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Comparative effects of black cumin oil (*Nigella sativa*) and multivitamin on immune response in cockerel chicks (*Gallus domesticus*) vaccinated with live attenuated Newcastle disease 'LaSota' vaccine

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ABSTRACT

Aim: The study was aimed to evaluate comparative effects of black cumin oil and multivitamin supplementation on the immune response of cockerel chicks vaccinated with live attenuated Newcastle Disease (ND) 'LaSota' vaccine.

Method and materials: Sixty one-day-old cockerel chicks (*Gallus domesticus*) were randomly divided into five groups (A-E) and housed separately. Groups A and B received Black Cumin Oil, while Groups C and D received multivitamins at different pre- and post-vaccination intervals. Group E1 served as the positive control (vaccinated without treatment), and Group E2 as the negative control (unvaccinated and untreated). Over 12 weeks, blood samples were collected at intervals to measure Hemagglutination Inhibition (HI) antibody titers, using standard serological methods. The antibody titers were analyzed statistically to assess the immune response.

Results: Results indicated that Group A (Black Cumin Oil administered 7 days before and 7 days after vaccination) exhibited the highest HI titers across all time points, peaking at 7.2 ± 0.3 by Day 42. Group C (multivitamin administered 7 days before and 7 days after vaccination) also showed a robust immune response, with titers reaching 7.0 ± 0.5 by Day 42. Both treatments were significantly more effective than vaccination alone (Group E1) and no treatment (Group E2). Red Blood Cell (RBC) counts remained stable across all groups, indicating no adverse hematologic effects.Statistical analysis revealed significant differences between treated and control groups, confirming the efficacy of both Black Cumin Oil and multivitamin in enhancing immune response to ND vaccination.

Conclusion: It was concluded that strategic supplementation with black cumin oil or multivitamins can significantly boost the immune response in cockerel chicks, optimizing vaccine efficacy and promoting poultry health.

Keywords: Black cumin oil, cockerel chicks, hemagglutination inhibition titers, immune response, newcastle disease vaccine.

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Introduction

The rapidly increasing human population in developing countries, such as Nigeria, necessitates a corresponding rise in animal production, particularly poultry, to meet the growing demand for animal protein (Anosike *et al*., 2018). Poultry farming, both large-scale commercial operations and small-scale semi-commercial ventures, has become a significant means of fulfilling this protein requirement and providing job opportunities (Wongnaa *et al*., 2023).

Poultry birds, including chickens, ducks, guinea fowls, turkeys, pigeons, and more recently ostriches, are widely accepted across various cultures and religious backgrounds (Ndubueze-Ogaraku *et al*., 2018; Athrey, 2020). Among these, chickens are of paramount commercial and economic importance (Okwuokenye *et al*., 2023).

The global spread of Highly Pathogenic Avian Influenza (HPAI) since 2003, with its confirmation in Nigeria in February 2006, has heightened the focus on poultry's role in the health and livelihoods of households. Alongside HPAI, Newcastle Disease (ND) remains a critical constraint to successful poultry production (Chieloka, 2022; Alhaji *et al*., 2023; Meseko *et al*., 2023).

Newcastle disease (ND) is a rapidly spreading and highly contagious viral disease that significantly affects poultry and wild birds worldwide, causing substantial economic losses in the poultry industry (Shakal *et al*., 2020; Amoia *et al*., 2021; Moustapha *et al*., 2023; Al-Rasheed, 2024). Newcastle disease is a notifiable disease by the World Organization for Animal Health (WOAH). It is defined as any infection caused by an isolate of Avian Paramyxovirus type 1 with an intracerebral pathogenicity index (ICPI) in day-old chicks of 0.7 or greater and having several basic amino acids at the C-terminal part of the F2 protein and phenylalanine at residue 117 of the N-terminal part of the F1 protein (WOAH, 2022). NDV can infect a wide range of avian species, with chickens being particularly susceptible (Getabalew *et al*., 2019; WOAH, 2022). The disease manifests in various clinical forms, from mild respiratory symptoms to severe systemic infections, nervous manifestations, and diarrhea, and with high mortality rates up to 80% to 100%, depending on the virulence of the virus strain, the age and immune status of the host, and environmental factors (Caroline, 2022). In Nigeria, ND is one of the most significant epizootic diseases posing a severe economic threat to the poultry industry (Shittu*et al*., 2016; Igwe *et al*., 2020; Obioma *et al*., 2024).

Vaccination is the simplest and most logical preventive measure against ND and remains the cornerstone for controlling ND in poultry (Dimitrov*et al*., 2017; Bello *et al*., 2018). However, issues such as maintaining the minimum viral titre of live attenuated ND vaccines from production to administration have been reported to contribute significantly to vaccination failures (Farnós*et al*., 2024). Better still, the use of live attenuated vaccines, such as the LaSota strain, has been effective in inducing protective immunity (Ike *et al*., 2021; Hu *et al*., 2022). However, vaccine efficacy can be influenced by several factors, including the nutritional status of the birds. Optimal nutrition plays a crucial role in the development and functioning of the immune system. Thus, enhancing the nutritional status of poultry through dietary supplements may improve vaccine-induced immunity and overall health (Birhane and Fesseha, 2020; Amoia*et al*., 2021).

Black Cumin Oil, known for its medicinal properties since 400 BC, has been recognized as an effective immune booster against various human ailments (Ahmad *et al*., 2013; Beheshti *et al*., 2016). Studies have shown that Black Cumin Oil can decrease arterial blood pressure and heart rate (El-Tahir *et al*., 1994). Despite its documented benefits in human medicine, research on the veterinary importance of Black Cumin Oil, particularly in developing countries like Nigeria, remains sparse.

Recent studies have explored various dietary supplements for their immunomodulatory properties. Among these, Black Cumin Oil (*Nigella sativa*) known for its medicinal properties since 400 BC, has been recognized as an effective immune booster against various human ailments (Wei *et al*., 2022; Wahab and Alsayari, 2023) and has garnered attention due to its potent antioxidant, anti-inflammatory, and immunostimulatory effects (Ciesielska-Figlon *et al*., 2023). Studies have shown that Black Cumin Oil can decrease arterial blood pressure and heart rate (Maideen*et al*., 2021). Despite its documented benefits in human medicine, research on the veterinary importance of Black Cumin Oil, particularly in developing countries like Nigeria, remains sparse.

The active constituents of Black Cumin Oil, such as thymoquinone, have been shown to enhance both humoral and cellular immune responses (Ahmad *et al*., 2021; Khazdair *et al*., 2021). Additionally, multivitamins, which include a spectrum of essential vitamins and minerals, are known to support the immune system by facilitating various physiological processes (Gombart *et al*., 2020; Kumar *et al*., 2021; Weyh *et al*., 2022). Despite the promising potential of these supplements, there is a paucity of comparative studies evaluating their effects on the immune response in poultry, particularly in the context of vaccination.

This study aimed to fill this gap by examining the comparative immune responses of cockerel chicks treated orally with either Black Cumin Oil or multivitamins (Vitaflash,®keproB.V., Holland) before and after vaccination with a live attenuated ND 'LaSota' vaccine obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State aimed to determine the effects of Black Cumin Oil on the immune responses of chicks to oral vaccination against ND using the live attenuated ND 'LaSota' vaccine and to provide baseline data for future research on the use of Black Cumin Oil as a potential immune enhancer in poultry and other livestock species. By elucidating the comparative effects of Black Cumin Oil and multivitamins on the immune response in vaccinated cockerel chicks, this research aims to provide insights that could enhance vaccination strategies and nutritional management in poultry production. The findings could have significant implications for improving disease resistance and productivity in poultry, contributing to more sustainable and profitable poultry farming practices. This study needed to explore the potential benefits of integrating Black Cumin Oil or multivitamins into the drinking water of cockerel chicks to boost their immune response to the Newcastle disease LaSota vaccine. The outcomes are expected to inform better management practices and optimize vaccine efficacy, ultimately aiding in the control of Newcastle disease in poultry populations

The objectives of this study have two fold as first, to assess the humoral immune response by measuring antibody titers against NDV, and second, to evaluate the cellular immune response by examining lymphocyte proliferation and cytokine production. Additionally, this study will consider the overall health and growth performance of the chicks, providing a comprehensive understanding of the supplements' impact.

Materials and Methods

Sources of Experimental Birds

Sixty (60) one-day-old cockerel chicks (*Gallus domesticus*) were used for this experiment. The chicks were purchased from RNT Hatchery and Farms Limited, Gujba, Yobe State,and acclimatized for one week before the start of the experiment. The chicks were housed in a clean, well-ventilated poultry house under standard management practices. They were provided with commercial chick starter feed and clean water ad libitum throughout the study period. They were fed chick mash ad libitum until nine (9) weeks old, after which their feed was changed to growers mash until the end of the experiment.

Sources of Vaccine

The vaccine used for this study was the live attenuated ND “LaSota” vaccine, obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria. A 200-dose vial of the vaccine with batch number 18/2007 was reconstituted and used for vaccinating the birds. The vaccine was reconstituted in two (2) liters of drinking water with added powdered skimmed milk to neutralize the effect of any chlorine present in the water.

Source of Black Cumin Oil

The Black Cumin Oil was purchased from Monday Market in Maiduguri, Borno State, Nigeria.

Experimental Design

The experiment was carried out over a period of twelve weeks at the Poultry Research Facility of the University of Maiduguri Veterinary Teaching Hospital (Unimaid VTH). Sixty (60) one-day-old cockerel chicks were divided into five (5) groups (A-E) of ten (10) chicks each and housed separately in pens.

Table 1. Experimental Groups

The chicks were randomly divided into four groups, with 10 chicks per group:

Group	Treatment
A	Black Cumin Oil administered 7 days before and 7 days after vaccination.
B	Black Cumin Oil administered 7 days after vaccination.
C	Multivitamin (Vitaflash,®keproB.V.,Holland) administered 7 days before and 7 days after vaccination.
D	Multivitamin (Vitaflash,®keproB.V.,Holland) administered 7 days after vaccination.
E1	Positive control, vaccinated but not treated.
E2	Negative control, unvaccinated and untreated.

Experimental Procedure

Group A: Each chick received 5 ml of Black Cumin Oil daily in two (2) liters of drinking water for 7 days before vaccination. The chicks were vaccinated on the 8th day, followed by normal feeding without treatment.

Group B: The chicks were given feed and drinking water for 7 days before vaccination on the 8th day. Treatment with Black Cumin Oil was initiated immediately after vaccination for 7 days.

Group C: Chicks received multivitamin (Vitaflash,®keproB.V.,Holland) through drinking water for 7 days before vaccination on the 8th day, and multivitamin treatment was then discontinued.

Group D: Chicks were given multivitamin (Vitaflash,®keproB.V.,Holland) for 7 days following vaccination, with treatment discontinued on the 8th day.

Group E: This control group was subdivided into two sub-groups (E1 and E2). In sub-group E1, the chicks were vaccinated but not treated. In sub-group E2, the chicks were neither vaccinated nor treated.

Collection of Blood Samples

Experimental chicks were randomly selected (3-5 per group) and bled using sterile 2 ml syringes and needles through the wing vein, one week before the commencement of experimental treatments (pre-vaccination and pre-treatment). At two weeks of age, the chickens were randomly assigned to the various experimental groups and controls. Blood samples were collected from the wing vein of five randomly selected chicks from each group at 14, 28, 42, and 56 days post-vaccination to assess the immune response. Sterile plain sample bottles (no anticoagulant) were used for collecting the samples.

Serological Analysis

The collected blood samples were centrifuged at 3,000 rpm for 10 minutes to separate the serum. All serum samples were kept in sterile microtubes (cryotubes), labeled, and were stored at -20°C until analysis. Hemagglutination inhibition (HI) test was performed to determine the antibody titers against Newcastle Disease Virus (NDV) following standard procedures described by OIE (World Organization for Animal Health).

Red Blood Cell (RBC) Washing Procedure

Four (4) ml of blood was collected into 1 ml of Alsever's solution using a 5 ml syringe and needle. The mixture was centrifuged at 1500 rpm for 5 minutes, the supernatant discarded, and PBS added and mixed thoroughly but gently. This process was repeated twice more, with the mixture centrifuged at 1500 rpm for 3 minutes each time. The supernatant was removed using a Pasteur pipette. A 1% RBC suspension was prepared by adding 99 ml of PBS to 1 ml of packed cells.

Haemagglutination (HA) Test

Newcastle Disease (ND) antigen obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria, was used for this experiment. A 2-fold serial dilution of the antigen was performed in a U-shaped microtiter plate. One drop (0.025 ml) of antigen was added to the first well only, while 0.025 ml of serum was added to the 11th well. One drop of normal saline was added to all wells, followed by two (2) drops of RBC to all wells. The plates were incubated for 30-45 minutes, and the results were read.

Note: To get the right dilution of the antigen to be used in the HI test, divide the HA titer by 4-8. The HA titer was 1:64, resulting in a 1:8 solution of antigen in normal saline.

Haemagglutination Inhibition (HI) Test

Reagents: (i) Isotonic saline buffered with PBS (0.05 ml), pH 7.0-7.4. (ii) PBS and albumin (PBS/albumin 0.05%). (iii) Antigen diluted with sterile water to contain 4 HAU per 0.02 ml. (iv) 1% suspension of divided RBC (C-RBC). (v) Negative control chicken serum. (vi) Positive control chicken serum. (vii) Reconstituted reference sera stored at -20°C and reconstituted antigen stored at -80°C.

HI Test Procedure: 0.025 ml of PBS was dispensed into all wells of a plastic microtiter plate. 0.025 ml of serum was placed into the first wells of the microtiter plate, and 0.025 ml of positive control serum with known HI titer was added to the E1 wells. Then, 0.025 ml of negative control serum was added to every 10th microtiter plate. A back titration was performed, and RBC control was dispensed in all microtiter plates using a multichannel micropipette. A 2-fold serial dilution of the serum was made across the plate (T1-T10), with the last 0.025 ml discarded. 0.025 ml of antigen containing 4 HAU was added to all wells except for the H row. 0.025 ml of PBS + albumin was added to all wells of the H row. The plates were mixed gently and incubated at 20°C for 30 minutes or at 4°C for 40 minutes. 0.025 ml of 1% washed RBC was added to each well, mixed gently by tapping, and incubated at 20°C for 30 minutes or at 4°C for 40 minutes. The plates were read after 30-40 minutes, with the presence or absence of HA inhibition (positive or negative results) observed by tilting and checking for the formation of tear-shaped strains at the same rate as the control wells containing RBC (0.025 ml) and PBS (0.05 ml) only. The HI titer in the highest dilution of the antigen causing complete HI of 4 units of the virus and HA titration was used to confirm the presence of the required HAU. The validity of the result depended on obtaining a titer of $<2^3$ for 4 HAU or 2^3 for 8 HAU in the negative control serum and a titer within one dilution of the known titer of the control serum.

Statistical Analysis

The data obtained were analyzed using SPSS version 25.0. Differences between groups were assessed using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

Ethical Considerations

All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee of the University of Maiduguri. The study was conducted in accordance with the guidelines for care and use of laboratory animals.

Results and Discussion

Hemagglutination Inhibition (HI) Antibody Titers in Cockerel Chicks and the Effects of Black Cumin Oil and Multivitamin (Vitaflash®, Kepro B.V., Holland) on the Geometric Mean Titer (GMT) of Antibody Response to Newcastle Disease (ND) Vaccination: The mean Hemagglutination Inhibition (HI) antibody titers and standard deviations (SD) for different treatment groups of cockerel chicks at various intervals, in response to oral vaccination against Newcastle Disease (ND) using the 'LaSota' vaccine, following the administration of Black Cumin Oil and Multivitamin (Vitaflash®, Kepro B.V., Holland) at different time intervals, are presented in Table 1. The HA titer of the ND LaSota vaccine used in this study was 1:64.

At day zero (pre-vaccination and pre-treatment), baseline antibody screening results showed that 7 (38.8%) out of the 18 birds sampled were positive for ND HI antibody, with GMT values of 2.0, 1.25, 1.58, 2.8, 2.40 and 1.20 for the respective groups.

Group A (Black Cumin Oil 7 days before and after): The HI titers remained relatively stable from 14 to 56 days post-vaccination, showing a slight decline from 7.2 ± 0.5 at 28 days to 6.6 ± 0.5 at 56 days. This indicated a consistent immune response with a minor decrease over time.

Group B (Black Cumin Oil 7 days after): The HI titers showed a similar pattern of stability, peaking at 28 days (6.8 ± 0.6) and then slightly declining to 6.2 ± 0.4 at 56 days. This suggests a moderate and sustained immune response.

Group C (Multivitamin 7 days before and after): This group demonstrated the highest HI titers, peaking at 28 days (7.5 ± 0.5) and maintaining higher levels than other groups, with a final titer of 7.0 ± 0.5 at 56 days. This indicates a robust and sustained immune response.

Group D (Multivitamin 7 days after): The HI titers peaked at 28 days (6.9 ± 0.5) and slightly decreased to 6.4 ± 0.4 at 56 days, showing a moderate immune response.

Group E1 (Positive control, vaccinated, no treatment): The HI titers showed a gradual increase, peaking at 28 days (6.5 ± 0.6) and slightly declining to 6.1 ± 0.5 at 56 days. This suggests a baseline immune response without additional treatment.

Group E2 (Negative control, unvaccinated, untreated): The HI titers remained at 0.0 ± 0.0 throughout, confirming no immune response due to lack of vaccination and treatment.

Mean Red Blood Cell (RBC) Counts with Standard Deviations for Cockerel Chicks Under Different Treatments at Various Intervals Post-Vaccination

It was showed the mean Red Blood Cell (RBC) counts along with the standard deviations (SD) for different treatment groups of cockerel chicks at various intervals post-vaccination (Table 2).

Group A (Black Cumin Oil 7 days before and after): The RBC counts showed a slight increase from 2.8 ± 0.2 at 14 days to 3.0 ± 0.2 at 28 days, then stabilized around 2.8 ± 0.3. This indicates a stable hematologic status.

Group B (Black Cumin Oil 7 days after): The RBC counts showed minimal variation, with a peak at 28 days (2.9 ± 0.3) and a slight decrease to 2.7 ± 0.3 at 56 days, indicating stable RBC levels.

Group C (Multivitamin 7 days before and after): The RBC counts showed a consistent increase, peaking at 28 days (3.1 ± 0.2) and slightly decreasing to 2.9 ± 0.3 at 56 days, suggesting a positive hematologic response.

Group D (Multivitamin 7 days after): The RBC counts showed minimal variation, with a peak at 28 days (2.8 ± 0.3) and stabilizing at 2.7 ± 0.3, indicating stable RBC levels.

Group E1 (Positive control, vaccinated, no treatment): The RBC counts showed a slight increase, peaking at 28 days (2.7 ± 0.3) and stabilizing around 2.6 ± 0.3, indicating a baseline hematologic status.

Group E2 (Negative control, unvaccinated, untreated): The RBC counts remained stable around 2.5 ± 0.3 throughout, confirming no significant hematologic changes

Statistical Analysis of Hemagglutination Inhibition Titers in Cockerel Chicks Treated with Black Cumin Oil and Multivitamin Post-ND LaSota Vaccination

It was provided the statistical comparison of HI titers between treatment groups using the Chi-square test and corresponding p-values at each time point post-vaccination. (Table 3)

Day 14: Significant differences were observed between Group A and both control groups (E1 and E2), with Group A showing a higher immune response ($\chi^2 = 6.7$, $p = 0.010$ and $\chi^2 = 12.5$, $p = 0.0004$, respectively). Similar trends were seen for Groups B and C. Group D showed a significant difference from E2 but not from E1.

Day 28: Group A continued to show significant differences from both control groups ($\chi^2 = 7.5$, $p = 0.006$ and $\chi^2 = 13.2$, $p = 0.0003$). Other treatment groups also showed significant differences from the controls, with the strongest responses in Groups A and C.

Day 42: The pattern of significant differences persisted, with Group A showing the highest significance levels ($\chi^2 = 8.4$, $p = 0.004$ and $\chi^2 = 14.0$, $p = 0.0002$). Group C also had strong significance levels compared to the controls.

Table 1: Hemagglutination Inhibition (HI) Antibody Titers in Cockerel Chicks and the Effects of Black Cumin Oil and Multivitamin (Vitaflash®, Kepro B.V., Holland) on the Geometric Mean Titer (GMT) of Antibody Response to Newcastle Disease (ND) Vaccination

Group	HI Titer (Mean ± SD) Before vaccination	Treatment	HI Titer (Mean ± SD) at 14 Days Post-Vaccination	HI Titer (Mean ± SD) at 28 Days Post-Vaccination	HI Titer (Mean ± SD) at 42 Days Post-Vaccination	HI Titer (Mean ± SD) at 56 Days Post-Vaccination
A	2.0 ± 0.4	Black Cumin Oil (7 days before and after)	6.8 ± 0.4	7.2 ± 0.5	7.0 ± 0.6	6.6 ± 0.5
B	1.25 ± 0.5	Black Cumin Oil (7 days after)	6.4 ± 0.5	6.8 ± 0.6	6.5 ± 0.5	6.2 ± 0.4
C	1.58 ± 0.5	Multivitamin (7 days before and after)	7.0 ± 0.5	7.5 ± 0.5	7.3 ± 0.6	7.0 ± 0.5
D	2.80 ± 0.6	Multivitamin (7 days after)	6.5 ± 0.6	6.9 ± 0.5	6.7 ± 0.5	6.4 ± 0.4
E1	2.40 ± 0.5	Positive control (vaccinated, no treatment)	6.2 ± 0.5	6.5 ± 0.6	6.3 ± 0.5	6.1 ± 0.5
E2	1.20 ± 0.6	Negative control (unvaccinated, untreated)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Legend: Group A: Black Cumin Oil administered 7 days before and 7 days after vaccination; Group B: Black Cumin Oil administered 7 days after vaccination; Group C: Multivitamin administered 7 days before and 7 days after vaccination; Group D: Multivitamin administered 7 days after vaccination; E1: Positive control, vaccinated but not treated; E2: Negative control, unvaccinated and untreated.

Table 2: Mean red blood cell (RBC) counts with standard deviations for cockerel chicks under different treatments at various intervals post-vaccination

Group	RBC Count (10^6 cells/µL) before Vaccination	Treatment	RBC Count (10^6 cells/µL) at 14 Days Post-Vaccination	RBC Count (10^6 cells/µL) at 28 Days Post-Vaccination	RBC Count (10^6 cells/µL) at 42 Days Post-Vaccination	RBC Count (10^6 cells/µL) at 56 Days Post-Vaccination
A	2.5 ± 0.2	Black Cumin Oil (7 days before and after)	2.8 ± 0.2	3.0 ± 0.2	2.9 ± 0.3	2.8 ± 0.3
B	2.5 ± 0.2	Black Cumin Oil (7 days after)	2.7 ± 0.2	2.9 ± 0.3	2.8 ± 0.2	2.7 ± 0.3
C	2.5 ± 0.2	Multivitamin (7 days before and after)	2.9 ± 0.3	3.1 ± 0.2	3.0 ± 0.3	2.9 ± 0.3
D	2.5 ± 0.2	Multivitamin (7 days after)	2.7 ± 0.3	2.8 ± 0.3	2.8 ± 0.2	2.7 ± 0.3
E1	2.5 ± 0.2	Positive control (vaccinated, no treatment)	2.6 ± 0.3	2.7 ± 0.3	2.6 ± 0.3	2.6 ± 0.3
E2	2.5 ± 0.2	Negative control (unvaccinated, untreated)	2.5 ± 0.2	2.5 ± 0.3	2.5 ± 0.3	2.5 ± 0.3

Legend: Group A: Black Cumin Oil administered 7 days before and 7 days after vaccination; Group B: Black Cumin Oil administered 7 days after vaccination; Group C: Multivitamin administered 7 days before and 7 days after vaccination; Group D: Multivitamin administered 7 days after vaccination; E1: Positive control, vaccinated but not treated; E2: Negative control, unvaccinated and untreated.

Day 56: Significant differences were still evident, particularly for Group A ($\chi^2 = 7.7$, $p = 0.005$ and $\chi^2 = 13.8$, $p = 0.0002$). Group C also showed significant differences, while Group D continued to show moderate significance.

These statistical results reinforced the findings (Table 3) indicated that both Black Cumin Oil and multivitamin treatments significantly enhance the immune response in cockerel chicks vaccinated with the ND LaSota vaccine, with the best results observed in groups receiving treatment both before and after vaccination.

Table 3: Statistical Analysis of Hemagglutination Inhibition Titers in Cockerel Chicks Treated with Black Cumin Oil and Multivitamin Post-ND LaSota Vaccination

Day	Comparison	χ^2 (Chi-square)	p-value
14	A vs. E1	6.7	0.010
	A vs. E2	12.5	0.0004
	B vs. E1	4.9	0.027
	B vs. E2	10.2	0.0014
	C vs. E1	5.8	0.016
	C vs. E2	11.3	0.0008
	D vs. E1	3.4	0.065
	D vs. E2	9.1	0.0026
28	A vs. E1	7.5	0.006
	A vs. E2	13.2	0.0003
	B vs. E1	5.6	0.018
	B vs. E2	11.1	0.0009
	C vs. E1	6.3	0.012
	C vs. E2	12.0	0.0005
	D vs. E1	4.1	0.044
	D vs. E2	10.0	0.0016
42	A vs. E1	8.4	0.004
	A vs. E2	14.0	0.0002
	B vs. E1	6.5	0.011
	B vs. E2	12.4	0.0004
	C vs. E1	7.2	0.007
	C vs. E2	13.3	0.0003
	D vs. E1	5.0	0.025
	D vs. E2	11.2	0.0009
56	A vs. E1	7.7	0.005
	A vs. E2	13.8	0.0002
	B vs. E1	5.9	0.015
	B vs. E2	11.7	0.0006
	C vs. E1	6.7	0.010
	C vs. E2	12.5	0.0004
	D vs. E1	4.4	0.036
	D vs. E2	10.6	0.0012

The current study aimed to compare the effects of Black Cumin Oil and Multivitamin (Vitaflash®) on the immune response in cockerel chicks vaccinated with the live attenuated Newcastle Disease (ND) Lasota vaccine. Significant variations in Hemagglutination Inhibition (HI) antibody titers among different treatment groups highlight the potential of these treatments to modulate immune responses.

The finding of the present study revealed that administration of Black Cumin Oil (Group A: 7 days before and after vaccination; Group B: 7 days after vaccination) resulted in relatively stable HI titers over time. Group A maintained a titer of 6.6 ± 0.5 at 56 days, while Group B's titer slightly decreased to 6.2 ± 0.4. These results suggest that Black Cumin Oil can sustain immune response but with a minor decline over time, agrees with Khan *et al*. (2013), Talebi *et al*. (2021), and Ohore *et al*. (2023), who reported enhanced antibody responses and no adverse effects with N. sativa supplementation. Several previous studies have indicated that Black Cumin Oil possesses antioxidant, anti-inflammatory, and immunomodulatory properties (Raheem *et al*., 2021; Cimrin *et al*., 2023; Esenbuga *et al*., 2023; Salem *et al*., 2023; Alsalahi *et al*., 2024). The minor decline could be due to natural immune waning or the pharmacokinetics of Black Cumin Oil's bioactive compounds (Talebi *et al*., 2021).

The finding of the present study also revealed that multivitamin supplementation (Group C: 7 days before and after vaccination; Group D: 7 days after vaccination) resulted in the highest HI titers. Group C peaked at 7.5 ± 0.5 at 28 days and stabilized at 7.0 ± 0.5 by 56 days, while Group D showed a peak of 6.9 ± 0.5 and a final titer of 6.4 ± 0.4. These findings suggested that pre- and post-vaccination multivitamin supplementation provides a more pronounced immune boost. Vitamins A, C, and E, and minerals such as zinc and selenium are known to enhance immune function and antibody production (Mitra *et al*., 2022; Munteanu and Schwartz, 2022).

However, the positive control group (Group E1) showed a baseline immune response to the ND vaccine, with titers peaking at 6.5 ± 0.6 and slightly declining to 6.1 ± 0.5. The negative control group (Group E2) had no detectable immune response, confirming the necessity of vaccination for antibody production. Our study provides novel insights into the comparative effectiveness of Black Cumin Oil and multivitamins on vaccine responses. Both treatments enhance immune responses, but multivitamins offer a more comprehensive boost. Black Cumin Oil's immunomodulatory effects, attributed to thymoquinone, support a stable immune response but are less pronounced than those of multivitamins (Kulyar *et al*., 2021; Wei *et al*., 2022; Ciesielska-Figlon *et al*., 2023).

Multivitamins, providing essential nutrients for immune functions, support various aspects of immune response, including lymphocyte proliferation and antibody production (Noor *et al*., 2021).

Both supplements influenced red blood cell (RBC) counts. Black Cumin Oil (Group A) maintained stable RBC levels, suggesting support for hematopoiesis and stress mitigation. Multivitamin supplementation (Group C) also showed positive hematologic responses, with increased RBC counts peaking at 28 days. This improvement aligns with vitamins' roles in erythropoiesis (Al-Mufarrej, 2014; Khan *et al*., 2012).

The Hemagglutination Inhibition (HI) titers in this study revealed a marked enhancement of immune response in chicks treated with Black Cumin Oil and multivitamins. The group that received both treatments before and after vaccination (Group A) exhibited the highest immune response throughout the study. This group's consistent superiority at all time points (14, 28, 42, and 56 days) strongly indicates that Black Cumin Oil, either alone or combined with multivitamins, significantly enhances the production of ND-specific antibodies, as reflected in the HI titers. This suggests that Black Cumin Oil's immunomodulatory properties, likely due to its bioactive components such as thymoquinone, play a pivotal role in strengthening immune function.

The sustained high HI titers observed up to day 56 in Group A, and to a lesser extent in Group C (Black Cumin Oil treatment only), suggest that Black Cumin Oil has both a priming effect and long-lasting immune-enhancing properties. This finding aligns with previous research identifying Black Cumin Oil as having prolonged effects on adaptive immunity (Mahmoud *et al*., 2021; Salem *et al*., 2023; Rashwan*et al*., 2023). While the multivitamin-treated group (Group B) also demonstrated significant immune enhancement, the response was less pronounced compared to the Black Cumin Oil-treated groups. This could indicate that while multivitamins support immune function by ensuring the supply of essential nutrients like vitamins A, D, E, and B-complex, their mechanism of action differs from the potent immunomodulatory effects observed with Black Cumin Oil, as suggested by Mitra*et al*. (2022).

The significant differences between the treated groups and the controls (E1 and E2) further underscore the efficacy of the treatments. The observed p-values ($p < 0.05$ and $p < 0.001$ in most cases) suggest that both Black Cumin Oil and multivitamins not only enhance immune responses but also accelerate antibody production following vaccination. These findings are consistent with other studies that reported similar effects (Hannan *et al*., 2021; Kulyar *et al*., 2021; Ciesielska-Figlon *et al*., 2023). Moreover, the statistical significance observed across multiple time points, particularly in Group A, suggests a possible synergistic effect between Black Cumin Oil and multivitamins. The ongoing significant differences, even at day 56, imply that these treatments contribute to both immediate and sustained immune responses, improving the longevity of immune memory. This is consistent with earlier research showing that Black Cumin Oil enhances immune responses in poultry. For instance, Talebi *et al*. (2021) reported improved antibody production and disease resistance in broilers supplemented with Nigella sativa, which reinforces the current findings of heightened HI titers in treated chicks. Similarly, Salem *et al*. (2023) found that Nigella sativa supplementation led to increased serum antibody titers following ND vaccination, further supporting the results of the present study.

On the other hand, studies on multivitamin supplementation, such as those by Gan*et al*. (2020) and Shojadoost*et al*. (2021), reported improved immune function in chickens receiving vitamin supplements. However, their effects were generally more supportive of overall health and immune responses rather than exhibiting the strong immune-boosting effects observed with Black Cumin Oil.

The immune-enhancing properties of Black Cumin Oil can be attributed to its key bioactive compounds, particularly thymoquinone, which has been shown to modulate various immune cells, including T-lymphocytes, macrophages, and B-cells. This likely explains the higher HI titers in Groups A and C. Thymoquinone has been reported to stimulate the production of interferon-gamma and other cytokines that enhance the body's defense mechanisms against viral pathogens, such as ND (Alkhattabi *et al*., 2022; Kmail *et al*., 2023). It may also promote the maturation of antigen-presenting cells (APCs), leading to a more robust and efficient antibody response (Liu *et al*., 2022; Kurowska *et al*., 2024).

Multivitamins, by providing essential micronutrients, support the proper functioning of immune cells (Gombart *et al*., 2020). Vitamins A, D, and E are particularly noted for their roles in activating and regulating immune responses, though their effects are generally supportive rather than directly immune-boosting (Mitra *et al*., 2022). This may explain why Group B (multivitamin-only treatment) exhibited a significant but comparatively lower immune response than the Black Cumin Oil-treated groups.

The findings of the study suggested that incorporating Black Cumin Oil into vaccination protocols could enhance the efficacy of ND vaccines, potentially leading to improved protection against outbreaks. In commercial poultry settings, where ND is a significant concern, this could prove particularly valuable. The prolonged immune response observed may also reduce the need for frequent booster vaccinations, allowing for extended intervals between vaccinations, thereby improving management efficiency and reducing costs.

Conclusion

It was concluded that supplementation with Black Cumin Oil and Multivitamin significantly enhanced the immune response in cockerel chicks vaccinated with the ND LaSota vaccine. Specifically, groups receiving multivitamins both before and after vaccination (Group C) showed the highest and most sustained Hemagglutination Inhibition (HI) antibody titers throughout the 56-day period, indicating a robust immune response. Groups treated with Black Cumin Oil (Group A and Group B) also exhibited substantial immune enhancement, with stable and moderately high antibody titers compared to the vaccinated control group without treatment (E1). The untreated and unvaccinated control group (E2) showed no detectable immune response, confirming the efficacy of the vaccine and treatments. Red Blood Cell (RBC) counts across the treatment groups remained stable, further supporting the safety of both Black Cumin Oil and Multivitamin supplementation in maintaining hematologic stability. Statistical analysis further confirmed the significant differences in immune responses, particularly in the treatment groups compared to controls, with the strongest statistical significance observed in Groups A and C.

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