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Influence of Different Immunostimulants on Growth, Serological Response and Histological Changes of Newcastle Disease Virus-vaccinated Chicks

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ABSTRACT

Newcastle disease (ND) vaccination was targeted to reduce mortality, viral load in the environment, and eradication of positive cases. Many immunostimulants have been used to improve the immune response of vaccinated chickens. The current study was designed to compare the effect of different immunostimulants on immune response of NDV vaccinated chicks. A total of 150 one-day old Cobb chicks were divided into 5 equal groups. Group 1 was lifted as non-vaccinated negative control, whereas other groups were vaccinated against NDV. The used immune stimulants under test were given to groups 3, 4, and 5 as follows *N. sativa* 6%, curcumin1%, and orego sol. respectively at 7th day old continuously till the end of experiment. The results showed high antibody titers, low mortality rates and better body performance in the groups treated with these immunostimulants than the other groups. However, chicks treated with *N. sativa* showed renal water cyst formation compared with other groups. In conclusion, application of immunostimulants can stimulate the immune response and reduce the pathogenicity of ND in chickens.

Key words: Histopathology, Immune response, Immunostimulants, NDV.

INTRODUCTION

NDV is a significant entry in the conversion resources or production elements into products, goods, and services that are available to consumers; it results in direct economic losses for the producer as well as a potentially loss of value in the consumer's eyes. The vaccine's aim is to increase immunity against virus infection and replication, and also protect birds against the more adverse effects of NDV infection and clinical indications. Vaccinated birds, however, may still be infected, reproduce, and excreted with lethal ND strains (Amer et al., 2017).The effect of a mixture of immunostimulatory compounds on the immune response of broiler chicks to ND vaccine is significant because the injected immunostimulant combination with the ND vaccine provides 100% protection against

the challenge of NDV, whereas chicks vaccinated with killed ND vaccine or live ND vaccines obtained 80 and 60% protection, respectively (Hussein et al., 2009).Antioxidant treatment enhanced immunological response, as revealed by significantly higher HI titers of the ND virus or relative weights of lymphoid organs such as thymus, bursa of Fabricius, and spleen (Tollba et al., 2007). Due to the obvious variations in the composition of the immune stimulants utilized, which included lectin, a vitamin combination, Beta-glucan, and other elements, had different mechanisms of action as immune stimulants. The goal of our research was to test the effect of some immune stimulants in increasing the chick's immunological response and performance following ND vaccination.

MATERIAL AND METHODS

Chickens

A total of 150 one-day-old cobb chicks were obtained from a commercial hatchery. The chicks will be reared on the floor and fed a well-balanced commercial feed. Four birds were slaughtered on their first day of birth (0 day) to obtain individual blood samples for serum analysis to assess maternal derived antibodies to ND.

Ration:

The chicks were fed a prepared ration in accordance with the Ross broiler management manual and the NRC (1994) broiler requirement. All chickens in cages were provided ad libitum rations and water.

Vaccine Strains:

Nobilis® ND Clone 30vaccine (7th day): Active components per dose:Live ND strain Clone 30: $\geq 6.0 \log_{10} EID_{50}$.

Nobilis® Gumboro D78 (10th day): Active components per dose:Live I.B.D. virus strain D78: $\geq 4.0 \log_{10} TCID5$.

Nobilis ND Lasota (14th day): Active components per dose: Live ND strain LaSota: $\geq 6.0 \log_{10} EID_{50}$.

Challenge virus:

The virus was isolated from the field, identified, and sequenced as velogenic ND (vNDV) genotype VII (Chicken/ Giza/Egypt/2020) with the accession number (OM243951) (Zain El deen et al., 2022). Viral stocks were titrated in 9–10-day-old chicken embryonated eggs, and the embryo infectious dose (EID_{50}) was estimated according to (Reed and Muench, 1938).

Hemagglutinating antigen for HI:

By passages in an allantoic sac of 9-10 days-old embryonated chicken eggs, NDV HI antigens were produced from the La Sota vaccinal strain of NDV vaccine. Infected embryos' amnio-allantoic fluids were collected aseptically and utilized as antigens in the HI test (Amer, 1984).

Immunostimulants:

Nigella sativa (NS) Seeds: *N.sativa* seeds were obtained from a local herbal store. The taxonomic identity of the plant seeds was verified at the National research center, Dokki, Egypt. The seeds were then carefully washed with distilled water to remove any extraneous materials, dried under shade at room temperature, ground into a coarse powder using an electronic grinder, weighed by analytical balance, and added to the diets at the rate of 6% of total ration according to (Al-Mufarrej,2014).

Orego solution: it was containingthymol (*thymus vulgaris oil* 2500mg/L,Oregano oil 40000(carvacol)mg/L, Glyceryl polyethylene glycol ricinoleate 220000 mg/l Water) CCPA international company in France. it was added in drinking water(1.25ml/L).

Commercial curcumin: (bio curcumin 1300mg, thereof curcuminoides1235 mg, and bio pepper 90 mg**)** German company (bio -grade curcumin in vegan capsules(1%), it was added at 1% in ration.

Experimental Design:

One hundred and fifty chicks were divided into 5 equal groups (1-5); each 30 chicks.

Each group was kept on the floor in a separate clean disinfected pen and commercial ration and water provided adlibitum. All chicken groups were vaccinated against ND. The used immune stimulants under test were given to groups3, 4, and 5 as followsN.Sativa (6%), curcumin (1%), and orego sol (1.25ml/litre)in drinking water, respectively at 7 th day old continuously till the end of experiment . At the third week of life, vNDV (10^6 EID_{50}/100 mL/bird) was injected intra-nasally (IN) into groups 2, 3, 4, and 5. All the groups were subjected to daily observation with recording of weekly body weight to calculate weekly and total feed conversion rate (FCR). Weekly individual blood samples were taken to separate serum for serological testing using HI at 0,21, days of age and 3,5,7 days' post infection (DPI). At 3, 5 and7 DPI; 4 birds were killed for collection of blood for clinicopathological examination and also cecal tonsils, proventriculus, and spleen tissues in formal saline for histopathology.

Histopathological Examination:

Tissue specimens from cecal tonsils, proventriculus, and spleen of experimentally infected and control chicks was fixed in 10% formalin buffered saline for 24 hours. Sections of an average thickness of 3 microns was prepared and stained with hematoxyline and eosin according to Banchroft et al. (1996).

Statistical Analysis:

The results were analyzed using Microsoft excel statistical package (SPSS 20.0).
ANOVA was used to compare means among different groups. P values of 0.05 or less than were considered significant.

RESULTS

Mean body weight, feed conversion rate (FCR) of commercial chicks vaccinated with ND:

The mean body weight, and feed conversion rate (FCR) of commercial chicks vaccinated ND are shown in Table (1).

Weight gain:

The body weight was recorded in all group (Fig. 1). The control positive group 2 (vaccinated and un-treated) showed the lowest body weight gain. The body weight gain was improved in the additive supplemented birds groups 3, 4, and 5compared to the control positive group 2. When compared to the other treated groups, group 5 (orego sol treated) showed the highest body weight gain.

Morbidity and mortality rate:

Regarding challenge test using vNDV showed that (Table 2) control –ve group 1 showed 0% morbidity and 0% mortality. Control positive vaccinated group is showed morbidity rate 26.6%, while diseased birds were detected earlier than treated control. Morbidity rate was shown 20%,13.3%,6%in *N.sativa*, Curcumin, Orego sol, receptively. No mortality was detected in Curcumin and Oregosolu groups while N.sativa and control vaccinated groups showed 13.3%, 10% mortality rate, receptively.

Serological test:

Our findings in (Fig.2) indicated that there are significant variations ($p<0.05$) in serological response between various groups and also between different days following treatment. Furthermore, group 5 had the highest antibodies titer compared to other groups.

Water cyst formation:

Dead birds were examined for postmortem lesions such as proventricular hemorrhage, tracheitis, cecal tonsil hemorrhage, and congested pectoral muscles and tracheal mucosa. Four chicks treated with *N.sativa* were shown renal water cyst while other groups were not shown any cyst.

Histopathological examination:

Spleen:

Negative control showed the normal architecture of splenic tissue (Fig. 1 A), while the positive control in group 2 on the 5th day post challenge showed lymphoid depletion (necrosis of lymphocytic cells), congestion and hemorrhages of red pulp, associated with severe reduction of lymphocytic cells distinguished by the lymphoid depletion of splenic pulps (Fig. 1 B and C). Treatment with Orego sol and *N. Sativa* in both group 5 and 3 ameliorated these histopathological lesions with mild depletion and mild congestion (Fig. 4 D and E). On the other hand, group, 4 treated with Curcumin showed moderate lymphoid depletion and moderate congestion of the red pulp (Fig. 4 F). On the 7th day post-challenge, sections of groups 3, 4, and 5 revealed lesions similar to those observed in 5th day post-challenge.

Cecal tonsils:

Negative control group showed the normal structure of tonsilar lymphoid nodules, normal structure of lieberkuhn glands (Fig. 5 A), while the positive controls in group 2 on the 5th day post challenge showed aggregation of inflammatory cells, congested small blood vessels with edematous enlargement of cecal tonsils. Glands of lieberkuhn showed degenerative changes with (Fig. 5 B and C). Treatment with Orego sol and *N. Sativa* in both group5 and 3 ameliorated these histopathological lesions with mild depletion and mild congestion (Fig. 5 D and E). On the other hand, group 4 treated with Curcumin showed moderate depletion of lymphoid nodules with mild degeneration in glands of Lieberkuhn (Fig. 5 F). On the 7th day post-challenge, sections of groups3, 4, and 5 revealed lesions similar to those observed in 5th day post-challenge.

Proventriculus:

Proventriculus (avian true stomach) showed normal histology of mucosal layer and glandular layer on 3rd day post-challenge for 1, 3, 4, and 5groups compared with the positive control. On the 5th day post-challenge, the proventriculus of control positive group showed severe inflammation of mucosal epithelium with fusion and thickening of mucosa due to infiltrations with inflammatory cells (figure 6.A). The treated groups showed mild congestion, (Fig. 6) compared to the positive control group. On the 7th day post-challenge, proventricular sections from all groups revealed changes similar to those observed in 5th day post- challenge.

Table (1): Mean body weight, feed conversion rate (FCR) of commercial chicks vaccinated with ND:

GR. NO	VACCINE	IMMUNE STIMULANT	AGE / WEEK	Mean of BW /GM	FCR
1	non vaccinated	(Negative control) Non treated, non-infected	1	159.45	3.58
			2	432.24	3.40
			3	881.89	3.21
			4	1227.83	3.19
		Positive control) Non treated	1	159.45	3.58
			2	389.12	3.17
			3	552. 15	2.89

2	Nobilis® ND Clone 30vaccine (7th day), Nobilis® Gumboro D78 (10thday), Lasota vaccine (14th day).	Challenged with 106 EID50/ml (IN) at 21 th day old	4	873.46	2.65
3		Treated withN.Sativa (6%) in ration at 7 th day Challenged with 106 EID50/ml (IN) at 21 th day old	1	159.45	3.58
			2	390.22	3.21
			3	583.89	3.07
			4	898.75	2.88
4		treated with curcumin (1%) in ration. Challenged with 106 EID50/ml (IN) at 21 th day old	1	159.45	3.58
			2	400.12	3.23
			3	795.87	2.67
			4	986.07	3.60
5		treated with orego (1.25ml/litre) in drinking water. Challenged with 106 EID50/ml (IN) at 21 th day old	1	159.45	3.58
			2	421.98	3.42
			3	890.22	3.64
			4	1254.04	3.88

IN= intranasal.

Table (2): Daily distribution of morbidity and mortality in challenged chickens:

Gr. NO.	Vaccine	Immune Stimulant	Case No.	Days post-challenge			Total NO	Percentage (%)	Protection (%)
				3	5	7			
1	non vaccinated	Negative control non treated	Dis.	0	0	0	0	0	100%
			Died	0	0	0	0	0	
2	Nobilis® ND Clone 30vaccine (7th day), Nobilis® Gumboro D78 (10thday), Lasota vaccine (14 day).	Positive control Non treated	Dis.	3	2	1	8	26.6	73.40%
			Died	2	2	0	4	13.3	
3		Treated with N.Sativa (6%)in ration at 7 th day	Dis.	1	2	3	6	20	80%
			Died	0	1	2	3	10	
4		treated with curcumin (1%) in ration	Dis.	0	1	1	4	13.3	86.60%
			Died	0	0	0	0	0	
5		treated with orego (1.25ml/litre) in drinking water	Dis.	0	0	1	2	6.6	93.30%
			Died	0	0	0	0	0	

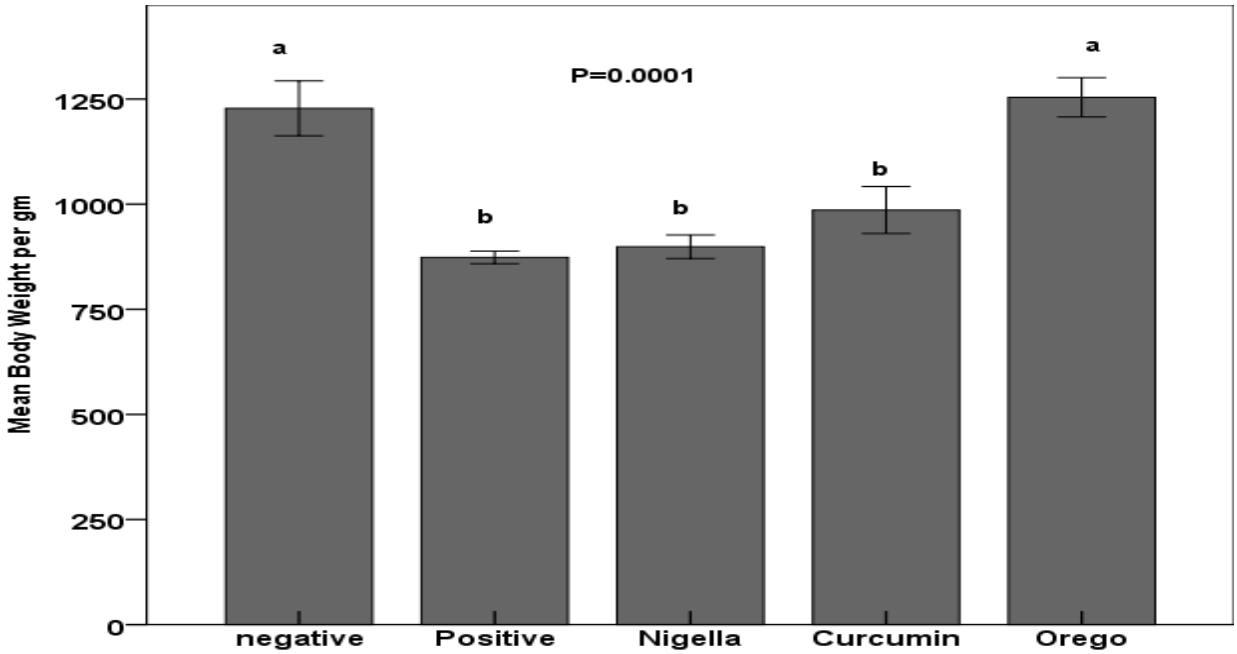

Figure (1): Effects of N. sativa, curcumin and orego sol. on the body weight of chickens at 4th weeks. Different alphabetical letters indicate significant (p = 0.0001) differences between the means of the experimental groups.

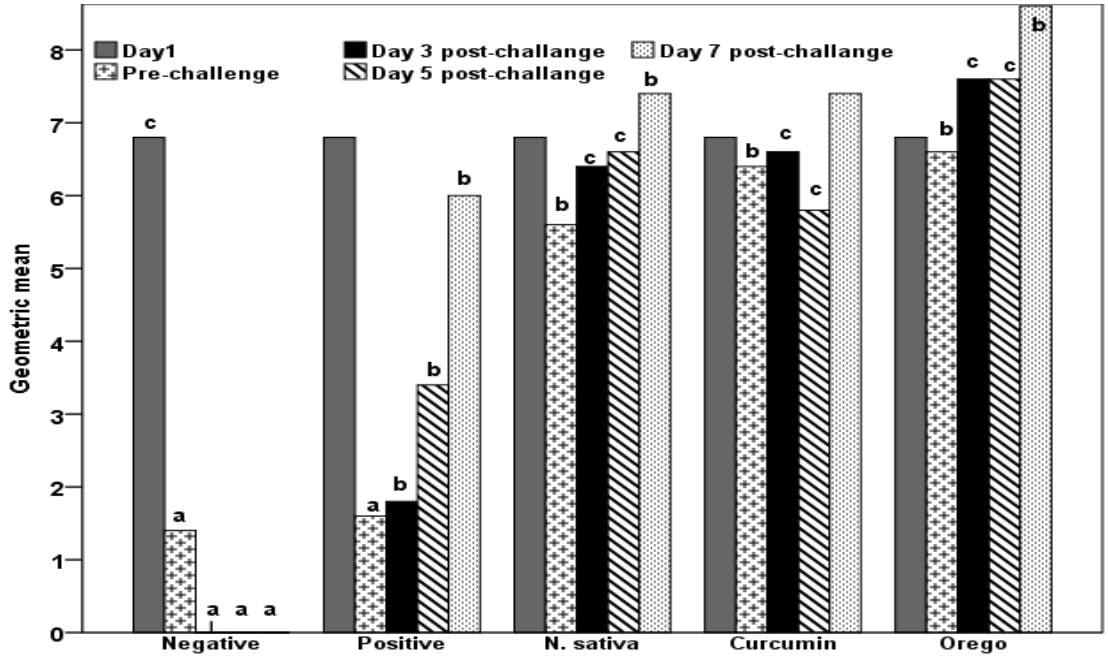

Figure (2): Hemagglutination inhibition (HI) titre (log 2) of Newcastle disease antibodies throughout the experimental period. Geometric mean of 9 serum samples per group at each observation time. Different letters at each observation time indicate to significant difference (P<0.05).

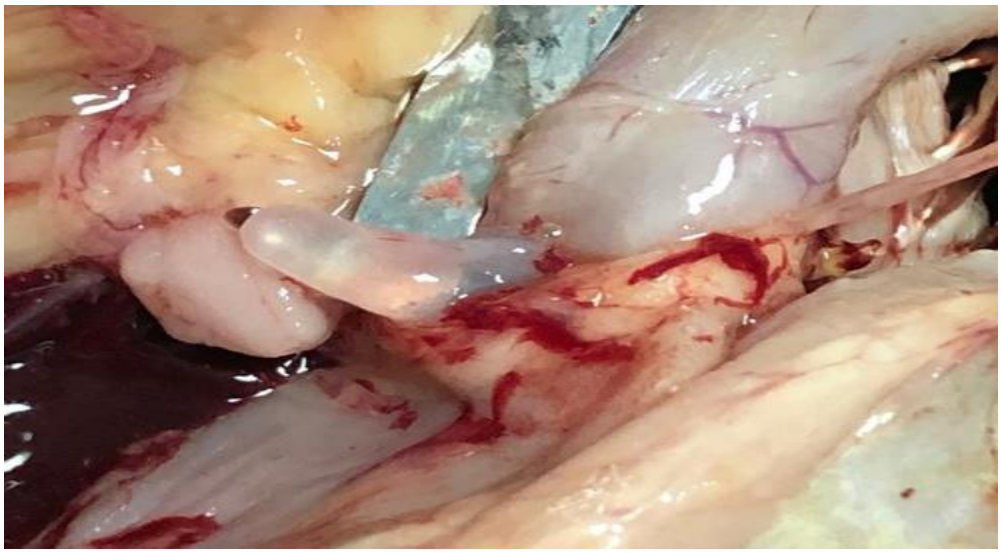

Figure (3): showed formation of renal water cyst in group 3(which treated with N.sativa) only.

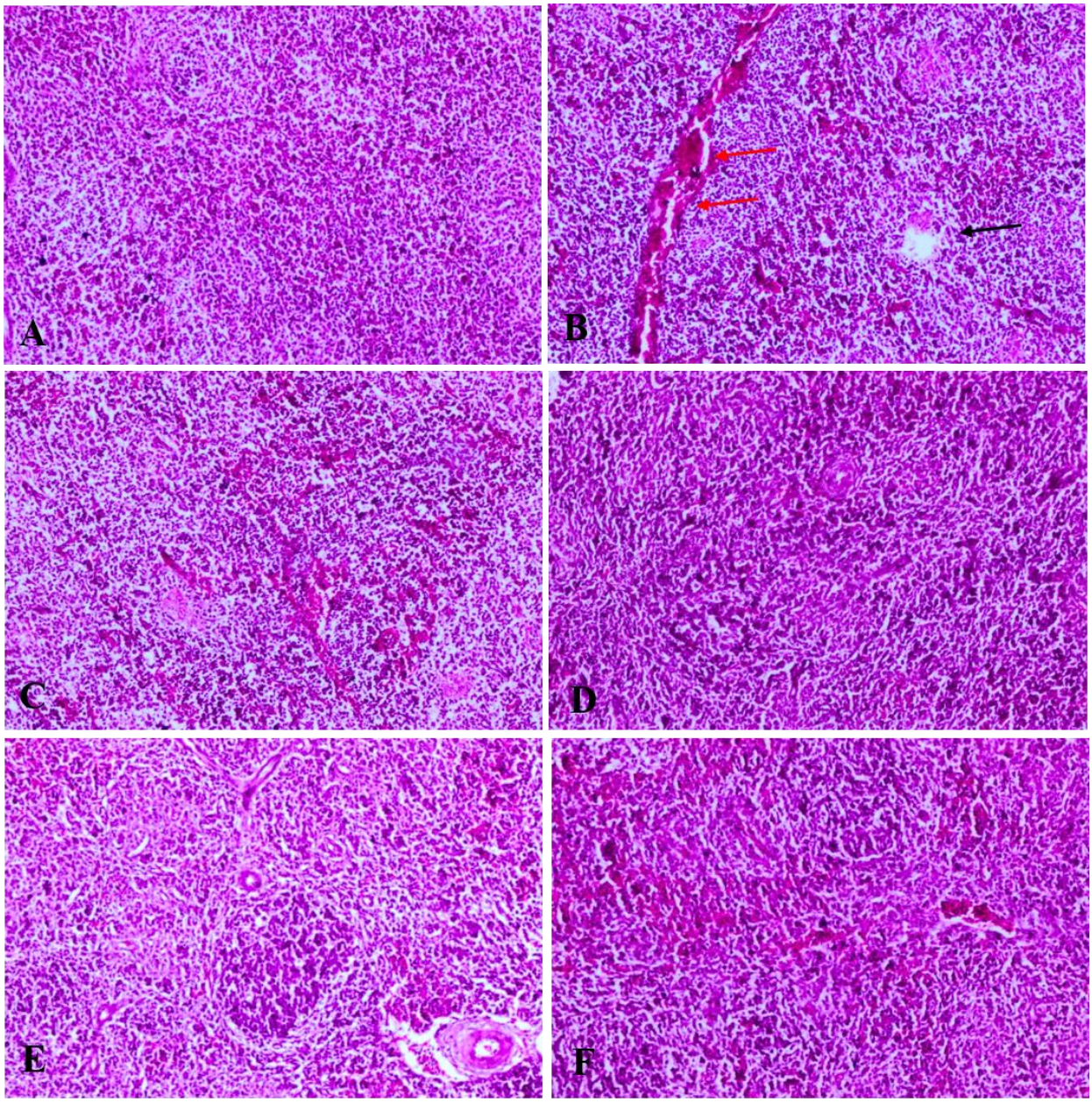

Figure (4): Photomicrograph of splenic tissue sections stained with H&E (X 100) showing (A) Normal histological picture in the control group. (B and C) Severe depletion of lymphoid cells (black arrows) with severe congestion and hemorrhages of the red pulp (red arrows) in the positive control. (D and E) Mild lymphoid depletion and mild congestion of red pulp in-group 3 and 5 (F) Moderate lymphoid depletion with moderate congestion of the red pulp in group 5.

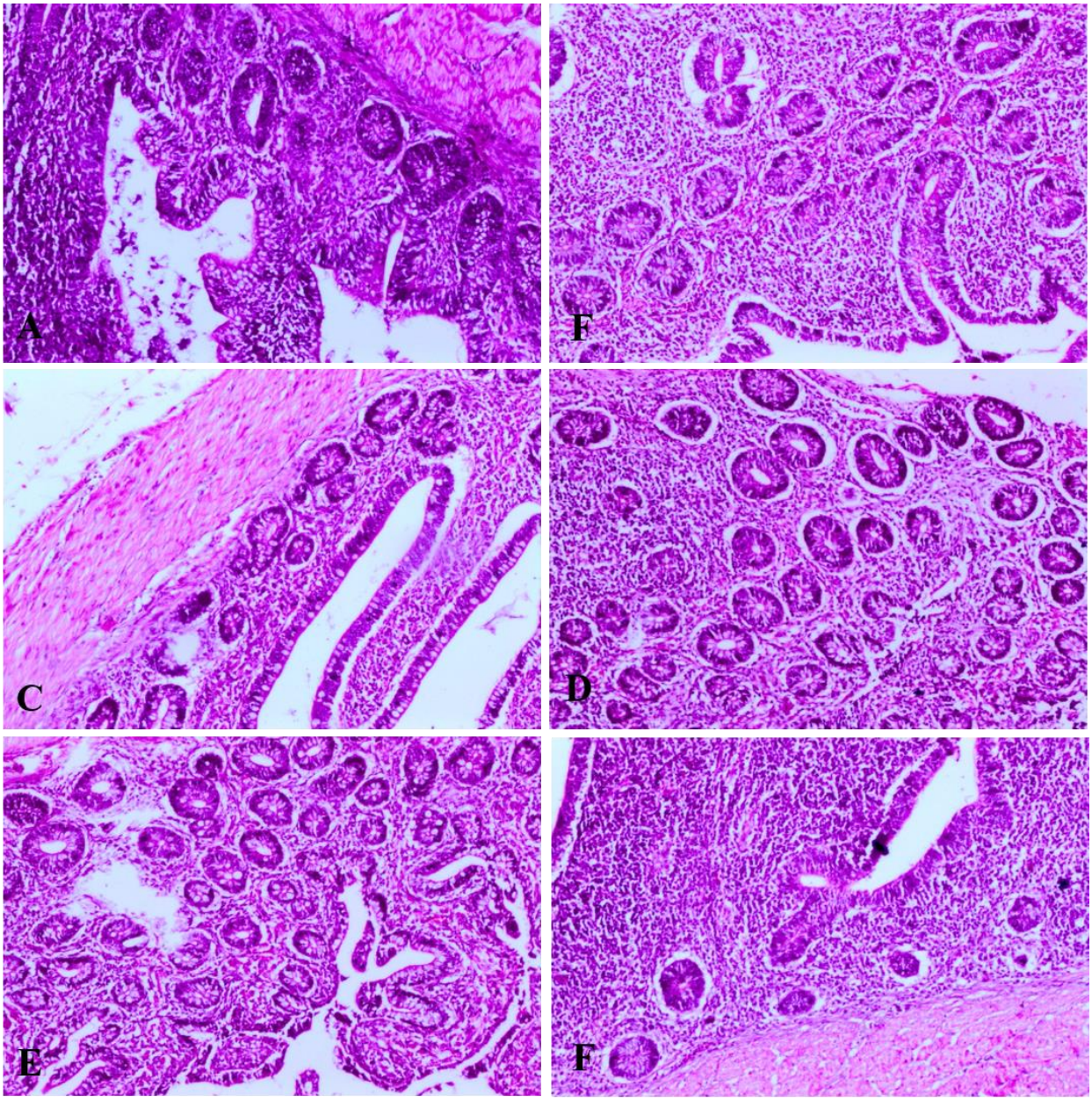

Figure (5): Photomicrograph of cecal tonsils tissue sections stained with H&E (X 100) showing A. control negative with normal tonsilar lymphoid nodules with glands of lieberkuhn. B and C. control positive showing aggregation of inflammatory cells with focal hemorrhages, degeneration of glands of Lieberkuhn with necrotic changes. D and E. group 3 and 5 showing mild tonsilar lymphoid depletion with focal hemorrhages. F. group 4 showing moderate depletion of lymphoid nodules with mild degeneration glands of Lieberkuhn.

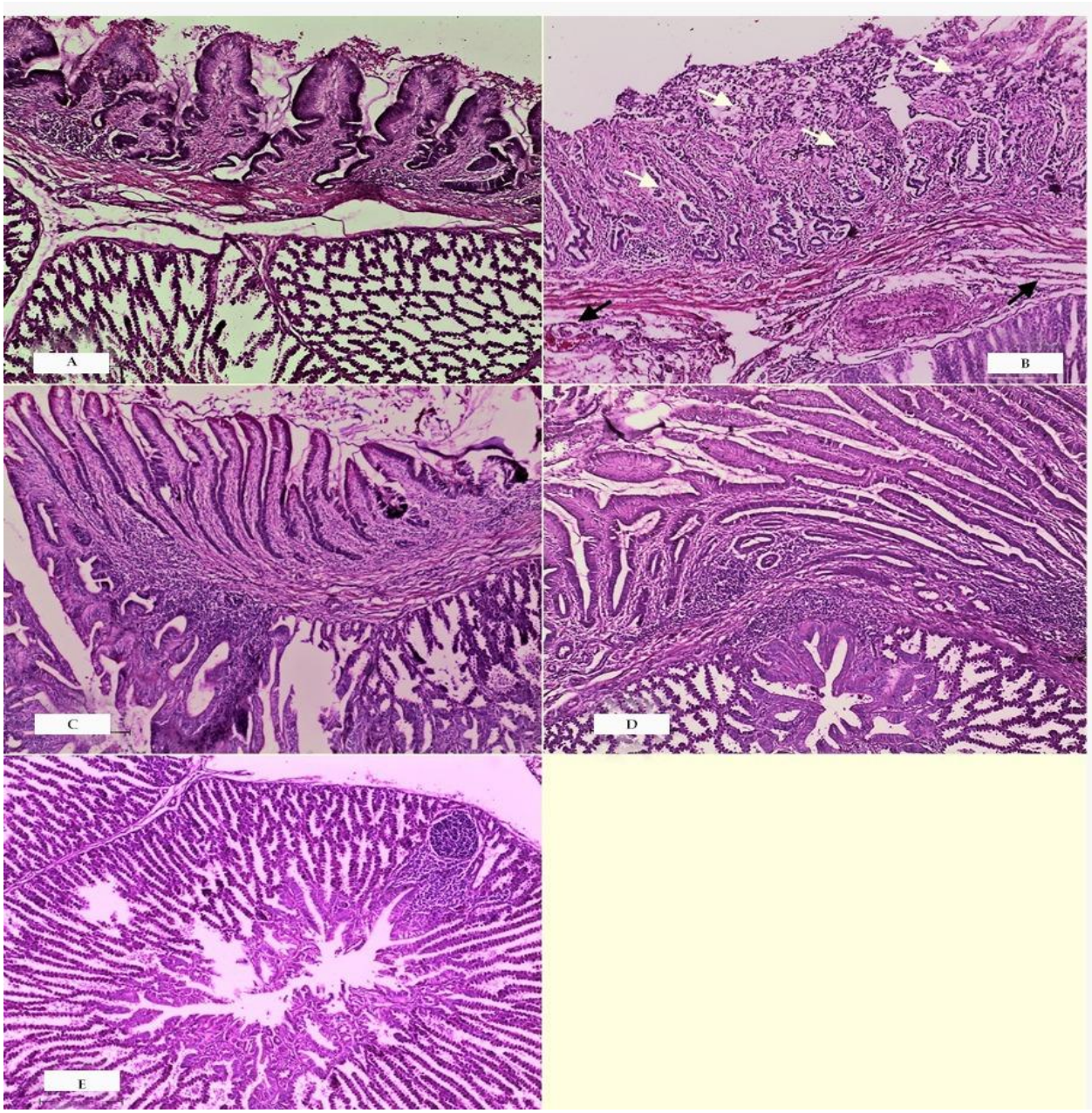

Figure (6): A. control negative (1): proventriculus showed normal histology of mucosal layer and glandular layer. B. control positive (2): proventriculus showed mucosal inflammation cause thickening of mucosal layer due to profuse infiltration with inflammatory cells (white arrows) congestion and submucosal edema (black arrows).C. group (3): showed mild inflammation of glandular layer with lymphoid aggregation D. group (4): showed normal focal mononuclear cell aggregation in mucosal and glandular layer .E. group (5): showed mild inflammation in glandular layer.

DISCUSSION

Newcastle disease is a major epidemic. The severity of the infection is determined by the virulence of the infecting virus and the susceptibility of the host. The disease spreads rapidly, with symptoms appearing throughout the flock. Young birds are the most susceptible. The symptoms that appear

depend on whether the infectious virus prefers to infect the respiratory, digestive, or neurologic systems. Respiratory symptoms, partial or complete stoppage of egg production may occur, resulting in direct economic losses (Otte and Chilonda, 2000).

The effects of oral supplementation with *N. sativa*, Curcumin, and orego sol. on body performance were investigated. Three sets of birds were evaluated and compared, including non-treated infected birds and non-treated vaccinated birds. At the fourth week, the mean body weight of the vaccinated groups with ND provided withorego sol was higher (1254.04gm) than the other groups, whereas curcumin (986.07gm) was higher than *N.sativa* (898.75gm each) and control positive group. Compared to control birds, oral supplementation with N. sativa, Curcumin, and orego solresulted in a substantial improvement in FCR at 30 days of age, as well as a higher and statistically significant weight gain (P =0.0001).Improvements in food conversion (Mocar et al., 2010), which promote better sedimentation of muscle proteins (Zheng et al., 2009), stimulation of appetite, digestive and absorption enzymes (Christaki et al., 2011), or the stimulating effect on Lactobacillus proliferation may be the mechanism of phytobiotic essential oils for improving broiler productivity performance (Roofchaee et al., 2011).The findings of this study were agreed by otherstudies like Hashemipour et al. (2013), who found that including thymol plus carvacrol in the diet improves body weight gain and feed efficiency. As a result, thymol and carvacrol can improve avian performance by lowering digesta viscosity, in addition to their antimicrobial activities (Wenk, 2000; Hashemipour et al., 2014).

According to the current investigation, the strongest NDV antibody responses were elicited in the birds supplemented orally with orego sol, followed by Curcumin, and finally N. sativa. Furthermore, Zataria multiflora essential oil (a mixture of thymol and carvarcol) caused a dose dependent increase in NDVHI specific Ab titers in broiler chickens that was greater than that of the levamisole medication. These findings could be explained by the potent antioxidant properties of thymol and carvarcol, which stimulate the chicks' immune responses (Gabor et al., 2010; Feizi and Nazeri, 2011).

Thymoquinone, the major active component in *N. sativa*, exhibits antioxidant properties (Al-Mufarrej, 2014). It inhibits tissue damage by neutralizing free oxygen radicals. Many research studies have been conducted to see how *N. sativa* affects ischemia or drug-induced hepatotoxicity and nephrotoxicity. Undesirable effect of *N. sativa* in our study (GIT problems and the formation of water cysts on the kidneys) could be related to concentration of *N. sativa* in ration. Other investigations have found that when N. sativa is applied topically, it might cause skin irritation. If ingested in large amounts, black seed oil might harm the liver and kidneys. Black seed oil slows blood coagulation, increasing the risk of bleeding (Ijaz et al., 2017). Despite the fact that no previous research had been done on this subject, another study concluded that using N. Sativa tablets at doses of 2 000 to 2 500 mg/d in diabetic patients could be nephrotoxic. Herbal medicine is indicated, especially for diabetic patients with acute renal failure who have no other underlying reason (Arslan et al., 2013). After applying Nigella sativa oil to the skin, fluid-filled skin blisters occurred, according to a case report. Because the oil was consumed, the blisters could have been part of a systemic reaction (such as toxic epidermal necrolysis) (Gelot et al., 2012).

The histopathology of the organs after NDV infection varies depending on the clinical symptoms and gross lesions, and is affected greatly by the viral strain and host

immunity. Solid immunity to NDV does not develop despite immunization. Vaccination protects against disease, but it does not prevent infection. (Mohammadamin and Qubih, 2011). When vaccinated birds are challenged with virulent ND viruses, the virus replicates and sheds (Hamid et al., 1990). At 5 and 7 days after the challenge, tissue sections from the spleen, proventriculus, and cecal tonsils revealed histological alterations, which were primarily characterized by lymphocyte depletion in all challenged groups. Lymphocytic depletion is a typical characteristic of aggressive NDV strains (Brown et al., 1999 and Kommers et al., 2003). In comparison to the 3, 4, and 5 groups, the histopathological alterations were more pronounced in group 2 (positive challenged vaccinated control). Low antibody levels in group 2 exacerbated the pathogenicity of the challenge virus, resulting in more severe histopathological abnormalities (Alexander, 2003). The histopathological changes in the *N. sativa*, Curcumin, and orego sol. groups were unique from the non-treated groups. In chickens challenged with virulent Salmonella enteretidis, Al-Dalo (2007) found that feeding Biomin®C-X (natural growth promotor) increased humoral immunity to NDV but did not improve histopathological lesions when compared to control.

N. sativa should be questioned.

The present study indicated the importance of using immunostimulants to boost immune responses during Newcastle disease virus vaccinations, because they result in higher antibody levels and increase protection rate up to 100% and low mortality rates, in addition to their effect on improving body performance.

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