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Effect of turmeric and ginger supplementation on immunity, antioxidant, liver enzyme activity, gut bacterial load and histopathology of broilers

N SAHOO[1], S K MISHRA[2], R K SWAIN[3], A P ACHARYA[4], S PATTNAIK[5], K SETHY[6] and L SAHOO[7]

Orrisa University of Agriculture and Technology, Bhubaneshwar, Odisha 751 003 India

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ABSTRACT

Day-old broiler chicks (182) were distributed randomly to 7 treatments with 2 replicates. Treatments were T_1 (control), basal diet; T_2, basal diet + turmeric powder (TP) (0.5% of basal diet); T_3, basal diet + TP (1% of basal diet); T_4, basal diet + ginger powder (GP) (0.5% of basal diet); T_5, basal diet + GP (1% of basal diet); T_6, basal diet + TP + GP (0.5% TP + 0.5% GP); T_7, basal diet + TP + GP (1% TP + 1.0% GP). The experiment was continued for 35 days. Immunity, antioxidant, liver enzyme activity, gut bacterial load and histopathology of broilers were conducted at fifth week of age**.** Higher cellular response against PHA-P was recorded in T_3 and T_7. Higher antibody titre against SRBC was recorded in T_3. The weight of lymphoid organs did not differ significantly. Significantly higher erythrocyte malondialdehyde (MDA) level was recorded in T_1. Significantly higher alanine amino transferase (ALT) levels were found in T_1 and T_7. Significantly higher aspartate amino transferase (AST) level was found in T_1. Higher total bacterial count and lower *E. coli* count were recorded in group T_3 and lower total bacterial count was recorded in T_7. In group T_1, liver showed mild congestion to mild cellular swelling and varying degree of vacuolar degeneration. From this study, it may be concluded that supplementation of 1% turmeric in ration either alone or in combination with 1% ginger improved the immunity, antioxidant status and gut health of broilers.

Key words: Antioxidant, Broilers, Ginger, Gut bacterial load, Histopathology, Immunity, Liver enzyme, Turmeric

The use of growth-promoting antibiotics as feed additives associated with storage of undesirable residues in the meat and eggs of poultry products, which may be harmful to man when consumed have been banned or limited in many countries due to these suspected residual effects (Diarra *et al*. 2011). The herbs and medicinal plants have attracted attention due to their wide range of potential beneficial effects (Manesh *et al*. 2012). These alternatives should be safe for animals and humans, environment friendly and address organic livestock issue (Cabuk *et al*. 2006). Phytobiotics, compounds of plant origin, are incorporated into animal feed to enhance livestock productivity through the improvement of digestibility, nutrient absorption and elimination of pathogens in the animal gut (Athanasiadou *et al*. 2007). Plants (e.g. turmeric and ginger) under *Zingiberaceae* family possess phenolics substances which have strong anti-inflammatory and anti-oxidative properties and exert substantial anti-carcinogenic and anti-mutagenic activities (Lee and Surh 1998). Turmeric (*Curcuma longa*) contains bioactive substances such as curcumin, bisdemethoxycurcumin, demethoxycurcumin, which are mostly isolated from rhizome of turmeric and present at the level of 2–5% of total spice in turmeric powder (Nouzarian *et al*. 2011). The major bio-active ingredient of turmeric is curcumin (diferuloylmethane), a lipophili-cpolyphenol that is practically insoluble in water but quite stable in the acidic pH of the stomach (Jurenka 2009). Curcumin, demethoxycurcumin and bisdemethoxy-curcumin are yellowish turmeric pigments and possess many pharmacological activities including antioxidative, anticarcinogenic, anti-inflammatory, antihepatotoxic and hypocholesterolemic activities (Nishiyama *et al*. 2005). Kim *et al*. (2013) showed that the chickens fed turmeric supplemented diets had enhanced systemic humoral immunity as assessed by greater levels of serum antibodies to an Eimeria micro-neme protein, MIC2, and enhanced cellular immunity as measured by concanavalin A-induced spleen cell proliferation. Turmeric supplementation improved the antioxidant capacity of broilers by increasing SOD and GSH-Px activities and decreasing serum MDA concentrations in broilers (Wang *et al*. 2015). Active compounds present in ginger (*Zingiber officinale*) have many such as atsiri oil, bornoeol, kamfen, limonene, humulen, gingibrol, gingiberen and gingerdiol (Rismunandar 1988). Gingerol, gingerdiol and gingerdione,

Present address: [1]Ex-Post Graduate Scholar (nibedita71.ns @gmail.com), [2]Professor (sumantkmishra @gmail.com), [3]Professor and Head (rkswainovc@yahoo.com), [6]Assistant Professor (babuivri@gmail.com), Department of Animal Nutrition; [4]Associate Professor (email..??), Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry. [5]PhD Scholar (email..??), Department of Microbiology. [7]Scientist, ICAR-Central Institute of Freshwater Aquaculture, Puri, Odisha.

the main important compounds in ginger, have the ability to stimulate digestive enzymes and affect the microbial and antioxidative activities (Dieumou *et al*. 2009). Ginger essential oils have ability to control pathogens owing to their antimicrobial activity (Dorman and Deans 2000). The aqueous extract of ginger rhizome mixed in water plays better performance as immune stimulant against ND, IB, IBD and coccidiosis (Nidaullah *et al.* 2010). Reported work on combined effect of supplementation of ginger and turmeric on the studied parameters are limited. The objectives of this study were to investigate the effect of turmeric and ginger in broiler birds on immunity, antioxidant, liver enzyme activity, gut bacterial load and histopathology of liver.

MATERIALS AND METHODS

Animal diet and experimental procedure: Commercial (BV 400) day-old broiler chicks (182) were distributed randomly in 7 dietary treatments. Each treatment group had 2 replicates containing 13 chicks in each replicate. The chicks were provided 24 h free access to clean drinking water. Starter feed was given up to 3 weeks and finisher feed was offered till 35th day. Turmeric and ginger was purchased from local market. The ginger used was observed for any physical defect, cleaned and washed in running tap water to remove adhering debris. It was cut into small pieces, sun-dried, and ground into powder form. Turmeric was sun-dried and ground into powder form. The ginger and turmeric were analyzed for proximate composition (AOAC 1995; Table 1). Basal diets were prepared to meet the nutrient requirement of starter and finisher phases of broilers birds (BIS 1992). The dietary treatments of the experiment were: T_1, basal diet (broiler starter/ broiler finisher); T_2, basal diet + TP (0.5% of basal diet); T_3, basal diet + TP (1% of basal diet); T_4, basal diet + GP (0.5% of basal diet); T_5, basal diet + GP (1% of basal diet); $T_{6,}$ basal diet + TP + GP (0.5% TP + 0.5% GP); T_7, basal diet + TP + GP (1% TP + 1.0% GP).

Measure of cellular immunity: At fifth weeks of age, 2 birds from each replicate in each dietary treatment were injected intra-dermally in the comb with 100 micro gram of phytohaemaglutinin-P (PHAP) in 0.1 ml of normal saline to measure the cellular immune response by cutaneous basophillc hyper sensitivity (CBH) test (Edelman *et al*. 1986). The thickness of comb was measured using digital calliper before inoculation and 24 h post inoculation and CBH response was calculated using the formula:

$$\text{CBS response} = \frac{\text{Post-injection skin thickness}}{\text{Pre-injection skin thickness}} \times 100$$

Pre-injection thickness

Measure of humoral immunity: The measure of humoral immunity was carried out as per Abdallah *et al*. (2009). Sheep red blood cells (SRBC) were used as test antigens to quantitatively analyse specific antibody response as measure of humoral immunity. At fifth week of age, 2 birds from each replicate in each dietary treatment were immunized intravenously via a wing vein with 0.07 ml packed RBC mixed with 0.93 ml physiological saline (0.9% NaCl) for measurement of primary response. The SRBCs were obtained in heparin solution from local sheep (reared at Instructional Livestock Farm, Bhubaneswar, Odisha) and washed 3 times in physiological saline. Seven days following the antigen challenge, blood samples were collected and serum samples were used to measure humoral immunity. Antibody production to SRBC was measured according to Bachman and Mashaly (1986) and Kai *et al*. (1988). All SRBC antibody titres were expressed as $\log_2$ of the reciprocal of the highest serum dilution causing agglutination of SRBC.

Processing of immune organs: At fifth week of post feeing, 4 birds from each treatment were randomly chosen and slaughtered for collection of spleen, bursa of fabricius and thymus. The birds were kept off fed overnight and live weights of the birds were recorded before slaughter. The birds were bled by modified Kosher's method (Panda and Mohapatra 1989). Spleen, bursa of fabricius and thymus were weighed in a top pan electronic balance.

Estimation of lipid peroxidation (LPO): The lipid peroxide level in the RBC haemolysate was determined as per Placer *et al*. (1966). A blank was run simultaneously by incorporating 0.2 ml of distilled water instead of the RBC haemolysate. The absorbance was read at 548 nm using a spectrophotometer. The concentration of malondialdehyde (MDA) per mg of Hb was calculated using the extinction coefficient of 1.56×10^8 L/mmol/cm (Utley *et al*. 1967). This was also expressed in µmol of MDA/mg haemoglobin.

$$\text{LPO (µmol MDA formed/mg Hb)} = [(\text{ODT} / \bullet) \times (\text{TV} / \text{VT}) \times \text{df} \times (1/ \text{mg Hb})] \times 10^9$$

where, ODT, absorbance of test; •, molar extinction coefficient (1.5×10^5)/m/cm; TV, total volume of reagent with sample taken; VT, volume of sample taken; df, dilution factor.

Estimation of liver enzyme: At the end of fifth week, blood was collected from 4 randomly selected birds from each treatment and serum samples were prepared for determination of AST and ALT levels. The serum AST and ALT were determined by using Crest biosystems (Goa, India) Kit.

Collection of intestinal content and estimation of bacterial load: Birds were sacrificed and eviscerated. The ileum was separated aseptically and the digesta within the 2/3rd caudal area of the ileum was collected aseptically

Table 1. Proximate composition of turmeric and ginger

Parameter (% on dry matter basis)	Turmeric	Ginger
Moisture	10.96	9.66
Crude protein	11.37	7.87
Crude fibre	4.7	8.75
Ether extract	9.73	6.64
Total Ash	6.73	6.46
NFE*	56.51	60.62

*calculated

into sterile bottles containing normal saline solution. Spread plate technique was used in bacterial load determination. Digesta (1 ml) was used for serial dilution in sterile test tubes, containing 9 ml normal saline solution. Serial dilution of digesta was made to 10^{-10} dilution level. The dilution (0.1ml) was pipetted out and inoculated on nutrient agar and MacConkey agar. The sample was spread evenly over the surface of agar using the sterile glass spreader and carefully rotating the petridish underneath at the same time. The plate is incubated at 37°C for 24 h. Discrete colonies on plates were counted using colony counter and estimated in $\log_{10}$ CFU/ml.

Histopathology of liver: For histological study, representative portion of liver of 5-week-old layer chicks, were collected in 10% formal saline solution during carcass characteristics study. The formalin fixed tissues were processed by routine histological techniques. The fixed tissues were washed over night in running tap water and dehydrated in ascending grades of alcohol and cleared in xylene. Paraffin blocks were prepared as per the routine procedure and sections were cut at 5 micron thickness and stained by routine haematoxylin and eosin method. Stained slides were examined under microscope for histological interpretations.

Statistical analysis: Data retrieved from the experiment was subjected to statistical analysis wherever required. The statistical analysis of the data was done according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Cellular (CBH) response against PHA-P, humoral immune response against SRBC, MDA levels, AST and ALT levels, gut bacterial count and weight of lymphoid organs of experimental broilers under different dietary treatments at fifth weeks of age are presented in the Table 2.

Immunity status: The cutaneous basophillc hyper sensitivity (CBH) response against PHA-P of broiler birds at fifth week of age were 109.82±9.35, 136.65±7.00, 156.91±12.88, 133.29±3.88, 137.50±13.88, 126.31±8.83 and 155.77±11.72 in T_1, T_2, T_3, T_4, T_5, T_6 and T_7, groups, respectively. Significantly (P<0.05) higher cutaneous basophillc hyper sensitivity (CBH) response against PHA-P was recorded in group T_3 and T_7. This implied that turmeric and ginger at 1% level of supplementation in combination and turmeric at 1% level showed higher cellular immune response of broiler birds to PHA-P. Kim *et al*. (2013) reported that the chickens fed turmeric supplemented diets had enhanced systemic humoral and cellular immune responses compared with controls. The better cellular response that observed in T_7 than that of T_6 might be owing to the combined effect of ginger and turmeric at higher levels. Similarly, Abou-Elkhair *et al*. (2014) reported significant rise in the antibody titre value against newcastle disease when black pepper, turmeric, and coriander seeds were included together but not separately, which they opined might be due to the collaborative effect of active components in black pepper, turmeric, and coriander seeds. On ginger supplementation (T_4 and T_5) in broiler ration, the cellular immunity was significantly higher than that of control group but no significant effect was observed in humoral immunity.

The Log_2 value HA titre against SRBCs of broiler birds at fifth week of age were 5.00±0.41, 6.75±0.48, 7.50±0.65, 5.75±0.48, 6.00±0.41, 6.50±0.29 and 6.75±0.48 in T_1, T_2, T_3, T_4, T_5, T_6 and T_7 respectively. Significantly (P<0.05) higher HA titre against sheep red blood cell (SRBC) was

Table 2. Cellular and humoral immune responses, MDA levels, AST and ALT levels, gut bacterial counts and weight of lymphoid organs of experimental broilers under different dietary treatments at fifth weeks of age

Parameter	Treatment							P value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	
Immunity								
Cutaneous basophilic hypersensitivity response	109.82^{c}± 9.35	136.65^{b}± 7.00	156.91^{a}± 12.88	133.29^{b}± 3.88	137.50^{ab}± 13.88	126.31^{bc}± 8.83	155.77^{a}± 11.72	0.04
Log2 value HA titre	5.00^{d}±0.41	6.75^{ab}±0.48	7.50^{a}±0.65	5.75^{cd}±0.48	6.00^{cd}±0.41	6.50^{bc}±0.29	6.75^{ab}±0.48	0.02
MDA levels								
MDA (µmol /mg Hb)	3.90^{a}±0.21	2.82^{b}±0.18	2.3^{b}±0.08	2.3^{b}±0.27	2.8^{b}±0.21	2.7^{b}±0.19	2.4^{b}±0.13	<0.05
AST and ALT levels								
ALT (U/L)	27.85^{a}±0.43	24.74^{b}±0.37	24.73^{b}±0.71	25.06^{b}±0.28	24.53^{b}±0.79	24.86^{b}±0.51	24.83^{b}±0.57	<0.05
AST (U/L)	111.45^{a}±0.84	100.62^{b}±0.75	101.56^{b}±0.67	102.09^{b}±1.01	101.70^{b}±1.88	101.86^{b}±0.73	101.90^{b}±1.18	<0.05
Logarithmic value (cfu/ml) of total bacterial and *E. coli* count								
Total bacterial count (cfu/ml)	5.86^{c}±0.13	7.79^{b}±0.02	8.55^{a}±0.09	5.81^{c}±0.03	5.76^{c}±0.05	7.85^{b}±0.11	4.93^{d}±0.03	<0.05
E. coli count (cfu/ml)	4.69^{a}±0.11	4.44^{b}±0.13	3.70^{c}±0.05	4.65^{ab}±0.05	4.76^{a}±0.05	4.33^{b}±0.13	3.28^{d}±0.15	<0.05
Weight of lymphoid organs (% of body weight)								
Spleen	0.23±0.01	0.22±0.01	0.23±0.02	0.25±0.02	0.25±0.01	0.23±0.01	0.23±0.02	0.78
Bursa of fabricius	0.16±0.01	0.17±0.01	0.18±0.01	0.20±0.01	0.17±0.01	0.17±0.01	0.18±0.02	0.46
Thymus	0.50±0.04	0.52±0.01	0.53±0.03	0.60±0.05	0.56±0.02	0.50±0.04	0.53±0.02	0.51

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

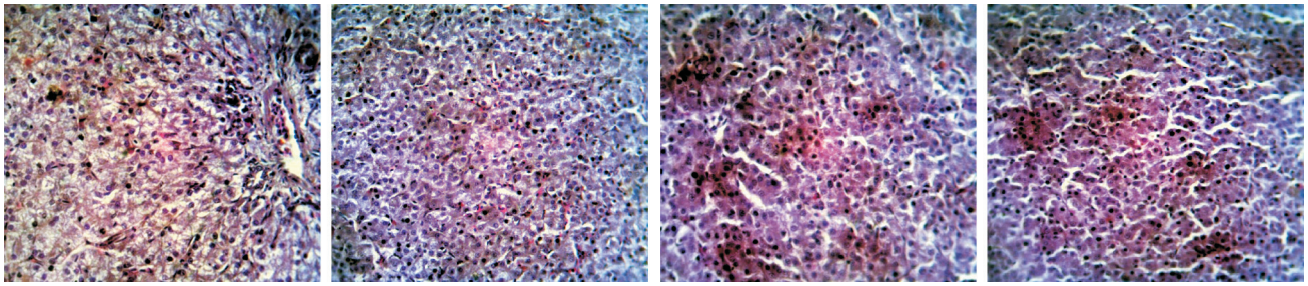
recorded in group T_3 and lowest in group T_1. This implied that turmeric supplementation resulted in higher humoral immune responses in broiler birds. Emadi and Kermanshahi (2007b) found that serum immunologlobulins of chickens were also affected by inclusion of turmeric powder into the diets, correspondingly, IgA and IgM at 21 days of age and IgG at 21 and 42 days of age significantly increased in birds fed different turmeric levels. The elevated antibody titer production and consequently better immune responses as observed in this study might be due to turmeric supplementation to broiler birds, which could be explained as it has immunomodulatory action that could modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells and dendritic cells (Ganesh and Bharat 2007). Ginger supplementation (T_4 and T_5) had no effect on humoral response, the present finding corroborated with the findings of Farhumand and Fazli (2009). They reported that adding ginger powder in broiler ration did not affect the titer of antibody against Newcastle disease. In contradiction, Nidaullah *et al.* (2010) reported that aqueous extract of ginger rhizome mixed in water in broilers plays better performance as immune stimulant against Newcastle disease, infectious bronchitis, infectious bursal disease and coccidiosis.

Weight of lymphoid organs: The average weight of lymphoid organ (% of body weight), viz. spleen, bursa of fabricius and thymus of experimental broiler birds ranged from 0.22±0.01 to 0.25±0.02, 0.16±0.01 to 0.20±0.01 and 0.50±0.04 to 0.60±0.05. From the present study, it was found that supplementation of turmeric and ginger, either alone or in combination at 0.5% and 1% level had no significant effect on the weight of the lymphoid organs of the broiler birds. Abou-Elkhair *et al.* (2014) reported no significant differences in the spleen, thymus gland and bursa of fabricius relative weight (% body weight) on supplementation of 0.5% turmeric in broiler diet. In contradiction to these findings, AL-Sultan (2003) reported higher bursa and thymus weight indices in birds those received diet containing 0.5% turmeric. Golshan *et al.* (2015) reported that ginger supplementation had significant effect on bursa weight in broiler.

Antioxidant status: The erythrocyte malondialdehyde (MDA) levels of broiler birds at fifth week of age were 3.90±0.21, 2.82±0.18, 2.3±0.08, 2.3±0.27, 2.8±0.21, 2.7±0.19 and 2.4±0.13 in T_1, T_2, T_3, T_4, T_5, T_6 and T_7 respectively. The erythrocyte malondialdehyde (MDA) level in other treatment groups were comparable and significantly lower than that of the control groups (T_1). Supplementation of turmeric either at 0.5% or 1% level alone or in combination decreased the LPO levels of experimental broiler birds. Turmeric contained phenolic compounds, mainly curcumin and xanthorrhizol and these might had contributed beneficial effect on the antioxidant system of the broilers (Rukayadi and Hwang 2013). Thring *et al.* (2011) reported that phenolic compounds can support antioxidant system possibly through the following: direct scavenging of ROS produced after oxidative stress and/or prevent the formation of ROS by inhibiting enzymes. Similarly, supplementation of ginger either at 0.5% or 1% level alone or in combination decreased the LPO levels of experimental broiler birds. Sadeghi *et al.* (2013) reported that supplementation of ginger powder significantly reduced MDA content in the serum of broilers at the ages of 21 and 42 day of age, which was corroborated to the present findings.

Liver enzyme activity: Across the group, the serum ALT levels (U/L) of broiler birds at fifth week of age were 27.85^{a}±0.43, 24.74^{b}±0.37, 24.73^{b}±0.71, 25.06^{b}±0.28, 24.53^{b}±0.79, 24.86^{b}±0.51 and 24.83^{b}±0.57 in T_1, T_2, T_3, T_4, T_5 T_6 and T_7 respectively. The serum AST levels (U/L) of broiler birds at fifth week of age were 111.45±0.84, 100.62±0.75, 101.56±0.67, 102.09±1.01, 101.7±1.88, 101.86±0.73 and 101.90±1.18 in T_1, T_2, T_3, T_4, T_5 T_6 and T_7 respectively. The serum ALT and AST levels of the experimental broiler birds in the supplemented groups differed significantly from control and no significant differences were found between the supplemented groups. It implied of better liver function due to ginger and turmeric supplementation alone or in combination to the broiler birds. Malekizadeh *et al.* (2012) in their experiment on feeding birds with a corn-soybean meal based diet containing different concentrations of ginger (1 and 3%) and turmeric (1 and 3%) and control (0%) for 9 weeks reported significant effect on SGOT and SGPT levels. In contradiction these findings, Hussein (2013) reported no significant difference with respect to ALT and AST concentration on turmeric supplementation at 0, 5, 7 and 9g/ kg of broiler diet. This might be due the fact that the decrease in the levels of AST, ALT and ALP in serum was dose dependent of curcumin (Kaur *et al.* 2006). George *et al.* (2015) reported that on ginger supplementation at the rate of 5, 10 and 15 g/kg in broiler ration no significant effect on alkaline phosphate, ALT and AST levels in broiler birds.

Gut bacterial load: The total bacterial count of broiler birds at fifth week of age were 5.86±0.13, 7.79±0.02, 8.55±0.09, 5.81±0.03, 5.76±0.05, 7.85±0.11 and 4.93±0.03 in T_1, T_2, T_3, T_4, T_5, T_6 and T_7 respectively. The *E. coli* counts of broiler birds at fifth week of age were 4.69±0.11, 4.44±0.13, 3.70±0.05, 4.65±0.05, 4.76±0.05, 4.33±0.13 and 3.28±0.15 in T_1, T_2, T_3, T_4, T_5, T_6 and T_7 respectively. Bacterial load in T_3 group was significantly higher than rest of the treated groups but the *E. coli* count was lowest among all the treated groups. Following T_3, higher gut bacterial load and lower *E. coli* counts were in T_2 and T_6 group. This implied that turmeric supplementation at 1% and 0.5% level could limit the growth of pathogenic bacteria, i.e. *E. coli*. Al-Mashhadani (2015) in his experiment on feeding 0, 0.2%, 0.4% and 0.6% turmeric powder in broiler diet and reported that turmeric could control and limit the growth and colonization of numerous pathogenic and non-pathogenic species of bacteria in the chicken's gut resulting in balanced gut microbial ecosystem that leads to better feed utilization reflected by live body weight and weight gain. They observed significantly higher



Figs 1–4. Vacuolar degeneration of hepatocytes in periportal areas in Gr_{T1} (40×). 2. Mild congestion, mild cellular swelling and vacuolation in GrT_1 (40×). 3. Hepatocytes showing regular pattern of arrangement in GrT3 (40×). 4. Hepatocytes showing regular pattern of arrangement in GrT6 (40×).

lactobacillus count in turmeric fed groups than that of control groups but no significant differences was reported in *E. coli* count. In group T_7, though turmeric was include at 1% level, but the colony count of total bacteria and coliform bacteria was lower. This could be due to synergetic effect of turmeric and ginger on limiting the growth of pathogenic and non-pathogenic species of bacteria. The colony count of total bacteria and coliform bacteria of T_4 and T_5 groups did not differ significantly (P>0.05) from control. This implied low role of ginger in limiting the gut bacterial population in broiler birds. Similar findings were reported by Adeyemo *et al*. (2016). They, in their experiment on feeding 0, 1, 1.5 and 2% ginger in broiler diet, observed no significant (P>0.05) variations observed for the total aerobic count and the total coliform count of the broilers fed dietary treatments.

Histopathological of liver: Histopathological changes in liver of experimental broiler birds under different dietary system are presented in Figs 1–4. In the group T_1 birds, liver showed diversified picture starting from mild congestion to mild cellular swelling and varying degree of vacuolar degeneration. The treatment groups showed improvement of liver status as indicated by presence of healthy hepatocytes with regular arrangement and proper staining properties due to hepatoprotective effect of added turmeric and ginger at the level of 0.5% and 1% alone or in combinations. There was not much difference of liver health and the histological pictures of different treatment groups were found to be comparable. Gowda *et al*. (2008) reported supplementation of turmeric in the broilers diet decreased aflatoxin induced necropsy lesion in liver. Turmeric improved the bile flow that might be the reason for improvement in liver health (Ahmadi 2010). Kumari *et al*. (2007) reported that turmeric supplementation at 1g/kg for 42 days did not cause any significant gross and histopathological changes in experimental birds. In our experiment, ginger also showed hepatoprotective effect at 0.5% and 1% in feed when administered for the period of 5 weeks. El-kott *et al*. (2010) in their experiment concluded that ginger could prevent the chemicals-induced diabetes mellitus toxicity and cell damage in liver and kidneys by enhancing insulin level and sensitivity and anti-oxidant capacity.

Supplementation of 1% turmeric in ration either alone or in combination with 1% ginger improved the immunity, antioxidant status and gut health of broilers. Though supplementation of ginger powder at 0.5% and 1.0% levels improved the immunity and antioxidant status in broiler birds but significant effect on improvement in gut microbial population were not found.

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