed at inclusion rates of 1 g/kg and 2 g/kg of the basal diet and administered orally throughout the experimental period.

Although the oregano used was locally sourced, future studies should include detailed phytochemical profiling to quantify major constituents such as carvacrol and thymol, as their concentrations can significantly influence biological outcomes.

Sample collection

Blood samples were collected from all groups on days 15, 22, 30, 37, and 42. The collected serum from the blood was transferred into labeled vials and the vials were stored at -20 ˚C until further processing. Post-mortem samples of the trachea, proventriculus, intestines, and cecum were collected at 37 days of the study for histopathological analysis. Sampling was performed on day 37 to allow sufficient time post-vaccination (14 days after booster) for peak antibody response and tissue recovery to be assessed.

Parameters assessed

Immune response: Antibody titers were evaluated using the enzyme-linked immunosorbent assay (ELISA) assay to measure the antibody titers against the ND vaccine in the broiler chickens. This quantitative test relies on enzyme-linked immunosorbent detection, where specific antibodies in the chicken serum bind to ND antigens coated on the microplate wells. A secondary enzyme-conjugated antibody reacts with the bound primary antibodies, producing a colorimetric change upon substrate addition. The intensity of the color, measured as optical density (OD) at a specific wavelength using a microplate reader, correlates with the antibody concentration. The ELISA kit provides a sensitive and reliable method for monitoring the immune response to ND vaccination. The ELISA kit used in this study was the ID Screen® ND Indirect ELISA kit (manufactured by IDvet, France).

The immune response was assessed using ELISA assay for antibody titers. However, cell-mediated immunity parameters, such as splenic lymphocyte proliferation or cytokine gene expression were not assessed and represent a limitation.

Histopathological changes: organ samples were examined for signs of inflammation, tissue damage, or protective effects of oregano. Histological tissues were fixed in the 10% neutral buffered formalin until the histological preparation was done. Following the routine procedures, 5-mm thick paraffin sectioning of organs were sectioned then stained with hematoxylin and eosin. Using a light microscope, the sections were investigated for pathological changes then photographed by a microscope camera (USB 2.0 digital image camera (Omax ToupView 9.0-Megapixel China).

Growth performance: Body weight gain was recorded at day 1, 21, and 45 of the study to assess the impact of oregano at two different doses on chicken growth and its protective effect against vaccination.

Histopathological scoring criteria

Scores were assigned manually based on microscopic examination. Histological sections of the trachea, proventriculus, intestines, and cecum were evaluated semi-quantitatively by two independent veterinary pathologists who were blinded to group assignments. A standardized lesion scoring system was used to grade the severity of histopathological changes as follows: 0=normal (no lesion), 1=mild (slight inflammatory infiltration or epithelial degeneration), 2=moderate (moderate mucosal disruption, cellular degeneration, or vascular congestion), 3=severe (extensive necrosis, severe inflammatory infiltration, or loss of tissue architecture). The final scores were averaged for each group and tissue and are presented as Mean±SD. This method ensures a consistent and comparative assessment of tissue damage across treatment groups.

Statistical analysis

The statistical analysis for this study was conducted using a one-way ANOVA to compare the means of different treatment groups at various time points. The data are presented as Mean±SD, with significance levels set at $P \leq 0.05$. Post hoc comparisons were performed using Tukey test to identify significant differences between groups.

For the semi-quantitative histopathological data (scored manually by blinded observers), values are expressed as Mean±SD. Group comparisons were analyzed using one-way ANOVA followed by Tukey post hoc test. A $P \leq 0.05$ was considered statistically significant. This approach allowed objective comparison of lesion severity among experimental groups.

Results

Immune response

At day 15, antibody titers differed significantly between groups ($F_{3, 36}=6.72$, P=0.001). The oregano 1 g/kg group (1676.6±274.4) and positive control (1528±319.9) exhibited significantly higher titers compared to the negative control (1072.4±241.3). At day 22, the oregano 1 g/kg group had the highest antibody response (2509.6±496.9), significantly greater than both the 2 g/kg group and the positive control ($F_{3, 36}=9.11$, P<0.001) (Table 1).

Table 1. The ELISA titers of the ND vaccine antibodies of the broiler chicken groups at day 15, 22, 30, 37, and 42 of the study

Group	Mean±SD				
	Day 15	Day 22	Day 30	Day 37	Day 42
Negative control	1072.4±241.3^{C}	1203.3±115.8^{C}	1244±173.4^{B}	1296±238^{C}	1355.1±349.7^{D}
Positive control	1528±319.9^{AB}	2135.6±182.9^{B}	3008.2±477.1^{A}	3285.2±363.5^{B}	4171±965.3^{B}
Oregano 1 g\kg treatment	1676.6±274.4^{A}	2509.6±496.9^{A}	2969±541.5^{A}	3770±534.4^{A}	4764.9±463^{A}
Oregano 2 g\kg treatment	1493.8±296.2^{B}	2188±417.8^{B}	2771.2±287.9^{A}	3031.6± 643.8^{B}	3310±858.5^{C}

Note: The different letters mean there are significant differences among groups at P≤0.05, (chickens=5).

Body weight analysis

No initial differences were shown among groups at day 1. At day 45, significant differences were observed ($F_{3, 36}$=18.54, P<0.001). The oregano 2 g/kg group recorded the highest body weight (3386±74.44 g), significantly greater than the vaccine-only group (2880±200.34 g). Tukey's test confirmed that both oregano-supplemented groups had significantly higher weights compared to the positive control.

Histopathological scores

Demonstrated clear differences (F values ranging from 22.41 to 35.78 across organs, all P<0.001). Oregano-treated groups exhibited significantly lower lesion scores compared to the vaccine-only group, with the 2 g/kg group approaching near-normal values.

Body weights

Day 1: No significant differences were observed among the groups. The initial body weights were comparable across all groups, indicating uniformity in starting conditions.

Day 21: There were no statistically significant differences in body weights among the groups. The group treated with 1 mg/kg oregano (368.6±19.96) showed the highest weight, while the vaccinated-only group had the lowest (316±9.46).

Day 45: Significant differences were observed in body weights. The highest body weight was recorded in the group treated with 2 mg/kg oregano (3386±74.44), followed closely by the negative control group (3369.4±71.99) and the 1 mg/kg oregano group (3298.6±40.13). The vaccinated-only group had the lowest body weight (2880±200.34), which was significantly lower than the other groups (Table 2).

Histological sections of chicken trachea

Histopathological examination focused on epithelial integrity, inflammatory infiltration, goblet cell status, and submucosal (SM) changes.

Control group revealed normal architecture of mucosa (M), SM and cartilage (C). Whereas ND vaccine treated group showed necrosis of the epithelial cells lining M, infiltration of inflammatory cells, and degeneration and necrosis of the chondrocytes in the cartilages. Oregano 1% and ND vaccine treated groups demonstrated intact

Table 2. Body weights of the broiler chicken groups at day 1, 21 and 45 of the study

Group	Mean±SD		
	Day 1	Day 21	Day 45
Negative control	44.2±1.15^{A}	351.2±5.11^{A}	3369.4±71.99^{A}
Positive control	43.4±0.92^{A}	316±9.46^{A}	2880±200.34^{B}
Oregano 1 g\kg treatment	43.4±0.87^{A}	368.6±19.96^{A}	3298.6±40.13^{A}
Oregano 2 g\kg treatment	43.4±0.92^{A}	334.6±18.14^{A}	3386±74.44^{A}

Note: The different letters mean there are significant differences among groups at P≤0.05 (chickens=5).

Table 3. Semi-quantitative histopathological scoring of trachea, proventriculus, intestine, and cecum across treatment groups (n=5)

Tissue	Mean±SD			
	Group A (Negative Control)	Group B (Positive Control)	Group C (Oregano 1 g/kg)	Group D (Oregano 2 g/kg)
Trachea	0±0	2.8±0.4	1.2±0.3	0.4±0.2
Proventriculus	0±0	2.6±0.5	1.4±0.3	0.6±0.3
Intestine	0±0	2.7±0.3	1.1±0.4	0.5±0.2
Cecum	0±0	2.5±0.4	1±0.3	0.3±0.1

Note: Scoring system: 0=normal; 1=mild changes; 2=moderate changes; 3=severe changes. Statistical significance evaluated using ANOVA followed by Tukey test. Values with different superscripts within rows differ significantly at P≤0.05.

M, and SM with mild degeneration of the chondrocytes in the cartilages. Oregano 2% and ND vaccine treated groups revealed intact M, SM and C (Figure 1).

Histological sections of chicken proventriculus

Control group showed normal architecture of M, SM, and gastric glands (GGs). ND vaccine treated group recorded necrosis of the epithelial cells lining M, infiltration of inflammatory cells, and necrosis of the GGs. In oregano 1 g/kg and ND vaccine-treated groups there were disorganized M with degeneration and infiltration of inflammatory cells. Also in oregano 2 g\kg and ND vaccine treated groups they showed intact M with mild degeneration, intact SM, and GGs (Figure 2).

Histological sections of chicken intestine

In the control group, there was normal architecture of M, SM, and intestinal glands (iG). In the ND vaccine-treated group, disorganized villi and necrosis of the epithelial cells lining M, infiltration of inflammatory cells, necrosis of iG, and blood vessels congestion in muscularis (Ms). Oregano 1 g\kg and ND vaccine-treated groups showed intact M, SM, and iG. In oregano 2 g\kg and ND vaccine-treated group, intact M with mild degeneration, intact SM, and iG (Figure 3).

Histological sections of chicken cecum

Control group: Normal architecture of M, SM, and Ms. ND vaccine treated group appears shortening of M, edema in the SM, and Ms. In oregano 1 g\kg and ND vaccine-treated group there were degeneration in M, intact SM, and edema in the Ms. In oregano 2 g\kg and ND vaccine-treated groups there were recorded intact M, SM, and Ms (Figure 4).

To provide a more objective and comparative assessment, semi-quantitative scoring of histopathological lesions was conducted for all tissues and is summarized in Table 3.

Discussion

The study demonstrates the potential benefits of oregano supplementation on immune response, organs protection, and growth performance in chickens, particularly when used alongside the ND vaccine. The results highlight the synergistic effect of oregano as an immunomodulator and its protective properties against vaccine-induced organs damage. The results also showed that oregano is effective in supporting the immune system when taken as a dietary supplement and enhanced the antibody levels in the group supplemented with 1% oregano more than the groups that did not receive the supplement or vaccinated only.

The oregano 2 g/kg group showed a significant result for antibody production since the early period after vaccination till the end of the experiment, especially on days 15, 22, 37, and 42 which proves its high immunostimulatory potential at this particular dose. The antibody levels in the vaccinated positive control group were also well developed but the levels were lower as compared to the group that was vaccinated along with 1% oregano supplement.

O. syriacum was selected due to its high content of bioactive compounds such as carvacrol and thymol, which exhibit known antimicrobial and immunomodulatory effects.

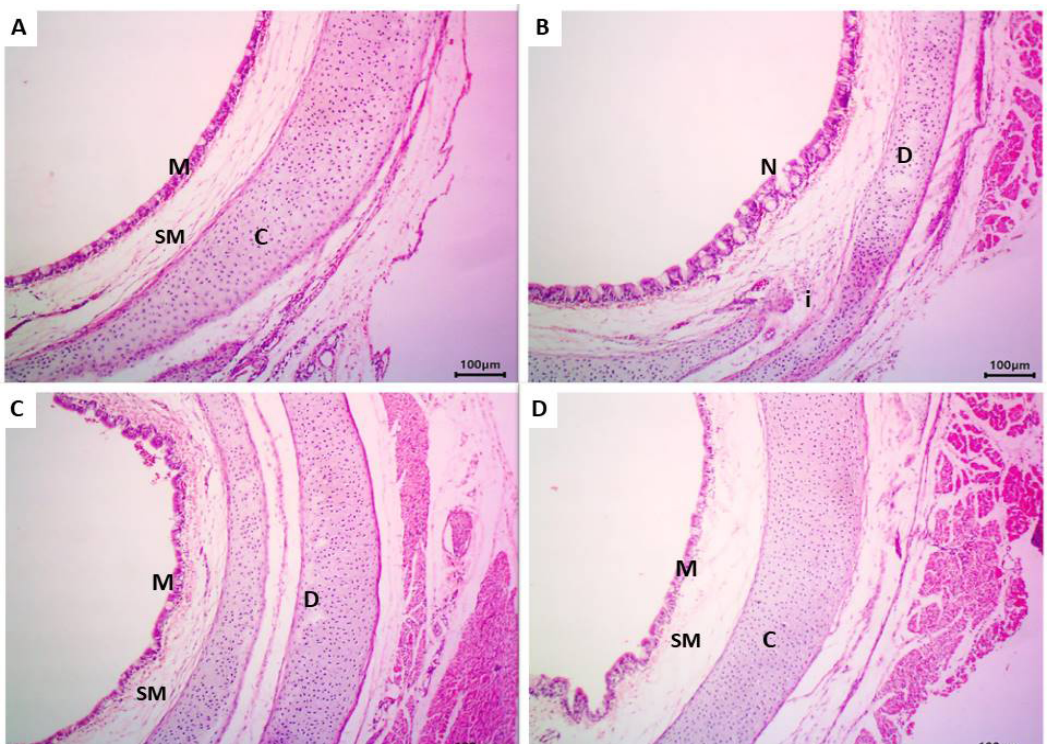

Figure 1. Histological sections of chicken trachea

Abbreviations: M: Mocus, SM; Submocusal; C: Cartilages.

Note: A) Control (oregano-treated) group: Normal architecture of M, SM, and C; B) ND vaccine-treated group: Necrosis of the epithelial cells lining mucosa (N), infiltration of inflammatory cells (i), and degeneration and necrosis of the chondrocytes in the Cs (D); C) Oregano 1% and ND vaccine-treated group: Intact M, and SM with mild degeneration of the chondrocytes in the Cs (D); D) Oregano 2% and ND vaccine-treated group: Intact M, SM and C. H&E stain, ×100. Scale-bar=100 µm.

The improvement in antibody titers in the 1% oregano-supplemented group supports the claim that oregano enhances immunity. The immunomodulatory effects of oregano may be due to the presence of bioactive compounds such as carvacrol and thymol, which are known to stimulate lymphocyte proliferation and increase antibody production (Gholami-Ahangaran et al., 2022). The results obtained in this study are in accordance with previous studies that pointed out that oregano and its phytochemicals have immunostimulatory effects (Mohamed et al., 2023).

Interestingly, the antibody levels in the group supplemented with 2% oregano at the end of the study were significantly less than the 1% oregano group and the positive control group, which could indicate that oregano at this dose gains its antiviral properties where it could reduce the vaccine-viral load. This is in agreement with Lelešius et al. (2019), Zhang et al. (2022), Deng et al. (2024), who demonstrated that oregano oil at certain doses reduced and eliminated the avian infectious bronchitis viral load in vitro.

This result was supported by the histopathological results, as oregano at a 2% dose protected the organs more efficiently than the 1% dose, in addition to the body weight results of this group which had the best body weight.

The 2 g/kg group may have exerted anti-inflammatory and antioxidant effects that suppressed local tissue damage, independent of humoral antibody levels, which were relatively lower.

The present findings demonstrate that oregano supplementation has a positive impact on body weight and protects the birds from the weight loss resulting from vaccination. The positive control group, which was vaccinated twice with ND live vaccine and did not receive oregano, had a significantly lower body weight than the vaccinated oregano-supplemented groups (1 g/kg and 2 g/kg) and the negative control group.

The observed decrease in antibody titers at 2 g/kg may indicate a dose-dependent antiviral suppression. Future investigations should include viral quantification and cy-

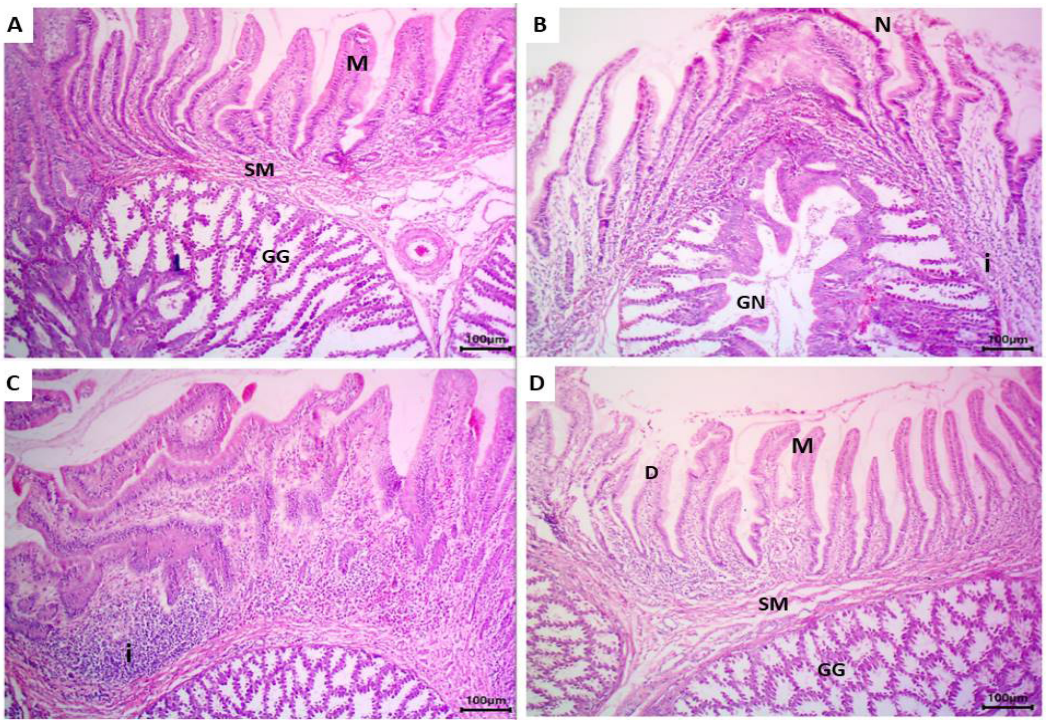

Figure 2. Histological sections of chicken proventriculus

Abbreviations: M: Mocus, SM; Submocusal; C: Cartilages; GG Gastric glands; I: Inflammatory cells; D: degeneration.

Note: A) Control (oregano-treated) group: Normal architecture of M, SM and GG; B) ND vaccine-treated group: Necrosis of the epithelial cells lining mucosa (N), infiltration of i, and necrosis of the GG (GG); C) Oregano 1% and ND vaccine-treated group: Disorganized of mucosa with D, and infiltration of i; D) Oregano 2% and ND vaccine treated group: Intact M, with mild D, intact SM and GG. H&E stain, ×100. Scale-bar=100 µm.

tokine profiling to confirm this hypothesis and delineate the underlying mechanisms.

The results suggest that oregano helped reduce possible stress or any adverse impact of vaccination on the birds, thereby enhancing growth. However, this interpretation requires further validation using cytokine profiling and viral quantification.

There were no significant differences in body weight at day 21; instead, the positive control group had the lower weight, while weight increased over time in the oregano-supplemented groups. The negative control group showed similar results to the groups given oregano, as oregano improved the body's ability as a growth-promoting factor in chickens. The analysis of body weight gain in oregano-treated groups reveals that the herb has a positive impact on the growth rate of the birds, which can be attributed to its antimicrobial and digestive properties (Inala & Ikpesu, 2022). Oregano has the potential to regulate the gut microbiota and enhance the absorption of nutrients; hence, it has potential to improve feed conversion ratio and growth rates (Jia et al., 2022). This effect may be useful in reducing the growth inhibition that is sometimes seen following vaccination and the associated stress.

The findings of this study have great significance in poultry farming. Oregano supplementation is therefore advantageous in that it enhances immune response and protects the organs that are vital to the animal's health and performance. Oregano is a natural animal growth stimulant that can be used in the production of poultry antibiotics (Patience & Ramirez, 2022).

To the best of our knowledge, there is a scarcity of studies concerning the use of dried oregano powder in relation to ND vaccination, to compare. Previous studies focus on oregano oil or oregano extract using different doses with different results (Galal et al., 2016; El-Shall et al., 2020; Abdulkadhim et al., 2022).

However, this study agrees with previous studies that showed oregano supplementation as essential oil (Abd Al Haleem et al., 2020) or aqueous extract (Zhang et al., 2021) increased daily weight gain, growth performance, and improved feed conversion rates in poultry. This was attributed to the enhancement of the microbial balance

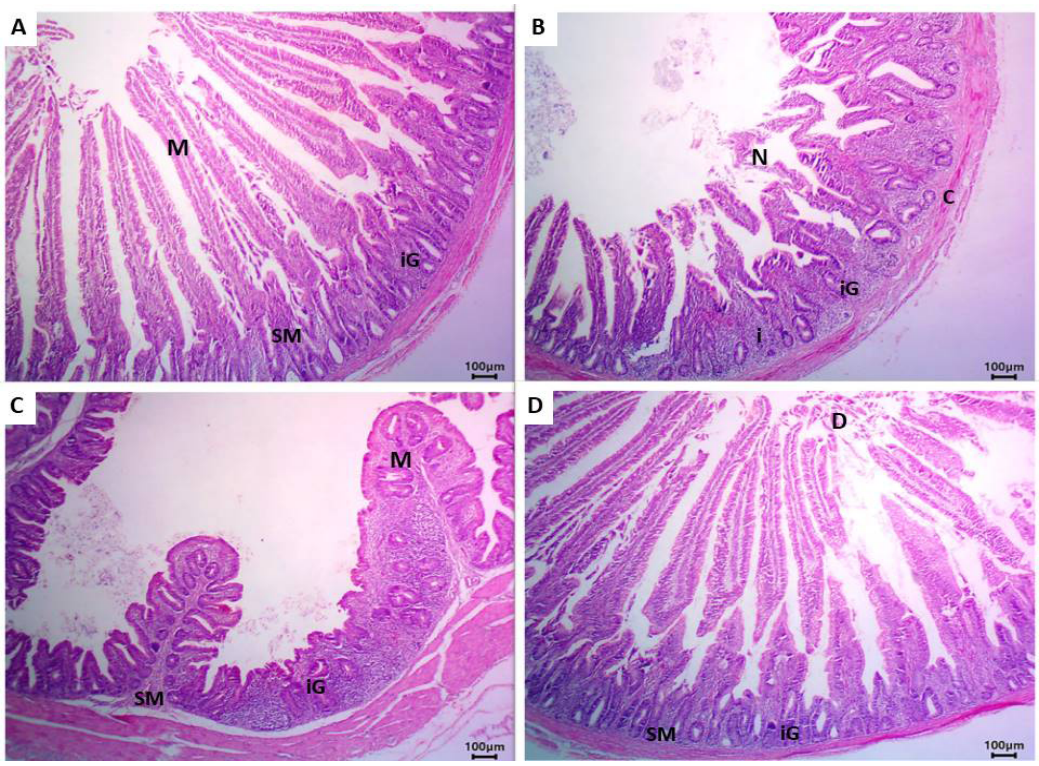

Figure 3. Histological sections of chicken intestine

Abbreviations: M: Mocus, SM; Submocusal; Ms: Muscularis: iG: Intestinal glands; cells; GN: Gastric glands; D: Degeneration.

Note: A) Control (oregano-treated) group: Normal architecture of M, SM and iG; B) ND vaccine-treated group: Disorganized of villi and necrosis of the epithelial cells lining mucosa (N), infiltration of inflammatory cells (i), necrosis of and intestinal glands (D), muscularis (C); C) Oregano 1% and ND vaccine-treated group: Intact M, and SM and iG; D) Oregano 2% and ND vaccine-treated group: Intact M with mild D, and intact SM and iG. H&E stain, ×100. Scale-bar=100 µm.

in the gut, maintaining intestinal health, leading to improved digestion and secretion of digestive enzymes. However, this study is not consistent with another study that showed no significant differences in growth performance between the oregano powder-supplemented group at a dose of 150 mg/kg diet and the control group (Ri et al., 2017).

Adding oregano to feed enhances the body's antioxidant activity, such as increasing glutathione peroxidase (GPx) levels, and improves immunity by enhancing the production of antibodies (IgA) and the gene expression of immune elements in the gut.

This act reduces the risk of inflammation and strengthens the intestinal barrier (Zhang et al., 2021). In addition, Oregano has shown strong properties against harmful bacteria such as *E. coli* and *Salmonella*, making it a natural option to reduce reliance on conventional antibiotics (Balta et al., 2021; Ijeh et al., 2024), thus reducing the bacterial impact on birds.

While the discussion references studies evaluating IgA and antioxidant enzymes, these markers were not directly measured in the present study, and conclusions remain inferential.

The histopathological findings show the protective effects of oregano against ND vaccine-induced organ lesions. Group 5 almost had smooth and intact M and SM of the proventriculus, intestines, and cecum with minimal degeneration. This finding indicates that oregano extract's anti-inflammatory and antioxidant properties help in reducing tissue damage due to vaccination-induced immune response (Badi et al., 2023; Montin et al., 2024). The decreased infiltration of inflammatory cells and the integrity of gastric and iG also support its protective effect. These findings are in accordance with research that has demonstrated that Oregano's phenolic compounds are free radical absorbers, reducing oxidative stress that may lead to inflammation and tissue injury (Bautista-Hernández et al., 2021). Oregano elements, carvacrol and thymol, have antioxidant, antimicrobial, and anti-inflammatory characteristics that lower

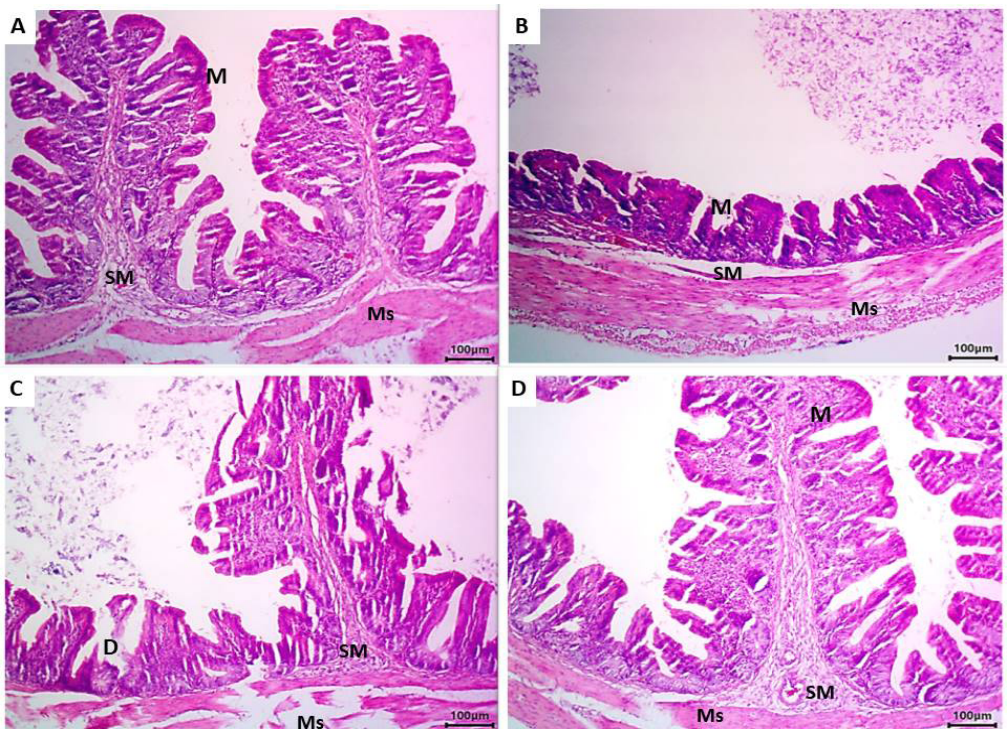

Figure 4. Histological sections of chicken cecum

Abbreviations: M: Mocus, SM; Submocusal; Ms: Muscularis.

Note: A) Control (oregano-treated) group: Normal architecture of M, SM and Ms; B) ND vaccine-treated group: Shortening of M, edema in the SM and Ms; C) Oregano 1% and ND vaccine-treated group: Degeneration in M, intact SM and edema in the Ms; D) Oregano 2% and ND vaccine-treated group: Intact M, SM and Ms. H&E stain, ×100. Scale-bar=100 µm.

the chances of post-vaccination issues like tissue inflammation or hindered growth rates (Mesmar et al., 2022; Mithuna et al., 2024).

The semi-quantitative histopathological scoring confirmed the protective effects of oregano, especially at the 2 g/kg dose. Compared to the positive control group, which exhibited severe lesions in all examined tissues, both oregano-supplemented groups showed significantly reduced histopathological scores, with the 2 g/kg group approaching near-normal values. These findings align with previous reports on the anti-inflammatory and antioxidant roles of oregano bioactive compounds (Gholami-Ahangaran et al., 2022; Bautista-Hernández et al., 2021), and reinforce its tissue-protective efficacy when used as a dietary supplement during ND vaccination.

To fully elucidate the immunomodulatory and antioxidant effects of oregano, subsequent studies should include cytokine assays and oxidative stress markers, such as GPx and malondialdehyde (MDA) levels.

The specific ways that oregano exerts its immunomodulatory and protective effects were not clearly explained. It is, therefore, suggested that more research be conducted to establish the effects of oregano supplementation in the future and also to other poultry diseases. It would also be useful to assess its economic impact when used in large scale poultry production.

Despite the observed improvements in body weight, feed intake and feed conversion ratio (FCR) were not recorded. These parameters should be prioritized in future research to determine the economic feasibility of oregano supplementation.

Future research should explore cellular-mediated immune responses and compare oregano administration methods (e.g. aqueous extract vs feed additive) to optimize its application.

Conclusion

The current findings reveal a dose-dependent, functionally distinct effect of *O. syriacum*. Supplementation at 1 g/kg enhanced humoral immunity, evidenced by higher antibody titers, whereas 2 g/kg conferred superior histological protection and growth performance. This

finding suggests that the higher dose may act via anti-inflammatory or tissue-preserving pathways independent of antibody levels. However, the lack of cellular immunity markers limits mechanistic insight. Further studies integrating cytokine profiling and splenic immune assessment are needed to optimize oregano's role in ND vaccination programs.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Animal Care and the Ethics Committee in the College of Veterinary Medicine, University of Mosul, Mosul, Iraq (Code: UM.VET. 2022.087P).

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Conceptualization, investigation, validation and final approval: All authors; Methodology, formal analysis, software, visualization, and writing: Mohammed Ghassan Saeed; Supervision, resources, project administration, and funding acquisition: Omar Bassim Al-Tayyar.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The researchers are grateful to the College of Veterinary Medicine, University of Mosul, Mosul, Iraq, and everyone who contributed to the completion of this study.

References

AbdAlHaleem, H.A., Elsayed, O.A., Fadl, A., & AbdEltoab, F. (2020). Effect of dietary oregano supplementation on productive, physiological and immunological performance of broiler chicks. *Egyptian Poultry Science Journal, 40*(2), 507-524. [DOI:10.21608/epsj.2020.96098]

Abdulkadhim, A. A., Mohammed Dughaim, F., & Ibrahim Ahmed, A. (2022). Effect of oregano essential oil combined with live and killed Newcastle disease vaccines on immune response in broilers chicks in Erbil, Iraq: A comparative study. *Archives of Razi Institute, 77*(3), 1303–1309. [DOI:10.22092/ARI.2022.357614.2067] [PMID]

Amerah, A. M., & Ouwehand, A. C. (2016). Use of essential oils in poultry production. In V. R. Preedy(ED.), *Essential Oils in Food Preservation, Flavor and Safety* (pp. 101-110). Washington, DC: Academic Press. [DOI:10.1016/B978-0-12-416641-7.00010-9]

Attia, Y. A., Rahman, M. T., Hossain, M. J., Basiouni, S., Khafaga, A. F., & Shehata, A. A., et al. (2022). Poultry production and sustainability in developing countries under the COVID-19 crisis: Lessons learned. *Animals : An open access Journal from MDPI, 12*(5), 644. [DOI:10.3390/ani12050644] [PMID]

Badi, F. A., Al-Kubati, A. A. G., & Al-Gabri, N. (2023). Promising immunomodulation potential of Ganoderma lucidium and Oregano as mixture on broiler chicken. *Advancements in Animal and Veterinary Sciences, 11*(12), 1968-1977. [DOI:10.17582/journal.aavs/2023/11.12.1968.1977]

Balmer, O., & Tanner, M. (2011). Prevalence and implications of multiple-strain infections. *The Lancet. Infectious Diseases, 11*(11), 868–878. [DOI:10.1016/S1473-3099(11)70241-9] [PMID]

Balta, I., Linton, M., Pinkerton, L., Kelly, C., Stef, L. & Pet, I., et al. (2021). The effect of natural antimicrobials against Campylobacter spp. and its similarities to Salmonella spp., Listeria spp., Escherichia coli, Vibrio spp., Clostridium spp. and Staphylococcus spp. *Food Control, 121*, 107745. [DOI:10.1016/j.foodcont.2020.107745]

Bautista-Hernández, I., Aguilar, C. N., Martínez-Ávila, G. C. G., Torres-León, C., Ilina, A., & Flores-Gallegos, A. C., et al. (2021). Mexican Oregano (Lippia graveolens Kunth) as Source of Bioactive Compounds: A Review. *Molecules (Basel, Switzerland), 26*(17), 5156. [DOI:10.3390/molecules26175156] [PMID]

Deng, H., Deng, Y., Song, T., Pang, L., Zhu, S., & Ren, Z., et al. (2024). Evaluation of the activity and mechanisms of oregano essential oil against PRV in vivo and in vitro. *Microbial Pathogenesis, 194*, 106791. [DOI:10.1016/j.micpath.2024.106791] [PMID]

El-Shall, N. A., Shewita, R. S., Abd El-Hack, M. E., AlKahtane, A., Alarifi, S., & Alkahtani, S., et al. (2020). Effect of essential oils on the immune response to some viral vaccines in broiler chickens, with special reference to Newcastle disease virus. *Poultry Science, 99*(6), 2944–2954. [DOI:10.1016/j.psj.2020.03.008] [PMID]

Galal, A. A. A. E., El-Araby, I. E., Hassanin, O., & Omar, A. E. (2016). Positive impact of Oregano essential oil on growth performance, humoral immune responses and chicken interferon alpha signalling pathway in broilers. *Advances in Animal and Veterinary Sciences, 4*(1), 57-65. [DOI:10.14737/journal.aavs/2016/4.1.57.65]

Gholami-Ahangaran, M., Ahmadi-Dastgerdi, A., Azizi, S., Basiratpour, A., Zokaei, M., & Derakhshan, M. (2022). Thymol and carvacrol supplementation in poultry health and performance. *Veterinary Medicine and Science, 8*(1), 267-288. [DOI:10.1002/vms3.663] [PMID]

Ijeh, S., Okolo, C. A., Arowoogun, J. O., Adeniyi, A. O. & Omotayo, O. (2024). Predictive modeling for disease outbreaks: a review of data sources and accuracy. *International Medical Science Research Journal, 4*(4), 406-419. [DOI:10.51594/imsrj.v4i4.999]

Inala, E. R., & Ikpesu, T. O. (2022). Assessment of the therapeutic potential of the synergy of solenostemous monostachyus and vernonia amygdalina in fish induced with oxide of calcium. *Nigerian Journal of Pharmaceutical and Applied Science Research, 11*(3). [DOI:10.60787/nijophasr-v11-i3-491]

Jia, L., Wu, J., Lei, Y., Kong, F., Zhang, R., & Sun, J., et al. (2022). Oregano essential oils mediated intestinal microbiota and metabolites and improved growth performance and intestinal barrier function in sheep. *Frontiers in Immunology, 13,* 908015. [DOI:10.3389/fimmu.2022.908015] [PMID]

Lelešius, R., Karpovaitė, A., Mickienė, R., Drevinskas, T., Tiso, N., & Ragažinskienė, O., et al. (2019). In vitro antiviral activity of fifteen plant extracts against avian infectious bronchitis virus. *BMC Veterinary Research, 15*(1), 178. [DOI:10.1186/s12917-019-1925-6] [PMID]

Mithuna, R., Tharanyalakshmi, R., Jain, I., Singhal, S., Sikarwar, D., & Das, S., et al. (2024). Emergence of antibiotic resistance due to the excessive use of antibiotics in medicines and feed additives: a global scenario with emphasis on the Indian perspective. *Emerging Contaminants, 10*(4), 100389. [DOI:10.1016/j.emcon.2024.100389]

Masoud, F., Ashraf, M. A., Usmani, M. W., Rafique, A., & Aslam, R. (2024). Newcastle Disease Virus as a Viral Vector Platform for Poultry Vaccines: A Review. *Pakistan Veterinary Journal, 44*(3), 539-546. [Link]

Mesmar, J., Abdallah, R., Badran, A., Maresca, M., & Baydoun, E. (2022). Origanum syriacum phytochemistry and pharmacological properties: A comprehensive review. *Molecules (Basel, Switzerland), 27*(13), 4272. [DOI:10.3390/molecules27134272] [PMID]

Mohamed, M. A., El-Mleeh, A. A., Hamad, R. T., Abu-Alya, I. S., El-Hewaity, M. H., & Elbestawy, A. R., et al. (2023). Immunostimulant potential of Moringa Oleifera leaves alcoholic extract versus Oregano Essential Oil (OEO) against cyclophosphamide-induced immunosuppression in broilers chicks. *Tropical Animal Health and Production, 55*(3), 209. [DOI:10.1007/s11250-023-03620-5] [PMID]

Montin, D., Santilli, V., Beni, A., Costagliola, G., Martire, B., & Mastrototaro, M. F., et al. (2024). Towards personalized vaccines. *Frontiers in Immunology, 15,* 1436108. [DOI:10.3389/fimmu.2024.1436108] [PMID]

Patience, J. F., & Ramirez, A. (2022). Invited review: Strategic adoption of antibiotic-free pork production: the importance of a holistic approach. *Translational Animal Science, 6*(3), txac063. [DOI:10.1093/tas/txac063] [PMID]

Picoli, T., Waller, S. B., Hoffmann, J. F., Peter, C. M., Barcelos, L. S., & Lopes, M. G., et al. (2021). Antiviral and virucidal potential of Origanum vulgare Linn. (Oregano) extracts against Bovine alphaherpesvirus 1 (BoHV-1). *Research, Society and Development, 10*(5), e28410514979. [DOI:10.33448/rsd-v10i5.14979]

Ri, C. S., Jiang, X. R., Kim, M. H., Wang, J., Zhang, H. J., & Wu, S. G. (2017). Effects of dietary oregano powder supplementation on the growth performance, antioxidant status and meat quality of broiler chicks. *Italian Journal of Animal Science, 16*(2), pp.246-252. [DOI:10.1080/1828051X.2016.1274243]

Ruan, D., Fan, Q., Fouad, A. M., Sun, Y., Huang, S., & Wu, A., et al. (2021). Effects of dietary oregano essential oil supplementation on growth performance, intestinal antioxidative capacity, immunity, and intestinal microbiota in yellow-feathered chickens. *Journal of Animal Science, 99*(2), skab033. [DOI:10.1093/jas/skab033] [PMID]

Suarez, D. L., Miller, P. J., Koch, G., Mundt, E., & Rautenschlein, S. (2020). Newcastle disease, other avian paramyxoviruses, and avian metapneumovirus infections. In: D. E. Swayne, M. Boulianne, C. M. Logue, L. R. McDougald, V. Nair & D. L. Suarez (Eds.), *Diseases of Poultry* (pp.109-166). New Jersey: John Wiley & Sons, Inc. [DOI:10.1002/9781119371199.ch3]

Toka, T., & Geinoro, T. (2023). Vaccine failure and its control methods in poultry production. *British Journal of Poultry Sciences, 12*(2), 63-73. [Link]

Vimalanathan, S., & Hudson, J. (2012). Anti-Influenza virus activities of commercial oregano oils and their carriers. *Journal of Applied Pharmaceutical Science, 2*(7), 214-218. [DOI:10.7324/JAPS.2012.2734]

Zhang, L. Y., Peng, Q. Y., Liu, Y. R., Ma, Q. G., Zhang, J. Y., & Guo, Y. P., et al. (2021). Effects of oregano essential oil as an antibiotic growth promoter alternative on growth performance, antioxidant status, and intestinal health of broilers. *Poultry Science, 100*(7), 101163. [DOI:10.1016/j.psj.2021.101163] [PMID]

Zhang, Y., Li, X. Y., Zhang, B. S., Ren, L. N., Lu, Y. P., & Tang, J. W., et al. (2022). In vivo antiviral effect of plant essential oils against avian infectious bronchitis virus. B*MC Veterinary Research, 18*(1), 90. [DOI:10.1186/s12917-022-03183-x] [PMID]

This Page Intentionally Left Blank