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Original Research Article

Nigella Sativa Seed Powder Pre-Administration Decreased Clinical Signs, Morbidity and Mortality Rates in Cockerels Challenged with a very Virulent Infectious Bursal Disease Virus

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Abstract: As infectious bursal disease (IBD), a viral disease, has no specific antiviral treatment, the use of herbal remedies with immunomodulatory and antiviral properties presents a valuable complementary approach to enhance host defenses and reduce disease severity. The aim of this study was to assess the clinical signs, morbidity and mortality rates due to *Nigella sativa* seed powder (NSSP) pre-administration in cockerels challenged with a very virulent IBD virus (vvIBDV). One hundred, one-day-old Dominant black marshal cockerel (DBMC) chicks were randomly divided into 5 groups (A, B, C, D and E) of 20 birds as follows: A and B were administered feed only from 1 to 42 days of age (doa); C and D administered the NSSP+feed from 21 to 27 doa; E was administered the NSSP+feed from 1 to 42 doa. All the chicks were vaccinated against ND at 7 and 17 days of age. At 28 doa, groups B, D and E were challenged with a vvIBDV orally. The chickens were monitored daily for clinical signs, and morbidity and mortality rates were calculated. Results revealed mild overall clinical sign in groups D (13.0%) and E (9.6%), and severe signs in group B (22.6%). There were lower morbidity and mortality rates in groups D (70.0%; 30.0%) and E (60.0%; 10.0%) than in group B (80.0%; 55.0%). In conclusion, pre-administration with NSSP decreased clinical signs, morbidity and mortality rates in the vvIBDV-challenged cockerels. Hence, there is need for assessment of the effects of NSSP on other parameters in vvIBDV-challenged birds.

Keywords: *Nigella sativa*, IBD, clinical signs, morbidity, mortality.

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious immunosuppressive disease that predominantly affects young chickens, and poses a major threat to the poultry industry worldwide. It is caused by the IBD virus (IBDV), and results in significant economic losses due to increased mortality, poor feed conversion, decreased weight gain, and increased susceptibility to secondary infections and vaccination failures. The very virulent strain of IBDV (vvIBDV) has exacerbated these challenges, causing severe outbreaks even in vaccinated flocks and threatening the sustainability of poultry production, particularly in developing countries where backyard and commercial poultry farming are critical sources of livelihood (Orakpoghenor *et al*., 2020; 2021; Wagari, 2021; Lawal and Bello, 2021; Beshah *et al*., 2024).

Clinically, IBD is characterized by depression, ruffled feathers, anorexia, diarrhea, and reluctance to move, often progressing to high morbidity and mortality within 3 to 5 days post-infection (Orakpoghenor *et al*., 2021; Andamin *et al*., 2023). The virus primarily targets the bursa of Fabricius, leading to its inflammation, haemorrhages, and eventual atrophy, which impairs ability of the bird to mount an effective immune response. The immunosuppressive nature of vvIBDV not only complicates disease management but also undermines the effectiveness of routine vaccinations against other pathogens (Orakpoghenor *et al*., 2020; Gao *et al*., 2024).

Despite the availability of conventional live and inactivated vaccines, the control of IBD, remains a challenge due to antigenic variation, poor vaccine coverage, and maternal antibody interference (Ramon *et*

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al., 2022; Orakpoghenor *et al*., 2023; Ungsyani *et al*., 2025). These limitations have resulted in interest in alternative and complementary strategies that can enhance immune resilience and reduce disease impact, especially in environments where biosecurity and vaccine delivery may be suboptimal. One of such promising approach involves the use of natural immunostimulants and antiviral agents derived from medicinal plants (Tahir and Alsayeqh, 2024).

Herbal remedies have long been recognized for their potential in the management of infectious diseases in both humans and animals. Among these, *Nigella sativa* (black seed) has gained considerable attention due to its wide range of pharmacological properties, including antioxidant, anti-inflammatory, immunomodulatory, and antiviral effects (Hannan *et al*., 2021; Wahab and Alsayari, 2023). Studies have demonstrated that active constituents such as thymoquinone play a critical role in modulation of immune response and provision of protection against various pathogens, thus, making *N. sativa* a viable candidate for the management of viral infections (Fatima Shad *et al*., 2021; Khazdair *et al*., 2021; Shoaib *et al*., 2023). Hence, in this study, we investigated the effects of *N. sativa* seed powder pre-administration on clinical signs, morbidity, and mortality rates in cockerels challenged with a vvIBDV.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this study was obtained from the Ahmadu Bello University committee on Animal Use and Care (ABUCAUC).

Source of Birds

One hundred, one-day-old Dominant black marshal cockerel (DBMC) chicks were purchased from a Hatchery (Zartech, Ibadan) and transported to the Research Pen of the Department of Veterinary Pathology, A.B.U. Zaria.

Housing and Management of Birds

The pen used for housing of the birds was thorough cleaned, washed with water and detergents, and disinfected. The pen was fumigated twice at two-week intervals before the arrival of the chicks. After the arrival of the chicks, the surrounding of the pen was fumigated with disinfectant twice per week till the end of the study. The chicks were brooded on deep litter with a floor space of 0.1 square meters per bird. Wood shavings served as the litter material, and feeders and drinkers each was provided, one for each group. The chicks had access to feed and water *ad libitum*. Rodents, and insects were controlled by the application of rodenticide, and insecticide, respectively, twice at one-week intervals.

Source of Feed

The feed used in this study was Vital feed® chick mash which was purchased from a commercial sales outlet in Zaria.

Sources of Challenge Virus and Vaccine

Very virulent strain of infectious bursal disease virus (vvIBDV) was obtained from Viral Research Department of National Veterinary Research Institute (NVRI), Vom, Plateau State. Vaccines against Newcastle disease (ND): (ND La Sota), produced by NVRI, Vom, Plateau State, was purchased from a commercial sales outlet in Kaduna.

Source of *Nigella sativa* Seed Powder

Nigella sativa seeds was obtained from Herbal Point, Samaru, Zaria, Kaduna State, and was taken to the Department of Botany, Faculty of Life Sciences, A.B.U. Zaria for identification. Thereafter, the seeds were grinded into the powdered form.

Preparation of *Nigella sativa* Seed Powder and Feed Mixture

The *Nigella sativa* seed powder (NSSP) and feed mixture was prepared by mixing 2.8 g of NSSP with 1 kg of feed (Al-Mufarrej, 2014).

Vaccination of Birds against Newcastle Disease

All the chicks were vaccinated against ND using ND vaccine La Sota at 7 and 17 days of age (doa).

Grouping of Birds

The chicks were randomly divided into 5 groups, A, B, C, D and E, of 20 birds each. Birds in groups A (Negative control) and B (Positive control) were administered feed only; C (NSSP from 21 to 27 doa) and D (NSSP from 21 to 27 doa + vvIBDV) were administered the NSSP + feed consecutively from 21 to 27 doa, while E (NSSP from 1 to 42 doa + vvIBDV) was administered the NSSP + feed consecutively for from 1 to 42 doa. At 28 doa, only birds in groups B, D and E were challenged with a vvIBDV orally.

Preliminary Assessment for Presence of Infectious Bursal Disease Antibody

Prior to challenge with vvIBDV, blood was collected on 1, 7, 14, 21 and 28 doa from all chickens via the brachial vein and serum was harvested. The serum was assayed for antibodies against IBDV using enzyme linked immunosorbent assay (ELISA). The antibodies were found to be below protective level at 28 doa.

Challenge of Birds with Very Virulent Infectious Bursal Disease Virus

Each bird was challenged by oral administration of 0.2 mL of vvIBDV suspension with virus titre of $10^{8.50}$ CID_{50}/mL.

Observation of Birds for Signs of Infectious Bursal Disease

Following challenge, the chickens were monitored daily for clinical signs associated with IBDV infection. The number of birds that presented each clinical sign per day was counted and expressed as a percentage (%) of the total number of birds in the group. The per cent of clinical signs per day was then calculated by dividing the individual percentages of clinical signs by the total number of clinical signs for that day. After that, the average per cent clinical signs was calculated by dividing the sum of daily % clinical signs by the total number of days clinical signs were observed. The severity of clinical signs for each species of bird per group was graded on a scale of 0-5 using the average % clinical signs as follows: 0 = no clinical signs (0%), 1 = mild (1-20%), 2 = moderate (21-40%), 3 = severe (41-60%), 4 = very severe (61-80%) and 5 = grave (81-100%) (Orakpoghenor *et al*., 2021).

The morbidity and mortality rates will be determined using the formulae as follows;

$$\text{Morbidity rate} = \frac{\text{Number of sick birds}}{\text{Number of challenged birds}} \times 100\%$$

$$\text{Mortality rate} = \frac{\text{Number of dead birds}}{\text{Number of challenged birds}} \times 100\%$$

Data Analysis

Chart and photograph were used for data presentation. Clinical signs were graded and presented in percentage. Also, morbidity and mortality rates were presented using percentages.

RESULTS

Clinical signs, morbidity and mortality (0.0%) were absent in chickens in groups A and C. The clinical signs observed in chickens challenged with vvIBDV (groups B, D and E) were anorexia, ruffled feathers, somnolence, huddling, prostration and whitish diarrhoea (Figure 1 and Plate I).

The overall clinical sign was severe in chickens of group B (22.6%), and mild in groups D (13.0%) and E (9.6%). The morbidity rates recorded in groups B, D and E were 80.0%, 70.0% and 60.0%, respectively; and the mortality rates were 55.0%, 30.0% and 10.0%, respectively (Figure 1 and Plate I).

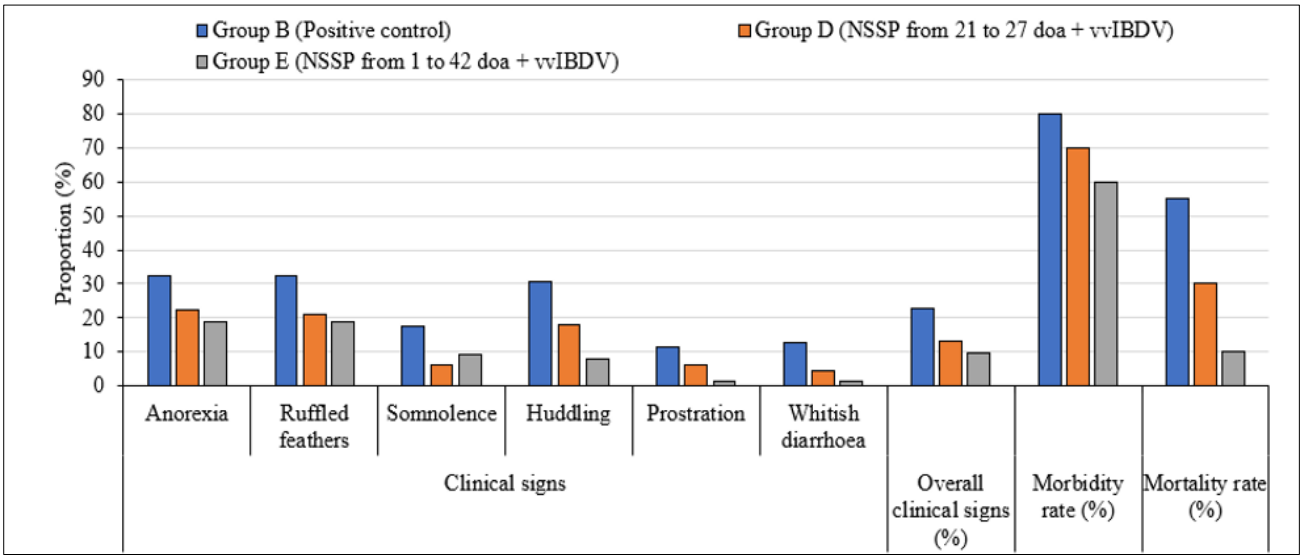

Figure 1: Clinical signs, morbidity, and mortality rates of chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age

NSSP-*Nigella sativa* seed powder; doa-days of age

Plate I: Photographs of chickens, challenged with a very virulent infectious bursal disease virus, in groups B, D and E. Note huddled chickens with ruffled feathers (arrows) in B and D; dead bird in B (db); chickens with ruffled feathers in E (arrows)

DISCUSSION

In this study, chickens in groups A and C, which were not challenged with the vvIBDV, showed no clinical signs or mortality, thus, indicating that the absence of viral infection allows for normal health status in chickens. In contrast, groups B, D, and E, which were challenged with vvIBDV, demonstrated various clinical signs, high morbidity and mortality rates, and these are consistent with previous reports (Orakpoghenor *et al*., 2021; Andamin *et al*., 2023; Shallmizhili *et al*., 2023). However, severity of clinical signs, morbidity and mortality rates were lower in groups E and D compared to group B. This may suggest that the pre-administration of *N. sativa* probably mitigated some of the adverse effects associated with the vvIBDV infection. The mechanisms underlying these findings may involve several factors related to both the viral pathogenesis and the immunological effects of *N. sativa*. The presence of vvIBDV disrupts normal immune function by targeting lymphoid tissues such as the BF, leading to immunosuppression and increased susceptibility to secondary infections (Wagari, 2021). On the other hand, bioactive compounds found in *N. sativa* are known for their antioxidant and anti-inflammatory properties, which could favourably modulate immune responses during viral challenges (Basiouni *et al*., 2023; Hassan and Hassan, 2023; Abbas *et al*., 2024; Alsalahi *et al*., 2024). Therefore, the combined action of these bioactive compounds may be responsible for the mild clinical signs, and lower morbidity and mortality rates in groups D and E, compared to group A. In addition, a previous report suggested major histocompatibility complex class II (MHC II) as a prime molecular target of IBDV (Saxena and Kaur, 2020). The clinical signs and morbidity due to IBDV infection were therefore associated with increased expression of MHC II in target cells (Orakpoghenor *et al*., 2021). Interference with and/or alteration of the expression levels of these molecules in the target cells, by bioactive compounds in the NSSP, might be another possible reason for the milder clinical signs, and lower morbidity and mortality rates in chickens in groups D and E compared to group B.

Moreover, the clinical signs, morbidity and mortality rates were lower in group E compared to group D. This could be attributed to the duration of *N. sativa* seed powder administration prior to the challenge with vvIBDV. The prolonged administration in group E might have enhanced the immune response through sustained activation of antioxidant defenses and immunomodulatory effects (Hannan *et al*., 2021;

Basiouni *et al*., 2023; Alsalahi *et al*., 2024), thus, resulting in milder clinical signs and lower morbidity and mortality rates. Also, interaction with MHC II expression pattern due to their prolonged administration might constitute another possible mechanism. This therefore, suggest that the continuous presence of the bioactive compounds in *N. sativa* may have effectively enhanced resilience of the chickens against the vvIBDV infection, and consequently allowed for improved survival rates despite exposure to the virus.

CONCLUSION

Pre-administration with *Nigella sativa* seed powder decreased the clinical signs, morbidity and mortality rates challenged with a vvIBDV. Hence, the assessment of the effects on other parameters is therefore recommended.

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