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Research Article

EFFECT OF LABORATORY ISOLATED *Lactobacillus reuteri* LRJFCM30 FROM GASTROINTESTINAL TRACT OF RED JUNGLE FOWL ON HEMATOLOGY AND SERUM BIOCHEMISTRY OF BROILER CHICKEN

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Abstract- The study was conducted to evaluate the effects of dietary supplementation of red jungle fowl specific laboratory isolated *Lactobacillus reuteri* (LRJFCM30) on hematology and serum biochemistry of broiler chicken. A total 360 CARIBRO-Vishal broiler chicks were weighed individually and randomly allocated to nine treatment groups, each having five replicates with eight chicks in each. The dietary treatments were T1 (basal diet, control), T2 (20 g BMD/100 kg feed), T3 (1 g commercial probiotic/kg feed), T4 (*L. reuteri* @1x 106 cfu/g/day), T5 (*L. reuteri* @1x 107 cfu/g/day), T6 (*L. reuteri* @1x 108 cfu/g/day), T7 (*L. reuteri* @1x 106 cfu/g/day + 0.1%MOS), T8 (*L. reuteri* @1x 107 cfu/g/day + 0.1% MOS), and T9 (*L. reuteri* @1x 108 cfu/g/day + 0.1% MOS). The birds were fed ad libitum for 42 days. The results revealed significant increase (P<0.05) in the serum total protein, albumin, calcium, and phosphors levels, whereas, significant (P<0.05) decrease in the serum glucose, cholesterol, and triglycerides levels were observed in birds fed diet T9 compared to diet T1 and T2, whereas, other dietary treatments yielded intermediate values. No significant dietary effects were observed in serum globulin and albumin: globulin ratio. The hematological parameters viz. total erythrocyte count (TEC), total leucocytes count (TLC), hemoglobin (Hb), and packed cell volume (PCV) were significantly (P<0.05) higher in dietary treatment T9 compared to treatment T1 and T2, whereas, other dietary treatments yielded intermediate values. From the results it can be concluded that the supplementation of laboratory isolated *Lactobacillus reuteri* @1x108cfu/g/day, along with 0.1% MOS in broiler chicken ration significantly improved the hematological and serum biochemical parameters of broiler chicken.

Keywords- *Lactobacillus reuteri, Laboratory isolate, Probiotic, Hematology, Serum biochemical*

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Introduction

The microbial population in the gastro-intestinal (GI) tract is very complex and consists of different groups of microbes and the GI system is the place where complex interactions occur between feed, microbes and host cells [1]. The health and nutritional status of poultry is largely interlinked with the gastrointestinal (GI) microflora, which directly or indirectly affects gut morphology, nutrition, the pathogenesis of intestinal disease, and immune responses [2] coined the term 'Probiotics' in 1965, which is derived from a Greek word 'biotikos' meaning 'for life'. Probiotics are live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host [3]. According to Fuller, 1989, probiotic is a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance. Bacteria are more commonly reported as probiotic than fungi. Two genera of bacteria are mostly reported including lactic acid bacteria of the genus *Lactobacllus* [4-8] and Bifodobacteria [9]. It was reported that probiotics have a good impact on the poultry performance [10], improve microbial balance, synthesize vitamins [11], decrease pH and release bacteriocins [12], improve feed consumption in layers and broilers [13]. The prebiotics, which are non-digestible feed supplements, are selectively fermented by beneficial micro flora and are utilized by them to exclude the pathogenic microbes. The combination of a prebiotic and probiotic as a single administration is called synbiotic which is characterized by antimicrobial, anticarcinogenic, antiallergic and immune stimulating actions. It also improves the absorption of minerals, protects from diarrhea and optimizes nutrient digestion processes [14]. In recent year's use of probiotics, prebiotics and synbiotics that enrich certain bacterial population in the digestive system are considered as alternatives to antibiotic growth promoters in poultry nutrition [9].

This study was aimed to evaluate the effect of different inclusion levels of laboratory isolated *Lactobacillus reuteri* with or without adding prebiotic (MOS) on hematological and serum biochemical parameters of 42 days old broiler chicken.

Materials and Methods

Experimental design and birds

This experiment was conducted in accordance with the guidelines of the Institute Animal Ethics Committee (I.A.E.C.) of CARI, Indian Council of Agricultural Research. A total of 360 CARIBRO Vishal broiler chicks were randomly assigned to nine dietary treatments and housed in specially designed battery brooder cages with standard watering and feeding facilities. Basal feed compositions used in the trial are given in [Table-1]. Various dietary treatment groups were T1 (basal diet, control), T2 (BMD 20 g/ 100 kg feed), T3 (0.1 % commercial probiotic), T4 (1x 106 cfu/g/day*L. reuteri*), T5 (1x 107 cfu/g/day of *L. reuteri*), T6 (1x 108 cfu/g/day of *L. reuteri*), T7 (1x 106 cfu*L. reuteri* + 0.1% MOS), T8 (1x 107 cfu/g/day of *L. reuteri* + 0.1% MOS) and T9 (1x 108 cfu/g/day*L. reuteri* + 0.1% MOS). Each treatment was allocated 5 replicates of chicks, with 8 birds in each. The feeding trial was conducted for six weeks and the feed as well as drinking water were provided ad libitum to the birds during the entire experimental period.

Dose standardization

Sub-culturing of isolates was performed in lactobacillus selection MRS broth by incubating at 37°C. for 24h under 5% CO_2 and anaerobic condition. Titration of *L. reuteri* LRJFCM30 isolate for dose standardization was carried out by serial dilution, plating and counting on MRS agar plates. Thereafter, the aliquots were adjusted to 106, 107 and 108cfu/ml using sterile PBS.

Table-1 Composition of basal diet used in feeding trials of broiler chicken

Ingredient Composition/ 100 kg feed	Broiler starter (0-3 week)	Broiler finisher (4-6 week)
Maize	55.5	62.425
DORB	2.14	1.55
Soyabean	30.6	20.5
Gour korma	4	4
RSM	4	4
Fish meal	0	4
Marble chips	0	0.6
Lime stone	0.8	0.5
DCP	2	1.6
Salt	0.3	0.3
DL- Methionine	0.1	0.07
Lysine	0.135	0.07
CP%	22.1586	19.711
ME	2816.66	2878.785
Calcium	1.06984	1.05871
Available P	0.4906	0.4138
Lysine	1.2408	1.0038
Methionine	0.4907	0.4294

(TM premix-0.1%, vit.premix-0.15%, Bcomplex-0.015%, Ch.chloride 0.05%, Toxine bind-0.05%, coccidiostate-0.05%, Soda. Bicarb-0.5%, Composition of trace minerals includes $FeSO_4$ 80 mg/kg of diet, $ZnSO_4$ and $CuSO_4$ 8mg/kg of diet, $MnSO_4$ 65 mg/kg of diet and KI 1.2 mg/kg diet. Composition of Vitamin Premix includes Choline chloride 500 mg/kg, Niacin 12mg/kg of diet, Pyridoxine hydrochloride 1.6 mg/kg of diet, Vitamin A 8250 IU/kg diet, Vitamin B 10.8 mg/kg of diet, Vitamin B2 8mg/kg of diet, Vitamin D3 1200 IU kg diet, Vitamin E 10mg/kg of diet and Vitamin K 1 mg/kg of diet.)

Table-2 Effect of different dietary treatments on serum biochemical parameters of broiler chicken

Treatment	Glucose (mg/dl)	Total Protein(g/dl)	Albumin (A) (g/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)	Globulin (G) (g/dl)	A/G ratio	Calcium (mg/dl)	Phosphorus (mg/dl)
T1	219.72^{b} ±15.68	4.76^{a} ± 0.10	1.68^{a} ± 0.08	85.55^{c}±4.85	208.83^{c}±11.26	3.08±0.11	0.55±0.04	10.46^{ab}±0.22	5.60^{ab}±0.12
T2	217.78^{b} ±14.30	4.36^{a} ± 0.15	1.62^{a} ± 0.04	82.01^{bc}±2.86	187.96^{bc}± 9.67	2.74±0.14	0.60±0.03	9.60^{a}±0.33	5.16^{a}±0.23
T3	140.28^{a} ±12.59	5.26^{bc} ± 0.20	1.93^{b} ± 0.07	66.81^{a}±4.66	168.71^{ab}±6.64	3.33±0.22	0.61±0.06	11.57^{bc}±0.43	6.21^{bc}±0.18
T4	158.33^{a} ±13.76	5.30^{bc} ± 0.12	1.93^{b} ± 0.07	79.93^{bc}±3.19	183.45^{abc}±11.07	3.56±0.20	0.56±0.04	11.28^{bc}±0.54	6.13^{bc}±0.21
T5	168.89^{a} ± 8.44	5.25^{bc} ± 0.25	1.93^{b} ± 0.07	77.33^{abc}±3.48	180.94^{abc} ±9.10	3.32±0.20	0.60±0.03	11.11^{bc}±0.36	6.08^{bc}±0.21
T6	136.39^{a} ± 7.50	5.02^{bc} ± 0.07	1.93^{b} ± 0.07	75.15^{abc}±3.54	171.01^{ab} ±9.66	3.08±0.10	0.64±0.04	11.04^{bc}±0.15	6.02^{b}±0.08
T7	136.53^{a} ± 7.55	5.33^{bc} ± 0.34	1.94^{b} ± 0.07	77.51^{abc}±4.16	175.03^{ab} ±13.56	3.39±0.33	0.62±0.07	11.73^{bc}±0.76	6.18^{bc}±0.35
T8	149.44^{a} ± 9.92	4.99^{bc} ± 0.10	1.91^{b} ± 0.04	71.34^{ab}±3.50	161.89^{ab} ±13.08	3.08± 0.08	0.62±0.02	11.19^{bc}±0.29	5.98^{b}±0.12
T9	169.03^{a} ± 10.60	5.48^{c} ± 0.21	2.05^{c} ± 0.02	67.35^{a}±4.26	150.25^{a}±9.33	3.35±0.12	0.59±0.03	12.00^{c}±0.24	6.69^{c}±0.13
P-value	0.003	0.002	0.001	0.011	0.018	0.105	0.872	0.04	0.03

Means with different superscript within column differ significantly (P<0.05)

Table-3 Effect of different dietary treatments on Haematological parameters of broiler chicken

Treatment	TEC ($\times10^{6}$ /µL)	TLC ($\times10^{3}$ /µL)	Hb (g/dl)	PCV (%)
T1	2.00^{a}±0.05	19.50^{a}±1.53	8.63^{a}±0.26	26.50^{a}±0.60
T2	2.15^{ab}±0.06	20.62^{ab}±1.23	8.69^{a}±0.28	26.63^{a}±0.47
T3	2.30^{b}±0.08	24.50^{c}±0.96	9.50^{b}±0.24	28.50^{ab}±0.71
T4	2.00^{a}±0.07	20.12^{ab}±0.78	8.81^{a}±0.19	26.50^{a}±0.41
T5	2.19^{ab}±0.06	21.75^{abc}±1.08	9.00^{ab}±0.20	26.88^{a}±0.50
T6	2.20^{ab}±0.06	22.12^{abc}±1.17	9.25^{ab}±0.14	27.94^{ab}±0.78
T7	2.22^{ab}±0.08	23.0^{abc}±0.92	8.94^{ab}±0.21	27.88^{ab}±0.73
T8	2.23^{b}±0.09	23.62^{bc}±1.04	9.63^{b}±0.20	28.06^{ab}±0.39
T9	2.30^{b}±0.08	25.0^{c}±1.22	9.56^{b}±0.21	29.13^{b}±0.79
P-value	0.021	0.032	0.045	0.023

Means with different superscript within column differ significantly (P<0.05)

Basal feed fermentation with titrated dose of Lactobacillus isolates was carried out in such a way that 20% of daily basal ration for broiler chicken was autoclaved and inoculated with 15% of *Lactobacillus* isolate broth culture having a viable count of 106, 107 and 108cfu/ml and fermented at 37°C for 24h before adding to daily ration afresh and was mixed well.

Sampling and Measurement

Hematology and blood biochemical parameters

At the end of 42 days six birds were randomly chosen and 2–3 ml of blood sample was collected aseptically from jugular vein of birds and hematology and serum biochemical parameters were evaluated by using Automated Hematology Blood Cell Counter and standard kits respectively. Total Erythrocyte count (TEC in ×106 /µl), Total leucocytes count (TLC in ×103 /µl), Haemoglobin (Hb in g/dl), Packed cell volume (PCV in %) whereas in serum biochemistry, estimation of glucose(mg/dl), total protein (g/dl), albumin (g/dl), globulin (g/dl), A/G ratio, triglycerides (mg/dl), cholesterol (mg/dl), calcium (mg/dl) and phosphorus (mg/dl).

Statistical analysis

All data collected were subjected to analysis using one-way ANOVA procedure of SPSS Version 20 followed by comparisons using Duncan's multiple range tests.

Results and Discussion

The effects of different dietary inclusion level of lab isolated *Lactobacillus reuteri* with or without MOS on serum biochemical and blood parameters are shown in [Table-2] and [Table-3] respectively.

Serum Biochemical parameters

Serum glucose

The serum glucose level was significantly (P<0.05) lower in different dietary inclusion level of lab isolated *Lactobacillus reuteri* with or without MOS and

commercial probiotic group in compare to T2 and T1 group. These observations indicate that the supplementation *Lactobacillus reuteri* with or without MOS and commercial probiotic reduce the serum glucose level of broiler chicken. The result of the present study was agreement with the findings of [15] and [16] who observed that supplementation of probiotic reduces the serum glucose level in broiler and quail respectively, whereas [17] reported that feeding probiotics increased serum glucose levels in broilers. These differences serum glucose levels among studies may be ascribed to dietary ingredients, nutrient composition, and probiotic effectiveness.

Total protein, Albumin, globulin and albumin globulin ratio

Mean± SE value of total protein and albumin was significantly (P<0.05) higher in T9 group and lower value was recorded in T2 and T1 group. The other treatment groups resulted in intermediate serum total protein which was statistically similar to T9 group. The serum albumin level was significantly (P<0.05) higher in T9 and lower value was recorded in T2 and T1 group. The other treatment groups resulted in intermediate serum albumin level. The feeding of different dietary treatment on serum globulin and albumin to globulin ratio was not differing significantly (P>0.05). These observations indicate that the supplementation of *Lectobacillus reuteri* with or with MOS increase the total protein and albumin level whereas no effect in level of globulin and albumin to globulin ratio in broiler chicken. The results of the present study are in agreement with the findings of [16] and [18] who showed that a probiotic-supplemented diet fed to Japanese quails increased serum total protein levels. However, [19-21] did not detect any influence of dietary probiotic supplementation on total protein and globulin levels in the serum of quails, broilers, and ducks, respectively. The higher serum total protein and albumin level detected in the birds fed, lab isolated *Lactobacillus reuteri* at 1x 108 along with MOS may be due to the better protein digestion.

Serum Cholesterol and Triglycerides

The serum triglycerides and cholesterol level were significantly (P<0.05) lower in either different inclusion level of lab isolated *Lactobacillus reuteri* with or without MOS and commercial probiotic group compared with antibiotic supplemented (T2) group and control (T1) group. These observations indicate that the supplementation of *Lectobacillus reuteri* with or with MOS decreases the serum cholesterol and triglycerides level on broiler chicken. This observation is in agreement with numbers of previous literature, [22-24] reported that probiotic supplementation resulted in lowering of the serum cholesterol level in broilers. [21] also supported the fact that supplementation of probiotic (*Pediococcus acidilactici*) to broilers diet reduces serum cholesterol in broiler chickens. Similar results were reported by [25] found that serum total cholesterol and triglycerides were reduced significantly by dietary supplementation of probiotic containing *Lactobacillus sporogenes at* 100 mg per kg diet. The significant reduction in serum cholesterol of broiler chickens fed probiotic supplemented diet could be attributed to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation [26]. Also, it was speculated that *Lactobacillus acidophillus* reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis [27]. Due to high bile salt hydrolytic activity lactobacillus have a property of deconjugation of bile salts [28]. Deconjugated bile acids are less soluble at low pH and less absorbed in the intestine and is more likely to excrete in faeces [29]. This could be the case in the present study as the probiotic utilized in the study (*Lactobacillus reuteri*) is acidophilic and it lowers the pH of the environment it occupies.

Serum Calcium and Phosphorus

The serum calcium level was significantly (P<0.05) higher T9 group and lower serum calcium level was observed in group T2. The other treatment groups resulted in intermediate serum calcium which was statistically similar to T9 and T1 group. The result of present experiment was agreement with the [30] explained that probiotics may increase the intestinal absorption of calcium, because the short- chain fatty acids produced by some probiotic bacteria reduce gastrointestinal pH, thereby increasing calcium solubility and presumably, calcium absorption. The serum phosphorus level was significantly (P<0.05) higher T9 group and lower serum phosphorus level was observed in group T2. The other treatment groups resulted in intermediate serum calcium which was statistically similar to T9 and T1 group. These observations indicate that the supplementation of *Lectobacillus reuteri* with or with MOS increases the serum calcium and phosphorus level in broiler chicken. These findings are comparable with the results of [31], who found higher serum phosphorus levels in broilers fed a probiotic-supplemented diet compared with those fed a control diet. [32] reported that probiotics reduced intestinal pH in humans, improving the absorption of minerals by enhancing their solubility.

Hematological parameters

Total erythrocytes count

The total erythrocytes count was significantly (P<0.05) higher in T3, T9, and T8 groups and lower count was observed in group T4 and T1. The other treatment groups resulted in intermediate TEC which were statistically similar to T1, T3, T4, T9, and T8 groups. These observations indicate that the supplementation of 0.1% MOS along with either 1x107cfu *Lactobacillus reuteri* or commercial probiotic (0.1%) in broiler chicken ration significantly increase the TEC of broiler chicken. These results are in agreement with observation of [33] who observed that the probiotic supplementation caused statistically significant increase in the erythrocyte count of Turkeys. However, in contrast to our results [34 and 21] found that the probiotic supplementation did not affect the blood constituents comprising, haemoglobin concentrations. The differences may be attributed to type and number of species of bacteria present in probiotics.

Total leucocytes count

The result of total leucocytes count was significantly (P<0.05) higher in T9 and T3 groups and lower count was observed in T1 group. The other treatment groups resulted in intermediate TEC which were statistically similar to T1, T3 and T9 groups. These observations indicate that the supplementation of 0.1% MOS along with either 1x108cfu *Lactobacillus reuteri* or commercial probiotic (0.1%) in broiler chicken ration significantly increase the TLC count of broiler chicken. The result of present study was agreement with the findings of [35], who obtained significantly higher WBC counts in broilers fed probiotics than in those fed a control diet.

Hemoglobin

The mean value of hemoglobin was significantly(P<0.05) higher in T9, T8 and T3 group and lower hemoglobin T1, T2 and T4 group. The other treatment groups resulted in intermediate hemoglobin count which was statistically similar to T1, T9, T8, T3, T2, and T4 groups. These observations indicate that the supplementation of 0.1% MOS along with either 1x107cfu *Lactobacillus reuteri* or commercial probiotic (0.1%) in broiler chicken ration significantly increase the hemoglobin level of broiler chicken. The present study was agreement with the findings of [15] who found that the supplementation of probiotic to the broiler diet significantly increased hemoglobin concentration at 42 days old of chicks compared to the control. The higher Hb concentration in the chicks received probiotics and synbiotic may be due to the acidic media of the alimentary tract caused by prebiotic fermentation which resulted in better iron salt absorption from the small intestine. This may also cause better vitamins B complex production by useful bacteria which may results in positively affecting blood-forming processes [36]. Meanwhile, normal iron supply leading to intensification of erythropoiesis was probably as a consequence of hyper synthesis of erythropoietin [37].

Packed cell volume

The mean value of packed cell volume was significantly(P<0.05) higher in T9 group and lower packed cell volume T1, T2, T4 and T5 group. The other groups resulted in intermediate PCV value which was statistically similar to T9, T1, T2, T4 and T5 groups. These observations indicate that the supplementation of 0.1% MOS along with 1x108cfu *Lactobacillus reuteri* in broiler chicken ration significantly increase the packed cell volume of broiler chicken. The finding of present study was agreement with observation of [37] findings, who observed that the probiotic supplementation caused statistically significant increase in the

haematocrit values of Turkeys.

Conclusion

From the results of the present study it can be concluded that the red jungle fowl specific laboratory isolated *Lactobacillus reuteri* @1x108cfu/g/day, along with 0.1% MOS in broiler chicken ration significantly improved the haematological and serum biochemical parameters of broiler chicken.

Application of research: Production of Safe and High-quality Poultry Meat

Research Category: Poultry Nutrition

Abbreviations:

LRJFCM30: Lactobacillus Red Jungle Fowl Ceacum30
BMD: Bacitracin methylene disalicylate
MOS: Mannan-oligosaccharides
ANOVA: Analysis of Variance
CFU: Colony forming unit

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