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Nutrient digestibility and growth promoting potential of *Lactobacillus plantarum* culture as probiotics in broilers#

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Abstract

This study aimed to assess the impact of supplementing Lactobacillus plantarum as probiotic, Mannan oligosaccharides (MOS) as prebiotic, and combination of probiotic and prebiotic on growth performance and ileal digestibility of nutrients in broiler chickens. A total of 128 Vencobb 430Y chicks were randomly allocated to four treatment groups, each with four replicates, and eight birds per replicate. The control diet was formulated in accordance with BIS recommendations and was fed to birds in T1. T2 birds received a basal diet, in addition 0.6 per cent L. plantarum as probiotic, T3 was supplemented with 0.4 per cent MOS extra, and T4 group added with both probiotic (0.6 per cent) and prebiotic (0.4 per cent) in basal diet. One bird from each replicate was randomly chosen to evaluate ileal nutrient digestibility on the 36th day using Titanium dioxide (TiO_2) as an external indicator, mixed at a rate of 5 g/kg of feed for six days. On 42nd day, birds were slaughtered, and ileal contents were collected to determine the titanium dioxide concentration. The results indicated that the inclusion of probiotics, prebiotics, and their combinations did not have a significant increase on the ileal digestibility of dry matter, crude protein, ether extract, crude fibre, organic matter, and nitrogen-free extract in broiler chickens. Significantly increased cumulative body weight and body weight gain was noted in T2 and T4 birds compared to control.

Keywords: *Broiler chicken, growth performance, ileal digestibility, nutrients, titanium dioxide*

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Probiotics are commonly used feed additives that are primarily used as growth promoters, alternative to antibiotics, in poultry. In recent years, an increase in awareness among consumers about antibiotic resistance has resulted in a reducing in the inclusion of antibiotics as growth promoters among the nutritionists. Probiotics are live microorganisms that, when administered in sufficient quantities, confer specific health benefits upon the host. Different modes of action of probiotics are listed by researchers include lowering the pH by acid fermentation, maintaining normal intestinal microflora by competitive exclusion antagonism through mucosal attachment and nutrients, producing bacteriocins and stimulating the immune system associated with the gut (Sarangi et al., 2016). Non-digestible non-starch polysaccharides or oligosaccharides, such as fructo-oligosaccharide (FOS), inulin, stachyose, oligo-fructose, mannan oligosaccharide (MOS), gluco-oligosaccharides, xylo-oligosaccharides, chito oligosaccharides, malto-oligosaccharides, isomalto-oligosaccharides and oligochitosan have been considered as prebiotics (Huang et al., 2007). The yeast cell wall fragment derived MOS from Saccharomyces cerevisiae contains glucan (30 per cent), mannan (30 per cent) and protein (12.5 per cent) and is used extensively in poultry farms (Song and Li, 2001). Different types of microorganisms are considered probiotics; more commonly lactic acid bacteria strains, which are natural inhabitants of the gastrointestinal tract (Fuller, 1989). Among these, a facultative, heterofermentative group of Lactobacilli viz., Lactobacillus plantarum was considered in this study to ascertain its potential effect on growth performance and ileal nutrient digestibility of broiler chicken and also its Combination with prebiotics.

Materials and methods

Source and preparation of probiotics

The freeze-dried culture of *L. plantarum* NCIM 2374 was purchased from National Collection of Industrial Microorganisms, Pune. The freeze-dried culture was revived by adding 20 mL of de Man, Rogosa and Sharpe (MRS) broth (Himedia M369) and marked as master culture. A working culture was prepared from master culture and adjusted to 10^9 colony-forming units (CFU)/mL by ten-fold serial dilution. Then, culture was centrifuged at 3200 x *g* for 15 minutes at 4 °C in a refrigerated centrifuge, the supernatant was removed, and cell pellets were washed with 0.1 M phosphate-buffered saline (PBS) (Kuriakose *et al*., 2023). Cell pellets were then resuspended in one mL of PBS containing 10^9 CFU was mixed thoroughly on a daily basis, ensuring uniform mixing by sprayer in the basal diet (v/w) as per the experimental design.

Housing, birds and experimental design

The experiment was undertaken following the guidelines approved by Institutional Animal Ethical Committee of Kerala Veterinary and Animal Sciences University (Reference number: IAEC/COVAS/PKD/20/6/2023) at Poultry Farm, Instructional Livestock Farm Complex, College of Veterinary and Animal Sciences, Pookode, Wayanad. One hundred and twenty-eight, day-old Vencobb-430Y broiler chicks were purchased from a local hatchery and randomly distributed to four groups *viz* T1, T2, T3 and T4 with four replicates having eight chicks each. The basal diet (broiler pre-starter, starter and finisher) was prepared as per BIS (IS 1374: 2007) and fed to T1; other treatment group rations were prepared as basal diet + 0.6 per cent probiotic (R2), basal diet + 0.4 per cent mannooligosaccharides as prebiotics (R3) and basal diet + 0.6 per cent probiotic and 0.4 per cent prebiotic (R4). The ingredient composition of experiment diets is presented in Table 1.

Growth performance measurements

The live body weights of all birds were recorded at weekly intervals in the morning hours after withdrawing feeders. Weekly weight gain and body weight were calculated for all replicates from the data collected. Daily feed consumption was calculated by the amount of feed consumed by all the birds from each replicate and by subtracting the leftover feed from total feed offered in a day. Weekly feed consumption was calculated by adding up the daily average feed consumption for the particular week. The weekly feed conversion ratio was calculated by dividing the weekly feed consumption by the weekly weight gain.

Table 1. Ingredient composition of experimental diets (%)

Ingredients	Period		
	Pre-starter (0-7 days)	**Starter (8-21 days)**	**Finisher (22-42 days)**
Maize	51.90	52.80	56.80
Soya bean meal	41.50	39.20	34.10
Di-calcium Phosphate	1.80	1.80	1.90
Calcite powder	1.40	1.40	1.40
Salt	0.38	0.38	0.38
Vegetable oil	2.64	4.10	5.21
Total	**100**	**100**	**100**
Trace mineral mix[1]	0.10	0.10	0.10
Vitamin premix[2]	0.08	0.08	0.08
L-Lysine[3]	0.06	0.18	0.06
DL-Methionine[4]	0.20	0.20	0.20
Choline Chloride[5]	0.10	0.10	0.10
Toxin binder[6]	0.10	0.10	0.10
Liver stimulant Powder[7]	0.03	0.03	0.03
Coccidiostat[8]	0.05	0.05	0.05
Chemical composition of basal diet (%, on dry matter basis*)			
Dry matter*	89.28 ± 0.07	88.82 ± 0.17	88.39 ± 0.16
Crude Protein*	23.34 ± 0.22	22.83 ± 0.06	20.48 ± 0.10
Ether extract*	3.59 ± 0.25	4.48 ± 0.14	5.66 ± 0.07
Crude fibre*	5.14 ± 0.15	4.95 ± 0.05	4.81 ± 0.04
Total ash*	7.20 ± 0.04	7.52 ± 0.05	7.49 ± 0.17
Calcium*	1.07 ± 0.01	1.08 ± 0.03	1.06 ± 0.02
Total phosphorus*	0.79 ± 0.02	0.71 ± 0.01	0.83 ± 0.01
Metabolizable energy (Kcal/kg) #	3000	3100	3200
Available Phosphorus #	0.45	0.45	0.45

** Estimated values; # calculated values*

Additives added to the basal diet contains

1. *Each kilo gram of mineral mixture contains- Manganese-100 g, Zinc-85 g, Iron- 90 g, Copper-15 g, Iodine-1.8 g, Selenium-0.45 g, Organic chromium- 0.15 g.*
2. *Each kilo gram of vitamin premix supplement contains Vitamin A - 82,500 IU, Vitamin D 3 – 12000 IU, Vitamin B 2 – 50 mg, Vitamin K – 10 mg, Vitamin B 1 – 4.0 mg, Vitamin B6 – 8.0 mg, Vitamin B 12 – 40 mcg, Niacin 60 mg, Calcium pantothenate – 40 mg, Vitamin E – 40 mg*
3. *L-Lysine mono-hydrochloride 98.5%. (Feed grade)*
4. *DL-Methionine 99%. (Feed grade)*
5. *Choline chloride 60%. (Feed grade)*
6. *Toxin binder containing a blend of Hydrated Sodium Aluminosilicate, organic acids, activated charcoal and natural herbal Ingredients*
7. *Liver Tonic Powder- hepatic stimulant and production enhancer*
8. *Coccidiostat- Diclazuril- (0.5 %) - each kg preparation containing 5 g of diclazuril*

Feed conversion ratio = Total feed consumed (g)/ Gain in weight (g)

Ileal digestibility trial

At 36th day of the experimental period, one bird from each replicate (four birds per treatment) was fed with Titanium dioxide (TiO_2) as an external marker mixed at the rate of 5 g/kg (Short *et al*., 1996) fed for six days and on day six, birds were slaughtered and the ileal content was collected by flushing with water and immediately stored at -20°C. The level of titanium dioxide was estimated with a spectrophotometer at 410 nm (Short *et al*., 1996; Myers *et al*., 2004). Proximate principles of experimental rations and ileal contents were analysed as per AOAC (2016). The digestibility of nutrients was calculated based on the formula given below.

Ileal Digestibility = 100 – [100 × (% Marker in Diet) / (% Marker in Ileal Contents) × (% Nutrients in Ileal Contents) / (% Nutrient in Diet)]

Statistical analysis

The experiment data collected on the ileal digestibility of nutrient parameters were analysed statistically for analysis of variance by One-way ANOVA for linear terms and means were compared for their significance at 99.5 per cent confidence level by Duncan's multiple range tests using the General Linear Model (GLM) of multivariate in statistical package IBM SPSS 20.0.

Results and discussion

Growth performance

The initial body weight, final body weight, cumulative body weight gain, cumulative feed intake and feed conversion ratio of broilers fed on different treatments are presented in Table 2 which revealed that the inclusion of probiotics, prebiotics or their combinations had no impact on the cumulative feed intake of broilers. In accordance with the present findings, Peng *et al*. (2016), reported supplementation of *L. plantarum* B1 probiotics (2 x 10^{12} CFU/ kg) added at the rate of 1g/ kg of basal feed and found similar feed intake in all groups during the experimental period. The dietary inclusion of MOS as prebiotics did not influence the feed intake of broilers in this study and our results are consistent with the findings of Zhou *et al*. (2019). Murshed and Abudabos (2015) demonstrated that the inclusion of prebiotics at different levels had no significant influence on cumulative feed intake and ADFI in broilers. However, in contrast to the present finding, Rokade *et al*. (2018) reported that prebiotic- dose-dependent variations were observed in feed intake of broilers.

Salehimanesh *et al*. (2016) evaluated supplementation of commercial multi-strain probiotics (minimum of 1 × 10^8 CFU/g) and MOS at 0.9 g/kg of broiler diets and found similar cumulative feed intake between the groups and similar findings were also observed by Wang *et al*. (2016). In contrary, Song *et al*. (2022) studied synbiotics supplementation (mixture of *L. Plantarum*- 1.0 x 10^{10} CFU/g product and FOS) in Arbor Acres broilers and

Table 2. Growth performance of broilers

Parameters	Groups				SEM	p-value
	T1	T2	T3	T4		
Initial body weight (g)	45.76	45.91	46.06	45.82	0.85	0.987^{ns}
Final body weight (g)	2285.76^a	2413.61^b	2254.06^a	2410.18^b	50.93	0.002*
Cumulative body weight gain (g)	2240.00^a	2367.70^b	2208.00^a	2364.36^b	22.55	0.000*
Cumulative feed intake (g)	3959.50	4004.00	3975.75	4039.25	47.06	0.656^{ns}
Feed conversion ratio	1.77	1.69	1.80	1.71	0.031	0.074^{ns}

$^{a, b}$ *Means with different superscripts within a column differ significantly*

**Significance at p<0.05, $^{ns-}$Non significant* showed significantly increased average daily feed intake during 1–21 days of its age.

In the present study, final body weight and cumulative body weight gain were significantly higher in T2 which was supplemented with 0.6 per cent *L. plantarum* when compared to the control. This finding is consistent with the results reported by Sampath *et al*. (2021), who examined the growth performance of broilers fed with 0.1 per cent *Lactobacillus* containing 1.2 x 10^9 CFU/kg.

In this study, the final body weight and cumulative body weight gain were similar between the control and 0.4 per cent MOS (T3) group. Similar to our findings, Khalaji *et al*. (2011), examined the effect of MOS supplementation at 0, 0.5, 1, and 1.5 g/kg of feed and found no significant improvement in their body weight and body weight gain, even at 1.5 g/kg inclusion. In contrast, Rokade *et al*. (2018) mentioned the inclusion of MOS at 0.5 per cent in broiler diets from 14 to 42 days of age, significantly increased body weight gain, compared to the control. In this study, when compared to the prebiotic group (T3), birds in the T2 and T4 groups had significantly higher body weight gain and similar results were also reported by Rajgor *et al*. (2015) who mentioned that the inclusion of prebiotics (500 g/ton of MOS) did not influence the body weight compared to control and had lower body weight when compared with birds supplemented with probiotics and combination of probiotics and prebiotics.

The findings of this study revealed that the birds fed with probiotics (T2) showed better FCR when compared to the control, however, they were statistically similar (P>0.05) to each other. These findings are in agreement with the findings of Sampath *et al*. (2021) who reported that the inclusion of 0.1 per cent *L. plantarum* containing 1.2 x 10^9 CFU/kg did not influence the FCR of broilers. In this study, FCR calculated in the control group was numerically better than birds that were fed with prebiotics (T3) and statistically did not differ. These present study findings are following the results reported by Khalaji *et al*. (2011) that inclusion of MOS in broiler diets at 0, 0.5, 1, and 1.5 g/kg did not influence the FCR of broilers.

In contrast to these findings, Rokade *et al.* (2018) reported a dose-dependent improvement in the FCR of broilers, with a significant improvement observed in the 0.5 per cent MOS group, followed by the 0.3 per cent MOS group, and finally in the control group.

The present study revealed T2 and T4 group birds showed significantly (P<0.05) improved FCR compared to birds fed with prebiotics (T3). These results are in accordance with Wang *et al*. (2016), who found that the inclusion of *Bacillus subtilis* spores as probiotics and probiotics along with prebiotics that contain MOS and β-glucans significantly improved the FCR of broilers when compared to both control and the birds fed with prebiotics. In contrast to our results, other researchers like Sarangi *et al*. (2016) and Salehimanesh *et al*. (2016) reported that supplementation of probiotics, prebiotics and synbiotics did not influence the FCR of broiler chicken during 1-42 days of its age.

Table 3. Ileal digestibility of nutrients

Attributes	Groups				SEM	p-value
	T1	T2	T3	T4		
Dry matter	75.57	77.75	76.45	78.07	0.60	0.060^{ns}
Crude protein	71.53	72.05	71.16	72.17	0.33	0.194^{ns}
Ether extract	77.06^{b}	77.11^{b}	75.99^{a}	77.43^{b}	0.24	0.013^{*}
Crude fibre	34.29	34.55	34.13	33.91	0.49	0.820^{ns}
Nitrogen free extract	72.24	71.30	73.41	72.57	0.63	0.206^{ns}
Organic matter	71.61	70.75	71.59	71.66	0.38	0.288^{ns}

a, b Means with different superscripts within a column differ significantly

**Significance at p<0.05, ^{ns}-Non significant*

Ileal digestibility of nutrients

The data on the ileal digestibility of nutrients are presented in Table 3 which revealed supplementation of probiotic, prebiotic, and combinations of probiotic with prebiotic had no influence on ileal digestibility of dry matter (DM), organic matter (OM), crude fibre (CF), and nitrogen-free extract (NFE) and the values obtained are presented in Table 2. However, the digestibility of ether extract (EE) showed significant differences among the treatments.

In this study, supplementation of probiotic did not significantly increase the ileal CP digestion compared to control, similar findings were reported by Sampath *et al*. (2021), while 0.1 per cent *L. plantarum* was added to broiler diet. These results suggest a potential dose-dependent influence of probiotic on nutrient digestibility, as previously indicated by Mountzouris *et al*. (2010), where an increased concentration of probiotics led to a reduction in the total tract digestibility of CP. Contrarily, He *et al*. (2019) showed a significant increase in the apparent total tract digestibility (ATTD) of DM, CP, and OM when *Bacillus subtilis*, *Bacillus licheniformis*, and *Saccharomyces cerevisiae* were added as probiotics. Mountzouris *et al*. (2010) reported significant improvements in the ileal digestibility of CP and EE when multi-strain probiotics were included at different levels (10^8 CFU/kg, 10^9 CFU/kg, and 10^{10} CFU/kg).

Ileal digestibility of CP was reported to be significantly increased by inclusion of MOS as prebiotic in broiler diets (Jahanian and Ashnagar, 2015) but in the present study, it was not evidenced. The present study findings evidenced that, ileal digestibility of EE was significantly ($p<0.05$) lower in prebiotic supplemented group than other groups.

Conclusion

This study reflects a complex interplay of probiotics and prebiotics on broiler growth and nutrient digestibility. In this study probiotics *L. plantarum* did not impact feed intake but showed potential for enhancing final weight and weight gain. However, prebiotics such as MOS did not notably affect intake or weight gain. Regarding nutrient digestibility, probiotics inconsistently enhanced certain nutrients, while prebiotics did not have an impact on nutrient digestibility. These mixed results underscore the variability influenced by dosage, specific bacteria used as probiotics, and the intricate relationship between probiotics and prebiotics.

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Conflict of interest

The authors declare that they have no conflict of interest

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