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Research Article

Black Cumin (*Nigella sativa*) Immunopotentiates Humoral Response in Commercial Broiler Chickens

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ABSTRACT

*Nigella sativa (*Black cumin) is a widely used medicinal plant for the treatment of various diseases and ailments in humans and animals, but its use in poultry husbandry is limited. This study investigated the clinicopathological and humoral immune responses of commercial broiler chickens administered with *Nigella sativa* extracts and also vaccinated against Newcastle disease virus (NDV). There were five experimental groups A-E; A-were administered with *Nigella sativa* only (6). B- were vaccinated with Newcastle disease vaccine and administered with low dose of *Nigella sativa L (6),* C- were vaccinated with Newcastle disease vaccine and administered with high dose of *Nigella sativa L, (6)*. D- those that were vaccinated with Newcastle disease vaccine only *(6)*. E- negative control *(6)*. The birds were monitored for weight changes, while blood and sera samples were collected for haematological and serological analysis following standard procedures. Birds administered with *Nigella sativa* only (A) and those vaccinated alone (D) and those with NDV-*Nigella sativa* combination *(B, C)* have higher weight gains compared to negative control (group E). Those vaccinated with NDV only (D) had higher antibody titre against ND virus at weeks 1, 2, and 3, compared to those birds vaccinated with low *Nigella sativa*, while in the 4th and 5th week, Groups with NDV vaccine-*Nigella sativa* combination (B&C) expressed a more significantly higher titre values. Group C birds vaccinated and administered with high dose of *Nigella sativa* had the highest antibody titre against ND virus throughout the weeks of experiment until week 5 when Group A (*Nigella sativa* only) showed a much higher NDV titre compared to other groups. In conclusion, administration of *Nigella sativa* improved body weight of chickens through feed conversion and also causes a more enhanced antibody response to NDV.

Keywords: *Nigella sativa, Enhance Humoral Response, Newcastle disease, Virus, Vaccine*

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INTRODUCTION

Newcastle disease is an acute, highly contagious rapidly spreading viral disease affecting birds of all ages (Abdu, 2007); with many breeds of domesticated and wild birds susceptible. Newcastle disease belongs to OIE listed diseases and is characterized as 'a transmissible disease that has the potential for very rapid spread irrespective of national borders; a disease of serious socio-economic or public health consequence and of major importance in the international trade of animals and animal products (Cattoli *et al*., 20011, OIE 2012). Newcastle disease is caused by Newcastle disease virus (NDV), a non-segmented, single stranded, negative sense RNA virus that belongs to the family paramyxoviridae and genus orthoavulavirus (Aldous and Alexander, 2001; Alexander 2008, Absalon *et al*, 2019; Okechukwu *et al*, 2020). Its effects are most notable in domestic poultry due to their high susceptibility and the potential for severe impacts of an epizootic on the poultry industries (Talebi, 2006: Yongola *et al*, 2006).

The only effective means of preventing Newcastle disease is through vaccination commonly given by the oral, ocular and intranasal routes (Wambura, 2009, Oyebanji *et al* 2017). The basis of immunity to Newcastle disease virus is based on exposure to low virulence strain, which induces immune responses in the birds. Chickens that survive infection with virulent Newcastle disease virus may develop long lasting immunity to the infection. However, most of the commercial chickens get infected with very virulent field (velogenic) strains. The ability of the killed virus to infect cells has been destroyed by treatment with a chemical, radiation or heat to make it suitable for vaccination. These vaccines invoke only a circulating antibody response. Some strains of Newcastle disease virus used in live vaccines include F, B1, Lasota, V4 (Saidu *et al*. 2006).

Nigella sativa (*N.sativa)*, also known as black seed or black cumin belongs to the family Ranunculaceae, and it is

native to Meditarenean, commonly grown in the Eastern Europe, Middle East and Western Asian, it is a small shrub with tapering green leaves with rosaceous white and purplish flowers (Badary *et al*., 1998). Its ripe fruit contains tiny dark coloured seed known as Habba Al Sauda or Habba Al – Barakah in Arabic and black cumin in English. Its seeds and oil have been used for various therapeutic purposes (Mansour *et al* 2001). Active component of *N. sativa* are numerous, and most of them are isolated from seeds and oil of *N. sativa* and they include thymoquinone, dithymoquinone, thymohydroquinone, nigellidine, nigellicine, alpha–hederin, p-cymene, carvacrol, 4-terpinol, t-anethol etc (Alemi *et al*., 2013). Benefits and biological properties of *N. sativa* have been reported such as anti-inflammatory (Pichette *et al*., 2012), antioxidative (Buntols and Bucan, 2000), neuroprotective (Kanter *et al*., 2006). Acute and chronic toxicity on laboratory animals have shown that *N. sativa* seed, and oil contents are safe, particularly when given orally (Ali-Ali *et al*., 2008). In spite its wide usage; there is a dearth of information on its use in poultry husbandry.

This study investigated the clinicopathological and humoral immune responses of commercial broiler chickens to the administration of *Nigella sativa* (black cumin) and also vaccinated against Newcastle disease (ND)..

MATERIALS AND METHODS

Experimental birds and vaccination; Thirty (30) birds, three weeks old broiler chickens were acquired for this experiment. This age group was selected because by three to four weeks, it is expected that the maternal antibody would have waned. There were 5 experimental groups comprising six (6) birds per group. A group was vaccinated with Nigella sativa alone, while other groups were vaccinated with the mixture of *Nigella sativa* (varying doses) and lasota vaccine and the last group received neither the vaccine nor *Nigella sativa* extract (Negative control). The groupings are as elucidated below:

Group A- Those administered *Nigella sativa* only (6).

B-Those vaccinated with Newcastle disease vaccine and administered with low dose of *Nigella sativa L (6),*

C-. Those vaccinated with Newcastle disease vaccine and administered with high dose of *Nigella sativa L, (6).*

D-Those that were vaccinated with Newcastle disease vaccine only *(6).*

E- not administered anything (negative control) *(6).*

This procedure (vaccination and administration of extract) was carried out three times, two weeks apart. The first vaccination was carried out exactly when the birds were 3 weeks old and another was done when they were 5 weeks old and the experiment was terminated at the 6th week. 100 doses of lasota live vaccine was diluted with 4mls sterile chlorine-free water, since the birds to be vaccinated are 24; 1ml per bird was therefore administered orally and thereafter, those to be administered *Nigella sativa* were hereafter given varying doses of the extract in drinking water.

Preparation and Administration of Nigella sativa L: The design of this research is such that, varying doses of *Nigella sativa* were administered and assayed in different groups of birds. Group A (*Nigella sativa* alone) was getting a sachet of Soul (*Nigella sativa L*) per week in 0.9 litres of water, while Group B (*Nigella sativa* low dose) was getting a satchet of the extract per week in 1.5 litres of water, shortly after vaccination. The third group (group C, *Nigella sativa* high dose) was administered two sachets of the extracts in 1.5 litres of water, shortly after vaccination. The birds usually finish the entire volume of the preparations within an hour.

Test samples; The test samples used for this study were blood and sera collected from commercial broilers chicken. A total 271 samples were collected, from which 151 serum samples were used for haemagluttinin-inhibition test, in order to know the response of the birds to Newcastle disease vaccination and also to *Nigella sativa L* extracts administration. 120 blood samples were collected for haematology, in order to know effects of *Nigella sativa* extracts on blood components of the birds. The birds were also weighed to know effects of *Nigella sativa L* on broiler growth and weight gain.

Blood collection: Blood was collected by veni-puncture of the external jugular vein using 21G needle on 5ml syringe. Firstly, the site was located and swabbed with methylated moist cotton wool. The needle was inserted into the vein, and about 4 mls of blood was collected and 2mls of it was dispensed into labelled EDTA bottle and the remaining 2mls was dispensed into labelled universal bottle and tilted on its side to enhance easy separation of serum from clotted blood. After 30 minutes, blood in universal bottle was centrifuged using macro-centrifuge machine. Immediately after the centrifuging, sera were decanted into appropriately labelled ependorf tubes and kept in the freezer.

Haemaglutination-inhibition (HI) test; The modified microhaemagglutination inhibition test previously described by Allan and Gough (1974) was carried out using the serum samples obtained, then local chicken red blood cell (RBC) as indicator. The test was performed following OIE laid standard protocols. Briefly, 0.025 µl of PBS was dispensed into each well of a plastic U-bottomed microtiter plate then 0.025 µl of serum was placed into the first well of the plate. Two-fold dilutions of 0.025 µl serum were then made across the plate. ND viral antigen (0.025 µl) was added to each well and the plate was left for 30 minutes at room temperature. Washed chicken Red Blood Cells (RBCs) (0.025 µl of 1% (v/v) was then added to each well and, after gentle mixing, the RBCs were allowed to settle for about 30 minutes at room temperature. Agglutination inhibition was assessed by examination of the plates under white floodlight for presence of neat buttons (absence of agglutination). The dilution of the last well where the neat button is observed is taken as the highest antibody titer present to inhibit agglutination.

Haematological evaluation; Tip of one end of the capillary tube containing dried heparin was inserted to the blood (mixed with anticoagulant). The capillary tube automatically aspirates the blood by capillary action. About three-quarter of the tube is filled and the end of the tube, which has not come into contact with blood, was plugged with plasticine. The capillary tubes were then placed in the microhaematocrit centrifuge with the sealed ends facing the outside of the centrifuge. It was then spinned for 3 minutes and the capillary tubes were removed. The percent packed cell volume was read using a micro-hematocrit reader.

Complete Blood Count; Clean slides were used for smear making. Small drop of blood was placed one slide (the sample slide), the second slide was used as a spreader to spread the blood across the surface of the slide. After a good smear was made, the sample slide was air dried, and then fixed using alcohol and stained with Giemsa. The slides were viewed under the microscope at low power (10x ocular/objective), this is to evaluate the quality of smear, the number of white blood counts, red blood counts, cell distribution and morphology. And then to the 40x objective, in order to count the number of leukocyte in at least 3 fields and calculate and calculate the average number per field. The number gotten was multiplied by 1500 to get the approximate total WBC counts. Also to evaluate White Blood Cell morphology, the size, shape, Type neutrophil, lymphocyte, eosinophil).

Platelet Estimation; The number of platelets in at least 5 fields was counted and the average was found. The result gotten was multiply by 15,000 to get the approximate total number of platelets.

Total Protein Estimation: Refractometer was used to estimate plasma protein, this was done by breaking the capillary tube at the top of the thin buffy coat layer to get the plasma protein, a drop of plasma protein was placed on the refractometer and viewed via ocular aperture. And the values were gotten from the point where there was brightness contrast.

Haemoglobin count was by sahli's haemoglobinometer (acid haematin method) as as described by Jain (1986)**.** Briefly, 0.1N HCL was placed in diluting tube up to mark 20.Anti-coagulated blood was taken in the haemoglobin pipette up to 20-cubic-mm-mark and blown into diluting tube and was thoroughly rinsed and kept for 7 minutes to form acid haematin. After 10 minutes, distilled water was added in drops and HCL and blood was mixed properly with stirrer. The colour of acid haematin was allowed to match with the solution, present in the calibration tube, and the final reading was directly noted from the graduation in the calibration tube. Descriptive Statistics was used to summarise data as Mean±Standard Deviation, while one way analysis of variance (ANOVA) was used for comparison across group at α=0.05.

RESULTS

Effects on body weight: As shown in Table 1, towards the sixth week when the experiment was to be terminated, Group C (high dose *Nigella sativa*-vaccine combination) had the most noticeable weight gain (1.86kg), followed by Group B (0.94g), A (0.56g), D (0.48g) and E (0.36g) in that order. Untreated group (Group E) has the lowest weight gain, while those that have *Nigella sativa* only or at higher doses had higher weight gains. Birds in group A, those administered with *Nigella sativa L* extract only, have a considerably high weight gains in weeks 3 and week 4. And birds of group C, those that were vaccinated and administered with high dose of *Nigella sativa L* extracts have increased body weights in weeks 2 and 5.

Table 1
Weekly Mean Weight Gains (kg) In Chickens Given *Nigella sativa* Extract

Group	Week 1	Week 2	Week 3	Week 4	Week 5
A: (extract)	1.07 ±0.2	1.32 ±O.2	1.47 ±0.2	1.53 ±0.3	1.51 ±0.2
B: (low extract + ND vaccine)	1.04 ±0.2	1.36 ±0.2	1.25 ±0.2	1.31 ±0.2	1.34 ±0.2
C: (High extract + ND vaccine)	0.99 ±0.2	1.21 ±0.2	1.21 ±0.2	1.43 ±0.2	1.36 ±0.2
D: (ND vaccine)	1.08 ±0.1	1.25 ±0.4	1.25 0.1	1.41 ±0.1	1.37 ±0.1
E: (Negative control)	1.08 ±0.1	1.11 ±0.2	1.18 ±0.2	1.28 ±0.2	1.32 ±0.2

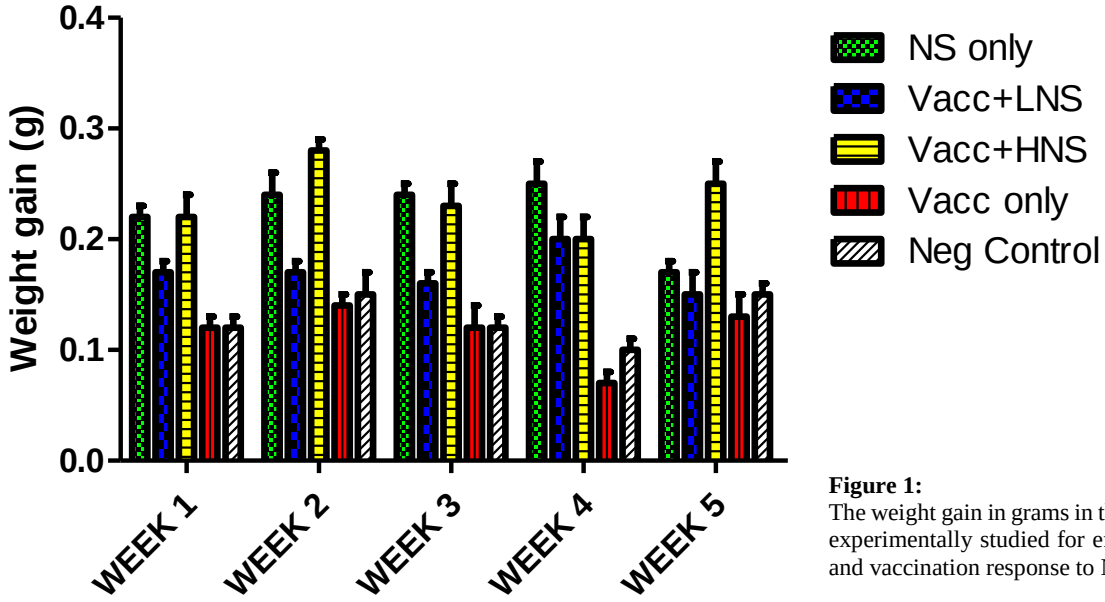

Figure 1:
The weight gain in grams in the five groups of birds experimentally studied for effect of *Nigella sativa* and vaccination response to Newcastle disease

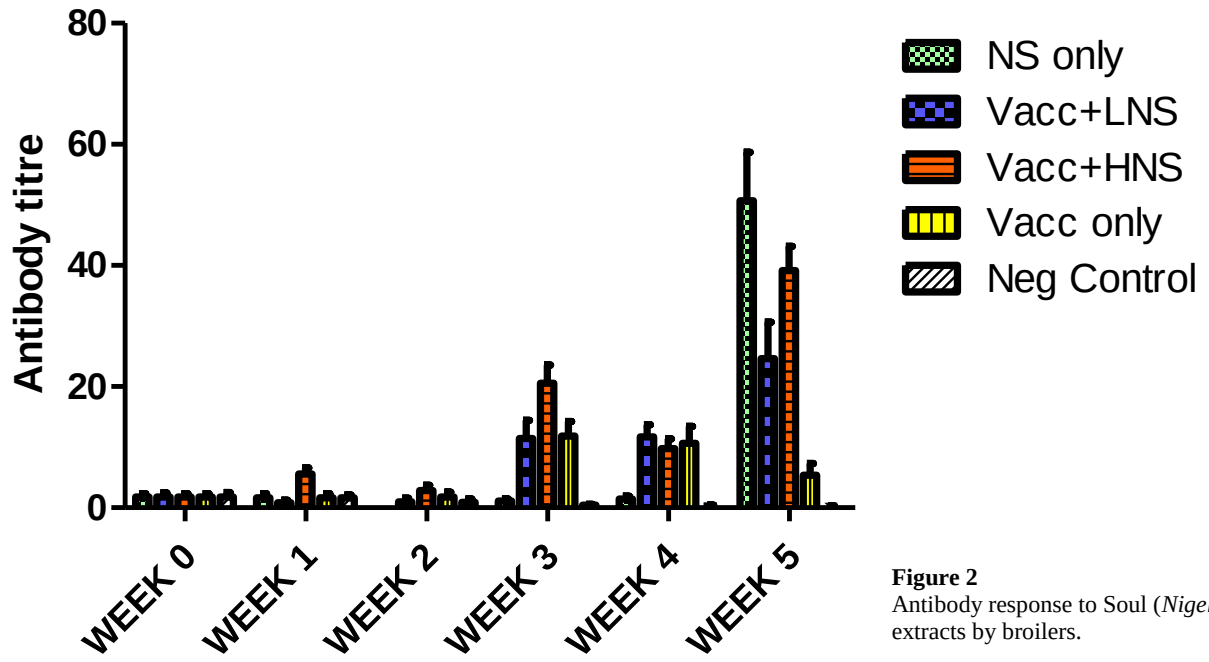

Figure 2
Antibody response to Soul (*Nigella Sativa*) extracts by broilers.

Serological Response: The antibody response of the five groups of birds experimentally studied is shown in Figure 1 below. Group A (birds administered with only *Nigella sativa L* extracts) has no titre against Newcastle disease virus at week 2,which shows that antibody against Newcastle vaccine through vaccination against Newcastle disease at day old has waned, antibody titre against Newcastle disease virus was observed at week 3 of administration of *Nigella sativa L* and gradual increase in the titre values of antibody against Newcastle disease virus was observed at week 4,compared to week 3. At week five, there was significant increase in the titre value of group A birds. Group B, birds vaccinated and administered with low dose of *Nigella sativa L* has low antibody titre compared to Group C. Group C, birds that were vaccinated and were administered with high dose of *Nigella sativa L* extract were observed to have higher antibody titre than other treated groups up to week 4, however in week 5, group A with *Nigella sativa L* only had slight higher titre than that of group C. Group D, the birds vaccinated only, this group has low antibody titre at week 4 and week 5 compare to those vaccinated and administered with *Nigella sativa L* extracts.

Haematology: There was no significantly difference in the PCV, HB and RBC across the groups (Table 2), but there was a transient leucopaenia in the birds vaccinated with Newcastle disease vaccine and administered with high dose of *Nigella sativa* extract *(Group C)* when compared to the other birds ($p<0.05$) in week 1. There was mild thrmbocytopaenia in birds administered with *Nigella sativa* only (Group A) in week 2 but returned to normal platelet counts in the following weeks. The plasma protein levels were consistently low ($p<0.05$) in the negative control birds without the *N. sativa* and Newcastle disease vaccination (group E).

DISCUSSION

The present study shows that at both high and low doses of N. sativa administration (Groups A-C), there was an increased weight gain and improved feed conversion rate, compared to untreated groups (D&E). The weight changes observed in birds in group A, those administered with *Nigella sativa L* extract only, in weeks 3 and 4, birds in group C, those that were vaccinated and administered with high dose of *Nigella sativa L* extract in weeks 2 and 5 and also birds in group B, that were vaccinated and administered with low dose of *Nigella sativa L* shows that *Nigella sativa L* extract administration increase feed intake, digestibility and an improved feed conversion rate of the broiler birds. This result does not totally agree with Shewita and Taha (2011) that shows that administration of *Nigella sativa L* extract, only improve body weight and feed conversion rate at low doses of administration.

In this study, antibody titre against Newcastle disease virus was observed at week 3 of administration of Nigella sativa L and gradual increase in the titre values of antibody against Newcastle disease virus through the weeks; this may be because Nigella sativa L extract elicits immune response from birds, but needs long period of time and continual administration for desired effect. And this might be advantageous in endemic areas since Newcastle disease outbreak might occur before the 5th week of administration and wipe the whole flock before Nigella sativa L extracts confers immunity on the birds. Overall the study showed that vaccination of the birds and administration of high dose of Nigella sativa L extracts boost birds antibody titre significantly against Newcastle disease virus and this agree with Al-Beltawi *et al*.,(2009). It also showed that the results obtained is dose-dependent which concur with other workers (Durrani *et al*., 2007; Shewita and Taha (2011).

Table 2:
Haemogram from experimental administration of *Nigella sativa* and Newcastle disease vaccine in birds

Week	Group	PCV (%)	HB (g/dl)	RBCx10^{3}µl	WBC x10^3 µl	PLT x10^3 µl	LYM (%)	HET (%)	MON (%)	EOS (%)	BAS (%)	PP (g/dl)
1	A	30.5±1.7^a	9.8±0.8^a	3.4±0.2^a	18.0±4.1^a	196.0±71.0^a	69.3±6.7^a	24.8±5.4^a	2.0±0.8^a	4.0±0.8^a	0.0±0.0^a	5.0±0.4^a
	B	29.5±3.3^a	9.8±1.3^a	3.2±0.4^a	17.5±2.9^a	162.5±33.5^b	69.8±3.8^a	24.5±4.1^a	2.5±0.8^a	2.7±0.5^b	0.0±0.0^a	5.1±0.5^a
	C	29.0±2.7^a	9.5±0.9^a	3.2±0.7^a	7.9±1.1^b	183.7±47.5^b	66.3±3.9^a	27.8±3.8^b	2.8±0.5^a	3.3±1.7^a	0.0±0.0^a	5.2±0.4^a
	D	31.0±1.8^a	9.7±0.8^a	3.4±0.1^a	15.6±0.4^c	151.5±18.4^b	67.0±3.9^a	26.3±3.0^b	3.0±0.8^a	3.4±1.3^a	0.0±0.0^a	5.3±0.4^a
	E	29.5±1.9^a	8.9±2.2^b	3.3±0.1^a	14.5±2.2^c	235.5±83.0^e	68.6±4.7^a	25±4.8^a	3.2±0.8^a	3.0±1.4^a	0.3±0.5^b	4.8±0.4^a
2	A	24.7±1.2^b	8.4±0.6^b	2.0±0.6^b	13.1±2.3^c	115.7±9.5^d	51.7±1.5^b	41.0±2.0^c	3.7±1.2^b	3.3±2.1^a	0.3±0.1^b	7.0±0.5^c
	B	27.1±2.7^b	9.2±1.9^b	3.0±0.8^a	12.9±0.2^c	175.0±1.8^a	57.6±5.3^b	35.8±4.8^c	3.1±1.0^a	3.1±1.4^a	0.4±0.2^b	6.9±0.3^c
	C	28.1±0.7^b	9.4±0.3^b	3.5±0.1^c	13.7±2.5^c	193.3±41.3^a	67.0±6.0^a	26.6±5.7^b	3.4±1.3^a	2.9±1.8^a	0.2±0.1^c	7.5±0.6^c
	D	27.5±3.1^b	9.1±1.0^b	2.9±1.1^a	13.9±2.4^c	174.3±34.3^a	63.5±7.9^a	29.3±7.8^b	3.2±0.8^a	3.5±2.2^b	0.2±0.1^c	7.4±0.9^c
	E	29.5±1.9^a	8.9±2.2^b	3.3±0.1^a	14.5±2.2^c	235.5±83.0^e	68.6±4.7^a	25±4.8^a	3.2±0.8^a	3.0±1.4^a	0.3±0.5^b	4.8±0.4^b
3	A	30.0±4.3^a	10.0±1.5^a	3.2±0.9^a	13.9±2.4^c	200.0±24.8^a	68.0±6.2^a	25.3±6.3^a	2.5±1.0^a	4.3±1.0^a	0.3±0.5^b	7.4±0.1^c
	B	28.4±3.2^a	9.4±1.2^b	3.2±0.8^a	13.2±2.5^c	207.4±19.0^a	66.9±6.8^a	26.3±7.0^a	2.7±1.0^a	3.7±0.8^a	0.4±0.5^b	7.2±0.6^c
	C	28.5±3.0^a	3.5±1.2^c	3.2±0.8^a	13.6±1.9^c	210.0±13.1^a	71.2±2.6^a	21.5±2.6^d	3.7±1.0^b	3.7±1.2^a	0.0±0.0^a	7.5±0.8^c
	D	29.8±3.6^a	10.3±1.2^a	3.2±0.7^a	14.7±2.4^c	196.2±33.5^a	69.8±4.4^a	24.0±6.0^a	3.2±1.1^a	3.0±2.2^a	0.4±0.6^b	6.9±0.7^c
	E	29.5±1.9^a	8.9±2.2^b	3.3±0.1^a	14.5±2.2^c	235.5±83.0^e	68.6±4.7^a	25±4.8^a	3.2±0.8^a	3.0±1.4^a	0.3±0.5^b	4.8±0.4^a
4	A	27.6±3.1^b	9.3±1.2^b	3.1±0.8^a	15.0±3.1^c	225.8±78.7^e	62.4±4.9^b	30.4±4.5^b	3.8±1.3^b	3.2±1.9^a	0.2±0.4^c	6.1±0.6^b
	B	26.6±1.3^b	8.6±0.4^b	2.8±0.4^a	13.7±2.9^c	179.8±25.0^a	67.0±4.2^a	28.6±6.1^b	3.2±0.8^a	3.0±1.9^a	0.4±0.6^b	7.0±0.9^c
	C	28.0±2.3^a	9.4±1.0^b	3.1±0.6^a	15.4±1.8^c	207.5±28.2^a	65.0±7.4^a	30.3±12.2^b	2.3±1.0^c	4.8±1.9^c	0.3±0.5^b	6.9±0.8^c
	D	32.0±6.5^c	10.8±1.9^c	3.5±0.5^c	14.3±2.2^c	161.2±21.0^b	60.3±6.8^b	33.3±6.3^b	3.5±1.3^b	3.0±1.6^b	0.0±0.0^a	7.0±1.0^c
	E	29.5±1.9^a	8.9±2.2^b	3.3±0.1^a	14.5±2.2^c	235.5±83.0^e	68.6±4.7^a	25±4.8^a	3.2±0.8^a	3.0±1.4^b	0.3±0.5^b	4.8±0.4^a
5	A	26.6±3.8^a	9.0±1.3^b	3.0±0.8^a	10.5±0.5^b	171.0±32.5^a	69.6±3.1^a	23.0±3.1^a	3.8±0.8^c	3.4±1.1^b	0.2±0.1^c	4.7±1.7^a
	B	30.7±1.8^a	9.9±0.6	3.5±0.1^c	11.1±1.3^b	144.7±34.2^d	68.2±5.4^a	25.5±5.1^a	2.7±1.0^a	3.3±1.6^b	0.3±0.1^a	6.6±0.5^c
	C	28.9±3.9^a	9.3±1.4	1.3±0.7^d	12.8±1.7^c	174.7±41.3^a	66.1±4.1^a	27.3±4.9^b	3.1±0.7^a	3.3±1.1^b	0.1±0.0^c	6.0±0.8^c
	D	31.8±3.3^a	10.5±0.9	3.4±0.1^a	12.3±0.8^c	145.3±25.4^d	63.3±2.5^b	30.3±4.4^b	2.0±0.8^c	4.5±1.3^c	0.0±0.0^a	6.3±0.4
	E	29.5±1.9^a	8.9±2.2	3.3±0.1^a	14.5±2.2^a	235.5±83.0^e	68.6±4.7^a	25.0±4.8^a	3.2±0.8^a	3.0±1.4^a	0.3±0.5^b	4.8±0.4

GROUP A: birds administered with Soul only;
GROUP B: birds that were vaccinated and administered with low dose of Soul extracts;
GROUP C: birds that were vaccinated and administered with high dose of Soul extracts;
GROUP D: birds that were vaccinated only.
WEEK 0: Control; WEEK 1: Week 0 post vaccination; WEEK 2: Week 1 post vaccination; WEEK 3: Week 0 post 1stbooster vaccination, WEEK 4: Week 1 post 1stbooster vaccination, WEEK 5: Week 0 post 2nd booster vaccination

Beneficial effects of *N. sativa* can also be deduced from the haemogram, because there were no significant disruptions in the normal values in the birds. In conclusion, administration of Nigella sativa to chickens improved their body weight gains and causes an enhanced/improved antibody response to Newcastle disease.

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