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Effect of dietary supplementation of ginger, garlic and turmeric on humoral immune response, antioxidant property and carcass traits of broilers

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Abstract
This experiment was conducted to examine the effects of ginger, garlic and turmeric supplementation on serum biochemical parameters, humoral immune response and carcass traits of broiler production. A total of 135, day old Cobb broilers were randomly allocated in to 9 experimental groups (T1, T2, T3, T4, T5, T6, T7, T8, T9), each consisting of 3 replicates of 5 chicks each. The standard broiler diets (T1) were formulated as per commercial feed specifications for Pre-starter: 0-14 days (22.5% CP and 3000 kcal ME/kg); Starter: 15-28 days (21.0% CP and 3125 kcal ME/kg) and Finisher: 29-42 days (19.5% CP and 3250 kcal ME/kg). Other diets were same as T1 except various levels of ginger, garlic and turmeric were supplemented in treatments groups. Diets T2, T3 and T4 were supplemented with 0.5% each of ginger, garlic and turmeric, respectively, T5 with 0.25% each of ginger and garlic, T6 with 0.25% each of turmeric and ginger, T7 with 0.25% each of turmeric and garlic, diet T8 with a combination of ginger, garlic and turmeric @ 0.25% each while, diet T9 with 0.5% each of ginger and garlic and 0.25% of turmeric. Experiment was conducted for six weeks. The results (0-6 weeks) of present study indicated that supplementation of 0.5% garlic to the basal diet of broilers (T3) significantly improved ($p<0.05$) serum biochemical parameters, humoral immune response and carcass traits of broilers followed by broilers fed diet supplemented with combination of 0.25% ginger and 0.25% garlic (T5). It could be concluded that supplementation of 0.5% garlic improved antioxidant status, immune response and carcass traits of broilers.

Keywords: Biochemical, carcass traits, garlic, ginger, humoral immune response, turmeric

1. Introduction
In India broiler production has taken a giant stride in the last decennium, evolving into commercial production system from an unscientific farming with growth rate of 11% in broiler production (Annual Report, 2016-17) [3]. Over the decades the commercially available antibiotics have been used in poultry feed. These antibiotics helps to overcome the morbidity and mortality issues in poultry farming, however, can lead to develop drug resistant microflora (Casewell *et al*., 2003) [9]. Recognizing increasing antimicrobial resistance as a major public health issue, hence, WHO strongly recommended ban on the use of antibiotics as feed additives (Antimicrobial Resistance, 2014) [4]. Hence, in order to enhance the rate of production, feeding trials have been carried out to develop other practical alternatives (Cabuk *et al*., 2006) [8]. Various bioactive products such as prebiotics, probiotics, symbiotic, enzymes, plant extracts etc. are intensively researched to boost the performance of poultry (Cabuk *et al*., 2006) [8]. So, to prevalent market contests and customer demand phytobiotics are believed to be excellent choice (Onu, 2010) [24].
Spices as an additive in the diet of chickens is very common. Among them very important are Ginger, Garlic and Turmeric. Reportedly, ginger (Zingiber officinale) possess pharmacologicaly potent substances like gingerol, gingerdiol and gingerdione which has been shown to enhance poultry production (Akhtar *et al*., 1984) as they have antioxidants, anti-inflammatory, antibacterial, anti-parasitic, antiseptic and immunomodulatory properties (Dieumou *et al*., 2009) [12]. Garlic (Allium sativum) is considered as wonder drug as it possessantioxidant antibacterial, antiparasitic, antifungal, anti-cholesteremic, antiviral, vasodilator and anticancerous characteristics (Hanieh *et al*., 2010) [16]. Allicin is the active key ingredient in garlic, which decompose rapidly to several organo-sulphur compounds which are volatile and bioactive also, it consists of allin, ajoene, diallylpolysulfides, vinyldithins, S-allylcysteine, various enzymes, amino acids and minerals (Chang and Cheong, 2008) [10].

Turmeric (Curcuma longa) also possess various phenolic compounds, such as demethoxycurcumin, curcumin, bisdemethoxycurcumin and tetrahydrocurcumin metabolites (Roughley and Whiting, 1973) [27]. These compounds have broad range of biological properties such as antioxidant, antimicrobial, antihypertensive, anti-carcinogenic, and anti-inflammatory activities (Aggarwal and Harikumar, 2009) [1]. It is also, reported that turmeric supplementation enhances growth rate as it stimulates digestive system by promoting intestinal lipase, maltase, and sucrases activities also secretion of pancreatic amylase, lipase, chymotrypsin and trypsin enhanced (Platel and Srinivasan, 2000) [26]. It was hypothesised that supplementation of ginger, garlic and turmeric will improve immune status of broilers. Limited research has been done on the effect of ginger, garlic and turmeric supplementation on immunity and carcass trait of broilers. Therefore, aim of present study was to evaluate the effect of ginger, garlic and turmeric on biochemical parameters, humoral immune response and carcass traits of broilers.

2. Materials and Methods

The study programme was conducted in the Animal Nutrition Department of college of veterinary Science and Animal Husbandry, Jabalpur (M.P.). The experiment was designed to see the efficacy of varying levels of ginger, garlic, turmeric and their combinations onantioxidant status, immune response and carcass trait. Experiment was conducted for a period of six weeks. The feed ingredients were procured from the market and examined for their proximate composition before formulation of diets. Experimental diets were formulated as per commercial chick feed specification (2011) specifications and mineral mixture was added @ 3.0% of the diet. The analyzed protein and ME values of feed ingredients were used for computation of rations. Composition of experimental broiler diets used in the study is shown in Table 1. One thirty five day old chicks were distributed randomly into nine dietary treatments each having 3 replicates of five chicks.

Table 1: Ingredient composition of broiler experimental diets

Feed ingredient (%)	Experimental diets (%)		
	Pre-starter (0-14 days)	Starter (14-28 days)	Finisher (28- 42 days)
Maize	55.214	57.332	59.273
Soybean meal	38.830	35.100	31.520
Oil	2.287	3.919	5.609
Calcite/LSP	0.627	0.663	0.698
Dicalcium phosphate	1.658	1.644	1.634
Methionine	0.223	0.223	0.213
Lysine	0.281	0.280	0.222
SodaBicarb	0.100	0.100	0.100
Salt	0.240	0.220	0.200
Trace mineral premix[1]	0.060	0.050	0.050
Vitamin premix[2]	0.060	0.050	0.050
Threonine	0.075	0.050	0.025
Maduramicin	0.000	0.000	0.050
Robimidine	0.033	0.033	0.000
Choline chloride (60%)	0.100	0.120	0.150
Liver tonic	0.050	0.050	0.050
Emulsifier	0.050	0.050	0.050
Toxin binder	0.100	0.100	0.100
Total	100	100	100

Different dietary treatments which were used in the study were T1: standard broiler diet as per commercial chick feed specifications (Control), T2: T1+ Ginger powder (GP) 0.5%, T3: T1 + Garlic powder (GAP) 0.5%, T4: T1 + Turmeric powder (TP) 0.5%; T5: T1+ GP 0.25% + GAP 0.25%, T6: T1 + GP 0.25% + TP 0.25%, T7: T1 + TP 0.25% + GAP 0.25%; T8: T1 + GP 0.25% + GAP 0.25% + TP 0.25%, T9: T1 + GP 0.5% + GAP 0.5% + TP 0.25%. Design of the experiment was completely randomized design as per Snedecor and Cochran (1994) [30].

Immune response: Humoral immune response Haemagglutination inhibition titre against Newcastle disease

Broiler birds were vaccinated on day 7th with F_1 strain, 14th day with infectious bursal disease and 21st of age with Lasota strain of Newcastle disease. Humoral immunity towards Newcastle disease was measured on day 30th and 42nd by the haemagglutination inhibition test according to the method of Office International des Epizooties.

Antioxidant property

At end of trial (42 day), birds was scarified for performing carcass traits, simultaneously from these birds parts from breast muscle and liver tissue samples (one from each replicate) was taken and analyzed for extent of lipid peroxidation. After storage at -20 °C for 30 days lipid oxidation was determined as thiobarbituric acid reactive substances (TBARS) according to the extraction method described by Witte *et al.* (1970). TBARS was expressed as mg of malondialdehyde (MDA) per kg of tissue.

Briefly, 10 g of sample was triturated with 25 ml of pre-cooled 20% trichloroacetic acid (TCA) in 2 M orthophosphoric acid solution for 2 min. The content was then quantitatively transferred into a beaker by rinsing with 25 ml of chilled distilled water. They were well mixed and filtered through ash less filter paper (Whatman filter paper No. 1). Then 3 ml of TCA extract (filtrate) was mixed with 3 ml of TBA reagent (0.005 M) in test tubes and placed in a boiling water bath for 30 min. A blank sample was made by mixing 3 ml of 10% TCA and 3 ml of 0.005 M TBA reagent. Absorbance (O.D.) was measured at fixed wavelength of 532 nm with a scanning range of 531 nm to 533 nm using UV-VIS spectrophotometer. TBARS value was calculated as mg malonaldehyde per kg of sample by multiplying O.D. value with a factor 5.2.

Carcass traits

To study the carcass traits, one broiler in each replicate were slaughtered on completion of experiment. Broilers were kept off feed for twelve hour before slaughter. During this period, they were provided fresh and clean drinking water *ad-libitum*. Each broiler was weighed before slaughter, then given severe cut to the jugular vein and allowed to bleed completely by hanging on the iron rails in inverted position. Thereafter, weight was recorded. After manual defeathering using hot water (50-55 °C) again weight was recorded. Thus dressed weight was then calculatedby subtracting weight loss as blood, head, feathers, shank and wingtips from live weight. After recording the dressed weight, a horizontal cut was applied posterior to keel bone. Breast was pushed forward to expose the viscera which were then pulled out followed by weighing of carcass. Various visceral organs like liver, heart, giblet, gizzard and pancreas were weighed. The eviscerated weight was calculated by subtracting weight of viscera from dressed weight and drawn weight was calculatedby adding weight of giblet with eviscerated weight. Various processing losses such as blood, head, feathers, shank, separable fat and wing tips were recorded replicate wise during the study. Replicate wise organs (liver, heart, gizzard, spleen and pancreas) were collected at the time of slaughter and their weight was recorded during the study.

Statistical analysis

Data was analysed statistically by analysis of variance using completely randomized design (CRD) as per Snedecor and Cochran (1994) [30]. Differences among the treatments were tested for significance by Duncan's Multiple Range Test (1955).

3. Results and Discussion

Humoral immune response

In present study significantly higher titer of antibody against Newcastle virus were observed in broilers fed on basal diets supplemented with T3 and T5 diets (Table 2). However, on 42ndday, more improved antibody titers against newcastle virus were observed in comparison to 30th day were observed.

Table 2: Antibody titer (log_{10}) against Newcastle disease virus in broilers under different treatment groups

Treatment	Days	
	30th	42nd
T1	2.07^{d}	2.28^{e}
T2	2.31^{cd}	2.61^{d}
T3	2.90^{a}	3.12^{a}
T4	2.31^{cd}	2.61^{d}
T5	2.90^{a}	3.11^{a}
T6	2.50^{bc}	2.80^{c}
T7	2.71^{ab}	2.96^{b}
T8	2.50^{bc}	2.80^{c}
T9	2.50^{bc}	2.80^{c}
SEM	0.06	0.05

Treatment means in column with different superscript differed significantly (p<0.05)

In support to our findings, Hanieh *et al*. (2010) [16] indicated that garlic supplementation in broilers diet increased anti-NDV antibody production which might be because of ameliorated immune cell functions besides improved functioning of immune cells by protecting them from oxidative stress. Similalry, Azhir *et al*. (2012) also indicated that incorporation of ginger root powder in broilers diet increased humoral immunity at 35th day of age. Likewise, Valiollahi *et al*. (2014) also showed that the antibody titers were significantly higher (*P*<0.05) at 21, 35 and 42 days when broilers fed with 2% ginger than control. In variance to our findings, Toghyani *et al*. (2010) reported that lymphoid organs weight and antibody titers against Newcastle and influenza viruses were not affected significantly by dietary supplementation of garlic powder.

Antioxidant status

Thiobarbituric acid reactive substances (TBARS; mg Malonaldialdehyde/ kg) in fresh liver tissue and breast muscle of broilers in different treatments areshown in Table 3. The treatment means indicated that TBARS in liver as well as breast muscle tissue was significantly higher in broilers assigned T1 diet.

Table 3: Thiobarbituric acid reactive substances (mg Malonaldialdehyde kg^{-1}) in fresh liver tissue or breast muscle of broilers in different treatment groups

Treatment	Liver tissue	Breast muscle
T1	0.91^{a}	0.41^{a}
T2	0.87^{b}	0.38^{b}
T3	0.80^{d}	0.32^{d}
T4	0.87^{b}	0.37^{b}
T5	0.81^{d}	0.33^{d}
T6	0.83^{c}	0.36^{bc}
T7	0.82^{d}	0.35^{c}
T8	0.83^{c}	0.36^{bc}
T9	0.83^{c}	0.36^{bc}
SEM	0.01	0.01

Treatment means in column with different superscript differed significantly (*p*<0.05)

It was significantly lower (*P*<0.05) in the broilers allotted T3 and T5 diet. In conformity with our findings, Kim *et al*. (2009) reported significant reduction (p<0.05) of liver TBARS in muscles of broilers fed on different level of garlic incomparison to that of control. Similarly, Choi *et al*. (2010) indicated reduced malondialdehyde (MDA) and increased glutathione peroxidase (GPx) values in liver after 42 days feeding of garlic powder and α-tocopherol. Similarly, Habibi *et al*. (2014) and Sahoo *et al*. (2019) indicated decreased level of MDA in serum on inclusion of ginger and 0.5-1% ginger respectively in broiler diet.

Carcass traits of broilers

The carcass yields (% of live weight) of broilers offered different diets are given in Table 4. The percent dressed weight was maximum in broilers fed T5 diet. However, statistically it was comparable to those allotted T2, T4, T7 and T8 diets. Minimum and significantly lower (*P*<0.05) percent dressed weight was recorded in broilers offered T1 diet. The percent eviscerated weight was also having more or less similar trend. Maximum percent eviscerated weight recorded in broilers assigned T7 diet was statistically similar with those allotted T2, T4, T5, T6 and T8 diets. Minimum and significantly lower (p<0.05) eviscerated weight was recorded in broilers assigned T1 diet. The percent drawn weight was recorded maximum in broilers fed T5 diet. However, statistically it was similar to those offered T6 and T7 diets. It was followed by those allotted T7, T8, T6 and T4 diets. Minimum and significantly lower (*P*<0.05) percent drawn weight was recorded in broilers allotted T1 diet. Our findings indicated that supplementation of ginger, garlic and turmeric

in broilers diet improved their carcass yield and among the supplemented group highest dressing percentage was observed in broilers offered T5 diet. However, no significant pattern was observed in processing losses.

Table 4: Carcass yields (% of live weight) of broilers in different treatment groups

Treatment	Parameters		
	Dressed weight (%)	Eviscerated weight (%)	Drawn weight (%)
T1	77.67^{d}	71.44^{d}	74.60^{e}
T2	79.93^{ab}	74.76^{ab}	78.56^{c}
T3	79.53^{bc}	74.44^{b}	78.76^{c}
T4	79.95^{ab}	75.09^{ab}	78.90^{bc}
T5	80.74^{a}	75.84^{a}	80.62^{a}
T6	80.50^{ab}	75.24^{ab}	79.74^{abc}
T7	80.53^{ab}	75.98^{a}	80.11^{ab}
T8	80.10^{ab}	74.97^{ab}	79.01^{bc}
T9	78.71^{c}	73.02^{c}	77.40^{d}
SEM	0.20	0.29	0.34

Treatment means in column with different superscript differed significantly (p<0.05)

Our results are in accordance with the findings of Eltazi (2014) [3] who showed that mixture of ginger and garlic powder in broilers diet caused significant (P<0.05) increase in dressing percentage with the highest commercial cuts percentages (breast, drumstick, and thigh). Similarly, Oleforuh-Okoleh *et al*. (2015) [23] observed significant effect (P<0.05) on abdominal fat weight and dressing percentage on supplementation of ginger and garlic. Makwana *et al*. (2015) also recorded significant (p<0.05) improvement in dressed yield along with non-significant effect on shrinkage loss, blood loss, feather loss, eviscerated yield and relative weight of giblet supplementation of 0.1% garlic. In contrast to our study Patel *et al*. (2017) [25] and Khaidem *et al*. (2019) showed that garlic had no significant (P>0.05) effect on the carcass characteristics.

4. Conclusion

Supplementation of 0.5% garlic in the diet of broilers improved humoral immune response, antioxidant status andcarcass traits. It was followed by those fed combination of 0.25% each of ginger and garlic.

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