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Effect of Diets Supplemented with Bacterial Culture (*Lactobacillus bulgaricus* + *Lactococcus lactis lactis*) on the Performances, Haemato-Biochemical Parameters and Carcass Characteristics of Broiler Chicken

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ABSTRACT

The effect of three levels of probiotic bacterial culture (*Lactobacillus bulgaricus* + *Lactococcus lactis lactis*) was studied in 240, day-old *Ven Cobb* broiler chicks. Chicks were randomly allocated to three groups (4 replicates per group; 10 chicks per replicate) following randomized block design. The starter (0-14 day) diets contained 23% CP and 2978 kcal ME/kg feed. Grower's (14 -28 day) diet contained 22.5% CP and 3141 kcal ME/kg while finisher's (28-42 day) diet contained 20.10% CP and 3241 kcal ME/kg feed. Treatment T_1 was control (basal diet without probiotic and E-Care-Se) and T_2 and T_3. Treatment were supplemented with probiotic bacterial culture: *Lactobacillus bulgaricus* + *Lactococcus lactis lactis* @ $2.7 \times 10^5 + 2.7 \times 10^5$ and $5.4 \times 10^5 + 5.4 \times 10^5$ CFU / g feed, respectively. Significant ($p<0.05$) reduction in DM intake and FCR in T_3 was observed. In spite of lower intake of DMI and FCR from T_2 to T_3 showed significant ($p<0.05$)) increase in body weight gain, calcium and phosphorus balances and efficiency of utilization of protein and energy for gained biomass in broilers. The non- significantly higher increased in weight of different cuts of carcass were measured in broilers of T_3. With regards to the hemato-biochemical profile there was significant ($p<0.05$) increased in the number of lymphocytes and HDL and significantly ($p<0.05$) decreases in the total serum cholesterol in the broilers of T_3 decreased. The higher performances and immunological responses ($p<0.05$) were noticed the in broilers treatment T_3 supplemented with 5.4×10^5CFU/g+$5.4x10^5$CFU/g bacterial culture of *Lactobacillus bulgaricus* + *lactococcus lactis lactis*.

Keywords: Probiotics, Dry matter intake, FCR, Gain, Chicken

The broiler chickens are succumbed to various kinds of stresses due to the intensive production pressure in the present farming system, which adversely affect their performance. Under such circumstances antibiotics and synthetic antimicrobial agents are often used for alleviating stress and to improve growth and feed efficiency. However, continuous use of sub-therapeutic levels of antibiotics in animal feed resulted in the presence of antibiotic residues in animal products and development of drug resistant microorganisms in human (Jin *et al*., 1997). Dietary use of probiotics is thus preferred to antibiotics to enhance nutrient utilization, improve feed efficiency and maintain health status because of their non-harmful effects on the consumers (Onifade *et al*., 1999; Falaki *et al*., 2011). Probiotics are live microbial feed supplements, which beneficially affects the host by improving its intestinal microbial balance (Fuller, 1989) resulting in improved performance of chicks (Mohan *et al*., 1996; Panda *et al*., 1999; Huang *et al*., 2004). The constant effort to produce human foods from animal sources has stimulated continued research for more suitable combinations of new additives, which increase the efficiency, rate of growth and the level of animal production. These wide spread efforts have led to the present use of probiotics in animal production (Dhama *et al.*, 2011). Thus, while the probiotics are not nutrient and cannot be considered as dietary essential, it is important

to understand their effects on animals and on meat produced. To increase the growth rate, feed utilization and to promote good health various useful bacterial cells are added to the animal feeds (Lee *et al.*, 2010). These include *Lactobacilli*, *Lactococcus, Bifidobacterium* and others and yeast such as *Saccharomyces* spp. The growth stimulants are distributed either through feed suppliers or they are to be cultured in laboratory to use as feed supplement. The experiment was conducted to evaluate the effect of live culture on the performance, hematological, biochemical parameters and carcass characters in broiler chicken.

MATERIALS AND METHODS

Preparation of probiotic culture

Pure culture of two bacterial species viz. *Lactobacillus bulgaricus* and *Lactococcus lactis lactis* were procured from the Department of Dairy Microbiology, NDRI Karnal. Elliker broth media was used for multiplication of bacterial cells under controlled condition and culture was incubated for 24 hours at 37° and 30°C *Lactobacillus bulgaricus* and *Lactococcus lactis lactis* respectively Elliker broth) contained (g/l) : casein enymic hydrolysate 20, yeast extract 5, gelatin 2.5, dextrose 5, lactose5, saccharose 5, sodium chloride 4, Sodium acetate 1.5, ascorbic acid 0.5. Viable bacterial counts was done by pour plate technique as described by Cruickshank *et al*. (1975). Three levels of bacterial culture were decided i.e., 0, 2.7×10^5 and 5.4×10^5 CFU of both *Lactobacillus bulgaricus* and *Lactococcus lactis lactis* /g feed.

Experimental design and diets

Two hundred forty, day-old *Ven Cobb* broiler chicks were allocated to three groups (4 replicates per group; 20 chicks per replicate) following randomized design. Three diets i.e. starter, grower and finisher were prepared for the study prepared periods. Diets were consisted of commonly available feed ingredients viz., maize, rice polish, de oiled soybean meal and fish meal along with vitamin premix, minerals and feed additives (Table 1 and 2). The starter (0-14 day) diets contained 23% CP and 2978 kcal ME/kg feed. Grower's (14 -28 day) diet contained 22.5% CP and 3141 kcal ME/kg while finisher's (28-42 day) diet contained 20.10% CP and 3241 kcal ME/kg feed. Probiotic bacterial culture with three levels in broiler chicken was supplemented in the diets. Group T_1 was control (basal diet without probiotic) for T_2 and T_3 were supplemented with probiotic bacterial culture: *Lactobacillus bulgaricus* + *Lactococcus lactis lactis* @ $2.7 \times 10^5 + 2.7 \times 10^5$ and $5.4 \times 10^5 + 5.4 \times 10^5$ CFU / g feed, respectively.

Housing and management

Chicks were reared in the deep litter under uniform system of housing and management. Wholesome neat and clean medicated drinking water was provided to all throughout the experimental periods. Artificial light was provided during night hours in order to extend 24 hrs photo period during the brooding period and similar and uniform standard management practices were followed throughout the experiments for all the treatments. Chicks were wing banded as identification marks from serial number 1 to 240. All birds were fed for 42 days and daily amount of feed intake was recorded. Chicken were weighed weekly throughout the experiment and weekly gain in body weight was calculated to determine the growth pattern and feed conversion ratio (FCR) of birds.

Metabolic trial and sampling

A metabolic trial of 3 days was conducted at the end of experiment (42-44 days). During trial excreta from each group was collected replicate wise. Daily feed offered and left over were recorded and a representative sample of feed offered and left over was collected daily for laboratory analysis. The excreta were collected once at the end of trial and oven dried at 60°C for the determination of proximate principles and energy contents. Other portions of fresh samples were subjected to analysis for nitrogen, calcium and phosphorus content. The gross energy value of feed, excreta and tissue were calculated using Bomb calorimeter (C-S123).

The feed ingredients, diets and excreta were analyzed for various proximate principles: dry matter, crude protein, ether extract, crude fiber, nitrogen free extract and total ash (AOAC, 1984) and NFE was calculated by difference. The calcium content in the feed ingredients, diets and excreta was determined by the precipitation method (Clark and Collip, 1925) and Phosphorus was determined by colorimetric method of Fiske and Subbarao (1925). The

Table 1: Ingredient composition of broiler starter (0-14 days), broiler grower (14-28 days) and broiler finisher (28-42 days) diet (on %DM basis)

Feed ingredient	Starter (0-14 days)			Grower (14-28 days)			Finisher (28-42 days)		
	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
Yellow maize	53	53	53	56.40	56.40	56.40	59.70	59.70	59.70
Deoiled soybean meal	36.40	36.40	36.40	33.40	33.40	33.40	26.30	26.30	26.30
Rice polish	2.50	2.50	2.50	—	—	—	—	—	—
Fish meal	2	2	2	2	2	2	5	5	5
Soybean oil	2.40	2.40	2.40	4.60	4.60	4.60	5.50	5.50	5.50
Dicalcium phosphate	1.70	1.70	1.70	1.60	1.60	1.60	1.30	1.30	1.30
Limestone powder	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
DL-methionine	0.28	0.28	0.28	0.26	0.26	0.26	0.22	0.22	0.22
Lysine	0.02	0.02	0.02	—	—	—	0.17	0.17	0.17
Soda	0.17	0.17	0.17	0.16	0.16	0.16	0.23	0.23	0.23
Salt	0.28	0.28	0.28	0.29	0.29	0.29	0.26	0.26	0.26
Premix**	0.56	0.56	0.56	0.58	0.58	0.58	0.62	0.62	0.62
Probiotic - *Lactobacillus bulgaricus* (*CFU/g)	—	2.7×10^5	5.4×10^5	—	2.7×10^5	5.4×10^5	—	2.7×10^5	5.4×10^5
Probiotic -*Lactococcus lactis lactis* (*CFU/g)	—	2.7×10^5	5.4×10^5	—	2.7×10^5	5.4×10^5	—	2.7×10^5	5.4×10^5
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

*CFU= Colony forming unit

** Trace mineral premix mg/kg diet: Mg 300, Mn 55, Fe 56, Zn 30, Cu 4; vitamin premix per kg diet: vit. A 8250IU, vit. K 1mg, vit. E 26.84 mg, vit. B_1 2 mg, vit. B_2 4 mg, vit. B_{12} 100mg, Niacin 60 mg, pantothenic acid 10 mg; choline 500 mg and 30 ppm salinomycin (Coxistac 12%), 55 ppm bacitracin methylene di salicyclate (BMD110,)

Table 2: Chemical composition of starter, grower and finisher broiler diet (on % DM basis)

Particulars	Moisture	CP	CF	EE	ME (kcal/ kg)	Calorie : Protein	Ash	AIA	NFE	Ca	P	Cu (ppm)	Zn (ppm)	Co (ppm)
Starter	10.51	22.97	3.67	5.8	2978	129.64	6.57	1.78	60.99	0.88	0.77	0.565	38.65	0.352
Grower	10.9	22.05	3.35	7.32	3141	142.44	7.21	2.03	60.07	0.79	0.82	0.834	25.36	0.494
Finisher	10.72	20.1	3.98	7.95	3241	162.24	6.98	1.57	60.99	0.88	0.84	0.109	28.57	0.477

microelement (Zn, Se, and Cu) in the diets (starter, grower and finisher), excreta, and meat tissue was determined by Atomic Absorption Spectrophotometer (Electronics Corporation of India Ltd. AAS 4141).

Carcass cuts measures

The birds were slaughtered by 'Halal' method at the end of metabolism trial. Prior to slaughter, birds were offered no feed for 6 hours and then weighed individually (pre-slaughter weight). By the 'Halal' method the birds were completely bleed and head was detached and skin with feather was removed. Both the legs were knuckled from hock joint. The carcass with viscera was weighed accurately. Abdomen was opened for evisceration and carefully all the viscera including organs of alimentary tract, air sacs, giblets (gizzard, liver and heart) and spleen were separated from carcass. The organs like gizzard, liver,

heart, spleen and different cuts of carcass like thigh, wing, back and neck and breast were weighed separately using sensitive balance. Lastly eviscerated carcass along with giblets and spleen were weighed for calculating dressing percentage (edible carcass yield). Protein and energy retention in the gained biomass of birds were determined following the method of Zaniecka (1969).

Heamato- biochemical analysis

Blood was collected from jugular vein in non heparinised and clean test tubes from three birds of each treatment on day 42. The serum was separated as per the standard procedure and stored under deep freezing temperature awaiting analysis. These samples were analysed for albumin, cholesterol, HDL- cholesterol and alkaline phosphatase in semi-automated analyzer by using diagnostic kits (Bayer Autopk biochemistry kits- Baroda) and methodology recommended by manufacturer. Blood samples were collected from jugular vein in heparinised vials (heparin @10 IU/ ml of blood) for hematological studies. The hematological observations were recorded in 3 birds randomly selected from each replicate on day 42. Hemoglobin, packed cell volume (PCV) and differential leukocyte counts (DLC) were performed as per the method described by Jain (1986).

Statistical analysis

For interpretation of the results, the data were subjected to one way ANOVA for analysis of variance following standard method suggested by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Chemical composition and growth performance

The effect of supplementing bacterial culture (*Lactobacillus bulgaricus* + *Lactococcus lactis lactis*) with three level in the broiler chicken on dry matter intake, body weight gain and FCR for 0-7, 7-14, 14- 21, 21-28, 28-35, and 35-42 days have been given in table 3. There was significant (($p<0.05$) difference on dry matter intake throughout the experimental period due to supplementation of three level of bacterial culture. The significantly ($p<0.05$) lowest dry matter intake was observed in T_3 group than T_2 and T_1 during entire period of experiment.

No significant difference on body weight gain among the groups was observed with bacterial culture except 3rd and final (6th) week. On 3rd week, highest gain was observed in the T^2 group than control, whereas T^3 group was comparable with T_2 and higher than control group. During 6th week, higher gain in body weight was recorded in T^3 group than T_2 and T_1, however T_2 and T_1 groups were comparable with each other.

The significantly ($p<0.05$) highest (1.5) FCR was recorded in T_1 and lowest (1.32) in T_3 when diet was supplemented with probiotic on 2nd, 3th, 5th and 6th week, however no significance difference was noticed during first and fourth week of experiment.

Bilgili and Moran (1990) and Awad *et al*. (2009) reported that dietary supplementation of dried whey up to 15g/ kg significantly reduced the feed intake of chicks as compared to the control. In the current study too the supplementation of *Lactobaccilus bulgaricus* and *Lactococcus lactis lactis* reduced the feed intake. This was possible due to better feed conversion efficiency and the nutrient requirements of birds could be met in relatively less feed than the control one. Similarly supplementation of another probiotic, pronifer and biogen at the rate of 1-2 g/kg in the diet of Ross strain broiler reduced the feed consumption. It was further stressed that as the level of these probiotic increased in the diet the feed intake reduced to a substantial amount (Shoeib and Madian, 2002; Nikpiran *et al*., 2013).

The improvements in BW and feed to gain ratio of broilers fed *Lactobacillus* supplement were probably due to the colonization of bacteria in the gastro intestinal tract which has the ability to attach to the intestinal epithelium of broiler and perform the beneficial functions pertaining to body weight gain and overall FCR (Jin *et al*., 1996d). These bacterial culture which were also used in the current experiment are resistant to the bile and acidic conditions and are able to antagonize and competitively exclude some pathogenic bacteria *in vitro* (Jin *et al*., 1996b,c) favoring the growth of beneficial ones. There are many reports about the supplementary effect of probiotic on growth of broilers which are in accordance with the current result (Tortuero 1973; Vogt *et al*., 1981; Vladimirova and Sourdjiyska, 1996; Runho *et al*., 1997; Jin *et al*., 1998; Dizajij *et al*., 2012). However, there are several studies in which no

Table 3: The effect of supplementation of probiotic bacterial culture on average weekly feed intake (g), weight gain (g) and feed conversion ratio in Broilers

Period (day)	Treatment			Significance
	T_1	T_2	T_3	
		Weekly feed intake (g)		
0-7	170.47^{b}±0.54	167.58^{ab}±1.61	165.61^{a}±1.31	0.05
7-14	370.8^{c}±1.22	354.4^{b}±0.99	334.68^{a}±1.31	0.05
14-21	712.83^{c}±2.13	696.2^{b}±2.51	687.09^{a}±0.97	0.05
21-28	901.11^{c}±1.62	886.48^{b}±1.84	865.29^{a}±2.72	0.05
28-35	1152.24^{c}±2.73	1133.55^{b}±3.49	1108.37^{a}±1.48	0.05
35-42	1048.68^{c}±2.42	999.07^{b}±4.01	928.92^{a}±1.86	0.05
		Average weekly weight gain (g)		
0-7	124.35±0.99	126.11±2.17	127.59±2.66	NS
7-14	246.11±0.74	248.92±3.79	252.65±2.63	NS
14-21	483.51^{a}±3.11	493.57^{b}±2.25	491.68^{ab}±2.85	0.05
21-28	558.2±8.44	549.83±3.87	551.27±1.58	NS
28-35	407.59±8.49	411.95±1.76	426±6.14	NS
35-42	434.25^{a}±1.79	440.49^{a}±0.74	452.44^{b}±5.76	0.05
		Average weekly feed conversion ratio (FCR)		
0-7	1.36±0.08	1.32±0.03	1.29±0.02	NS
7-14	1.50^{c}±0.04	1.42^{b}±0.02	1.32^{a}±0.01	0.05
14-21	1.47^{b}±0.09	1.40^{a}±0.07	1.39^{a}±0.07	0.05
21-28	1.61±0.02	1.6±0.01	1.53±0.03	NS
28-35	2.82^{b}±0.06	2.74^{b}±0.01	2.59^{a}±0.04	0.05
35-42	2.41^{c}±0.01	2.26^{b}±0.01	2.05^{a}±0.02	0.05

Mean ^{abc} having different superscripts in row wise differ significantly (P< 0.05)

Table 4: Performance of broiler due to supplementation of probiotic culture

Particulars	Treatments			Significance
	T_1	T_2	T_3	
Total Feed Consumed (g)	4355.93^{c}±7.01	4232.05^{b}±8.67	4090.46^{a}±4.09	0.05
Total BW Gain (g)	2301.21^{a}±1.66	2315.97^{b}±0.78	2349.11^{c}±1.08	0.05
Feed Conversion Ratio	1.89^{b}±0.03	1.82^{b}±0.02	1.74^{a}±0.06	0.05
Nitrogen intake (g/day)	3.51^{b}±0.06	3.32^{a}±0.03	3.67^{c}±0.03	0.05
Nitrogen retention (%)	76.58±1.53	78.08±1.53	75.43±1.15	NS
Calcium intake (g/day)	1.08±0.04	1.09±0.09	1.03±0.06	0.05
Calcium retention (%)	39.19^{a}±5.77	38.34^{a}±2.55	41.33^{b}±2.89	0.05
Phosphorus intake (g/day)	0.49^{b}±0.03	0.52^{b}±0.02	0.39^{a}±0.01	0.05
Phosphorus Retention (%)	38.44^{a}±1.69	40.52^{b}±1 .02	41.15^{c}±2.19	0.05
Crude protein intake (g)	916.65±0.40	896.34±0.05	866.94±0.01	NS
Crude protein deposited (g)	324.72^{a}±0.10	338.98^{b}±0.15	339.52^{b}±0.12	0.05
Conversion efficiency of dietary protein into meat (%)	35.42^{a}±0.11	37.81^{b}±0.21	39.16^{c}±0.14	0.05
Calorific value of feed intake (Mcal)	37.27±0.01	35.06±0.02	32.39±0.13	NS
Calorific value of meat energy (Mcal)	2.78±0.04	2.93±0.06	2.86±0.09	NS
Efficiency of conversion of GE into product (%)	7.45^{a}±0.02	8.35^{b}±0.03	8.82^{b}±0.06	0.05

Mean ^{abc} having different superscripts in row wise differ significantly (P< 0.05).

positive results were found (Watkins and Kratzer, 1984; Maiolino *et al.* 1992; Vargas *et al*., 2013) when host-specific and non-host specific strains of *Lactobacillus,* commercial product of *Lactobacillu*s, *L. acidophilus* and *S. faecium* were used in the diet of chicken from 8 to 60 days. However, some reports showed positive results in body weight gain and feed conversion efficiency when various strains of bacteria as probiotics were supplemented in the different diets of chicken at varying environmental conditions (Han *et al*. 1984; Jin *et al*., 1996; Ahmad, 2006; Chen *et al*., 2013). Other workers failed to report the positive effect of probiotic supplementation on FCR, body weight gain, PER and nitrogen balance in broilers (Ladukar *et al*., 2001; Karaoglu and Durdag, 2005; Wolde *et al*., 2011).

It was hypothesised that bacterial culture contains *Lactobacillus bulgaricus and Lactococcus lactis lactis* possesses mechanism of the action which favours the absorption of nutrient and ultimately increases the gain of birds. These micro-organisms, when fed along with the basal feed ingredients, might be inhibited the growth of pathogenic microbes (entered in the gastro intestinal tract through feed, water or air) by increasing acidity of the intestinal contents and by thinning the wall of small intestine, which might favored the absorption of nutrient . Thus, the microbes of probiotic may be helpful to provide pathogen free intestinal medium for the proper functioning of endogenous enzymes to break down the energy nutrients of the experimental rations. This in turn may improve gain and feed conversion efficiency of the chicks fed treated rations as compared to the chicks fed control diets. These microbes also have been reported to secrete enzymes such as proteases, amylase, cellulase, hemicellulase and lipase; therefore these enzymes may compensate the catalytic or hydrolytic activities of the endogenous enzymes. Such compensation or cooperation of enzymes, secreted by the microbes present in the probiotic may enhance rate of digestion of feed nutrients, such as protein, starch, cellulose, hemicellulose and lipids or fats for improving the overall performance of broiler chicks in terms of increasing gain and feed conversion ratio as compared to the chicks fed control diets.

Retention of Nitrogen, Calcium and Phosphorus

There was no significant difference amongst groups in percent retention of nitrogen fed diet supplemented with probiotic (Table 4). However, significant difference on calcium and Phosphorus retention percent was recorded among the groups due to supplementation of bacterial culture. Highest Ca and P retention was observed in the T^3 groups than T^2 and T^1. It indicates that the level of probiotic is important in exerting its effect on overall calcium balance in the body.

Microbial probiotics for poultry have been extensively reviewed by Simmering and Blaut (2001), Patterson and Burkhokder (2003) and Sahil *et al.* (2017). According to these review articles, it is concluded that there has not been a well-established link between microbial probiotics and mineral absorption, or bone development. Nahashon *et al*. (1994) reported positive correlations between *Lactobacillus* diets (1,100 and 2,200 ppm) and P and Ca retentions. In the current experiment effect of microbial probiotic showed positive effect as for as the absorption and retention of Ca and P are concerned.

Carcass characteristics

Non-significant difference in percentage weight of cuts (liver, gizzard, heart, spleen, wing piece, thigh, breast, back and neck), %giblet yield, abdominal fat (%), dressing percentage moisture, and protein percent of meat among the treatments supplemented with different levels of probiotic culture were observed due to dietary supplementation of probiotics (Table 5).

The better immunological responses ($p<0.05$) were noticed in broilers due to the supplementation probiotics. The most important effected organs were liver, thymus, spleen and bursa of fabricius and these organs were significantly increased in their weights in which number of lymphocytes reflect the immune response.

Present results are in accordance with the findings of Kabir *et al.* (2004) and Omar 2014 who found significantly ($P<0.01$) higher carcass yield in broilers fed probiotic. This result is in agreement with Shoeib and Madian, 2002 and Faseleh *et al*. (2016). However Murry *et al*. (2006) found that probiotic did not affect the carcass characteristics significantly in broilers.

Heamato and biochemical parameters

With regards to the hematological profile there was increase in the number of lymphocytes in the T_3 group

Table 5: The effect of supplementation of probiotic bacterial culture on weight of various cuts of carcass of broiler chicken at 42 days

Particulars	Treatments			Significance
	T_1	T_2	T_3	
Live weight (g)	2317.47±16.27	2344.52±23.77	2337.90±32.01	NS
Liver (g)	51.80±0.40	52.10±0.63	52.12±1.00	NS
Gizzard (g)	34.47±0.70	34.80±0.79	34.97±0.97	NS
Heart (g)	9.80±0.17	10.17±0.21	10.07±0.40	NS
Spleen (g)	2.50±0.10	2.55±0.06	2.52±0.04	NS
Wing piece (g)	431.52±3.93	433.15±3.83	446.0±15.49	NS
Thigh (g)	437.15±3.92	441.57±4.68	455.82±18.48	NS
Breast (g)	206.32±3.61	212.15±5.67	212.75±7.52	NS
Back and neck (g)	278.65±2.06	279.02±1.61	277.05±1.57	NS
Giblet yield (g)	96.10±1.28	96.97±1.37	97.17±2.23	NS
Abdominal fat (g)	47.62±1.21	47.60±0.92	47.05±1.12	NS
Dressed meat (g)	1499.87±15.94	1573.02±17.85	1538.42±42.31	NS
Bursa of fabricius(g)	2.77^{a}±0.01	2.92^{b}±0.01	2.82^{a}±0.04	0.05
Spleen(g)	1.07±0.03	1.08±0.01	1.07±0.03	NS
Liver(g)	22.34±0.01	22.21±0.09	21.99±0.33	NS
Thymus(g)	4.09±0.01	3.80±0.15	3.94±0.07	NS
Moisture (%)	64.49±0.02	65.07±0.03	66.10±0.01	NS
Protein (%)	21.65±0.03	21.55±0.02	22.07±0.05	NS
Ready to cook yield (%)	64.71±0.30	64.52±0.13	65.76±0.92	NS

Mean ^{abc} having different superscripts in row wise differ significantly (P< 0.05).

Table 6: The effect of supplementation of probiotic bacterial culture on certain blood biochemical, hematological and serum mineral of broiler at 42 days

Particulars	T_1	T_2	T_3	Significance
Biochemical				
Albumin (g/dl)	1.63±0.01	1.62±0.05	1.63±0.05	NS
Alkaline phosphatase (U/l)	562.74±1.02	560.93±0.75	560.78±0.86	NS
Cholesterol (mg/dl)	166.58^{c}±0.72	162.66^{b}±0.33	157.91^{a}±1.25	0.05
HDL cholesterol (mg/dl)	93.08^{a}±0.16	95.33^{b}±0.71	98.41^{c}±0.15	0.05
Hematological				
Hb (g/dl)	7.26±0.16	7.35±0.12	7.25±0.09	NS
PCV (%)	30.75±0.92	29.12±0.68	29.37±0.55	NS
Lymphocyte	56.50^{a}±0.28	56.96^{a}±0.42	58.21^{b}±0.09	0.05
Heterophil	32.83±0.21	32.41±0.43	32.58±0.28	NS
Monocyte	7.74±0.20	7.49±0.28	7.66±0.27	NS
Eosinophil	3.08±0.20	2.74±0.16	2.49±0.21	NS
Basophil	0	0	0	NS
Serum Mineral				
Cu (ppm)	0.16±0.04	0.17±0.07	0.16±0.06	NS
Zn (ppm)	1.59±0.01	1.58±0.04	1.63±0.12	NS
Se (ppm)	0.17±0.02	0.16±0.03	0.16±0.02	NS
Ca (mg/dl)	10.41±0.15	10.72±0.10	10.64±0.05	NS
P (mg/dl)	10.69±0.06	10.75±0.06	10.54±0.13	NS

Means ^{abc} having different superscripts in row wise differ significantly (P< 0.05)

of the broilers due to supplementation of probiotic, however other haematological parameters i.e. Hb, PVC, hetrophil, eosinophil, monocyte and basophil were not influenced due to supplementation of probiotics (Table 6). The biochemical parameters like activity of alkaline phosphatase and albumin concentration were not affected due to dietary supplementation of probiotic, however cholesterol was significantly decreased and HDL was increased in the T_3 where 5.4×10^5CFU/g+5.4×10^5CFU/g bacterial culture of *Lactobacillus bulgaricus* + *lactococcus lactis lactis* was supplemented in the diet. Probiotic supplementation did not influence significantly the level of serum mineral level in the broiler chicken throughout the experimental periods (Table 6).

The cause of reduction of cholesterol in probiotic supplemented group might be due to direct absorption of cholesterol by *Lactobacillus bulgaricus* and *Lactococcus lactis lactis* microbes and thereby reduction of serum cholesterol in the broiler chicken. The higher level of HDL in probiotic supplemented groups broiler chicken might be due to secretion of various microbial enzyme like proteases, amylase, and lipase, which could favor the digestion of protein and fat in supplemented groups. Probiotics increases the immunological response of chicken and there could be acceleration of immunological activity in system, due to that lymphocytes might be increased in the supplemented groups to kill the pathogenic microbes of chicken.

The present findings are in an accordance with Ashayerizadeh *et al*. (2011) reported that dietary supplementation with probiotic decrease cholesterol concentration when compared with birds fed basal diet. Similarly Apata (2008) reported that the supplementation of probiotic (*Lactobacillus acidophilus, Bacillus subtilis, Saccharomyces cerevisiae and Aspergillus oryza*e) indicated significant decrease in serum cholesterol concentration after 6 weeks of experiment with probiotic treatment.

CONCLUSION

The dietary supplementation of probiotic bacterial culture in the broiler diet revealed significant reduction in DM intake, FCR and total serum cholesterol, and increase in body weight gain, calcium and phosphorus balances, HDL cholesterol and efficiency of utilization of protein and energy for gained biomass in broilers of the group with 5.4×10^5CFU/g+5.4×10^5CFU/g bacterial culture of *Lactobacillus bulgaricus* + *Lactococcus lactis lactis*.

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