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IMMUNOMODULATORY EFFECT AND HISTOPATHOLOGICAL CHANGES OF SPIRULINA PLANTENSIS AGAINST INFECTIOUS BURSAL DISEASE (IBD) IN POULTRY

ABEER S. HAFEZ¹, HANAA A. ELSAMADONY², ASMAA IBRAHIM ABDELAZIZ ZIN ELDIN³, AHMED EL-SHEMY⁴, ABDELFATTAH I. EL-ZANATY², ENAS HAMAD⁵, HEMAT S. EL-SAYED⁶, AALAA S. A. SAAD⁷*

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ABSTRACT

Infectious bursal disease (IBD) continues to pose a considerable risk to poultry health and productivity, especially in areas with elevated poultry production, such as Egypt. This study assessed the immunomodulatory effects of Spirulina platensis (SP) supplementation in broiler chickens vaccinated with an intermediate-plus IBDV vaccine and challenged with virulent IBDV. Birds were divided into four groups: negative control (G1), vaccinated only (G2), vaccinated + 2% SP (G3), and vaccinated + 3% SP (G4). The results demonstrated that SP supplementation, especially at a 3% concentration, enhanced immune responses, reduced clinical signs, and mitigated the effects of IBDV. SP-supplemented birds exhibited elevated levels of IFN-y and proinflammatory cytokines, improved antibody titers, and better cellular immune function, including increased macrophage activity and lysozyme concentration. Moreover, SP reduced nitric oxide (NO) levels and oxidative stress markers, while considerably improving total antioxidant capacity. qRT-PCR revealed that 3% SP supplementation (G4) led to a notable decrease in viral load and shedding. Histopathological findings supported these results, with G3 and G4 showing less severe bursal damage than the vaccinated-only group. These findings suggest that SP can serve as a natural immunostimulant, improving vaccine efficacy, enhancing immune protection, and reducing viral dissemination in broiler chickens. Incorporating SP into poultry diets may offer a promising strategy for controlling IBD in commercial operations.

Keywords: Infectious bursal disease, Spirulina platensis, Immunomodulatory, Histopathology.

Corresponding author: AALAA S. A. SAAD E-mail address: alaa.samir87@yahoo.com

Present address: Biotechnology Department, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Giza. P.O. 12618, Egypt

¹ Immunology Department, Animal Health Research Institute (AHRI), Agriculture Research Centre (ARC), Dokki, Giza P.O. 12618, Egypt.

² Poultry Diseases Diagnosis and Research Department, Animal Health Research Institute (AHRI), Agriculture Research Centre (ARC), Giza P.O. 12618, Egypt.

³ Microbiology and Immunology Department, Veterinary Research Institute, National Research Centre, P.O. 12622, Egypt.

⁴ Department of Parasitology and Animal Diseases, Veterinary Research Institute, National Research Centre, P.O. 12622, Egypt.

⁵ Poultry Diseases Department, Mansoura Lab Animal Health Research Institute (AHRI), Agriculture Research Centre (ARC), 12618, Egypt.

⁶ Poultry Diseases Department, Benha-Branch Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), 12618, Egypt.

⁷ Biotechnology Department, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Giza. P.O. 12618, Egypt.

INTRODUCTION

Infectious bursal disease (IBD) is a global economic threat to the chicken industry. The illness is caused by a highly contagious, bisegmented, double-stranded RNA virus called infectious bursal disease virus (IBDV) (Lefkowitz et al., 2018). IBDV is a member of the *Avibirnavirus* genus within the Bernardina family. There are two serologically defined serotypes: serotype 1, including pathogenic strains affecting chickens, and serotype 2, which consists of naturally occurring non-pathogenic strains found in turkeys (Cubas-Gaona et al., 2022).

IBDV primarily affects young chickens between ages of two and six weeks. The disease has a morbidity rate of 100% and can result in mortality rates of up to 90%. Clinically, infected birds exhibit symptoms, such as diarrhoea, vent picking, anorexia, and urates around the vent feathers. These symptoms typically appear two to three days after infection, and can persist for three to four days even after the birds start to recover (Getachew and Fesseha, 2020).

IBDV propagates in the bursa of Fabricius, differentiating lymphocytes, destroying large quantities of growing B-lymphocytes, and impeding immunological maturation. This pronounced immunosuppression renders the diseased chickens more vulnerable to other infectious diseases (El-Samadony et al., 2019; Shahat et al., 2023).

Although vaccination is crucial for effectively controlling the disease, reports of vaccination failure have also surfaced in recent years due to the emergence of variants or antigenic drift of the virus (Boudaoud et al., 2016). Furthermore, attenuated live vaccinations for IBD can lead. to varving levels. ofimmunosuppression in vaccinated birds, increasing their susceptibility to other infections (Lupini et al., 2020; Kumari et al., 2019).

Spirulina platensis (SP), the blue-green algae, is rich in phycocyanins and flavonoids, as well as several other beneficial compounds. These bioactive exhibit anti-inflammatory, compounds antiviral, immunomodulatory, antioxidant effects (Abdel-Moneim et al., 2022; Aladaileh et al., 2020; Masih, 2025). SP has been shown to enhance the overall health and growth efficiency of broilers by improving the structure of the intestinal tissue and inducing positive changes in the population of intestinal microbial flora (Mamashli et al., 2025). Numerous studies investigated the connection between spirulina consumption and improvements in the immune system (Vasudevan et al., 2021). Notably, Spirulina serves as a significant immunomodulator, enhancing both the innate and adaptive immune responses to IBDV. Additionally, the bioactive proteins in Spirulina stimulate the gut immune system, which improves the vaccination response (Lestingi et al., 2024).

This study investigates the effects of administering different concentrations (2% and 3%) of SP on the efficacy of an extreme intermediate plus IBDV vaccine and its anti-inflammatory properties in birds challenged with IBDV. To achieve this, we evaluated several factors, including virus shedding, total antioxidant capacity (TAC), lysozyme activity, serum NO levels, antibody titers against IBDV, and the relative expressions of IL1 β , IL6, and INF γ , as well as histopathological changes.

MATERIALS AND METHODS

1. Spirulina platensis (SP) is a crumbled, dark blue-green powder with a mild seaweed smell. It was obtained from the National Research Centre, Dokki, Giza, Egypt, by spray drying the cyanobacterium's biomass.

2. IBD virus titration in SPF-ECE

The viral stock was serially diluted 10 times using five eggs per dilution and injected into 10-day-old SPF embryonated eggs. Then, the eggs were incubated at 37°C for seven days. Mortality within 24 h after injection was considered nonspecific and was not included in the calculations. Using the Reed–Muench method, the virus titre was determined as the median embryo infectious dose per milliliter (EID50/mL).

3. Chicken experiment and design protocol

The Agricultural Research Center, Animal Health Research Institute, Egypt's ethical committee approved the experimental procedures with permission (number ARC-AHRI-160-24).

A total of 80 one-day-old Cobb500 were divided into four groups (20 birds per group): group 1 was used as a negative control (non-vaccinated, non-infected), group 2 (G2) was vaccinated and challenged with vvIBDV, group 3 (G3) was vaccinated and supplemented with 2% SP, challenged with vvIBDV, and group 4 (G4) was vaccinated and supplemented with 3% SP and challenged with vvIBDV.

Groups 2, 3, and 4 received the vaccination as follows: Birds were immunized with IBD extreme intermediate plus, Winterfield 2512 strain (CEVAC IBDL®, Ceva Sante Animale, France) at 11 days old. The vaccine was administered through drinking water.

4. Spirulina platensis supplementation in G3 and G4 was synchronized with IBD vaccination at 11 days of age and persisted for seven successive days. At 22 days of age, the vvIBDV (OQ999393.1/Delt-11) challenge virus was inoculated into G2, G3, and G4 via the oculonasal route (50 μ L) and orally (50 μ L) using 10⁵ EID50/mL/ bird (OIE, 2018).

The chickens were placed in an isolator with unrestricted access to both feed and water throughout the experiment. They began with a starter diet consisting of crumbled feed.

5. Evaluation of immune response and protective effects

5.1. Blood samples

Blood samples were collected from the wing vein for serum separation, with five samples taken from each group at one day, the day before vaccination, the day before challenge, and days 3, 7, and 14 after infection. Serum was stored at -20°C until analysis. Before testing, the sera were inactivated by heating them to 56°C for 30 minutes.

5.2. ELISA (Enzyme-Linked Immunosorbent Assay)

Serum samples were analyzed to monitor the development of IBDV-specific antibodies using commercial indirect ELISA kits BD-plus (ProFLOK® IBD Ab, Canada) in compliance with the manufacturer's guidelines.

5.3. Assay of nitric oxide (NO)

The concentrations of NO were calculated using the standard curve with sodium nitrate and the NO values were ascertained, as reported by Lee et al. (2011).

5.4. Lysozyme activity assay

The assay was ascertained as Schultz (1987) described. A concentration of 500 mg/L of *Micrococcus lysodeikticus* was employed to dissolve 1% agarose in 0.06 M PBS at pH 6.3 to prepare the lysoplates. The lysozyme concentrations were established using the logarithmic curve from a standard lysozyme assay.

5.5. Total antioxidant capacity (TAC)

TAC was calculated with biodiagnostics kits using the colorimetric approach.

6. Tissue samples

At 3, 7, and 14 days post-challenge (DPC), five chicks from each experimental group were euthanized and then killed by cervical dislocation to collect the bursa of Fabricius, kidneys, spleen, and thymus. Bursal tissues were analyzed to assess viral load and the expression of cytokine genes (IL1β, IL6,

and INFγ) at both time points. For histopathological examination, bursa, kidneys, spleen, and thymus tissues were immediately fixed in 10% buffered formalin at 7 DPC.

7. Evaluation of the virus load in bursal tissues

The collected tissues were extensively homogenized and then centrifuged to obtain the supernatants.

Following the COL-NA-kit (REME-D, Egypt) guidelines, the RNA was extracted from supernatants. Samples analysis was performed using ABT 2X one-step qRT-PCR mix (probe) using one-step TaqMan real-time qRT-PCR with the following thermal profile: one cycle of 42°C for 20 min and 95°C for 3 min, followed by 40 cycles of 95°C for 15 sec, 53°C for 30 sec, and 72 °C for 30 sec.

8. Viral shedding

Cloacal swabs were collected from five birds per group at 3, 7, and 14 DPC to determine viral shedding, and were analyzed using rRT-PCR (Mosad, 2024).

9. Cytokine gene expression in the bursa Real-time PCR analysis, using aV2.2.2 software (AB Applied Biosystems). Duplicate sets of each reaction sample were performed using ABT 2X qRT-PCR mix (SYBR) with the following thermal profile: one cycle of 42°C for 20 min and 95°C for 3 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 30 sec, and 72 °C for 30 sec.

Amplification data of cytokines were normalized against 28S RNA, and the fold change of cytokine gene expression was calculated as previously described $2^{(-\Delta\Delta CT)}$ (Livak and Schmittgen, 2001).

10. Histopathology

Samples were processed for histology using routine procedures and stained with Hematoxylin and Eosin (H&E) (Suvarna et al., 2019). We used a microscope (Carl Zeiss, 37081 GmbH, Germany) and a camera (Carl Zeiss, 37081 GmbH, Germany) for imaging. Lesions were scored

as 0 = normal, 1 = mild, 2 = moderate, and 3 = severe (Gibson et al., 2013).

11. Statistical analysis

All data were expressed as the mean \pm standard deviation (SD), with Student's t-test employed as specified in each test. Statistical significance was defined as a probability (P) value of less than 0.05 (P<0.05).

RESULTS

1. Clinical signs, mortalities, and gross lesions

Birds in G2 (vaccinated, not supplemented) exhibited classical clinical signs of IBD, including depression, reduced activity, inappetence, reluctance to respond to stimuli, watery white diarrhoea, and slight huddling. The signs continued up to seven days post-inoculation (DPI). By 14 DPI, most birds showed clinical recovery, with illness signs largely subsiding and a return to normal behaviour and feed intake. In G3 (2% SP), the birds exhibited mild lethargy and slight ruffling in their feathers, along with a temporary dip in feed intake. No signs of diarrhoea or severe depression were observed. However, in G4 (3% SP), the clinical signs were minimal or completely absent. As time progressed, the G3 birds continued their path to recovery. Some delays in growth and slight variations in body size were noted; however, there were no deaths.

Post-mortem examination at 3 DPI revealed lesions, including hemorrhages in the thigh and breast muscles, bursal congestion, petechial hemorrhages on mucosal surfaces, and enlarged kidneys. G3 (2% SP) exhibited mild bursal congestion. Group 4 (3% SP) displayed near-normal bursal architecture, with only minimal lymphoid depletion, and no hemorrhages were observed.

2. Evaluation of Immune Responses and Protective Advantages

2.1. Serum lysozyme concentration

Data in Fig. 1a illustrated that before challenge (BC), Group 1 (G1) had the lowest lysozyme concentration (16.34 \pm 1.48), while G2, G3, and G4 had significantly higher scores (19.40 \pm 4.22, 20.54 \pm 1.74, and 22.35 \pm 3.47, respectively).

After the challenge, all groups showed a progressive increase in serum lysozyme concentration at all time intervals. The findings revealed significant differences between G1 and G2. Although the difference in lysozyme concentration between G3 and G4 was numerical, it was substantial between G3 and G4 and that of G2. G4 recorded the highest lysozyme concentration among all groups, reaching 25.26 ± 10.7 , 29.00 ± 8.6 , and 29.99 ± 4.9 at 3, 7, and 14 DPC, respectively.

2.2. Serum nitric oxide (NO) levels

Figure 2b displays that before challenge, the NO concentrations were relatively similar across groups: 5.96 ± 0.26 in G1; 6.79 ± 0.7 in G2; 6.61 ± 0.39 in G3; and 6.54 ± 0.29 in G4. After the challenge, G2 exhibited the most significant and sustained difference in NO at all time intervals, particularly at 7 DPC, where it reached 8.91 ± 1.5 . Similar values in NO concentration were noted between G3 and G4 after the challenge, indicating the same effect of both SP concentrations. The protective role of SP with its two concentrations succeeded in returning NO to its normal values, making them similar to those of G1.

2.3. Serum total antioxidant capacity (TAC)

The recorded data (Figure 1c) indicated that before the challenge, G1 exhibited the lowest levels of TAC at 1.21 ± 0.1 , followed by G2 (1.52 ± 0.15), G3 (1.80 ± 0.18), and G4 (2.14 ± 0.08).

A consistent improvement in serum total antioxidant capacity over time was observed after the challenge in G3 and G4 compared to G2. The superior outcomes in TAC in G4 suggest a significant response to a high concentration of SP, where it recorded 2.37 ± 0.03 , 2.44 ± 0.12 , and 2.53

 \pm 0.12 compared to those of G3, which scored 2.12 \pm 0.09, 2.17 \pm 0.15, and 2.19 \pm 0.14 at 3, 7, and 14 DPC.

2.4. ELISA (Enzyme-Linked Immunosorbent Assay)

IBDV-specific antibody titers were measured before challenge and at 3, 7, and 14 days DPC across four groups (G1–G4). In G1, the IBDV-specific antibody titer at one day of age was 2800, but it was not detected the day before vaccination.

The negative control showed no detectable antibody response throughout experiment, maintaining a zero value at all time points. In contrast, G2, G3, and G4 demonstrated varying degrees of immune response. Before the challenge, antibody titers were 2548 ± 148 in G2, 2790 ± 390 in G3, and 2921.90 ± 345 in G4. By 3 DPC, titers declined across these groups, but remained detectable, with G2 at 900 ± 201 , G3 at 1200 ± 232 , and G4 at 1400 ± 427 . Titers then began to rise again, reaching 1400 ± 140 , 2,000 ± 150 , and 2400 ± 155 in G2, G3, and G4, respectively, by 7 DPC. At 14 DPC, a marked increase was observed, with G2 at 2400 ± 341 , G3 at 3000 ± 124 , and G4 achieving the highest response at 3400 ± 213 . These results indicated that G3 and G4 elicited the strongest and most sustained antibody responses following the challenge (Figure 2).

3. Viral Load in the Bursa Tissues

qRT-PCR was conducted to assess the viral load in the bursal tissue of infected chickens (Figure 3). The results indicated that IBDV replication in the bursal tissue of vaccinated chickens challenged and supplemented with 3% SP (G4) $(4.6 \pm 0.4, 4.9 \pm 0.4, 4.3 \pm 0.5)$ log10 EID50/mL) differed significantly than the vaccinated group supplemented) (G2) (5.61 \pm 0.3, 5.80 \pm 0.16, $4.9 \pm 0.12 \log 10 EID50/mL$) in 3, 7, and 14 DPC, respectively. Meanwhile, IBDV replication in the bursal tissue of vaccinated chickens challenged supplemented with 2% SP (G3) was $5.11 \pm$ 0.5, 5.41 ± 0.49 , and $4.6 \pm 0.38 \log 10$ EID50/mL in 3, 7, and 14 DPC, respectively, with no significant difference compared to G2 and G4. The IBDV nucleic acid was not detected in the control negative group (G1).

4. Viral Shedding

qRT-PCR was conducted to quantify virus shedding from the cloaca. Compared to the vaccinated, challenged, non-supplemented group (G2) $(5.11 \pm 0.1, 5.5 \pm 0.17, \text{ and } 4.6)$ \pm 0.15), G4 that includes vaccinated chickens challenged and supplemented with 3% SP showed significantly (P < 0.05) decreased viral shedding (4.35 \pm 0.2, 4.6 \pm 0.4 and $3.8 \pm 0.66 \log 10 \text{ EID50/mL}$) at 3 and 7 DPC, respectively (Figure 4). In contrast, the shedding from vaccinated chickens in the group supplemented with 2% SP (G3) was 4.81 ± 0.3 , 5.01 ± 0.5 , and $4.31 \pm 0.73 \log 10 EID50/mL$ in 3, 7, and 14 DPC, respectively, with no significant difference compared to the other two groups (G2, G4). Virus shedding was not detected in the negative control group (G1).

5. Cytokine Gene Expression in the Bursa

IFN-γ is a crucial cytokine for both innate and adaptive immunity. It primarily activates macrophages and stimulates natural killer cells and neutrophils. In the vaccinated group (G2), the mRNA transcript of IFN-y was upregulated, showing levels of 2.3 ± 0.82 at 3 DPC and 3.4 ± 0.67 in the SP-treated G3 and G4, respectively. The most significant increase $(P \le 0.05)$ was observed in the 3% SP group, which recorded 4.3 ± 0.34 at 3 DPC and 6.1 ± 0.56 at 7 DPC. The 2% SP group also exhibited increases, with levels of 3.4 \pm 0.39 at 3 DPC and 4.4 \pm 0.44 at 7 DPC (Figure 5).

Proinflammatory cytokines, particularly IL- 1β and IL-6, play a crucial role in regulating the growth, activation, differentiation, and migration of immune cells to sites of infection, which is essential for combating intracellular pathogens such as viruses. In the bursa, the mRNA expression of IL- 1β significantly increased in the 3% SP group

(G4), reaching 4.6 ± 0.63 at 3 DPC and 7.6 ± 0.63 at 7 DPC. In contrast, the vaccinated (not supplemented) group (G2) and 2% SP group (G3) had lower levels of 3.8 ± 0.1 and 2.9 ± 0.79 at 3 DPC, and 5.7 ± 0.73 and 4.7 ± 0.73 at 7 DPC, respectively (Figure 5).

Similarly, IL-6 mRNA expression was notably higher in the 3% SP group (G4), measuring 4.3 ± 0.39 at 3 DPC and 5.3 ± 0.32 at 7 DPC. In comparison, the 2% SP (G3) and vaccinated (not supplemented) group (G2) showed expressions of 3.8 ± 0.83 and 2.8 ± 0.81 at 3 DPC and 4.5 ± 0.52 and 3.2 ± 0.21 at 7 DPC, respectively (Figure 5).

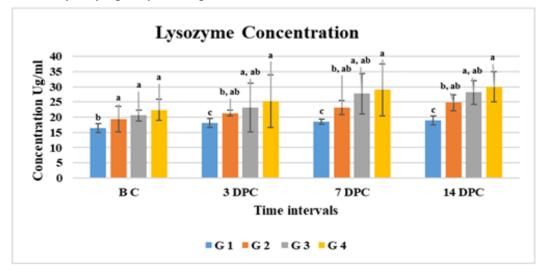
6. Histopathological Analysis

The histological structure of the bursa in G1 (non-vaccinated, non-challenged) showed normal bursal follicle structure (Figure 6a) compared to moderate lymphocyte depletion and atrophy of follicles (Figure 6b) in G2 (vaccinated, challenged), in addition to cyst formation and epithelialization. However, mild lymphocyte depletion and atrophy of follicles were observed in G3 (2% SP) (Figure 6c), while mildest depletion of follicle lymphocytes was seen in G4 (3% SP) (Figure 6d) (Table 2).

Regarding kidney histopathology, a normal histological structure of the kidneys was noted in G1 (Figure 7a), but moderate interstitial nephritis and congestion of blood vessels were observed in G2 (Figure 7b). Mild interstitial nephritis, congestion of blood vessels, and intertubular blood capillaries were noted in G3 and G4 (Figure 7c-d; Table 2).

The normal histological structure of the spleen's white and red pulp was observed in G1 (Figure 8a), while the spleen in G2 showed depletion of lymphocytes (Figure 8b). The depletion of lymphocytes was mild in the spleens of SP-supplemented G3 and G4 (Figures 8 c-d) (Table 2). The normal histological structure of the thymus cortex and medulla was observed in G1 (Figure 9a), while moderate medullary lymphocyte

depletion was noted in the thymus of group 2 (Figure 9b). Normal cortex with mild medullary lymphocyte depletion was shown in SP-supplemented G3 and G4 (Figures 9c-d; Table 2).



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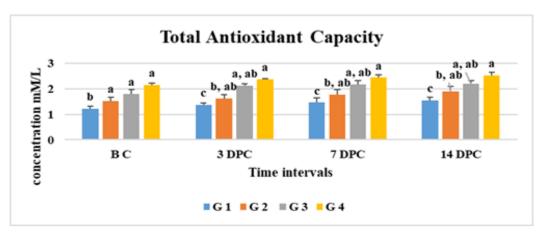


Figure 1. Evaluation of immune responses and protective advantages. a. Effect of *Spirulina platensis* on lysozyme concentration. b. Effect of *Spirulina platensis* on nitric oxide concentration. c. Effect of *Spirulina platensis* on total antioxidant capacity. Data were expressed as mean ± standard error. Different small letters indicate significance between groups within the same interval at P ≤ 0.05. BC: before challenge; DPC: days post challenge. G1: (control negative) non-vaccinated and non-infected; G2: vaccinated, non-supplemented, and infected with IBDV; G3: vaccinated and supplemented with 2% *Spirulina platensis* and infected with IBDV.

a

b

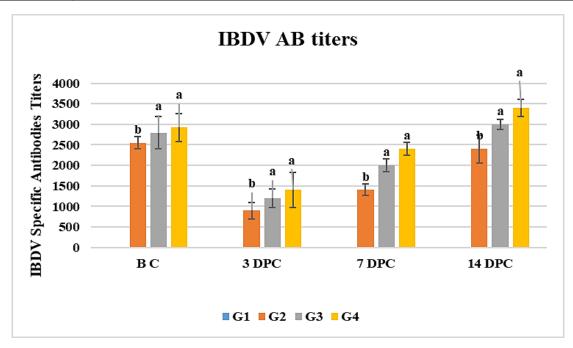


Figure 2: IBDV-specific antibody titre in the serum at: BC: before challenge, 3 DPC: 3 days post challenge, 7 DPC: 7 days post challenge, 14 DPC: 3 days post challenge. G1: (control negative) non-vaccinated non-infected; G2: vaccinated, non-supplemented, and infected with IBDV; G3: vaccinated, supplemented with 2% *Spirulina platensis*, and infected with IBDV; G4: vaccinated, supplemented with 3% *Spirulina platensis*, and infected with IBDV. Each bar represents the mean \pm SD (n = 5). The Student's *t*-test was used to calculate the *P* values. Different small letters indicate significance between groups within the same interval at P≤ 0.05.

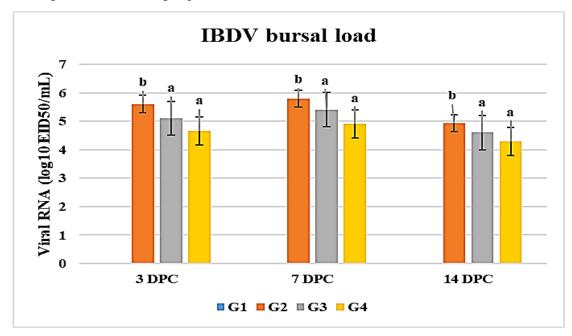


Figure 3: Effect of *Spirulina platensis* on the IBD viral load in the bursa tissues in infected chickens. The viral RNA load was measured by real-time quantitative polymerase chain reaction (real-time qRT-PCR). G1: (control negative) non-vaccinated non-infected; G2: vaccinated, non-supplemented, and infected with IBDV; G3: vaccinated, supplemented with 2% *Spirulina platensis*, and infected with IBDV. Each bar represents the mean \pm SD (n = 5). The Student's *t*-test was used to calculate the *P* values. Different small letters indicate significance between groups within the same interval at $P \le 0.05$.

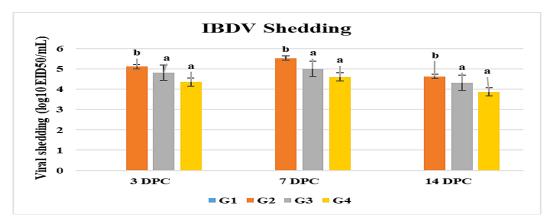


Figure 4. Effect of *Spirulina platensis* (2% and 3%) on the IBD viral cloacal shedding in infected chickens. The viral RNA load was measured by real-time quantitative polymerase chain reaction (real-time qRT-PCR). G1: (control negative) non-vaccinated non-infected; G2: vaccinated, non-supplemented, and infected with IBDV; G3: vaccinated, supplemented with 2% *Spirulina platensis*, and infected with IBDV. G4: vaccinated, supplemented with 3% *Spirulina platensis*, and infected with IBDV. Each bar represents the mean \pm SD (n = 5). The Student's *t*-test was used to calculate the *P* values. Different small letters indicate significance between groups within the same interval at $P \le 0.05$.

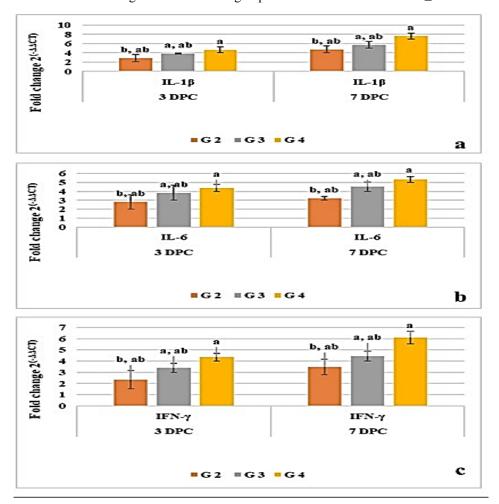


Figure 5. Dynamic changes in immune-related genes in the bursa of chickens. The bursae of inoculated chickens were collected at 3 and 7 DPC in each group (G2) vaccinated, non-supplemented and infected with IBDV. G3: vaccinated, supplemented with 2% *Spirulina platensis*, and infected with IBDV; G4: vaccinated, supplemented with 3% *Spirulina platensis*, and infected with IBDV. Total RNA was extracted, and cDNA was prepared. Cytokine expression levels were measured by the $2^{-\Delta\Delta Ct}$ method, with relative quantification by real-time quantitative polymerase chain reaction (real-time qRT-PCR). Differences in expression levels of the various genes between various groups were analyzed using a Student's *t*-test and considered significant (P < 0.05).

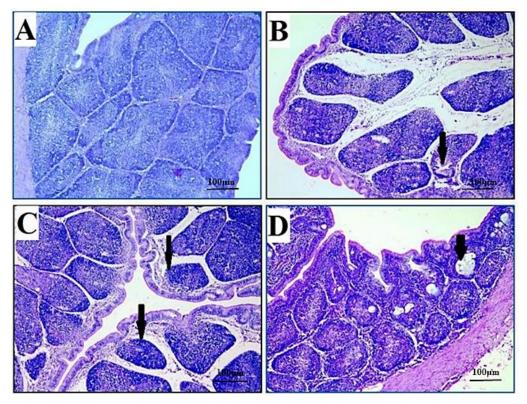


Figure 6. Histopathology of chicken bursa at 8 DPC. (A) G1 with apparently normal tissue histology. (B) bursa of G2 showing lymphocyte depletion, follicle atrophy, and epithelization (arrow); (C) bursa of G3 showing lymphocyte follicle atrophy; (D) bursa of G4 with depletion of lymphocytes of the medulla of lymphoid follicle with cyst formation (arrow) (H&E X100, 100μm).

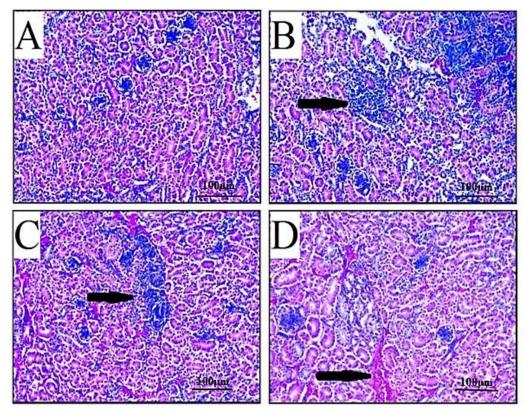


Figure 7: Histopathology of chicken kidney at 8 DPC. (A) G1 with apparently normal architecture; (B) kidney of G2 showing lymphocyte aggregation (arrow); (C) kidney of G3 showing lymphocyte aggregation (arrow); (D) kidney of G4 with congestion of blood vessels (arrow), H&E X100, 100 μm.

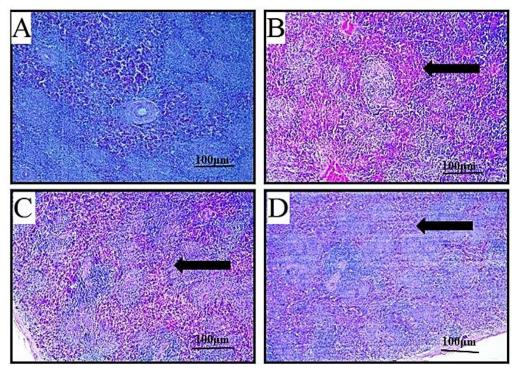


Figure 8. Histopathology of chicken Spleen at 8 DPC. (A) Spleen of G1 with apparently normal architecture; (B) Spleen of G2 showing depletion of lymphocytes; (C, D) spleen of G3 and G4 showing mild depletion of lymphocytes. H&E X100, 100 μm.

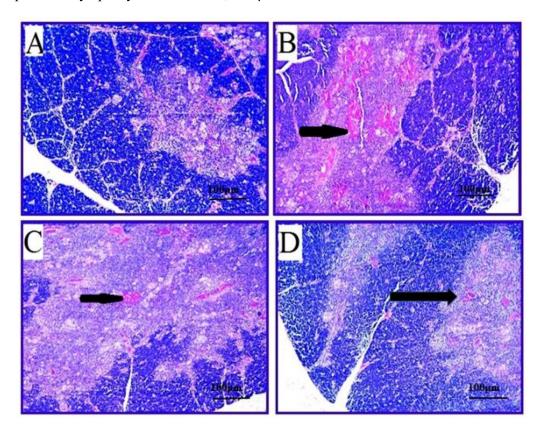


Figure 9. Histopathology of chicken thymus at 8 DPC. (A) Thymus of G1 with apparently normal architecture; (B) Thymus of G2 showing depletion of lymphocytes in the medulla (arrow); (C) Thymus of G3 showing depletion of lymphocytes in the medulla (arrow), (D) thymus of G4 with congestion of blood vessels, H&E X100, 100 μm.

Table 1: Primers used in the quantitative real-time PCR assay for various cytokine genes and IBDV.

RNA target	Sequence (5'-3')	
IL-1β	F: GCTCTACATGTCGTGTGTGATGAG	Giotis et al., 2015
	R: TGTCGATGTCCCGCATGA	_
IL-6	F: GCTCGCCGGCTTCGA	_
	R: GGTAGGTCTGAAAGGCGAACAG	_
IFN-γ	F: GTGAAGAAGGTGAAAGATATCATGGA	_
	R: GCTTTGCGCTGGATTCTCA	_
28S	F: GGCGAAGCCAGAGGAAACT	_
	R: GACGACCGATTTGCACGTC	
IBDV	Common-F: GCCGAGAAACTCCACAAGTC	Elmasry et al., 2025
	Common –R: TGTTTCCACGAGTGCTGAGT	
	Probe: FAM-GAC CCC GAT GCA GAC TGG TTT GA- TAMRA	

Table 2: Histopathological lesion scores of different organs.

Organ/Group	Group 1 (non-vaccinated, non-challenged)	Group 2 (vaccinated, challenged)	Group 3 (2% SP)	Group 4 (3% SP)
Bursa of Fabricius	0	2	1	1
Kidney	0	2	1	1
Spleen	0	2	1	1
Thymus	0	2	1	1

DISCUSSION

Gumboro disease, also known as infectious bursal disease (IBD), is one of the most critical epidemiological and economic challenges facing large-scale poultry production worldwide. It leads to direct losses, such as morbidity, mortality. It also indirect losses through immunosuppression, making birds more susceptible to secondary infections (Ramzy et al., 2024). Despite the widespread use of vaccination and biosecurity measures, controlling IBD remains challenging (Ramzy et al., 2024; Abd El-Fatah et al., 2024). This needs a requirement for natural immunostimulants that can enhance the efficacy of existing vaccines.

In the present study, *Spirulina platensis* (SP), a blue-green microalgae known for its immunomodulatory, antioxidant, and antiviral properties, was evaluated as a dietary supplement to boost immune response in broiler chickens vaccinated and challenged with IBDV. The findings demonstrated that SP supplementation at

both 2% and 3% dietary levels had a significant immune-enhancing effect.

Groups supplemented with SP (G3: 2%; G4: 3%) exhibited elevated levels of proinflammatory cytokines and interferongamma (IFN- γ) compared to the vaccinated-only group (G2). These immune markers are critical in initiating and regulating antiviral responses. Consistent with previous studies, the upregulation of IFN- γ suggests enhanced activation of natural killer (NK) cells and increased antiviral activity (Bax et al., 2023).

Humoral immunity was considerably improved in the SP-supplemented groups. G3 and G4 exhibited higher antibody titers at all post-challenge time points compared to G2, with G4 demonstrating the most robust and sustained response. These results suggest that SP enhances both the magnitude and duration of the humoral response, likely due to its rich content of bioactive compounds, including phycocyanin, polysaccharides, vitamins, minerals, and essential amino acids

(Spínola, 2024). This dose-dependent enhancement confirms the immunostimulatory potential of SP, supporting earlier findings by Abotaleb et al. (2020).

The cellular immune response, particularly activity heterophils of macrophages, also positively was influenced by SP. The immunosuppressive effect on lysozyme concentration in IBDVchallenged birds (G2) reflects the virus's ability to target heterophils as well as dividing IgM+ B lymphocytes in the bursa of Fabricius. Moreover, the consequence of heterophil infection is associated with the suppression of heterophil migration, a decrease in their phagocytic capacity, and a reduction in the lysozyme content located in the granules of heterophils (Rehman et al., 2016). The destructive effect on lysozyme concentration is mitigated by supplementation at its two concentrations in G3 and G4 in a dose-dependent manner. These results are similar to those reported by Abdel-Moneim et al. (2022), Aladaileh et al. (2020), and Rosas et al. (2019). Additionally, SP enhances the function of innate immunity by increasing the count of white blood cells and improving both phagocytic activity and the phagocytic index of macrophages (Salaha et al., 2025).

Moreover, NO levels were elevated in G2, reflecting increased oxidative stress and inflammatory damage following IBDV infection. This elevation is attributed to IFN-γ-induced upregulation of inducible nitric oxide synthase (iNOS), promoting apoptosis and tissue damage to the bursal tissues (Khatri and Sharma, 2006). SP supplementation reduced NO levels in G3 and G4, indicating its anti-inflammatory effect. This is likely due to the inhibition of the NF-κB pathway and iNOS expression by SP-derived lipids and heptadecane, as reported by Ku et al. (2013) and El-Shall et (2023). Furthermore, NO has a detrimental role in viral clearance through the prevention of nucleic acid replication (Ganguly et al., 2018).

Total antioxidant capacity (TAC) was also markedly higher in SP-treated groups. These findings align with studies in other poultry models, which demonstrated SP's ability to scavenge reactive oxygen species, reduce lipid peroxidation, and protect against oxidative DNA damage (Farag et al., 2016; Attia et al., 2023; Agustini et al., 2015). Furthermore, additional research by Al-Khalaifah (2022) and El-Shall et al. (2023) verified that phycocyanin, sulfated polysaccharides, and essential fatty acids—the bioactive components of SP—have been shown to strengthen both the innate and adaptive immune systems of poultry.

The qRT-PCR data on viral load and shedding further confirm SP's antiviral properties. G4 (3% SP) showed the most significant reduction in IBDV load at 3 and 7 days after the challenge, indicating enhanced viral clearance. G3 demonstrated reduced, but not statistically significant differences compared to G2, suggesting a threshold effect for SP's antiviral action. These effects may be attributed to SP's role in improving mucosal immunity and limiting viral replication (Al-Khalaifah, 2022).

The decrease in viral shedding may also be due to the antioxidant activity of SP, since it can potentially reduce oxidative stress and improve the mucosal immune response, decreasing viral replication and shedding. In contrast, 2% SP supplementation (G3) did not produce a statistically significant decrease in viral shedding compared with G2. This implies that a dose-dependent manner may be necessary for enhancing immunoprotective effects. This outcome of response proportionality is consistent with earlier reports that higher doses of SP boosted the immune response decreased viral load (Qureshi et al., 2010). There was no viral shedding in the control group (G1), confirming the specificity of the qRT-PCR assay and that there was no external contamination.

Histopathological examination supported the molecular and immunological findings. The bursa of Fabricius in G2 showed severe lymphoid depletion, interstitial fibrosis, and necrosis. These lesions were markedly milder in G3 and G4, indicating the protective effects of SP on lymphoid architecture. Similarly, the spleen and thymus of SP-supplemented birds exhibited improved histological profiles, suggesting enhanced systemic immunity (Gameiro et al., 2010; Sedeik et al., 2023).

These data strengthen the argument that SP may be beneficial as a supplementary feed ingredient for enhancing the response of birds to IBD vaccines and decreasing viral shedding and the spread of IBDV in commercial poultry farms. Further research, particularly involving cytokine profiling, is warranted for understanding the mechanisms underlying the observed antiviral actions.

CONCLUSION

Dietary supplementation with Spirulina significantly platensis improves immune response to IBD vaccination in broiler chickens, particularly at a 3% supplementation rate. SP enhances humoral and cellular immunity, reduces oxidative stress and inflammatory markers, limits viral replication and shedding, and protects lymphoid organs from structural damage. These findings provide strong evidence for incorporating SP into vaccination protocols in order to improve disease resistance and reduce economic losses in poultry farms. Further investigations focusing on cytokine profiling and performance metrics are recommended to fully elucidate mechanisms of action and optimize dosage levels for commercial application.

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التأثير المناعي والتغيرات النسيجية المرضية لسبيرولينا بلانتنس ضد مرض الجراب المأثير الماعي والتغيرات المعدى (IBD) في الدواجن

عبير حافظ , هناء السمدوني , اسماء زين الدين , احمد الشيمي , عبدالفتاح الزناتي , ايناس حماد , همت السيد , الاء سعد

ا قسم المناعة، معهد بحوث صحة الحيوان، مركز بحوث الزراعة، الدقي، الجيزة، ص.ب. ١٢٦١٨، مصر. قسم بحوث وتشخيص أمراض الدواجن، معهد بحوث صحة الحيوان، مركز بحوث الزراعة، الجيزة ص.ب. ١٢٦١٨، مصر. تقسم بحوث وتشخيص أمراض الدواجن، معهد البحوث البيطرية، المركز القومي للبحوث، ص.ب. ١٢٦٢٢، مصر. قسم الطفيليات وأمراض الحيوان، معهد البحوث البيطرية، المركز القومي للبحوث، ص.ب. ١٢٦٢٢، مصر. قسم أمراض الدواجن، معهد بحوث صحة الحيوان، معمل المنصورة، مركز البحوث الزراعية، ١٢٦١٨، مصر. آقسم أمراض الدواجن، معهد بحوث صحة الحيوان، فرع بنها، مركز بحوث الزراعة، ١٢٦١٨، مصر. ٧ قسم البيوتكنولوجي، معهد بحوث صحة الحيوان، مركز بحوث الزراعة، الجيزة. ص.ب. ١٢٦١٨، مصر.

Email: alaa.samir87@yahoo.com Assiut University web-site: www.aun.edu.eg

لا يزال داء الجراب المعدي (IBD) يُشكل تهديدًا كبيرًا الصحة الدواجن وإنتاجيتها، لا سيما في المناطق ذات الإنتاج المرتفع للدواجن مثل مصر. قيّمت هذه الدراسة التأثيرات المُعدّلة للمناعة لمُكمّلات سبيرولينا بلاتنسيس (SP) في دجاج التسمين المُلقّح بلقاح IBDV متوسط الفعالية ومُقاوم لفيروس IBDV شديد الضراوة. قُسّمت الطيور إلى أربع مجموعة المُلقّحة الضبط السلبية (G1) ، مجموعة المُلقّحة فقط (G2)، مجموعة المُلقّحة بجرعة ٢٪ من SP (G3) هومهوعة المُلقّحة بجرعة ٣٪ من SP (G3) هومهوعة المُلقّحة بجرعة ٣٪ من SP (G3) هومهوعة المُلقّحة بعرعة ٣٪ من الله المناعية، وخفّضت الأعراض السريرية، وخفّفت من آثار IBDV . أظهرت الطيور المُلقّحة بجرعة PS مستويات مرتفعة من إنترفيرون-جاما والسيتوكينات المُحفّرة للالتهابات، وحسّنت عيارات الأجسام المُضادة، وحسّنت وظائف المناعة الخلوية، بما في ذلك زيادة والسيتوكينات المُحفّرة للالتهابات، وحسّنت عيارات الأجسام المُضادة، وحسّنت وظائف المناعة الخلوية، بما في ذلك زيادة التاكسدي، مع تحسين كبير في القدرة الكلية لمضادات الأكسدة. وكشفت تقنية تفاعل البوليميراز المتسلسل الكمي - PCR التائج النسيجية المرضية هذه النتائج، حيث أظهرت المجموعتان G3 و G4 تلقًا أقل حدة في الجراب مقارنة بالمجموعة التي تلقت اللقاح فقط. وتشير هذه النتائج إلى أن سبيرولينا بلاتنسيس في علائق التهارية. ويُولى من انتشار الفيروس في دجاج التسمين. قد يُقدم دمج سبيرولينا بلاتنسيس في علائق اللقاح، ويعزز الحماية المناعية، ويُقلل من انتشار الفيروس في دجاج التسمين. قد يُقدم دمج سبيرولينا بلاتنسيس في علائق الدواجن استراتيجية واعدة للسيطرة على داء الأمعاء الالتهابي في العمليات التجارية. ويُوصى بإجراء المزيد من الدراسات الدواجن استراتيجية واعدة السيطرة على داء الأمعاء الالتهابي في العمليات التجارية. ويُوسى وفوائدها طويلة المدى.

الكلمات الدالة: مرض الجراب المعدى، سبير ولينا بالتينسيس، تعديل المناعة، علم الأمراض النسيجي.