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Effect of Dietary Supplementation of Garlic Along with Multi-strain Probiotic on Growth Performance, Relative Organ Weights, Intestinal Morphology, Caecal Microflora and Serum Profile of Broiler Chicken

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ABSTRACT

The present study was conducted to evaluate the effects of feeding garlic along with multi-strain probiotics on performance of broilers. One hundred eighty no. (N=180) of day old commercial Ven Cobb 50 broiler chicks 45 chicks in each group with 3 replicates of 15 chicks each by following Randomized Block Design (RBD) and chicks were randomly distributed into 4 treatment groups *i.e.* T_0, T_1, T_2 and T_3 on the basis of their body weight. Experimental diets were formulated for starter and finisher phases to meet the nutrient requirement as per ICAR (2013) using commonly available ingredient. A corn and soybean meal based diet was used as the control diet (T_0). The experimental diets were formulated by mixing the basal diet with garlic powder (GP) and multi-strain probiotics (MSP) at 0.25, 0.50 or 0.75% (GP+MSP) in diets of groups T_1, T_2 and T_3, respectively. daily weight gain and feed conversion ratio (FCR) was significantly (Pd"0.05) improved in T_2 group as compared to other group. Jejunal villus height and villus width was significantly ($P<0.05$) higher in T_2 group. The population of cecal microflora was not altered by dietary treatments. Broiler chickens fed the 0.50% (MSP+GP) diet exhibited ($P<0.05$) lower levels of serum triglycerides and cholesterol compared to control group. Collectively, the results show that dietary supplementation of GP+MSP at 0.50% can be used as a synbiotic for better production and health status in commercial broilers birds.

Key words: Chicken, Caecal microflora, Gut morphology, Garlic powder, Multi strain probiotic, Relative organ weight

INTRODUCTION

India ranks fourth in world of poultry production. Due to emergence of the anti-microbial resistance (AMR), the use of antibiotic growth promoter (AGP) are now banned in many countries. In this situation probiotics and phytogenic feed additives have emerged as an alternative to AGP. Garlic exhibit nutraceutical property as it contains aromatic oils which enhances digestion activity and positively influence respiratory system being inhaled into airsacs and lungs of bird (Gardzielewska *et al.,*2003). Beneficial effect of probiotics have been reviewed (Dowarah *et al.,* 2017).Current trends of research in animal nutrition advocates the concept of symbiotic to reap the maximum benefits (Singh *et al.,* 2021). Interestingly, it was reported functional properties of garlic was enhance whilst it was fermented with *L.mesenteroides.* Higher sulphur related metabolites such as 3-vinyl-4H-1,2-dithiin, all ylmethyltrisulfide, diallyl disulphide diallyltrisulfide, but lower alliin was produced as compared to fresh garlic (Yan *et al.,* 2012; Hussain *et al.,* 2014). Considering these synergistic effects we hypothesized that feeding of garlic along with probiotic would improve performance of commercial broilers. If so, it would also be desirable to standardize the dose of the synbiotic. The specific objective of the present study was to evaluate the effect of supplementation of different levels of a synbiotic containing garlic powder and multi-strain probiotic on growth performance, relative organ weights, intestinal morphology, caecal microflora and serum profile of broiler chicken.

MATERIALS AND METHODS

The experiment was conducted in the experimental poultry shed of the Department of Animal Nutrition, College of Veterinary Science, Guwahati, India. The experimental protocol was approved by the institutional

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animal ethics committee (IAEC) with approval No. 770/ GO/Re/S/03/ CPCSEA/FVSc/AAU/IAEC/18-19/674 and carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Environment, Forests and Climate Change, Government of India.

The best LAB was identified as *Pediococcus acidilactici* FT28 by 16S rRNA sequencing and was used as probiotic in the present experiment (Accession No. KU837246) obtained from IVRI, Izatnagar screened from faeces of pigs. *Lactobacillus acidophilus* NCDC-15 was obtained from the culture collection (Dairy Microbiology Department, NDRI, Karnal, India). The cultures were inoculated in MRS (Man Rogosa and Sharpe) broth (Difco) and incubated at 37°C for overnight in BOD incubator under aerobic condition. One kilogram maize was mixed with 500ml distill water and inoculated with 24 hr old culture (100ml) of either *L. acidophilus* NCDC15 + *P. acidolactici* KD22 + *Enterococcus faecalis* 114 and kept for 24h at 37ºC for fermentation. Next day, a good quality fermented product was ready and the same fermented maize 10% (w/w) was used as inoculums for preparation of fermented feed for the next coming days and such process was continued for 10 days. After 10 days, a new vial of culture was used to prepare fermented feed. The $CFUg^{-1}$ in fermented feed was counted at every alternate day to check the viability of bacterial cell and was maintained at the level of $1\text{-}2 \times 10^9$.

Garlic was procured from the nearby market, peeled, sun dried, made paste of it in the mixture grinder. It was then allowed to get dried in the hot air oven for next two days at temp 60 ± 5ºC. After drying the product was again put in the Willy grinder and fine powder was produced. The garlic powder was then put in the air tight container and used in feed formulation at the rate of 0.25%, 0.5%, 0.75% level (on dry matter basis).One of the active principle present in garlic is Inulin. It was estimated with slight modification of standard method by Sharma (2009). Inulin is a polymer of fructose β-alpha-2-1 linkage. It was extracted first by washing the sample powder with 80% alcohol to remove soluble sugars.

Table 1. Ingredients and chemical composition of basal diets for grower and finisher stage of chicken

Items	Grower (0-21 days)	Finisher (22-35 days)
Ingredient, %		
Maize	44.3	51
Soyabean meal	21.5	22
Rice polish	10	10
Groundnut cake	18.5	11.4
Vegetable oil	3.5	3.5
Common salt	0.5	0.5
Mineral mixture	1.5	1.5
Lysine	0.067	-
Methionine	0.133	0.1
Total	100	100
Calculated chemical composition		
Crude protein, %	21.56	19.64
Metabolizable energy (kcal/kg)**	3048	3129.3
Lysine	1.2	0.99
Methionine	0.5	0.4

*T_0, control (basal diet), T_1, basal diet + 0.25% garlic powder (GP)+ multi-strain probiotic (MSP), T_2, basal diet + 0.50% GP + MSP, T_3= basal diet + 0.75% GP + MSP

The basal diet was formulated as per the ICAR (2013) recommendation (Table-1). The control group was fed with basal diet during starter and finisher phase and designated as T_0. Whereas, the treatment groups were fed basal diet (T_0) supplemented with GP+MSP at 0.25% (T_1), 0.50% (T_2) and 0.75% (T_3), respectively during starter and finisher phases (Table 1). The probiotic product (2.0×10^9 CFU/g) was mixed in the basal diet. Fresh feed and clean water were offered *ad libitum* throughout the experimental period.

One hundred eighty no. (N=180) of day old commercial Ven Cobb 50 broiler chicks were randomly distributed into 4 treatment groups *i.e.*, T_0, T_1, T_2 and T_3 on the basis of their body weight. Each treatment group having three replicates of 15chicks each (n=45 chicks/ treatment). Experimental diets were formulated for starter and finisher phases to meet the nutrient requirement as per ICAR (2013) using commonly available ingredients (Table1). The control group was fed with a basal diet during starter and finisher phase and designated as T_0. Birds of the treatment groups were supplemented with a synbiotic preparation GP+MSP at 0.25, 050 and 0.75% in groups T_0, T_1, T_2 and T_3 -, respectively (Table 1).

Body weight and feed intake were measured by pen on a weekly basis and feed conversion ratio (FCR) was calculated as kg of feed consumed to gain one kg of body mass. On day 35, three birds per pen were randomly selected for blood sampling after euthanasia. Immediately after blood sampling, the liver, spleen, heart and abdominal fat were excised, weighed and expressed as relative weight in grams per 100 g of body weight. For the morphological determination of the villi, 1-cm long sections from mid-jejunum were sampled. Caecal content was sampled and kept on ice until measurement of Microflora on the same day of sampling. Intestinal samples were fixed in 10% phosphate buffered formalin for a minimum of 48 h, and 4.0ìm sections were prepared as described elsewhere (Kim *et al.,* 2015). The sections were stained with standard hematoxylin-eosin solution and villus height, villus width (mid-point), and crypt depth were measured at 100×magnification under light microscopy. Twenty well-oriented villi per bird were measured and averaged for each bird. For enumeration of the caecal micro flora, approximately 1 g of caecal contents were mixed with 9 volumes of sterilized ice-cold saline solution (w/v), vortexed, and serially diluted. A volume of 100 ìL of caecal suspension was plated on the respective agar plates and incubated at 37ºC for 48 h. Colony was read and expressed as colony forming unit (cfu) per gram of caecal contents. The media used were MRS (Difco, USA), EMB (BBL™ Eosin Methylene Blue Agar, USA), and SS-agar (BBL™ Salmonella Shigella Agar, USA). No colonies were detected in SS-agar. Colonies in MRS and EMB agars were selected and confirmed

Table 2. Nutrient composition of experimental diets (DM basis)

Nutrients (%)	Dietary Treatments*			
	T_0	T_1	T_2	T_3
Dry matter	90.32	90.48	90.57	90.62
Crude protein	21.32	21.52	21.50	21.30
Ether extract	4.50	4.4	4.10	4.20
Crude fibre	4.30	4.35	4.30	4.35
Total ash	4.20	4.3	4.00	4.3
Nitrogen free extract	65.68	65.93	66.1	65.85
Calcium	1.4	1.33	1.38	1.50
Phosphorus	0.42	0.4	0.45	0.52
ME (kcal/kg)**	3048	3042.5	3034.17	3025.8

*T_0, control (basal diet); T_1, basal diet + 0.25% garlic powder (GP)+ multi-strain probiotic (MSP); T_2, basal diet + 0.50% GP + MSP; T_3,basal diet + 0.75% GP + MSP; [a,b,c]Means bearing different superscript in a row differ significantly.

Table 3. Effect of supplementation of garlic with multi-strain probiotic on the growth performance of broiler chickens

Attributes	Dietary treatments*				P
	T_0	T_1	T_2	T_3	
Initial body weight (g)	115.75±4.17	118.37±0.16	112.33±0.00	117.11±0.59	0.111
Final body weight (g)	1676^{b}±0.11	1725^{c} ±0.13	1765^{d}±0.16	1520^{a}±0.03	<0.001
Total net gain(g)	1570^{b}±4.16	1607^{c}±0.21	1653^{d}±0.16	1403^{a}±0.59	<0.001
Total feed intake	2929±39.36	2954±2096	3019±1.83	2935±4.53	0.097
Feed conversion ratio	1.80^{a}±0.03	1.81^{a}±0.02	1.78^{a}±0.01	2.07^{b}±0.01	0.010
BPEI	68.26^{ab}±4.52	82.63^{b}±5.90	76.75^{ab}±7.91	62.01^{a}±1.48	0.011

*T_0, control (basal diet);T_1, basal diet + 0.25% garlic powder (GP)+ multi-strain probiotic (MSP), T_2, basal diet + 0.50% GP + MSP,T_3,basal diet + 0.75% GP + MSP; [abc]Means bearing different superscript in a row differ significantly; BPEI, broiler performance efficiency index

by 16S rRNA by PCR analysis using commercial primers (forward primer 5'-AGA GTT TGA TCC TGG CTC AG3' and reverse primer 5'-AAG GAG GTG ATC CAN CCR CA-3'; Lane, 1991). Nucleotide sequence from colonies in MRS exhibited 100% homology with *Bacillus licheniformis* and those in EMB with *Escherichia coli*.

Plasma was used to measure albumin, total protein, glucose, total cholesterol. All analysis were performed by commercial kits manufactured by Aspen Laboratories as per protocol provided by the manufacturer. The statistical analysis of the experimental data was carried out using statistical package for the social science (version 17.0 for Windows; SPSS, Chicago, III., U.S.A.). Data were subjected analysis of variance (ANOVA), and Duncan's multiple range test was performed to compare the means, significance was declared at 5% level of significance according to Duncan's multiple range test.

RESULTS AND DISCUSSION

Data pertaining to final BW, total gain in body weight, cumulative feed intake (CFI), feed conversion ratio and broiler performance efficiency index (BPEI) of broiler chicken which shown in the Table 3.

In the present study, gain in weight and daily weight gain in growing bird were improved by the addition of garlic+MSP at 0.50% in the diet. The results of this present study are in good agreement with those of Toghyani *et al.* (2011) and Elagib *et al.* (2013). A positive effect was also observed on FCR in treatment groups (Table-2), the best response was observed in group T_2 in which garlic+MSP was supplemented at 0.5% of the diet. The result of the present experiment are in good agreement with earlier reports (Fadlalla*et al.* 2010; Mansoub*et al.,*2011; Ramiah*et al.,*2014; Lukanov *et al.,* 2018).

Similarly, Lewis *et al.* (2003) and Demir *et al.* (2003) reported improved FCR and body weight gain in

Table 4. Effect of supplementation of garlic with multi-strain probiotic on the relative organ weights of broiler chickens

Attributes	Dietary treatments				SEM	P value
	T_0	T_1	T_2	T_3		
Heart	0.58±0.04	0.63±0.03	0.59±0.03	0.67±0.03	0.02	0.419
Liver	0.64±0.04	0.62±0.01	0.58±0.02	0.55±0.01	0.01	0.135
Gizzard	1.94±0.01	1.83±0.06	1.91±0.05	2.1±0.04	0.04	0.148
Abdominal Fat	1.1±0.17	1.1±0.17	0.90±0.15	0.90±0.15	0.06	0.658

*T_0, control (basal diet);T_1, basal diet + 0.25% garlic powder (GP)+multi-strain probiotic (MSP); T_2, basal diet + 0.50% GP + MSP;T_3,basal diet + 0.75% GP + MSP

Table 5. Effect of supplementation of garlic with multi-strain probiotic on caecalmicroflora of broiler chickens

Attributes	Dietary treatments				SEM	P value
	T_0	T_1	T_2	T_3		
LAB	6.31[a]±0.16	7.76[b]±0.05	8.18[c]±0.03	8.13[c]±0.02	0.16	<0.001
E.coli	5.91[b]±0.07	4.43[a]±0.27	4.24[a]±0.17	4.91[a]±0.41	0.18	0.001

*T_0, control (basal diet);T_1, basal diet + 0.25% garlic powder (GP)+ multi-strain probiotic (MSP), T_2, basal diet + 0.50% GP + MSP, T_3= basal diet + 0.75% GP + MSP; [a,b,c]Means bearing different superscript in a row differ significantly.

broilers fed garlic. The improvement in weight gain of the birds fed garlic in their rations may probably due to the fact that allicin (an antibiotic substance found in garlic) inhibits growth of intestinal bacteria such as *S. aureus* and *E. coli* (Meraj, 1998). When the load of these bacteria in the intestine is low, birds may absorb more nutrients, thus leading to the improvement in weight gain of the birds using rations supplemented with garlic. Addition of 0.6 per cent of garlic powder did not influence the body weight, feed intake and FCR. However, Konjufca *et al.* (1997) reported that feeding of dietary garlic did not affect the body weight gain and FCR. There are many reports which indicate that supplementation multi-strain probiotic positively influenced performance of broilers (Aalaei *et al.,* 2019). The results clearly indicate supplementation of synbiotics can improve the performance of broilers. Similarly, supplementation of synbiotic preparation improved performance of broilers (Lukanov *et al.,* 2018).

Perusals of the data revealed that relative organ weight was similar among the groups. However, supplementation of commercial broiler feeds with fermented garlic preparation did not significantly affect the relative organ weight of broiler chickens(Anjum *et al.* 2005, Mehr *et al.* 2007, Santoso *et al.* 1995).Similar study, Abbas, (2010) and Awadein *et al.* (2010) reported that weights of liver, gizzard, heart and spleen of broilers was not influenced by garlic and fenugreek supplemented. Makwana *et al.* (2015) reported that 0.1% and 0.5% garlic supplementation did not influence on shrinkage loss, blood loss, feather loss, eviscerated yield and relative weights of heart, liver, gizzard and giblets. In contrast to the present findings, Ashayerizadeh *et al.* (2009) and Makwana *et al.* (2015) reported significant increase in dressing percentage on garlic supplementation in broilers.

The mean abdominal fat percent under different treatment groups were recorded as 1.1±0.17, 1.1±0.17, 0.90±0.15 and 0.90±0.15 for groups T_0, T_1, T_2 and T_3, respectively. The values were similar among the groups. Results corroborate well with earlier reports (Anjum *et al.,* 2005; Abdullah *et al.* 2010; Pourakbari *et al.*

Table 6. Effect of supplementation of garlic with multi-strain probiotic on the serum profile of broiler chickens

Attributes	Dietary treatments*				P
	T_0	T_1	T_2	T_3	
Glucose(mg/dl)	126±11.6	121±20.4	119±26.4	127±29.0	0.149
Total Protein (g/dl)	5.53±0.321	5.66±0.37	5.76±0.25	5.42±0.50	0.852
Albumin (g/dl)	3.39±0.36	3.48±0.196	3.49±0.233	3.35±0.347	0.970
Globulin (g/dl)	2.13±1.17	2.17±1.21	2.27±0.98	2.07±1.69	0.886
Cholesterol (mg/dl)	110[ab]±37.8	117[ab]±26.6	95.8[a]±28.2	129[b] ±30.5	0.278
Triglycerides (mg/dl)	150[b]±38.3	134[ab]±20.6	120[a]±28.9	138 [ab]±20.2	<0.001

*T_0, control (basal diet); T_1, basal diet + 0.25% garlic powder (GP)+ multi-strain probiotic (MSP), T_2, basal diet + 0.50% GP + MSP,T_3= basal diet + 0.75% GP + MSP; [a,b,c]Means bearing different superscript in a row differ significantly.

Table 7. Effect of supplementation of garlic with multi-strain probiotic on intestinal morphology of broilers

Attributes	Dietary treatments*				P
	T_0	T_1	T_2	T_3	
Villi height (mm)	764[a]±43.2	798[a]±31.59	1052[b]±27.8	1016[b]±58.61	<0.001
Crypt depth(mm)	437±77.5	311±49.5	466±51.2	354±38.7	0.241
Crypt width (mm)	12.8±0.93	11.9±0.60	14.2±1.41	14.4±0.69	0.526

*T_0, control (basal diet); T_1, basal diet + 0.25% garlic powder (GP)+ multi-strain probiotic (MSP), T_2, basal diet + 0.50% GP + MSP,T_3= basal diet + 0.75% GP + MSP; [a,b,c]Means bearing different superscript in a row differ significantly.

2016) that indicate no influence of garlic or ginger as phytogenic feed additives on abdominal fat content of chicken.

Caecal micro-flora was positively modulated by the supplementation of the synbiotic (Table 5). The highest concentration of lactic acid bacteria (LAB) was observed in T_2 group (8.18±0.03) and the lowest concentration was found in T_0 group (6.31±0.16 $\log_{10}$cfu/g caecal content). The *E. coli* count followed the reverse trend. Similar results have been reported earlier (Ramiah *et al.,*2014; Yin bo Li *et al.,* 2014).

Data pertaining to serum biochemistry are presented in Table 6. Serum concentration glucose, albumin, globulin and ratio of albumin: were similar among the groups. Similarly, Karim *et al.* (2017) reported no significant effect of garlic supplementation on serum glucose level. Pourakbari *et al. (*2016) that probiotic feeding showed no significant effect on these parameters. Fadlalla *et al.* (2010) also reported that while incorporating graded levels (0, 0.15, 0.45, 0.3, 0.6%) of garlic to the basal diets of broiler birds, no significant difference in serum total protein, albumin and globulin was observed. Serum concentration of cholesterol and triglycerides were lower (P<0.01) in group T_2 as compared to other groups. It is also possible that some of the microorganisms present in the probiotic preparation could assimilate the cholesterol present in the GIT for their own cellular metabolism and these probiotic bacteria precipitate the cholesterol with de-conjugated bile salts by the ability of probiotics to produce bile salt hydrolase enzyme (BSH) (EC 3.5.1.24) for bile salt deconjugation in the enterohepatic circulation, retardation of cholesterol synthesis via the inhibition of hydroxy methylglutaryl coenzyme A (HMG CoA) reductase and conversion of cholesterol by probiotics in the intestine into coprostanol, which is directly excreted with the faeces (Ooi and Liong, 2010), thereby preventing them from acting as precursors in cholesterol synthesis.

Data pertaining to intestinal morphology with respectto villi height (mm), crypt depth (mm) and crypt width are presented in Table 7. Villi height (mm) was significantly (P<0.001) higher in T_2 group. In a previous study it was reported that feeding of *P. acidilactici* and *L. acidophilus*, increased height, crypt depth, and V:C ratio in grower-finisher pigs (Dowarah *et al.,* 2017).

CONCLUSIONS

The result of the present investigation revealed that supplementation of a synbiotic containing garlic powder and multi-strain probiotic at 0.50% improved growth performance, feed conversion ratio and intestinal morphology.

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