



Supplementation of *Lactobacillus reuteri* isolated from red jungle fowl along with mannanoligosaccharide improves growth performance, immune response and gut health in broiler chicken

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ABSTRACT

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, following complete randomized block design (CRD). The experiment was conducted for 6 weeks duration. The nine treatment groups were control fed basal diet (T1), basal diet+bacitracin methylene disalicylate @ 20 mg/kg feed (T2), basal diet+commercial probiotic @ 0.1 g/kg feed (T3), basal diet + Lab isolated *Lactobacillus reuteri* (LLR) @ 1×10^6 CFU/g of fermented feed (T4), basal diet+LLR @ 1×10^7 CFU/g of fermented feed (T5), basal diet+LLR @ 1×10^8 CFU/g of fermented feed (T6), basal diet+LLR @ 1×10^6 CFU/g of fermented+0.1% MOS (T7), basal diet+LLR @ 1×10^7 CFU/g of fermented+0.1% MOS (T8), and basal diet+LLR @ 1×10^8 CFU/g of fermented feed+ 0.1% MOS (T9). 20% of daily basal ration for broiler chicken was autoclaved and inoculated with 15% of *Lactobacillus* isolate broth culture having a viable count of 10^6 , 10^7 , and 10^8 CFU/ml and fermented at 37°C for 24 h before adding to daily ration afresh and was mixed well. Results of the present study revealed that body weight, body weight gain, immune response both humoral and cell mediated was significantly higher in T9 group. Also the pathogenic bacteria count (*Salmonella* and *E.coli*) was significantly lower in the GIT of T9 group as compared to other groups. The significantly higher relative expression of growth related genes, IGF-1 and IGF-1R and immune related gene, IL-6 whereas IL-10 and TLR-4 expression were significantly downregulated in T9 group. So, it can be concluded from the present study that *Lactobacillus reuteri* isolated from the GIT of the red jungle fowl along with MOS is effective in improving the growth performance, immune response and gut health of commercial CARIBRO-Vishal broiler chicken.

Keywords: Broiler, Body weight gain, Genes, Immunity, *Lactobacillus reuteri*, Mannanoligosaccharide (MOS)

The chicken gastrointestinal tract (GIT) is rich in microbial biodiversity of more than 500 phylotypes or over 1 million bacterial genes, which equates to 40-50 times of number in the chicken nuclear genome. This microflora has a role in nutrition, detoxification of certain compounds, growth performance, and protection against colonization of pathogens and influences health and well-being of host animals (Paul *et al.* 2022). When these live microorganisms are administered in adequate amounts, they confer a health benefit to the host and with this, the concept of probiotic has been evolved (Chaudhari *et al.* 2022). Antibiotic growth promoters (AGP) have been used in poultry diets to prevent diseases and to promote growth performance for many decades but since the ban imposed by European Union in 2006 on the use of these AGP in farm animals alternatives to the use of AGP must be found to promote

growth or production at or near the genetic potential of the modern day poultry. Uses of probiotics that enrich certain bacterial population in the digestive system are considered as alternatives to antibiotic growth promoters in poultry nutrition (Rivera-Pérez *et al.* 2021). Domestic birds raised under commercial conditions are vulnerable to a number of pathogens (Paul *et al.* 2021). The World Health Organization (WHO) has now urged egg and meat producers to use environment friendly alternative methods to control diseases. Phasing out of antibiotic growth promoters from poultry diets in Europe and recent moves toward reduction or removal of these compounds in other parts of the world will likely change the microbial profile of the GIT environment in commercial poultry. Hence every effort would be made to improve the gut efficiency through natural microflora and fauna for better nutrient utilization. Assuming that the Red Jungle Fowl being raised in natural habitats could represent the most natural GIT environment and may be harbouring certain uncharacterized strains of microbes imparting better immunity and adaptability to these birds. Keeping above facts in view, the present

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study has been designed to effect of supplementation of *Lactobacillus reuteri* isolated from the intestine of red jungle fowl along with mannanoligosaccharide (MOS) on growth performance, intestinal microbial count, immunity and expression of growth and immune related genes in broiler chicken raised under intensive system.

MATERIAL AND METHODS

Isolation of *Lactobacillus* by cultural methods: Samples were collected from adult Red Jungle fowl housed under uniform management and feeding conditions. Gut samples from 4 birds were collected separately from crop, proventriculus, ileum and caecum for *Lactobacillus* species isolation. The contents were collected into a sterile 15 ml tube containing about 10 ml of sterile PBS and pooled as per different segments of GI tract. The sample contents in the tubes were homogenized by vortexing and centrifuged at 700× for 5 min for removing debris. Supernatant was collected from each tube and aliquots (1 ml) from each sample were enriched in MRS broth and incubated for 48 h at 37°C in anaerobic jars. After that aliquots (100 µl) from each sample previously enriched with 9 ml of MRS broth were plated on MRS agar. Thirty-two colonies, eight colonies each from crop, proventriculus, ileum and caecum were randomly picked up based on colonial morphology, i.e. selection based on colour, texture, size and shape of colony. Then each isolate was inoculated to MRS broth and incubated at 37°C for 48 h; subcultured in MRS broth two times for purification of *Lactobacilli* isolates. Then a loopful of inoculums of each isolate was streaked onto MRS agar and incubated at 37°C for 48 h, single colony from each plate was picked up and sub-cultured in MRS broth twice. The purified cultures were stored as 50% glycerol stock mixture (250 µl of broth culture in 1.75 ml of 50% glycerol+50% MRS broth medium) at -80°C until further use. The isolates were sub-cultured at least twice before all of the assays. 1 ml from each isolate culture was used for CFU enumeration. After isolation of *Lactobacillus*, characterization was carried out by morphological, biochemical and molecular methods.

Basal feed fermentation with different titrated dose of *Lactobacillus* isolate: Broiler (starter and finisher) ration were fermented with selected *Lactobacillus* isolate. 20% of daily ration was autoclaved and daily inoculated with 15% of *Lactobacillus* isolate broth culture having viable count of 10⁶, 10⁷ and 10⁸ CFU/ml and fermented at 37°C for 24 h before adding to daily ration afresh and mixed well. Different experimental dietary treatments were used in the experiment.

Experimental birds and housing management: 360 day old CARIBRO-Vishal broiler birds were procured from ICAR-CARI, Izatnagar hatchery. Each bird was weighed on arrival and randomly assigned to nine groups using completely randomized design (CRD). Each dietary treatment had five replicates having eight broiler birds in each replicate. All the experimental groups of birds were reared in the battery brooder fitted with waterer and

feeder from day one to 42nd day of age. Twenty-three-hour light was provided throughout the experimental period. Fresh and clean water were offered *ad lib.* throughout the experimental period. Proper ventilation was maintained in the shed. Daily feed intake, weekly body weight and Mortality, if any was recorded throughout the experimental feeding. The birds were vaccinated for common diseases following standard vaccination schedule.

Experimental feeding of birds: All the experimental birds fed basal diet composed of maize, soybean meal, de-oiled rice bran and fish meal formulated to target the requirement of the essential nutrients for broiler chickens as per ICAR (2013). T1 fed basal diet (BD), T2 BD + Antibiotic BMD (Bacitracin Methylene Di-salicylate) @ 20 mg/kg diet, T3 BD + Commercial multi-strain probiotic (0.1 g/kg feed), T4 BD+ Lab isolated *Lactobacillus reuteri* (1×10⁶ CFU/g fermented feed), T5 BD+ Lab isolated *Lactobacillus reuteri* (1×10⁷ CFU/g fermented feed), T6 BD + Lab isolated *Lactobacillus reuteri* (1×10⁸ CFU/g fermented feed), T7 BD + Lab isolated *Lactobacillus reuteri* (1×10⁶ CFU/g fermented feed)+MOS (0.1 g /kg feed), T8 BD + Lab isolated *Lactobacillus reuteri* (1×10⁷ CFU/g fermented feed)+MOS (0.1 g/kg feed), T9 BD+ Lab isolated *Lactobacillus reuteri* (1×10⁸ CFU/g fermented feed)+MOS (0.1 g/kg feed). Weighed amount of each test diet used during the starting period (0-3 wks) and finishing period (4-6 wks) were offered daily in five replicates of eight chicks each to ensure *ad lib.* feeding at all the time.

Growth performance and feed intake: Body weights of the birds were recorded at 0th day, 1st, 2nd, 3rd, 4th, 5th and 6th week in the morning before offering the feed to birds. Body weights were taken by using platform digital balance. To measure weekly feed intake of the birds weighed quantity of respective diet was offered *ad lib.* daily to quadruplicate groups of each dietary regimen in the morning and the residue was weighed at the end of every week.

Immune response: Immune responses of the experimental birds as affected by different dietary dose of lab isolated *Lactobacillus reuteri* treatments were evaluated in terms of humoral and cell mediated immune response.

Humoral immune response: Humoral immunity was evaluated as antibody titre (HA) against 1% sheep red blood cells (SRBC) suspension. The microtitre haemagglutinin (HA) procedure was followed to measure total HA antibody titre in chickens on 5th and 10th day of post inoculation

In vivo cell mediated immune response (CMI): The CMI response to phytohaemagglutinin-P (PHA-P) mitogen was evaluated by the method of Cheng and Lamont (1988). PHA-P (1 mg/ml of PBS) was injected intra-dermally in the left foot web of 8 birds/groups. Right foot web of the same birds received 0.1 ml sterile PBS and thus served as control. The skin thickness of foot webs (Right and Left) of injected birds of each group was measured by a micrometer at 0 and 24 h after injection of mitogen. The foot web swelling was calculated by subtracting the skin thickness at 24 hours from that of 0 hours of injection of both feet. Foot web index (FWI) was calculated by subtracting the

difference in thickness at 0 and 24 hrs of mitogen injected foot web with the difference in thickness of control foot web at 0 and 24 hrs.

GI tract microflora population: GIT contents were collected separately from crop, ileum and caecum at the end of experiment from broilers for bacterial enumeration, viz. *Lactobacilli*, *Salmonella* and *E. coli*. GIT contents were diluted 10-fold with buffered peptone water and vortexed for 2 min; 100 microliters of supernatant was smeared onto appropriate selective media in duplicate plates and incubated under optimum time-temperature combinations. After incubation period bacterial counts were recorded as CFU/ml.

Statistical analysis: The data generated in the above experiment were statistically analyzed using IBM SPSS version 20 computer package. For comparison of groups, generalized linear model ANOVA procedure and Duncan's multiple range tests were used (Snedecor and Cochran 1994). The fold expression of gene was calculated by using the $2^{-\Delta\Delta C_t}$ method and analyzed by one way ANOVA.

RESULTS AND DISCUSSION

Growth performance: The data pertaining to growth performance of experimental birds as influenced by various dietary treatments has been presented in Table 1. The cumulative body weight at 6 week of age was significantly ($P<0.05$) higher in T9 followed by statistically similar T8, T2, T7, T6 and T3 whereas significantly lower body weight was recorded in T1. No significant difference ($P>0.05$) was observed in average body weight gain among the nine different dietary treatment groups in 0-3 and 4-6 week of age. The body weight gain (0-6 week) was significantly ($P<0.05$) higher in T9 followed by statistically similar T8, T7, T6, T3 and T2 whereas significantly ($P<0.05$) lower body weight was recorded in T1 followed by statistically similar T8, T7, T6, T3 and T2 group. The beneficial effect of any supplement is primarily judged through examination of the response in terms of growth of birds for which it is intended to be offered as a part of the diet. Accordingly, growth response of broiler chicken to dietary supplement was an important part of the study. From this study it can be concluded that dietary supplementation

Table 1. Body weight and body weight gain in broilers fed on different dietary treatments

Treatment	Body weight gain (g)		
	0-3 weeks	4-6 weeks	0-6 weeks
T1	493.70±6.24	676.71±13.44	1514.82 ^a ±23.11
T2	515.43±7.94	722.96±18.30	1591.41 ^{bc} ±27.74
T3	503.36±7.16	689.34±15.51	1536.21 ^{abc} ±12.13
T4	499.25±6.65	675.37±15.95	1520.91 ^{ab} ±26.07
T5	506.26±7.77	681.07±16.54	1529.97 ^{ab} ±20.60
T6	513.05±6.18	695.50±15.16	1546.81 ^{abc} ±22.09
T7	515.37±10.62	706.91±12.79	1559.68 ^{abc} ±23.03
T8	516.82±6.62	717.53±9.00	1591.50 ^{bc} ±22.40
T9	521.88±6.94	724.32±9.99	1605.99 ^c ±16.61
P-value	0.166	0.051	0.033

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

of lab isolated *Lactobacillus reuteri* @ 1×10^8 CFU along with MOS (0.1%) increases body weight and body weight gain at 6 week of age with no effect on feed intake and feed conversion ratio. This finding is in agreement with several reports demonstrating that probiotic supplemented to the birds improve the body weight, body weight gain and feed conversion ratio (Qiu *et al.* 2022). However, contrary to our observation body weight, daily weight gain and FCR of broilers were not influenced by the dietary supplementation with probiotics (Gyawali *et al.* 2022). The improvement in the body weight, daily weight gain, feed consumption and feed conversion ratio in the present study may be due to the increase in the digestion and absorption of the nutrients by increasing the secretion and activity of the digestive enzymes processes and reduction of the population of pathogenic bacteria by decreasing the intestinal pH due to presence of the *Lactobacillus reuteri* bacteria and also maintain the balance between beneficial and harmful bacteria which is required for sound gut health and optimum growth performance (Yaqoob *et al.* 2022).

Feed intake and feed conversion ratio (FCR): The data pertaining to feed intake (g) and FCR is presented in Table 2. The results indicate that no significant ($P>0.05$) difference was observed in feed intake among the different dietary supplemented groups during 0-3, 4-6 and 0-6

Table 2. Feed intake and feed conversion ratio (FCR) in broilers fed on different dietary treatments

Treatment	FCR			Feed Intake (g)		
	0-3 Weeks	4-6 Weeks	0-6 Weeks	0-3 Weeks	4-6 Weeks	0-6 Weeks
T1	1.65±0.03	3.23±0.04	1.98±0.03	813.73±19.31	2182.44±49.98	2946.17±68.86
T2	1.61±0.03	2.97±0.14	1.87±0.06	831.42±16.45	2133.75±53.25	2965.17±61.59
T3	1.62±0.05	3.09±0.05	1.92±0.03	816.24±18.07	2132.63±28.01	2948.86±41.09
T4	1.68±0.04	3.19±0.13	1.97±0.07	838.02±20.98	2145.94±53.03	2983.96±67.61
T5	1.63±0.04	3.17±0.06	1.95±0.03	824.72±14.85	2151.21±19.37	2975.93±18.75
T6	1.59±0.03	3.12±0.07	1.93±0.03	817.77±21.71	2169.46±32.12	2987.23±50.74
T7	1.60±0.06	3.06±0.11	1.91±0.06	822.46±16.33	2157.32±41.17	2979.79±56.68
T8	1.60±0.03	3.02±0.06	1.88±0.04	826.84±17.21	2163.77±47.02	2990.61±63.15
T9	1.56±0.04	2.97±0.08	1.85±0.04	814.30±16.15	2148.09±46.50	2982.40±56.84
P-value	0.609	0.354	0.452	0.987	0.996	1.00

Table 3. Mean log₁₀ value of *Lactobacillus* spp. *Salmonella* spp. and *E.coli* isolated from broiler chicken intestine

Treatment	<i>Lactobacillus</i> spp			<i>Salmonella</i> spp			<i>E. coli</i>		
	Crop	Ileum	Caeca	Crop	Ileum	Caeca	Crop	Ileum	Caeca
T1	8.38 ^a ±0.04	8.61 ^a ±0.04	8.40 ^a ±0.02	7.31 ^c ±0.03	7.14 ^c ±0.03	7.16 ^b ±0.03	7.35 ^b ±0.03	7.17 ^c ±0.03	7.23 ^c ±0.04
T2	8.58 ^b ±0.04	8.69 ^{ab} ±0.03	8.35 ^a ±0.04	6.94 ^{ab} ±0.03	6.99 ^{ab} ±0.03	6.92 ^a ±0.03	7.19 ^a ±0.02	7.00 ^a ±0.06	6.98 ^a ±0.04
T3	8.84 ^d ±0.05	8.87 ^d ±0.01	8.71 ^b ±0.04	6.92 ^a ±0.04	7.07 ^{abc} ±0.04	7.13 ^b ±0.04	7.26 ^{ab} ±0.02	7.02 ^{ab} ±0.04	7.10 ^{abc} ±0.04
T4	8.69 ^c ±0.05	8.75 ^{bc} ±0.04	8.61 ^b ±0.04	7.28 ^c ±0.05	7.11 ^{bc} ±0.04	7.12 ^b ±0.04	7.33 ^b ±0.02	7.14 ^{bc} ±0.03	7.21 ^{bc} ±0.05
T5	8.81 ^d ±0.05	8.77 ^{bcd} ±0.05	8.65 ^b ±0.04	7.25 ^c ±0.04	7.10 ^{bc} ±0.05	7.10 ^b ±0.03	7.31 ^b ±0.03	7.13 ^{abc} ±0.04	7.17 ^{bc} ±0.05
T6	8.84 ^d ±0.02	8.80 ^{cd} ±0.04	8.70 ^b ±0.03	7.23 ^c ±0.02	7.06 ^{abc} ±0.04	7.07 ^b ±0.05	7.25 ^{ab} ±0.05	7.13 ^{abc} ±0.04	7.14 ^{bc} ±0.07
T7	8.75 ^{cd} ±0.03	8.86 ^d ±0.03	8.64 ^b ±0.05	7.26 ^c ±0.03	7.05 ^{abc} ±0.04	6.95 ^a ±0.04	7.31 ^b ±0.05	7.12 ^{abc} ±0.04	7.12 ^{abc} ±0.04
T8	8.83 ^d ±0.02	8.97 ^c ±0.03	8.70 ^b ±0.05	7.03 ^b ±0.02	7.02 ^{ab} ±0.04	6.94 ^a ±0.03	7.27 ^{ab} ±0.03	7.10 ^{abc} ±0.03	7.10 ^{abc} ±0.05
T9	9.03 ^e ±0.03	9.03 ^c ±0.02	8.93 ^c ±0.02	6.91 ^a ±0.05	6.95 ^a ±0.04	6.92 ^a ±0.05	7.20 ^a ±0.03	7.03 ^{ab} ±0.05	7.07 ^{ab} ±0.05
P-value	0.000	0.000	0.000	0.000	0.024	0.00	0.014	0.052	0.023

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

week of age. The result of FCR showed that there were no significant (P>0.05) differences between the different dietary groups and control group at 0-3, 4-6 and 0-6 week of age. The T9 group showed numerically better FCR than the other groups whereas, the control group showed highest feed conversion ratio than others dietary treatment groups at all times

Intestinal microbial count: The least squares analysis of variance for log₁₀ CFU/g of *Lactobacillus*, *Salmonella* and *E. coli* in crop, ileum and caecum of Broiler fed with different treatments is presented in Table 3. Enumeration of *Lactobacillus* spp. *Salmonella* spp. and *E.coli* was conducted at 42th day of feeding trial in Broiler. The *Lactobacillus* count in crop was significantly (P<0.05) higher in T9 group and significantly lower in T1 group whereas other treatments resulted in intermediate *Lactobacillus* count in crop at 6th week of age. The *Lactobacillus* count in ileum was significantly (P<0.05) higher in T9 followed by statistically similar T8 and lower in T1 and T2 whereas other treatments resulted in intermediate *Lactobacillus* count in ileum at 6th week of age. In caecum also T9 showed higher (P<0.05) *Lactobacillus* count than control and other dietary treatment groups.

Significantly (P<0.05) lower *Salmonella* count was observed in the crop and ileum of the broilers in T9 group as compared to all the treatment groups at 6th week of age. *Salmonella* count in caecum was significantly (P<0.05) lower in T9 followed by statistically similar in T8, T7 and highest *Salmonella* count was observed in T1 group at 6th week of age. The *E.coli* count in crop was significantly (P<0.05) lower in T9 group followed by statistically similar in T8, T3 and T6 groups whereas significantly (P<0.05) higher in T1 followed by statistically similar T7, T8, T3, T4, T5, T6 and T8 at 6 week of age. In ileum *E. coli* count was significantly (P<0.05) lower in all dietary treatments except T4 and T1. Similar finding was also observed in caecum with significantly (P<0.05) lower *E. coli* count in T9 group followed by statistically similar T8, T7, T6, T5, T4, T3 and higher count observed in T1 group. The results obtained in this work revealed that 1×10⁸ CFU of *Lactobacillus reuteri* along with 0.1% MOS significantly increased *Lactobacillus*

count and reduced *Salmonella* and *E. coli* count in crop, ileum, and caecum. Feeding of lab isolated *Lactobacillus reuteri* 1×10⁸ CFU/g along with MOS (0.1%) to broiler chicken for 6 weeks significantly (P<0.05) increases *Lactobacillus* count in different parts of GIT (crop, ileum and caecum) with concomitant decrease in pathogenic organisms such as *E. coli* and *Salmonella* count. The results were in accordance with study of Chen *et al.* (2020) who reported that reduction of *E. coli* and *Salmonella* count in intestine upon *Lactobacillus* feeding. The mechanism of the antimicrobial action may be due to the potential of *Lactobacillus* isolates to produce fatty acids, lactic acids, hydrogen peroxide diacetyl acetoin and the small, heat-stable inhibitory peptides called bacteriocins (Monika *et al.* 2021). Other proposed mechanisms of pathogen inhibition by the probiotic micro-organisms include competition for nutrients, and by modifying the structure and function of the intestinal epithelium. Prebiotics such as MOS can also flush out pathogens from GIT serving as receptor sites for their adherence (Vineetha *et al.* 2017).

Immune response: The results of immune response (humoral and cell mediated immune response) as affected by different treatments are presented in Table 4. The antibody titre (humoral immunity) was significantly (P<0.05) lower in T1 group followed by statistically

Table 4. Humoral and cell mediated immune response of broilers under different dietary treatments

Treatment	HA titre	CMI (mm)
T1	6.25 ^a ±0.40	0.46±0.07 ^a
T2	8.38 ^{cd} ±0.37	0.52±0.04 ^a
T3	8.00 ^{bcd} ±0.67	0.51±0.05 ^a
T4	6.38 ^a ±0.37	0.46±0.03 ^a
T5	6.63 ^{ab} ±0.44	0.51±0.05 ^a
T6	7.75 ^{bc} ±0.55	0.56±0.04 ^{ab}
T7	8.00 ^{bcd} ±0.41	0.59±0.04 ^{ab}
T8	8.75 ^{cd} ±0.32	0.72±0.10 ^b
T9	9.25 ^d ±0.43	0.75±0.10 ^b
P-Value	0.000	0.012

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

similar T4 and T5, whereas significantly ($P < 0.05$) higher antibody titre was obtained in T9 group which did not differ significantly from treatment T8, T2, T7, T3, and T6 groups. The antibody titre revealed that dietary inclusion of lab isolated *Lactobacillus* along with MOS significantly ($P < 0.05$) enhanced the antibody titre values of broilers, more prominently in groups T9 and T8. The cell mediated immunity was significantly higher in T9 group followed by statistically similar T8 group, whereas significantly ($P < 0.05$) lower cell mediated immunity was obtained in control (T1) group which did not differ significantly from treatment T4, T3, T5, T2, T6 and T7 group. In Chicken IL-6, secreted by T cells and macrophages, acts as a pro-inflammatory cytokine in association of the production of acute phase proteins. The IL-6 upregulation in chicken has been associated with *Salmonella* and *Eimeria* infection (Park *et al.* 2022). Therefore, a downregulation of IL-6 in the present study favours an anti-inflammatory response and shows that *Lactobacillus reuteri* had an anti-inflammatory effect in the gut. The results of present study are in agreement with the finding of Lei *et al.* (2009) who reported downregulation of IL-6 in the chicken gut when their diet was supplemented with probiotic. The probiotic bacteria could act in stabilizing intestinal inflammation by balancing the intestinal microflora, maintaining mucosal barrier, modulating and improving the intestinal mucosal immune system, especially by keeping the balance of pro-inflammatory and anti-inflammatory cytokines and production of intestinal IgA (Zagato *et al.* 2014). The relative fold expression of IL-10 gene were significantly ($P < 0.05$) up regulated in T9 Group. The IL-10 act as anti-inflammatory cytokine that controls the nature and extent of inflammatory responses to various microbial infections (Couper *et al.* 2008) and is involved in intestinal immunity and homeostasis (Manzanillo *et al.* 2015). Similar to the results of the present study, Chen *et al.* (2012) observed significant increase in IL-10 expression following the *Lactobacillus* supplementation in broiler chicken. The TLR-4 is the principal receptor for lipopolysaccharide, which is a major component of the outer membrane of gram-negative bacteria (Kannaki *et al.* 2010). The decline in the expression of TLR-4 expression in T9 group in the present study means lesser receptor binding sites for the gram negative bacteria which results in the expulsion of pathogens like *Salmonella* and *E. coli* from the gut. The downregulation of TLR-4 expression would be expected in the intestine of broilers fed the probiotic-supplemented diet because dietary inclusion of the probiotics decreased the population of gram-negative bacteria such as Coliform in the rectum of broilers (Lei *et al.* 2009).

In the present study, supplementation of *Lactobacillus reuteri* sourced from red jungle fowl improved growth performance (body weight gain), immune response (humoral and cell mediated immune response), reduced pathogenic gut bacteria (*Salmonella* and *E. coli*)

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REFERENCES

- Alayande K A, Aiyegoro O A and Ateba C N. 2020. Probiotics in animal husbandry: Applicability and associated risk factors. *Sustainability* **12**(3): 1087.
- Castanon J I R. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science* **86**(11): 2466–71.
- Chaudhari A and Dwivedi M K. 2022. The concept of probiotics, prebiotics, postbiotics, synbiotics, nutraceuticals, and pharma-biotics. *Probiotics in the Prevention and Management of Human Diseases*. Academic Press.
- Chen C Y, Tsen H Y, Lin C L, Yu B and Chen C S. 2012. Oral administration of a combination of select lactic acid bacteria strains to reduce the *Salmonella* invasion and inflammation of broiler chicks. *Poultry Science* **91**(9): 2139–47.
- Chen C, Li J, Zhang H, Xie Y, Xiong L, Liu H and Wang F. 2020. Effects of a probiotic on the growth performance, intestinal flora, and immune function of chicks infected with *Salmonella pullorum*. *Poultry science* **99**(11): 5316–23.
- Cheng S and Lamont S J. 1988. Genetic analysis of immunocompetence measures in a white leghorn chicken line. *Poultry Science* **67**(7): 989–95.
- Couper K N, Blount D G and Riley E M. 2008. IL-10: The master regulator of immunity to infection. *Journal of Immunology* **180**(9): 5771–77.
- Duclos M J, Chevalier B, Goddard C and Simmon J. 1993. Regulation of amino acid transport and protein metabolism in myotubes derived from chicken muscle satellite cells by insulin like growth factor-I. *Journal of Cell Physiology* **157**(3): 650–57.
- Garriga M, Pascual M, Monfort J M and Hugas M. 1998. Selection of lactobacilli for chicken probiotic adjuncts. *Journal of Applied Microbiology* **84**: 125–32.
- Guobin C, Xiangping L and Jing L. 2011. Temporal and spatial expression of the pax-7 gene during chicken embryo and postnatal development. *Journal of Animal and Veterinary Advance* **10**(1): 1785–88.
- Gayawali I, Zeng Y, Zhou J, Li J, Wu T, Jiang Q and Zhu C. 2022. Effect of novel *Lactobacillus paracasei* microcapsule on growth performance, gut health and microbiome community of broiler chickens. *Poultry Science* 101912.
- Haghighi H R, Gong J, Gyles C, Hayes M A, Zhou H, Sanei B and Sharif S. 2006. Probiotics stimulate production of natural antibodies in chickens. *Clinical Vaccine Immunology* **13**(9): 975–80.
- Jha R, Das R, Oak S and Mishra P. 2020. Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: A systematic review. *Animals* **10**(10): 1863.
- Kaiser M G, Cheeseman J H, Kaiser P and Lamont S J. 2006. Cytokine expression in chicken peripheral blood mononuclear cells after *in vitro* exposure to *Salmonella enterica* serovar Enteritidis. *Poultry Science* **85**(11): 1907–11.
- Kannaki T R, Reddy M R, Shanmugam M, Verma P C and Sharma R P. 2010. Chicken toll-like receptors and their role

- in immunity. *Worlds Poultry Science Journal* **66**(4): 727–38.
- Khaksefidi A and Ghoorchi T. 2006. Effect of probiotic on performance and immunocompetence in broiler chicks. *Journal of Poultry Science* **43**(3): 296–300.
- Kita K, Kato S, Yaman M A, Okumura J and Yokota H. 2002. Dietary L-carnitine increases plasma insulin-like growth factor-I concentration in chicks fed a diet with adequate dietary protein level. *British Poultry Science* **43**(1): 117–21.
- Lei Y, Zhang K Y, Ding X M, Bai S P and Jung S C. 2009. Effect of probiotics on growth performance, development of small intestinal tract and microbial populations in broilers. *Agriculture Science and Technology* **3**: 24–31.
- Li SP, Zhao X J and Wang J Y. 2009. Synergy of Astragalus polysaccharides and probiotics (*Lactobacillus* and *Bacillus cereus*) on immunity and intestinal microbiota in chicks. *Poultry Science* **88**: 519–25.
- Mahmoud S, Shukry M and Saad M. 2013. Lymphocytic proliferation and interleukin-2 production in chickens supplemented with growth promoters. *Research Opinions in Animal and Veterinary Sciences* **3**(3): 68–72.
- Manzanillo P, Eidenschenk C and Ouyang W. 2015. Deciphering the crosstalk among IL-1 and IL-10 family cytokines in intestinal immunity. *Trends in Immunology* **36**(8): 471–78.
- Monika K, Malik T, Gehlot R, Rekha K, Kumari A, Sindhu R and Rohilla P. 2021. Antimicrobial property of probiotics. *Environment Conservation Journal* **22**(SE): 33–48.
- Okumura J and Kita K. 1999. Recent advance in the relationship between endocrine status and nutrition in chickens. *Asian-Australasian Journal of Animal Sciences* **12**: 1135–41.
- Park I, Oh S, Goo D, Celi P and Lillehoj H S. 2022. Effect of dietary sphorolipids on growth performance and gastrointestinal functionality of broiler chickens infected with *Eimeria maxima*. *Poultry Science* **101**(7): 101944.
- Paul S S, Chatterjee R N, Raju M V N, Prakas B, Rao S V R, Yadav S P and Kannan A. 2021. Gut microbial composition differs extensively among Indian native chicken breeds originated in different geographical locations and a commercial broiler line, but breed-specific, as well as across-breed core microbiomes, are found. *Microorganisms* **9**: 391.
- Qiu K, Wang X, Zhang H, Wang J, Qi, G and Wu S. 2022. Dietary supplementation of a new probiotic compound improves the growth performance and health of broilers by altering the composition of cecal microflora. *Biology* **11**(5): 633.
- Rivera-Pérez W, Barquero-Calvo E and Chaves A J. 2021. Effect of the use of probiotic *Bacillus subtilis* (QST 713) as a growth promoter in broilers: An alternative to bacitracin methylene disalicylate. *Poultry Science* **100**(9): 101372.
- Saleh A A, Amber K, El-Magd M A, Atta M S, Mohammed AA, Ragab M M and El-Kader H A. 2014. Integrative Effects of feeding *Aspergillus awamori* and fructooligosaccharide on growth performance and digestibility in broilers: Promotion muscle protein metabolism. *BioMed Research International* **21**(2): 41–50.
- Siegel P B and Gross W B. 1980. Production and persistence of antibodies in chickens to sheep erythrocytes. 1. Directional selection. *Poultry Science* **59**(1): 1–5.
- Snedecor G W and Cochran W G. 1994. *Statistical Method*, 6th ed. The Iowa State University Press, Ames, Iowa, USA.
- Stringfellow K, Caldwell D, Lee J, Mohnl M, Beltran R, Schatzmayr G and Farnell M. 2011. Evaluation of probiotic administration on the immune response of coccidiosis-vaccinated broilers. *Poultry Science* **90**(8): 1652–58.
- Vineetha P G, Tomar S, Saxena V K, Kapgate M, Suvarna A and Adil K. 2017. Effect of laboratory isolated *Lactobacillus plantarum* LGFCP 4 from gastrointestinal tract of guinea fowl on growth performance, carcass traits, intestinal histomorphometry and gastrointestinal microflora population in broiler chicken. *Journal of Animal Physiology and Animal Nutrition* **101**(5): 362–70.
- Yakar S, Rosen C J, Beamer W G, Ackert-Bicknell C L, Wu Y, Liu J L and LeRoith D. 2002. Circulating levels of IGF-1 directly regulate bone growth and density. *Journal of Clinical Investigation* **110**(6): 771–81.
- Yaqoob M U, Wang G and Wang M. 2022. An updated review on probiotics as an alternative of antibiotics in poultry. *Animal Bioscience* **35**(8):1109–20
- Zagato E, Mileti E, Massimiliano L, Fasano F, Budelli A, Penna G and Rescigno M. 2014. *Lactobacillus paracasei* CBA L74 Metabolic products and fermented milk for infant formula have anti-inflammatory activity on dendritic cells *in vitro* and protective effects against colitis and an enteric pathogen *in vivo*. *PloS One* **9**(2): 87615.
- Zhang B, Zhang H, Yu Y, Zhang R, Wu Y, Yue M and Yang C. 2021. Effects of *Bacillus coagulans* on growth performance, antioxidant capacity, immunity function, and gut health in broilers. *Poultry Science* **100**(6): 101168.