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Effect of garlic powder supplementation on gut bacterial load, histopathology and immunity of colour synthetic broilers

SWETAPATRA BISWAL[1], S K MISHRA[1]✉, K SETHY[1], A P ACHARYA[1], N C BEHURA[1] and D P SAMANTRAY[1]

Odisha University of Agriculture and Technology, Bhubaneswar, Odisha 751 003 India

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ABSTRACT

To study the effect of garlic supplementation on gut bacterial load, histopathology and immunity of colour synthetic broilers, 100 day old colour synthetic broiler chicks were randomly distributed into five dietary treatments. The dietary treatments for this experiment were: T1: Basal diet, T2: Basal diet + Probiotic, T3: Basal diet + Garlic powder (0.5% of Basal diet), T4: Basal diet + Garlic powder (0.75% of Basal diet) and T5: Basal diet + Garlic powder (1% of Basal diet). Cellular immunity, weight of lymphoid organs and histopathology were performed at 35th day of experimental feeding period. The faecal bacterial load was performed at 14th and 28th day of experiment and intestinal bacterial load at 35th day. The weight of lymphoid organs, CBH response of different treated groups did not differ significantly. The total plate count (log_{10} cfu/ml) in the faeces of birds at 14th day of experiment of group T1 was found to be significantly higher than that of garlic and probiotic fed groups. The total plate count and *E. coli* count in faeces at 28th day and intestine (35th day) of birds in garlic supplemented groups were found to be significantly lower than the control group. The total plate count and *E. coli* count in the faces (28th day) and intestine (35th day) of the birds of 0.75 and 1.0% levels of garlic feeding had no significant difference between the treated groups. From this experiment, it may be concluded that supplementation of garlic at 0.75% in colour synthetic broiler ration reduced the gut microbial load.

Keywords: Bacterial load, Broilers, Faecal microbial load, Garlic, Histopathology, Immunity

Antibiotics, at sub-therapeutic dose, have been observed to produce remarkable effect in augmenting the growth and feed efficiency in poultry, cattle and swine along with reducing the susceptibility to diseases and enhancing the egg and meat production. The nutrient absorption and assimilation are diminished by the gastro-intestinal micro-flora through increase in GIT thickness, rate of passage of digesta whereas the nutriment requirement is enhanced by greater yield of gut mucus and competition with host for specific part of energy and protein in diet (Ravindran *et al.* 1984, Apajalahti *et al*. 2004). An unprecedented over-use of antibiotics have resulted in prevalence of antibiotic resistance of enteric pathogens like *E.coli, Salmonella* and *Campylobacter* worldwide and loss of the efficacy of antibiotics posing potential threat to public health. Due to the hazardously rising trends of antibiotic resistance, European Union has banned (2006) and eventually some other countries have voluntarily restricted the unabated use of antimicrobial growth promoters. Thus, it created avenues for development of non-conventional growth promoters. The most studied non-conventional growth promoters are: probiotics; other safer alternatives include prebiotics, synbiotics, enzymes, organic acids, toxin binders, etc. Phytobiotics are the object of ever growing interest across poultry during the last two decades. Phytobiotics can be defined as "plant derived products added to feed in order to improve health and performance" (Windisch *et al*. 2008). These are the product from roots, leaves, fruits or tubers of herbs, spices and other parts of plant. They produce innumerable beneficial effects such as stimulation of feed intake and digestive enzyme secretion; antimicrobial, coccidiostatic, antiviral or inflammatory activity; antioxidant properties and stimulation of immune system.

Among many medicinal plants available as phytobiotics, garlic (*Allium sativum*) is one of the most widely used ones and is considered as the king of medicinal plants as growth promoter in chickens. Garlic has been reported to decrease serum cholesterol as well as liver cholesterol (Qureshi *et al*. 1983), reduce aggregation of platelets (Apitz-Castro *et al*. 1983), impede multiplication of bacteria and other pathogens (Cavallito and Bailey 1944), inhibit stress due to oxidation (Horie *et al*. 1992), reduce arterial blood pressure (McMohan and Vargas 1993) and lower fatty infiltration of liver (Sand *et al*. 1995). Animal studies have shown that garlic has 'hypo-lipidemic (lipid-lowering agent), hypo-tensive (lowering blood pressure), hypo-glycemic (lowering glucose level), hypo-thrombotic (reducing thrombin production) and hypo-atherogenic

Present address: [1]Odisha University of Agriculture and Technology, Bhubaneswar, Odisha. ✉Corresponding author email: sumantkmishra@gmail.com

(reducing production of fatty deposits in arteries) effects (Bordia *et al*. 1975).

The main bioactive compound present in garlic is allicin (chemically allicin is allyl 2-propene thiosulfinate or diallylthiosulfinate). Alliin (present in fresh raw garlic) is converted to allicin by allinase enzyme which gets activated by chopping, slicing or crushing (Rybak *et al*. 2004). The frequency of research and experimentation on garlic is quite high for obvious reasons-its easy availability, low cost and effective anti-microbial property therefore, it is readily used as growth promoter in poultry. An experiment by Harris *et al*. (2001) revealed that garlic extract in general and allicin in particular produced bacteriostatic effect on some vancomycin-resistant enterococci. Moreover, various animal trials have elucidated its marvelous effects on digestibility, nutrient absorption and carcass traits as well as evaluated garlic as an herbal alternative of growth promoter in poultry. Though a number of studies and experiments have been conducted on the impact of garlic and garlic-derived products on various aspects of chicken, frequent inconsistencies have been reported in outcomes. The objective of this experiment is to ascertain the comparative effect of garlic powder and probiotics supplementation on gut bacterial load, histopathology and immunity of colour synthetic broilers.

MATERALS AND METHODS

In this experiment, day old colour synthetic broiler chicks (100) were randomly distributed into five dietary treatments containing 20 chicks in each group. Each group had 2 replicates containing 10 chicks in each. The dietary treatments of the present experiment were: T1, Basal diet; T2, Basal diet + Probiotics; T3, Basal diet + Garlic powder (0.5% of Basal diet); T4, Basal diet + Garlic powder (0.75% of Basal diet); T5, Basal diet + Garlic powder (1% of Basal diet).

The experimental diets were prepared as per BIS (2007). The ingredient compositions of basal feeds (T1) are presented in Table 1 and proximate compositions of basal feeds is presented in Table 2. The chicks were reared in cages. In T2 group, the same feed that provided to T1 group was provided with addition of probiotics over and above the composition of feed. In T3, T4 and T5 groups, 0.5, 0.75 and 1% feed was removed from the feed provided to group T1 and the same quantities were replaced with addition of 0.5, 0.75 and 1% garlic powder to the respective groups. Before experiment, the poultry shed along with the cages, feeders and waterers were cleaned thoroughly with water and disinfected with Germex (Vetneeds lab). On arrival of chicks, they were weighed, wing banded, randomly allotted to different treatment groups and electrolyte was offered to them in drinking water. Adequate light, heat during brooding period and proper ventilation was provided to chicks. On 7th day chicks were vaccinated with RD (lasota strain) vaccine, IBD (intermediate strain) vaccine on 14th day and booster RD on 22nd day were provided.

Table 1. Ingredients composition of experimental diets

Ingredient	Treatment		
	Pre starter	Starter	Finisher
Maize	50.50	52.50	57.00
Soybean meal	42.00	39.00	34.00
Vegetable oil	4.00	5.00	5.50
Dicalcium phosphate	1.82	1.82	1.82
Limestone	0.95	0.95	0.95
Salt	0.50	0.50	0.50
L-lysine	0.03	0.03	0.03
DL-methionine	0.05	0.05	0.05
Vitamin and trace mineral mix	0.15	0.15	0.15
Additives	*	*	*

*Biocholine, Biobantox, Layvit and K-zyme @ 0.50 kg each/ 100 kg feed. Livoline @ 0.25 kg/100 kg feed.

Table 2. Proximate composition of the basal diet

Ingredient	Treatment		
	Pre starter	Starter	Finisher
Crude protein	22.81	21.93	20.15
Ether extract	3.99	2.75	2.91
Crude fibre	4.83	4.67	4.61
Total ash	11.03	10.94	11.26
NFE	57.34	59.71	61.07
Calcium	1.19	1.07	1.12
Phosphorus	0.64	0.67	0.74
Metabolizable energy[#]	2971.00	3060.00	3141.00

[#]calculated.

Estimation of bacterial load: Two birds from each replicate of each treatment, faecal samples collection was done. It was collected directly from cloaca ascetically and transferred to clean and autoclaved vials. For collection of caecal content, the birds were sacrified at 35th day. The caeca were separated and the caecal content was collected asceptically into sterile bottles containing normal saline and covered with aluminium foil. Miles-Misra technique (1983) was used for bacterial cell counting. The inoculums were serially diluted 1 ml suspension with 9 ml normal saline in sterile ependymal tube. Serial 10-fold dilution of faecal samples was carried out to 10^{-6} dilution levels. Then 0.1 ml dilution was pipatted out and incubated in Mc Conkey's agar and Eosin-Methylene blue agar for *Salmonella* and *E.coli*, respectively. Then the samples were spread over the surface of agar by sterile glass spreader and rotating the petridish underneath at the same time. The plates were incubated at 37°C for 24 h and discrete colonies were counted by colony counter and estimated as CFU/ml = (No. of colonies × dilution factor) / volume of culture plate.

Determination of cellular immunity: The cellular immunity of experimental birds was determined at 5th weeks of age as per the method described by Edelman *et al*. (1986). Two birds from each replicate were injected with phytohaemaglutinin- P (PHA-P) (100 microgram in 0.1 ml of normal saline) intradermally. The thickness of the web

was measured by digital slide calliper before and 24 h after inoculation. The CBH response was calculated by using the formula:

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

Processing of immune organs: At 5th week of post feeing, two 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.

Histopathology examination: At 5th week of experimental period, one bird from each replicate was taken for study. Portion of duodenum, jejunum and ileum from experimental birds were collected in 10% normal saline. Then the samples were processed and formalin fixed tissues were prepared. These formalin fixed tissues were processed for histological study. The formalin fixed tissues were first washed in water and dehydrated in alcohol and cleared in xylene. Paraffin blocks were prepared and sections were cut at 5 micron thickness and stained by haematoxylin and eosin method. The stained slides were examined under microscope for histological study.

RESULTS AND DISCUSSION

Intestinal and faecal bacterial load: The $\log_{10}$ (cfu/ml) values of faecal bacterial load and intestinal bacterial load of experimental birds of all treated groups are presented in Tables 3 and 4, respectively. The total plate count ($\log_{10}$ cfu/ml) in the faeces of birds at 14th day of experiment of group T1 group was found to be significantly higher than that of garlic and probiotic fed groups. Similar observations were also recorded at 28th day of experiment. This implied that supplementation of probiotics or garlic reduced the total plate count of faeces of experimental birds. The intestinal total plate count ($\log_{10}$ cfu/ml) and *E. coli* count in the intestine of the birds were found to be higher in control group than rest of the treated groups. This implied that supplementation of probitic reduced the total plate count and *E. coli* count in faeces and intestine. The *E. coli* count in the faeces was observed to be significantly (P<0.05) higher than rest of the treated groups. Al-Al-Khalaifa *et al*. (2019) in their experiment, supplemented probiotics to broiler birds and they reported that the growth of *E. coli* was reduced significantly on probiotics supplementation. Kralik *et al*. (2004) reported that supplementation of probitic to birds decrease the number of bacteria after 42 days of supplementation. Jin *et al*. (1998) supplementation of *Lactobacillus* bacteria in chicken diet significantly reduced the number of *coli* bacteria in the caecum in comparison to control group.

The total plate count and *E. coli* count in faeces and intestine of birds in garlic supplemented groups were found to be significantly lower than the control group. This implied that garlic have antibacterial characteristics (Hanieh *et al*. 2010) and it act against the gram negative and gram positive bacteria (Harris *et al*. 2001). Sarica *et al*. (2005) reported that supplementation of garlic reduced the concentration of total aerobic bacteria and *E. coli* content of the small intestine of the broiler birds. Sugiharto *et al*. (2018) observed significant effect of garlic on the gut microbial

Table 3. Logarithmic$_{10}$ (cfu/ml) values of faecal bacterial load of experimental birds

Day	Parameter	Treatment					P value
		T_1	T_2	T_3	T_4	T_5	
14th	Total plate count	$8.44^{a}\pm0.04$	$8.28^{b}\pm0.02$	$8.14^{c}\pm0.02$	$8.04^{d}\pm0.04$	$7.99^{d}\pm0.06$	<0.05
	E. coli count	$8.35^{a}\pm0.05$	$8.12^{bc}\pm0.05$	$7.96^{d}\pm0.07$	$8.21^{b}\pm0.06$	$8.08^{cd}\pm0.05$	0.04
	Salmonella count	0	0	0	0	0	
28th	Total plate count	$8.41^{a}\pm0.02$	$8.25^{b}\pm0.03$	$8.29^{b}\pm0.02$	$8.05^{c}\pm0.02$	$8.01^{c}\pm0.03$	<0.05
	E. coli count	$6.42^{a}\pm0.02$	$6.24^{b}\pm0.05$	$6.15^{c}\pm0.04$	$6.11^{c}\pm0.04$	$6.01^{d}\pm0.03$	<0.05
	Salmonella count	0	0	0	0	0	

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

Table 4. Logarithmic$_{10}$ (cfu/ml) values of intestinal bacterial load of experimental birds

Parameter	Treatment					P value
	T_1	T_2	T_3	T_4	T_5	
Total plate count	$6.37^{a}\pm0.05$	$5.86^{b}\pm0.04$	$6.20^{c}\pm0.05$	0^{d}	0^{d}	<0.05
E. coli count	$6.03^{a}\pm0.01$	$5.67^{b}\pm0.04$	$5.81^{c}\pm0.03$	0^{d}	0^{d}	<0.05
Salmonella count	5.07±0.01	0	0	0	0	

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

Table 5. Weight of lymphoid organs (% of body weight) and CBH response of experimental birds at 5th weeks of age

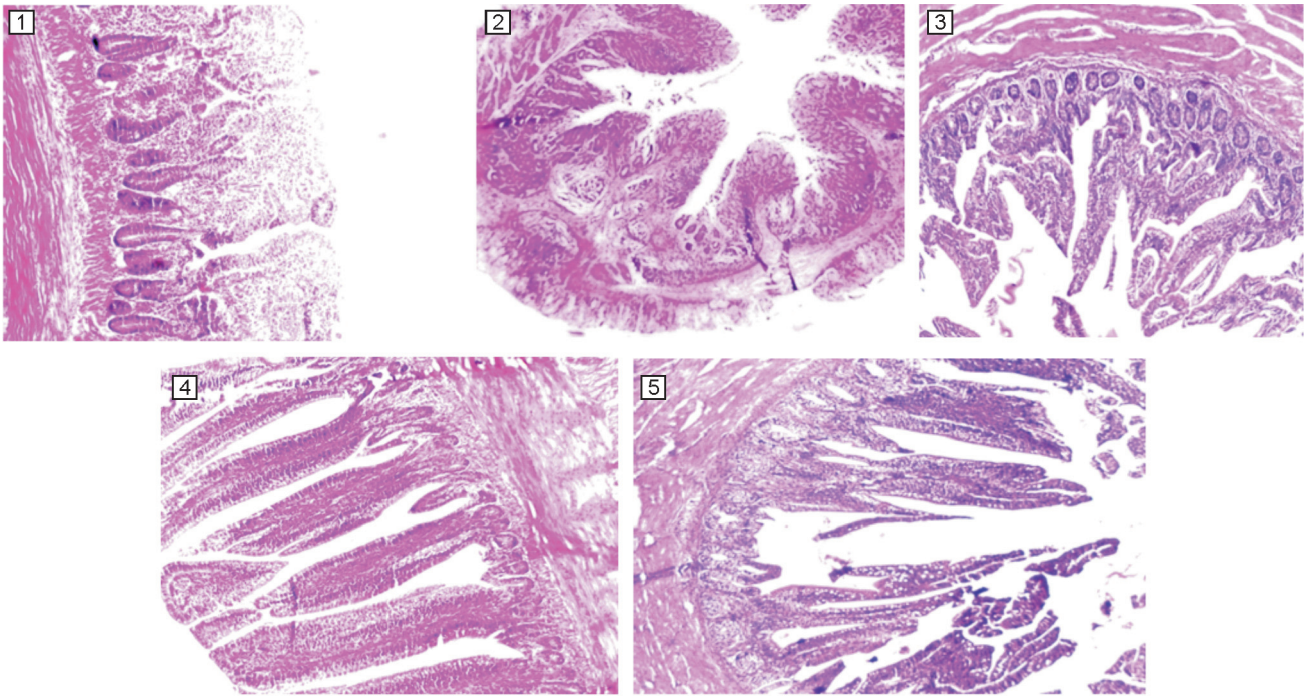
Parameter	Treatment					P value
	T_1	T_2	T_3	T_4	T_5	
Spleen	0.22±0.010	0.24±0.030	0.25±0.020	0.24±0.020	0.24±0.010	0.87
Bursa of fabricius	0.22±0.010	0.22±0.010	0.24±0.010	0.25±0.020	0.23±0.020	0.75
Thymus	0.55±0.020	0.59±0.030	0.57±0.020	0.52±0.010	0.53±0.020	0.17
Cutaneous basophilic hypersensitivity (CBH) response	126.90±5.39	125.83±6.24	133.30±10.38	131.06±8.74	136.71±9.43	0.88

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

population of broiler birds at 28th day of experiment. They observed bacterial population like coliform in the illeal digesta of broiler chicken reduced significantly on garlic supplementation. Dieumou *et al*. (2009) reported that on supplementation of garlic extract to birds, the *E. coli* and *Staphylococcus aureus* load of the intestine and caecum reduced significantly.

From this study, it was observed that the garlic had bactericidal action for which significantly reduced levels of total plate count and *E. coli* count was observed in garlic supplemented groups of birds. Peinado *et al.* (2012) reported that presence of lower levels of enteropathogens in broiler birds might be due to presence of sulphur derivatives in garlic. *Allicin* present in the garlic might be have antibacterial activity (Ross *et al.* 2001) and it acts against gram positive and gram negative bacteria (Chang and Cheong 2008). In this study, it was observed that the total plate count and *E. coli* count in the faeces and intestine of the birds of 0.75 and 1.0% levels of garlic feeding had no significance between the treated groups.

Effect on cellular immunity and weight of lymphoid organs: The weight of lymphoid organs (% of body weight) and cutaneous basophilic hypersensitivity response (CBH) of experimental birds at 5th weeks of age is presented in Table 5. The weight of lymphoid organs (% of body weight) of different treated groups did not differ significantly (P>0.05). This implied that supplementation of garlic at different levels and probiotics had no significant effect on weight of lymphoid organs. El-katcha *et al*. (2016) supplemented garlic extract at 0.1, 0.2, 0.3 and 0.4 mg/kg of diet to broiler birds. They reported non-significant effect of garlic supplementation on spleen weight of birds. But they observed increase in bursa of fabricius and thymus on 0.24 mg/kg garlic extract supplemented group. Lee *et al*. (2016) reported decrease in weight of bursa of fabricus on increasing fermented garlic in broiler ration. No significant



Figs 1–5. **1**. Photomicrograph of small intestine showing normal morphology in T1. **2**. Photomicrograph of small intestine showing normal morphology in T2. **3**. Photomicrograph of small intestine showing in increase in villi height in T3. **4**. Photomicrograph of small intestine showing in marked increase in villi height in T4. **5**. Photomicrograph of small intestine showing in marked increase in villi height in T5.

difference was observed in spleen, thymus and bursa of fabricus of broilers between control and probiotics supplemented groups. Seidavi *et al*. (2017) supplemented a mixture of probiotics and enzymes to broiler birds and observed no significant difference in the relative weight of bursa of fabricus and thymus of supplemented group and control group. But they observed significant difference in the weight of spleen of supplemented and unsupplemented groups. In contradiction to this present study, Awad *et al*. (2009) reported increase in relative weight of spleen, thymus and bursa of fabricus of broilers on supplementation of *Lactobacillus* type probiotics. Similar results were also reported by Teo and Tan (2007).

The CBH response to PHA-P of experimental birds in all the experimental groups did not differ significantly (P>0.05). PHA-P, a lectin, causes agglutination of erythrocyte and non-specific activation of T-cells (Shokrollahi *et al.* 2016). Sugiharto *et al*. (2018) reported no significant effect of dietary supplementation of 1% garlic to broiler birds on antibody titer against NDV. But Shokrollahi *et al*. (2016) reported that interdemal injection of PHA-P to goats supplemented with garlic had better response to PHA-P compared with the control group of goats.

Histopathology examination: The photographs of intestinal histological sections are presented in Figs 1 to 5. From the figures it was revealed that the height of villi was higher in group T4 containing 0.75% garlic powder and T5 containing 1.0% garlic powder than that of T3 group. In rest of the groups, no such changes were noticed.

From this experiment, it may be concluded that supplementation of garlic at 0.75% in colour synthetic broiler ration reduced the gut microbial load.

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