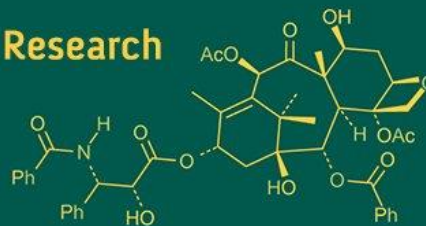


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Effect of *Spirulina* supplementation on immune and antioxidant parameters of commercial broilers

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Abstract

An experiment was conducted to evaluate the effect of dietary *Spirulina platensis* supplementation on performance and gut health of commercial broilers. A total of 300 day old chicks were randomly allocated to five dietary treatments with 12 replicates of five birds each. Birds were reared under uniform management conditions and fed corn-soybean meal-based isocaloric and isonitrogenous diets from 0 to 42 days of age. The treatments included a basal diet without additives (T₁, negative control), basal diet with lincomycin @ 100 g/MT (T₂, positive control), and basal diet supplemented with *Spirulina platensis* at 0.5% (T₃), 0.75% (T₄), and 1.0% (T₅). Feed and water were provided ad libitum. Supplementation of *Spirulina platensis* significantly ($p < 0.05$) improved the antioxidant enzyme activity of broilers. Superoxide dismutase and glutathione peroxidase levels were significantly higher, while lipid peroxidation levels were significantly lower in *Spirulina* supplemented groups compared to control and antibiotic groups, with the highest improvement observed in the 1.0% *Spirulina* group. There was no significant ($p > 0.05$) difference in the immune parameters among all the treatments. Based on the results, it was concluded that dietary supplementation of *Spirulina platensis*, particularly at 0.75% and 1.0% inclusion levels, can be used as a potential natural alternative to antibiotic growth promoters to improve overall performance of broilers.

Keywords: *Spirulina*, immune, antioxidant, performance

1. Introduction

Poultry nutrition is undergoing a significant transition, shaped by evolving production demands and increasing scrutiny of conventional feeding practices. Historically, synthetic antimicrobials and chemical feed additives were widely incorporated into intensive poultry diets to promote rapid growth, improve feed efficiency, and provide effective disease prevention. However, rising concerns regarding antimicrobial resistance, consumer safety, and sustainability have driven the search for safer and more natural nutritional alternatives.

In this context, growing research evidence supports the inclusion of naturally derived bioactive substances such as phytochemicals, essential oils, organic acids, probiotics, and microalgae in poultry feeding programs. These compounds exhibit multiple functional roles, contributing to enhanced growth performance, improved nutrient utilization, strengthened immune responses, increased antioxidant defense, and better intestinal health. Among these alternatives, microalgae have emerged as promising candidates for future-oriented poultry nutrition strategies (Rafiq *et al.*, 2022) ^[15].

One microalga of particular interest is *Arthrospira platensis*, commonly known as *Spirulina*, a filamentous cyanobacterium belonging to the genus *Arthrospira*, which also comprises *A. maxima* and *A. fusiformis*. *Spirulina* is recognized for its exceptionally high protein content, richness in essential vitamins, and abundance of bioactive pigments such as phycocyanin with strong antioxidant properties (Ogbuewu *et al.*, 2025) ^[11]. Due to these attributes, *Spirulina* has attracted considerable attention as a functional feed ingredient in poultry diets. Previous studies have demonstrated that its dietary inclusion in broilers can positively influence meat quality, nutritional composition, sensory characteristics, and the accumulation of health-promoting bioactive compounds (Toyomizu *et al.*, 2001) ^[16].

The functional value of *Spirulina* is further enhanced by its diverse phenolic profile, including salicylic, trans-cinnamic, sinapic, chlorogenic, quinic, and caffeic acids, which

collectively contribute to its pronounced antioxidant activity (Lassiter *et al.*, 2024) [8]. Additionally, its rapid growth rate and relatively uncomplicated cultivation process have facilitated its commercial-scale production in several regions across the globe.

Despite the extensive documentation of *Spirulina*'s nutritional richness and biofunctional properties, research focused on its optimal dietary inclusion and targeted effects in broiler chickens remains limited and fragmented. Hence, the present study was undertaken to evaluate the influence of *Spirulina* supplementation on growth performance and gut health in broiler chickens, with the aim of generating evidence to support its effective utilization under commercial poultry production conditions.

2. Materials and Methods

Three hundred (300) day old broiler chicks were randomly allocated to five dietary treatment groups with twelve (12) replicates of five (5) birds each and all the experimental groups were reared under unvarying uniform managemental conditions. The birds were vaccinated against RD with a live vaccine of La Sota on 7th and 28th day and against IBD on 14th and 21st day of the experiment. Corn and Soyabean meal based basal diet was formulated for birds to meet the nutrient requirements during pre-starter, starter and finisher phases. Treatment groups included were T₁ as control which is basal diet without any feed additive, T₂ with antibiotic (Lincomycin-Positive control) @ 100 g/MT diet, T₃ with (*Spirulina platensis*) @ 0.5%, T₄ with (*Spirulina platensis*) @ 0.75% and T₅ with (*Spirulina platensis*) @ 1.0%. Broiler pre-starter, starter and finisher diets were formulated to contain 22%, 20.5% and 19% crude protein and metabolizable energy was 3000, 3100 and 3200 kcal/kg respectively.

Table 1: Ingredients and nutrient composition of control diets (%) fed to the commercial broilers at different phases of growth.

Ingredient name	Pre starter (0-2 weeks)	Starter (3-4 weeks)	Finisher (5-6 weeks)
Maize	55.6487	58.381	61.7103
Vegetable oil	3.461	4.5663	5.4676
Soyabean meal	36.4079	32.8216	28.9622
Stone grit	1.2739	1.2777	1.2742
DCP	1.8926	1.7518	1.4354
Salt	0.421	0.4222	0.3981
DL-Methionine	0.2663	0.2402	0.2188
L-Lysine HCl	0.2189	0.1393	0.1334
L-Threonine	0.0097	0	0
Trace Mineral Mixture*	0.1	0.1	0.1
Vitamin Premix**	0.05	0.05	0.05
Choline chloride 60%	0.1	0.1	0.1
Toxin binder	0.1	0.1	0.1
Coccidiostat	0.05	0.05	0.05
Total	100	100	100
Nutrient composition (calculated values)			
ME (kcal/kg)	3000	3100	3200
Crude protein (%)	22	20.5	19
Dig Lysine (%)	1.25	1.1	1
Dig Methionine (%)	0.57	0.528	0.49
Calcium (%)	0.94	0.9	0.82
Available phosphorous (%)	0.45	0.42	0.36

*Trace minerals provided per kg diet: Manganese 120 mg, Zinc 80 mg, Iron 25 mg, Copper 10 mg, Iodine 1 mg and Selenium 0.1 mg.

**Vitamin premix provided per kg diet: Vitamin A 200000 IU, Vitamin D3 3000 IU, Vitamin E 10 mg, Vitamin K 2 mg, Riboflavin 25 mg, Vitamin B1 1 mg, Vitamin B6 2 mg, Vitamin B12 40 mg and Niacin 15 mg.

2.1 Immune Parameters

2.1.1 Cell mediated Immune response

Cell-mediated immune response was evaluated using the cutaneous basophilic hypersensitivity (CBH) reaction to phytohaemagglutinin-phosphate (PHA-P). On the 40th day of the experimental period, one bird was randomly selected from each replicate for the assessment. The thickness of the skin web between the third and fourth digits of both the left and right feet was measured using a micrometer prior to injection. The right foot web was inoculated intradermally with 100 µg of PHA-P dissolved in 0.1 ml of phosphate-buffered saline (PBS), whereas the left foot web served as the control and received 0.1 ml of PBS alone. After 24 hours, the thickness of both webs was again measured with a micrometer, and the CBH response was calculated using the standard formula.

2.2 Antioxidant response

2.2.1 Glutathione Peroxidase (GSHPx) Enzyme Activity in Serum

Glutathione peroxidase (GSH-Px) activity was determined according to the method described by Paglia and Valentine (1967), with minor modifications for microplate analysis [13]. The assay was performed in 96-well microtiter plates. Each reaction well contained 12.5 µl of serum, 250 µl of 0.1 mM phosphate-buffered saline (PBS; pH 7.4), 12.5 µl of hydrogen peroxide (H₂O₂), and 12.5 µl of reduced glutathione. The reaction mixture was allowed to incubate at room temperature for 5 minutes. Subsequently, 12.5 µl of nicotinamide adenine dinucleotide phosphate (NADPH) was added to initiate the enzymatic reaction. The decrease in absorbance was monitored at 340 nm using an ELISA microplate reader for a duration of 5 minutes, with readings recorded at 60-second intervals. Enzyme activity was expressed as units per milligram of protein.

2.2.2 Superoxide Dismutase (SOD) Enzyme Assay in Serum

Serum superoxide dismutase (SOD) activity was determined using a microplate-based assay following the procedure described by Madesh and Balasubramanian (1998) [9]. The assay was carried out in 96-well microtiter plates, wherein 100 µl of serum sample was dispensed into each well. This was followed by the addition of 6 µl of 1.25 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution, 15 µl of freshly prepared 100 µM pyrogallol, and 29 µl of 25 mM phosphate-buffered saline (PBS), resulting in a final reaction volume of 150 µl per well. Pyrogallol was prepared immediately prior to use and added after all other reagents had been introduced. The reaction mixture was incubated at room temperature for 10 minutes. Thereafter, 150 µl of dimethyl sulfoxide (DMSO) was added to each well to terminate the reaction and solubilize the MTT formazan crystals. The plates were shaken thoroughly, and absorbance was recorded at 570 nm using an ELISA microplate reader (BioTek Instruments µQuant).

2.2.3 Lipid Peroxidation in Serum

Lipid peroxidation in serum was estimated by measuring malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) method as described by Ohkawa *et al.* (1979) [12]. In this assay, MDA formed during lipid peroxidation reacts with thiobarbituric acid (TBA)

under acidic and high-temperature conditions to produce a pink-colored chromogen, which is subsequently extracted into butan-1-ol. The intensity of the resulting chromogen was measured spectrophotometrically to quantify lipid peroxide concentration. Results were expressed as nanomoles of MDA per milligram of protein. For the assay, 100 μ l of serum was transferred into a clean, dry test tube, followed by the addition of 100 μ l of distilled water and 50 μ l of 8% (w/v) sodium dodecyl sulfate. The mixture was allowed to stand at room temperature for 10 minutes. Thereafter, 375 μ l each of 20% acetic acid and TBA reagent were added, and the samples were heated in a boiling water bath for 60 minutes to facilitate color development. After cooling to room temperature, 2.5 ml of distilled water and 1.25 ml of a butanol-pyridine mixture (15:1, v/v) were added, followed by thorough mixing. The absorbance of the organic phase was recorded at 542 nm using a spectrophotometer. To minimize interference from residual chromogenic components and improve photometric clarity, alkaline pyridine-butanol was used to achieve a clear solution suitable for direct absorbance measurement.

2.4 Statistical analysis

The data was analyzed using the General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 20th version and comparison of means was done using

Duncan's multiple range test and significance was considered at $p < 0.05$.

3. Results and Discussion

3.1 Immune Parameters

Different dietary supplementations did not show significant ($p > 0.05$) effect on the cell mediated immune response to Phytohemagglutinin-P (PHA-P). Khan *et al.* (2020) reported that *Spirulina* at 1.0 g/kg (SPA1), 1.5 g/kg (SPA2), numerically improved lymphoproliferative skin response compared with the control group (SPA0), whereas birds fed SPA2 and SPA3 showed the greatest increase in skin thickness after 24 hr, confirming enhanced CMI in SPA3 but non-significant ($p > 0.05$) [7]. Contrary to the present finding, Khadanga *et al.* (2023) demonstrated significant improvements in cellular immunity with *Spirulina* at 0.5-1.5%, including increased CBH response and increased lymphoid organ weights [6].

The immunomodulatory effects of *Spirulina* are attributed to bioactive compounds including phycocyanin, β -carotene, polysaccharides and essential fatty acids, which stimulate macrophage activity, enhance natural killer cell function and promote antibody production. The lack of significant effects in the present study's cell-mediated immunity assessment may be attributed to differences in *Spirulina* dosage, duration of supplementation, or the specific immune challenge protocol employed.

Table 2: Effect of *Spirulina platensis* supplementation on immune responses in broiler chicken at 42 days of age

Trt code	Treatments	Cutaneous basophilic hypersensitivity
T ₁	Control	132.3
T ₂	Antibiotic	128.2
T ₃	<i>Spirulina</i> (0.5%)	135.5
T ₄	<i>Spirulina</i> (0.75%)	137.8
T ₅	<i>Spirulina</i> (1.0%)	140.7
P-value		0.85
SEM		2.55
N		6

^{a,b,c}Means having different superscripts within a column are significantly ($p < 0.05$) different

P value = probability value;

N = number of replicates (5 birds in each replicate);

SEM = Standard Error Mean

3.2 Serum Antioxidant Enzyme Responses

3.2.1 Glutathione Peroxidase (GPx)

The concentration of glutathione peroxidase differed significantly ($p < 0.05$) among the dietary treatments. Birds fed *Spirulina* (1.0%) recorded the highest GPx activity, which was significantly higher than the values observed in the T₁ and T₂ groups. The GPx activity in T₃ and T₄ remained intermediate and did not differ significantly from either T₅ or the lower-performing groups. The lowest GPx concentration was recorded in the control (T₁) group.

These findings are in agreement with Abdelfatah *et al.* (2024) who reported that *Spirulina* supplementation at 0.1%, 0.3% and 0.5% significantly enhanced GPx activity, with 0.5% inclusion producing the strongest antioxidant response [1]. Park *et al.* (2018) observed that dietary *Spirulina* up to 1.0% significantly elevated serum GPx activities, reflecting enhanced systemic antioxidant capacity [14]. However, in contrast Ahmed *et al.* (2022) findings did not show any significance at 3% and 5% [3].

3.2.2 Lipid Peroxidation (MDA)

Lipid peroxidation levels, expressed as nmol MDA/mg protein, were significantly ($p < 0.05$) reduced in birds receiving *Spirulina* supplementation compared with the control group. The lowest MDA concentration was observed in T₅, followed by T₄, both of which differed significantly from the control (T₁). The T₂ and T₃ groups showed intermediate values that did not differ significantly from the higher *Spirulina* fed groups.

The reduction in lipid peroxidation is crucial for maintaining cell membrane integrity, protecting cellular components from oxidative damage and supporting overall metabolic efficiency. Moustafa *et al.* (2021) reported significant enhancements in antioxidant status under cyclic heat stress, with *Spirulina* reducing MDA and increasing antioxidant capacity [10].

3.2.3 Superoxide Dismutase (SOD)

A significant ($p < 0.05$) increase in superoxide dismutase concentration was noted with *Spirulina* supplementation.

Birds supplemented with 1% *Spirulina* exhibited the highest SOD activity, which was significantly higher than that of control group and Antibiotic group. The T₃ and T₄ groups showed intermediate values that did not differ significantly from either the highest (T₅) or the lower performing treatments.

Abdel-Moneim *et al.* (2022) reported that SOD activity increased significantly with *Spirulina* inclusion, with both linear and quadratic components evident, demonstrating strong antioxidant enzyme stimulation [2]. Alaqil *et al.*

(2023) reported that supplementation with *Spirulina* increased antioxidant markers including elevated SOD levels while reducing oxidative stress metabolites [4]. Hassan *et al.* (2024) observed strong improvements with significantly increased SOD activity MDA levels [5]. The enhanced antioxidant status observed in the present study likely contributes to improved growth performance and feed efficiency, as reduced oxidative stress supports optimal cellular function, protein synthesis and energy metabolism.

Table 3: Effect of *Spirulina platensis* supplementation on the antioxidant enzymes response of broiler chicken at 42 days of age

Trt code	Treatments	Glutathione peroxidase (units/mg)	Lipid peroxidation (units/mg protein)	Superoxide dismutase (nmol MDA/mg protein)
T ₁	Control	249.55 ^b	3.57 ^a	6.24 ^b
T ₂	Antibiotic	363.92 ^b	2.70 ^{ab}	7.16 ^b
T ₃	<i>Spirulina</i> (0.5%)	505.39 ^{ab}	2.48 ^{ab}	7.74 ^{ab}
T ₄	<i>Spirulina</i> (0.75%)	572.19 ^{ab}	1.68 ^{bc}	7.99 ^{ab}
T ₅	<i>Spirulina</i> (1.0%)	757.14 ^a	1.24 ^c	8.19 ^a
	P-value	0.02	0.001	0.004
	SEM	63.82	0.19	0.53
	N	12	12	12

^{a,b,c}Means having different superscripts within a column are significantly ($p < 0.05$) different

P value = probability value;

N = number of replicates (5 birds in each replicate); SEM = Standard Error Mean

4. Conclusion

Based on the observed results, it was concluded that *Spirulina* supplementation at higher inclusion levels, particularly 1%, exerted a greater synergistic influence on the evaluated parameters. This level was found to be the most effective in enhancing immunity, strengthening antioxidant defense mechanisms, thereby supporting its use as a natural alternative to antibiotic growth promoters in broiler diets.

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