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SYNERGISTIC ANTIVIRAL EFFECTS OF CURCUMA LONGA EXTRACT AND VACCINATION ON HISTOPATHOLOGICAL, HEMATOLOGICAL, AND BIOCHEMICAL RESPONSES IN BROILER CHICKENS

Saqlain Atta Panezai[*1], Aisha Khatoon[2], Syed Abdul Hadi[3], Shakil Ahmed[4], Zaira Sharif[5], Farooque Ahmed Khushk[6], Muhammad Ismail[7], Rameez Raja Kaleri[8]

*[*1,3,4,7]Livestock and Dairy Development Department, Government of Balochistan*
[2]Department of Pathology, Faculty of Veterinary Sciences, Agriculture University of Faisalabad
[5,8]Directorate of Veterinary Research and Diagnosis, (CVDL), Tandojam
[6]Department of Poultry Husbandry, Sindh Agriculture University, Tandojam

[2]aisha.khatoon@uaf.edu.pk

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Corresponding Author: *
Saqlain Atta Panezai

Abstract

This study aimed to evaluate the immunomodulatory and antiviral potential of Curcuma longa (turmeric) extract in broiler chicks challenged with Infectious Bursal Disease Virus (IBDV). A total of 120-day-old broiler chicks were procured from a local hatchery and randomly allocated into four groups with five replicates each. Group A served as the negative control (NC), Group B as the positive control (PC), Group C received curcumin extract at 2g/kg feed, and Group D administered both curcumin extract (2g/kg) and IBDV. Daily records of morbidity and mortality were maintained. The feed conversion ratio (FCR) significantly improved (P<0.05) in Groups C and D compared to Groups A and B. Both humoral and cellular immune responses were assessed through lymphoproliferation assays using tuberculin and Sheep Red Blood Cells (SRBCs). Group D showed a marked increase in absorbance values and SRBC response compared to Groups A and B. The packed cell volume (PCV) was significantly higher (P<0.05) in Group D compared to Group B, with no notable difference from Group A. Serological analysis revealed a significant rise (P<0.05) in antibody titters in Group D relative to controls. Additionally, Groups C and D demonstrated elevated total protein, albumin, and globulin concentrations. Liver function enzymes were notably reduced in Group D, and serum urea and creatinine levels were also suppressed. Gross and histopathological evaluations of lymphoid organs (bursa of Fabricius and thymus) indicated significant improvements in size and cellular integrity in Group D. In conclusion, dietary inclusion of Curcuma longa extract at 2g/kg of body weight enhanced immune responses and offered protective effects against IBDV in broilers. The findings suggest that curcumin not only complements vaccination but may also serve as a natural alternative to conventional antiviral therapies, potentially reducing resistance concerns. These results highlight curcumin's role as a promising immunostimulant in poultry health management.

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INTRODUCTION

Poultry production plays a vital role in Pakistan and a growing sector of agriculture that contributes remarkably at the rate of (1.3%) of the total GDP (Aritonang et al., 2024). Unfortunately, the poultry industry is facing plenty of challenges and emergency situations, including a variety of irresistible threats in the shape of diseases and infections (Abdelaziz et al., 2024). One such disease is IBD (Infectious Bursal Disease), IBDV is the common reason for commercial losses in the poultry industry. Infectious bursal disease Virus is an immunosuppressive virus that affects the immature B-lymphocytes in bursa of Fabricius (Zhang et al., 2024). IBDV is a virus with two strands of RNA, with a non-enveloped icosahedral capsid that measures 60 nm in diameter and is primarily responsible for immunosuppression in birds, IBDV is more susceptible to rapid genetic changes due to its segmented double-stranded RNA genome. As a result, IBDV has a lot of genetic and phenotypic variability (Niu et al., 2024).

The virion key structural proteins are VP2 and VP3. In birds, VP2 is thought to be responsible for inducing protective immunity. Much work has gone into developing 'VP2' as a vaccination to avoid the disease. VP2 subunit vaccines have been created, as well as live reunification to transmit the viral vaccines containing the VP2 to be inserted alone or in combination with other viral segments of polypeptides (Hu et al., 2023). Most of these immunizations induce a better anti-IBDV antibiotic that gives an effective response, but their protection against severe IBDV exposure varies and can be inadequate. IBDV infects most commercial hens at a young age (Criado et al., 2023). The oral route appears to be the most common method of infection in natural situations. Phagocytic cells, most likely to be the permanent stayers of macrophages, transfer the virus from the gut to other regions. Although viral antigen is discovered in first few hours within the liver and kidney while affected by the infection, the bursa of Fabricius is the site of considerable viral replication (Babiker and Tawfeeg, 2008). The virus which contains cells form the bursa within hours after exposure, and the virus exploits quickly via the bursal follicles. In the sections of medullary and cortical of the follicles, virus replication causes widespread lymphoid cell death (Orakpoghenor et al., 2020). Apoptosis of virus-free bystander cells may amplify the cellular destructive process. Although there is no discernible decline in circulating immunoglobulins, the virus acute lytic phase is associated with a decline in circulating IgM+ cells (Igs). IBDV infection does not affect T cells (Mahgoub, 2012).

Even though the thymus undergoes considerable thymocyte shrinkage and death also in the duration of the severe stage of viral infection, there is no proof that the virus increases in thymic cells. Within a few days of infection, gross and microscopic lesions in the thymus are resolved, Recent findings shed light on the pathophysiology of IBDV as well as the mechanism of recovery from severe infection (Ji et al., 2023). Herbal extracts are being employed as feed supplements to help animals perform better, Fenugreek seeds have been reported to be high in protein, fat, total carbs, and minerals like calcium, phosphorus, iron, zinc, and magnesium. Fenugreek also helps the digestive system (Bulucea et al., 2023).

Curcumin is a significant component in curcuminoid combinations and has a greater solubility in organic solvents like ethanol (Nur et al., 2023). By comparing crystals formed from a curcuminoid mixture to pure curcumin, the single phase and its growth kinetics at different temperatures were examined (Eleiwa et al., 2023). Curcuminoid molecules are important because they play a crucial role in the bioactive activities of turmeric and its derivatives (Aqeel et al., 2024; Eleiwa et al., 2023).

Materials and Methods

Bird Procurement and Experimental Design

A total of 120-day-old commercial broiler chicks were sourced from a certified local hatchery. The trial was conducted over a period of 37 days under controlled environmental and management conditions. Birds were randomly distributed into four experimental groups, each with five replicates:

- Group A (Negative Control – NC): Uninfected and received a standard basal diet.
- Group B (Positive Control – PC): Infected with IBDV without curcumin supplementation.
- Group C: Supplemented with *Curcuma longa* extract at 1 g/kg of feed, uninfected.
- Group D: Supplemented with *Curcuma longa* extract at 1 g/kg of feed and challenged with IBDV.

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The curcumin extract was incorporated into the feed starting on Day 3 and continued until the end of the study (Day 37). On Day 9, birds in Groups B and D were challenged with the Infectious Bursal Disease Virus (IBDV) at a dose of 0.1 CFU/mL per bird via the subcutaneous route.

Monitoring and Sampling Protocol

Birds were observed twice daily for clinical signs of disease, behavioral changes, and mortality. Body weight was recorded weekly to assess growth performance and feed conversion.

To evaluate immunological and pathological responses, four birds per group were randomly selected and humanely sacrificed on Days 7, 14, 21, and 28 post-infections. Blood samples were collected via cardiac puncture for hematological and biochemical analyses. Postmortem examinations were conducted immediately after euthanasia, and gross lesions in vital organs, particularly the bursa of Fabricius, thymus, spleen, and liver were recorded. Tissues were preserved in 10% buffered formalin for subsequent histopathological evaluation (Bancroft and Gamble, 2008).

Curcumin extraction procedure: (Conventional Soxhlet Method)

Curcumin extract was prepared by the following method as described by (Jiang et al., 2021).

General parameters

The following parameters were studied**,** clinical signs were observed twice a day, mortality was recorded daily, feed intake was recorded daily, and body weight gain was recorded.

Hematological studies

The following blood parameters were determined.

Erythrocytes count, Hemoglobin (HB) concentration. (Sahli's Method), PCV (Hematocrit method) Total Leukocytes count by a method described by (Nayaka et al., 2013).

Serum biochemistry

Serum biochemistry of Total protein through, albumin, Alanine aminotransferases linear

Aspartate aminotransferases (AST), Blood urea nitrogen and Creatinine through Randox laboratories Ltd-CR510 and Globulins concentration were determined by subtracting the albumin concentration from total protein.

Immunological studies

Antibody response to sheep red blood cells described by (Nolin et al., 2024). Carbon clearance assay by (Hussain et al., 2023). and Lymphoproliferative response to avian tuberculin by (Maqbool et al., 2023).

Histopathological parameters

The following organs bursa of Fabricius, thymus, liver, and kidney were collected and preserved in 10% neutral buffered formalin. Organs were processed for histopathological examinations by the method of (Bancroft and Gamble, 2008).

Growth performance parameters

Every week, the weight of the birds was measured, all birds were weighed to determine their average weight gain every week. The feed intake of treated birds was recorded on the daily basis by using following formula:

Feed Intake (FI) = Feed given to birds in 24 hours – Feed left after 24 hours

The feed intake per bird in a group was calculated. Morbidity, mortality, and case-fatality were monitored daily, and necropsy of dead birds was performed to investigate macroscopic gross lesions and tissues were taken for histological alterations. After the birds were slaughtered, the liver and immune organs (spleen, thymus, and bursa) were extracted and used to obtain relative organ weight from absolute organ weight and live bird weight using the formula below.

Relative organ weight = Absolute organ weight (g) / Total body weight (g) X 100

Hematological parameters

The blood samples were collected at the time of slaughtering in tubes containing EDTA to note down the following hematological parameters.

RBCs counting, WBCs counting, PCV (Packed cell Volume), Hemoglobin concentration.

Serum biochemical parameters

Blood was collected at the time of slaughtering without anticoagulant and serum was separated and stored for serum biochemical evaluation. Aspartate

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aminotransferases (AST), Alanine aminotransferases (ALT) through Merck commercial kit, Blood urea (mg/dL) Urea concentration in the blood was measured by using commercially available kits of Merck company catalogue no. 5.17611.0002. Creatinine (mg/dL) Urea concentration in the blood was measured by using commercially available kits of Merck company catalogue no. 5.17530.0002. Total proteins and Serum albumin Bioclin® kit was used for serum total proteins. Serum globulins were calculated by differentiation method.

Serum globulins = Total serum protein – Serum albumin

Carbon clearance assay

The carbon clearance assay or mononuclear phagocytic system function assay was used to determine the ability of macrophages present in the bird's body to phagocytose the foreign particles injected into the blood of birds and effect of copper nanoparticles toxicity on the ability of this system was also observed.

Avian tuberculin test

The avian tuberculin test was performed to assess the hypersensitivity of basophils in response (CBH response) to avian tuberculin injected in the skin of birds. The method of Corrier (1990).

CBH Response = Thickness at specific time (after injection) of right claw – thickness before injection (0 hrs.) of right claw

Antibodies response against sheep RBCs

The production of antibodies against sheep RBCs injected in the birds was assessed.

Histopathology

On day 14 and 37 of the experimental trial the birds were humanely slaughtered according to Islamic procedure and the immune organs like kidney, bursa, thymus, and liver were collected and before slaughtering the live weight of birds were calculated. Both the absolute organ weight and the live weight were noted to find out the relative organ weight. Histopathology was performed to observe the alteration in the microarchitecture of the tissues and cells.

Results

Weekly body weight

During the first there weeks were non-significant differences among all the groups. During the 4th, 5th, and 6th week of trial the Curcumin extract supplemented groups G-C and G-D significantly increased body weight from all other groups. During the fourth week, group G-D and G-C significantly increased from control positive while there were non-significant differences between groups G-B and control negative group.

Daily feed intake

The feed intake of birds from each group was assessed daily both refusal feed and newly provided feeds were observed. Birds from each group on an average basis were subjected to feed calculations. G-B has significantly improved feed intake as compared to G-D during the start of the trial till the end. However, non-significance was observed between G-A and G-D. Same wise G-C has significantly improved FCR value than G-A.

Mortality

During the trial the highest mortality 53.669% was observed in G-B group which is the control positive group while in groups G-A, G-C and in Group-D mortality was observed 3.33%, 0.00% and 6.66% respectively.

Relative organ weight

Bursa of Fabricius

On 14th post-infection day, the mass of the bursa in the influence negative showed a significant increase than that of the influence treated groups, while the G-C group was not substantially different from the control negative group but substantially higher than G-B and the other G-D groups were considerably different from the control favorable but dramatically decrease than the regulate negative group. The weight of bursa of Fabricius of G-D was significantly greater than the weight of G-B IN experiment two.

Thymus

T-lymphocytes are created in the thymus and play a crucial part in the immune system's development. In experiment I, which took place on the 14th day after

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infection, the weight of thymus in groupings G-C and G-A was considerably higher than in the control positive group, although group G-C was not statistically different from the G.A, in experiment 2 the weight of the thymus in groupings G-D was considerably higher than that of the control group, which was G-C.

Kidney

During a load of infection, the kidney was bulging and was out of the socket in the first Slaughtering in G-B and G-D. Regressed kidneys were observed in G-A and G-C. In the second experiment at 37th days of trial G-D kidneys were apparently less swollen due to use of curcumin as compared to group-2, however non-significant result was seen in G-A and G-C in both experiments.

Liver

The weight of the liver in group G-D was considerably higher than in the control positive group, but not statistically different from the control negative group. In experiment 2 (at 37 days), the weight of the liver in G-C and G-D was considerably higher than in the control positive, although the weight of the liver in G-B was not statistically different from the control negative. The size of the liver was increased and brittle in G-B and G-D in the first killing, but not in G-A and G-C. In the second slaughtering, the G4 liver differs significantly from G-B liver.

Hematological parameters

Total leukocyte count

White blood cells (WBCs) in slaughtering G-A were considerably fewer in groups G-A and G-C compared to the control positive group, whereas (TLC) were significantly higher in the control negative group. While in slaughtering G-B, G-C was not substantially different from the control negative group and G-D was much lower than in the G.B indicating that the infection had eradicated.

Hemoglobin concentration (Hb)

In slaughtering G-A the Hemoglobin concentration in group G-D was significantly increased from the G.B. while the group G-D significantly decreased from the control negative group while there were observed non-significant relationships between G-C and control negative group. In slaughtering G-B Hemoglobin concentration in groups G4, were significantly increased from the control G.B. G-C has a non-significant difference from the control negative group.

Packed cell volume (PCV)

PCV in group G-D was significantly increased from the control positive while no significant difference from the control negative while G-D has significantly decreased and increased G-C from the control negative in slaughtering G-A and slaughtering G-B.

Serum biochemistry parameters

ALT and AST

ALT and AST were significantly lowered in bird's supplemented Curcumin extract compared to other groups. G-D fed with curcumin shows considerably lower plasma AST activity as compared to G-B. This was observed in both slaughtering.

Urea and Creatinine

The results indicated that G-D had considerably higher urea and creatinine levels than G-B. G-D, on the other hand, has a non-significant value with the control being negative. Serum biochemistry depicts nutrition metabolism in the body and emphasizes variations caused by internal and external causes.

Serum proteins

The results showed that the influence of various curcumin supplements in G-D and G-C has significantly improved protein serum ratio as compared to G-B. This was measured at both experiments. Total protein ratio and globulin ratio was significantly improved in the curcumin group.

Carbon clearance assay

As the Indian ink was injected in 3 minutes and at 15 minutes the blood samples were collected, then these samples were centrifuged and then checked for increased absorbance by a photo spectrometer at 640 nm wavelength. Increased absorbance %age at 3 minutes in G-D was significantly decreased from the control positive while there were non-significant occurrences among G-C and control negative groups. At 15 minutes the increased absorbance percentage in groups G-D was significantly decreased from the

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control positive. The G-C was significantly increased from the control negative while there is non-significant difference between the G-C and the control negative group.

Lymphoproliferative Response to Avian Tuberculin

As 0.1 ml avian tuberculin was injected between the third and fourth interdigital space of birds and after injection, the birds were marked. After 24 hours the inflammatory response in G-D was significantly increased from the control positive while there was a non-significant difference among G-B. At 48 hours the inflammatory response in group G-D was significantly increased from the control positive while there is non-significant difference among these G-A and G-C groups and the G-B.

Response of antibodies against RBCs (sheep red blood cells)

The immunity of RBCs after the first and second weeks of primary injection.

There was no significant difference among the groups during the first and second weeks of RBC primary infusion, after booster administration, RBC antibody response was measured. Antibody IgA and IgG titers were substantially greater in groups G-C and G-D than in the positive control group, but not substantially higher in the negative controls.

Histopathology

Thymus

Microscopic analysis of the thymus has revealed the negative group number of empty spaces increased to some extent in Hassall's corpuscles. Vacuolation was also increased to some level; there were non-significant changes identified in group G-C while in group G-D the lymphocytic depletion was more than in the G-C group. In groups G-B and G-D congestion and lymphocytic depletion were observed. In the control positive group G-B due to lymphocytic depletion, a significant rise in empty spaces has been found. Increased number of vacuoles in the Hassall's capsule. Pinkish myelin bodies filled the medullary region.

Bursa of Fabricius

A microscopic examination of the bursa revealed medulla was darkly pigmented and intact, these alterations were as noticeable as those seen in interfollicular connective tissue. There was a significant increase in interfollicular connective tissue in the control positive group. Increased empty spaces are caused by lymphocytic loss in the cortex and medulla. The surface epithelium remained intact, although there were many tiny crypts.

Liver

There are no microscopic lesions in the livers of control negative birds or control diet turmeric in G-C chickens. The level of liver damage varies, with severe to moderate hepatic congestion, hepatocyte vacuolar degeneration, hepatocyte necrosis, and cellular infiltration observed in G-B. In the livers of birds given curcumin extract in G-D, histopathologic lesions exhibited moderate and severe congestion, vacuolar degeneration, and severe necrosis.

Kidney

G-B birds caused significant kidney congestion, as well as degeneration and necrosis of tubular epithelial cells. Turmeric supplementation in G-D caused lesions progression from moderate to mild. G-B was found to have renal deterioration and necrosis. In the same G-B group, some renal tubules demonstrate vacuolar degeneration and epithelial necrosis. There were no microscopic alterations in the kidneys of G-C or control negative birds.

Discussion

Poultry production in Pakistan plays a vital role and is a growing sector of agriculture that contributes remarkably at the rate of (1.3%) of the total GDP (Mohamed et al., 2023). Herbal extracts are one of the veterinary medicine choices for supplementing immunizations. comprehensive procedure for detention of IBDV is using herbs *Curcuma long. Linn* extract @2gm /kg body weight has better antiviral effects and shows better results as compensatory to vaccination (Dhama et al., 2018).

The current research are line with Arslan et al. (2022) tested all degrees of supplementation, his report confirms improved feed conversion quality. Curcumin can help broilers develop faster, have better immune responses, have a higher dressing percentage, and have a lower cholesterol profile (Mondal et al., 2015). Similarly the use of turmeric at 0.50 percent in

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the diets that can improved body weight gain and feed conversion ratio and there was a substantial improvement in feed intake, enhance immune response, histopathological change in broiler chicken (Khan et al., 2012a). Simultaneously, there was no significant difference for edible components, but there was a significant difference (P>0.05) for dressing percent for all treatments compared to the control group. The impact of turmeric (*Curcuma longa)* supplementation on broiler chicken development and blood chemistry was investigated, and the study is line with (Javed et al., 2009). The inclusion of Curcuma longa powder resulted in a substantial (P>0.05) increase in body weight growth and serum indices content (Arslan et al., 2017).

Curcuma longa supplementation, on the other hand, had no influence on the blood biochemical markers of birds (P>0.05). The current findings verified the positive benefits of dietary *Curcuma longa* powder on broiler chicken body weight and Hb content. The presence of active chemicals in turmeric, namely antioxidant activity, which increases protein synthesis by the avian enzymatic system, that may increase growth performance (Sugiharto et al., 2011).

In slaughtering G-A and slaughtering G-B, the weightage of bursa of the control –VE group was significantly higher than that of the control positive group, Current results show significant relation with G1 and G3. In the 5th and 6th week kidney weight drastically decreased in G2 and G4 showed a significant relation comparatively to group 1st and 3rd these results are line with Khan et al. (2012b) investigated the effects of rhizome powder and black pepper on adult broiler chickens. dietary treatment has substantial improvement in organs weightage especially lymphatic organs. demonstrated that a TRE-supplemented diet at 2% increased the weight of the thymus, bursa, and liver, portrayed the optimal concentration of curcumin supplementation and its influence on broiler chick, organ weight, and nutrient utilization. Dietary turmeric powder increased live body mass, relative immunological organ (bursa, thymus) weight and feeding efficiency (Shohe and Vidyarthi, 2020). Mazhar *et al.* (2001) studied that Turmeric supplementation improved growth performance and carcass weight. According to the findings, Body weight gain and bursa size were enhanced by supplementation at 1% and 1.5 percent curcumin powder. During the trial, the daily feed intake was evaluated G-C has an overall improved FCR rate as compared to the controls groups and other groups. G-D has shown significantly improved daily feed intake than the control positive and non-significant relation was seen between G-C and the control negative. Suharto *et al*. (2020) conducted an experiment that showed that FCR greatly improved in birds fed with curcumin extract and non-significantly decreased feed intake in non-curcumin fed groups.

The RBCs in slaughtering one there were significantly increased in G-D as compared to the control positive while the groups G-A and G-C were non-significantly different. In slaughtering two same patterns were observed. The WBCs in G-C and G-D were significantly lower from the control positive group and G-C and G-D had significantly increased from the control negative group. While in second slaughtering G-C and G-D were non-significantly different from the control negative group and significantly decreased from the control positive group. In slaughtering 1 Hb conc in G-C and G-D were significantly improved from the control positive while G-C and G-D had non-significant difference from the control negative group. In slaughtering 2 the Hb conc G-C and G-D were significantly increased from the control positive where G-C and G-D had non-significant relation with the control negative group. Current research is line with Rajput and Wang (2013) investigated the effects of neem and turmeric on performance of hematological markers in broiler birds. Neem fed broilers showed significantly improved hemoglobin concentration than non-fed. Current results are line with Baran *et al.* (2016) demonstrated when neem along turmeric were given birds showed increased PCV volume.

The hematocrit values of G-C and G-D were substantially higher than those of the control positive group, however G-C and G-D had no significant relationship with the control negative group. The Hematocrit value of G-C and G-D in the second slaughtering follows the same pattern as in the first slaughtering. Osman *et al.* (2012) investigated the effect of curcumin extract on albino rat and rabbit blood parameters. He claimed that the group G-C in which 2mg curcumin extract has been given has more platelets, RBCs count the same for Hb. G-D RBCs and Hb also significantly increased while the other groups had non-significant differences. Church *et al.*

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(1984) has suggested that curcumin extract has significant effects on blood components. David *et al.* (2002) has reported that the supplementation of curcumin causes the Hb, lymphocytes, platelets, and RBCs to increase significantly in rats whereas PCV, RBCs and Hb was significantly increased in rabbits while all other parameters indicated unchanged. Adgebite *et al.* (2016) reported that there were non-significant increments in RBCs and Hb concentration in the group given curcumin. Also, there was a non-significant increment in Hct. Hb and PCV. WBC counts were high in the given group. Rajpoot and Wang (2013) investigated the effects of neem, turmeric, vitamin E, and their combinations on performance and hematological in broilers, hematological examinations revealed that the presence of hemoglobin in the neem-fed group was significantly (P0.05) greater (10.5 g/dL) than in the turmeric-fed group (9.85 g/dL); in contrast, the diet that contains turmeric alone had the least hemoglobin level.

ALT and AST were significantly lowered in birds supplemented with Curcumin Extract G-C and G-D compared to other groups. G-D fed with curcumin shows considerably lower plasma AST activity as compared to G-B, this was observed in both slaughtering and line with Adegoki and Iposa (2018) looked at the plasma biochemistry review and reference of broiler chicks given turmeric and cayenne pepper as antioxidants. Yildrim *et al.* (2004) found that the curcumin diet has improved liver function. These results indicate that the beneficial effect of curcumin is that it improves the integrity of hepatic cells due to hydrophobic compounds. Qasem *et al.* (2016) examined various stages of turmeric powder in mixed broiler chicken.The activity of liver enzymes were high in non-fed groups. Odetola *et al.* (2012) demonstrated that a graduated suspension of curcumin dramatically reduced ALT activity in broiler chicks. Oloruntola *et al.* (2002) discovered that dietary curcumin extract substantially reduced ALT activity in broiler chicken. The results indicated that G-D had considerably higher urea and creatinine levels than G-B. G-D, on the other hand, has a non-significant value with a control negative.

Total protein ratio and globulin ratio were significantly improved in the curcumin group are linn Russel *et al.* (1999) concluded that curcumin supplemented diets in broilers increased protein activity, increased the percentage of albumin and globulin and improved T and B cells proliferation.

The macrophages were tested by an assay of carbon clearance. Increased absorbance percentage in three minutes were observed in G-C and G-D and was significantly decreased from the control positive are line with Park *et al.* (1992) used the Carbon clearance test to demonstrate the immunomodulatory effects of curcumin extract in male mice. He found CCA decreased with passing minutes, indicating that phagocytic activity increased in every curcumin extract supplanted group. The current results are in line with Akhouri *et al.* (2014) Curcumin is a great source of protein, carotenes, Vitamin C, calcium, potassium, and functions as natural antioxidants. Hanna *et al.* (2002) observed phenolic compounds are present in turmeric that can help to prevent oxidative stress by scavenging free radicals and bio-activating carcinogens for liver excretion. To determine cell-mediated immunity, delayed hypersensitivity was tested by injecting sheep red blood cells as an allergen. Titers were measured after the first and second weeks of initial injection to assess RBC antibody response are line Deshmukh *et al.* (2015) conducted a similar investigation on group rats, supplementing groups with turmeric extract (aqueous + ethanolic).as per his findings Polyphenol compounds have been closely involved in eliminating NO molecules, preventing cholesterol and oxidative tissue damage caused by low density lipoproteins.

Deshmukh *et al.* (2015) reported that the lymphoproliferative response significantly increased in aqueous and ethanolic extract supplemented groups are line with Ferreria *et al.* (2008) indicated Phenylalanine, lysine, arginine, methionine, and histidine are present in *Curcuma longa*. Arginine is reported to stimulate cell immunity and increase lymphoproliferation and macrophage proliferation.

Histopathological data indicated that the thymus and bursa of birds in group G-B had severe lymphocytic depletion, whereas treated group G-C had mild depletion when compared to the control negative group and considerably better than all other groups except G-B. Groups G-C and G-D showed moderate lymphocytic depletion are line with Youxiang and Qiang (2011) published research on microscopic data that revealed a large decrease in lymphocytes, a

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reduction in lymph follicles, reticuloendothelial cell hyperplasia, and distortion owing to congestion and hemorrhage. Atif *et al.* (2014) depicted thymic lobular dysplasia, reduced lymphocytes in the medulla, resulting in an increase of epithelial reticular cells and fat cell infiltrate into the thymus material were also observed, all of which contributed to thymus degeneration. Moderate lymphocytic depletion and epithelial cell hyperplasia in the bursa, as well as mild hemorrhages and congestion in the thymus. The current research is in line with Uddin *et* al. (2012) notified cells, resulting in severe immunological suppression by damaging both B lymphocytes of the bursa and T lymphocytes of the thymus. Murmur *et al.* (2014) discovered similar histological bursal lesions, including moderate to severe lymphoid depletion in the bursal follicle, cyst formation, follicular necrosis, and follicular bleeding.

The current research is lined with liao and Liu (2012) showed tubular degeneration, necrosis and dilation. Majeed and Gholami-Ahangaran *et al.* (2016) displaying the liver mild degenerative alterations like congestion and perivascular cellular infiltration. Soto *et al.* (2007) showed fatty alteration with lymphoid cellular infiltration, suggesting the participation of mycotoxin-induced damage. Haghighi *et al.* (2004) credited liver to be hyperemic with circular borders.

Conclusion

This current research concluded that *Curcuma longa L.* extract at 1mg /kg body weight has better antiviral effects. It can give the best results when used with vaccination. Curcumin can be used as a growth promoter and immunizer in broiler chicks. Moreover, Toxicity chances are rare. In association with vaccination, it works somehow as a replacement to antiviral drugs to overcome the antiviral resistance. Curcumin, an active ingredient of *Curcuma longa*, has immunity-boosting effects.

Table 1. Relative organ weight and hematology of broiler birds infected with infectious bursal disease virus, treated with Curcumin extract in different groups in slaughtering-one (Mean ± SD)

Groups	Group A	Group B	Group C	Group D	P-valve
Relative Organs Weight at first experiment					
Bursa	0.298 ±0.010[b]	0.175 ±0.01[cd]	0.322 ±0.001[a]	0.189 ±0.03[c]	0.002
Liver	3.53±1.52	2.76±2.38	5.23±1.52	3.71±3.36	0.011
Kidney	0.37±0.07[c]	0.63±0.06[a]	0.39±0.09[c]	0.42±0.10[b]	0.001
Thymus	0.073 ±0.015[b]	0.047 ±0.030[d]	0.094 ±0.023[a]	0.065 ±0.001[c]	0.001
Relative Organs Weight at 2nd experiment					
Bursa	0.176 ±0.08[b]	0.124 ±0.01[d]	0.322 ±0.01[a]	0.171 ±0.05[bc]	0.012
Liver	2.75±0.72	1.80±0.04	3.79±0.19	3.00±0.39	0.021
Kidney	0.53±0.11[b]	0.71±0.05[a]	0.56±0.04[b]	0.64±0.09[ab]	0.001
Thymus	0.157 ±0.021[b]	0.085 ±0.02[d]	0.190 ±0.01[a]	0.109 ±0.08[c]	0.001
Hematology of broiler in first experiment					
Hb/ g/dl	11.26 ±0.70[a]	9.03 ±0.40[b]	11.5 ±0.86[a]	9.3 ±0.52[b]	0.012
Hct/ %	35.67 ±4.93[ab]	27.66 ±1.52[b]	27.66 ±1.73[a]	31.67 ±3.06[ab]	0.001
RBC/ 10^6/ul	35.67 ±0.2[b]	27.66 ±0.30[c]	38 ±0.32[a]	31.67 ±0.30[b]	0.001
WBC/ cell/ul	6.73 ±1.07[b]	16.93 ±0.90[a]	5.13 ±0.58[b]	5.13 ±0.63[b]	0.000
Hematology of broiler in 2nd experiment					
Hb/ g/dl	9.9 ±0.14[b]	6.4 ±0.56[c]	11.6 ±0.57[a]	9.9 ±0.14[b]	0.001
Hct/ %	32.5 ±2.12[b]	28.5 ±0.70[b]	39 ±0.73[a]	37.5 ±0.75[a]	0.011
RBC/ 10^6/ul	3.2 ±0.28[bc]	2.35 ±0.63[c]	4.85 ±0.07[a]	4.35 ±0.71[ab]	0.001
WBC/ cell/ul	6.4 ±0.56[b]	14.65 ±0.21[a]	5.8 ±0.41[b]	7.2 ±0.70[b]	0.001

Values (Means ± SD) with different letters are significant statistically different from control positive group ($p > 0.05$). Values (Means ± SD) with different lettering are significant statistically different from control negative group ($p > 0.05$).

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Table 2. Growth performance and mortality percentage of broiler birds infected with infectious bursal disease virus and treated with Curcumin extract (Mean ± SD)

Groups	Group A	Group B	Group C	Group D	P-valve
Feed intake from week first to sixth					
Week 1	45.71±5.70	38.71±3.54	41.00±5.59	41.57±8.22	**0.23**
Week 2	49.85±5.66	48.74±6.26	61.51±2.08	55.09±3.67	**0.78**
Week 3	87.43±4.99[b]	78.15±3.65[c]	95.84±3.86[a]	91.26±3.69[ab]	**0.001**
Week 4	135.97±18.63[a]	107.02±9.35[b]	131.40±15.08[a]	142.19±9.48[a]	**0.021**
Week 5	147.09±5.01[ab]	139.71±9.79[b]	156.92±9.56[a]	149.20±3.88[ab]	**0.001**
Week 6	147.09±5.01[b]	140.42±10.7[a]	161.71±9.06[a]	149.85±4.70[b]	**0.001**
Live body weight from week first to sixth					
Groups	Group A	Group B	Group C	Group D	P-valve
Week 1	75.64±3.54[b]	73.15±4.72[b]	89.89±5.84[a]	79.67±13.13[b]	**0.892**
Week 2	169.11±10.96[b]	161.33±18.02[b]	190.33±19.55[a]	194.7±11.71[a]	**0.789**
Week 3	551.7±61.10[b]	393.22±35.97[d]	627.11±40.69[a]	462.00±21.33[c]	**0.001**
Week 4	832.11±53.91[b]	676.8±34.26[d]	963.1±38.70[a]	796.9±52.20[c]	**0.011**
Week 5	1557.12±83.5[b]	1109.2±51.27[d]	1687.79±62.39[a]	1378.61±79.51[c]	**0.001**
Week 6	1937.80±63.94[b]	1573.16±85.7[b]	2115.36±91.84[a]	1886±80.95[bc]	**0.001**
Mortality rate of birds for 7 weeks each group having 30 birds					
Groups	Group A	Group B	Group C	Group D	%
Week 1	-	-	-	-	--
Week 2	-	-	-	-	--
Week 3	-	-	-	-	--
Week 4	1	10	-	3	**53.55**
Week 5	0	6	-	2	**16.34**
Week 6	0	0	-	-	--

Values (Means ± SD) with different letters are significant statistically different from control positive group (p> 0.05). Values (Means ± SD) with different lettering are significant statistically different from control negative group (p> 0.05).

Table 3. Serum indices of broiler birds infected with infectious bursal disease virus, treated with Curcumin extract in different groups in experiment-1 and experiment-2(Mean ± SD)

Groups	Group A	Group B	Group C	Group D	P-valve
S-1 ALT	14.307±0.428[c]	33.045±0.468[a]	18.326±0.351[b]	15.745±0.421[c]	0.001
S-2 ALT	13.703±0.259[c]	32.124±0.638[a]	17.983±0.323[b]	15.066±0.187[c]	0.01
S-1 AST	125.64±1.411[c]	190.46±2.645[a]	164.35±11.292[b]	140.81±5.040[bc]	0.01
S-2 AST	111.28±4.161[d]	206.53±5.514[a]	171.45±4.198[b]	131.44±1.438[c]	0.000
S-1 urea	25.518±0.580[c]	38.644±0.154[a]	31.809±0.842[b]	27.577±0.453[c]	0.001
S-2 urea	26.033±0.284[c]	39.923±0.266[a]	29.387±0.535[b]	27.646±0.799[bc]	0.003
S-1 creatinine	1.588±0.02[c]	2.676±0.03[a]	2.227±0.13[b]	1.747±0.004[c]	0.001
S-2 creatinine	1.495±0.06[d]	3.127±0.06[a]	2.407±0.03[b]	1.778±0.008[c]	0.002
S-1 BUN	11.992±0.18[d]	18.188±0.07[a]	14.995±0.39[b]	13.017±0.21[c]	0.001
S-2 BUN	12.296±0.13[c]	18.786±0.12[a]	13.863±0.25[b]	13.049±0.37[bc]	0.002
S-1 TP	2.80±0[a]	1.95±0.21[b]	2.45±0.49[a]	2.65±0.14[a]	0.001
S-2 TP	3.00±0.14[a]	1.64±0.07[c]	3.33±0.16[a]	2.16±0.09[b]	0.001

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S-1 Albumin	1.42±0.01[ab]	1.11±0.16[b]	1.76±0.02[a]	1.84±0.24[a]	0.000
S-2 Albumin	1.94±0.05[a]	0.77±0.14[a]	2.54±0.31[a]	0.93±1.02[a]	0.002
S-1 Globulin	1.38±0.01[a]	0.84±0.04[a]	0.68±0.47[a]	0.81±0.09[a]	0.002
S-2 Globulin	1.06±0.08[a]	0.87±0.07[a]	0.78±0.14[a]	1.23±0.93[a]	0.001
CLA-3 minutes	158.39 ± 14.8[c]	225.17 ± 26.3[a]	129.67 ± 17.6[d]	182.08 ± 13.9[b]	0.001
CLA-15 minutes	58.20 ± 3.5[c]	130.97 ± 7.3[a]	42.90 ±5.6[d]	95.55 ± 9.7[b]	0.000
LPR-24 hours	0.173±0.01[b]	0.098±0.04[d]	0.201±0.06[a]	0.113±0.02[c]	0.000
LPR-48 hours	0.107±0.03[ab]	0.048±0.02[c]	0.132±0.02[a]	0.098±0.04[b]	0.000

Values (Means ± SD) with different letters are significant statistically different from control positive group (p> 0.05).
Values (Means ± SD) with different lettering are statistically different from control negative group (p> 0.05).
TP; total protein, CLA; carbon clearance assay, LPR; lymph proliferation response

Table 4. Total antibody titer of broiler birds infected with infectious bursal disease virus, treated with Curcumin extract in different groups in experiment 1 and experiment 2 (Mean ± SD)

Experiment-1	Group A	Group B	Group C	Group D	p-valve
Total Ig	7.50±0.707[a]	4.50±0.707[b]	6.50±0.707[ab]	5.50±0.707[ab]	0.01
IgG	5.50±0.707[a]	3.50±0.707[c]	4.00±0.707[b]	4.00±0[b]	0.01
IgM	2.00±0[a]	1.00±0[c]	2.00±0[a]	1.50±0.707[b]	0.01
Experiment-2					
Total Ig	5.50±0.707[a]	4.00±0[a]	4.50±0.707[a]	4.50±2.121[a]	0.00
IgG	4.50±0.707[a]	3.00±0[a]	3.50±0.707[a]	3.00±1.414[a]	0.00
IgM	1.00±0[a]	1.00±0[a]	1.00±0[a]	1.50±0.707[a]	0.00
Experiment-1					
Total Ig	9.00±0.70[a]	4.00±1.414[b]	8.00±0707[ab]	5.00±1.414[ab]	0.02
IgG	7.50±0.707[a]	3.00±1.414[b]	6.50±0.707[ab]	3.50±0.707[b]	0.01
IgM	1.50±0.707[a]	1.00±1.41[b]	1.50±0.707[a]	1.50±0.707[a]	0.01
Experiment-2					
Total Ig	7.00±0[a]	3.50±0.707[b]	7.50±0.707[a]	5.50±0.707[ab]	0.01
IgG	5.50±0.707[a]	2.50±0.707[a]	5.00±1.414[a]	3.50±0.707[a]	0.00
IgM	1.50±0.707[b]	1.00±0[c]	2.50±0.707[a]	2.00±1.414[a]	0.01

Values (Means ± SD) with different letters are significant statistically different from control positive group (p> 0.05). Values (Means ± SD) with different lettering are significant statistically different from control negative group (p> 0.05).

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