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RESEARCH ARTICLE

Pre-Administration with *Nigella sativa* Seed Powder Caused Alterations in Antibody and Serum Interferon Gamma Profiles in Cockerels Challenged with a Very Virulent Infectious Bursal Disease Virus

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Abstract

Infectious bursal disease (IBD) compromises the immune system of young birds, lowering protective antibody levels. This study assessed antibody and serum interferon-gamma (INF-γ) responses in cockerels pre-treated with *Nigella sativa* seed powder (NSSP) and challenged with very virulent IBD virus (vvIBDV). One hundred one-day-old Dominant Black Marshal Cockerels were assigned to five groups (A-E, 20 birds each). Groups A and B received standard feed from 1-42 days of age; groups C and D received NSSP from 21-27 days of age; group E received NSSP continuously from 1-42 days of age. All birds were vaccinated against Newcastle disease. On day 28, groups B, D, and E were orally challenged with vvIBDV. Blood samples were analyzed for IBD antibody and INF-γ. Maternal antibody titres declined below protective levels by 14 days in groups A-D but remained protective in group E. Post-challenge, groups D and E showed significantly higher antibody titres, with group E maintaining the highest levels. Serum INF-γ increased from 7 to 21 days of age in all groups, and from 29 to 42 days of age, group E exhibited the highest levels compared to D and B. Pre-administration of NSSP slowed maternal antibody decay and enhanced post-challenge immune responses. Dietary inclusion of NSSP may therefore provide a natural strategy to sustain IBD antibody protection and improve immune performance in poultry.

Keywords: Antibody, Infectious bursal disease, Interferon gamma, *Nigella sativa*

Introduction

Poultry health is a critical component of global food security and agricultural sustainability, with infectious diseases posing substantial economic and welfare challenges. One of the most devastating diseases affecting young chickens is infectious bursal disease (IBD), an acute and highly contagious viral infection caused by the infectious bursal disease virus (IBDV), particularly its very virulent strains (vvIBDV) [1,2]. Infectious bursal disease primarily targets the bursa of Fabricius leading to immunosuppression and increased susceptibility to secondary infections or vaccine failures [3,4]. The decay of maternally derived antibodies (MAB) over time without adequate immunological response has been identified as a major vulnerability point in early chick development [5]. In addition to antibody-mediated protection, cell-mediated immunity plays a vital role in the host's defense against IBDV infection. Serum interferon-gamma (INF-γ), a key cytokine involved in antiviral and immunomodulatory activities, serves as an indicator of immune activation and resistance in infected or immunostimulated birds [6,7].

Conventional vaccination programmes remain the mainstay for IBD prevention; however, maternal antibody interference and the timing of vaccination present persistent limitations. As maternal antibodies wane, typically within the first two to three weeks of life, chicks become increasingly vulnerable to vvIBDV infections [5,8].

Therefore, understanding and managing the dynamics of maternal antibody decay is essential to optimize vaccination efficacy and ensuring robust immune protection. Also, the use of adjunct strategies that can stabilize or extend protective antibody levels during this critical window are needed to supplement conventional immunization protocols.

Nigella sativa (black seed), a traditional medicinal plant widely studied for its antioxidant, anti-inflammatory, and immunomodulatory properties, has gained attention in veterinary immunonutrition [9-11]. Bioactive compounds in *N. sativa*, particularly thymoquinone, have been shown to modulate immune responses and improve antibody production in various animal models [12,13]. These immunomodulatory effects have been demonstrated across a range of disease conditions, including viral infections such as Newcastle disease and infectious bursal disease, bacterial infections, and inflammatory disorders. In poultry, *N. sativa* supplementation has been associated with enhanced humoral immune responses following vaccination and experimental infections, suggesting its potential role in improving resistance to immunosuppressive diseases such as IBD. Thymoquinone, the major bioactive component, is believed to enhance B-cell activity, cytokine production, and antioxidant defense mechanisms, thereby supporting both humoral and cell-mediated immunity [14-16].

This study was designed to evaluate the impact of pre-administration with *N. sativa* seed powder (NSSP) on the maternal antibody decay and the antibody response in cockerels experimentally challenged with a vvIBDV strain. The study hypothesized that continuous or strategic inclusion of NSSP in chick diets may prolong the protective duration of maternally derived antibodies and enhance post-challenge immune responses. The study aims to provide insights into the potential role of NSSP as a natural immunomodulatory agent through evaluation of the kinetics of antibody titres and serum INF-γ levels across different time points and feeding regimens. This would be significant in the design of better nutritional and health strategies for poultry, and also contribute to the growing interest in phytogenic feed additives as sustainable alternatives to synthetic drugs.

Material and Methods

Ethical Approval

The Ahmadu Bello University committee on Animal Use and Care (ABUCAUC) granted the ethical approval for this study with approval number ABUCAUC/2024/79.

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, Ahmadu Bello University, Zaria.

Housing and Management of Birds

The poultry housing unit was thoroughly cleaned using water and detergent, followed by disinfection. Fumigation of the pen was carried out twice at two-week intervals prior to the arrival of the chicks. Upon their arrival, the area surrounding the pen was fumigated twice weekly with disinfectants throughout the duration of the study. The chicks were brooded on deep litter, and wood shavings were used as bedding material. Each group was provided with its own feeder and drinker. Feed and water were made available to the chicks at all times. Control of rodents and insects was achieved through the application of rodenticides and insecticides, administered twice at one-week intervals.

Source of Feed

Vital feed® chick mash was used in this study, and it was purchased from a commercial sales outlet in Zaria.

Sources of Challenge Virus and Vaccine

The Viral Research Department of National Veterinary Research Institute (NVRI), Vom, Plateau State provided the vvIBDV used. Also, the vaccines against Newcastle disease (ND): (ND La Sota) was produced by NVRI, Vom, and was purchased from a commercial sales outlet in Kaduna.

Source of *Nigella sativa* Seed Powder

Nigella sativa seeds were 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 [14].

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.

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 days of age) and D (NSSP from 21 to 27 days of age + vvIBDV) were administered the NSSP + feed consecutively from 21 to 27 days of age,

while E (NSSP from 1 to 42 days of age + vvIBDV) was administered the NSSP + feed consecutively for from 1 to 42 days of age. At 28 days old, only birds in groups B, D, and E were challenged with a vvIBDV orally.

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.

Collection of Blood

Blood was collected on 1, 7, 14, 21, 28, 29, 30, 31, 32, 33, 34, 35, 38, and 42 days of age from all chickens via the brachial vein and serum was harvested. The serum was assayed for antibodies against IBDV and serum INF-γ using enzyme linked immunosorbent assay (ELISA).

Enzyme Linked Immunosorbent Assays

ELISA was used to detect IBDV antibody and INF-γ in the sera of the cockerels using IBDV antibody test kit (ID Screen® IBD VP2 - ID.vet Innovative Diagnostics, France) and chicken INF-γ ELISA kit (Pioway Medical Lab Equipment Co., Ltd. Nanjing, China), respectively by following the manufacturers' instructions. The optical density (OD) values at 450 nm wavelength were measured using ELISA microtitre plate reader (Thermo Scientific, Multiskan Ex). Thereafter, the absolute level of antibody and INF-γ in the sample was calculated according to the manufacturer's recommendation. For the IBD antibody, the breakthrough titre was 845, and antibody titre ≥845 was considered protective, while titre <845 was considered not protective.

Histopathological evaluation of the Bursa of Fabricius was not included in this study, as the focus was primarily on functional immune responses (antibody titres and serum IFN-γ levels).

Data Analysis

Chart was used for data presentation. Data was expressed as mean ± SEM and subjected to repeated measure One-way analysis of variance (One-way ANOVA) followed by Turkey's *post hoc* test. Graphpad prism version 8.0 (San Diego California, USA) was used for the analysis, and values of P≤0.05 were considered significant.

Results

No mortality was recorded in any of the experimental groups throughout the study period, including after challenge with vvIBDV.

Decay in Maternal Antibody

The mean IBD maternal antibody titre decreased significantly (P<0.05) from protective level (at 1 days of age) to below breakthrough titre (<845) at 14 days of age in chickens of groups A (2067±87.62; 726.2±93.26), B (2121±63.76; 740.6±64.12), C (2107.4±51.05; 716.9±65.43) and D (2132.8±97.74; 738.1±31.99) *(Fig. 1)*. There was a significant (P<0.05) decrease in the mean IBD maternal antibody titre in groups E (2076±63.98; 1008.6±84.03), from 1 to 14 days of age, but the titres were within the protective level (>845). At 21 days of age, the mean IBD maternal antibody titre increased in all groups, but this was significantly (P<0.05) higher and within protective level in group E (1036±61.84) compared to groups A (815.9±46.83), B (797.2±73.67), C (786.7±49.14), and D (786.4±42.56). At 28 days of age, the mean IBD maternal antibody titre was significantly (P<0.05) higher and within protective level in chickens of group E (975.4±47.63) compared to A (576.7±93.26), B (594.6±64.12), C (651.3±51.15), and D (675.3±39.26) *(Fig. 1)*.

Antibody Response Post-Challenge

From 28 to 42 days of age, there was a significant (P<0.05) decrease in the mean IBD antibody titre in groups A (576.7±93.26; 85.25±14.74) and C (651.3±51.15; 60±9.14) *(Fig. 1)*. The mean antibody titre increased (P<0.05) significantly from below protective level (from 28 to 31 days of age) to protective level (from 32 to 38 days of age), and followed by a decrease within protective level (at 42 days of age) in group B (594.6±64.12; 1294±73.29; 917±44.18). In groups D (675.3±39.26; 1585±48.76; 1361±69.95) and E (975.4±47.63; 2118±31.45; 1976±61.63), the mean antibody titre increased (P<0.05) significantly from 28 to 38 days of age, followed by a decrease within protective level at 42 days of age, but the titre was significantly (P<0.05) higher in group E compared to D *(Fig. 1)*.

Changes in Serum Interferon Gamma Level

The mean (±SEM) serum interferon gamma (INF-γ) level showed no significant (P>0.05) difference in all the

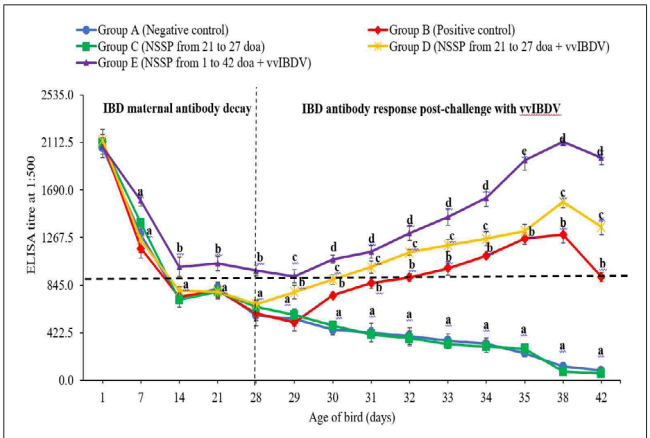

Fig 1. Infectious bursal disease maternal antibody profile of chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age. Values with different alphabets in the same day differ significantly at P<0.05. NSSP-*Nigella sativa* seed powder; doa-days of age

groups of chickens from 1 to 28 days of age, but was non-significantly (P>0.05) higher in group E at 7, 14, 21, and 28 days of age. In all the groups, the mean serum INF-γ level increased (P<0.05) significantly from 7 to 21 days of age, followed by a decrease at 28 days of age, but with no significant difference between the groups *(Fig. 2)*.

From 29 to 35 days of age, the mean serum INF-γ level increased significantly (P<0.05) in groups B, D and E, but was significantly (P<0.05) higher in group E (19.76±5.72; 88.92±5.65 pg/mL) followed by group D (21.27±2.95; 63.32±4.24 pg/mL) compared to group B (9.76±4.83; 51.41±4.60 pg/mL), A (5.68±0.95; 2.93±0.75 pg/mL) and C (7.14±2.83; 3.91±0.89 pg/mL). This was followed by a decrease in the mean serum INF-γ level, at 38 and 42 days of age, but with a significantly (P<0.05) higher value in group E (78.26±4.53; 70.68±7.95 pg/mL) followed by group D (58.05±3.65; 42.98±3.18 pg/mL) compared to group B (47.53±4.13; 36.38±5.66 pg/mL), A (1.27±0.57; 1.74±0.65 pg/mL) and C (2.74±0.92; 2.20±1.09 pg/mL) *(Fig. 2)*.

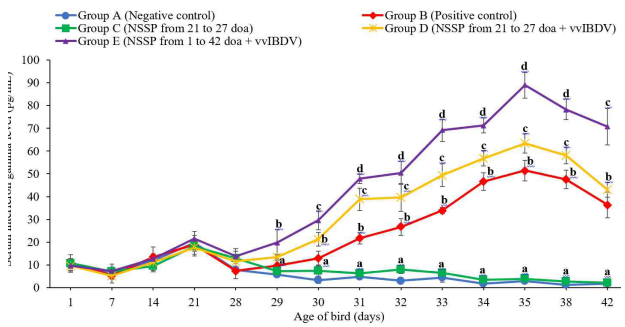

Fig 2. Mean (± SEM) serum interferon gamma level in chickens administered *Nigella sativa* seed powder and challenged with a very virulent infectious bursal disease virus at 28 days of age. Values with different alphabets on the same day differ significantly at P<0.05. NSSP-*Nigella sativa* seed powder; doa-days of age

Discussion

The significant decrease in mean IBD maternal antibody (MAB) in all the groups of chickens, from 1 to 14 days of age in this study is consistent with previous reports [8,17]; therefore, the eventual decline may support the need for timely vaccination strategies to enhance immunity as maternal protection wanes. This decrease over time could be associated with the spontaneous breakdown of MAB [5,18]. The decrease below breakthrough titre at 14 days of age in groups A, B, C, and D might suggest a loss of passive immunity provided by MAB. This decline is vital as it may indicate the high susceptibility of young chicks to IBD during this period, since existing literature has highlighted the role of MAB in providing initial protection against IBD in chickens [5,19].

Moreover, the MAB titres in group E remained at protective levels (compared to groups A and B) until 28 days of age. This may suggest that NSSP administration may delay MAB decay, possibly by preserving immune integrity through its antioxidant and anti-inflammatory effects. Possible mechanism for this decreased MAB decay might be linked to direct or indirect interference with the MAB breakdown processes, by bioactive compounds, such as thymoquinone in NSSP. However, in groups C and D, the MAB levels likely declined more rapidly compared to group E, and this could be associated with the duration of NSSP administration as previously suggested. Hence, this prolonged NSSP administration might have helped to maintain higher levels of MAB for a longer duration, thereby providing better protection against the decay of these antibodies. This may be due to the fact that the continuous presence of bioactive compounds of *N. sativa* might have enhanced the stability and longevity of MAB, and/or decreased their breakdown, thus, reducing their rate of decay. This phenomenon aligns with previous studies that indicated that dietary antioxidants from plants can support immune functions and prolong the effectiveness of MAB in poultry [20-23].

Post-challenge with vvIBDV, there was an increased IBD antibody titre in groups B, D, and E, and this is consistent with previous studies that reported increased IBD antibodies following challenge with IBDV in chickens [8,17,24]. Chickens in group B showed a significant increase in antibody titres from below breakthrough titre to protective levels between 32 to 38 days of age. This may indicate an effective immune response following viral exposure despite initial low titres. In contrast, groups D and E also demonstrated increased antibody titres but maintained significantly higher levels compared to group B throughout the observation period. This finding suggests that NSSP might have played a role in the enhancement of immune responses post-challenge, probably through its immunomodulatory properties [23], as previously suggested. This is supported by the observed quantitative differences, where chickens in group E consistently maintained significantly higher antibody titres compared to other challenged groups, remaining well above the protective threshold (>845) for a longer duration. However, the higher antibody response in group E compared to group D may be associated with a more effective priming of the immune system, and/or enhanced activation and proliferation of B cells, resulting from the sustained administration of NSSP. In addition, the limited administration of NSSP in group D might have resulted in a less effective immune activation following the vvIBDV challenge.

The serum INF-γ levels, from 1 to 28 days of age, suggests that the baseline immune status was similar among the

groups prior to the vvIBDV challenge. The increase in INF-γ levels from 7 to 21 days of age across all groups indicates an initial immune activation, likely as a result of vaccination against Newcastle disease (ND) at 7 and 17 days of age, which is known to stimulate cellular immunity and enhance the overall immune response. Following the vvIBDV challenge, groups B, D, and E demonstrated significant increases in serum INF-γ levels from 29 to 35 days of age, with group E showing the highest levels. This elevation in INF-γ may indicate a robust Th1-type immune response, which is crucial for combating viral infections. In the present study, this was evident from the significantly higher IFN-γ concentrations recorded in NSSP-treated groups, particularly group E, during the post-challenge period, indicating enhanced cellular immune activation. The significantly higher INF-γ levels in group E suggest that pre-administration of NSSP might have enhanced the immune response by promoting lymphocyte activation and proliferation. Previous studies have shown that *N. sativa* possesses immunomodulatory properties that could enhance cytokine production, thereby improving ability of the host to respond to infections [25,26].

At later time points (38 and 42 days of age) in this study, group E continued to show significantly higher INF-γ levels compared to other groups, and this may indicate sustained immune activation. This prolonged elevation may be attributed to the combined effects of *N. sativa* and prior vaccination against ND, which may have primed the immune system for a more effective response against vvIBDV. This suggests that the immune system was more effectively primed due to continuous dietary supplementation and initial ND vaccination, thus, led to a quicker and stronger antibody production in response to vvIBDV challenge. However, this assertion requires further investigation, and it highlights the importance of both vaccination and dietary supplementation in enhancing immune responses in poultry. Although the findings demonstrate clear immunomodulatory effects of NSSP, further investigations incorporating histopathological assessment of lymphoid organs (especially the Bursa of Fabricius), viral load quantification, and broader cytokine profiling would provide deeper insight into the mechanisms underlying these observations.

Pre-administration with *N. sativa* seed powder decreased the maternal antibody decay, and enhanced the antibody response in cockerels challenged with a vvIBDV. Hence, dietary inclusion of NSSP may constitute a natural strategy to sustain protective IBD maternal antibody levels and improve immune response in poultry production. However, further studies integrating pathological and molecular assessments are recommended to fully elucidate the mechanisms involved.

Declaration

Availability of Data and Materials: Data and materials for this research are available upon request.

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Conflict of Interest: The authors declared that there is no conflict of interest.

Declaration of Generative Artificial Intelligence (AI): The authors declare that the article, tables and figures were not written/created by AI and AI-assisted Technologies.

Author Contributions: SBO, BM and IWM contributed to the design of this study. MSM, OO, and MOE participated in the sample collection, data analysis. OO wrote the original draft. All authors contributed to data collection and discussion.

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