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Nigella sativa seed effect on immune response and broiler chick's performance

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A B S T R A C T

In recent years, the use of plant powder as a feed additive in poultry and animal production has increased. In the meantime, *the Nigella sativa* plant has analgesic, antibacterial, and immune-stimulating properties. In this, study the effect of *Nigella sativa* on the immune responses and the performance of vaccinated broiler chickens with Newcastle disease and infectious bursal disease was evaluated. The research was conducted on a poultry farm at the Middle Technical University, Kut Technical Institute, Iraq. One-hundred-and-eighty-day-old broiler chickens of Ross 308 were divided into three equal groups with four replications (subgroups included fifteen chickens). All groups were maintained under similar conditions. Food and water were provided ad libitum. Group one was the control, and group two and three birds were vaccinated against live attenuated ND and IBDV. *Nigella sativa* seeds were used as 1% powder in G3. Blood samples were collected from 15 birds of each group at 17 and 35 days of age. ELISA kits were used to detect the antibody titer against ND and IBDV. Body weight, mean weight gain, the feed, the mean daily feed intake, and Feed Conversion Rate (FCR) were determined weekly and over a period of 1–35 days. Statistical analysis of the data was performed using SAS 9.1. The antibody titer in G1 was not increased during the experiment. NDV and IBDV antibody titer in the G3 group was significantly more than group 2 ($p < 0.05$). The average body weight, feed consumption, and Weight gain during the five-week study in group 3 was significantly different and higher than other groups ($p<0.05$). The FCR in group 3 was significantly lower than in groups 1 and 2 ($p<0.05$). The present study confirmed that adding *Nigella sativa* to feed can be considered an alternative natural growth promoter for poultry instead of antibiotics and improve the immune system function against Newcastle disease and infectious bursal disease.

Keywords: *Nigella sativa, immunity, broiler, Newcastle disease, infectious bursal disease*

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1 Introduction

Newcastle disease virus (NDV) spreads rapidly via airborne droplets produced by infected birds' coughs or sneezes, and mortality is often 100 percent in young unvaccinated chickens. Birds of all ages can be affected, but young birds are more susceptible; the incubation period is three to five days, followed by fatigue, coughing, sneezing, and wheezing. A gurgling sound in the throat accompanies rapid breathing. Respiratory symptoms usually develop first and are sometimes followed by nervous symptoms characterized by tremors and convulsion, paralysis, twisting of wings and legs, torticollis, circling with spasms, complete paralysis, up to 100 percent morbidity, and up to 90 percent mortality (1, 2). Depending on the environment and the level of resistance of the birds, not all symptoms or digestion problems may occur, leading to slow-growing greenish feces, diarrhea, and loss of appetite. The economic impact of IBD on the poultry industry is challenging to estimate due to the complex nature of the losses associated with the disease. Immunodeficiency in chickens caused by IBDV infection leaves the flock vulnerable to other viral, bacterial, and parasitic infections, resulting in indirect losses. Because poultry houses infected with IBDV are resistant to most disinfectants and environmental factors, they remain contaminated with IBDV, which remains on the premises and reappears in subsequent flocks (3). *Nigella sativa* seeds have antimicrobial effects against pathogens, including bacteria, viruses, schistosomes, and fungi. *Nigella sativa* seed in traditional medicine, in the treatment of microbial diseases, has been used without any reported side effects (4) *Nigella sativa* is used as anti-nociceptive and anti-inflammatory effects, supporting the common folk perception of *Nigella sativa* as a potent analgesic and anti-inflammatory agent. Many protective properties are attributed to reproducible radical scavenging activity and interaction with numerous molecular targets involved in inflammation, including pro-inflammatory enzymes and cytokines (4). *Nigella sativa* powders did exert beneficial immune-modulatory effects by increasing antibody titters against important poultry diseases (5), and some researchers showed *Nigella sativa* effectiveness in the treatment of both infectious and non-infectious disease and reported *Nigella sativa* as a good natural antimicrobial (6). The present study was designed to obtain the maximum immune response and improve flock performance (weight gain, feed intake, and FCR).

2 Materials and methods

2.1 Experiment Design

The study was conducted at a Middle Technical University, Kut Technical Institute poultry farm. One hundred and eighty-day-old broiler chicks Ross 308 were divided into three equal groups, with four replicates each (each subgroup included fifteen chicks). All groups were kept under similar conditions; feed and water were provided ad libitum. The farm was meticulously cleaned and prepared. Before vaccination, a blood sample was collected to determine maternal antibody titer.

2.2 Preparation N. sativa

Nigella sativa seeds were purchased from Shree Herbals, a Shree AyurShree company in India, and after preparation, they were used as 1% powder in G3.

2.3 Vaccination and immune response of chicks

According to the manufacturer's instructions, Group G2 and G3 birds were vaccinated against ND on the first day with live attenuated vaccines via spray method and revaccinated on days 10 and 28 via drinking water. Groups G2 and G3 were vaccinated twice against IBDV at 14 and 24 days.

2.4 Blood samples

Blood samples were collected from 15 birds of each group at 17 and 35 days of age. To detect the titer against ND and IBDV, ELISA kits were used according to the manufacturer's instructions (Synbiotics Corporation, San Diego, US).

2.5 Chicken body weight measurements

Body weight was determined for all groups at 1, 7, 14, 21, 28, and 35 days of age, respectively and mean weight gain was calculated. The feed was re-weighed on the 7th, 14th, 21st, 28th, and 35th days, and the mean daily feed intake and FCR (g feed/g gain) was determined weekly and over a period of 1–35 days. Mortality was recorded daily during the experiment.

2.6 Statistical analysis

Statistical data analysis was performed using SAS (Statistical Analysis System, Version 9.1). Two-way

ANOVA and the Least Significant Difference (LSD) post hoc tests were performed to assess significant differences between means. Chi-square was used to evaluate significant differences between proportions. $P<0.05$ is considered statistically significant.

3 Results

Statistical analysis of total body weight, feed intake, weight gain, and FCR data during the five weeks of the study are shown in Table 1.

Table 1. Effect 1% Nigella sativa powder with and without vaccination at 1-35 days of age.

Group	Body Weight		Feed Intake		Weight Gain		FCR	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
(a)1	676.07^{bc}	101.54	442.51^{bc}	51.69	265.29^{bc}	26.12	1.58^{bc}	.048
(b)2	626.54^{ac}	91.89	399.43^{ac}	46.68	236.09^{ac}	22.37	1.65^{ac}	.072
(c)3	712.29^{ab}	107.60	416.01ab	53.47	282.74^{ab}	29.30	1.39^{bc}	.052

*The heterogenous letters in each means column indicate that there are significant differences between the two groups

The mean body weight in the three groups of chickens was significantly different from other groups ($P<0.05$), and the mean body weight in group 3 was significantly higher than in groups 1 and 2 ($p<0.05$). Statistical analysis of food consumption data during the 5-week study (Table 1) showed that the average food consumption between the three groups was significantly different ($p<0.05$). The average feed consumption in groups 1 and 3 was significantly higher than group 2 ($p<0.05$), and in group 1 was higher than group 3, but this difference was not significant ($P>0.05$). Statistical analysis of weight gain and feed conversion rate (FCR) during the five-week study (Table 1) showed that weight gain in group 3 was significantly higher than groups 1 and 2 ($p<0.05$), and FCR in group 3 was It was significantly less than group 1. and 2, ($p<0.05$). The details of the results of body weight assessment, feed intake, weight gain, and FCR per week are presented in tables below.

Table 2. Effect 1% Nigella sativa powder with and without vaccination at seven days of age.

Group	Body Weight		Feed Intake		Weight Gain		FCR	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1(a)	125.70^{bc}	.62	107.10b	2.11	85.50c	.520	1.25b	.032
2(b)	115.80^{ac}	.67	115.62a	2.40	81.92c	2.46	1.41ac	.026
3(c)	132.20^{ab}	.65	112.00	913	92.00ab	.66a	1.22b	.015

*The heterogenous letters in each means column indicate that there are significant differences between the two groups

Table 3. Effect 1% Nigella sativa powder with and without vaccination at second week of age.

Group	Body Weight		Feed Intake		Weight Gain		FCR	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1(a)	323.50bc	.975	287.50bc	4.25	197.80	.877	1.45c	.0275
2(b)	303.40ac	1.30	197.50ac	3.64	169.25c	18.15	1.22	.168
3(c)	344.57ab	1.64	221.27ab	3.43	212.02b	1.41	1.04a	.0192

*The heterogenous letters in each means column indicate that there are significant differences between the two groups

Table 4. Effect 1% Nigella sativa powder with and without vaccination at third week of age.

Group	Body Weight		Feed Intake		Weight Gain		FCR	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1(a)	616.85bc	2.15	486.48c	2.47	293.35	2.10	1.66bc	.007
2(b)	584.65ac	2.7	497.02c	7.69	306.55	24.28	1.77ac	.0314
3(c)	648.98ab	3.78	452.81ab	7.10	304.41	2.43	1.49ab	.0189

*The heterogenous letters in each means column indicate that there are significant differences between the two groups

Table 5. Effect 1% Nigella sativa powder with and without vaccination at fourth week of age.

Group	Body Weight	Feed Intake	Weight Gain	FCR

	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	950.12bc	3.57	577.39c	2.27	335.75b	3.06	1.72bc	.012
2	905.20ac	6.23	595.87c	8.18	320.00a	4.08	1.87ac	.032
3	981.48ab	2.83	523.87ab	9.89	332.50	6.43	1.57ab	.040

*The heterogenous letters in each means column indicate that there are significant differences between the two groups

Table 6. Effect 1% Nigella sativa powder with and without vaccination at fifth week of age.

Group	Body Weight		Feed Intake		Weight Gain		FCR	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	1364.17bc	3.39	754.08b	3.39	414.05bc	2.96	1.83bc	.013
2	1223.65ac	16.79	591.12ac	16.79	302.70ac	3.62	1.95ac	.023
3	1454.23ab	19.72	770.10b	19.73	472.75ab	17.96	1.63ab	.020

*The heterogenous letters in each means column indicate that there are significant differences between the two groups

3.1 Antibody titers against (ND) vaccine in broiler chicks

Antibody titers in the unvaccinated group (G1) did not increase during the experiment, confirming no environmental or cross-contamination (Table 7). Antibody titer increased in the NDV-vaccinated groups. In G3 (with the addition of *N. sativa*), the antibody titer was significantly higher than G2 ($p < 0.05$). However, all chickens in G2 and G3 recorded a significant ($p \leq 0.05$) increase in NDV antibody titer with increasing age (Table 7). This increase in the antibody is evident in the treatment groups compared to the control group at (35 days) compared to (17 days). The NDV antibody titer showed a higher value when broilers were fed *Nigella sativa*.

Table 7. Mean antibody titers against Infectious Bursal Diseases vaccination (Mean ± SE)

Groups	17 days	35 days
G1	A 0.20 ± 0.007c	A 0.20 ± 0.007h
G2	B 2050.80 ± 31.82f	A 3283.05 ± 32.98g
G3	B 3010.77 ± 55.85e	A 3787.12 ± 36.60e
LSD	135.50	—

Means with a different small letter in the same column are significantly different ($p \leq 0.05$)
Means with a different capital letter in the same row are significantly different ($p \leq 0.05$)

3.2 Antibody titers against Gumboro vaccine in broiler chicks

The IBDV antibody titer (Table 7) showed a significant difference between the weeks of the group. Also, the results showed that there was a significant increase ($p \leq 0.05$) of antibody in G3 compared to (G1, G2) in (17,35) days. It also showed a significant increase ($p \leq 0.05$) in antibodies in G2 compared to G1 in (17, 35) days.

4 Discussion

In recent years, the use of plant powder as a feed additive in poultry and livestock production has increased. Meanwhile, the *Nigella sativa* plant has analgesic, antibacterial, and immune-stimulating properties (7). It is a fatty plant of the *Ranunculaceae* family (8). in this study, the effect of one percent of *Nigella sativa* on the immune responses and the performance of vaccinated broiler chickens with Newcastle disease and IBD was evaluated. NDV and IBDV Antibody titer in the treated group was significantly higher than others. The average body weight, feed consumption, FCR, and weight gain during the five-week study in the treated group was significantly different and better than other groups.

Vaccination has an adverse effect on weight gain, feed consumption, and FCR, and 1% *N. sativa* powder has reduced the negative effect of vaccination and increased herd performance. The positive effect of 1% *N. sativa* powder may be related to improving digestion and absorption of nutrients, with a positive effect on intestinal activity and increasing digestive enzymes. In our study, antibody titers against Newcastle and Gamboro diseases increased in infected birds treated with *N. sativa* powder, and it was concluded that *N. sativa* powder has cytoprotective, antioxidant, and antiviral effects against Newcastle and Gamboro viruses (7). The effect of *N. sativa*

supplementation on growth performance and carcass characteristics of broiler chickens was investigated, and it was concluded that feeding low level of *N. sativa* tends to improve performance characteristics in terms of body weight, feed intake, and carcass characteristics (8). Researchers showed that a dietary supplement of black cumin seed (*Nigella sativa*) at 1% or 1.4% would enhance immune responsiveness in broiler chickens against NDV, IBV, and NDV vaccines (9). The effectiveness of Kalo Jeera seeds (*Nigella sativa*) and Thankuni (*Centella asiatica*) seeds on the growth performance of broiler chickens was evaluated by Ibrahim et al. in 2011, and better growth performance was observed. These plants have antimicrobial, immune-stimulating, anti-stress, antifungal, insecticidal, and liver tonic properties (10). The antimicrobial effect of *Nigella sativa seed* extract from different geographical regions was compared and concluded that the antimicrobial effect of *Nigella sativa* from India was much higher than other geographical regions (11). In one study, the effects of Dietary Black Cumin (*Nigella sativa*) on growth performance, nutrient utilization, blood biochemical profile, and carcass traits in broiler chickens. They concluded that dietary black cumin seed at 1.0% can be used as a cholesterol-lowering agent and an alternative to antibiotic growth promoters in broiler chickens (12). In a study, the antimicrobial activity of aqueous and oil extracts of *Nigella sativa* was evaluated, and both showed the highest inhibition zone against *E. coli* bacteria and the least inhibition against *S. pyogenes*. Black seed works against gram-positive and gram-negative bacteria (13). Supplementation of 1-2% *N. sativa* seed in broiler diets as a multifunctional natural growth promoter improves performance and enhances humoral immune responses. It affects the serum biochemical profiles of broiler chickens and causes changes in their hemogram and leucogram, while it has no residual or dangerous side effects (14). The addition of black cumin seeds (*Nigella Sativa*) to the performance of broiler chickens in summer was also evaluated, and it was concluded that using this seed as a vegetable feed additive helps improve the health status of birds (15). The effect of seasons and levels of black cumin powder (Nigella sativa) on the performance of broiler chickens stated that black cumin powder at a ratio of 2 and 3% on body weight, weight gain, feed consumption, and feed efficiency, performance index and carcass weight had a positive effect, especially in summer (16). Black seed is considered a safe medicinal plant as a dietary supplement. N. sativa contains a wide range of bioactive substances. The biological properties of *N. sativa* include antioxidant, antimicrobial, antifungal, anti-inflammatory, anti-cancer, anti-diabetic, antihypertensive, blood lipid-lowering, and antioxidant activities (17). In one study, supplementation of 1% black cumin seeds in broiler diets significantly improved body weight, FCR, and carcass yield of broilers after a growing period of 6 weeks (18). Finally, it should be said that using *N. Sativa* seeds as a plant feed additive helps to improve the health status of birds, showing positive effects on the performance and survival of broiler chickens raised under normal conditions. The highest body weight and the lowest FCR are considered an essential economic index in the ability of the bird to convert the ratio into live body weight. Herbal products affect chicken performance by improving the digestibility of dietary protein in the small intestine and have antibacterial and antifungal effects (19). The present study confirmed that adding *N. sativa* to feed can be considered an alternative natural growth promoter for poultry instead of antibiotics and improve the immune system function against Newcastle disease and IBD.

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Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

Every author contributed to the original idea, study design, writing, and editing of the manuscript, and the final draft was approved.

Data Availability Statement

The 1st author can provide the data upon reasonable request.

Ethical Considerations

This article is a review of existing research and adheres to all ethical guidelines concerning the use of texts and images.

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