

Nigella sativa Seed Powder Supplementation Mitigates Oxidative Stress Biomarkers Alterations in Very Virulent Infectious Bursal Disease Virus-Infected Chickens

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Abstract

Oxidative imbalance plays a key role in the pathology associated with highly virulent infectious bursal disease virus infection in chickens; however, nutritional strategies to limit this damage remain insufficiently explored. This study evaluated the influence of dietary *Nigella sativa* seed powder on selected oxidative biomarkers in experimentally infected cockerels. One-hundred-day-old birds were randomly distributed into five groups: uninfected controls, infected controls, seed powder supplementation alone, short-term supplementation with a viral challenge, and continuous supplementation with a viral challenge. The supplement was incorporated into the feed for either 7 or 42 days, while infection was induced orally at 28 days of age. Serum analysis revealed that viral infection reduced superoxide dismutase activity while increasing catalase activity, glutathione peroxidase activity, and lipid peroxidation levels. Birds receiving continuous supplementation showed markedly attenuated alterations compared to the untreated infected groups. These findings indicate that *Nigella sativa* seed powder effectively moderated virus-associated oxidative disturbances by supporting endogenous antioxidant defenses and limiting membrane lipid damage, highlighting its potential value as a functional dietary intervention in infected poultry.

Keywords

Bursal pathology, Enzymatic defense, Lipid peroxidation, Phyto-genic supplementation, Poultry health

Introduction

In commercial poultry production, young chickens infected with very virulent infectious bursal disease virus (vvIBDV) suffer from systemic oxidative stress in addition to immunosuppression and bursal damage (Abdu *et al.*, 2023; Wang *et al.*, 2024). Oxidative stress is characterized by an increased reactive oxygen species (ROS) level in relation to the antioxidant defense capacity of the body. This often leads to lipid, protein, and DNA damage, as well as impairment of cellular and immune functions (Hu *et al.*, 2023; Oke *et al.*, 2024). In the context of vvIBDV infection, such oxidative imbalance exacerbates tissue injury and compromises recovery. However, there is limited information describing alterations in antioxidant enzyme activity and lipid peroxidation markers in this disease model.

Considering the role of oxidative stress in disease progression, there is increasing interest in nutritional strategies to enhance antioxidant defenses in poultry. This involves the use of natural phytogetic supplements (plant-derived feed ingredients with bioactive properties). These supplements are considered useful because of their potential to support redox homeostasis, improve immune responses, and reduce reliance on synthetic antioxidants or drugs (Li *et al.*, 2024). One of the natural phytogetics that is commonly used is *Nigella sativa*. *Nigella sativa* (black cumin) seed and oil have been shown to increase the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and decrease malondialdehyde (MDA) concentrations in poultry under heat-stress or toxin-challenge conditions (Akter *et al.*, 2025; Elbaz *et al.*, 2025). However, most studies have focused on general production or stress models

rather than viral immunosuppressive challenges.

Although there are several reports on the positive impacts of *N. sativa* on poultry nutrition, there is a dearth of information regarding its role during viral infections, such as vvIBDV. Most existing studies have focused on growth performance, immune stimulation, or responses to environmental and dietary stressors (El-Kashef, 2020; Akter *et al.*, 2025; Khan *et al.*, 2025), hence, there is limited data on oxidative stress modulation in virus-infected cockerels. *N. sativa* supplementation has been shown to enhance antibody production and antioxidant capacity in vaccinated broilers; however, there is no report on the evaluation of oxidative stress biomarkers following vvIBDV challenge (Khalaf *et al.*, 2024). Moreover, differences in preparation methods, such as oil, extract, or nanoparticle forms, have produced inconsistent results, and few investigations have compared short-term and continuous supplementation using whole seed powder. The relationship between *N. sativa*-mediated changes in the activities of antioxidant enzymes, such as SOD, CAT, GPx, and markers of lipid peroxidation (MDA), during viral infection remains poorly defined. This therefore highlights the need for a controlled experimental assessment under standardized vvIBDV challenge conditions.

To address these gaps, this study investigated the effect of *N. sativa* seed powder (NSSP) supplementation on oxidative stress biomarkers in chickens experimentally challenged with vvIBDV. We compared two supplementation strategies, involving a short-course period and a continuous feeding period, to determine whether continuous inclusion better preserves redox homeostasis in the face

of viral-induced oxidative stress. Thus, we aimed to provide clearer mechanistic insights into how dietary NSSP may support antioxidant defense and disease resilience in poultry infected with vvIBDV.

Materials and Methods

Experimental cockerels and management

A total of 100 day-old cockerel chicks (Dominant Black Marshal) were procured from Zartech Hatchery, Ibadan, Nigeria. The cockerels were transported to the research facility of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Prior to chick arrival, the experimental pens and all equipment were thoroughly cleaned, disinfected, and fumigated. Upon arrival, the chicks were allowed a 7-day acclimatization period before the commencement of any experimental supplementation or sampling procedures. During this period, they were monitored to ensure health stability and adaptation to the housing environment. The brooding room was maintained at a temperature of 32–34 °C during the first week, after which the temperature was gradually reduced by 2–3 °C per week until it reached 24–26 °C. Relative humidity was maintained between 55% and 65% throughout the acclimatization and experimental periods. Strict hygiene and biosecurity measures were maintained throughout the experiment. The cockerels were raised on deep litter using wood shavings, provided with clean water and feed *ad libitum*, and reared under standard management conditions. Each treatment group was housed separately with dedicated feeders and drinkers to prevent cross-contamination.

Diet preparation of *Nigella sativa* seed powder (NSSP)

A commercial starter mash (Vital Feed® chick mash) was used as the basal diet. Dried *Nigella sativa* seeds were purchased from a local supplier (Herbal Point in Samaru, Zaria, Nigeria) and authenticated by the Department of Botany, Ahmadu Bello University. The seeds were cleaned and ground into fine powder using a laboratory milling machine. The resulting NSSP was incorporated into the basal diet at a fixed inclusion rate of 2.8 g/kg of feed (Al-Mufarrej, 2014). The treated feed was offered *ad libitum*, allowing the cockerels to consume it freely throughout the supplementation period. Feed batches were thoroughly mixed to ensure even distribution of the powder and stored in airtight containers until use.

Vaccination and viral inoculum

All birds were vaccinated against Newcastle disease using the La Sota strain at 7 and 17 days of age (doa), according to standard vaccination schedules (Oluwadare *et al.*, 2016). The challenge virus, a confirmed very virulent infectious bursal disease virus (vvIBDV) isolate, was supplied by the Viral Research Department of the National Veterinary Research Institute (NVRI), Vom, Nigeria. At 28 doa, birds to be challenged were administered 0.2 mL of vvIBDV suspension containing $10^{8.50}$ CID₅₀/mL orally. Control groups were handled similarly but were not exposed to the virus.

Study design and treatments

The 100 chicks were randomly divided into five groups of 20 birds each as follows:

- Group A: Healthy group
- Group B: Positive control – basal diet only, challenged with vvIBDV.

- Group C: NSSP short-course (2.8 g/kg feed from day 21–27), unchallenged.
- Group D: NSSP short-course (day 21–27) + vvIBDV challenge.
- Group E: Continuous NSSP supplementation (2.8 g/kg feed from day 1–42) + vvIBDV challenge.

Sampling

Blood was collected from the brachial vein into labeled plain tubes at predetermined ages: 1, 7, 14, 21, 28, 29, 30, 31, 32, 33, 34, 35, 38, and 42 days. The blood was allowed to clot, and the obtained serum was stored at -20°C until assayed for oxidative stress biomarkers.

Assay for oxidative stress biomarkers

Serum superoxide dismutase (SOD) activity was measured using a commercial kit (North West Life Science Specialist, Vancouver, Canada) according to the manufacturer's protocol. Catalase (CAT) activity was assayed using a CAT kit (Abcam Plc, Cambridge, UK) following the manufacturer's protocol. The activity of glutathione peroxidase (GPx) was measured using a standard microplate assay. Malondialdehyde (MDA) levels were evaluated using the thiobarbituric acid reactive substances (TBARS) method, as described by Draper and Hadley (1990), and modified by Yavuz *et al.* (2004). All assays were performed in duplicate, and the results were expressed as units per milliliter (U/mL) for enzyme activities and nanomoles per milliliter (nmol/mL) for MDA levels.

Data analysis

Mean $\pm$ standard error of the mean (SEM) was used to express the data. The data were subjected to a one-way repeated-measures analysis of variance (ANOVA), followed by Tukey's *post-hoc* test to compare means. GraphPad Prism (GraphPad Software v 8.0, San Diego, USA) was used for the analysis, and $P \leq 0.05$ was considered significant. Results are presented using graphs to illustrate temporal changes and intergroup differences.

Results and Discussion

Superoxide dismutase activity

There was no significant difference ($P > 0.05$) in mean serum SOD activity in chickens from 1 to 31 days of age (Figure 1). This was followed by significantly ($P < 0.05$) lower mean serum SOD activity in group B ($31.94 \pm 2.50 - 35.31 \pm 1.83$ U/mL), compared to groups A ($42.76 \pm 1.09 - 49.25 \pm 3.21$ U/mL), C ($41.32 \pm 2.64 - 51.36 \pm 4.17$ U/mL), D ($35.95 \pm 1.08 - 41.14 \pm 3.45$ U/mL) and E ($38.17 \pm 3.76 - 44.81 \pm 2.36$ U/mL), from 32 to 42 DOA (Figure 1).

No significant difference ($P > 0.05$) was observed for the mean serum CAT activity in all groups of chickens from days 1 to 33 DOA (Figure 2). At 34 and 35 DOA, CAT activity was significantly higher ($P < 0.05$) in group B ($40.85 \pm 1.48; 41.27 \pm 3.48$ U/mL) compared to groups A ($30.12 \pm 3.19; 28.27 \pm 2.70$ U/mL), C ($32.41 \pm 2.56; 31.64 \pm 1.23$ U/mL), D ($32.34 \pm 3.25; 38.26 \pm 4.58$ U/mL) and E ($30.36 \pm 3.48; 36.25 \pm 3.35$ U/mL). Subsequently, at 38 and 42 DOA, CAT activity was significantly higher ($P < 0.05$) in group B than in groups A, C, D, and E (Figure 2).

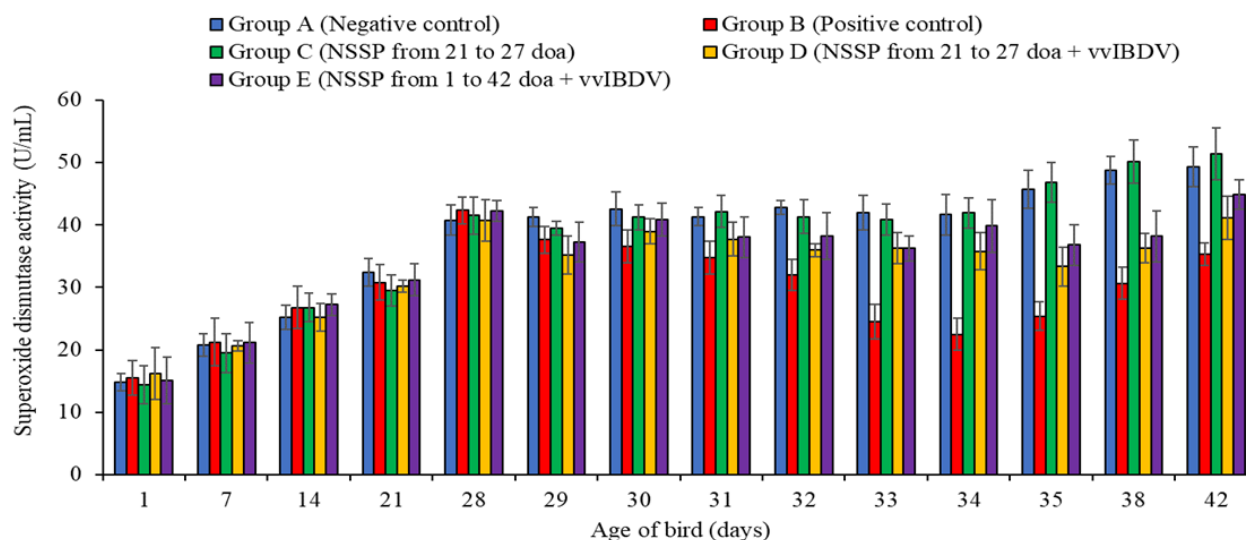


Figure 1. Mean (± SEM) serum superoxide dismutase activity in cockerels supplemented with *Nigella sativa* seed powder and challenged with a vvIBDV orally at 28 days of age. Values with different alphabets on the same day differ significantly at $P < 0.05$. NSSP, NSSP-*Nigella sativa* seed powder; vvIBDV, very virulent infectious bursal disease virus; doa, days of age.

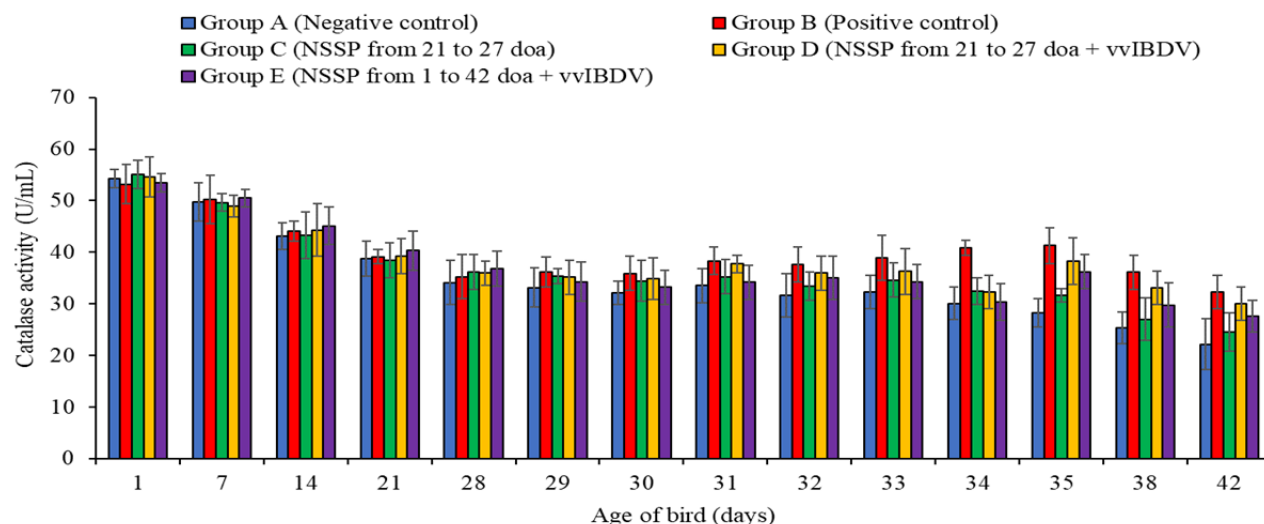


Figure 2. Mean (± SEM) serum catalase activity in cockerels supplemented with *Nigella sativa* seed powder and challenged with a vvIBDV orally at 28 days of age. Values with different alphabets on the same day differ significantly at $P < 0.05$. NSSP, NSSP-*Nigella sativa* seed powder; vvIBDV, very virulent infectious bursal disease virus; doa, days of age.

Glutathione peroxidase activity

The mean serum GPx activity was not significantly different ($P > 0.05$) in chickens from 0 to 28 days of age. At 29 and 30 days of age, GPx activity was non-significantly higher ($P > 0.05$) in groups B, D, and E (Figure 3). From 31 to 42 doa, GPx activity was significantly

higher ($P < 0.05$) in group B ($33.49 \pm 2.23 - 34.26 \pm 2.28$ U/mL) followed by groups D ($30.12 \pm 4.01 - 23.01 \pm 1.53$ U/mL) and E ($28.76 \pm 1.92 - 18.01 \pm 1.20$ U/mL), compared to groups A ($23.50 \pm 3.57 - 15.36 \pm 1.02$ U/mL) and C ($24.05 \pm 1.60 - 14.67 \pm 0.98$ U/mL) (Figure 3).

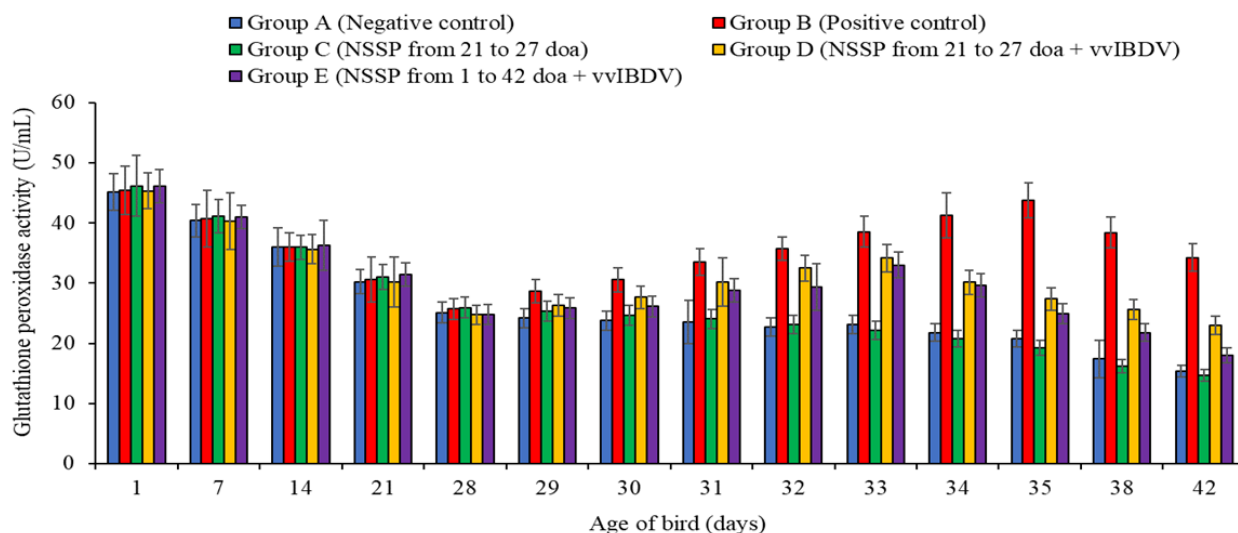


Figure 3. Mean ($\pm$ SEM) serum glutathione peroxidase activity in cockerels supplemented with *Nigella sativa* seed powder and challenged with a vvIBDV orally at 28 days of age. Values with different alphabets on the same day differ significantly at $P < 0.05$. NSSP, NSSP-*Nigella sativa* seed powder; vvIBDV, very virulent infectious bursal disease virus; doa, days of age.

Malondialdehyde concentration

There was no significant difference ($P > 0.05$) in the mean serum MDA level in all the groups of chickens from days 0 to 28 d (Figure 4). At 29 and 30 doa, MDA level was non-significantly ($P > 0.05$) higher in groups B, D and E than in groups A and C. This was followed by significantly ($P < 0.05$) higher MDA level, from 31 to 33 doa, in groups B ($43.93 \pm 1.83 - 46.91 \pm 1.97$ nmol/mL), D ($40.95 \pm 3.45 - 40.72 \pm 2.43$ nmol/mL) and E ($39.93 \pm$

$3.67 - 38.63 \pm 4.85$ nmol/mL) compared to groups A ($34.28 \pm 3.31 - 32.72 \pm 1.83$ nmol/mL) and C ($32.15 \pm 4.01 - 29.27 \pm 2.43$ nmol/mL). From 34 to 42 doa, the mean MDA level was significantly higher ($P < 0.05$) in group B ($48.35 \pm 3.95 - 37.25 \pm 4.18$ nmol/mL) compared to groups, A ($31.15 \pm 2.75 - 19.28 \pm 4.85$ nmol/mL), C ($28.75 \pm 3.31 - 17.25 \pm 3.18$ nmol/mL), D ($37.63 \pm 4.20 - 25.53 \pm 4.52$ nmol/mL) and E ($35.17 \pm 5.10 - 21.36 \pm 3.95$ nmol/mL) (Figure 4).

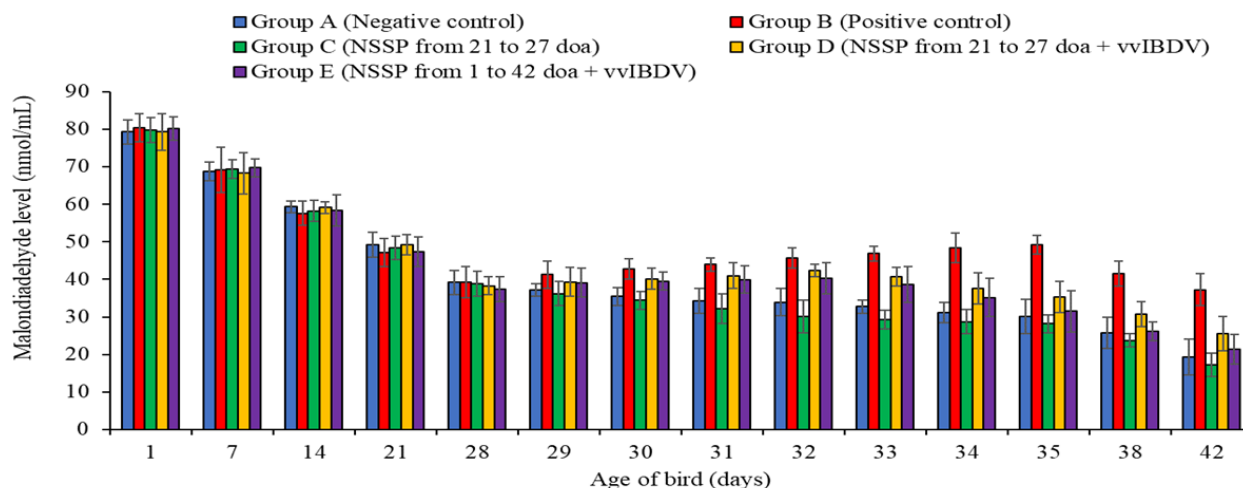


Figure 4. Mean ($\pm$ SEM) serum malondialdehyde level in cockerels supplemented with *Nigella sativa* seed powder and challenged with a vvIBDV orally at 28 days of age. Values with different alphabets on the same day differ significantly at $P < 0.05$. NSSP, NSSP-*Nigella sativa* seed powder; vvIBDV, very virulent infectious bursal disease virus; doa, days of age.

Discussion

In this study, the significantly reduced SOD activity observed in vvIBDV-infected chickens (Groups B, D, and E) compared with uninfected controls (Groups A and C) indicates that viral replication induced oxidative stress and compromised antioxidant defense mechanisms. This observation aligns with the findings of Abdu *et al.* (2023) and Wang *et al.* (2024), who reported that vvIBDV infection leads to a redox imbalance and depletion of endogenous antioxidants. The relatively higher SOD activity in groups pretreated or continuously fed with NSSP might indicate that the bioactive compounds of the plant, notably thymoquinone, enhanced the enzymatic antioxidant capacity, thus mitigating oxidative damage. This finding agrees with previous reports, which showed that *N. sativa* augments SOD activity in heat-stressed or toxin-exposed chickens (Bordoni

et al., 2019; Alrashidi *et al.*, 2022; Fathi *et al.*, 2024a; 2024b).

Catalase (CAT) decomposes hydrogen peroxide into water and oxygen; thus, it serves as a secondary defense against oxidative injury. The transient elevation in CAT activity in groups B, D, and E may represent a compensatory cellular response to increased reactive oxygen species. However, the decreased CAT activity in groups D and E compared to group B may suggest that *N. sativa* supplementation attenuated oxidative pressure, thereby reducing the need for overactivation of CAT. Similar modulatory effects on CAT have been reported in studies in which *N. sativa* improved the redox balance under oxidative stress conditions (Abdalbagemohammedabdalsadeg *et al.*, 2024; Anwar *et al.*, 2024).

For glutathione peroxidase (GPx) activity, the pattern observed in this study supports its role as a downstream antioxidant enzyme that complements SOD and CAT in

neutralizing peroxides. The higher GPx activity in vvIBDV-challenged chickens, particularly in the positive control group, may imply an adaptive response to elevated oxidative stress. However, in birds that received NSSP, there was moderate GPx activity, which is consistent with reduced oxidative challenge due to enhanced upstream antioxidant defenses. This finding agrees with previous reports showing that *N. sativa* components restore GPx activity by increasing intracellular glutathione and limiting lipid peroxidation (Ursini and Maiorino, 2020; Pei *et al.*, 2023).

The significantly increased MDA levels among vvIBDV-challenged birds in this study may indicate membrane lipid oxidation caused by excessive ROS generation. The lower MDA levels in the NSSP-treated groups, particularly in the continuously supplemented group, demonstrate the potential of the plant to inhibit lipid peroxidation. Thymoquinone and other phenolic constituents of *N. sativa* scavenge free radicals, enhance glutathione activity, and upregulate antioxidant gene expression (Balami *et al.*, 2021; Huang *et al.*, 2021). Therefore, continuous NSSP administration provided sustained antioxidant protection, whereas short-course feeding offered only partial mitigation.

In interpreting the present findings, it is important to note that the study focused solely on oxidative stress biomarkers. Immune parameters, such as antibody titers, lymphoid depletion, bursal histology, and cytokine profiles, were not evaluated; nor were indicators of viral replication (RT-qPCR, antigen ELISA, or bursal viral load) or clinical and pathological outcomes, including lesion

scoring or mortality. In addition, only one supplementation level (2.8 g/kg) was employed, limiting the ability to determine whether this represents the minimal effective or optimal dose, and future dose-response evaluations are warranted. Although the *Nigella sativa* seeds were botanically authenticated, phytochemical quantification (e.g., thymoquinone content) was not performed, and a single batch was used, which precludes the assessment of batch-to-batch variation. These considerations indicate that the present results support an improved oxidative stress status but do not allow conclusions regarding effects on immune function, viral replication, or disease resistance. Further studies integrating immunological, virological, pathological, and multi-dose assessments are necessary to clarify these mechanisms and enhance the practical relevance of *Nigella sativa* supplementation in poultry health.

Overall, the distinct oxidative stress biomarker profiles observed between short-term and continuous supplementation confirm that prolonged administration of *N. sativa* supports more stable antioxidant homeostasis and minimizes oxidative stress associated with vvIBDV infection. This indicates that the duration of supplementation is critical for maximizing the protective effects of the plant. These findings highlight the therapeutic potential of NSSP as a natural dietary additive to strengthen antioxidant defenses, support immune competence, and reduce the impact of oxidative injury in poultry diseases. Further studies assessing molecular pathways (such as Nrf2 and NF- κ B signaling) and field trials under commercial conditions

are warranted to optimize dosage, duration, and delivery methods for practical application in poultry health management.

Conclusion

This study demonstrated that dietary supplementation with NSSP effectively reduced oxidative stress in chickens challenged with vvIBDV. The continuous inclusion of NSSP enhanced antioxidant enzyme activities and lowered lipid peroxidation compared with unsupplemented birds, indicating an improved redox balance and cellular protection. These findings suggest that *N. sativa* possesses potent antioxidant properties that can support immune resilience and health in poultry exposed to oxidative challenges. Therefore, continuous supplementation may serve as a practical and natural feed additive strategy for improving disease resistance in chickens.

Approval of Ethical Commission

Ethical approval for this study was granted by the Ahmadu Bello University Committee on Animal Use and Care (approval number: ABUCAUC/2024/79).

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Author's Contribution

MSM: Performed the methodology, investigation, data curation, and provided resources; OO: Contributed to the methodology, investigation, data curation, formal analysis, and wrote the original draft of the manuscript; SBO: Was responsible for

the conceptualization, supervision, validation, and reviewing, and editing of the manuscript; BM: Contributed to the conceptualization, supervision, validation, reviewing, and editing of the manuscript; IWM: Contributed to the conceptualization, supervision, and reviewing, and editing of the manuscript. All authors have read and approved the final version of the manuscript.

Conflict of Interest

The authors declare no potential conflict of interest.

Data Availability Statement

Data are available upon request.

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