Bangl. J. Vet. Med. (2025). 23 (2): 39 – 47 ISSN: 1729-7893 (Print), 2308-0922 (Online)

Received: 23-11-2025; Accepted: 08-01-2026 DOI: https://doi.org/10.33109/bjvmjd2025am1

ORIGINAL ARTICLE

Dietary *Nigella sativa* as an Immunomodulator against Infectious Bursal Disease Virus: Gross and Histopathological Evaluation

M. S. Muhammed[1], O. Orakpoghenor[2*], S. B. Oladele[1], B. Mohammed[1], and I. W. Musa[3]

[1]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Abuja, Abuja, Nigeria
[3]Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

Abstract

Background: *Nigella sativa* (black cumin) possesses immunomodulatory and antioxidant properties that may protect lymphoid tissues from damage induced by very-virulent infectious bursal disease virus (vvIBDV) in poultry. This study investigated the gross and histopathological effects of dietary Nigella sativa seed powder (NSSP) supplementation in cockerels experimentally challenged with vvIBDV.

Methods: One hundred day-old Dominant Black Marshal cockerel chicks were randomly allocated into five experimental groups (A–E). Group A received a basal diet and remained unchallenged; group B received a basal diet and was challenged with vvIBDV; group C received NSSP (2.8 g/kg feed) from 21 to 27 days of age (doa) and was unchallenged; group D received NSSP from 21 to 27 doa and was challenged; and group E received NSSP continuously from 1 to 42 doa and was challenged with vvIBDV. Following viral challenge, gross lesions, carcass weight, and histopathological changes in the bursa of Fabricius, spleen, and thymus were evaluated.

Results: No gross or histopathological lesions were observed in groups A and C. Severe lesions were recorded in group B, moderate lesions in group D, and mild lesions in group E. Dietary NSSP supplementation reduced muscle and bursal hemorrhages, splenic congestion, and thymic lymphoid depletion. Although relative bursal and thymic weights were significantly increased in infected birds during the early post-challenge period, lesion severity decreased progressively in NSSP-treated groups, indicating improved lymphoid tissue protection and recovery.

Conclusion: Continuous dietary supplementation with *Nigella sativa* seed powder effectively mitigated vvIBDV-induced lymphoid damage in cockerels, supporting its potential use as a natural immunomodulatory agent in poultry health management.

Keywords: *Nigella sativa*, infectious bursal disease, gross lesions, histopathological lesions.

*Correspondence: ochuko.orakpoghenor@gmail.com

Introduction

Infectious bursal disease (IBD), caused by infectious bursal disease virus (IBDV), is an acute, highly contagious, and immunosuppressive disease of young chickens. It continues to cause substantial economic losses worldwide by impairing humoral immunity and predisposing affected flocks to secondary infections and vaccination failure (Orakpoghenor et al., 2020; Abd El-Fatah et al., 2024). In recent years, the emergence and circulation of very-virulent IBDV (vvIBDV) strains have been linked to more severe bursal lesions, increased morbidity and mortality, and reduced productivity across several regions, thereby highlighting persistent challenges in disease control within endemic production systems (Enyetornye et al., 2024; Tibebu et al., 2025).

Current IBD control strategies rely primarily on vaccination and biosecurity. However, vaccine efficacy against rapidly evolving and highly virulent strains remains inconsistent, and practical field limitations often compromise the success of vaccination programmes, particularly in low-resource production systems (Muneeb et al., 2024). Consequently, there is growing interest in complementary, cost-effective interventions capable of preserving lymphoid tissue integrity and enhancing host resistance to viral damage without adversely affecting productivity. Among such approaches, the use of herbal immunomodulators, including *Nigella sativa*, has gained increasing attention (Khan et al., 2025).

Nigella sativa (black cumin) and its principal bioactive compound, thymoquinone, possess well-documented antioxidant, anti-inflammatory, and immunomodulatory properties across multiple animal models and in vitro systems. Previous studies have demonstrated enhanced humoral immune responses and modulation of cellular immunity following dietary supplementation or extract administration (Badary et al., 2021; Ciesielska-Figlon et al., 2023; Alberts et al., 2024). In poultry, dietary *N. sativa* supplementation has been associated with improved immune indices and increased resistance to common avian pathogens, suggesting its potential role as a nutraceutical adjunct to conventional disease-control measures (Elbaz et al., 2025; Khan et al., 2025). Nevertheless, most existing studies have focused primarily on performance traits, serological responses, or general immune markers, with limited emphasis on direct pathological evaluation of lymphoid organs following controlled vvIBDV challenge.

This study hypothesized that dietary supplementation with *N. sativa* seed powder (NSSP), particularly when administered continuously before and during viral exposure, would attenuate vvIBDV-induced gross and histopathological damage in primary and secondary lymphoid organs. Unlike previous investigations, this study provides a targeted assessment of lesion severity, lymphoid architecture, and relative immune-organ weights following experimental vvIBDV infection. Therefore, the aim of this study was to evaluate the effects of dietary NSSP supplementation on gross and histopathological lesions in cockerels experimentally challenged with vvIBDV, with particular emphasis on lesion severity, relative lymphoid organ weights, and microscopic alterations of lymphoid tissues, to determine the potential of *N. sativa* as a practical immunomodulatory intervention against vvIBDV-associated lymphoid damage.

Materials and methods

Ethical approval

All experimental procedures involving animals were conducted in accordance with institutional and international guidelines for the care and use of laboratory animals. Ethical clearance was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC/2024/79).

Experimental design and birds

A total of one hundred (100) day-old Dominant Black Marshal cockerel chicks were procured from a reputable commercial hatchery. Upon arrival, the chicks were brooded and acclimatized for seven days under standard husbandry conditions, with ad libitum access to feed and clean water. They were then randomly assigned into five experimental groups (A–E) of twenty birds each, as outlined below:

- Group A (Negative Control): Fed a basal diet only and remained unchallenged.
- Group B (Positive Control): Fed a basal diet only and challenged with vvIBDV.

- Group C: Fed a diet supplemented with NSSP from 21 to 27 days of age (doa) and remained unchallenged.
- Group D: Fed NSSP from 21 to 27 doa and challenged with vvIBDV.
- Group E: Continuously fed NSSP from day 1 to 42 doa and challenged with vvIBDV.

Each bird was individually tagged for identification. The experimental layout allowed for both pre-exposure and continuous supplementation regimens to assess prophylactic and protective effects of NSSP.

Preparation and administration of *Nigella sativa* seed powder

Whole seeds of *N. sativa* were sourced from a verified herbal store in Zaria, Nigeria. The seeds were air-dried, milled into fine powder using a sterile electric grinder, and incorporated into the basal feed at 2.8 g per kilogram, following the dosage protocol described by Al-Mufarrej (2014). The supplemented feed was freshly prepared weekly to maintain nutrient stability and homogeneity.

Virus and challenge procedure

The challenge virus was a locally isolated very-virulent strain of IBDV obtained from the Viral Research Department, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The virus had a titre of $10^{8.50}$ CID_{50}/mL and was confirmed by reverse transcription–PCR before use. At 28 days of age, birds in groups B, D, and E were orally inoculated with 0.2 mL of the vvIBDV suspension. The unchallenged groups (A and C) received an equivalent volume of sterile phosphate-buffered saline (PBS).

Gross pathological evaluation

Post-challenge birds were observed for mortality, and dead birds were immediately necropsied. Also, three live birds per group were humanely euthanized on days 31, 32, 35, 38, and 42 doa for necropsy. Carcasses were examined for gross lesions in the pectoral and thigh muscles, bursa of Fabricius, thymus, and spleen. The severity of lesions was scored according to the scale described by Orakpoghenor et al. (2021). The bursa-to-body-weight ratio was calculated using the formula:

$$\text{Relative bursal weight} = \frac{\text{Weight of bursa (WB) in grams}}{Carcass\ weight\ (CW)\ in\ grams} \times 100$$

Similar indices were computed for the thymus and spleen.

Histopathological examination

Representative tissue samples of the bursa of Fabricius, thymus, and spleen were fixed in 10% neutral-buffered formalin for 48 hours, processed routinely, and embedded in paraffin wax. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H&E) following standard procedures. Microscopic lesions were graded semi-quantitatively as mild (+), moderate (++), or severe (+++), based on follicular depletion, necrosis, and interfollicular oedema (Orakpoghenor *et al*., 2021).

Data analysis

Gross and histopathological lesions were presented as photographs and photomicrographs respectively. Data on carcass and organ weights were expressed as mean ± standard error of the mean (SEM). Statistical differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons, employing GraphPad Prism version 10.0. Differences were considered statistically significant at $p < 0.05$.

Results

Gross pathological changes

Gross lesions were absent in groups A and C. At 31 and 32 days of age (doa), lesions were severe in group B, moderate in group D, and mild in group E. By 35, 38, and 42 doa, lesions were mild in groups B, D, and E. The principal gross lesions observed included severe hemorrhages in the pectoral, thigh, and leg muscles in group B; hemorrhages confined to the thigh and leg muscles in group D; and mild congestion of the thigh and leg muscles in group E (Figure 1).

The bursa of Fabricius was markedly enlarged and severely hemorrhagic in group B, enlarged with mild hemorrhage in group D, and mildly congested in group E (Figure 2). Hemorrhagic thymic lobes were observed in group B (Figure 3). The spleen was enlarged and markedly congested in group B, moderately congested

in group D, and slightly congested in group E (Figure 4).

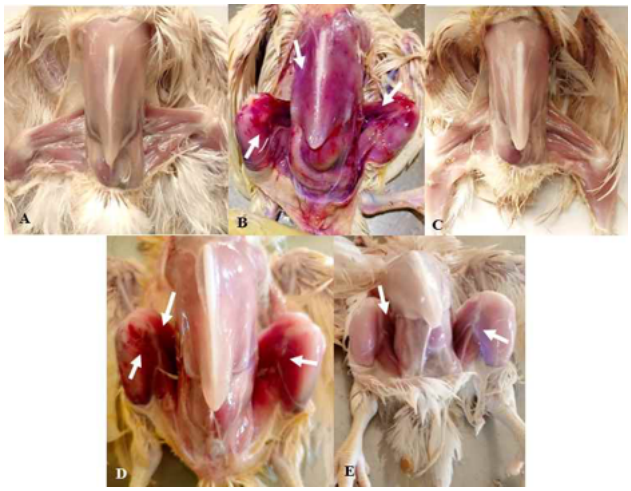

Figure 1: Photographs of carcasses of chickens administered Nigella sativa seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age. Note absence of lesion in groups A and C; severe haemorrhages of the pectoral, thigh and leg muscles in group B (arrows); haemorrhage of the leg and thigh muscles in group D (arrows); slight congestion of the leg and thigh muscles in group E (arrows).

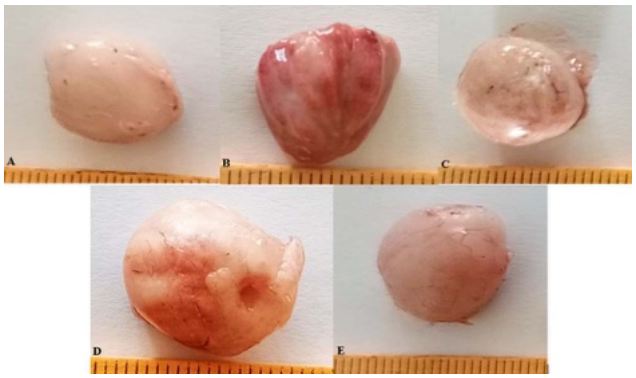

Figure 2: Photographs of bursae of Fabricius from chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age. Note intact BF in groups A and C; enlarged and severely haemorrhagic BF in group B; enlarged and slightly haemorrhagic BF in group D; slightly congested BF in group E.

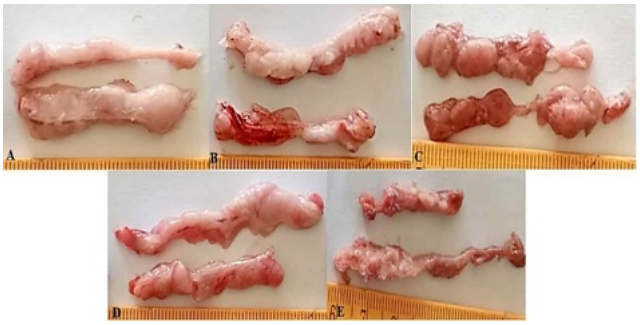

Figure 3: Photographs of thymus from chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age. Note intact thymic lobes in groups A, C, D and E; haemorrhages of the thymic lobes in group B (arrows).

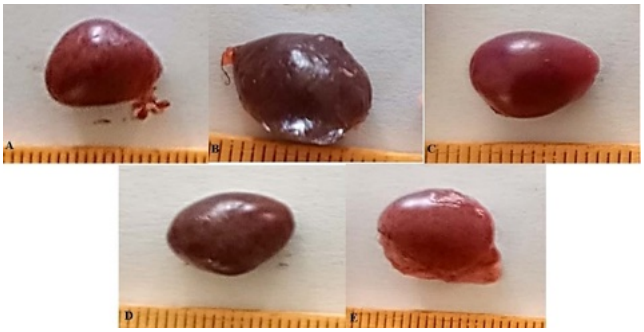

Figure 4: Photographs of spleen from chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age. Note intact spleen in groups A and C; enlargement and congestion in group B; congestion in group D; and slight congestion in group E.

Changes in carcass weight and relative weight of immune organs

The mean carcass weight showed no significant ($P > 0.05$) difference in all the groups from 31 to 42 doa, but was non-significantly ($P > 0.05$) higher in groups A and C, followed by in group D, and then in groups E and C (Table 1). The relative weight of the BF (RWBF) was significantly ($P < 0.05$) higher in groups B (0.72 ± 0.05; 0.68 ± 0.06), D (0.69 ± 0.02; 0.68 ± 0.09) and E (0.65 ± 0.05; 0.66 ± 0.05) compared to groups A (0.43 ± 0.04; 0.42 ± 0.04) and C (0.39 ± 0.03; 0.39 ± 0.04) at 31 and 32 doa. From 35 to 42 d0a, the RWBF was non-

significantly (P > 0.05) different between the groups of chickens (Table 2).

There was significantly (P < 0.05) higher mean RW of thymus (RWTY) in groups B (0.59 ± 0.02; 0.57 ± 0.05), D (0.54 ± 0.06; 0.55 ± 0.08) and E (0.52 ± 0.04; 0.51 ± 0.05) compared to groups A (0.41 ± 0.03; 0.42 ± 0.02) and C (0.41 ± 0.02; 0.39 ± 0.05) at 31 and 32 doa. No significant difference existed for the mean RWTY in all the groups at 35, 38 and 42 doa. The relative weight of the spleen (RWSP) was not significantly (P > 0.05) in all the groups from 31 to 42 doa (Table 2).

Table 1: Mean (± SEM) carcass weights (g) of chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age

Age of bird (days)	Group A (Negative control)	Group B (Positive control)	Group C (NSSP from 21 to 27 doa)	Group D (NSSP from 21 to 27 doa + vvIBDV)	Group E (NSSP from 1 to 42 doa + vvIBDV)
31	215.64 ± 25.05	194.25 ± 21.32	220.60 ± 13.58	199.17 ± 26.24	205.71 ± 18.11
32	217.57 ± 32.26	198.71 ± 27.41	223.45 ± 31.63	202.11 ± 25.72	209.43 ± 16.73
35	224.35 ± 26.97	201.87 ± 14.67	227.93 ± 23.21	207.95 ± 19.75	212.64 ± 28.14
38	235.19 ± 22.05	212.14 ± 19.62	237.47 ± 23.45	218.72 ± 32.46	223.18 ± 17.35
42	267.41 ± 28.42	227.32 ± 30.45	274.48 ± 21.64	235.41 ± 15.96	246.34 ± 23.47

Histopathological changes

There were no histopathologic changes in groups A and C; the changes were mild (groups D and E), and severe (group B), at 31 and 32 doa, and mild in groups B, D and E at 35, 38 and 42 doa. The changes observed in the BF in groups D and E were slightly depleted follicles; and in group B were depleted follicles, vacuolations and thickened interfollicular spaces (Figure 5). In the thymus, the changes observed were depleted medulla (Figure 6), and in the spleen, depleted white pulp (Figure 7).

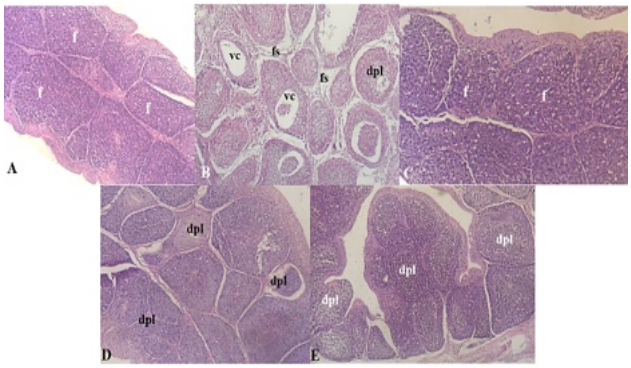

Figure 5: Photomicrographs of bursae of Fabricius from chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age. Note intact follicles (f) in groups A and C; depleted follicles (dpl), vacuolations (vc) and thickened interfollicular spaces (fs) in group B; slightly depleted follicles (dpl) in groups D and E. H & E × 200.

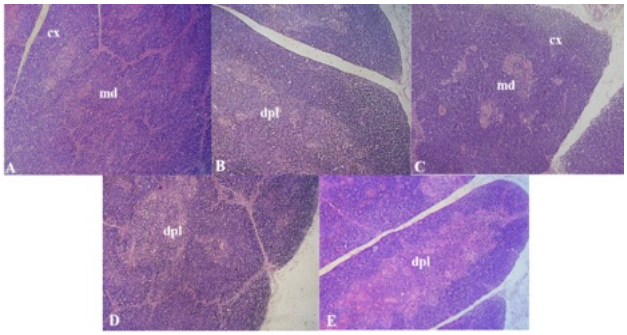

Figure 6: Photomicrographs of thymus from chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age. Note intact cortex (cx) and medulla (md) in groups A and C; depleted medulla (dpl) in groups B, D and E. H & E × 200.

Table 2: Mean (± SEM) relative weights of the bursa of Fabricius, thymus and spleen from chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age

Age of bird (days)	Immune organ	Group A (Negative control)	Group B (Positive control)	Group C (NSSP from 21 to 27 doa)	Group D (NSSP from 21 to 27 doa + vvIBDV)	Group E (NSSP from 1 to 42 doa + vvIBDV)
31	BF	0.43 ± 0.04[a]	0.72 ± 0.05[b]	0.39 ± 0.03[a]	0.69 ± 0.02[b]	0.68 ± 0.05[b]
	Thymus	0.41 ± 0.03[a]	0.59 ± 0.02[b]	0.41 ± 0.02[a]	0.54 ± 0.06[b]	0.52 ± 0.04[b]
	Spleen	0.32 ± 0.02	0.39 ± 0.04	0.32 ± 0.03	0.35 ± 0.04	0.34 ± 0.06
32	BF	0.42 ± 0.04[a]	0.68 ± 0.06[b]	0.39 ± 0.04[a]	0.68 ± 0.09[b]	0.66 ± 0.05[b]
	Thymus	0.42 ± 0.02[a]	0.57 ± 0.05[b]	0.39 ± 0.05[a]	0.55 ± 0.08[b]	0.51 ± 0.05[b]
	Spleen	0.31 ± 0.03	0.39 ± 0.03	0.32 ± 0.03	0.35 ± 0.04	0.34 ± 0.03
35	BF	0.29 ± 0.04	0.41 ± 0.05	0.26 ± 0.03	0.38 ± 0.05	0.38 ± 0.03
	Thymus	0.39 ± 0.06	0.43 ± 0.02	0.37 ± 0.05	0.41 ± 0.06	0.40 ± 0.04
	Spleen	0.28 ± 0.03	0.27 ± 0.03	0.26 ± 0.04	0.27 ± 0.04	0.28 ± 0.02
38	BF	0.27 ± 0.02	0.37 ± 0.02	0.25 ± 0.03	0.34 ± 0.06	0.31 ± 0.05
	Thymus	0.31 ± 0.02	0.31 ± 0.04	0.30 ± 0.04	0.29 ± 0.05	0.30 ± 0.03
	Spleen	0.24 ± 0.03	0.24 ± 0.03	0.22 ± 0.04	0.21 ± 0.04	0.22 ± 0.02
42	BF	0.18 ± 0.02	0.23 ± 0.02	0.19 ± 0.03	0.22 ± 0.02	0.21 ± 0.02
	Thymus	0.25 ± 0.02	0.22 ± 0.04	0.21 ± 0.02	0.23 ± 0.03	0.22 ± 0.4
	Spleen	0.21 ± 0.03	0.20 ± 0.02	0.19 ± 0.03	0.21 ± 0.04	0.22 ± 0.03

Values with different alphabets in the same row and on the same day differ significantly at $P < 0.05$.

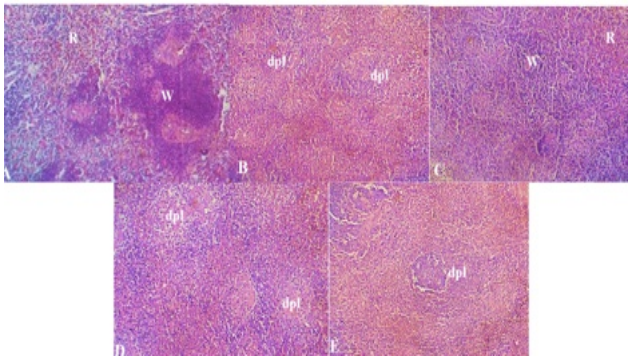

Figure 7: Photomicrographs of spleens from chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age. Note intact white (W) and red (R) pulps in groups A and C; depleted white pulps (dpl) in groups B, D and E. H & E × 200.

Discussion

In this study, the gross lesions observed in groups B, D, and E indicate that vvIBDV infection resulted in tissue damage and systemic pathological effects. These findings are consistent with previous reports describing characteristic gross lesions in chickens infected with vvIBDV, including muscular hemorrhages and lymphoid organ involvement (Zeryehun et al., 2012; Aliyu et al., 2016; Orakpoghenor et al., 2021; Damairia et al., 2023). The moderate lesions observed in group D and the mild lesions in group E suggest that prior administration of *Nigella sativa* seed powder (NSSP) conferred varying degrees of protection against vvIBDV-induced pathology. The immunomodulatory and anti-inflammatory properties of *N. sativa* may have enhanced host immune responses and reduced inflammatory damage, thereby limiting lesion severity. This interpretation aligns with earlier studies demonstrating the capacity of *N. sativa* to modulate immune function, enhance antioxidant defenses, and mitigate viral-induced tissue injury in poultry (Ciesielska-Figlon et al., 2023).

The persistence of hemorrhages in group D indicates that although short-term NSSP supplementation offered partial protection, the limited duration of administration may have been insufficient to fully counteract the effects of vvIBDV challenge. In contrast, group E exhibited only mild congestion and minimal lesions, supporting the notion that continuous dietary exposure to *N. sativa* before and during infection enhanced immune regulation and reduced inflammatory responses. These findings further corroborate existing evidence that sustained *N. sativa* supplementation promotes more effective immune modulation and protection against viral pathology.

Trends in mean carcass weight across groups from 31 to 42 days of age further reflect the impact of vvIBDV infection and the mitigating effects of NSSP. Group B, which experienced severe viral-induced pathology, likely suffered growth impairment, whereas groups D and E showed comparatively less reduction in carcass weight. This observation is consistent with previous studies reporting improved growth performance and body weight gain in poultry supplemented with *N. sativa* (Seidavi et al., 2020; Samy et al., 2023).

The significantly higher relative weight of the bursa of Fabricius (RWBF) in groups B, D, and E compared with groups A and C at 31 and 32 days of age is likely attributable to inflammation and lymphoid hyperplasia associated with viral infection and immune activation (Orakpoghenor et al., 2021c). Similarly, the increased relative weight of the thymus (RWTY) in infected groups at early post-challenge stages suggests heightened immune activity, given the thymus' role in T-cell maturation and proliferation during infection (Gulla et al., 2023). However, the comparatively lower RWBF and RWTY observed in group E relative to group D indicate that continuous NSSP supplementation may have moderated excessive inflammatory responses and reduced tissue swelling, reflecting improved immune regulation. In contrast, the elevated RWBF observed in group B likely represents an exaggerated inflammatory response in the absence of any protective dietary intervention.

No significant differences were observed in the relative weight of the spleen (RWSP) among groups, suggesting a broadly similar splenic response to vvIBDV challenge and NSSP supplementation. Nevertheless, the gross congestion noted in groups B, D, and E indicates ongoing immune activation. The comparatively mild congestion observed in group E further supports a protective effect of early and sustained NSSP supplementation, consistent with reports highlighting the role of antioxidant compounds in reducing splenic inflammation (Fan et al., 2020; Eleiwa et al., 2023; El-Kazaz et al., 2024; Fathi et al., 2024).

Histopathological findings in groups B, D, and E were consistent with previous descriptions of vvIBDV-

induced lesions in poultry, confirming the virulence of the challenge strain and its capacity to cause marked lymphoid damage (Orakpoghenor et al., 2021; Salaheldin et al., 2024; Liu and Huang, 2025). Groups D and E exhibited only mild histopathological alterations, including slight follicular depletion in the bursa of Fabricius. The presence of *N. sativa* prior to viral challenge in these groups likely contributed to a more controlled immune response and reduced tissue injury. Preservation of lymphoid architecture in NSSP-treated birds suggests that *N. sativa* supplementation may help maintain immune organ integrity through antioxidant activity and balanced immunomodulation.

Thymic medullary depletion observed across all vvIBDV-exposed groups indicates disruption of T-cell development due to viral infection. The severity of thymic depletion was likely greatest in group B, reflecting unmitigated viral effects in the absence of dietary supplementation. This observation aligns with previous studies demonstrating thymic damage and impaired cellular immunity following IBDV infection (Orakpoghenor et al., 2021; Salaheldin et al., 2024; Liu and Huang, 2025). In the spleen, depletion of white pulp in group B further illustrates the systemic immunosuppressive effects of vvIBDV. The less severe white pulp depletion observed in groups D and E suggests that NSSP supplementation may support splenic lymphoid preservation, potentially by enhancing lymphocyte survival and proliferation, as previously proposed.

Conclusion

Dietary supplementation with *Nigella sativa* seed powder demonstrated protective immunomodulatory effects against very-virulent infectious bursal disease virus infection in chickens. NSSP-treated groups exhibited reduced severity of gross and histopathological lesions, improved preservation of lymphoid organ architecture, and evidence of enhanced recovery compared with untreated infected controls. Continuous supplementation produced the most consistent protective effects, indicating a duration-dependent benefit. From a practical perspective, NSSP represents a feasible and low-cost dietary adjunct to existing IBD control strategies, particularly in regions where vaccine efficacy is inconsistent and vvIBDV remains endemic. Incorporation of *N. sativa* into poultry feeding programs may contribute to improved lymphoid resilience and reduced pathological consequences of IBDV under field conditions.

Acknowledgement

National Veterinary Research Institute (NVRI) Vom, Nigeria

Funding

None of the authors receive any fund for the study.

Data Availability Statement

Data are available upon request

Conflict of Interest

The authors declare no potential conflict of interest.

References

1. Abd El-Fatah AH, Ayman D, Samir M, Eid S, Elgamal M, El-Sanousi AA, et al. Molecular characterization of circulating infectious bursal disease viruses in chickens from different Egyptian governorates during 2023. Virol J. 2024;21:312.
2. Alberts A, Moldoveanu ET, Niculescu AG, Grumezescu AM. *Nigella sativa*: a comprehensive review of its therapeutic potential, pharmacological properties, and clinical applications. Int J Mol Sci. 2024;25:13410.
3. Aliyu HB, Sa'idu L, Jamilu A, Andamin AD, Akpavie SO. Outbreaks of virulent infectious bursal disease in flocks of battery cage brooding system of commercial chickens. J Vet Med. 2016;1:1–7.
4. Al-Mufarrej SI. Immune-responsiveness and performance of broiler chickens fed black cumin (*Nigella sativa*) powder. J Saudi Soc Agric Sci. 2014;13:75–80.
5. Badary OA, Hamza MS, Tikamdas R. Thymoquinone: a promising natural compound with potential benefits for COVID-19 prevention and cure. Drug Des Devel Ther. 2021;15:1819–33.
6. Ciesielska-Figlon K, Wojciechowicz K, Wardowska A, Lisowska KA. The immunomodulatory effect of *Nigella sativa*. Antioxidants. 2023;12:1340.

7. Damairia BA, Putri K, Wibowo MH. Examination of macroscopic and microscopic lesions in IBDV-infected organs and molecular characterization of IBDV VP1 gene fragments obtained from commercial broiler farms in Indonesia. Vet World. 2023;16:1061.
8. Elbaz AM, Ashmawy ES, Farahat MAA, Abdel-Maksoud A, Amin SA, Mohamed ZS. Dietary *Nigella sativa* nanoparticles enhance broiler growth performance, antioxidant capacity, immunity, gene expression modulation, and cecal microbiota during high ambient temperatures. Sci Rep. 2025;15:861.
9. Eleiwa NZ, El-Shabrawi AA, Ibrahim D, Abdelwarith AA, Younis EM, Davies SJ, et al. Dietary curcumin modulating effect on performance, antioxidant status, and immune-related response of broiler chickens exposed to imidacloprid insecticide. Animals. 2023;13:3650. El-Kazaz SE, Hafez MH, Albadrani GM, Al-Ghadi MQ, Abdel-Daim MM, El-Sayed YS. The influence of quercetin on behavior, performance and splenic immunity in broiler chickens. Vet Anim Sci. 2024;26:100398.
10. Enyetornye B, Abugri HA, Kusi-Appiah AK, Maboni G, Odoom T, Gottdenker NL, et al. Pathology and VP2-based characterization of infectious bursal disease virus associated with an outbreak in layer chickens in Ghana. Pathogens. 2024;13:1115.
11. Fan RF, Liu JX, Yan YX, Wang L, Wang ZY. Selenium relieves oxidative stress, inflammation, and apoptosis within spleen of chicken exposed to mercuric chloride. Poult Sci. 2020;99:5430–9.
12. Fathi M, Zarrinkavyani K, Biranvand Z, Mohmmadi Y, Hilal MJ. Improving antioxidant function, inflammatory responses, growth performance, and mortality by supplementing black cumin (*Nigella sativa*) seed in broiler chickens exposed to low ambient temperature. J Hell Vet Med Soc. 2024;75:7845–56.
13. Gulla S, Reddy MC, Reddy VC, Chitta S, Bhanoori M, Lomada D. Role of thymus in health and disease. Int Rev Immunol. 2023;42:347–63.
14. Khan MT, Xu C, Khan S, Ali F, Abbas G, Bughio E, et al. Dietary *Nigella sativa* supplementation enhances performance, carcass characteristics, meat quality, immune response, and gut health in broilers. Poult Sci. 2025;104:105626.
15. Liu W, Huang X. Very virulent infectious bursal disease virus infection triggered microscopic changes, apoptosis, and inflammatory cytokines imbalance in chicken spleen and thymus. Avian Pathol. 2025;54:62–75.
16. Muneeb JM, Pawaskar DK, Ullah-Rafiqui M, Siddiqui R, Shabir N, Pawaskar NK, et al. A comprehensive overview of infectious bursal disease, a major threat to poultry health and production. Int J Adv Biochem Res. 2024;8:154–63.
17. Orakpoghenor O, Oladele SB, Abdu PA. Infectious bursal disease: transmission, pathogenesis, pathology and prevention – an overview. Worlds Poult Sci J. 2020;76:292–303.
18. Orakpoghenor O, Oladele SB, Abdu PA, Markus TP, Andamin AD, Esievo KAN. Comparative pathological changes induced by very virulent infectious bursal disease virus infection in inoculated, sentinel pigeons and chickens. Open Vet Sci. 2021;2:55–64.
19. Salaheldin AH, Abd El-Hamid HS, Ellakany HF, Mohamed MA, Elbestawy AR. Isolation, molecular, and histopathological patterns of a novel variant of infectious bursal disease virus in chicken flocks in Egypt. Vet Sci. 2024;11:98.
20. Samy A, Hassan HMA, Kabary H, Elsherif HMR. The influence of *Nigella sativa* and curcumin extracts as phytogenic feed additives on broiler performance, blood parameters, antioxidant status and intestinal microbiota. Egypt J Nutr Feeds. 2023;26:353–63.
21. Seidavi AR, Laudadio V, Khazaei R, Puvača N, Selvaggi M, Tufarelli V. Feeding of black cumin (*Nigella sativa* L.) and its effects on poultry production and health. Worlds Poult Sci J. 2020;76:346–57.
22. Tibebu A, Bahiru A, Yitbarek T, Teshome Y, Tamrat H, Alemu YF, et al. Prevalence of infectious bursal disease in chickens in Ethiopia: a systematic review and meta-analysis. J Appl Poult Res. 2025;34:100617.
23. Zeryehun T, Hair-Bejo M, Rasedee A. Hemorrhagic and clotting abnormalities in infectious bursal disease in specific-pathogen-free birds. World Appl Sci J. 2012;16:1123–30.