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# **Evaluation of *Spirulina platensis* efficacy against local field vvIBDV challenge in broiler chickens**

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## **Abstract**

The antiviral and immunostimulant effect of dietary supplementation of *Spirulina platensis* (SP) against Infectious bursal disease (IBD) was assessed as follows; 126 one-day-old cobb broiler chicks were randomly distributed into six groups (21 birds/group with three replicates, 7 birds each): G1 (non-vaccinated, and non-challenged ; NC), G2 (non-vaccinated, and challenged; PC), vaccinated challenged G3 (vaccinated, and challenged; VC), Spirulina 0.1% (vaccinated, 0.1% SP-supplemented, and challenged; G4), Spirulina 0.3% (vaccinated, 0.3% SP-supplemented, and challenged; G5), and Spirulina 0.5% (vaccinated, 0.5% SP-supplemented, and challenged; G6). The chicks in NC, PC, and VC groups were not supplemented with SP. All vaccinated groups, regardless SP supplementation, showed significant improvement of body weight gain (19% in 0.3% SP) and FCR during IBDv challenge (10 dpc) ( $P < 0.05$ ). On the 10<sup>th</sup> dpc, IBD ELISA titer in SP-supplemented groups were decreased ( $P > 0.05$ ) than that of G3, suggesting an antiviral efficacy of SP. This antiviral activity was evident by the viral RNA load in the cloacal swabs as well. The viral shedding peak was on the 5<sup>th</sup> day pc which was significantly decreased on G4-6 ( $P < 0.05$ ) in comparison to G2 and G3. Also, the bursal ratio was improved in SP supplemented groups

comparable to G3. Moreover, in unchallenged broilers, the gene expression of INF- $\gamma$  and IL-18 were upregulated while that of IL-10 was downregulated in spleen of SP-supplemented chicks. Finally, severe histopathological lesions of kidneys, bursa, thymus, and spleen were observed in G2 which moderately minimized in G3 and were more alleviated in SP-supplemented groups. Collectively, SP supplementation improved body weight gain and decreasing viral shedding with antiviral activity. The mechanisms of SP against IBDV challenge may include cell-mediated immunity promoting effect, antiviral activity, and/or anti-inflammatory effect that should be further studied.

**Key words:** *Spirulina platensis*, Infectious Bursal Disease, immune response, viral shedding, broiler chickens, ELISA, challenge, efficacy

## 1. Introduction

Immunosuppression is a fundamental obstacle for the poultry industries, that caused by diverse factors including viral infections. The viral infections not only incremented the tendency to other microbial infections, but also taking charge of vaccination failure or effectiveness reduction that cause harmful losses on the bird's health and performance (**Ingrao et al., 2013**).

Infectious bursal disease (IBD), one of the immunosuppressive diseases, caused by IBD virus (IBDV) which targets the B lymphocyte not only the bursa of Fabricius but also spleen and thymus resulting in serious injurious effects (**Dey et al., 2019**). Two serotypes were identified of IBDV; serotype 1 strains are pathogenic in chickens and include vvIBDV, classical virulent IBDV, antigenic variant IBDV, and attenuated IBDV (**Bolis et al., 2003**). Very virulent and classical ones are the most serious strains causing severe lymphoid depletion of bursa, and marked humoral antibody response depletion (**Wagari, 2021**). While, a variant IBDV does not cause mortality, it is an immunosuppressive agent on NDV vaccination, the virus severely damaged the B lymphocytes of bursa, spleen and thymus with decreasing the body weight of broilers (**Fan et al., 2020**).

IBDV cause 100% morbidity in chicks between 3 and 6 weeks old with mortality rate reach up to 90% and the clinical symptoms appears 2-3 days after viral infection in the form of depression, ruffled feather, diarrhea, vent picking, anorexia, urates around vent feather, and dehydration which continue up to 3-4 days followed by recovery in the live birds (**Murphy et al., 1999**). IBDV is very resistant to environmental conditions and disinfectants, so the vaccination program is the best way for controlling the IBD (**Muller et al., 2012**). However, IBDV undergo continuous genetic mutations, and modification of genome segments which augmented the virulence and antigenicity of the virus which minimize the effectiveness of vaccine (**Jackwood et al., 2011**).

*Spirulina platensis* (SP), a blue green alga, rich in proteins, vitamins, minerals, and various bioactive compounds such as flavonoids, phenolic, terpenoids, phycocyanins, carotenes, and others (**Agustini et al., 2015**). In refer to its bioactive components, SP possess antioxidant, antiinflammatory, antiviral, immunomodulator, and hepatoprotective activities (**Abdel-Moneim et al. 2021; Aladaileh et al. 2020**). SP is free from algal toxin, and mineral toxins which rendering it as a safe nutritional feed ingredient (**Yang et al., 2011**). Phycocyanin, a characteristic component of SP, has hepatoprotective, antioxidant, immunomodulating, anti-inflammatory, and antitumor activities (**Xalxo et al., 2013**). To best of our knowledge, there is little literature regarding the effect of dietary supplementation of SP on IBDV challenge in broilers. Therefore, the current study focused to evaluate the anti-immunosuppressive efficacy of SP on IBDV challenge as well as evaluating its antiviral effect and growth promoting activity in commercial vaccinated broilers challenged with vvIBDV.

## **2. Material and methods**

### **2.1. *Spirulina platensis***

A free-flowing dark blue-green SP powder with characteristic seaweed smell, obtained from National Research Center, Al-Dokky, Giza, Egypt. It is analyzed for phenolic compounds and the essential minerals and toxic elements using High Performance Liquid Chromatography (HPLC), and atomic absorption spectroscopy respectively. The total phenolics is 830.7 mg%, Pb (0.01). Cd (0.08), Ni (10.45). Fe (210.7), Mn (44.8). Zn (200.4), Cr (2.6), Ca (121.6), Mg (30.3), K (261.3), and Na (217.1) ppm.

## 2.2. Vaccination and the challenge virus

Maternal antibody titer (MAb) was determined in the serum of ten random chickens at 1-day old for designing the vaccination program using commercial vaccines as follow:

Table (1): vaccination program

Vaccine	Company Country	Batch No	Type	Day of administration	Route of administration
<b>Combivac C</b>	Jovac Jordan	10D0121	Live attenuated NDV clone strain and IBV H120 strain	5 <sup>th</sup>	Eye drop
<b>BioShield ND-Flu 2 in1</b>	Harbin China	2021001	Bivalent inactivated H5N1 Egy/PR8-5 strain + La Sota strain	8 <sup>th</sup>	s/c
<b>Avishield</b>	Dechra UK	108021	Live attenuated La Sota strain	13 <sup>th</sup>	Eye drop
<b>Ornibur</b>	Bioveta Checz Republic	665726A	Live intermediated plus IBD vaccine	15 <sup>th</sup>	Eye drop

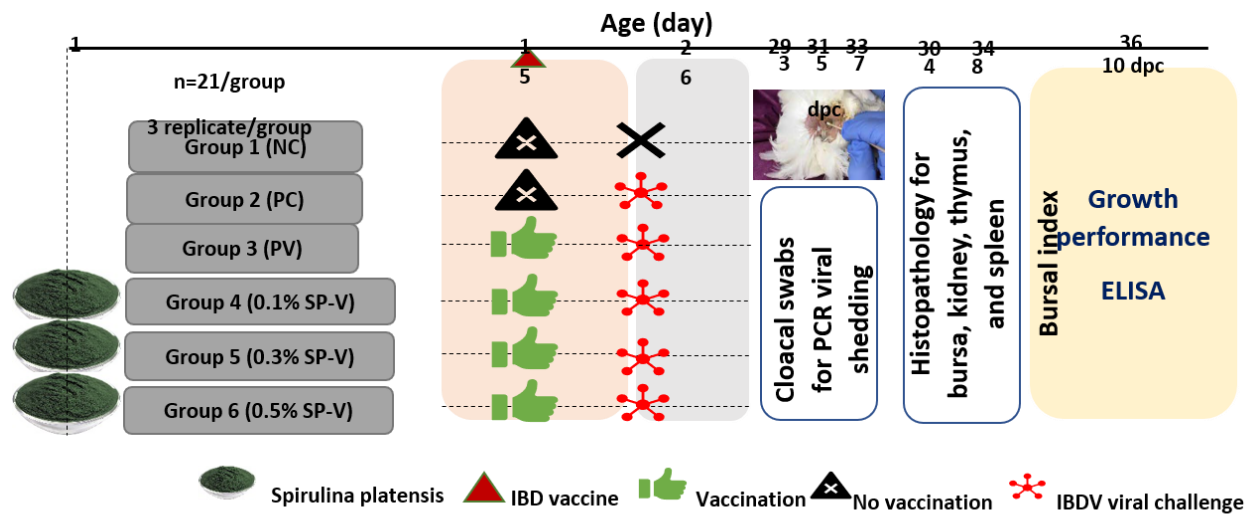
A local vvIBDV field virus with accession No. MG 599731 was isolated from SASO chicks at 25-day-old with a mortality rate of 6.7% and characterized as Egypt-Behira-29-2017 (**El-Shall et al., 2018**) through sequencing of VP2 gene. It was propagated and titrated by inoculation into CAM of SPF embryos and EID<sub>50</sub> was estimated according to **Reed and Muench, (1938)**. The challenge dose of 3.5 log 10 EID<sub>50</sub> /0.1 ml (100 µL / bird: 50 µL by eye drops and 50 µL using nasal drop route) at 26<sup>th</sup> day old.

## 2.3. Experimental chickens and design

A total 136 one-day-old cobb chicks were obtained from the local hatchery, reared in metal cages, and maintained at 32°C on day 1, then the temperature was linearly decreased by 2°C per week, and free access provided with feed and water. The experimental design and procedures were observed under the Local Experimental Animal Care Committee and approved by the institutional committee ethics of the Alexandria University, Egypt (ALEXU-IACUC-013-20-12-13-MD (2)-69). All efforts were made to minimize suffering.

The chicks were randomly allocated into six groups (21 chicks per group allocated into 3 replicates, 7 chicks each) as the following: G1 (non-vaccinated, and non-challenged ; NC), G2 (non-vaccinated, and challenged; PC), vaccinated challenged G3 (vaccinated, and challenged; VC), Spirulina 0.1% (vaccinated, 0.1% SP-supplemented, and challenged; G4), Spirulina 0.3% (vaccinated, 0.3% SP-supplemented, and challenged; G5), and Spirulina 0.5% (vaccinated, 0.5% SP-supplemented, and challenged; G6). The chicks in NC, PC, and VC groups were not supplemented with SP. All chickens were received the vaccination program provided in table (1) except NC and PC groups. The chemical composition of the diet was provided in table (2), and the SP powder was freshly mixed with feed daily and supplemented to the chicks from the first day till the end of experiment. The experimental design was summarized in Fig. 1.

Figure (1): Experimental design



## 2.4. Growth performance and serological testing

The chickens were individually weighted on the 1<sup>st</sup>, 21<sup>th</sup>, and 35<sup>th</sup> day of age and the final body weight and weight gain was calculated for each chick with determination of the average feed consumption per chick at the same intervals and calculation of the feed conversion ratio (FCR).

Ten chicks were slaughtered at one-day-old, and the blood samples were collected without anticoagulant for determination of IBDV-specific maternally derived antibodies. Also, blood samples were collected from the wing vein of randomly selected 9 birds per group (three birds/replicate) on the 10<sup>th</sup>, and 20<sup>th</sup> day of age, and on the 36<sup>th</sup> day (10<sup>th</sup> day post challenge pc).

The blood samples were centrifuged for 10 min. at 1500 xg and the sera were stored into Eppendorf tubes at -20°C until use. The concentration of anti-IBDV IgG titers was performed by ELISA using IDEXX IBV kits, USA where the results are negative at S/P ratio  $\leq 0.20$  and positive at S/P ratio  $>0.20$ .

## 2.5. Bursal index calculation

At day 30<sup>th</sup> day and at day 34<sup>th</sup> day (4<sup>th</sup> and 8<sup>th</sup> day pc), a total of 3/21 chicks from each group were selected randomly, weighted, scarified and the bursae were weighed individually (using digital balance) to calculate bursal body weight ratio and bursal body weight ratio according to the formulas of **Sharma et al., (1989)** and **Lucio and Hitchner, (1979)**, where bursa index calculated as weight of bursa in grams/ body weight of bird in grams X 1000 and bursa index calculated as bursa index of infected birds/bursa index of uninfected bird. Where chicken with bursa index lower than 0.7 were considered to have bursal atrophy.

## 2.6. Quantification of viral shedding by real time RT-PCR (rRT-PCR)

Cloacal swabs were taken from three chicks in each group at the third, fifth-, and seventh-day pc in 1.5 mL of phosphate buffer saline containing penicillin G (1000 units/mL) and gentamicin (200 mg/mL). Centrifugation of the collected swabs at 3000 rpm for 10 min; the supernatants were collected and stored at -80 °C until use. Extraction of RNA was performed using Thermo Scientific Gene JET Viral DNA and RNA Extraction Kits following the manufacturer's recommended procedures. The IBD virus V2 gene was amplified using the primers and probe (table. 2) with the Stratagene Real-Time PCR system. The reaction was composed of 5 mL of template RNA, 12.5 mL of rRT-PCR Master Mix, 1.25 mL of RT-enhancer, 0.25 mL of enzyme mix, 1 mL of forward primer (50 pmol), 1 mL of reverse primer (50 pmol), 0.25 mL of probe, and 3.75 mL of RNase free water. The PCR cycling conditions were 50 cycles at 94 °C for 10 s, 58 °C for 5 s, and 72 °C for 10 s preceded by an initial denaturation for 15 min at 95 °C.

Table 2: Primer sequences for IBDV

Primer sequence for IBDV		Reference
<b>F:</b>	5- GAGCCTTCTGATGCCAACAAAC-3	<b>Tomas et al., (2012)</b>
<b>R:</b>	3- AGTCTCTGGAGCTGGATGTAACT-5	
<b>Probe</b>	5-FAM-ACACCCTAGAGAAGC.MGB-3	

## **2.7. Histopathological examination**

On the 4<sup>th</sup> and 8<sup>th</sup> day pc, three chicks from each experimental group were euthanized then killed by cervical dislocation for collecting bursa of fabricius, spleen, thymus, and kidneys immediately and fixed in 10% formalin for histopathological examination. The organs were embedded in paraffin and sectioned using a microtome into slices of 4-6  $\mu$ m thickness and then stained with Hematoxylin and Eosin (H&E) stain.

### **Statistical analysis**

The results were expressed as means  $\pm$  standard error. Data were statistically analyzed using the statistical analysis program (SPSS, 2017) (IBM, 2017). Duncan's multiple range tests and Tukey's multiple comparison tests (post-hoc test) were used to compare the means at ( $P < 0.05$ ) of the treated groups.

## **3. Results**

### **3.1. Effect of SP on growth performance**

At the age of 21<sup>st</sup> day (3<sup>rd</sup> week), the average body weight gains (BWG) did not differ significantly among groups ( $P > 0.05$ ). While at 5<sup>th</sup> week (14 dpc), the BWG was significantly decreased in PC group compared to NC by 7%. The BWG in SP-supplemented groups was significantly higher than that in PC by 8% and VC by 4% at the same period at  $P \leq 0.05$ . Interestingly, the BWG during challenge period (from 3<sup>rd</sup> to 5<sup>th</sup> week) was significantly greater in SP-supplemented groups by 19% compared to PC and 6% compared to VC at  $P > 0.05$ . Furthermore, FCR was the best in SP-0.3 (1.28), followed by SP-0.1, and SP-0.5 (1.49, and 1.57 respectively) compared to NC (1.65), VC (1.64) and the bad FCR observed in PC (1.77) at  $P \leq 0.05$ , the data represented in figure 4.13.

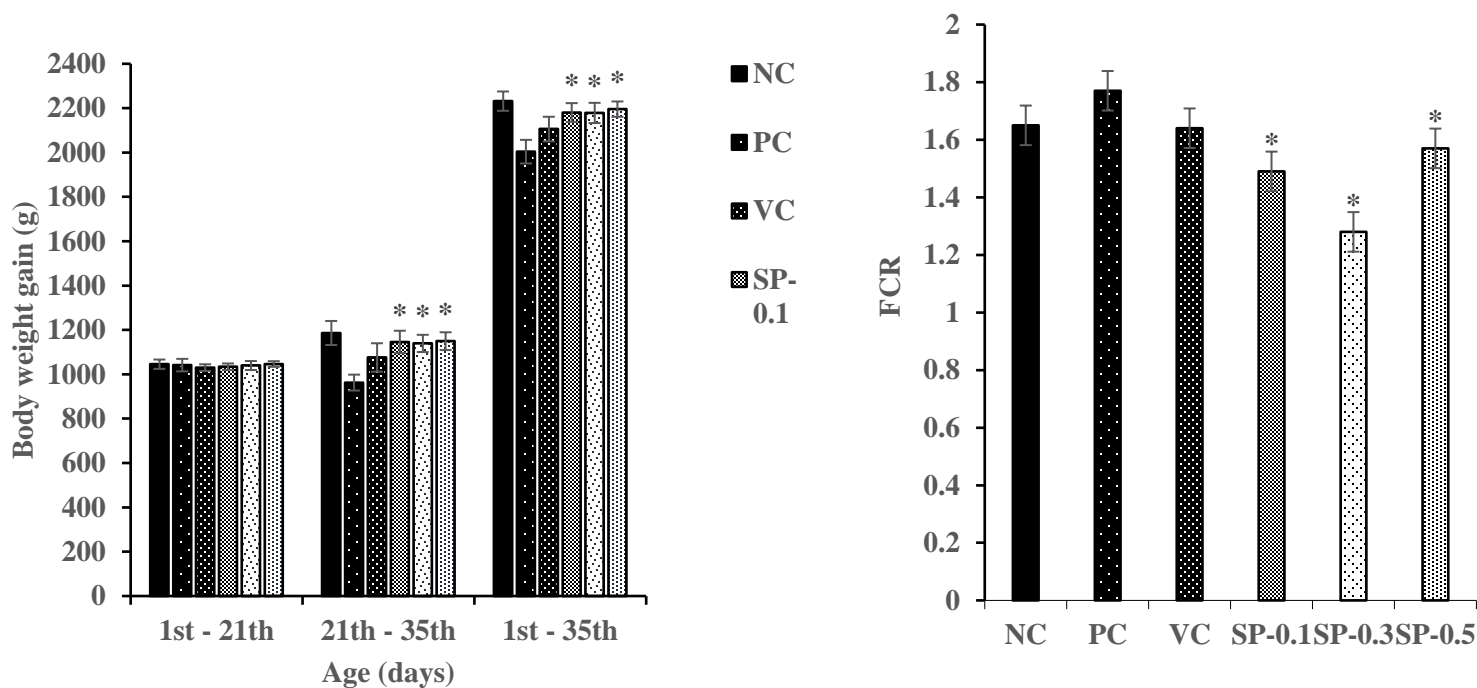


Fig (2): Body weight and FCR in broiler chicken treated with Spirulina and challenged with IBDV

### 3.2. Immune response to IBD vaccine and virus challenge

The concentration of MDA to IBDV measured by ELISA was  $7012.2 \pm 271.3$  ng/ml which was gradually decreased by the time in all experimental groups. At 10<sup>th</sup> day, insignificant ( $P > 0.05$ ) higher titer was observed in the SP-0.5% group ( $1802.00 \pm 70.42$  ng/ml) as compared to NC, PC, and VC ( $1539.98 \pm 74.12$ ,  $1546.60 \pm 92.31$ ,  $1519.77 \pm 105.48$  ng/ml) respectively, while significantly higher than those in SP-0.1 and SP-0.3 ( $1301.70 \pm 77.94$ ,  $1479.50 \pm 71.45$  ng/ml) respectively at  $P \leq 0.05$ . At 20<sup>th</sup> day, the concentration of IgG was decreased in all groups compared to those at 10<sup>th</sup> day with the highest significant titer observed in the SP-0.1 and SP-0.3% ( $343.70 \pm 14.97$ , and  $389.60 \pm 54.49$  ng/ml, respectively) when compared to other groups at the same period. Regarding the challenge period, the IgG titer was increased at 36<sup>th</sup> day (10<sup>th</sup> dpc) in all challenged groups where the greatest titer was observed in the VC group ( $5003.88 \pm 147.92$  ng/ml) as compared to other groups at the same period. The Ab titer of SP-treated groups (SP-0.1% and SP-0.5%) was ( $4125.20 \pm 233.71$  and  $4170.80 \pm 261.31$  ng/ml) significantly higher than the PC group ( $3606.60 \pm 97.73$  ng/ml) but still lower than the VC group. However, the Ab titer in SP-0.3 group ( $3452.20 \pm 134.26$  ng/ml) was not different from that in PC and lower than the other

challenged groups at  $P>0.05$ . The NC group showed the lowest Ab titer against IBDV at the same period of 10<sup>th</sup> dpc.

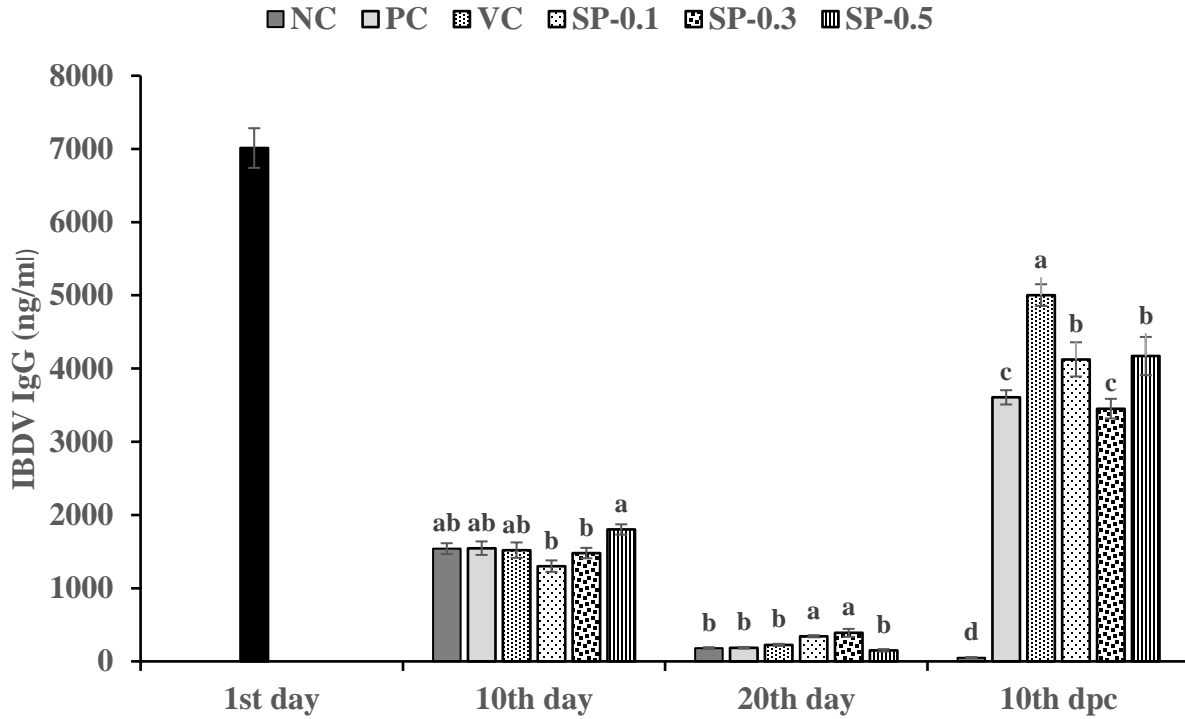


Fig (3): ELISA IBDV antibody titer in broiler chicken treated with Spirulina and challenged with IBDV

### 3.3. Bursal index

The bursa wt index and bursal ratio were significantly higher in the PC group indicating enlargement of bursa due to IBDV challenge along at the 4<sup>th</sup> dpc while significantly decreased at 8<sup>th</sup> dpc indicating the atrophy of bursa compared to the NC group. The improvement of bursa wt index and bursa ratio were significant in VC group and SP-treated groups (SP-0.1, SP-0.3, and SP-0.5) as compared to PC during the challenge period at  $P\leq 0.05$  which is more prevalent in SP-0.5.

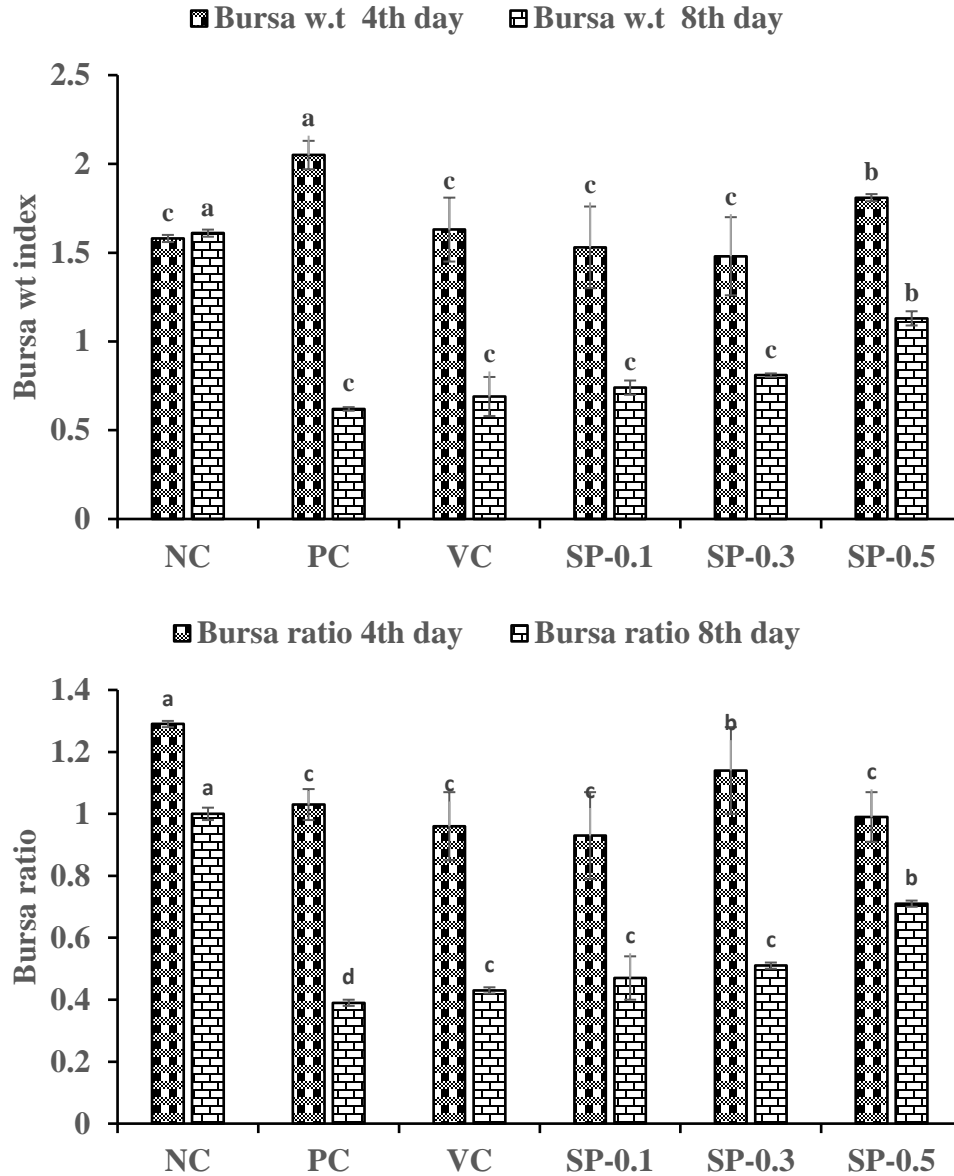


Fig (4): Bursal index and ratio in broiler chicken treated with Spirulina and challenged with IBDV

### 3.4. Cloacal IBDV shedding

There is no IBDV shedding in NC group. The viral RNA quantity in PC group was significantly higher ( $5.31 \pm 0.07 \log_{10}$ ) as compared to other groups at 3<sup>rd</sup> dpc. At the same period, the viral shedding was significantly decreased in VC and SP-treated groups when compared to PC with the minimal viral quantity shedding observed in SP-0.5 ( $4.22 \pm 0.07 \log_{10}$ ). The cloacal viral shedding at 5<sup>th</sup> dpc was statistically decreased in all groups as compared to the same groups at 3<sup>rd</sup> dpc in which the VC group ( $4.08 \pm 0.03 \log_{10}$ ) revealed a significant lower titer when compared

to PC ( $4.48 \pm 0.03 \log_{10}$ ) and the SP-treated groups ( $4.01 \pm 0.03$ ,  $4.01 \pm 0.03$ , and  $3.29 \pm 0.03 \log_{10}$ ) showed the least viral RNA quantity in dose dependent manner as compared to NC and PC at the same period. Finally, at 7<sup>th</sup> dpc, the viral shedding was significantly lower in all groups as compared to the previous period in which the VC group revealed significant lower titer when compared to PC while SP-treated groups showed the least viral RNA quantity in dose dependent manner as compared to PC and VC at the same period (table 3).

Table (3): Viral shedding quantity

Groups	Viral shedding quantity (Log <sub>10</sub> )		
	3 <sup>th</sup> dpc	5 <sup>th</sup> dpc	7 <sup>th</sup> dpc
G1	--	--	--
G2	$5.31 \pm 0.07^{aA}$	$4.48 \pm 0.03^{aB}$	$4.06 \pm 0.02^{aC}$
G3	$4.66 \pm 0.07^{bA}$	$4.08 \pm 0.03^{bB}$	$3.33 \pm 0.02^{bC}$
G4	$4.63 \pm 0.07^{bA}$	$4.01 \pm 0.03^{bB}$	$3.32 \pm 0.02^{bC}$
G5	$4.58 \pm 0.07^{bA}$	$4.01 \pm 0.03^{bB}$	$3.30 \pm 0.02^{bC}$
G6	$4.22 \pm 0.07^{cA}$	$3.29 \pm 0.03^{cB}$	$3.12 \pm 0.02^{cC}$

### 3.5. Splenocyte cytokine gene expression

Interleukin-18 (IL-18; known as INF- $\gamma$  inducing cytokine), which plays a role in regulation of both innate and acquired immune response, was significantly upregulated ( $\sim$  two-fold) in the birds of the VC, and SP-supplemented groups relative to NC group with the greatest fold change of expression observed in SP-0.3 and SP-0.5 groups ( $2.59 \pm 0.49$ ,  $2.69 \pm 0.34$ , respectively) at  $P \leq 0.05$ . On the same context, Interferon gamma (INF- $\gamma$ ), a cytokine critical to both innate and adaptive immunity, and functions as the primary activator of macrophages, in addition to stimulating natural killer cells and neutrophils. The INF- $\gamma$  mRNA transcript was also upregulated in the VC group ( $\sim$  two-fold), and SP-treated groups with the highest change recorded in SP-0.3 group ( $\sim$  2.5-fold) at  $P \leq 0.05$  relative to the NC group. However, Interleukin-10 (IL-10), a cytokine with potent anti-inflammatory properties that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis. The expression of IL-10 mRNA was significantly upregulated in the spleen of the VC group ( $\sim$  2.7-fold) relative to the NC group while was significantly downregulated in the SP-

supplemented groups in dose dependent manner (~ 1.9-, 1.6-, and 1.4-fold) respectively as compared to the VC group at  $P \leq 0.05$ .

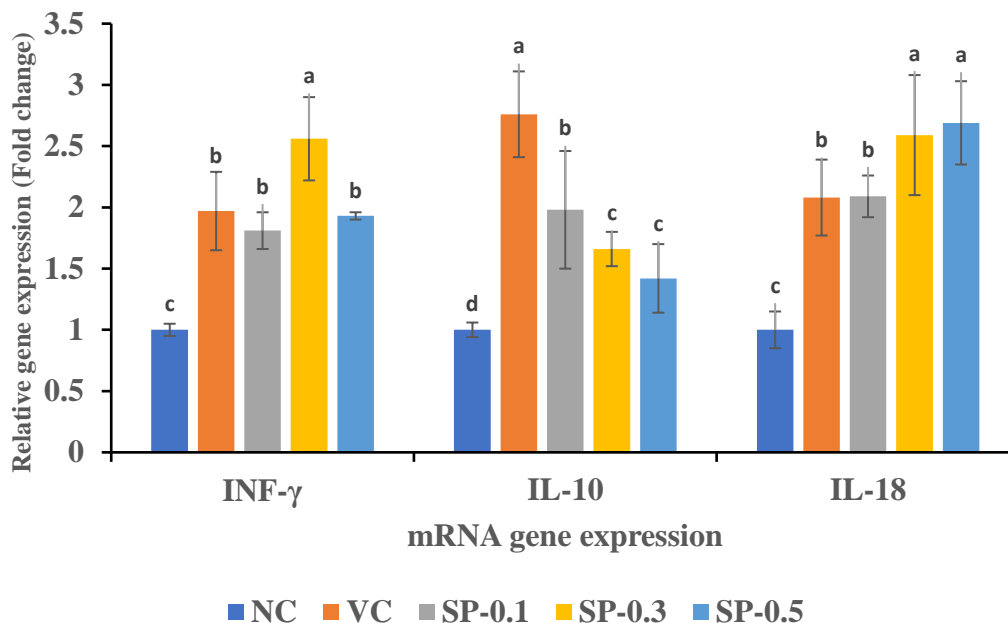


Fig (5): Immune related gene expression in broiler chicken spleen treated with Spirulina and challenged with IBDV

### 3.6. Histopathology

The histological structure of bursa in PC group showed severe lymphocyte depletion, and atrophy of follicles (photo 6B) compared to normal bursal follicle structure in NC (Fig 6A). However, moderate lymphocyte depletion, and atrophy of follicles was noticed in the VC group (Fig 6C), while only moderate depletion of bursal lymphocytes (Fig 6D) in SP-0.1 group, with mild depletion of lymphocytes (Fig 6E-F) in SP-0.3 and -0.5 groups.

The normal histological structure of thymus cortex and medulla in NC group (Fig 7A), while severe medullary lymphocyte depletion was noticed in PC group (Fig 7B), and the VC group (Fig 7C), while normal cortex with mild medullary lymphocyte depletion were observed in SP-supplemented groups dose dependently (Fig 7D-E-F). Regarding kidney histopathology, normal histological structure of kidneys in NC group (Fig 8A), severe interstitial nephritis and congestion of blood vessels in PC group (Fig 8B), severe interstitial nephritis in the VC group (photo 8C), moderate interstitial nephritis in SP-0.1 group (Fig 8D), mild interstitial nephritis in SP-0.3 group

(Fig 8E), congestion of blood vessels, and congestion of inter tubular blood capillaries in SP-0.5 group (Fig 8F).

The normal histological structure of spleen white and red pulp was observed in NC group (Fig 9A). The PC group showed depletion of white pulp, and lymphocyte in splenic nodules (Fig 9B), while atrophy of splenic nodules was noticed in the VC group (Fig 9C). However, SP-0.1, and -0.3 groups exhibited depletion of white pulp (Fig 9D-E), with normal splenic nodules was observed in SP-0.5 group (Fig 9F).

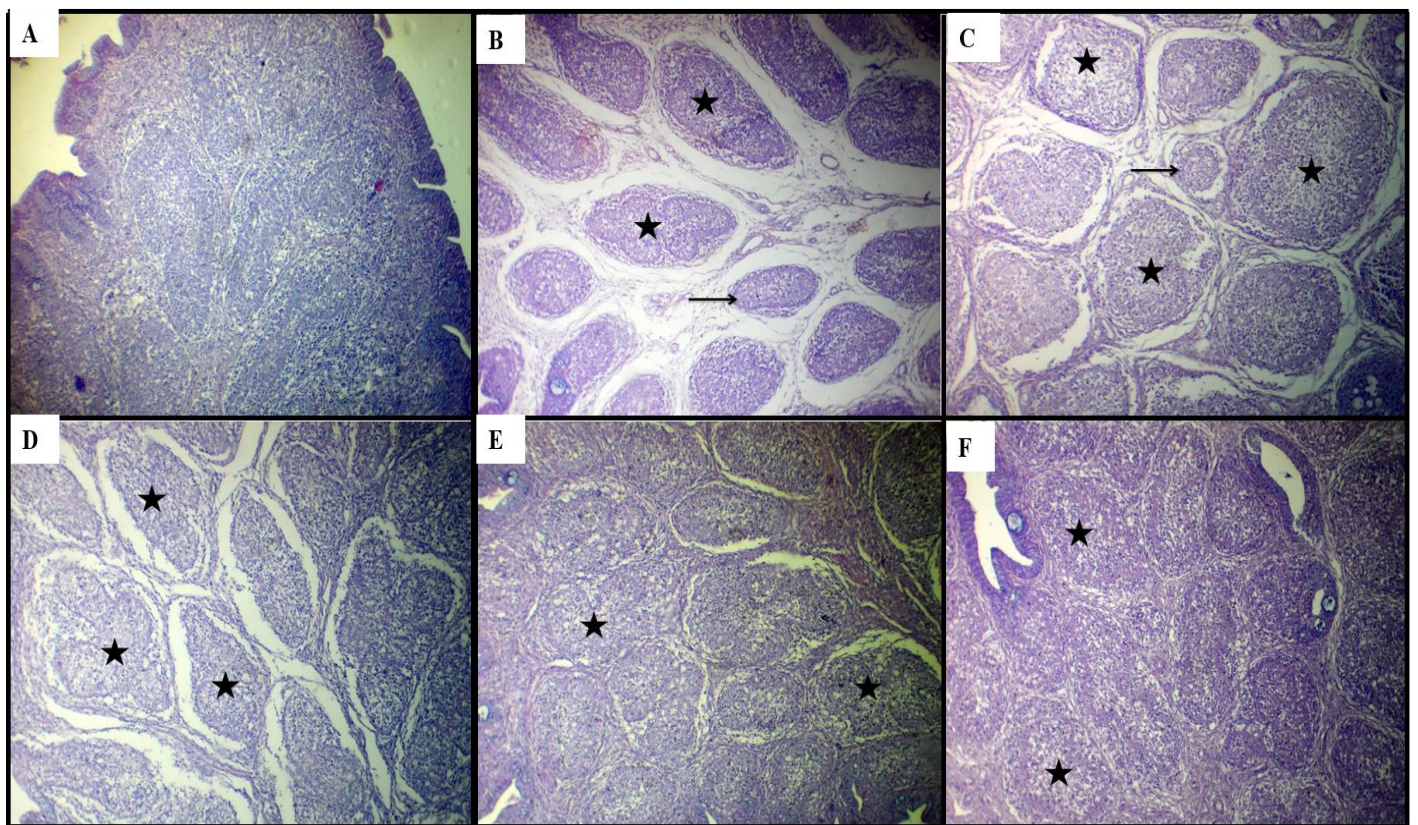


Figure 6: Light microscopy photomicrographs of H&E stained sections of bursa in broilers challenged with IBDV (X.100). A: normal structure bursal follicles of non-vaccinated untreated unchallenged group (G1), B: bursal lymphocyte depletion (stars), and atrophy of follicles (arrow) in non-vaccinated untreated challenged group (G2), C: bursal lymphocyte depletion (stars), and atrophy of follicles (arrow) in the vaccinated untreated challenged group (G3), D: depletion of bursal lymphocytes (stars) in 0.1% SP-treated group (G4), E-F: mild depletion of lymphocytes (stars) in 0.3% and 0.5% SP-treated groups (G5, G6).

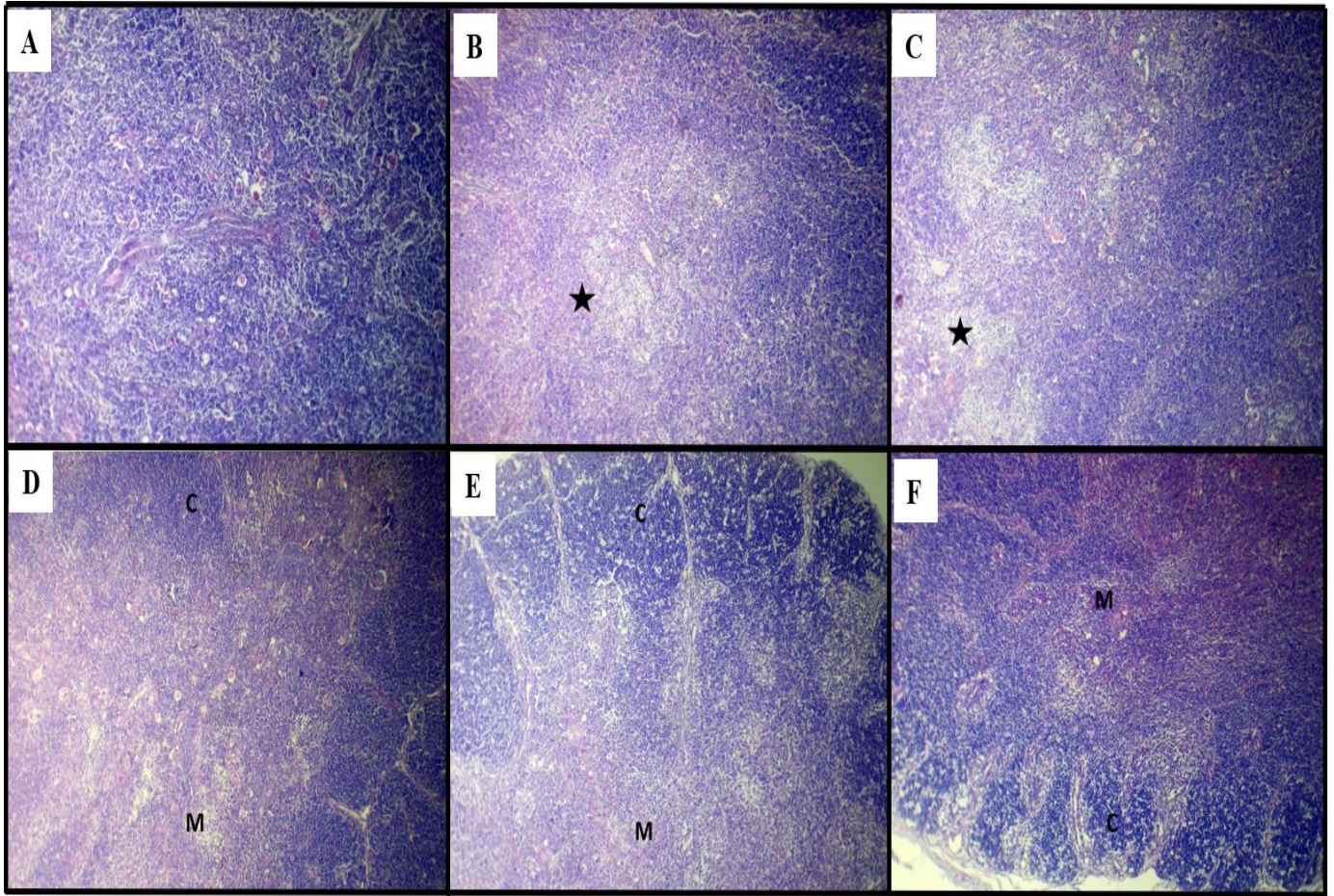


Figure 7: Light microscopy photomicrographs of H&E stained sections of thymus in broilers challenged with IBDV (X.100). A: normal histological structure of thymus cortex and medulla in non-vaccinated non-treated non-challenged group (G1), B-C: severe medullary lymphocyte depletion (stars) in non-vaccinated non-treated challenged group (G2), and the vaccinated non-treated challenged group (G3), D-E-F: normal cortex (C) with medullary lymphocyte depletion (M) in 0.1%, 0.3% and 0.5% SP-treated groups (G4, G5, G6).

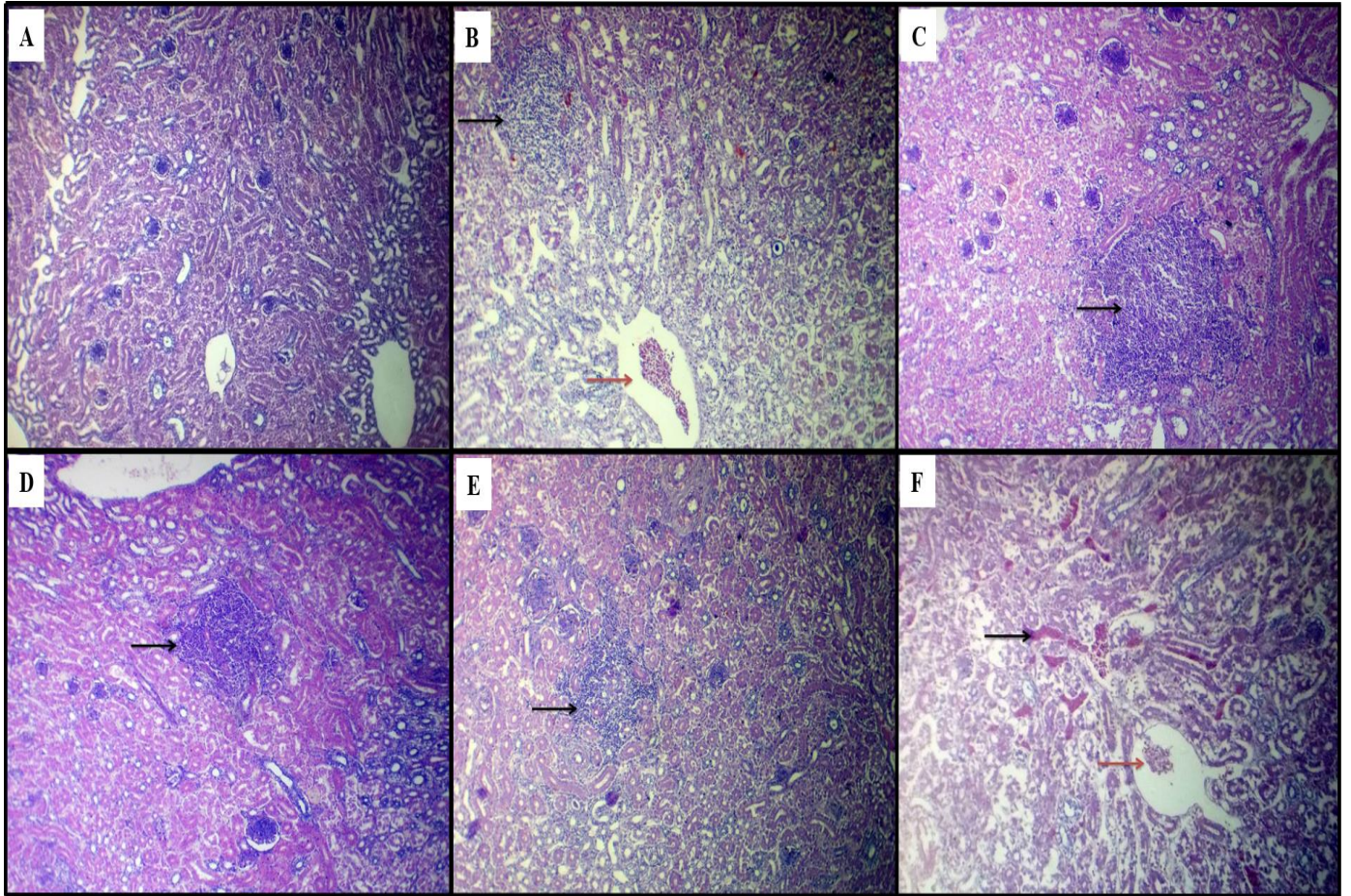


Figure 8: Light microscopy photomicrographs of H&E stained sections of kidneys in broilers challenged with IBDV (X.100). A: normal histological structure of kidneys in non-vaccinated non-treated non-challenged group (G1), B: severe interstitial nephritis (black arrow), and congestion of blood vessels (red arrow) in non-vaccinated non-treated challenged group (G2), C: severe interstitial nephritis (arrow) in the vaccinated non-treated challenged group (G3), D: moderate interstitial nephritis (arrow) in 0.1% SP-treated group (G4), E: mild interstitial nephritis (arrow) in 0.3% SP-treated group (G5), F: congestion of blood vessels (red arrow), and congestion of inter tubular blood capillaries (black arrow) in 0.5% SP-treated groups (G6).

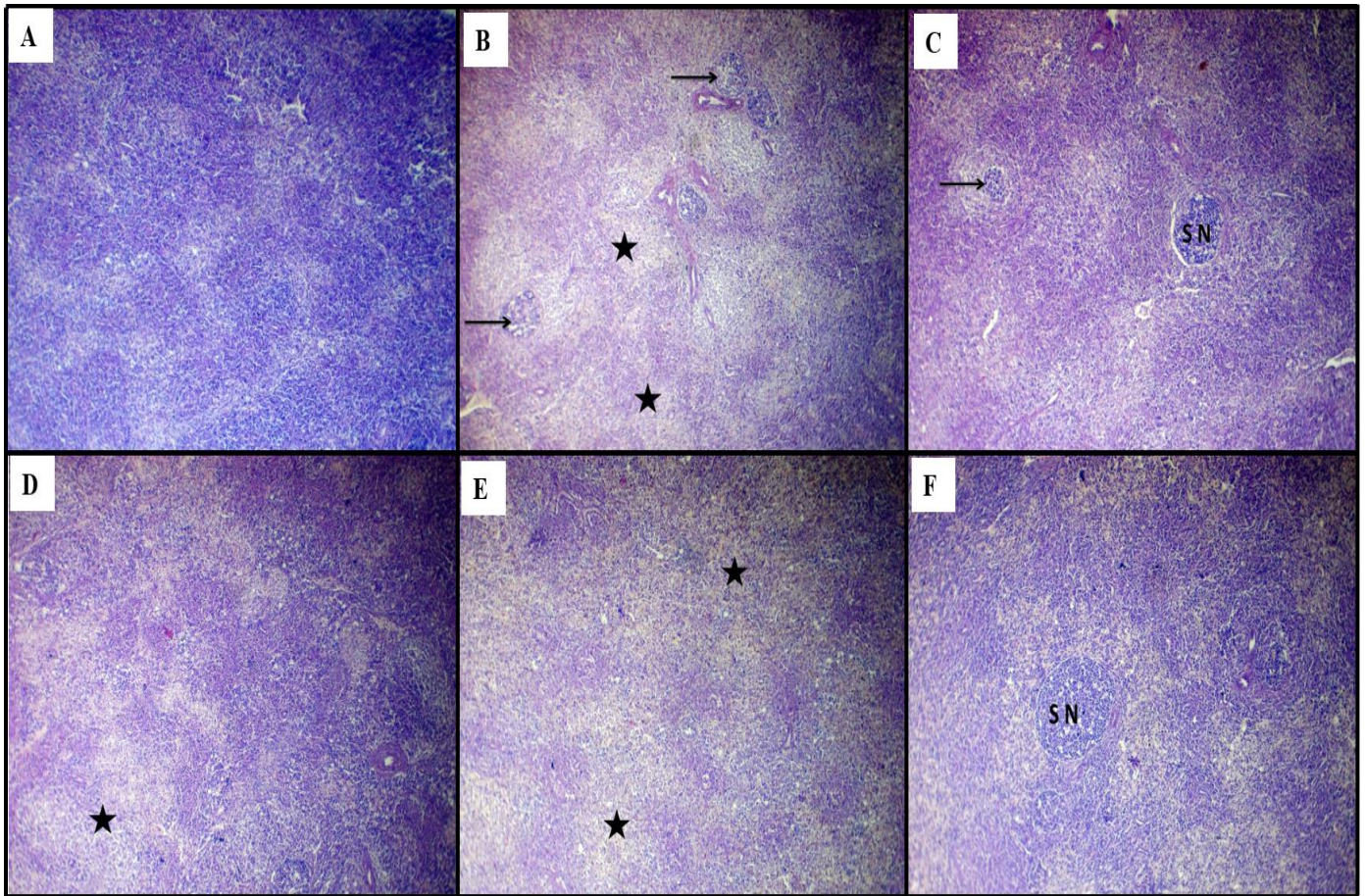


Figure 9: Light microscopy photomicrographs of H&E stained sections of spleen in broilers challenged with IBDV (X.100). A: normal histological structure of spleen white and red pulp in non-vaccinated non-treated non-challenged group (G1), B: depletion of white pulp (stars), and lymphocyte in splenic nodules (arrow), in non-vaccinated non-treated challenged group (G2), C: atrophy of splenic nodules (arrow) in the vaccinated non-treated challenged group (G3), D-E: depletion of white pulp in 0.1%, and 0.3% SP-treated groups (G4, G5), F: normal splenic nodules in 0.5% SP-treated group (G6).

#### 4. Discussion

Our results revealed the enhancement of growth performance by SP supplementation during IBDV challenge. The same result was recorded previously, SP supplement during IBDV challenge improved the body weight gain, FCR and decreased the feed intake (**Billah et al., 2022**). Also, **Hanafy, (2022)** reported that SP supplementation up to 0.7 g/kg had beneficial effect on the growth performance which recommend it as a promising growth promotor agent for poultry. The

enhanced growth performance induced by SP may be contributed to efficient nutrient utilization, and normalizing the gut microbiota via its antibacterial compounds; laminarin and fucoidan (**Alwaleed et al., 2021**). Furthermore, **Zeweil et al., (2016)** concluded that dietary SP supplementation for broilers under heat stress promising the growth traits via restoring the liver functions, modulation of lipid profile, and augmentation of total protein and albumin levels. Elaboration of cellular and humoral immune response, and refinement of gut histomorphology in broiler chickens by SP feeding reflected on improvement of body weight, feed intake, and FCR (**Khan et al., 2020**). In the same context, **Bonos et al., (2016)** reported that supplementation of 5 g SP/kg enhancing the quality of broiler meat through maximizing the omega-3 fatty acids concentration in the thigh muscles. Another explanation of growth promoting effect of SP is the elevation of lactobacillus probiotics and increasing the vitamin absorption (**Mariey et al., 2012**). In parallel line, **Park et al., (2018)** showed that increasing lactobacillus population, digestibility of dry matter, antioxidant enzymes while decreasing the ammonia gas emission in the excreta of broilers fed SP.

The SP-treated groups showed the least viral RNA quantity in dose dependent manner as compared to NC and PC at 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> dpc, vaccinated chicken groups consumed 5 gm of SP showed a significant decrease in cloacal viral shedding than other groups, the most promising active constituents of SP is a sulphated polysaccharides (calcium spirulan) is major polymer with a structure similar to glycogen possess antiviral activity (**Prasanna et al., 2010**).

Bursal index is a critical issue for determination of IBDV pathogenicity (**Ivan et al., 2001**). Our results revealed enlarged bursa at 4 dpc then become atrophied at 8 dpc, which in parallel with (**Van den berg and Meulemans, 1991**) who recorded severe lymphocyte depletion with hemorrhagic inflammatory condition in the bursa of chickens infected with vvIBDV. Also, this result was coincided with (**Murmu et al., 2014**) who confirmed an increasing bursa body weight ratio in the vaccinated and IBDV affected chickens compared to control one. However, (**Myint et al., 2021**) proved that variant IBDV caused bursa atrophy and reduction of bursa body weight ratio associated with depletion, atrophy, and fibrosis of lymphoid tissues, apoptosis, and follicle vacuolation. **Kumari et al., (2019)** mentioned that SP feeding to broilers inhibited the immunosuppressive effect of IBD vaccine, increased bursal index, and body weight.

The distribution of the observed lesions was variable. They were characterized by pyknotic nuclei in the medullary region of the lymphoid follicle, slight lymphoid depletion, infiltration of inflammatory cells in the interstitial connective tissue, degenerative alterations, edema, interstitial fibroplasia, medullary necrosis with cyst formation, fibrosis, absence of lymphoid follicle due to hemorrhage and other types of alterations in the lymphoid follicles (Aktar et al., 2020).

## 5. References

- El-shall, N. A., Sedeek, M. E., El-badawy, M. M., Hussein, E. G., Awad, A. M. 2018. Phylogenetic Characterization of Infectious Bursal Disease (IBD) Viruses Isolated From Field Outbreaks in Chickens From Behera And Alexandria Governorates, Egypt. *Alexandria Journal for Veterinary Sciences*, 56(1).
- Lucio, B., Hitchner, S. B. 1979. Infectious bursal disease emulsified vaccine: effect upon neutralizing-antibody levels in the dam and subsequent protection of the progeny. *Avian Diseases*, 466-478.
- Murphy, F.A., Gibbs, E.P.J., Horzinek, M.C., Studdert, M.J. 1999. *Veterinary virology*, 3rd edn. Academic Press, London, pp 405–409.
- Sharma, J. M., Dupuy, J. M., Lamontagne, L. (1989): Immunosuppression by avian infectious bursal disease virus and mouse hepatitis virus. In *Virus-Induced Immunosuppression* (pp. 201-216). Springer, Boston, MA.
- Ingrao, F., Rauw, F., Lambrecht, B., van den Berg, T. 2013. Infectious bursal disease: a complex host–pathogen interaction. *Developmental & Comparative Immunology*, 41(3), 429-438
- Myint, O., Suwanruengsri, M., Araki, K., Izzati, U. Z., Pornthummawat, A., Nueangphuet, P., Yamaguchi, R. 2021. Bursa atrophy at 28 days old caused by variant infectious bursal disease virus has a negative economic impact on broiler farms in Japan. *Avian Pathology*, 50(1), 6-17.
- Aktar, M. F., Noor, M., Kamal, A. H., Rahman, M. M. 2020. Scoring of bursal lesions in commercial broiler chickens infected with field IBD virus at Sylhet region of Bangladesh. *Int Clin Pathol J*, 8(1), 8-12.
- Murmu, R., Islam, M. N., Juli, M. S. B., Khan, M. A. S., Harun-ur-Rashid, S. M., Hossain, F. M. A., Rahman, M. M. 2014. Pathogenicity and immunosuppressive properties of GM-97 strain of infectious bursal disease virus in commercial broiler chickens. *Journal of Advanced Veterinary and Animal Research*, 1(1), 1-7.
- Van den Berg, T. P., Meulemans, G. 1991. Acute infectious bursal disease in poultry: protection afforded by maternally derived antibodies and interference with live vaccination. *Avian pathology*, 20(3), 409-421.
- Iván, J., Nagy, N., Magyar, A., Kacskovics, I., Mészáros, J. 2001. Functional restoration of the bursa of Fabricius following in ovo infectious bursal disease vaccination. *Veterinary Immunology and Immunopathology*, 79(3-4), 235-248.

Bonos, E., Kasapidou, E., Kargopoulos, A., Karampampas, A., Christaki, E., Florou-Paneri, P. Nikolakakis, I., 2016. Spirulina as a functional ingredient in broiler chicken diets. South African Journal of Animal Science, 46, 94-102.

Xalxo, R. K., Sao, S., Sahu, P. K. 2013. Effect of antibacterial properties of cyanobacterial *Spirulina platensis*. International Journal of Pharmacy & Life Sciences, 4(9).

KUMARI, P., NEHRA, V., LATHER, D., KUNDU, P., Narang, G. 2019. Effect of Spirulina on growth and bursal index of infectious bursal disease vaccinated chickens. Haryana Vet, 58(1), 70-72.

Park, J. H., Lee, S. I., Kim, I. H. 2018. Effect of dietary Spirulina (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. Poultry science, 97(7), 2451-2459.

Mariey, Y.A., Samak, H.R. Ibrahim, M.A., 2012. Effect of using spirulina platensis algae as a feed additive for poultry diets: 1- productive and reproductive performance of local laying hens. Egyptian Poultry Science Journal, 32, 201–215

Khan, S., Mobashar, M., Mahsood, F. K., Javaid, S., Abdel-Wareth, A. A., Ammanullah, H., Mahmood, A. 2020. Spirulina inclusion levels in a broiler ration: evaluation of growth performance, gut integrity, and immunity. Tropical Animal Health and Production, 52(6), 3233-3240.

Zeweil, H., Abaza, I. M., Zahran, S. M., Ahmed, M. H., AboulEla, H. M., Saad, A. A. 2016. Effect of Spirulina platensis as dietary supplement on some biological traits for chickens under heat stress condition. Asian Journal of Biomedical and Pharmaceutical Sciences, 6(56), 8-12.

Billah, M. M., Debi, M. R., Al-Mamun, M., Islam, K. M. S. 2022. Supplementation of Spirulina platensis in feed and water for the performance of broiler. Bangladesh Journal of Animal Science, 51(1), 17-24.

Alwaleed E, El-Sheekh M, Abdel-Daim M, Saber H. Effects of Spirulina platensis and Amphora coffeaeformis as dietary supplements on blood biochemical parameters, intestinal microbial population, and productive performance in broiler chickens. Environ Sci Pollut Res. 2021;28:1801–1811.

Agustini T, Suzery M, Sutrisnanto D, Ma'Ruf W, Comparative study of bioactive substances extracted from fresh and dried Spirulina sp. Procedia Environ Sci. 2015;23:282–289.

Abdel-Moneim A-ME, El-Saadony MT, Shehata AM, Saad AM, Aldhumri SA, Ouda SM, Mesalam NM (2021) Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi J Biol Sci 29:1197–1209.

Müller, H., Mundt, E., Eterradosi, N., Islam, M. R. 2012. Current status of vaccines against infectious bursal disease. Avian pathology, 41(2), 133-139.

Aladaileh SH, Khafaga AF, Abd El-Hack ME, Al-Gabri NA, Abukhalil MH, Alfwuaires MA, Bin-Jumah M, Alkahtani S, Abdel-Daim MM, Aleya L, Abdelnour SA 2020. Spirulina

platensis ameliorates the sub chronic toxicities of lead in rabbits via anti-oxidative, anti-inflammatory, and immune stimulatory properties. *Sci Total Environ* 701:134879.

Wagari, A. 2021. A review on infectious bursal disease in poultry. *Health Economics & Outcome Research: Open Access*, 7(2), 18-23.

Bolis, D. A., Paganini, F. J., Simon, V. A., Zuanaze, M. F., Scanavini Neto, H., Correa, A. R. A., Ito, N. M. K. 2003. Gumboro disease: evaluation of serological and anatomopathological responses in vaccinated broiler chickens challenged with very virulent virus strain. *Brazilian Journal of Poultry Science*, 5, 137-146.

Jackwood DJ, Sommer-Wagner SE 2011. Amino acids contributing to antigenic drift in the infectious bursal disease birnavirus (IBDV). *Virology* 409:33–37

Fan, L., Wang, Y., Jiang, N., Chen, M., Gao, L., Li, K., ... & Qi, X. (2020). Novel variant infectious bursal disease virus suppresses Newcastle disease vaccination in broiler and layer chickens. *Poultry science*, 99(12), 6542-6548.

Dey, S., Pathak, D. C., Ramamurthy, N., Maity, H. K., Chellappa, M. M. 2019. Infectious bursal disease virus in chickens: prevalence, impact, and management strategies. *Veterinary Medicine: Research and Reports*, 10, 85.

Yang, Y., Park, Y., Cassada, D. A., Snow, D. D., Rogers, D. G., Lee, J. 2011. In vitro and in vivo safety assessment of edible blue-green algae, *Nostoc commune* var. *sphaeroides* Kützinger and *Spirulina platensis*. *Food and chemical toxicology*, 49(7), 1560-1564.

Hanafy, A. 2022. *Spirulina platensis* a Promising Growth Promoter for Poultry Industry. *Asian Journal of Research in Animal and Veterinary Sciences*. 10 (1): 27-33.

Prasanna, R., Sood, A., Jaiswal, P., Nayak, S., Gupta, V., Chaudhary, V., Joshi, M., Natarajan, C. Rediscovering cyanobacteria as valuable sources of bioactive compounds. *Appl. Biochem. Microbiol.*, 2010, 46(2), 133-47.