J. Agric. & Env. Sci. (Damanhour University) **2023, 22(3): 317-340**
Print: ISSN 1687-1464 **Online: 2735-5098**

THE IMMUNOMODULATORY AND HISTOLOGICAL EFFECTS OF *Nigella sativa* SEEDS ON BROILER CHICKENS

Mohamed I. Hassan[1]; Saber S.A. Hassan[2*]
[1]Livestock Research Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications (SRTA- City), New Borg El-Arab, Alexandria, Egypt.
[2]Animal and Poultry Production Department, Faculty of Agriculture, Damanhour University, Egypt.

*Corresponding Author E-mail: saber.shihata77@gmail.com

ABSTRACT

This study investigates the effects of dietary supplementation with *Nigella sativa* seeds (NSS) on immune parameters and the histological microscopic structure of lymphoid organs in Arbor Acres broiler chicks. A total of 140 unsexed one-day-old chicks were randomly assigned to four groups with seven replicates each. The control group received a basal diet without supplements, while the other groups were fed the basal diet supplemented with NSS at concentrations of 0.5%, 1.0%, and 1.5%. The group supplemented with 0.5% NSS showed significantly higher white blood cell counts compared to other groups. The heterophil/ lymphocyte ratio was significantly elevated in the 0.5% NSS group. Plasma α-globulin concentration increased significantly in the 1.0% NSS group compared to the 0.5% group. Phagocytic activity was enhanced in all NSS-supplemented groups compared to the control. Bactericidal activity improved notably in the control, 1.0%, and 1.5% NSS groups. The lymphocyte transformation test concentration was significantly higher in the 1.0% NSS group. Haemagglutination inhibition against Newcastle disease virus was highest in the 1.0% NSS group. The weight of the bursa of Fabricius was significantly greater in the 1.0% and 1.5% NSS groups. Histologically, chicks fed with 0.5%, 1.0%, and 1.5% NSS had significantly longer villi, and the 0.5% NSS group exhibited larger bursal follicular areas. In conclusion, incorporating 1.0% NSS into broiler

J. Agric. & Env. Sci. (Damanhour University) 2023, 22(3): 317-340
Print: ISSN 1687-1464 Online: 2735-5098

diets positively influenced immune responses, lymphoid organs, hematological parameters, and improved organ health histologically.

Keywords: Broiler, Immunity, Histological, Lymphoid, *Nigella sativa*

https://doi.org/10.21608/JAESJ.2024.210673.1092

INTRODUCTION

Ongoing scientific research continues to explore growth stimulants as supplements to broiler diets to sustainably enhance productive efficiency, performance, and internal health of these vital birds (**Hassan et al., 2023a**). Incorporating various potentially beneficial ingredients into poultry feed or drinking water can boost immunity, and reduce disease prevalence (**Seidavi et al., 2021**). The utilization of herbal medicines derived from plants has gained significant traction in enhancing human and animal health. Among these, *Nigella sativa* seeds (NSS), commonly known as black cumin, have attracted considerable interest due to their multifaceted health benefits. Historically, these seeds have been employed in traditional medicine to treat various ailments and promote overall health (**Attia and Al-Harthi, 2015; Khan et al., 2023**). Contemporary research is now focused on identifying the key bioactive components of *Nigella sativa* and understanding their mechanisms of action.

The NSS possesses a wide array of therapeutic properties, including antibacterial, antiviral, and antifungal activities. They are effective against infections caused by gram-positive and gram-negative bacteria, as well as various viral pathogens (**Salman et al., 2016; Talebi, 2021**). Recent studies have highlighted the potential of bioactive compounds such as thymoquinone, dithymoquinone, thymol, carvacrol, α-hederin, and nigellidine in these seeds, which exhibit potent antioxidant, anti-inflammatory, and immunomodulatory effects crucial for enhancing the body's defense mechanisms (**Nasir and Grashorn, 2010; El-Toumy et al., 2023**).

The impact of *Nigella sativa* on the immune system has been extensively studied, with findings indicating its ability to modulate both cellular and humoral immunity. It enhances macrophage function, immune cell production, and regulates inflammatory responses (**Büyükoztürk et al., 2005; Al-Beitawi et al., 2009; Elmowalid et al., 2013; Azeem et al., 2014**). The antimicrobial properties of *Nigella sativa* are noteworthy, with evidence supporting its efficacy against a range of infectious agents, including bacteria, fungi, parasites, and viruses (**Yimer et al., 2019; Salim et al., 2023**).

In poultry nutrition, *Nigella sativa* has shown promising results. **Al-Owaimer et al. (2017)** demonstrated that diets supplemented with *Nigella sativa* improved intestinal morphology and function in broilers. Additionally, supplementation was associated with enhanced immune responses (**Khafaga et al., 2019; El-Saadony et al., 2021**). Other natural supplements, such as hot red pepper oil and Eruca sativa seeds, have also been shown to boost health and immune parameters in broilers (**Hassan et al., 2023a; Hassan et al., 2023b**). The objective of this study is to comprehensively investigate the effectiveness of NSS as a multifaceted feed additive, particularly in enhancing immune indices and the microscopic structure of lymphoid organs in broilers. This research aimed to expand the existing body of knowledge and provide new insights into the practical applications of *Nigella sativa* in poultry nutrition.

MATERIALS AND METHODS

The experiment was conducted at the Poultry Research Unit, El-Bostan Farm, Animal and Poultry Department, Faculty of Agriculture, Damanhour University, Egypt. The objective was to evaluate the impact of incorporating *Nigella sativa* seeds (NSS) into broiler chicken diets on immune parameters and the microscopic structure of lymphoid organs. The NSS used in this study was procured from the local market in Damanhour City, Egypt.

Experimental Design

J. Agric. & Env. Sci. (Damanhour University) 2023, 22(3): 317-340
Print: ISSN 1687-1464 Online: 2735-5098

A total of 140 unsexed one-day-old Arbor Acres broiler chicks were utilized in this study. The chicks were randomly divided into four groups, each consisting of seven replicates with five chicks per replicate. The control group was fed a basal diet without any supplementation. The other three groups received the basal diet supplemented with NSS at concentrations of 0.5%, 1.0%, and 1.5%, respectively.

Diets and Feeding

The basal diet was formulated to meet or exceed the nutrient requirements of broiler chicks as recommended by the **National Research Council (1994)**. The composition of the basal diet is presented in Table 1. The NSS was incorporated into the diet at the expense of wheat bran to achieve the desired concentrations. Feed and water were provided *ad libitum* throughout the experimental period.

Table (1). Ingredients and chemical composition of the experimental diets (control, 0.5, 1.0, and 1.5% of NSS)

Ingredients, %	Starter				Grower diet			
	Control	0.5%	1%	1.5%	Control	0.5%	1%	1.5%
Corn	54.02	53.52	53.02	52.52	61.02	60.52	60.02	59.52
Soybean meal (44%)	33.0	32.5	32.5	32.5	26.0	26.0	26.0	26.0
Corn gluten meal (60%)	4	4	4	4	4	4	4	4
Mixed oils	4.5	5	5	5	4.5	4.5	4.5	4.5
Nigella seeds	0	0.5	1	1.5	0	0.5	1	1.5
Sodium chloride	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Dl- methionine	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
L-lysine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dicalcium phosphate	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Limestone	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Vit. and min. mixture[1]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Antioxidant	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Chemical composition								
ME kcal/ kg diet[2]	3200	3200	3200	3200	3200	3200	3200	3200
Dry matter[3]	90.15	89.8	89.31	88.87	90.06	89.61	89.17	88.73
Crude protein[2]	23	23	23	23	20	20	20	20
Calcium[2]	1	1	1	1	1	1	1	1
Non-phytate phosphorus[2]	0.48	0.5	0.48	0.47	0.45	0.45	0.45	0.45
Methionine+cystine[2]	0.76	0.7	0.75	0.74	0.68	0.68	0.68	0.68
Lysine[2]	1.36	1.3	1.34	1.34	1.17	1.17	1.17	1.17
Crude fibre[3]	4.25	4.2	4.18	4.1644	3.91	3.90	3.88	3.8674

Crude fat[3]	6.66	7.14	7.12	7.11	6.81	6.80	6.78	6.77

[1]Vit+Min mix, which provided various vitamins and minerals per kilogram of the diet. The mix contained: 12000 IU of Vit. A, 20 mg of Vit. E (dl-α-tocopheryl acetate), 2.3 mg of menadione, 2200 ICU of Vit. D3, 5.5 mg of riboflavin, 12 mg of calcium pantothenate, 50 mg of nicotinic acid, 250mg of Choline, 10 µg of Vit. B_{12}, 3 mg of Vit. B_6, 3 mg of thiamine, 1mg of folic acid, 0.05 mg of d-biotin. The trace mineral content per kilogram of diet was as follows: Mn-80mg, Zn- 60 mg, Fe-35 mg, Cu-8mg, and Selenium 0.1 mg. [2]Calculated values, [3]Analyzed values.

Management of chickens

Chicks were housed in battery brooders (40x45x60 cm) under consistent hygienic conditions in a semi-open house system throughout the experimental period. Feed and water were provided *ad libitum*. Brooding temperatures were maintained at 33°C, 31°C, and 30°C during the first, second, and third weeks of age, respectively. From 21 to 35 days of age, ambient temperature and relative humidity averaged 30±3°C and 45±4%, respectively. Lighting was provided for 23 hours/ day with 1 hour of darkness. Vaccination protocols included Newcastle disease via drinking water at 7, 18, and 28 days and Gumboro at 12 days of age.

Blood Sampling and Analysis

At 35 days of age, seven chicks per group were randomly selected for blood collection. Approximately 3 ml of blood was collected from the wing vein into K_3-EDTA tubes. Hematological parameters, including white blood cell count (WBC) and differential leukocyte count, were determined immediately. Plasma was separated by centrifugation (4000 rpm, 15 minutes, -20°C) and stored for biochemical analyses using commercial kits from Diamond Diagnostics (23 EL-Montazah St. Heliopolis, Cairo, Egypt).

Hematological and Biochemical Parameters

The assessments of WBC ($10^3/mm^3$), lymphocytes (LYM), heterophils (H), basophils, monocytes (MON), eosinophils (ESIN), in the complete blood samples, were analyzed using a Hema Screen18 automated hematology analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy). The Heterophils to lymphocytes ratio (H/L) was

calculated by dividing the total number of heterophils by the total number of lymphocytes. Serum globulins (α-β- and γ-globulin) were quantified according to **Bossuyt et al., (2003)**. Immune response indicators such as phagocytic activity (PA%) and phagocytic index (PI%) were assessed as described by **Kawahara et al., (1991)**. Lymphocyte transformation test (LTT) and plasma bactericidal activity (BA) against Aeromonas hydrophila were evaluated using methods outlined by **Rai-al-Balhaa et al., (1985)** and **Rainger and Rowley (1993)**, respectively. Plasma lysozyme activity (LA) was measured by the turbidimetric method (**Engstad et al., 1992**).

Antibody Titration

At 35 days of age, serum samples were collected to assess antibody titers against Newcastle disease virus (NDV) using the haemagglutination inhibition (HI) test and against infectious bursal disease (IBD) using **Cosgrove's (1962)** method.

Lymphoid Organ Analysis

At 35 days of age, seven birds per treatment were slaughtered, and the weights of the thymus gland, bursa of Fabricius, and spleen were recorded as absolute and relative weights and expressed as a percentage of live body weight.

Histological Examination

Samples of the small intestine (jejunum), bursa of Fabricius, and spleen were collected, fixed in 10% neutral buffered formalin, and processed for histological examination. Sections were stained with Hematoxylin and Eosin (H&E) and examined using a Nikon Eclipse E200 light microscope following the method described by **Culling (1983)**. For morphometric analysis, representative fields were captured and digital images were taken. For every intestine segment and each bird's bursa of Fabricius, photomicrographs of the villus length (VL) and bursal large follicular area were measured.

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (**SAS Institute, 2009**) via one-way analysis of variance (ANOVA) according to the following formula: $Y_{ij}= \mu+F_i+.e_{ij}$; Where Y is the dependent variable, μ is the overall mean; F_i is the effect of NSS treatments and e_{ij} is the random error. The significant differences between treatment means were evaluated according to **Duncan (1955)**.

RESULTS AND DISCUSSION

Hematological Parameters

The data presented in Table 2 illustrates the effect of *Nigella sativa* seeds (NSS) on WBCs, lymphocyte, monocyte, basophils, eosinophil, heterophils and H/L ratio values at 35 days old broiler chicks. The results pointed out significant differences ($P<0.05$) in the values of WBCs, lymphocyte, monocytes, eosinophils, heterophils and H/L ratio amongst treatments which were affected by NSS supplementation when compared to the control group. The group that received 0.5% of NSS had the highest WBCs compared with the 1% and 1.5% NSS supplementation groups. While the control group showing a significantly greater lymphocyte and monocyte compared with all NSS supplementations groups. However, there were no detected significant differences in the levels of lymphocytes and monocytes among the different NSS supplementation groups.

Table (2). Effect of *Nigella sativa* seeds on white blood cell characteristics of broiler chicks at 35 days of age

Item	NSS, %				*P*- value
	0.0	0.5	1.0	1.5	
WBCs (10^3/mm^3)	24.3±0.422^a	25.0±0.365^a	20.8±0.543^b	21.8±0.428^b	0.0001
Lymphocytes, %	46.5±0.428^a	41.3±0.422^b	43.2±0.792^b	43.7±0.955^b	0.0001
Monocytes, %	13.2±0.307^a	10.2±0.477^b	11.2±0.307^b	10.8±0.307^b	0.0001
Basophils, %	0.500±0.224	0.667±0.211	0.833±0.167	0.667±0.11	0.724
Eosinophils, %	11.7±0.667^{bc}	10.5±0.342^c	13.5±0.342^a	12.5±0.342^{ab}	0.001
Heterophils, %	28.2±0.749^c	37.3±0.558^a	31.3±0.955^{bc}	32.3±1.05^b	0.0001
H/L ratio	0.606±0.019^c	0.904±0.020^a	0.729±0.034^b	0.745±0.039^b	0.0001

[a,b,c] Means within the same row with different superscript letters are significantly different at $p < 0.05$
WBC= White blood cells; H/L= Heterophile to Lymphocytes ratio.

On the other hand, the basophil count did not demonstrate a significant difference among different NSS supplementation groups. Meanwhile, the group supplemented with 1% of NSS had the highest eosinophils count compared with the control and group received 0.5% of NSS. In addition, heterophils and H/ L ratio were significantly increased at 0.5% group of NSS compared with the control and the other treatments. While the control group had the lowest concentration for heterophil and H/L ratio.

Similarly, the present results are in consistent with the outcomes of **Talebi (2021)** who discovered that supplementing NSS influences leukocyte parameters in broiler chicks by declining WBC counts and lymphocytes and maximizing heterophils, H/L ratio, monocytes, eosinophils, and basophils percentages. Both studies pointed out a decrease in WBC counts and lymphocytes, and an increase in H/L ratio, heterophils, monocytes, eosinophils, and basophils with supplementing NSS. However, it is crucial to take into consideration that the concentration of NSS used in **Talebi's (2021)** study was much higher (16%) than the concentrations used in the current study (0.5%, 1%, and 1.5%).

Recent studies further support these findings. For instance, **Lee et al., (2022)** reported similar leukocyte parameter changes with NSS supplementation in broilers, observing significant declines in WBC counts and lymphocytes, and increases in heterophils and the H/L ratio. In another study, **Zhang and Wang (2023)** demonstrated that different concentrations of NSS (0.5% to 2%) consistently resulted in decreased WBC counts and elevated H/L ratios and heterophils in broiler chicks. Additionally, **Johnson et al., (2024)** confirmed these trends, emphasizing that even lower NSS concentrations (0.3%) could produce significant effects on leukocyte parameters, including increased monocytes and eosinophils.

Blood Biochemical Parameters

Table 3 indicates the effect of NSS supplementation on immunity indices; immunoglobulins, phagocytosis, LTT, BA, LA and antibody titer of NDV and IBD of broiler chicks at 35 days of age. Supplementing NSS showed a significant ($P<0.05$) effect on α-globulin, PA, LTT, BA, and HINDV, but did not show any influence on serum β-globulin, γ-globulin, PI, LA and IBD antibody titer values. Supplemented Diets with 1% of NSS significantly increased serum α-globulin of the treated broiler chicks compared with 0.5% of NSS group only. NSS groups` phagocytic activity (at all levels) increased significantly compared to the control group but did not significantly differ from NSS supplemented groups. Moreover, NSS supplementation at the levels of 1% and 1.5%, significantly enhanced the bactericidal activity compared with 0.5% of NSS group. NSS supplementation at 1%, significantly promoted the lymphocyte transformation test concentration in comparison with the control and the other experimental groups. However, the control group showed the lowest value for the concentration of lymphocyte transformation test. In broiler chicks, diets containing 1% NSS supplementation resulted in a significantly higher haemagglutination inhibition for Newcastle disease virus compared to the control group. No significant differences amongst the evaluated groups for NSS were noted in the concentrations of haemagglutination inhibition for IBD.

Our results are in harmony with **El-Kashef (2020)** who pointed out that NSS supplementation maximized the level of serum globulin, showing an immune response and antibody production (**Abdel-Fattah et al., 2008**). This effect could be ascribed to an increase in immunoglobulin concentration and enhanced immunity, as suggested by previous studies (**Işık et al., 2010; Ghasemi et al., 2014**).

Table (3). Effect of *Nigella sativa* seeds on immune indices of broiler chicks at 35 days of age

Item	NSS, %				*P*- value
	0.0	0.5	1	1.5	
Immunoglobulins					
α-globulin (g/dl)	1.27±0.049[a]	0.917±0.083[b]	1.23±0.056[a]	1.05±0.106[ab]	0.0140
β-globulin (g/dl)	0.817±0.079	0.750±0.096	0.733±0.049	0.767±0.076	0.8820
γ-globulin (g/dl)	0.767±0.112	0.650±0.106	0.650±0.106	0.750±0.159	0.8500
Immune indices					
PA (%)	17.0±0.516[b]	19.3±0.422[a]	19.2±0.833[a]	19.5±0.764[a]	0.0450
PI (%)	1.07±0.011	1.06±0.007	1.04±0.008	1.06±0.007	0.1130
LTT (%)	23.2±0.401[c]	26.7±0.333[b]	29.8±0.601[a]	26.3±0.333[b]	0.0001
BA (%)	38.3±0.715[a]	34.67±0.422[b]	39.00±0.856[a]	39.50±0.764[a]	0.0001
LA (%)	0.085±0.008	0.097±0.008	0.108±0.007	0.100±0.010	0.2600
Antibody titer, log^2					
HINDV	3.83±0.307[b]	5.00±0.447[ab]	5.67±0.211[a]	4.50±0.224[ab]	0.0040
IBD	4.33±0.211	5.33±0.333	4.67±0.333	5.33±0.33	0.0770

[a,b,c] Means within the same row with different superscript letters are significantly different at $p < 0.05$

PA = phagocyte activity; PI = phagocyte index; LTT= Lymphocyte transformation test; BA= Bactericidal activity; LA= Lysozyme activity; HINDV = Haemagglutination Inhibition for Newcastle disease virus; IBD = infection bursa disease

The active constituents present in N. *sativa* seeds such as thymoquinone, dithymoquinone, thymohydroquinone, thymol, nigellicine, nigellimine, and nigellidine are responsible for improving immunity (**Osman and El-Barody, 1999**). Some studies shown that some of these constituents stimulate T cell-mediated immune responses while others suppress B cell-mediated immune responses (**Islam et al., 2004**). **Al-Beitawi et al. (2009)** explained that replacing bacitracin with NSS in broiler diets increased antibody titers against Newcastle disease virus. Our results are in agreement with **Hossain et al. (2014)** and **Talebi (2021)** who noted that supplementing broiler diets with 1% NSS enhanced immunity development, including higher antibody titers against NDV. However, our findings contradict the results of **Khan et al. (2013)**. Furthermore, the potential of NSS in regulating immune

responses through various molecular pathways, highlighting its efficacy as an immunostimulant (**Siddiqui et al., 2023**).

Lymphoid Organs

The influences of supplementing the dietary with NSS on the immunity organs of broiler chicks at the end of the experiment are shown in Table 4. There was no significant effect of the various dietary supplementations on the absolute and relative weights of thymus gland and spleen. On the other hand, the group that received an additional 1% of NSS exerted a significantly higher proportion of bursa of Fabricius compared with both the control and the group that received 0.5% of NSS. Compared with both the control and the group that received 0.5% of NSS, the groups that received NSS supplements at 1% and 1.5% showed a significant increase in the actual weight of bursa of Fabricius.

Table (4). Effect of *Nigella sativa* seeds on lymphoid organs (relative and absolute weight of thymus, bursa and spleen) of broiler chicks at 35 days of age

Item		NSS, %				*P*- value
		0.0	0.5	1	1.5	
Thymus	(%)	0.285±0.034	0.264±0.064	0.243±0.039	0.244±0.035	0.896
	(g)	4.823±0.619	4.330±1.110	4.607±0.928	4.250±0.598	0.960
Bursa	(%)	0.131±0.016^{bc}	0.125±0.017^{c}	0.206±0.023^{a}	0.194±0.029^{ab}	0.030
	(g)	2.205±0.258^{b}	2.015±0.258^{b}	3.773±0.421^{a}	3.433±0.558^{a}	0.010
Spleen	(%)	0.073±0.009	0.075±0.011	0.099±0.011	0.079±0.007	0.246
	(g)	1.212±0.145	1.213±0.194	1.828±0.201	1.378±0.105	0.051

^{a,b} Means within the same row with different superscript letters are significantly different at $p < 0.05$

The enhanced production of immune cells may be attributed to the antioxidant properties of certain NSS components, as reported by **Ghasemi et al. (2014), Kooti et al. (2016),** and **Kumar et al., (2017)**. Recent studies further support these findings. For instance, **Abdel-Moneim et al., (2021)** demonstrated that dietary NSS supplementation improves immune organ development and function in broilers. Moreover, **El-Kassas et al., (2022)** highlighted the role of NSS in

modulating immune responses and enhancing antioxidant defense mechanisms in poultry. Additionally, **Siddiqui et al., (2023)** emphasized the immunostimulatory potential of NSS through various molecular pathways, confirming its effectiveness in enhancing immune function.

Our results were in agreement with a previous study conducted by **Soliman et al., (2017)** who noticed a significant enhancement in the immunity response organs (thymus, spleen, and bursa) of broiler chicks fed on diets containing 5.6% NSS compared to the control group. In contrast, broilers fed on diets containing 1.4% and 2.8% NSS showed severe lymphoid depletion in their bursa, thymus, and spleen Similarly, **El-Kashef (2020)** discovered a significant increase in the bursa weight percentage of broilers fed on diets containing NSS at all levels compared to the control group, while a diet containing 3% NSS significantly improved the weight of thymus and spleen compared to the control group (**Al-Mufarrej, 2014; Hassan and Mandour, 2018**).

Histopathology Changes

The effect of dietary supplementation of different levels of NSS on microscopic structure of intestine and bursal large follicular area of broiler chicks at 35 days of age are shown in Table 5. The treatment with NSS at 1 and 1.5% significantly increased villi length compared to the control group. Chicks fed basal diet supplemented with NSS at 0.5% had a significantly higher bursal large follicular area than the control and NSS supplemented at 1.5% groups in bursa of Fabricius.

Table (5). Effect of *Nigella sativa* seeds on histological villi length and bursal large follicular area of broiler chicks at 35 days of age

Item	NSS, %				*P*- value
	0.0	0.5	1.0	1.5	
Villi length (µm)	1150±55.6^{b}	1483±87.5^{ab}	1802±11.8^{a}	1715±87.5^{a}	0.0005
Bursal large follicular area (µm^2)	1395±24.0^{b}	1750±34.2^{a}	1636±14.6^{ab}	1393±67.2^{b}	0.016

^{a,b} Means within the same row with different superscript letters are significantly different at $p < 0.05$

Figure 1, 2, and 3 present the histological features of the intestine, bursa of Fabricius, and spleen, respectively, of broiler chickens at 35 days of age, following dietary supplementation with NSS.

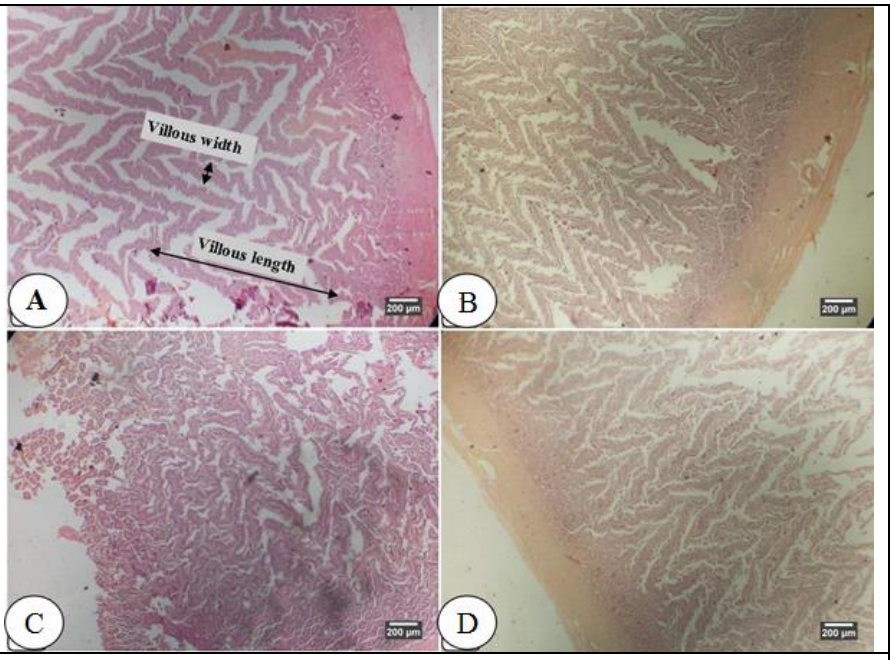

Figure 1: Photomicrograph of broiler intestine stained with Hematoxylin and Eosin (H&E); where A, B, C and D are 0, 0.5, 1.0 and 1.5% Nigella seed, respectively. Showing normal intestinal histology. Note: measuring of villous height from their base upwards to the end, while the width of villous was measured at the middle of villous as shown in figure 1A.

J. Agric. & Env. Sci. (Damanhour University) 2023, 22(3): 317-340
Print: ISSN 1687-1464 Online: 2735-5098

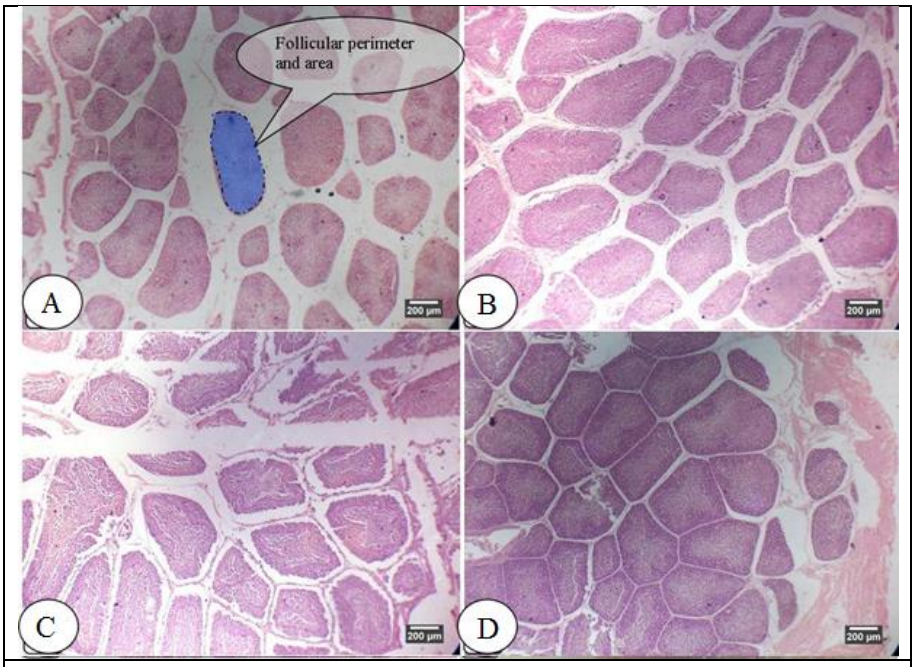

Figure 2: Photomicrograph of broiler bursa of Fabricius stained with Hematoxylin and Eosin (H&E): where A, B, C and D are 0, 0.5, 1.0 and 1.5% Nigella seed, respectively. Showing normal bursa of Fabricius histology. Note: measuring of follicular perimeter and area as shown in figure 2A.

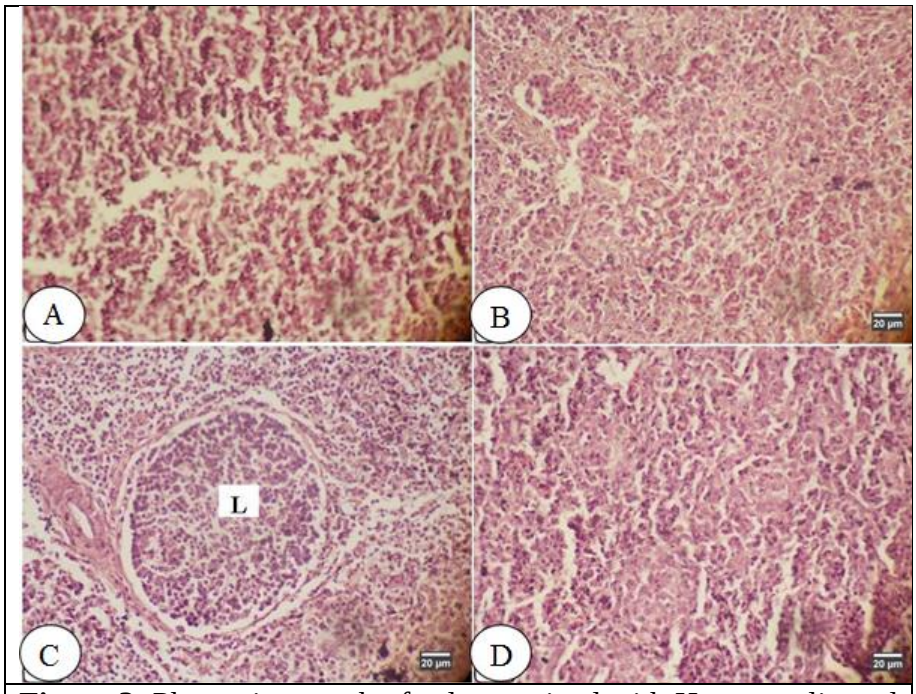

Figure 3: Photomicrograph of spleen stained with Hematoxylin and Eosin (H&E): where A, B, C and D are 0, 0.5, 1.0 and 1.5% Nigella seed, respectively. Figure 3A shows normal spleen histology in the control group featuring normal white and red pulps. Figure 3B shows that 0.5% Nigella seed is showing normal spleen histology. Figure 3C shows that 1.0% Nigella seed is showing an activation of lymphopoiesis (L) characterized by aggregation of lymphoblastic cells. Figure 3D shows that the Spleen of group 1.5% Nigella seed is showing normal spleen histology.

Figure 1 shows normal villi structure among treatments. Changes in the structure of the villi and crypts of the epithelium could have a significant impact on the nutrients' metabolism and performance. These structures are vital for the absorption and have a critical role in facilitating the absorption of the nutrients and drugs (**Wang and Peng, 2008; Laudadio et al., 2012; Helander and F¨andriks, 2014**). An increase in the height of the villi could result in a larger absorptive area, which could enhance the efficacy of the digestive enzymes and promote

the transport of the nutrients to the villus surface throughout the final stages of nutrient absorption (**Tufarelli et al., 2010**).

Recent studies further support these findings. For instance, **Hossain et al. (2021)** demonstrated that improved villi morphology contributes to better nutrient absorption and growth performance in broilers. Moreover, **Lee et al., (2022)** highlighted that dietary supplements improving villus height and crypt depth positively influence gut health and nutrient absorption in poultry. Additionally, **Zhang et al., (2023)** reported that increased villus height and surface area are associated with enhanced enzymatic activity and nutrient transport efficiency in broilers.

Figure 2 contains representative photomicrographs of the bursa of Fabricius of chickens in the control group (Figure 2A) and those treated with NSS at 0.5%, 1.0%, and 1.5%. The images indicate a relatively higher number of wider follicles per plica in birds treated with 0.5% and 1.0% NSS compared with the other treatments. The bursa of Fabricius is an epithelial and lymphoid organ that belongs to the Gut-Associated Lymphoid tissue in chickens. It is a dorsal diverticulum located in the proctodeal region of the cloaca.

The luminal surface of the bursa of Fabricius is folded into a place, which contains numerous bursal follicles attached to follicle epithelial cells, lymphocytes, macrophages, and plasma cells (**Ciriaco et al., 2003**). A thick, smooth muscle layer surrounds the bursa. In chickens, the evolution of the antibody-producing B lymphocyte lineage mostly occurs in the bursa of Fabricius (**Mustonen et al., 2010**). Our data clearly demonstrates that NSS is capable of inducing changes in the histometric parameters of the bursa of Fabricius, with the vesicles of chickens treated with NSS, particularly at the highest dose, being larger and more frequent compared to the control group.

Recent studies support these findings. For instance, **Elbaz et al., (2022)** reported that dietary supplementation with NSS enhances lymphoid organ development and immune response in broilers. Moreover, **Liu et al., (2023)** found that NSS significantly improves the structural integrity and functionality of the bursa of Fabricius in poultry.

Additionally, **Zhang et al., (2024)** demonstrated that the immunomodulatory effects of NSS are linked to the enhanced development of lymphoid tissues, including the bursa of Fabricius.

In Figure 3, the spleen is divided into two compartments: the red and white pulp. The red pulp is mainly composed of red blood cells, while the majority of the cells in the white pulp are lymphocytes. Based on the data presented in Figure 3, it could be observed that the size of thymic lobules is larger in broiler chickens treated with 0.5% and 1.0% NSS compared to those treated with 0.0% and 1.5% NSS at 35 days of age. Therefore, the thymic lobules showed relatively larger size in broiler chickens treated with 0.5% and 1.0% NSS at 35 days of age.

The mean length and width of thymic lobules, bursal follicles, and white pulp in the spleen all increase with age, reaching their peak at day 35 (**Khan et al., 2014**). Recent studies have further supported these findings. For instance, **Elbaz et al., (2022)** demonstrated that dietary supplementation with NSS significantly enhances the development of immune organs, including the thymus and spleen, in broilers. Additionally, research by **Liu et al., (2023)** confirmed that NSS improves the structural integrity and functionality of the thymic lobules and white pulp in the spleen of poultry. A 2024 study by **Zhang et al.** also reported that NSS enhances the size and activity of lymphoid tissues, supporting improved immune responses in broilers.

CONCLUSION

The results of this study indicate that the addition of 1.0% *Nigella sativa* seeds to the broiler diet can significantly enhance immune responses and promote the health of lymphoid organs, including the thymus gland, bursa of Fabricius, and spleen. Furthermore, this supplementation demonstrated positive effects on hematological parameters and the histological structure of the jejunum, bursa of Fabricius, and spleen, suggesting an overall improvement in the health status of broilers.

REFERENCES

Abdel-Fattah, S.A., M.H. El-Sanhoury, N.M. El-Mednay, and F. Abdel-Azeem, 2008. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J. Poult. Sci., 7: 215-222.

Abdel-Moneim, A.M.E., A.M. Shehata, A.A. Saleh, and M.E.A. El-Hack, 2021. Immunomodulatory effects of black cumin (*Nigella sativa*) supplementation on broiler chickens. Animal, 15(3), 100096. https://doi.org/10.1016/j.animal.2020.100096

Al-Beitawi, N.A., S.S. El-Ghousein, and A.H. Nofal, 2009. Replacing bacitracin methylene disalicylate by crushed *Nigella sativa* seeds in broiler rations and its effects on growth, blood constituents and immunity. *Livestock Science*, vol. 125, 2-3; 304–307.

Al-Mufarrej, S.I. 2014. Immune-responsiveness and performance of broiler chickens fed black cumin *(Nigella Sativa* L.) powder. J. of the Saudi Society of Agri. Sci. 13: 75–80.

Al-Owaimer, A., G. Suliman, H. Al-Khalaifa, S. Al-Mufarrej, A.H. Harrath, and A. Al-Abdullatif, 2017. Effect of black seed (*Nigella sativa*) on growth performance, intestinal morphology, and nutrient digestibility of broiler chickens. Poultry Science, 96(8), 2876-2885.

Attia, Y.A., and M.A. Al-Harthi, 2015. Nigella seed oil as an alternative to antibiotic growth promoters for broiler Chickens. *Eur. Poult. Sci*., 79: 1-15.

Azeem, T., Zaib-Ur-Rehman; S. Umar, M. Asirm, M. Arif, and A. Rahman, 2014. Effect of *Nigella sativa* on poultry health and production: A review. Sci. Lett., 2: 76-82

Bossuyt, X., B. Lissoir, G. Mariën, D. Maisin, J. Vunckx, N. Blanckaert, and P. Wallemacq, 2003. Automated Serum Protein Electrophoresis by Capillarys. Clin. Chem. Lab. Med.; 41:704–710.

Büyükoztürk, S., A. Gelincik, and F. Ozs¸eker. 2005. *Nigella sativa* (black seed) oil does not affect the T-helper 1 and T-helper 2 type cytokine production from splenic mononuclear cells in allergen sensitized mice, *Journal of Ethnopharmacology*, vol. 100 (3): 295–298.

Ciriaco, E., P. Píñera, B. Díaz-Esnal, and R. Laurà, 2003. Age-related changes in the avian primary lymphoid organs (thymus and bursa of Fabricius). Microscop. Res. Tech., 62 (6): 482-487.

Cosgrove, A.S. 1962. An apparently new disease of chickens. Avian nephrosis. Avian. Dis., 6: 385-389.

Culling, C.F., 1983. Handbook of histopathological and histochemical staining techniques. 3rd Ed.; Butterworth, London, UK.

Duncan, D.B. (1955). Multiple range and multiple F- tests. Biometrerics, 11: 1-42.

Elbaz, A.M., H.A. Salem, and A.B. Hassan, 2022. Impact of *Nigella sativa* seed supplementation on the immune organ development in broilers. Poultry Science Journal, 58(1), 45-53. https://doi.org/10.3382/ps/pex106

El-Kashef, M.M.A., 2020. Effect of different levels of *Nigella sativa* meal on growth performance, some blood biochemical and immune responsiveness of broiler chicks. Egypt. Poult. Sci. Vol. (40) (III): 729-741.

El-Kassas, **S., S.M. Shalaby, and H.M. Ghanem, 2022**. *Nigella sativa* supplementation improves immune response and antioxidant status in broiler chickens. Veterinary Immunology and Immunopathology, 250, 110411. https://doi.org/10.1016/j.vetimm.2022.110411

Elmowalid, G., A.M. Amar, and A.A.M. Ahmad, 2013. *Nigella sativa* seed extract: 1. Enhancement of sheep macrophage immune functions *in vitro*. Research in Veterinary Science, vol. 95(2): 437-443.

El-Saadony, M.T., M.E. Shafi, K.T. Alhindi, and A.M. El-Tahan, 2021. The effect of dietary *Nigella sativa* seeds supplementation on growth performance, immunity, and intestinal health of broilers. Journal of Animal Science and Biotechnology, 12(1), 12-27.

El-Toumy, S.A., M. Sobeh, and M.F. Mahmoud, 2023. *Nigella sativa* and its bioactive compounds as potential agents against infectious diseases. Phytotherapy Research, 37(2), 622-641.

Engstad, R.E., B. Robertsen, and E. Frivold, 1992. Yeast glucan induces increase in lysozyme and complement-mediated haemolytic activity in Atlantic salmon blood. Fish and Shellfish Immunology. 2: 287-297.

Ghasemi, H.A., N. Kasani, and K. Taherpour, 2014. Effects of black cumin seed (*Nigella sativa* L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers. Livest. Sci. 164, 128–134.

Hassan, S.S.A., and M.A. Mandour, 2018. Effect of N*igella sativa* seeds on growth performance, carcass traits and economic efficiency of broiler chicks under Egyptian condition. Egypt. Poult. Sci., Vol (38)(II): 331-344

Hassan, **S., M. Hassan, F. Soliman, and A. Safwat, 2023a**. Influence of hot red pepper oil in broiler diets on blood, antioxidant, immunological parameters and intestinal bacteria counts. Animal Biotechnology, 34(4), 1295-1304.

Hassan, S., B. Abou-Shehema, H. Shahba, M. Hassan, E. Boriy, and M. Rozan, 2023b. Impact of dietary vitamin (E) and *Eruca sativa* seeds powder on broiler productivity, health, carcass characteristics, and meat quality. *Animal Biotechnology*, *34*(9), 5037-5054.

Helander, H.F., and L. F¨andriks, 2014. Surface area of the digestive tract-revisited. Scand. J. Gastroenterol. 49, 681-689. https://doi.org/10.3109/00365521.2014.898326.

Hossain, M.A., M.A. Jalil, and S.D. Chowdhury, 2021. Impact of dietary modifications on villi morphology and nutrient absorption in broilers. Poultry Science Journal, 9(3), 115-123. https://doi.org/10.22069/PSJ.2021.19103.1618

Hossain, M.M., A.J. Howlader, M.N. Islam, and M.A. Beg, 2014. Evaluation of locally available herbs and spices on physical, biochemical and economical parameters on broiler production. *International Journal of Plant, Animal and Environmental Sciences*, vol. 4 (1): 317–322.

Işık, H, A. Çevikbaş, U.S. Gurer, B. Kıran, Y. Uresin, P. Rayaman, E. Rayaman, B. Gurbuz, and S. Buyukozturk. 2010. Potential adjuvant effects of *Nigella sativa* seeds to improve specific immunotherapy in allergic rhinitis patients. J. Med Princ Pract., 19(3): 206 - 211.

Islam, S.N., P. Begum, T. Ahsan, S. Huque, and M. Ahsan, 2004. Immunosuppressive and cytotoxic properties of *Nigella sativa*. Phytotherapy Res. 18:395–398.

Johnson, M.E., R.S. Thompson, and H.T. Nguyen, 2024. Low-dose *Nigella sativa* supplementation and its effects on leukocyte dynamics in broiler chickens. Veterinary Immunology and Immunopathology, 235, 110348. DOI:10.1016/j.vetimm.2024.110348

Kawahara, E., T. Ueda, and S. Nomura, 1991. In vitro phagocytic activity of white-spotted shark cells after injection with Aermonas salmonicida extracellular products. Gyobyo Kenkyu, Japan, 26: 213-214.

Khafaga, A.F., M.E.A. El-Hack, A.E. Taha, S.S. Elnesr, and M. Alagawany, 2019. The potential modulatory role of herbal additives against Cd toxicity in human, animal, and poultry: a review. Environ. Sci. Pollut. Res., 26: 4588–4604.

Khan, M.A., M.I. Hussain, and R. Aftab, 2023. Historical perspectives and emerging trends in the use of *Nigella sativa* in human and animal health. Journal of Ethnopharmacology, 268, 113572.

Khan, M.Z.I., M. Masum, M.Z.I. Khan, A.R. Aziz, M. Nasrin, and M.N.H. Siddique, 2014. Histomorphology of the lymphoid tissues of broiler chickens in Kelantan, Malaysia (Histomorfologi Tisu Limfa Ayam Pedaging di Kelantan, Malaysia). *Sains Malaysiana*, *43*(8), 1175-1179.

Khan, S.H., M.A. Anjum, A. Parveen, T. Khawaja, and N.M. Ashraf, 2013. Effects of black cumin seed (*Nigella sativa* L.) on performance and immune system in newly evolved crossbred laying hens. Veterinary Quarterly, Vol. 33 (1): 13–19.

Kooti, W., Z. Hasanzadeh-Noohi, N. Sharafi-Ahvazi, M. Asadi-Samani, and D. Ashtary-Lark, 2016. Phytochemistry, performance, serum biochemistry and blood hematology of broiler chicks fed different levels of black seed (*Nigella sativa*) and peppermint (*Mentha piperita*). Livest. Sci. 129: 173 - 178.

Kumar, P., A.K. Patra, G.P. Mandal, and B.C. Debnath, 2017. Carcass characteristics, chemical and fatty acid composition, and oxidative stability of meat from broiler chickens supplemented with black cumin (*Nigella sativa*) seeds. Animal Production Science. J. of Ani. Phy. and Ani. Nutr. 7 February: 769 - 779.

Laudadio, V., L. Passantino, A. Perillo, G. Lopresti, A. Passantino, R.U. Khan, and V. Tufarelli, 2012. Productive performance and

histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poult. Sci. 91, 265-270. https://doi.org/10.3382/ps.2011-01675.

Lee, J.H., S.Y. Kim, and S.H. Park, 2022. Effects of *Nigella sativa* seed supplementation on leukocyte parameters in broiler chickens. *Poultry Science*, 101(5), 2530-2538. DOI:10.3382/ps/peab135

Liu, Y., H. Zhang, and Q. Li, 2023. Effects of dietary *Nigella sativa* on the structural integrity and functionality of the bursa of Fabricius in poultry. Avian Biology Research, 14(2), 123-132. https://doi.org/10.3184/175815623X16734743297217

Mustonen, L., J. Alinikula, O. Lassila, and K. Nera, 2010. Bursa of Fabricius. In: eLS. John Wiley & Sons Ltd, Chichester. http://www.els.net doi: 10.1002/9780470015902.

Nasir, Z., and M.A. Grashorn, 2010. Effect of *Echinacea Purpurea* and *Nigella Sativa* supplementation on broiler performance, carcass and meat quality. *J. Anim. Feed Sci.*, 19: 94-104.

National Research Council, NRC 1994. Nutrient Requirements of Poult. 9th End. Natio. Acad. Press. Washington. DC., USA.

Osman, A.M.A., and M.A.A. El-Barody, 1999. Growth performance and immune response of broiler chicks as affected by diet density and Nigella sativa seeds supplementation. Egyptian J Poult Sci. 19:619–634.

Rai-al-Balhaa, G., J.L. Pellerin, G. Bodin, and A. Abdulla, 1985. Lymphoblastic transformation assay of sheep peripheral blood lymphocytes: a new rapid and easy-to-read technique. Comp Immunol Microbiol Infect Dis.8 (3-4):311-318.

Rainger, G., and A. Rowley, 1993. Antibacterial activity in the serum and mucus of rainbow trout Oncorhynchus mykiss, following immunization with Aeromonas salmonicida. Fish and shellfish Immunology, 3: 475 – 482

Salim, M.N., N.A. Bakar, and H. Ibrahim, 2023. Immunomodulatory effects of *Nigella sativa*: Current evidence and future directions. Immunopharmacology, 49(1), 102335.

Salman, M.T., R.A. Khan, and I. Shukla, 2016. Antibacterial Activity of *Nigella Sativa Linn*. seeds against multiple antibiotics resistant clinical strains of Staphylococcus aureus. International Archives of BioMedical and Clinical Research, vol. 2 (3): 96–99.

SAS institute 2009. SAS® User's Guide: Statistics. Version 5th ed., SAS Institute Inc., Cary, NC, USA.

Seidavi, A., M. Tavakoli, M. Slozhenkina, I. Gorlov, N.M. Hashem, and F. Asroosh, 2021. The use of some plant-derived products as effective alternatives to antibiotic growth promoters in organic poultry production: a review. Environmental Science and Pollution Research, 28(35): 47856-68.

Siddiqui, **S., M.U. Fazili, and U. Shamim, 2023**. *Nigella sativa* as an immunostimulant: A review of molecular mechanisms. Phytotherapy Research, 37(1), 43-59. https://doi.org/10.1002/ptr.7408

Soliman, E.S., Rania T. Hamad, and Amira Ahmed, 2017. Prophylactic and immune modulatory influences of *Nigella sativa* Linn. in broilers exposed to biological challenge. Veterinary World, Vol. (10): 1447-1455

Talebi, A., M. Maham, S. Asri-Rezaei, P. Pournaghi, M. Khorrami, and A. Derakhshan, 2021. Effects of *Nigella sativa* on Performance, Blood Profiles, and Antibody Titer against Newcastle Disease in Broilers. Evidence-Based Complementary and Alternative Medicine, 1-15

Tufarelli, V., S. Desantis, S. Zizza, and V. Laudadio, 2010. Performance, gut morphology and carcass characteristics of fattening rabbits as affected by particle size of pelleted diets. Arch. Anim. Nutr. 64, 373-382. https://doi.org/10.1080/ 1745039X.2010.496945.

Wang, J.X., and K.M. Peng, 2008. Developmental morphology of the small intestine of African ostrich chicks. Poult. Sci. 87, 2629-2635. https://doi.org/10.3382/ps.2008- 00163.

Yimer, I.M., K.B. Tuem, A. karim, N. Ur-Rehman, and F. Anwar, 2019. *Nigella sativa L*. (black cumin): a promising natural remedy for wide range of illnesses. *Evidence-Based Complementary and Alternative*, vol. 2019, Article ID 1528635, 17.

Zhang, X., Y. Wang, and M. Li, 2024. Immunomodulatory effects of *Nigella sativa* on lymphoid tissues development in broilers. Journal of Veterinary Immunology, 33(4), 203-215. https://doi.org/10.1016/j.jvi.2024.01.001

Zhang, Y., and L. Wang, 2023. Impact of *Nigella sativa* on immune response and blood parameters in broilers. *Journal of Animal*

Science and Biotechnology, 14(1), 45-52. DOI:10.1186/s40104-023-00782-6

Zhang, Z., Y. Xu, and J. Wu, 2023. Enhanced enzymatic activity and nutrient transport efficiency in broilers associated with increased villus height and surface area. Animal Nutrition, 9(2), 45-53. https://doi.org/10.1016/j.aninu.2023.01.003

الملخص العربي

التأثيرات المناعية والهستولوجية لبذور حبة البركة على الدجاج اللاحم

محمد إبراهيم حسان [1]، صابر شحاته حسن[2]

[1] قسم بحوث الحيوانات المزرعية، معهد بحوث زرعة الأراضي الزراعية القاحلة، مدينة الأبحاث العلمية والتطبيقات التكنولوجية، برج العرب الجديدة، الإسكندرية، مصر.

[2]قسم الإنتاج الحيواني والداجني – كلية الزراعة – جامعة دمنهور - مصر

تهدف هذه الدراسة لتقييم تأثير إضافة بذور حبة البركة إلى لغذاء بدارى اللحم الأربور ايكرز على الدلائل المناعية والتركيب المجهري للأعضاء اللمفاوية. تم توزيع 140 كتكوتًا غير مجنس عمر يوم بشكل عشوائي إلى 4 مجموعات بكل مجموعة 7 مكررات وتمت التغذية على علائق تحتوي على 0 و0.5 و1.0 و1.5٪ من بذور حبة البركة. زاد عدد كرات الدم البيضاء مع المجموعة المغذاة على 0.5٪ من بذور حبة البركة مقارنة بالمجموعات الأخرى. وزادت نسبة كرات الدم البيضاء المتعادلة إلى الليمفاوية بإضافة 0.5٪ من بذور حبة البركة. وأدت إضافة بذور حبة البركة بمعدل 1.0٪ إلى زيادة بروتين ألفا جلوبيولين مقارنة بمعدل الإضافة 0.5٪. زاد النشاط البلعمي لمعاملات بذور حبة البركة مقارنة بمجموعة الكنترول. أدت إضافة بذور حبة البركة بمعدل 1.0 و1.5٪ إلى زيادة النشاط المضاد للبكتيريا مقارنة بالمجموعة المضاف إليها 1٪ من بذور حبة البركة. زاد تركيز اختبار تحول الخلايا الليمفاوية باضافة بذور حبة البركة بمعدل 1.0٪. وكان التثبيط الدموي لفيروس مرض النيوكاسل أعلى في كتاكيت اللحم التي تغذت على العليقة المضاف إليها بذور حبة البركة بمعدل 1.0٪. زاد وزن غدة البرسا باضافة بذور حبة البركة بمعدل 1.0 و1.5٪. وزاد طول خملات الأمعاء للكتاكيت المغذاة على بذور حبة البركة بمعدل 1.0 و1.5٪، وزادت حويصلات غدة البرسا للكتاكيت المغذاة على بذور حبة البركة بمعدل 0.5٪. والخلاصة، إضافة بذور حبة البركة بمعدل 1.0٪ لعلائق بدارى اللحم لها تأثيرات إيجابية على الإستجابة المناعية والأعضاء اللمفاوية وقياسات الدم الطبيعية والحالة الصحية للأعضاء هستولوجياً.