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Impact of dietary *Spirulina (arthrospira) platensis* on growth performance, gene expression and antioxidant status of quail challenged with *Salmonella enteritidis*

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ABSTRACT

Objective: To investigate the effect of dietary *Spirulina platensis* supplementation on growth performance, organ colonization, expression of inflammatory-related genes, and antioxidant status in quail challenged with *Salmonella enteritidis.*

Design*:* Randomized controlled experimental study.

Animals: hundred eighty-seven-day-old Japanese quail.

Procedures: birds were randomly allocated into 6 equal groups. G1, G2, and G3: non challenged and fed abasal diet supplemented with *Spirulina* at 0, 1, and 2 g/kg diet, respectively. G4, G5, and G6: challenged and fed a basal diet supplemented with *Spirulina* at 0, 1, and 2 g/kg diet, respectively. At 21 days of age, all challenged groups were orally inoculated with 1ml of ($1.00x10^7$ *Salmonella enteritidis*/ml). The collected samples were serum for determining biochemical and antioxidant parameters, cecal tissue samples for determination of gene expression of inflammatory-related genes, and tissue samples from liver, heart, spleen, and caecum for bacterial reisolation.

Results*:* The dietary supplementation of *Spirulina* significantly improved growth performance parameters and reduced organ colonization. The cecal pro-inflammatory gene expressions (IL-6, IL1 ß and TNFα) were significantly downregulated while the anti-inflammatory cytokine (IL-10) was significantly increased. In addition, gene expression of cecal serum amyloid (SAA) was significantly down-regulated. The antioxidant and serum biochemical parameters were improved.

Conclusion and clinical relevance: dietary supplementation of *Spirulina* could be a helpful strategy for mitigating the harmful effects of *Salmonella enteritidis* in quail.

Keywords: *Salmonella enteritidis***,** quails, *Spirulina platensis***,** Growth performance, Cytokines**.**

1. INTRODUCTION

Foodborne illnesses in human caused by *Salmonella* represent a serious public health concern worldwide. *Salmonella enterica* serovar Enteritidis (*S.enteritidis*) was the most common serotype associated with *Salmonella* outbreaks in human and animals associated with consuming contaminated eggs and meat [1]. Furthermore, *Salmonellosis* causes significant economic losses in poultry production [2]. Therefore, control of *Salmonella* in poultry production is an issue of considerable concern. Different serotypes of *Salmonella* have been isolated from quails, including *S. Typhimurium, S. Enteritidis, and S. gallinarum* that could cause considerable losses in quails [3].

During the last several decades, antibiotics have been used in poultry production to prevent, control and treat diseases. Furthermore, subtherapeutic antibiotics are routinely added to chicken feed to promote growth performance [4]. With growing concerns about drug residues in meat products and the development of microbial resistance, modern countries have restricted or even banned the use of antibiotics in poultry flocks [5]. Consequently, researchers' rising interest to seek out natural products be suitable as an alternative to antibiotics.

Arthrospira platensis (*A. platensis*), known as *Spirulina* (blue-green alga), is one of the candidates expected to replace antibiotics. American food and drug administration and European food safety authority consider *Spirulina platensis* as generally recognized as safe (GRAS) [6]. *Spirulina (Arthrospira) platensis* is a filamentous spiral-shaped blue-green microalga that belongs to the class of Cyanophyta/Cyanobacteria. *Spirulina* is generally regarded as a promising nutrient source due to its high nutritional content of protein, vitamins, minerals, fatty acids, essential amino acids, and various photosynthetic pigments [7 and 8]. Previous studies suggested that bioactive constituents of *S. platensis*, like sulphated polysaccharides phycocyanin, γ-linolenic acid, ß-carotene, and phenolic compounds, give this type of microalgae its powerful antioxidant, antimicrobial, immunostimulant activities as well as resistance against diseases [9 and10].

Many researchers reported that the dietary supplementation of *Spirulina* in the quail diet improves body weight gain, feed conversion ratio, immune response, and antioxidant status [11 and 12]. However, limited information is available for the effect of *Spirulina platensis* as a dietary supplement on quail under infection. Therefore, this study was planned to assess the effects of *Spirulina* supplementation on growth performance, *Salmonella* colonization, inflammatory gene expression, and antioxidant status of quail experimentally challenged with *Salmonella*.

2. MATERIALS AND METHODS

2.1. Salmonella enteritidis infection

A serotype of *Salmonella enteritidis* resistance to novobiocin-nalidixic acid (NO 25 ug/ml, /NA 20 ug/ml) was obtained from, Animal Health Research Institute, Dokki, Giza, Egypt. Challenge inoculum was diluted and adjusted to $1.00x10^7$, and each bird was inoculated 1 ml into the lumen of the crop by oral gavages at 21 days of age [13].

2.2. *Spirulina platensis*

The blue-green alga was obtained from Algal Biotechnology Unit National Research Centre, Dokki, Giza, Egypt.

2.3. Experimental quail

A total of 180 seven-day-old Japanese quail were used in this experiment. Quail chicks were randomly assigned (30 birds/group in two replicates) to one of the following treatment groups: G1: non-challenged and fed a basal diet without any additives (negative control); G2: non-challenged and fed a basal diet supplemented with *Spirulina platensis* (1 g/kg diet); G3: Non-challenged and fed a basal diet supplemented with *Spirulina platensis* (2 g/kg diet); G4: Challenged and fed a a basal diet without any additives (positive control); G5: Challenged and fed a basal diet supplemented with *Spirulina platensis* (1 g/kg diet) and G6: Challenged and fed a basal diet supplemented with *Spirulina platensis* (2 g/kg diet).

Dietary supplementation of *Spirulina* was applied from seven days of age till the end of the experiment. Quails were raised under optimum environmental temperature and supplied with suitably formulated ration and kept in rooms under completely hygienic conditions in separate caged batteries.

2.4.Clinical investigation and mortality rate

Birds were observed daily from the start of the challenge to the end of the experiment for clinical signs and mortalities. Dead birds were submitted to necropsy for gross evaluation.

2.5.Growth Performance

Bodyweight (BW), body weight gain (BWG), and feed intake (FI) per cage was recorded weekly. The feed conversion ratio (FCR) was calculated based on feed intake divided by body weight gain (BWG).

2.5.1. *Salmonella recovery and counting*

On days 7, 14, and 21 days post-SE challenge, four birds from each group (2/replicate) were picked up randomly and sacrificed. Swabs from the liver, spleen, and heart were suspended in tetrathionate broth for enriched at 37 °C for 24 hours after enrichment, the homogenate (broth) was streaked on XLD plates containing novobiocin-nalidixic acid (NO 25 ug/ml, /NA 20 ug/ml) and incubated for 24 hours at 37 °C and examined for typical SE colonies. Morphological, biochemical, and serological identification of suspected colonies was performed as reported by [14]. The cecum from each chicken was removed aseptically. The contents were homogenized and diluted with sterile PBS (10% *w*/*v*), and the spread was plated onto XLD plates to enumerate *Salmonella enteritidis*, according to Fukata et al. [15].

2.5.2. Serum Biochemistry

Blood samples were obtained from the brachial vein of four birds/groups at 7 and 21day post-challenge. These samples were centrifuged at 3500 rpm for 15 min to obtain serum and stored at–20 °Ċ. Commercial diagnostic kits used to determine serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid, and creatinine were obtained from Bio-Med. Company Egypt. Level of Malondialdehyde (MDA), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were estimated using commercial diagnostic kits obtained from Bio diagnostic Co. (Egypt. Giza).

2.5.3. Cecal tissue sample collection

Four birds were randomly selected and sacrificed from each group for cecal tissue sample collection 3 days post-challenge. Samples from cecum tissue were sterile collected, washed in phosphate buffer saline (PBS), snap-frozen in liquid nitrogen, and stored at−80 °C for quantification of gene expression.

2.6. Gene mRNA Expression Assay

2.6.1. RNA extraction

RNA extraction from tissue samples was applied using QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH) when 200 µl of the sample were added to 600 µl RLT buffer containing 10 µl β-mercaptoethanol per 1 ml, incubated at room temperature for 10 min. One volume of 70% ethanol was added to the cleared lysate, and the steps were completed according to the Purification of Total RNA protocol of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH).

N. B: On column DNase, digestion was done to remove residual DNA.

2.6.2. Oligonucleotide Primers

Primers used were supplied from Metabion (Germany) are listed in the table (1).

2.6.3. SYBR green rt-PCR

Primers were utilized in a 25- µl reaction containing 10 µl of the 2x *HERA* SYBR® Green RT-qPCR Master Mix (Willowfort, UK), 1 µl of RT Enzyme Mix (20X), 0.5 µl of each

primer of 20 pmol concentration, 5 µl of water, and 3 µl of RNA template. The reaction was performed in a step one real-time PCR machine.

2.6.4. *Analysis of the SYBR green rt-PCR results*

Amplification curves and ct values were determined by the step one software. The relative expression of the gene in each sample versus a control compared to *B- actin* gene and calculated according to the $2^{-\Delta\Delta Ct}$ method Yuan et al. [16].

2.7. Statistical Analysis:

Data were analyzed using repeated-measures ANOVA, then one-way ANOVA at each time point with a post hoc Duncan multiple comparison test ($p< 0.05$).

Table1. Primers sequences, target genes, amplicon sizes and cycling conditions for SYBR green rt-PCR**.**

Target gene	Primers sequences	Reverse transcription	Primary denaturation	Amplification (40 cycles)			Reference
				Secondary denaturation	Annealing (Optics on)	Extension	
B- actin	CAACACAGTGCTGTCTGGTGG ATCGTACTCCTGCTTGCTGAT	50˚C 30 min.	94˚C 15 min.	94˚C 15 sec.	55˚C 30 sec.	72˚C 30 sec.	[17]
IL-1*β*	TGCTGGTTTCCATCTCGTATGTA CCCAGAGCGGCTATTCCA						[18]
IL-6	CCCGCTTCTGACTGTGTTT GCCGGTTTTGAAGTTAATCTTT						[18]
TNF-*α*	CCCCTACCCTGTCCCACAA ACTGCGGAGGGTTCATTCC						[18]
Serum amyloid (SSA)	TGCTTCGTGTTGCTCTCCAT CATGTCCCGGTATGCTCTCC						[19]

Table 3. Effect of *Spirulina platensis* on growth performance parameters in quails experimentally infected with *Salmonella enteritidis*

Parameter	G1	G2	G3	G4	G5	G6
1-3 week						
Initial weight	29.75±0.47^{a}	29±0.40^{a}	29.75±0.75^{a}	30.5±0.64^{a}	30±0.91^{a}	29.25±0.75^{a}
Final weight	106.25±0.85^{c}	117.75±0.62^{b}	123±0.70^{a}	103.75±0.47^{c}	119.25±0.478^{b}	121.75±0.853^{a}
Body weight gain	76.5±1.04^{c}	88.75±0.62^{b}	93.25±0.47^{a}	73.25±0.47^{c}	89.25±1.10^{b}	92.50±0.95^{a}
Feed intake	195	210	220	190	214	218
Feed conversion ratio	2.55±0.014^{a}	2.37±0.01^{b}	2.35±0.01^{a}	2.60 ±0.01^{a}	2.39±0.01^{b}	2.36±0.02^{a}
3-6 week						
Body weight gain	86.25±0.62^{c}	92.2 ±0.64^{b}	98±0.57^{a}	68.75±1.5^{f}	75.5±0.95^{e}	80±1.22^{d}
Feed intake	430	440	465	370	396	416
Feed conversion ratio	4.98±0.01^{b}	4.77 ±0.02^{c}	4.74±0.01^{c}	5.38 ±0.02^{a}	5.24 ±0.20^{b}	5.2 ±0. 070^{b}
1-6 week						
Final weight	192.5±1.45^{d}	210±1.52^{b}	221±2.02^{a}	172.5±1.55^{e}	194.75±1.73^{d}	201.5±1.15^{c}
Body weight gain	162.75±1.45^{d}	181±1.15^{b}	191.25±1.5^{a}	142± 0.76^{e}	164.75±2.08^{d}	172.25 ±1.18^{c}
Feed intake	625	650	685	560	610	634
Feed conversion ratio	3.84 ±0.02^{c}	3.59±0.01^{d}	3.58±0.02^{d}	3.94 ±0.00^{a}	3.70 ±0.01^{b}	3.68±0.01^{b}

SEM: Standard error of mean (n=4). Means within same row carrying different superscripts are significantly differ at (P<0.05).

3. RESULTS

3.1. Clinical signs and mortalities

No clinical signs or mortalities were observed in the non-challenged groups. While in the challenged groups (G4, G5, and G6), the clinical signs started 3rd-day post-challenge and were depression, ruffled feather, decreased feed intake, huddle together, and white diarrhea. Birds in challenged treated groups (G5 and G6) had milder clinical signs than the positive control (G4). The mortalities started on the 5th-day post-challenge. The mortality rate was reduced from 23.33% in the control positive group (G4) to 10% in both challenged treated groups (G5 and G6) (Table 2).

3.2. Gross lesions

Dead and sacrificed birds in challenged groups had severe congestion in the liver and spleen, enteritis, and nephritis. The severity of lesions in challenged treated

groups (G5 and G6) was less pronounced than positive control group(G4). Post mortem lesions were minimized by the 2nd week post-infection.

Table 4. Rate of reisolation of *salmonella enteritidis* from internal organs of experimentally infected quail and supplemented with *Siprulina platensis*.

Organs	DPI	Groups					
		G1	G2	G3	G4	G5	G6
Liver	7	0/4	0/4	0/4	4/4	3/4	3/4
	14	0/4	0/4	0/4	3/4	2/4	2/4
	21	0/4	0/4	0/4	1/4	0/4	0/4
	7-21	0/12(0%)	0/12(0%)	0/12(0%)	8/12(66.7%)	5/12(41.7%)	5/12(41.7%)
Spleen	7	0/4	0/4	0/4	2/4	2/4	2/4
	14	0/4	0/4	0/4	2/4	1/4	1/4
	21	0/4	0/4	0/4	1/4	0/4	0/4
	7-21	0/12(0%)	0/12(0%)	0/12(0%)	5/12(41.7%)	3/12(25%)	2/12(16.7%)
Heart	7	0/4	0/4	0/6	2/4	2/4	1/4
	14	0/4	0/4	0/6	2/4	0/4	0/4
	21	0/4	0/4	0/4	0/4	0/4	0/4
	7-21	0/12(0%)	0/12(0%)	0/12(0%)	4/12(33.3%)	2/12(16.7%)	1/12(16.7%)
Total recovery rate*		0/36 (0%)^{c}	0/36 (0%)^{c}	0/36 (0%)^{c}	17/36 (47.22%)^{a}	10/36 (27.78%)^{b}	9/36 (25%)^{b}

DPI: day post challenge *Total recovery rate: total positive samples /total examined samples.
Means within same row carrying different superscripts are significantly differ at (P<0.05).

Table 6. Effects of dietary supplementation of *Spirulina platensis* on serum parameters.

	ALT (U/ L)	AST (U/ l)	Creatinine (mg/dl)	Uric acid (mg/dl)	Total p (g/dl)	Albumin (g/dl)	Globulin (g/dl)
1WPI							
G1	20.10±0.93^{C}	122.21±0.94^{c}	0.34±0.01^{c}	2.13 ±0.01^{c}	4.90 ±0.01^{c}	3. 29±0.01^{b}	1.61 ±0.05^{c}
G2	20.56±0.88^{c}	124.99±1.55^{c}	0.33±0.01^{c}	2.09 ±0.03^{c}	4.97±0.01^{b}	3.28±0.01^{a}	1.69 ±0.02^{b}
G3	20.21±0.59^{c}	120.70±0.84^{c}	0.32±0.01^{c}	2.06±0.01^{c}	5. 04 ±0.02^{a}	3.29 ±0.03^{a}	1.75 ±0.03^{a}
G4	40.81±0.86^{a}	155.35±1.58^{a}	0.94±0.03^{a}	4.78±0.14^{a}	4.73 ±0.01^{f}	3.2 3±0.02^{c}	1.50 ±0.05^{e}
G5	32.14±1.89^{b}	135.51±0.73^{b}	0.53±0.01^{b}	3.50±0.13^{b}	4.79 ±0.06^{e}	3.25 ±0.06^{b}	1.5 5 ±0.02^{d}
G6	30.15±1.98^{b}	132.32±0.58^{b}	0.50 ±0.01^{b}	3.5 2±0.12^{b}	4.8 7±0.01^{d}	3.28 0.01^{b}	1.5 9±0.05^{c}
3WPI							
G1	21.40±1.08^{c}	121.08±0.85^{c}	0.35±0.01^{c}	2.15±0.05^{c}	5.19 ±0.01^{c}	3.3 3±0.06^{a}	1.8 6±0.02^{cb}
G2	22.67±0.88^{c}	117.54±2.53^{c}	0.38±0.13^{c}	2.17±0.02^{c}	5.26 ±0.01^{b}	3.3 6 ±0.02^{a}	1.9 0±0.02^{b}
G3	20. 81±0.91^{c}	119.11±1.81^{c}	0.36±0.01^{c}	2.18±0.02^{c}	5.30 ±0.06^{a}	3.35 ±0.01^{b}	1.9 5 ±0.01^{a}
G4	36.47±1.86^{a}	140.54±5.46^{a}	0.78±0.13^{a}	3.45±0.20^{a}	4.90 ±0.01^{e}	3.3 0±0.06^{c}	1.6 6±0.04^{d}
G5	25.72±0.29^{b}	130.31±1.14^{b}	0.43±0.01^{b}	2.33±0.09^{b}	5.08 ±0.04^{d}	3.3 0±0.04^{b}	1.78 ±0.01^{c}
G6	27.73±1.06^{b}	128.97±0.81^{b}	0.46±0.01^{b}	2. 31±0.04^{b}	5.16 ±0.03^{c}	3.3 3±0.03^{b}	1.8 3±0.02^{b}

Means within same column carrying different superscripts are significantly differ at (P<0.05).
WPI= weeks post infection

Table7. Effect of *Spirulina platensis* on antioxidant enzymes of quail experimentally infected with *salmonella enteritidis*.

GROUP	1WPI			3WPI		
	SOD (u/ L)	MDA (nmol/ml)	GPx (u/ L)	SOD (u/ L)	MDA (nmol/ml)	GPx (u/ L)
G1	13.5 2±0.16^{c}	33.06±0.40^{c}	1.89±0.02^{c}	13.16±0.17^{c}	31.38±0.53^{b}	1.90±0.04^{c}
G2	15.41±0.12^{b}	29.74±1.28^{d}	2.8±0.08^{b}	16.81±0.74^{b}	26.96±0.33^{d}	2.89±0.06^{b}
G3	17.50±0.18^{a}	27.63±0.82^{e}	3.43±0.06^{a}	18.37±0.66^{a}	24.73 ±0.63^{e}	3.61±0.11^{a}
G4	9.46±0.12^{e}	52.35±0.59^{a}	1.02±0.01^{e}	10.88±0.30^{e}	42.23±0.88^{a}	0.67±0.01^{e}
G5	11.70±0.13^{d}	42.67 ±1.55^{b}	1.22 ±0.03^{d}	12.44±0.24^{d}	34.23±0.88^{b}	1.24±0.01^{d}
G6	13.47±0.15^{c}	41.8 7 ±0.11^{b}	1.89±0.02^{c}	13.11±0.25^{c}	33.74±0.89^{b}	1.92±0.05^{c}

Means within same column carrying different superscripts are significantly differ at (P<0.05).

Table2. Mortality rate of quail experimentally infected by *Salmonella enteritidis* and supplemented with *spirulina platensis*.

Group	Total No of birds	No. of dead birds/WPI			Total No. of dead birds	Mortality rate
		1st	2nd	3rd		
G1	30	0	0	0	0	0
G2	30	0	0	0	0	0
G3	30	0	0	0	0	0
G4	30	4	2	1	7	23.33
5G	30	2	1	0	3	10
G6	30	2	1	0	3	10

WPI: week post infection

Table5. Cecal colonization of *Salmonella enteritidis* (log10 CFU).

Group	7DPI	14DPI	21DPI
G1	0	0	0
G2	0.	0	0
G3	0	0	0
G4	4.94±0.014^{a}	3.84±0.012^{a}	2.60±0.015^{a}
G5	4.93±0.003^{a}	3.80±002^{ab}	2.58±0.012^{ab}
G6	4.90±0.016^{a}	3.77±0.009^{b}	2.55±0.0.014^{b}

DPI: Days post challenge.
Means within same column carrying different superscripts are significantly differ at (P<0.05).

3.3. Growth performance

Data summarized in table (3) indicated that dietary *Spirulina* supplementation significantly increased ($p < 0.05$) the final body weights, body weight gain, and decreased ($p < 0.05$) feed conversion ratio in non-challenged treated groups (G2 and G3) as compared to control negative group (G1) and in challenged treated groups (G5 and G6) as compared to control positive group (G4). In addition, there was a significant increase ($p < 0.05$) in body weight and body weight gain with increasing dietary levels of *Spirulina* (from 1-2 g/kg).

3.4. *Recovery of Salmonella enteritidis from organs:*

Our results showed that the control positive group (G4) had the highest rate of reisolation from liver, spleen, and heart (45.8%), while in challenged treated groups (G5 and G6) was reduced to (27.1%) and (25%) respectively (Table 4).

3.5. *Salmonella enteritidis colonization in cecum*

No, salmonella colonies were recovered from the caecum of the non-challenged groups throughout the time of the experiment. On day 7 post-challenge, there was no significant difference ($p > 0.05$) in cecal *Salmonella enteritidis* count between challenged treated groups (G5 and G6) and the positive control group (G4). While at 14 and 21 dpi, there was a significant decrease ($p < 0.05$) in *Salmonella* count in the challenged group treated with 2 g/kg *Spirulina* (G6) as compared to the positive control (G4) and the another challenged treated group (G5). However, there was no significant difference ($P > 0.05$) in the cecal *Salmonella* colonization among challenged treated groups (Table 5).

3.6. Relative expression of cecal genes

Salmonella challenge induced significant ($p < 0.05$) up-regulation of pro-inflammatory cytokines IL-1 ß, IL-6, and TNF-α and a significant ($p < 0.05$) downregulation of anti-inflammatory cytokine IL-10 in the control positive group (G4) compared to the negative control group (G1). Dietary*Spirulina* supplementation was significantly ($p < 0.05$) down-regulated expression of IL-1 ß, IL-6, and TNF-α in (G5 and G6) and significantly increased ($p < 0.05$) IL-10 gene expression at 3rd-day post-challenge as compared to control positive (G4). In the non-challenged treated groups (G2 and G3), the gene expression of pro-inflammatory and anti-inflammatory cytokines did not show significant differences ($P > 0.05$) in comparison with the control negative (G1) (Fig. 1-4). In addition, our results revealed that SE challenge significantly ($p < 0.05$) upregulated expression of serum amyloid A (SAA) in the cecum, and dietary supplementation of *Spirulina* suppresses this expression at 3-day post challenge as compared to the control positive group (G4) (Fig.5).

3.7. *Results of biochemical parameters*

Our results showed that there was a significant increase ($p < 0.05$) in total protein and globulin levels in non-challenged treated groups (G2 and G3) as compared to the negative control group (G1). On the other hand, *Salmonella* infection induced a significant decrease ($p < 0.05$) in total protein and globulin level in the control positive group (G4) as compared to the negative control group (G1). Dietary *Spirulina* supplementation results in a significant increase in total protein and globulin levels in challenged treated groups (G5 and G6) compared to the control positive (G4).

There were no significant differences ($p > 0.05$) in ALT, AST, creatinine, and uric acid concentration in non-challenged treated groups (G2 and G3) compared to the negative control group (G1). *Salmonella* infection was significantly increased ($P < 0.05$) level of these parameters in challenged groups compared to non-challenged groups. Dietary *Spirulina* supplementation results in a significant reduction ($P < 0.05$) in the level of these parameters in challenged treated groups (G5 and G6) compared to the positive control group (G4). There was no significant difference ($p > 0.05$) with increase dietary level of *Spirulina* inclusion in all serum biochemical parameters in challenged treated groups (Table 6).

3.8. *Serum antioxidant status*

Dietary *Spirulina* supplementation significantly decreased the level of MDA values and increased activity of SOD and GP_X in both non challenged treated groups (G2 and G3) and in challenged treated groups (G5 and G6) as compared to the control negative group (G1) and control positive group (G4) respectively (Table 7). There was a significant increase ($p < 0.05$) in the level of SOD and GP_X

with an increased dietary level of spirulina inclusion from (1-2 g).

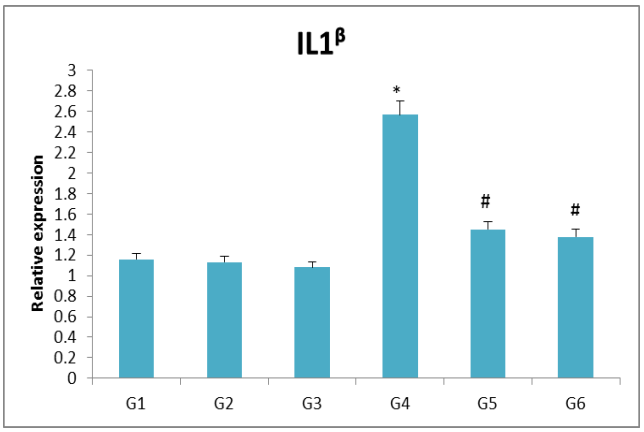

Figure 1. Gene expression of IL-1 *β* in the cecum of quail at 3day day post infection. The results are expressed as means ± SEM. *P<0.05 as compared with control negative group, #P<0.05 as compared with control positive group.

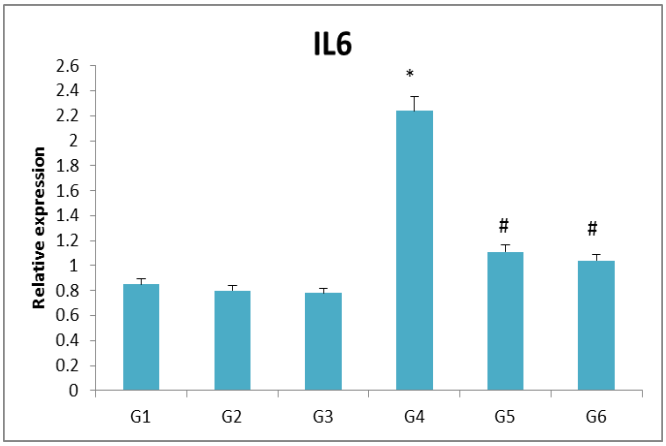

Figure 2. Gene expression of IL-6 in the cecum of quail at 3day day post infection. The results are expressed as means ± SEM. *P<0.05 as compared with control negative group, #P<0.05 as compared with control positive group.

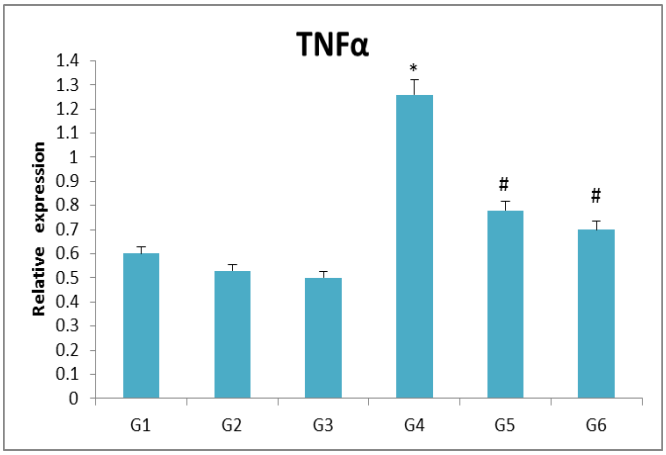

Figure 3. Gene expression of TNFα in the cecum of quail at 3day day post infection.. The results are expressed as means ± SEM. *P<0.05 as compared with control negative group, #P<0.05 as compared with control positive group.

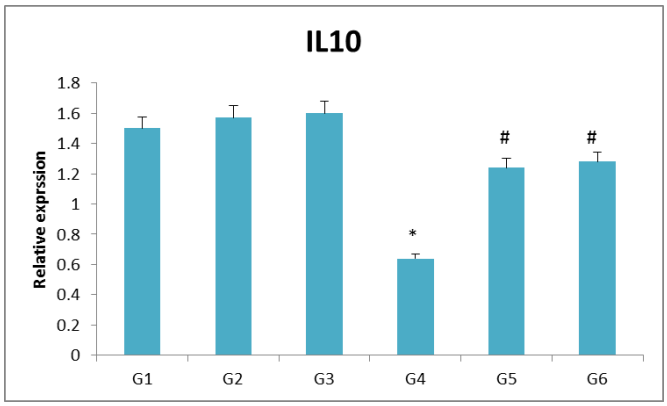

Figure 4. Gene expression of IL10 in the cecum of quail at 3day day post infection. The results are expressed as means ± SEM. *P<0.05 as compared with control negative group, #P<0.05 as compared with control positive group.

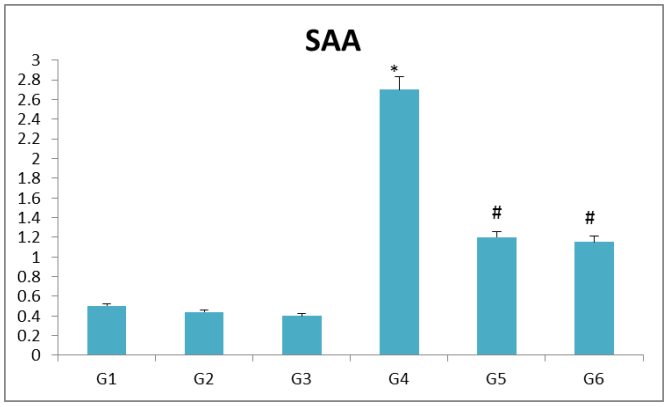

Figure 5. Gene expression of serum amyloid A (SAA) in the cecum of quail at 3day post-infection. The results are expressed as means ± SEM. * P < 0.05; compared with the control negative group, # P < 0.05; compared with the positive control group.

4. DISCUSSION

No clinical signs or gross pathological lesions were observed in non-challenged groups (G1, G2, and G3). Birds in challenged treated groups (G5 and G6) had less severe clinical signs and gross pathological lesions than the control positive group (G4). Also, challenged treated groups (G5 and G6) had a lower mortality rate (10% for both) as compared to the control positive group (G4). The improvement of the general health condition of birds could be attributed to the ability of phycocyanin and β carotene content of *Spirulina* to manage the inflammatory conditions and oxidative damages [20].

Regarding bird's performance, dietary supplementation of quails with different levels of *Spirulina* (1 and 2 gm/kg) displayed higher (P < 0.05) body weight (BW), body weight gain (BWG), and better FCR values in non-challenged treated groups (G2 and G3) and challenged treated groups throughout the entire experimental period versus those of the control groups (G1 and G4) respectively. In harmony with our findings, several studies clarified that dietary incorporation of *S. platensis* in non-challenged quails had significant beneficial effects on growth performance parameters (BWG and FCR) [12,21 and 22]. On the contrary, Dogan et al. [23] and Hajati et al. [24] reported no significant

difference in FCR in quails fed with different levels of *Spirulina* as compared to control. On the other hand, feed intake (FI) in groups supplemented with *Spirulina* was higher than that of the control group. Similar findings were reported by [22 and 25]. On the contrary, Yusuf et al. [21] reported that cumulative FI in *Spirulina* supplemented quail was lesser than that of the control group. There was a significant difference ($p < 0.05$) in (BW and BWG) and a non-significant difference in FCR ($p > 0.05$) with the increased level of inclusion of *Spirulina* from (1-2 g) in non-challenged treated groups and challenged treated groups. Similar finding reported by Fathi [26]. While**,** Park et al.[27] illustrated that BW, BWG and FCR significantly ($P < 0.05$) improved with increased level of *Spirulina platensis* inclusion in broiler diets.

The exact mechanism- by which *Spirulina* alleviates the negative effects of *Salmonella* infection on the performance of quail is unclear, and related studies on dietary *Spirulina* in birds under challenge are lacking. The improvement in growth performance may be a subsequence of its high nutrient composition, especially vitamins, minerals, essential fatty acids, amino acids, and other nutrients that positively affect metabolism systems related to growth performance [28]**.** Furthermore, *Spirulina* improves nutrient digestibility, minerals absorption and consequently increases the feed utilization efficiency.

Despite many previous studies documented in vitro antibacterial effect of *Spirulina* on many pathogenic bacteria such as *Salmonella typhi* and *Salmonella para typhi* [29 and 30]. We could not find any in vivo studies that address the antimicrobial activities of *Spirulina* against SE. In the present study, the recovery rate of SE from internal organs of challenged treated groups (G5 and G6) was reduced to (27 and 25%) as compared to the control positive group (45.8%). Regarding results of cecal SE count, our results revealed that dietary *Spirulina* significantly reduced SE count in the challenged group treated with 2 g/kg (G6) at 14 and 21dpi. There was no significant reduction in SE count in the challenged group treated with 1 g/kg (G5) compared to the infected treated group (G4)**.** Shanmugapriya et al. [31] and Alwaleed et al. [32] reported that supplementation of *Spirulina platensis* to diets might have a negative effect on *E. coli* count in the ileal and caecal digesta of broiler. Also, Yusuf et al. [21] reported that supplementation of SP in the diet at a dose of 2 g/kg lead to a significant reduction in both total bacterial and coliform counts. Hajati et al. [24] reported that different SP levels decreased *E. Coli* count in ileum content of the laying quails suffering heat stress condition. The antimicrobial activities of *Spirulina* have been attributed to the high content of tocopherols, C-phycocyanin, extracellular polysaccharides, γ-linolenic acid, active fatty acid lauric, and palmitoleic acid [33].

Resistance to *Salmonella enteritidis* is associated with the upregulation of pro-inflammatory cytokines and chemokines[34]. Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α regulate the immune response by inducing differentiation and proliferation of leukocytes to eliminate pathogens [35] and serve as significant factors for the initiation of the acute phase response (APR) [36]. IL-10, a critical anti-inflammatory cytokine, acts as an inflammation feedback factor to modulate the immune response [37]. *Spirulina* treatment had no significant effect on expression levels of IL-1β, IL-6, TNF-α and IL-10 in the uninfected quail in the present study. In contrast, the *Salmonella* challenge induced an inflammatory immune response that was characterized by significant ($P < 0.05$) upregulation in IL-1*β*, IL-6, and TNF*α* and significant downregulation of IL-10. Dietary supplementation of *Spirulina* in SE-challenged groups significantly suppressed ($P < 0.05$) the upregulation of inflammatory-related cytokine genes IL-1β, IL-6, TNF-α, and promoted the gene expressions of anti-inflammatory cytokines IL-10. Modulation of gene expression in challenged treated groups indicated the protective effects and anti-inflammatory properties of *Spirulina*. C-phycocyanins and β-carotene contained in *Spirulina* can block NF-κB activity and thus suppresses TNF-α [38]**.** Moreover, β-carotene also inhibits the expression of pro-inflammatory cytokines such as IL-1β and IL-6 in stimulated macrophages by suppressing their transcription [39].

Immune response in birds challenged with very immunogenic *Salmonella serovars*, such as *S. Enteritidis*, increase metabolism with a consequent increase in cell and protein production for acute-phase proteins [40]. The concentration of these proteins is related to the extent of infection and useful for monitoring poultry health [41]. Our results revealed that SE challenge significantly ($p < 0.05$) upregulated expression of serum amyloid A (SAA) in the cecum, and dietary supplementation of *Spirulina* suppresses this expression at 3-day PI as compared to the challenged treated group (G4). Similarly, Singh et al*.* [42] reported that expression of the SAA gene in quails challenged with *Salmonella* was significantly upregulated than control groups.

Serum total protein levels are normally used as humoral immunity indicators [43]**.** In the present study, there was a significant increase ($p < 0.05$) in total serum protein and globulin level in non-challenged treated groups (G3 and G4) as compared to the negative control (G1). In contrast, the *Salmonella* challenge induces a significant decrease in total protein and globulin level in the challenged non-treated group (G4) compared to the negative control group (G1). *Spirulina* treatment significantly increased the total protein and globulin level in challenged treated groups compared to the control group (G4). These results reflected that *Spirulina* had enhanced immunity and improved health. The elevation of globulin is attributed to the rich content of *Spirulina* with functional compounds, such as C-phycocyanins, β-carotene, minerals, and vitamins, which have an immunomodulatory role [44].

There was no significant change in ALT, AST, creatinine, and uric acid concentration in non-challenged treated groups (G2 and G3) compared to the control group (G1), indicating that *Spirulina* did not compromise the normal function of the liver or kidney. Consistent with another

study, feeding of *S. platensis* had no significant impact on AST, ALT, and uric acid levels in quail [45]. In contrast, there was a significant increase ($P < 0.05$) in ALT, AST, creatinine, and uric acid concentration after infection in all the infected groups compared to the negative control (G1). The pre-administration of *Spirulina* (1 and 2 gm) diminished the increase in serum hepatic and renal biomarkers in challenged treated groups (G5 and G6) as compared to a positive control (G4). Previous studies illustrated the hepatoprotective and nephroprotective impacts of *Spirulina* [46 and 47]. β-carotene and C-phycocyanins of *Spirulina* could regenerate damaged hepatic cells and protect renal tissues from toxicity by scavenging the ring of free radicals [48].

Regarding the antioxidant activity, dietary *Spirulina* supplementation enhanced antioxidant status by reducing MDA values, and increasing activity of SOD of GPx in non-challenged treated groups (G2 and G3) and in challenged treated groups (G5&G6) as compared to control negative (G1) and control positive group (G4) respectively. Therefore, our results support that *Spirulina* can exert beneficial antioxidant effects under both normal and challenging conditions.

Similar findings were reported by Park et al. [27] stated that dietary *Spirulina* supplementation caused a significant increase ($p < 0.05$) in the serum enzyme activity of superoxide dismutase and glutathione peroxidase. Also, Hajati et al. [24] reported that dietary *Spirulina* supplementation decreases malondialdehyde level in quail under heat stress compared to control. The positive effect of *Spirulina* on antioxidant markers attributed to high antioxidants content such as *β*-carotene, tocopherol, selenium, polypeptide pigment, phycocyanin, or phenolic acids [50].

Conclusion

Dietary supplementation of *Spirulina* was able to mitigate the adverse effect of *Salmonella* challenge in quail. So, it can be supposed that quail would be profited with pretreatment by *Spirulina*. However, additional researches are needed to confirm this hypothesis, especially on the standardization of the *Spirulina* level and periods of supplementation.

Conflict of interest statement

The authors declare that there is no conflict of interest in the current research work.

Research ethics committee permission

The current research work is permitted to be executed according to the standards of the Animal Health Research Institute(AHRI),Agriculture Research center(ARC),P.O.Box 246 Dokki,12618-Giza,Egypt.

Authors' contribution

All authors contributed equally to this work, whereas they designed, conducted the experiment. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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