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Immunomodulatory Effect of Turmeric (Curcuma longa) in *Escherichia coli* Induced Infected Broiler Chicks

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Abstract

Immune-modulatory effect of turmeric (*Curcuma longa*) was investigated in *Escherichia (E.) coli* induced infected broiler chicks. A flock of one hundred broiler birds were purchased and divided equally into four equal groups (A-D). Negative and positive control were assigned to groups A and B respectively. Birds of groups C and D were supplemented with turmeric at 10g/kg of feed from 10th to 42nd days of age. Birds of groups B and D were given a pathogenic strain of *E. coli* (1×10^4 CFU/ml/bird) on their 15th day of age. The hemagglutination inhibition (HI) assay was used to assess the humoral immune response to SRBCs and NDV. An avian tuberculin test and a carbon clearance assay were used for cellular immune response. Levels of IgA and IgG were determined through ELISA. Results showed that antibody titers against NDV and SRBCs were significantly higher ($p<0.05$) in the turmeric supplemented group. Similarly, levels of IgA and IgG were significantly higher ($p<0.05$) in birds supplemented with turmeric. The phagocytic index and lymphoproliferative response were significantly higher in the turmeric supplemented groups C and D as compared to control groups A and B. The findings of this study revealed that 10g/kg of dietary turmeric supplementation in broiler feed improves the cellular and humoral immune responses of broiler birds.

Keywords: *E. coli* • *Curcuma longa* • Phagocytic index • IgA • IgG

Introduction

Poultry is a well-recognized sector and plays a vital part to fulfil the supply and demand for protein in Pakistan. Its contribution to meat production is about 34%, whereas egg production showed a growth of 5.6% [1]. One of the main hindrances in the development of the poultry industry is the occurrence of infectious and non-infectious diseases [2]. *Colibacillosis* is caused by avian pathogenic *E. coli* (APEC) and is characterized by a variety of lesions. In poultry, it decreases egg production, hatchability, increases mortality and treatment expenses [3]. Disease severity is based on strain virulence, the presence of the causative agent, and environmental stress. *Colibacillosis* causes intestinal septicemia followed by localized inflammation of multiple organs [2]. Pathogenic strains of *E. coli* produce shiga toxins, responsible for disease production in birds. Typical lesions in this disease are pericarditis, perihepatitis, and air sacculitis [4].

Long term use of antibiotics to cure the infection increases the chances of antimicrobial resistance and their residues in eggs and meat are major concerns of livestock and poultry industry [5]. Therefore, the alternative of antibiotics, such as natural botanicals can be safely used in animal diets. Because their phytochemical components have antibacterial capabilities, nutritional supplementation of herbs and their essential oils has been proven to have beneficial benefits on poultry health and performance [6]. Biologically active plants in animal feed trigger immune response, showed antimicrobial, and antioxidant effects, and enhance digestive enzyme secretions [7].

In this regard, *Curcuma longa,* a rhizomatous ginger plant belonging to *Zingiberaceae* family has antibacterial and antioxidant properties [8]. Dried turmeric comprises of 69.4% carbohydrates, 6.3% protein, 5.1% fat, 3.5% minerals and 13.1% moisture. Curcumin, desmethoxycurcumin, and bisdemethoxycurcumin are three main bioactive compounds of *Curcuma longa* [9,10]. Curcumin is essential biologically active constituent that is responsible for biological activity. It is a polyphenol and known to have hypolipidemic, anti-inflammatory, immunomodulatory and antioxidant activity [11]. It has also been reported as a chemoprotective agent and used in respiratory disorders and GIT infections. Studies revealed that incorporation of turmeric in broilers diet enhance their performance [12]. Current study reports the antibacterial and immunomodulatory effects of turmeric powder in commercial poultry.

Material and Methods

Isolation of *E. coli*

Disease-suspected birds with clinical indications of *colibacillosis* were taken from the field to the Diagnostic Laboratory, University of Agriculture Faisalabad. Tissue samples were obtained from the liver, pericardium, intestine, heart, lungs, and cloaca of infected birds. Primary isolation was made by streaking on MacConkey agar, incubated aerobically at 37°C for 18 hours [2]. Morphological confirmation was done by Gram's staining [13]. Different biochemical tests including Citrate, Lactose fermentation, Indole, and Methyl red tests were performed for the identification of *E. coli* [14]. The pathogenicity of *E. coli* isolates was determined by using Congo red dye agar [15].

Procurement of Turmeric roots

Turmeric roots were procured from the local market and shade dried by regular turning using a clean muslin cloth. After that turmeric roots were ground into powder by using a food processor. Turmeric powder was mixed in broiler feed at 10g/kg feed from 10th to 42nd day of the experiment [16]. A total of 100-day-old broiler chickens were purchased from a local hatchery and kept under good management. After brooding, birds were divided into four groups

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each with 25 birds (Table 1). Birds were closely observed for 42 days. On the 15th day of age, birds were challenged with an enteropathogenic strain of *E. coli* (1×10^4 CFU/ml/bird) intramuscularly to induce *colibacillosis* [17].

Humoral Immunity

On day 12, seven birds from each group were injected intravenously with 0.25ml of 3% sheep red blood cells (SRBCs) to assess humoral immunity. Blood was collected and serum was separated and stored at -20°C for further use [18]. Avian tuberculin and carbon clearance assay (CCA) were performed at 37th day [19] and level of IgA and IgG were also checked [20]. Tissue samples from liver, heart and intestine was collected and fixed in 10% neutral buffered formalin solution immediately and embedded in paraffin. Transverse and longitudinal slices were cut with a microtome, stained with hematoxylin and eosin, and examined under the microscope [21].

Statistical Analysis: Data thus collected were statistically analyzed by using SPSS (analytical software). A significant difference between various groups was calculated by the least significant difference ($p<0.05$) between the groups.

Results

After 18, 24, 48, and 72 hours of incubation, the colonies were examined on Congo red agar plates. Pathogenic *E. coli* formed red colonies between 18 and 72 hours of incubation, while non-pathogenic *E. coli* greyish white and stayed unchanged throughout the incubation period (Figure 1). In the current study, a non-significant difference was observed in bodyweight of broiler birds up to 2nd week of age. From 3rd to 6th week of age, the body weight of turmeric supplemented group was significantly higher as compared to non-turmeric supplemented groups (Figure 2).

When compared to the control negative and turmeric supplemented groups, the lymphoproliferative response to avian tuberculin was significantly lower in the positive control group at 24, 48 and 72 hours post inoculation. At 24, 48, and 72 hours, there was no significant difference between the control negative and turmeric supplanted group infected with *E. coli* (Table 2). At 3 and 15 minutes after carbon ink inoculation, a significant decrease in phagocytic index was observed in the turmeric supplemented group as compared to all other groups, while a non-significant difference was observed between negative control and turmeric supplanted group infected with *E. coli* (Table 3).

On days 7 and 14, the turmeric supplemented group had a substantial rise in antibody titer against SRBCs as determined by HI test, whereas the positive control group infected with *E. coli* had considerably lower antibody titer than the negative control group and turmeric supplemented group infected with *E. coli* (Table 4). Turmeric supplemented broiler birds have significantly higher IgA and IgG level than the negative control group at 7th, 14th and 21st days post infection, while the positive control group had a significantly lower level of IgA and IgG than the turmeric supplemented group infected with *E. coli* (Table 5, 6).

Table 1. Experimental layout.

Experimental groups	No of Birds	Treatments
A	25	Negative Control
B	25	Positive Control (*E. coli* infection)
C	25	Turmeric powder at 10g/kg of feed + non-infected
D	25	Turmeric powder at 10g/kg of feed + *E. coli* infection

Negative control= no *E. coli* infection and no turmeric powder supplementation *E. coli* = 1× 10^4 CFU/ml

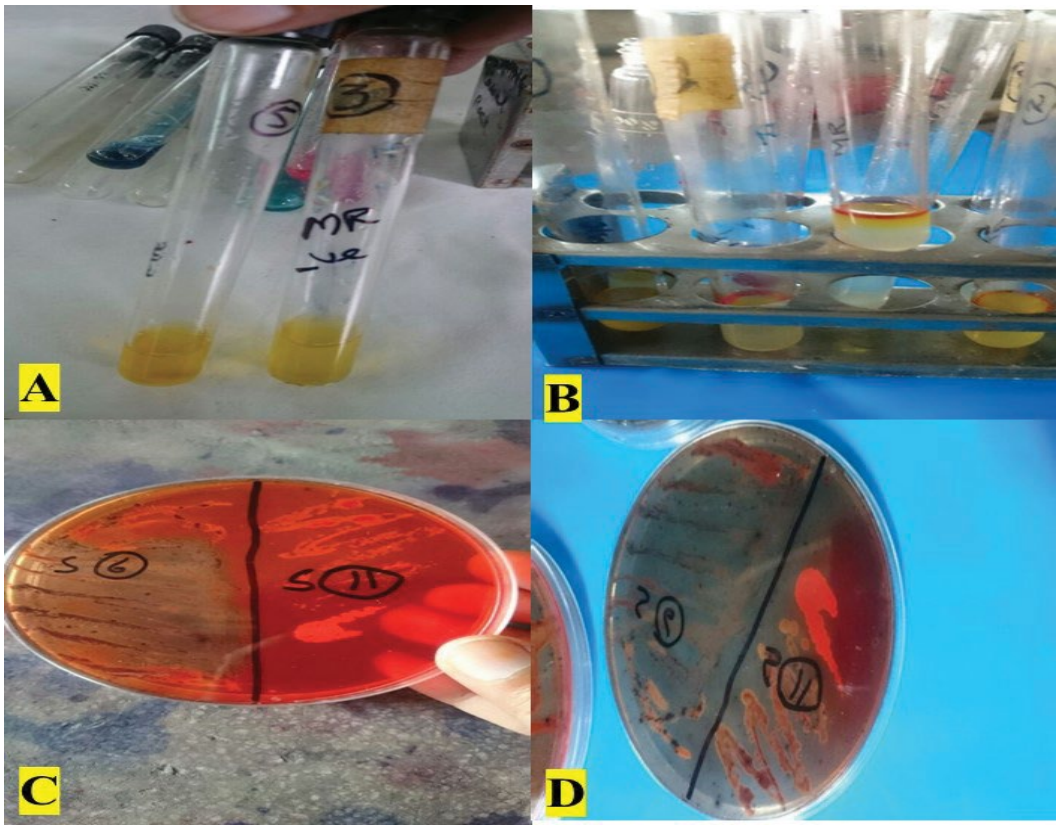

Figure 1. Biochemical test of *E. coli*, the **A)** showed Methyl red test **B)** showed Indole test with ring formation **C)** showed pathogenic *E. coli* bright red colonies after indole test **D)** pathogenic showed greyish white colonies.

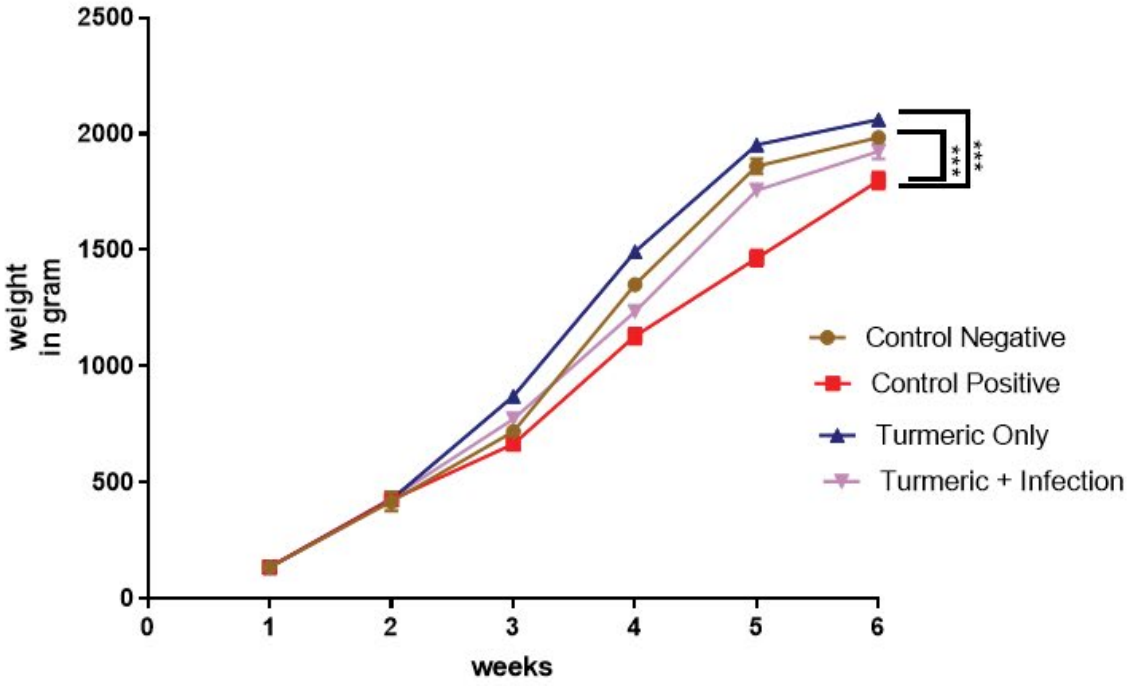

Figure 2. Bodyweight (g) of broiler birds experimentally infected with E. coli and supplemented with Turmeric powder.

Table 2. Lymphoproliferative response (mm) against avian tuberculin in *E. coli* induced infected broiler chicks supplemented with turmeric powder.

Hours	Negative control	Positive control (*E. coli* 1× 10^4 CFU/ml)	Turmeric (10g/kg feed)	Turmeric (10g/kg feed) + (*E. coli* 1× 10^4 CFU/ml)
0	3.053 ± 0.067^{a}	2.293 ± 0.025^{a}	2.743 ± 0.068^{a}	3.020 ± 0.040^{a}
24	2.897 ± 0.074^{ab}	2.157 ± 0.032^{b}	2.270 ± 0.098^{ab}	2.810 ± 0.089^{ab}
48	2.700 ± 0.149^{bc}	1.980 ± 0.060^{bc}	2.153 ± 0.097^{ab}	2.617 ± 0.031^{ab}
72	2.553 ± 0.097^{c}	1.847 ± 0.049^{c}	2.037 ± 0.067^{b}	2.357 ± 0.086^{b}

Mean ± SD having similar alphabets (superscript) in a row are non-significantly different ($p < 0.05$), Zero Hours= before tuberculin administration

Table 3. Macrophage activity of *E. coli* induced infected broiler chicks supplemented with turmeric powder.

Minutes	Negative control	Positive control (*E. coli* 1× 10^4 CFU/ml)	Turmeric (10g/kg feed)	Turmeric (10g/kg feed) + (*E. coli* 1× 10^4 CFU/ml)
0	0.237 ± 0.063^{a}	0.302 ± 0.055^{b}	0.126 ± 0.059^{b}	0.192 ± 0.054^{ab}
3	0.236 ± 0.057^{a}	0.337 ± 0.050^{ab}	0.142 ± 0.054^{a}	0.185 ± 0.057^{b}
15	0.234 ± 0.058^{a}	0.356 ± 0.041^{a}	0.146 ± 0.038^{a}	0.199 ± 0.056^{a}

Mean ± SD having similar alphabets (superscript) in a row are non-significantly different ($p < 0.05$), Zero Minute= before ink administration

Table 4. Antibody titer against sheep red blood cells in *E. coli* induced infected broiler chicks supplemented with turmeric powder.

DPI	Negative control	Positive control (*E. coli* 1× 10^4 CFU/ml)	Turmeric (10g/kg feed)	Turmeric (10g/kg feed) + (*E. coli* 1× 10^4 CFU/ml)
7^{th}	5.520 ± 0.259^{ab}	3.420 ± 0.249^{c}	6.500 ± 0.158^{a}	4.360 ± 0.241^{b}
14^{th}	4.560 ± 0.288^{b}	2.152 ± 0.215^{c}	5.440 ± 0.241^{a}	3.240 ± 0.270^{bc}

Mean ± SD having similar alphabets (superscript) in a row are non-significantly different ($p < 0.05$), DPI= after primary & booster dose of SRBCs injection

Microscopically, spleen showed lymphocyte proliferation and degeneration. Infected birds' spleens showed hemorrhagic patches and congestion, while their thymuses showed no significant histological changes. In the thymus, however, there was a modest reduction and loss of lymphocytes (Figure 3).

Discussion

A collection of bacteria known as avian pathogenic *E. coli* (APEC) causes avian *colibacillosis*, which is one of the most common endemic diseases affecting the poultry industry globally [22]. The severity of the disease is usually determined by the bacterial strain's virulence and host immunological status. Infection is mainly followed by localized inflammation in different organs or sudden death of the birds. The common signs associated with the disease are perihepatitis, pericarditis, and air sacculitis [23]. *Curcuma longa,* is known for its medicinal benefits and its major bioactive compound is curcumin which is responsible for its biological activities. It has antibacterial, immunomodulatory, and chemoprotective effects and is used in respiratory diseases and gastrointestinal tract infections [12]. Present study reports the effects of turmeric powder supplementation on body weight, gross and histopathological changes in lymphoid organs as well as immunomodulatory effects in *E. coli* infected broiler birds.

The turmeric supplemented group showed a considerable gain in body

Table 5. IgA level in *E. coli* induced infected broiler chicks supplemented with turmeric powder.

DPI	Negative control	Positive control (*E. coli* 1× 10^4 CFU/ml)	Turmeric (10g/kg feed)	Turmeric (10g/kg feed) + (*E. coli* 1× 10^4 CFU/ml)
7^{th}	0.386 ± 0.025^{b}	0.252 ± 0.031^{c}	0.472 ± 0.041^{a}	0.320 ± 0.016^{bc}
14^{th}	0.484 ± 0.034^{b}	0.356 ± 0.021^{c}	0.540 ± 0.016^{a}	0.436 ± 0.011^{bc}
21^{st}	0.588 ± 0.029^{ab}	0.424 ± .026^{c}	0.636 ± 0.030^{a}	0.508 ± 0.027^{ab}

Mean ± SD having similar alphabets (superscript) in a row are non-significantly different ($p < 0.05$), IgA= immunoglobulin A

Table 6. IgG level in *E. coli* induced infected broiler chicks supplemented with turmeric powder.

DPI	Negative control	Positive control (*E. coli* 1× 10^4 CFU/ml)	Turmeric (10g/kg feed)	Turmeric (10g/kg feed) + (*E. coli* 1× 10^4 CFU/ml)
7^{th}	4.140 ± 0.297^{ab}	2.820 ± 0.319^{c}	5.080 ± 0.192^{a}	3.444 ± 0.282^{b}
14^{th}	5.220 ± 0.259^{ab}	3.380 ± 0.335^{c}	6.200 ± 0.292^{a}	4.240 ± 0.251^{b}
21^{st}	6.340 ± 0.344^{ab}	4.180 ± 0.377^{c}	7.260 ± 0.241^{a}	5.240 ± 0.167^{b}

Mean ± SD having similar alphabets (superscript) in a row are non-significantly different ($p < 0.05$), IgG= immunoglobulin G

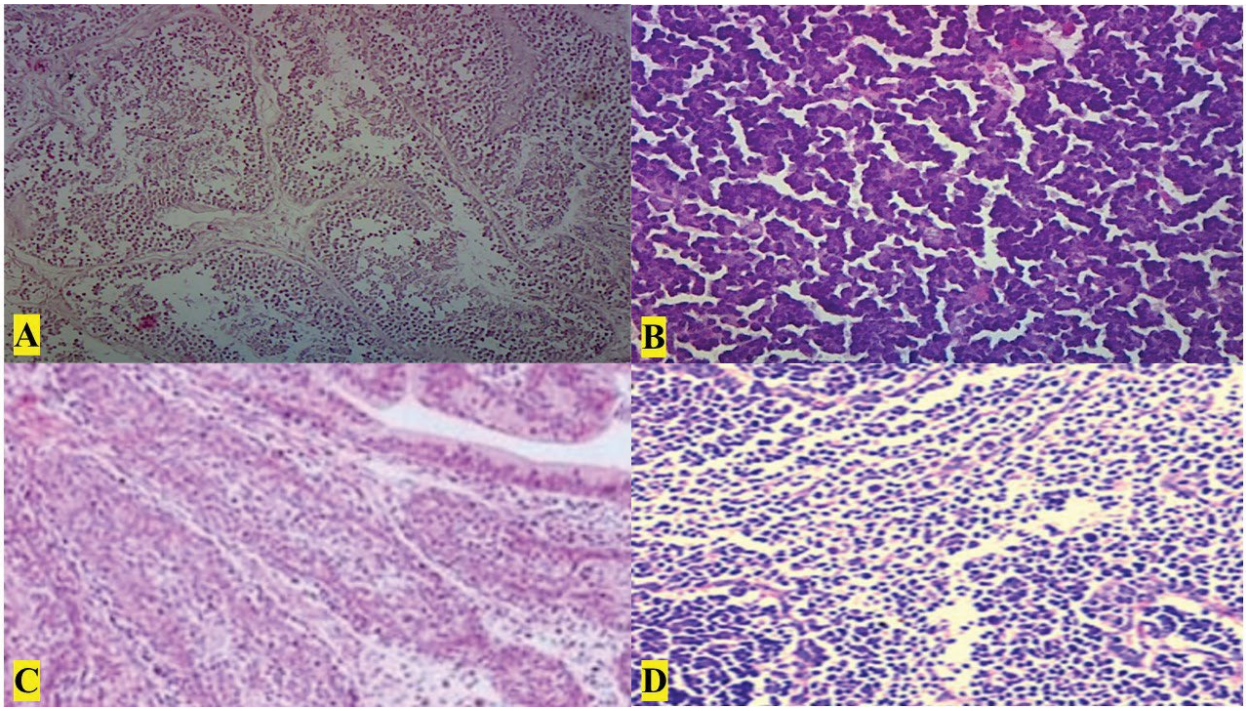

Figure 3. Photomicrograph of Thymus (A) and spleen (B) showing depletion of lymphocytes in centers of lymph nodules. Photomicrograph of Proventriculous (C) and liver (D) showing infiltration of lymphocytes (H&E stain, 100X)

weight. The increase in broiler performance and weight gain is responsible for this improvement [24]. Turmeric supplementation causes an increase in body weight because it increases feed digestibility by boosting intestinal lipase, maltase, and sucrose activity, as well as secretion of amylase, pancreatic lipase, trypsin, and chymotrypsin enzymes [12]. A recent study showed that turmeric increases the length of villus and width of duodenum, jejunum and caeca of broiler birds [25]. Due to the reason, turmeric supplementation increases the body weight, feed intake, and overall performance of birds by enhancing the assimilation of feed ingredients.

The presence of a sophisticated and highly categorized immune system in birds termed the lymphoid system is required for disease resistance. This system comprises of unique organs and divides into two components, which are morphologically and functionally different from each other's. B lymphocytes are produced from bursa of Fabricious responsible for humoral immunity while T lymphocytes are produced from thymus responsible for cellular immunity [26]. In current study, it was found that turmeric supplementation enhances the cellular and humoral immune responses. It also enhanced the antibody titer against SRBCs in broiler birds. The active compound of turmeric is curcumin which enhances the production of T lymphocytes, B lymphocytes and enhances the macrophages phagocytosis by production of reactive oxygen species [11].

Curcumin enhances the cell membrane integrity, increase stress-related proteins, and reduces the expression of pro-apoptotic signaling molecules [27]. These observations were similar to the findings of [28] that dietary turmeric supplementation enhanced the antibody titers. Moreover, high concentration of turmeric powder in diet enhanced the antibody production against SRBCs. Dietary supplementation boosted the immunological performance by improving the physiology of immune system which in turn improves humoral immunity by producing antibodies against SRBCs antigens [6]. Similarly, turmeric supplementation in broiler diets improves the cellular and humoral immune responses and dietary inclusion of turmeric powder reduces the *E. coli* count in intestinal contents of laying hens that improves the immune response to NDV and SRBCs antigens [7].

In the current study, circulatory macrophage concentration in blood was determined by phagocytic index known as carbon clearance assay. In this assay, macrophage engulfs the foreign particles to determine the phagocytic activity of macrophages [19]. The results showed that turmeric supplemented broiler birds cleared more carbon particles from their blood than control groups, indicating increased macrophage phagocytic activity. In the current study, significantly high levels of IgG and IgA were observed in turmeric supplemented group, when compared with infected and control groups. Turmeric powder

supplementation improves the laying hen's immunity by increasing the total immunoglobulins (Ig) level after SRBCs administration and suggested that dietary inclusion of turmeric powder improves the immune response to NDV and SRBCs antigens [7]. From the current study, it was concluded that turmeric supplementation had a favorable effect on broiler immune system by boosting the humoral and cellular immune response.

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