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

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ABSTRACT

Day-old broiler chicks (182) were distributed randomly to 7 treatments with 2 replicates. Treatments were T1 (control), basal diet; T2, basal diet + turmeric powder (TP) (0.5% of basal diet); T3, basal diet + TP (1% of basal diet); T4, basal diet + GP (0.5% of basal diet); T5, basal diet + GP (1% of basal diet); T6, basal diet + TP + GP (0.5% TP + 0.5% GP); T7, 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 T3 and T7. Higher antibody titre against SRBC was recorded in T3. The weight of lymphoid organs did not differ significantly. Significantly higher erythrocyte malondialdehyde (MDA) level was recorded in T1. Significantly higher alanine amino transferase (ALT) levels were found in T1 and T7. Significantly higher aspartate amino transferase (AST) level was found in T7. Higher total bacterial count and lower E. coli count were recorded in group T7 and lower total bacterial count was recorded in T1. In group T1, 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 lipophilicpolyphenol 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, gugiberen and gingerdiol (Rismunandar 1988). Gingerol, gingerdiol and gingerdione,
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. 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: T1, basal diet (broiler starter/broiler finisher); T2, basal diet + TP (0.5% of basal diet); T3, basal diet + GP (0.5% of basal diet); T4, basal diet + TP (1% of basal diet); T5, basal diet + GP (1% of basal diet); T6, basal diet + TP + GP (0.5% TP + 0.5% GP); T7, 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 injected intra-dermally in the comb with 100 micro gram. The thickness of comb was measured using digital calliper before inoculation and 24 h post inoculation and CBS 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 log2 of the reciprocal of the highest serum dilution causing agglutination of SRBC.

Processing of immune organs: At fifth week of post feeding, 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 Placert 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 × 10³ L/mmol/cm (Utley et al. 1967). This was also expressed in µmol of MDA/mg haemoglobin.

\[
\text{MDA (µmol MDA formed/mg Hb)} = \frac{[(\text{ODT /} \times \text{ (TV / VT}) \times \text{ df} \times (\text{1/mg Hb})] \times 10^9}{\text{where, ODT, absorbance of test; }, \text{ molar extinction coefficient (1.5 } \times 10^5)/\text{m/cm}; \text{ TV, total volume of reagent with sample taken; VT, volume of sample taken; df, dilution factor.}}
\]

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.
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 basophilic 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 T1, T2, T3, T4, T5, T6 and T7 groups, respectively. Significantly (P<0.05) higher cutaneous basophilic hyper sensitivity (CBH) response against PHA-P was recorded in group T3 and T7. 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 T3 than that of T5 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 (T4 and T5) 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 T1, T2, T3, T4, T5, T6 and T7 respectively. Significantly (P<0.05) higher HA titre against sheep red blood cell (SRBC) was observed in group T3 and T7.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cutaneous basophilic hypersensitivity response</td>
<td>109.82±9.35</td>
<td>136.65±7.00</td>
<td>156.91±12.88</td>
<td>133.29±3.88</td>
<td>137.50±13.88</td>
<td>126.31±8.83</td>
<td>155.77±11.72</td>
<td>0.04</td>
</tr>
<tr>
<td>Log$_2$ value HA titre</td>
<td>5.00±0.41</td>
<td>6.75±0.48</td>
<td>7.50±0.65</td>
<td>5.75±0.48</td>
<td>6.00±0.41</td>
<td>6.50±0.29</td>
<td>6.75±0.48</td>
<td>0.02</td>
</tr>
<tr>
<td>MDA levels (µmol /mg Hb)</td>
<td>3.90±0.21</td>
<td>2.82±0.18</td>
<td>2.3±0.08</td>
<td>2.3±0.27</td>
<td>2.8±0.21</td>
<td>2.7±0.19</td>
<td>2.4±0.13</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>27.85±0.43</td>
<td>24.74±0.37</td>
<td>24.73±0.71</td>
<td>25.06±0.28</td>
<td>24.53±0.79</td>
<td>24.86±0.51</td>
<td>24.83±0.57</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Logarithmic value (cfu/ml) of total bacterial and E. coli count</td>
<td>111.45±0.84</td>
<td>100.62±0.75</td>
<td>101.56±0.67</td>
<td>102.09±1.01</td>
<td>101.70±1.88</td>
<td>101.86±0.73</td>
<td>101.90±1.18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total bacterial count (cfu/ml)</td>
<td>5.86±0.13</td>
<td>7.79±0.02</td>
<td>8.55±0.09</td>
<td>5.81±0.03</td>
<td>5.76±0.05</td>
<td>7.85±0.11</td>
<td>4.93±0.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E. coli count (cfu/ml)</td>
<td>4.69±0.11</td>
<td>4.44±0.13</td>
<td>3.70±0.05</td>
<td>4.65±0.05</td>
<td>4.76±0.05</td>
<td>4.33±0.13</td>
<td>3.28±0.15</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Weight of lymphoid organs (% of body weight)</td>
<td>0.23±0.01</td>
<td>0.22±0.01</td>
<td>0.23±0.02</td>
<td>0.25±0.02</td>
<td>0.25±0.01</td>
<td>0.23±0.01</td>
<td>0.23±0.02</td>
<td>0.78</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.16±0.01</td>
<td>0.17±0.01</td>
<td>0.18±0.01</td>
<td>0.20±0.01</td>
<td>0.17±0.01</td>
<td>0.17±0.01</td>
<td>0.18±0.02</td>
<td>0.46</td>
</tr>
<tr>
<td>Bursa of fabricius</td>
<td>0.50±0.04</td>
<td>0.52±0.01</td>
<td>0.53±0.03</td>
<td>0.60±0.05</td>
<td>0.56±0.02</td>
<td>0.50±0.04</td>
<td>0.53±0.02</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Values bearing different superscripts in a row differ significantly (P<0.05).
recorded in group T₃ and lowest in group T₁. This implied that turmeric supplementation resulted in higher humoral immune responses in broiler birds. Emadi and Kermanshahi (2007b) found that serum immunoglobulins 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 (T₄ and T₅) 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 a beneficial effect on the antioxidant system of broiler birds. Abou-Elkhair et al. (2015) reported that ginger supplementation had significant effect on the weight of the lymphoid organs of the broiler birds. Al-Mashhadani (2015) in his experiment on feeding broiler ration no significant effect on alkaline phosphate, ALT and AST 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.

**Liver enzyme activity:** Across the group, the serum ALT levels (U/L) of broiler birds at fifth week of age were 27.85±0.43, 24.74±0.37, 24.73±0.71, 25.06±0.28, 24.53±0.79, 24.86±0.51 and 24.83±0.57 in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ 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 T₁, T₂, T₃, T₄, T₅, T₆ and T₇ 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 9 g/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 turmeric supplementation at the rate of 1%, 2%, 3% and 4% in broiler ration no significant effect on 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.

**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 T₁, T₂, T₃, T₄, T₅, T₆ and T₇ 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 T₁, T₂, T₃, T₄, T₅, T₆ and T₇ respectively. Bacterial load in T₃ group was significantly higher than rest of the treated groups but the E. coli count was lowest among all the treated groups. Following T₃, higher gut bacterial load and lower E. coli counts were in T₂ and T₅ 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...
lactobacillus count in turmeric fed groups than that of control groups but no significant differences was reported in *E. coli* count. In group T7, 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 T4 and T6 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 T1 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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