B-5414
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RESEARCH ARTICLE Indian Journal of Animal Research

Dietary Spirulina Supplementation Enhanced Performance, Immune Response and Reduced Oxidative Stress in Broiler Chicken

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10.18805/IJAR.B-5414

ABSTRACT

Background: *Spirulina* have antimicrobial, antioxidant, anticancer, anti-inflammatory and immune-enhancing properties, which improved the performance of birds. It has also got metallo protective, radio protective, hypocholesterolemic and antiviral properties.
Methods: A 35-day study was conducted, taking one hundred sixty Vencobb broiler birds divided into four groups. Each group consists of four replicates with 10 birds each. The dietary treatments were: T0: basal diet; T0.5: basal diet + 0.5% *Spirulina* powder; T1.0: basal diet + 1.0% *Spirulina* powder; and T1.5: basal diet + 1.5% *Spirulina* powder. Parameters under study included body weight gain, cumulative FCR, antioxidants, immunity status, and intestinal E. coli, *Salmonella,* and *Lactobacillus* counts.
Result: *Spirulina* supplementation at 1.5% significantly ($P<0.05$) improved the body weight gain, FCR, antioxidant status, immunity, and intestinal *Lactobacillus* count in broiler chickens compared to other treatments. Supplementation of *Spirulina* at 0.5% and 1% showed better performance compared to un-supplemented control birds.

Key words: Antioxidants, Body weight, Chicken, Immunity, *Spirulina*.

INTRODUCTION

Microorganisms like bacteria, fungi, microalgae, *etc.* play a great role in meeting the nutritional requirements of human beings, livestock, and poultry. Among all the microorganisms, algae are the chief photosynthetic producers. The most commonly used algae in animal and poultry feed is *Spirulina platensis* (Seyidoglu *et al.,* 2020). It is a unicellular filamentous cyanobacterium that generally occurs in a spiral shape (Holman *et al.,* 2012).The excellent nutrient profile of *Spirulina* in terms of high carotenoid, iron, and phosphorus content in addition to protein is responsible for its potential growth-promoting effect (Solanki *et al.,* 2023). *Spirulina* is also a non-toxic, nutritious feed for reproduction and immune enhancement in animals and poultry. It is also rich in all essential amino acids, vitamins, and gamma-linolenic acids (GLA), which are highly required for the health benefits of livestock (Howe *et al.,* 2006).

Spirulina also produces different biologically active products that are responsible for anti-oxidant, anti-inflammatory, and anti-cancerous effects (Khanna *et al.,* 2016). These bioactive products are phenolic compounds, like phycobillin proteins and phycocyanin C (Khadanga *et al.,* 2023). Supplementation with *Spirulina* enhanced cell-mediated and humoral immunity in birds (Khadanga *et al.,* 2023). It improves the mononuclear phagocytic potential in chickens for better resistance to various diseases (Seyidoglu *et al.,* 2017). It also enhances the defense mechanism of the organism by increasing the phagocytic cell number, which ultimately increases the phagocytic activity and decreases the local infection without interfering with the natural defense system (Finamore *et al.,* 2017). It also enhances feed conversion efficiency, average daily weight gain and carcass yield percentage in chickens (Kharde *et al.,* 2012).

During recent research, a carbohydrate extracted from *Spirulina platensis,* known as "spirulin," was found to popularly act as a prebiotic for the beneficial microbiota present inside the body of the organism (Sugiharto *et al.,* 2018). It inhibits as well as promotes different microorganisms in the body due to the production of different acids and bioactive products. It inhibits several bacteria like *Staphylococcus faecalis*, *S.epidermitis*, *Candida albicans*, gram +ve bacteria like *S. aureus*, gram -ve bacteria *E.coli* and promotes the probiotic effect enhancing bacteria widely known as lactic acid bacteria which include *L. lactis, L. casei*, *L. acidophilus, L.bulgaricus* (Finamore *et al.,* 2017).

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How to cite this article: Naik, M., Sethy, K., Panda, N., Mishra, S.K., Andhale, V.T. and Thakur, D. (2024). Dietary Spirulina Supplementation Enhanced Performance, Immune Response and Reduced Oxidative Stress in Broiler Chicken. Indian Journal of Animal Research. 1-6. doi: 10.18805/IJAR.B-5414.

Submitted: 29-05-2024 **Accepted:** 16-10-2024 **Online:** 26-12-2024

It also improves the absorption of minerals, protects from diarrhea, and optimizes nutrient digestion processes (Gruzauskas *et al.,* 2004). However, the supplementation of *Spirulina* and its effect on broiler chickens are relatively scanty and largely unknown in Indian context. Therefore; the present experiment was designed to assess the effect of *Spirulina* supplementation on the performance of Vencobb broiler chickens.

MATERIALS AND METHODS

Selection of birds and dietary treatments

The present study of 35 days duration was carried out as per the guidelines laid down by Institutional Animal Ethics Committee, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar during the month of March and April, 2024. 160-day-old Vencobb broiler chicks were randomly assigned to four dietary treatments. Each treatment had four replicas, each with 10 chicks. In the T0 group, birds were fed the regular basal diet. In $T_{0.5}$, $T_{1.0}$, and $T_{1.5}$, birds were fed a basal diet similar to that of the control in addition to *Spirulina* powder at 0.5%, 1.0%, and 1.50% of the basal diet, respectively. A deep litter system was used to rear the chicks. The experimental diets were made in accordance with BIS (2007). Before the experiment, the pen, feeders, and waterers were properly cleaned with water, followed by disinfection with Germex (Vetneeds Lab). Birds were given the RD (Lasota strain) vaccination on the seventh day, the IBD (intermediate strain) vaccine on the 14th day, and a booster dose of RD on the 21_{st} day.

Preparation of *spirulina* powder

Spirulina was cultivated in the month of July, 2023 in a modified Zarrouk's medium (N_aCl 1.0 g/L; $C_aCl_2.2H_2O$ 0.04 g/L; KNO_3 2.5 g/L; $FeSO_4.7H_2O$ 0.01 g/L; EDTA (Na) 0.08 g/L; K_2SO_4 1.0 g/L; $MgSO_4.7H_2O$ 0.2 g/L; $NaHCO_3$ 16.8 g/L and K_2HPO_4 0.5 g/L). Algae were incubated in an artificial pond at mean temperature of 30°C. Harvesting was performed after 12-14 days. It was dried at 40°C and the composition of *Spirulina* powder was measured by AOAC (1995).

Body weight gain and FCR

Individual body weights of the birds were measured using an electric pan balance at different weeks of the experiments. The initial body weight was subtracted from the final to get the BW gain. Feed intake was calculated at a weekly interval by subtracting the leftover feed from the total feed supplied during that week. FCR was calculated using a standard formula.

Estimation of antioxidant enzymes

Lipid peroxidation in RBC hemolyzate was determined by Placer *et al.* (1966), wherein the concentration of malonaldialdehyde (MDA) in nmol of MDA/mg hemoglobin was calculated using the extinction coefficient of 1.56×108/M/cm (Utely *et al.* 1967). Catalase (CAT) was assayed in erythrocytes by the method of Bergmeyer (1983). The superoxide dismutase (SOD) activity of RBC haemolyzate samples was measured using nitro blue tetrazolium as a substrate after suitable dilution according to Marklund and Marklund (1974) with certain modifications as suggested by Minami and Yoshikawa (1979).

Immunity study

The cellular immunity was assessed at the age of five weeks (Edelman *et al.,* 1986) by injecting PHA-P intradermally in the foot web of two birds from each replicate (eight birds in each treatment). The humoral immunity was determined after the fifth week of the experiment as per the method of Khalifeh *et al.* (2009).

Estimation of bacterial load

Caecal samples collected from experimental birds were serially diluted up to a level of 10^{-6}. Then, 0.1 ml of the dilution was pipetted out and inoculated in McConkey's agar, Eosin-Methylene blue agar, and *Lactobacillus* MRS agar for the growth of *Salmonella, E. coli,* and *Lactobacillus,* respectively. The samples were applied to the agar surface using a sterilized glass spreader while simultaneously rotating the petri dish beneath. The agar containing petridish was incubated at 37°C for 24 h, and individual colonies were counted with a colony counter and quantified as:

$$\text{CFU/ml} = \frac{(\text{no. of colonies} \times \text{dilution factor})}{\text{volume of culture plate.}}$$

Statistical analysis

Statistical analysis was done using Software Package for Social Sciences (SPSS) Version 17.0 (2008), and one-way analysis of variance (generalized linear model, ANOVA) with comparison among means was made by Duncan's multiple range test (Duncan, 1955) with a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Chemical composition of basal feed and *spirulina*

The broiler pre-starter, starter, and finisher rations had crude protein contents (%) of 22.50, 21.30 and 19.60, respectively (Table 1). The protein and energy needs were in line with those outlined in BIS (2007). *Spirulina* powder contained 46.50% CP and 7.40% EE. Similar to our result, Becker (2007) reported 46-63% crude protein and 4.0-9.0% ether extract in dry *Spirulina*. Ali (2017) observed higher protein content (61.8%) than our finding. This may be due to different growth mediums and climatic conditions during the cultivation.

Body weight and feed conversion ratio

The day-old live body weight of experimental broiler chicks was almost alike, indicating the well-randomized distribution of chicks among the experimental treatments. It was seen that the body weight at the starter and finisher phases was significantly higher in all supplemented birds compared to the control. It was also observed that supplementation of *Spirulina* at 1.5% significantly improved body weight gain compared to 0.5% and 1.0% supplemented birds. Similarly, a better feed conversion ratio (FCR) was observed in supplemented birds compared to control during the starter and finisher phases (Table 2). This implies that supplementation of *Spirulina*1.5% in the ration of broilers had a significant ($P<0.05$) effect on body weight gain and FCR in birds.

Similar to our results, significant improvement in body weight gain and FCR was observed in birds supplemented with varied levels of *Spirulina* (Jamil *et al.,* 2015; Mirzaie *et al.,* 2018; Park *et al.,* 2018; Khan *et al.,* 2021). El-Dayem *et al.* (2021) and Ibrahim *et al.* (2018) also observed a significant ($P<0.05$) increase in body weight gain and a decreased feed conversion ratio when *Spirulina* was added at doses of 1 and 2 g/kg to the quail diet. Increased feed conversion efficiency in terms of lowered FCR is corroborated by the findings of Khan *et al.* (2021) and El-Dayem *et al.*(2021). The increase in body weight in birds supplemented with *Spirulina platensis* may be due to improved feed utilization efficiency, vitamin and mineral absorption, and optimizing the nutrient digestion process (Gruzauskas *et al.,* 2004). *Spirulina* have pre and probiotic effects, which enhances digestion, absorption and assimilation of various nutrients (El-Dayem *et al.,* 2021).

Contrary to this, when *Spirulina* was provided to the broiler diet as a 10-20% replacement for soyabean meal, it showed a gradual decrease in growth rate (Toyomizu *et al.,* 2001). The decreased body weight may be due to different strains, a high dose rate, and environmental conditions compared to the pre sent experiment.

Table 1: Composition of basal diet.

Particulars	Prestarter (%) (0-7th day)	Starter (%) (8-21st day)	Finisher (%) (22-35th day)
Maize	51.00	53.00	56.00
Soyabean meal	43.50	39.50	35.50
Vegetable oil	2.00	4.00	5.00
Dicalcium phosphate	1.80	1.80	1.80
Limestone	0.96	0.96	0.96
DL-Methionine	0.05	0.05	0.05
L-Lysine	0.03	0.03	0.03
Vitamin and mineral premix	0.15	0.15	0.15
Common salt	0.51	0.51	0.51
Chemical composition (% DM)			
Crude protein	22.50	21.30	19.60
Ether Extract	2.6	5.35	5.87
Crude fibre	4.9	5.2	5.45
Total Ash	9.1	8.3	8.95
Nitrogen free extract*	60.90	59.85	60.13
Calcium	1.50	1.40	1.32
Phosphorus	0.58	0.60	0.55
Metabolisable energy (Kcal/kg)*	2980	3090	3210

*Calculated values.

Table 2: Body weight and FCR of experimental birds.

Parameters	Treatments					
	T_0	$T_{0.5}$	$T_{1.0}$	$T_{1.5}$	SEM	P Value
Cumulative BW gain						
Prestarter	105.95	108.30	107.10	107.85	1.25	0.390
Starter	675.80^{a}	724.70^{b}	732.20^{b}	740.20^{b}	14.60	0.016
Finisher	1765.50^{a}	1839.40^{b}	1846.80^{b}	1875.60^{b}	22.20	0.006
Cumulative FCR						
Prestarter	1.11	1.12	1.14	1.12	0.02	0.762
Starter	1.51^{b}	1.40^{a}	1.40^{a}	1.41^{a}	0.03	0.024
Finisher	1.88^{b}	1.79^{ab}	1.77^{ab}	1.74^{a}	0.02	<0.01

[ab]Values bearing different superscripts in a row differ significantly ($P<0.05$).

Antioxidant status

Spirulina at different concentrations significantly ($P<0.05$) increased catalase and SOD levels and decreased ($P<0.05$) lipid peroxidation, as indicated by the fall in MDA levels in all the treatments compared to control birds (Table 3). Similarly, Moustafa *et al.* (2021) and Abd El-Hady *et al.* (2022) observed increased antioxidant enzyme activity and decreased malondialdehyde levels in chicken supplemented with *Sprirulina*. Omar *et al.* (2022) also observed that catalase and superoxide dismutase activity increased linearly and malondialdehyde (MDA) concentration decreased linearly by increasing the *Spirulina* level in chickens. The antioxidant activity of *Spirulina* is attributed to the presence of total phenols and flavonoids. *Spirulina* also contains C-phycocyanin, a potent antioxidant, as one of its major constituents (Mirzaie *et al.,* 2018).

Immunity of birds

Spirulina supplementation enhanced the humoral and cellular immunity in broiler birds (Table 4). Similarly, Qureshi *et al.* (1996) and Lokapirnasari *et al.* (2016) observed significant improvement in immunity in birds supplemented with *Spirulina*. *Spirulina* enhances the function of macrophages and increases the potency of the mononuclear phagocyte system (Finamore *et al.,* 2017). Active principles like β-glucan and phycocyanin pre sent in *Spirulina* enhanced the production and maturation of antibodies in birds (Seyidoglu *et al.,* 2017). Supplementation of *Spirulina platensis* to K strain chicks at 0, 10, 100, 1,000, and 10,000 ppm improved the IgG and total anti-SRBC titers ($P<0.05$) over the controls. Supplementation of *Spirulina* significantly reduces the expression of cytokines like IL-1β, IL-6, and TNF-α ; C-phycocyanins and β-carotene pre sent in *Spirulina* block

Table 3: Antioxidant status (U/mg haemoglobin) in experimental birds.

Parameters	Treatments					
	T0	T0.5	T1.0	T1.5	SEM	P Value
Catalase	14.96^{a}	25.42^{b}	30.39^{c}	33.69^{cd}	2.28	<0.01
SOD	19.07^{a}	27.59^{b}	35.90^{c}	39.49^{d}	3.30	<0.01
LPO	2.97^{c}	2.28^{b}	1.97^{b}	1.62^{a}	0.07	0.036

^{abcd}Values bearing different superscripts in a row differ significantly ($P<0.05$).

Table 4: Immune status of experimental birds.

Attributes	Treatments					
	T_0	$T_{0.5}$	$T_{1.0}$	$T_{1.5}$	SEM	P value
CBH (%)	160.52^{a}	185.43^{ab}	199.37^{b}	225.30^{c}	9.75	0.013
HI titre (Log_2)	4.33^{a}	5.66^{b}	6.20^{c}	6.86^{d}	0.04	<0.01

^{abcd}Values bearing different superscripts in a row differ significantly ($P<0.05$)

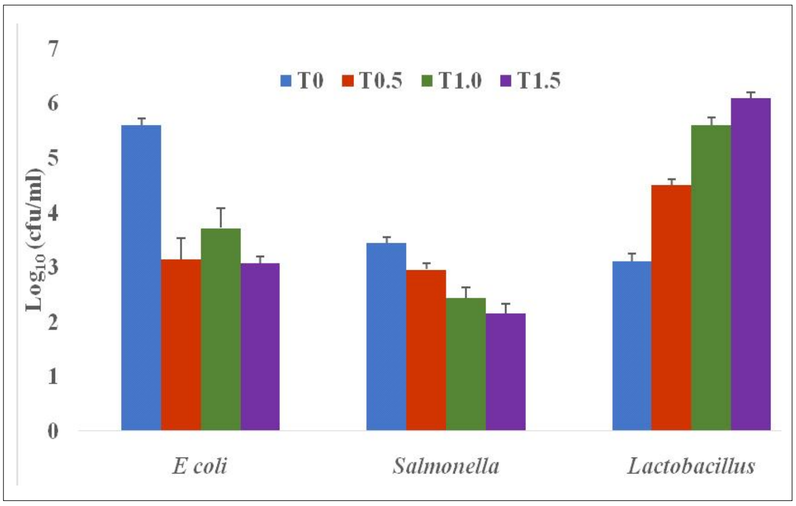

Fig 1: Intestinal bacterial load of experimental birds at 35th day.

the activity of NF-κB and suppress TNF-α, leading to enhanced immunity in birds (El-Dayem *et al.,* 2021).

Intestinal microbial load

Log_{10} values of intestinal *E. coli* and *Salmonella* count were found to decrease significantly (P<0.05) in all the treated birds compared to the control (Fig 1). Earlier studies have suggested that *Spirulina*and its extract are effective antimicrobial agents against bacteria, fungi, and viruses (Shanmugapriya *et al.,* 2015; Fathi *et al.,* 2018). The intestinal *Lactobacillus* count was found to be significantly (P<0.05) higher in all *Spirulina*-treated groups than in control birds.

Similarly, Park *et al.* (2018) observed that supplementation with 1% *Spirulina* significantly increased the *Lactobacillus* count in birds. Fathi *et al.*(2018) observed that supplementation of *Spirulina* at 0.7 and 0.9 g/kg diet showed decreased microbial load and increased *Lactobacillus* count in the intestinal content. The non-digestible part of the cell wall of *Spirulina* acts as a source of prebiotics, so it helps in the growth of *Lactobacillus*.

CONCLUSION

Dietary supplementation of *Spirulina* at 1.5% resulted in maximum body weight gain, immunity and intestinal *Lactobacillus* count and reduced stress in birds. Addition of 0.5 % and 1.0 % *Spirulina* in the diet found to be beneficial for birds compared to control.

ACKNOWLEDGEMENT

The authors are thankful to the Vice Chancellor, Odisha University of Agriculture and Technology, Bhubaneswar for providing necessary funds and facilities to carry out this research.

Conflict of interest

The authors declare that they have no conflicts of interest.

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