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Research Article

Effect of prebiotic (fructooligosaccharide) and probiotic (*Lactobacillus rhamnosus* NCDC 298) as alternative to antibiotics on the growth performance, gut health, and immune response in broiler chickens

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Abstract

This study aimed to evaluate the effects of dietary supplementation with prebiotic (fructooligosaccharide) and probiotic (*Lactobacillus rhamnosus* NCDC 298) on broiler chickens' growth performance and gut health. A total of 216 Ven Cobb 400 day old broiler chicks were randomly assigned to four dietary groups: basal diet (control), antibiotic at 500 g/ton feed (AGP), prebiotic (PRE), and probiotic (PRO) @ 1 mL/day/bird in drinking water. Body weight, feed intake, and feed conversion ratios were monitored weekly for 42 days. Blood biochemistry and immune response were assessed at specific time points. Carcass traits, gut microbiome, and gut morphology were examined at the end of the trial. The results showed that probiotic and prebiotic supplementation significantly increased final body weight and average daily gain compared to the control group. The probiotic group also had an improved feed conversion ratio. Feed intake, carcass traits, blood biochemistry, and antibody titers for infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV) were not affected by the treatments. Total *E. coli* counts were lower in the probiotic and prebiotic groups, while total *Salmonella* counts were lower and *Lactobacillus* counts were higher in the probiotic group. Crypt depth in the duodenum was lower in the probiotic group, while the villi height to crypt depth ratio was higher. In conclusion, probiotic and prebiotic supplementation could be a promising alternative to antimicrobials in broiler production.

Keywords: Antibiotic, Broiler, Growth performance, Gut health, Prebiotic, Probiotic

Highlights

- *Lactobacillus rhamnosus* NCDC 298 was used as probiotics in drinking water.
- The probiotic and prebiotic groups showed improved feed conversion ratio compared to the other groups
- Total *E. coli* and *Salmonella* counts were significantly lower in the probiotic supplemented group.
- *Lactobacillus* counts were significantly higher in the probiotic supplemented group.

INTRODUCTION

The broiler chicken industry is expanding rapidly to meet the increasing demand for chicken (Ghosh *et al*., 2019). However, the excessive use of antibiotics as growth promoters in broilers poses serious risks such as drug resistance and residues in livestock products, threatening food security (Rana *et al*., 2019). Many countries have banned the use of antibiotics as growth promoters in animal feed, leading to a shift towards alternative growth promoters in feed research.

Prebiotics are selectively fermented in the colon by native gut microbiota, targeting lactic acid bacterial genera such as *Bifidobacterium* and *Lactobacillus*. These bacterial species produce bacteriocins that combat pathogenic microbes like *Salmonella* sp. and *Escherichia coli*, improving chicken health (Shang *et al*., 2018; Al-Khalaifa *et al*., 2019; Omar *et al*., 2021). Fructooligosaccharide (FOS) is a prebiotic commonly found in plants, obtained through enzymatic synthesis using sucrose (Delbès *et al*., 2018). FOS is known for its antioxidant and immunomodulatory properties in both humans and animals (Dankowiakowska *et al*., 2019; Amer *et al*., 2020; Ricke *et al*., 2020; Namted *et al*., 2025). Studies have shown that dietary FOS supplementation improves physio-biochemical traits, antioxidant capacity, and carcass characteristics in broiler chickens (Al-Khalaifah and Al-Nasser, 2021; Ibrahim *et al*., 2021, Yan *et al*., 2025). Supplementation with FOS enhanced gut health by increasing the ratio of villus height to crypt depth, stimulating the growth

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of *Lactobacillus* and decreasing the presence of *E coli* (Phungkeha *et al.,* 2025).

Probiotics like *Lactobacillus, Lactococcus, Bifidobacterium*, and *Saccharomyces* are added to feed to improve growth performance, nutrient digestibility, gut microecology, immunity, and inhibit pathogenic bacteria in broiler chickens (Zhao *et al*., 2020; Rashidi *et al*., 2020; Inatomi and Otomaru, 2018; Ghasemi-Sadabadi *et al*., 2019, Hossain *et al*., 2024, Xu *et al*., 2023, Khan *et al*., 2024). Substituting veterinary drugs with probiotics is essential for sustainable broiler production.

Table 1. Composition of basal diets.

SL. No.	Ingredients	Pre Starter (Day 1-14)	Starter (Day 15-28)	Finisher (Day 29-42)
1	Maize	57.289	59.381	62.519
2	Soya DOC (45%).	37.247	34.035	30.003
3	Oil-veg	1.841	3.143	4.208
4	DCP	1.503	1.375	1.261
5	LSP powder	0.756	0.835	0.828
6	Salt	0.322	0.324	0.326
7	DL-methionine[1]	0.314	0.260	0.231
8	L-Lysine HCL[2]	0.226	0.154	0.131
9	L-Threonine[3]	0.084	0.055	0.055
10	Toxin Binder[4]	0.050	0.050	0.050
11	Sodium bi-carbonate	0.100	0.100	0.100
12	Choline Chloride, 60%[5]	0.050	0.070	0.070
13	Trace mineral mixture[6]	0.100	0.100	0.100
14	Vitamin premix[7]	0.100	0.100	0.100
15	Antioxidant[8]	0.010	0.010	0.010
16	Phytase 5000[9]	0.010	0.010	0.010
Nutrient composition				
1	Metabolizable energy (kcal/kg)[10]	3000.00	3100.00	3200.00
2	Crude protein (%)[11]	22.19	20.80	19.20
3	Ether extract (%)[11]	4.49	5.83	6.97
4	Crude fiber (%)[11]	4.00	4.24	5.36
5	Dry matter (%)[11]	90.32	91.29	90.28
6	Total Ash (%)[11]	11.48	13.51	11.54
7	Acid Insoluble Ash (%)[11]	0.82	0.82	0.66
8	Calcium (%)[11]	0.92	0.91	0.87
9	Total phosphorus (%)[10]	0.79	0.76	0.72
10	Available phosphorus (%)[10]	0.45	0.42	0.39
11	Lysine (%)[10]	1.22	1.09	0.98
12	Methionine (%)[10]	0.6	0.53	0.49
13	Methionine + cysteine (%)[10]	0.88	0.80	0.74
14	Threonine (%)[10]	0.77	0.70	0.65
15	Sodium (%)[10]	0.16	0.16	0.16
16	Chloride (%)[10]	0.18	0.18	0.18

[1]Evonik Methinine SEA Pte. Ltd. Singapore; [2]Qiqihar Longjiang Fufeng Biotechnologies Co., Ltd. Heilongjiang, China; [3]Xinjiang Meihua Amino Acid Co., Ltd, Xinjiang, China; [4]Niltox™, Zeus Biotech Limited, Mysore, India. (Toxin binder); [5]Indian Herbs Specialitis Pvt. Ltd. Solan, Himachal Pradesh; [6]Contains zinc 4.0%, manganese 4.0%, iron 1.5%, copper 0.8%, iodine 0.4%, selenium 300 ppm, chromium 200ppm (Zenex animal health India Pvt. Ltd, Patiya, Ahmedabad, India); [7]Contains vitamin E100 g, vitamin A 40000000 IU, vitamin D_3 12000000 IU, pantothenic acid 60 g, vitamin K 8 g, vitamin B_1 120 g, vitamin B_2 24 g, vitamin B_6 10 g, vitamin B_{12} 0.10 g, biotin 0.40 g, folic acid 4 g, niacin 100 g (DSM Nutritional Products India Pvt. Ltd. Mahabubnagar, Telangana, India); [8]Endox, Kemin Industries, Inc., USA; [9]Quantamblue, AB Vista, Pune, India; [10]Calculated values (based on the Asia South feed ingredients report 2016, Evonik Pvt Ltd, Singapore); [11]Analysed values (average of triplicate values).

The combination of probiotics and prebiotics can enhance the intestinal microbiome, absorption, and overall performance of broiler chickens. This study aims to investigate the impact of probiotic (*Lactobacillus rhamnosus* NCDC 298) and prebiotic (Fructooli-gosaccharide) supplements at different growth stages on growth performance, carcass traits, immune function, gut microbiota, and gut morphology of broiler chickens as an alternative to antimicrobials in poultry production to safeguard global health security.

MATERIALS AND METHODS

Broiler chickens, experimental design and diets: A total of 216 one-day-old mixed-sex commercial broiler chickens (Vencobb 400, Venkys, Pune, India) were randomly divided into four dietary groups, each consisting of six replicated pens (n=6) with nine broiler chickens per pen. The dietary groups included: 1) basal diet without any growth promoter (CON), 2) basal diet with antibiotic (Bacitracin methylene disalicylate - BMD) at 500 g/ton feed (AGP), 3) basal diet with prebiotic (fructooligosaccharide) at 1 mL/day/bird in drinking water (PRE), and 4) basal diet with probiotic(*Lactobacillus rhamnosus* NCDC 298) at 1 mL/ day/ bird in drinking water (PRO). The basal diet (Table 1) was formulated in mash form using maize and soybean to meet or exceed the nutritional requirements of broiler chickens at different stages (starter, grower, and finisher) based on the recommendations for Vencobb 400 broiler chickens (Venkys, 2017). The experimental diets were prepared weekly, packed in high-density polyethylene bags with inner liners, and provided ad libitum along with water.

Preparation of probiotics and prebiotics: The active probiotic culture Lactobacillus rhamnosus NCDC 298 was sourced from the Department of Dairy Microbiology, Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences in India. The culture was sub-cultured in MRS broth at 37°C for 15-18 hours for inoculation. To make it suitable for feeding to broiler chickens, the probiotics were prepared in liquid form using a skim milk-based medium. Skim milk powder (30 g) was mixed with distilled water (1000 mL), dextrose (10 g), and a nutrient mix (2.5 g). The mixture was sterilized at 121°C for 5 minutes in conical flasks. After cooling, the active MRS broth culture of Lactobacillus rhamnosus NCDC 298 was added at a rate of 1% and incubated at 37°C for 15-18 hours to create a probiotic fermented preparation. This probiotic preparation was stored at 5-7°C for up to 7 days.

For easier administration, the prebiotics were also prepared in liquid form. Skim milk powder (30 g), dextrose (10 g), fructooligosaccharide (50 g), and a supplement mixture (2.5 g) were reconstituted in 1000 mL distilled water in a conical flask and sterilized at 121°C for 5 minutes. After cooling, the prebiotic preparation was stored at 7°C for up to 7 days. This prebiotic preparation was then used for feeding to broiler chickens.

Management and rearing of birds: Before the chicks were introduced, the experimental house, feeding, and watering troughs were thoroughly cleaned and disinfected. The chicks were housed in floor pens (1.22 m × 0.76 m) separated by plastic wire netting. Rice husk and chopped paddy straw were used as litter, and each pen was equipped with sterile plastic feeders and water troughs. Initially, the chicks were provided with continuous lighting from fluorescent lamps for the first two days, followed by a lighting schedule of 23 hours of light and one hour of darkness each night. The temperature in the poultry house was regulated using heating elements, starting at 32°C on day 1 and gradually decreasing to 24°C by day 22. Proper ventilation was ensured with exhaust fans throughout the trial. All birds received vaccinations against Newcastle Disease virus (NDV) at 5 and 21 days of age and infectious bursal disease virus (IBDV) at 12 days of age.

Measurement of performance traits: The initial body weight (BW) of all chickens was recorded on the first day of the trial. Weekly weight measurements were taken, with a final measurement on the last day in the morning. Average daily gain (ADG) was calculated for each replicate. Weekly feed intake was determined by subtracting the remaining feed from the total offered per pen. Average daily feed intake (ADFI) was calculated by dividing the total feed consumed per day by the number of chickens in each pen. Feed conversion ratio (FCR) was calculated by considering cumulative feed intake and weight gain for each replicate pen. Daily mortality was recorded, and post-mortem examinations were conducted to determine the cause of death. Mortality percentage in each replicate was calculated at the end of the trial and used to adjust BW, ADG, ADFI, and FCR calculations.

Measurement of serum biochemical and antibody titre against Newcastle disease virus and Infectious bursal disease virus: Twelve birds per treatment were randomly selected, with two birds chosen from each pen. Blood samples were collected from broiler chickens on day 35 after a 12 hour fasting period. The samples were drawn from the wing vein without anticoagulant, and the serum was stored at -20°C until analysis. The concentrations of glucose, total protein, albumin, uric acid, triglycerides, and cholesterol in the serum were measured using commercial kits from DiaSys Diagnostic India Pvt. Ltd., Mumbai, India. Antibody titers for NDV and IBDV were determined using an ELISA kit from IDEXX Laboratories Inc. USA. The optical density (OD) was measured twice for each sample in a Microplate reader from Meril Life, India, and the mean OD values were used for analysis.

Measurements of carcass characteristics: On day 42, two birds (one male and one female with body weights close to the average for that replicate) were randomly chosen per replicate and slaughtered by cervical disarticulation for carcass trait evaluation. Various body portions were accurately weighed and recorded using a digital scale.

Enumeration of pre-caecal bacterial count: The caecal contents of chickens slaughtered on day 42 were collected aseptically in a sterile sample collection bag (HiMedia, India) and processed on the same day for bacteriological analysis. A standard colony counting procedure was used to quantify *E. coli, Salmonella*, and *Lactobacillus* in the caecal content (Quinn *et al*., 1994). One gram of caecal content was diluted tenfold with sterile phosphate-buffered saline (PBS). Subsequently, 10 ìL of the diluted sample was spread onto specific agar plates for each bacterium: *E. coli* on sorbitol-MacConkey agar, *Salmonella* on Xylose Lysine Deoxycholate agar, and *Lactobacillus* on Lactobacillus agar (all from HiMedia, India). The agar plates were incubated at 37°C for 24 to 48 hours, and the characteristic colonies for each bacterial group were counted using a digital colony counter (HiMedia, India). The results were expressed as Log10 colony-forming units (cfu) per gram of the sample.

Morphological study of small intestine: Small intestinal tissue samples were collected from slaughtered chickens of each dietary group to measure villus height, villus width, and crypt depth. Sections of the duodenum, jejunum, and ileum were taken and fixed in buffered formaldehyde solution, embedded in paraffin wax, stained with Delafield's Hematoxylin and Eosin, and mounted on DPX. Measurements were taken using an ocular micrometer and image analysis software. Villus height was measured from the tip to the villus-crypt junction, and crypt depth was measured as the depth between two villi. Twelve intact villi per section were selected based on the lamina propria. Each sample was observed in at least three sections with ten measurements each, and the values were averaged for analysis.

Chemical analysis of feeds samples: The feed samples were analyzed according to AOAC, (1995) methods. Dry matter (DM) was determined using method 934.01. Crude protein (CP) was analyzed using method 968.06 with Kelplus equipment from Pelican Equipments, Chennai, India. Crude fiber (CF) was measured using the Foss Fiber Cap 2021 Fiber Analysis System from Foss Analytical, Hilleroed, Denmark. Ether extract (EE) was determined using method 920.39 with Socsplus equipment from Pelican Equipments, Chennai, India. Calcium content was determined following the procedure described by Talapatra *et al.* (1940). Additionally, the AIA levels of the diets were measured using the technique outlined by Furuichi and Takahashi (1981).

Statistical analysis: The data were analyzed using one-way analysis of variance (ANOVA) with the statistical software SPSS 22 (SPSS Inc, 1997). The study followed a completely randomized design, with treatment as the main factor and pen as the experimental unit for body weight gain, feed intake, and FCR. Individual birds were considered as the experimental units for other parameters. Mortality data met homogeneity criteria and did not require transformation for statistical analysis. A significance level of P≤0.05 was used to determine significance, while P≤0.01 was considered a trend. When treatment had a significant effect, post-hoc Duncan's Multiple Test was used to detect differences among treatment means.

Table 2. Effect of prebiotic (Fructooligosaccharide) and probiotic (*Lactobacillus rhamnosus* NCDC 298) on average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of broiler chickens.

	Treatment[1]					
Attribute	CON	AGP	PRE	PRO	SEM[2]	P-Value
ADG (g/d)						
Day 1-14	25.26	25.51	25.26	25.41	0.162	0.940
Day15-28	74.80	76.43	76.67	76.79	0.630	0.680
Day 29-42	67.31	71.04	76.13	78.78	1.760	0.084
Day 1-42	55.79^{b}	57.67^{ab}	59.35^{a}	60.33^{a}	0.610	0.032
Final Body weight	2385.68^{b}	2464.55^{ab}	2535.56^{a}	2576.10^{a}	25.568	0.032
ADFI (g/d)						
Day 1-14	29.09	29.00	28.84	29.01	0.047	0.300
Day15-28	105.60	104.50	102.95	102.36	0.892	0.599
Day 29-42	133.13	131.04	132.07	132.11	1.079	0.936
Day 1-42	89.26	88.18	87.95	87.83	0.486	0.743
FCR (g intake/g gain)						
Day 1-14	1.16	1.14	1.14	1.14	0.008	0.852
Day15-28	1.41	1.37	1.34	1.33	0.015	0.221
Day 29-42	1.99	1.85	1.75	1.70	0.044	0.083
Day 1-42	1.60^{b}	1.53^{b}	1.49^{ab}	1.46^{a}	0.017	0.011

^{abc}means bearing different superscripts in the same row differ significantly (p≤0.05); [1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with prebiotic (Fructooligosaccharide) at the rate of 1 mL/per bird/ day (PRE), control diet with probiotic (*Lactobacillus rhamnosus* NCDC 298) at the rate of 1 mL/per bird/day; [2]SEM, standard error of means (n=6).

RESULTS

Growth performance: Table 2 presents the final body weight, average daily gain, feed intake, and feed efficiency data. The final body weight of birds significantly increased in the probiotic and prebiotic groups compared to the control group, but did not differ with antibiotic group. The average daily gain (ADG) was significantly higher in the probiotic and prebiotic groups throughout the 42-day experiment period compared to the control group, but did not differ with antibiotic group. However, no significant differences were observed during the starter (1-14 days), grower (15-28 days), and finisher (29-42 days) phases. The average daily feed intake (ADFI) did not vary significantly among the treatment groups during different phases or over the entire 42-day period. Feed conversion ratio (FCR) showed no significant differences during the starter, grower, and finisher phases but was significantly improved in the probiotic group compared to the control and antibiotic groups over the entire 42-day period, however did not differ with prebiotic supplemented group.

Blood serum biochemical profile: The concentration of glucose, total protein, albumin, uric acid, triglyceride, and cholesterol in serum did not vary significantly (P>0.05) based on dietary treatments in this study (Table 3).

Immune response: There were no significant differences (p>0.05) in antibody titers to the Newcastle disease (ND) and infectious bursal disease (IBD) vaccines between the dietary treatment groups on days 28 and 35 (Table 4).

Carcass characteristics: No significant differences (P>0.05) were observed in slaughter body weight, eviscerated carcass weight, dressing percentage, breast, frame, thigh, drumstick, wing, neck, gizzard, liver, heart, spleen, bursa, and abdominal fat weight in grams across the various treatment groups (Table 5).

Gut microbes: The count of *E. coli* was significantly lower (P<0.05) in the probiotic and prebiotic

Table 3. Effect of prebiotic (Fructooligosaccharide) and probiotic (*Lactobacillus rhamnosus* NCDC 298) on blood biochemical profile of broiler chickens.

	Treatment[1]					
Attribute	**CON**	**AGP**	**PRE**	**PRO**	**SEM[2]**	**P-Value**
Glucose (mg/dL)	282.61	240.01	244.41	254.49	11.991	0.627
Total protein (mg/dL)	3.34	3.77	3.38	3.64	0.097	0.367
Albumin (mg/dL)	2.99	3.24	3.14	3.25	0.051	0.241
Uric acid (mg/dL)	3.26	3.11	3.23	3.19	0.081	0.933
Triglyceride (mg/dL)	159.90	170.43	176.89	151.78	7.053	0.636
Cholesterol (mg/dL)	171.12	157.89	160.54	160.48	6.772	0.920

[1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with prebiotic (Fructooligosaccharide) at the rate of 1 mL/per bird/day (PRE), control diet with probiotic (*Lactobacillus rhamnosus* NCDC 298) at the rate of 1 mL/per bird/day; [2]SEM, standard error of means (n=6).

Table 4. Effect of prebiotic (Fructooligosaccharide) and probiotic (*Lactobacillus rhamnosus* NCDC 298) on antibody titer ($\log_{10}$) against New castle disease vaccine (NDV) and infectious bursal disease vaccine (IBDV) of broiler chickens.

	Treatment[1]					
Attribute	**CON**	**AGP**	**PRE**	**PRO**	**SEM[2]**	**P-Value**
NDV						
Day 28	2.50	2.68	2.62	2.69	0.047	0.478
Day 35	2.80	2.82	2.81	2.87	0.042	0.959
IBDV						
Day 28	3.09	3.12	3.15	3.16	0.040	0.917
Day 35	3.15	3.27	3.23	3.29	0.041	0.673

[1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with prebiotic (Fructooligosaccharide) at the rate of 1 mL/per bird/day (PRE), control diet with probiotic (*Lactobacillus rhamnosus* NCDC 298) at the rate of 1 mL/ per bird/day; [2]SEM, standard error of means (n=6).

Table 5. Effect of prebiotic (Fructooligosaccharide) and probiotic (*Lactobacillus rhamnosus* NCDC 298) on carcass characteristics of broiler chickens.

	Treatment[1]					
Attributes	**CON**	**AGP**	**PRE**	**PRO**	**SEM[2]**	**P-Value**
Slaughter BW (g)	2443.75	2462.27	2458.15	2482.29	17.424	0.920
Eviscerated BW (g)	1664.85	1684.91	1654.01	1673.14	14.409	0.921
Dressing Percentage (%)	68.12	68.45	67.27	67.40	0.278	0.420
Breast (g)	598.67	606.67	586.00	631.67	12.569	0.688
Frame (g)	307.00	285.67	296.67	302.33	5.092	0.546

Cont. Table 5.

Table 5., Cont. ...

Attributes	Treatment[1] CON	AGP	PRE	PRO	SEM[2]	P-Value
Thigh (g)	227.67	222.00	220.33	195.67	4.996	0.081
Drumstick (g)	217.00	228.33	219.00	219.00	2.759	0.533
Wing (g)	126.33	134.67	135.00	124.67	2.878	0.502
Neck (g)	58.17	60.67	62.00	55.83	1.522	0.552
Gizzard (g)	44.51	47.43	47.34	46.20	1.207	0.858
Liver (g)	33.07	39.51	37.16	41.86	1.589	0.257
Heart (g)	10.29	10.67	10.09	9.69	0.146	0.088
Spleen (g)	1.94	2.26	2.77	2.03	0.201	0.524
Bursa (g)	1.43	1.43	0.58	0.10	0.199	0.418
Abdominal Fat (g)	38.04	35.93	36.12	41.30	1.732	0.733

[1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with prebiotic (Fructooligosaccharide) at the rate of 1 mL/per bird/day (PRE), control diet with probiotic (*Lactobacillus rhamnosus* NCDC 298) at the rate of 1 mL/per bird/day; [2]SEM, standard error of means (n=6).

groups compared to the control group, but did not differ with antibiotic supplemented group. The count of *Salmonella* was significantly lower (P<0.05) in the probiotic group compared to the control and antibiotic groups, while there was no significant difference between probiotic and prebiotic supplemented groups. Additionally, the count of *Lactobacillus* was significantly higher (p<0.05) in the probiotic group compared to the prebiotic, control and antibiotic supplemented groups (Table 6).

Table 6. Effect of prebiotic (Fructooligosaccharide) and probiotic (*Lactobacillus rhamnosus* NCDC 298) on viable bacteria numbers (log_{10}cfu/g) in caecal content of broiler chickens.

Attribute	Treatment[1] CON	AGP	PRE	PRO	SEM[2]	P-Value
Escherichia coli	6.81^{a}	6.66^{ab}	6.55^{b}	6.43^{b}	0.046	0.014
Salmonella sp	6.70^{a}	6.57^{ab}	6.35^{bc}	6.23^{c}	0.056	0.005
Lactobacillus sp	7.12^{c}	7.16^{bc}	7.27^{b}	7.50^{a}	0.035	0.000

^{ab}means bearing different superscripts in the same row differ significantly (p≤0.05); [1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with prebiotic (Fructooligosaccharide) at the rate of 1 mL/per bird/day (PRE), control diet with probiotic (*Lactobacillus rhamnosus* NCDC 298) at the rate of 1 mL/ per bird/day; [2]SEM, standard error of means (n=6).

Gut morphology: There were no significant differences (p>0.05) in villi height (VH) duodenum, jejunum and ileum; and crypt depth (CD), and the ratio of villi height to crypt depth (VH/CD) in the jejunum and ileum among the treatment groups. However, the probiotic group exhibited significantly higher crypt depth in the duodenum compared to the control and antibiotic-treated groups. Furthermore, an improved ratio of villi height to crypt depth in the duodenum was observed in the probiotic group compared to the control group (Table 7).

Table 7. Effect of prebiotic (Fructooligosaccharide) and probiotic (*Lactobacillus rhamnosus* NCDC 298) on gut morphology of broiler chickens.

Attributes	Treatment[1] CON	AGP	PRE	PRO	SEM[2]	P-Value
Duodenum						
Villi height (VH; µm)	1552.83	1547.17	1507.67	1613.50	37.094	0.814
Crypt depth (CD; µm)	189.83	178.50	133.00	90.17	14.826	0.056
VH/CD ratio	8.24^{b}	14.23^{ab}	11.59^{ab}	18.56^{a}	1.366	0.041
Jejunum						
Villi height (VH; µm)	955.67	964.83	991.33	1012.50	38.689	0.960
Crypt depth (CD; µm)	123.33	117.17	111.83	107.00	5.494	0.772
VH/CD ratio	8.13	8.36	9.13	10.03	0.497	0.554

Cont. Table 7.

Table 7., Cont. ...

Attributes	Treatment[1]				SEM[2]	P-Value
	CON	AGP	PRE	PRO		
Ileum						
Villi height (VH; µm)	661.33	670.00	672.00	675.00	22.069	0.997
Crypt depth (CD; µm)	134.33	128.50	123.67	122.00	6.134	0.907
VH/CD ratio	5.00	5.36	5.48	6.04	0.256	0.572

[ab]means bearing different superscripts in the same row differ significantly ($p \leq 0.05$); [1]The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200 mg/MT feed (AGP), control diet with prebiotic (Fructooligosaccharide) at the rate of 1 mL/per bird/day (PRE), control diet with probiotic (*Lactobacillus rhamnosus* NCDC 298) at the rate of 1 mL/per bird/day; [2]SEM, standard error of means (n=6).

DISCUSSION

In this study, it was observed that supplementing probiotics significantly influenced the final body weight and average daily gain throughout the 42-day experiment. Additionally, feed conversion ratios were significantly improved in the prebiotic-supplemented group during the entire experimental period. These findings confirm the positive effects of probiotics on the growth performance of broilers, consistent with previous studies (Mookiah *et al*., 2014; Kaushal *et al*., 2019; Tarabees *et al*., 2019; Tsega *et al*., 2024; Liu *et al.,* 2025). Moreover, the supplementation of single probiotic or mixed probiotic feed additives could remarkably enhanced broilers' growth performance and their feed conversion ratio under normal, stressful, diseased, and other challenging conditions (Attia *et al*., 2013). Broilers fed with *L. casei* and *L. acidophilus* showed an increase in average daily gain (Drissi *et al*., 2017). Conversely, others have stated that probiotics had no positive eûect on broilers' performance (Fujiwara *et al*., 2009). The inconsistent roles of probiotics among these studies including the present study may be related to the type and dosage of probiotics, and the breed of the broilers. It is pertinent to mention that FCR is very crucial in the poultry sector as it reûects the efficient utilization of nutrients (Dixon, 2020). The amylase, protease, and lipase produced by probiotic preparation could degrade plant carbohydrates and complex substances in the feed, so that the carbohydrates were better absorbed and utilized by the intestine, thereby improving the feed efficiency (Liao and Nyachoti, 2017).

In this study, the concentrations of glucose, total protein, albumin, uric acid, triglyceride, and cholesterol in serum showed no significant variations ($P>0.05$) across different dietary treatments. This finding is consistent with previous research by Abdel-Hafeez *et al*. (2017), who also found no impact of prebiotics and probiotics on these serum markers. Similarly, Karunanayaka *et al*. (2020) observed no significant differences in serum cholesterol and triglyceride levels when comparing the use of probiotics, prebiotics, and synbiotics to a control group. Additionally, Sun *et al*. (2019), Chuang *et al*. (2021) and Liza *et al*. (2022) reported that the broiler diet did not have a significant effect on the blood biochemical profile.

In this study, there were no significant differences ($p>0.05$) in antibody titers to the infectious bursal disease (IBD) and Newcastle disease (ND) vaccines among the dietary treatment groups on day 28 and day 35, as well as for the Newcastle disease (ND) vaccine on day 35. This result aligns with previous research by Rehman *et al*. (2020), who found that the antibody titers against Newcastle disease were not influenced by probiotics, prebiotics, or their interactions ($P>0.05$). In contrast, Ghasemi-Sadabadi *et al*. (2019) reported conflicting results, showing that antibody titers against Newcastle and Influenza viruses significantly increased ($P<0.05$) when birds were fed diets containing prebiotics and probiotics compared to the control diet.

The current study did not show any statistically significant differences in carcass characteristics when probiotics and prebiotics were added to the diet of broiler chickens. These results are consistent with previous research. Sarangi *et al*. (2016) found no significant differences in carcass traits in broiler chickens due to prebiotic supplementation. Rehman *et al*. (2020) also reported no notable changes in organ weights and lengths with prebiotic supplementation. Askri *et al*. (2022) observed no significant differences in carcass yields, breast muscle, and thigh weights ($P>0.05$). These results align with studies by Mahmud *et al*. (2005), Midilli *et al*. (2008), Chumpawadee *et al*. (2009), Konca *et al*. (2009), Wang *et al*. (2015), Salehimanesh *et al*. (2016) and Abu Shulukh *et al*. (2017) indicating that prebiotic supplementation did not significantly impact carcass characteristics or meat quality. Tang *et al*. (2021) and Yadav *et al*. (2018) found no significant differences in carcass traits with probiotic supplementation in broiler chickens. Islam and Nishibori, (2023) also reported no significant effects on carcass traits with varying levels of probiotic supplementation. However, Abdel-Hafeez *et al*. (2017) observed a significant increase in carcass traits in broiler chickens supplemented with probiotic at a specific concentration.

This study found that probiotic supplementation increased Lactobacillus levels and decreased *E. coli* and *Salmonella* levels compared to antibiotic and control groups. Previous research has shown that probiotics, especially lactic acid bacteria, can effectively inhibit *Salmonella*, *Enteritidis*, and *E. coli* infections in poultry. Rivera-Pérez *et al*. (2021) studied the use of *Bacillus subtilis* as an alternative to antibiotic growth promoters (AGP). They observed lower E. coli and Enterococcus counts and higher Lactobacillus counts in the probiotic group compared to the control and AGP groups. Qiu *et al*. (2021) also found that *B. subtilis* supplementation increased *Lactobacillus* and *Bifidobacteria* levels while decreasing *Coliforms* and *Clostridium perfringens*. Jeong and Kim, (2014) showed that *B. subtilis* supplementation increased *Lactobacillus* counts and reduced *E. coli* counts in various parts of the gut. It also tended to decrease *Clostridium perfringens* and *Salmonella* counts. Gao *et al*. (2017) reported that *Bacillus subtilis* supplementation promotes beneficial bacteria like Lactobacillus and *Bifidobacteria* while reducing pathogenic bacteria such as *E. coli*, *Coliform*, *Enterobacteriaceae*, and *Salmonella*.

The present study did not show any significant effect on gut morphology in the treatment groups except probiotic shows significantly improved the crypt depth and ration of villi height to the crypt depth in duodenum, while other treatment groups did not show significant effects on gut morphology. These results are consistent with previous studies by Salehimanesh *et al*. (2016) and Çalýk *et al*. (2017), which also found no changes in gut morphology with probiotics and prebiotics. However, studies by Al-Baadani *et al*. (2016) and Al-Sultan *et al*. (2016) reported significant improvements in villi length and surface area with probiotics, prebiotics, and synbiotics supplementation. In contrast, Bogus³awska-Tryk *et al*. (2021) and Ghahri *et al*. (2013) did not observe significant changes in gut morphology with probiotics, prebiotics, and synbiotics supplementation in broiler chickens.

Birds given probiotics had a better feed conversion ratio (FCR) throughout the experiment compared to the control group. Probiotic supplementation also led to lower levels of *E. coli* and *Salmonella* in the ceca compared to other groups. Lactobacillus levels were significantly higher in birds that received probiotics compared to those in the control and antibiotic groups. These findings suggest that probiotics could serve as effective alternatives to antibiotics in poultry farming, particularly for broilers on an antibiotic-free diet, promoting overall bird health. Further research is necessary to fully explore and optimize the use of probiotics in poultry production. This study underscores the potential of probiotics as a promising option for improving gut health in poultry without the need for antibiotics.

Conflict of interest: We wish to confirm that there is no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

Author's contribution: SS: Involved in the investigation, data generation, and preparing original draft; GPM: Engaged in conceptualization, data curation, supervision and final editing; BR: Methodology editing; SaM: Interpretation of gut morphology; SuM: Concept for preparation of probiotic; IS: Supervised the gut microbiota and ELISA.

Data availability statement: Raw data of this study are available from the corresponding author.

Ethical statement: The study protocol was approved by Intuitional Animal Ethics Committee of West Bengal University of Animal and Fishery Sciences, (Ethics Approval Number: WBUAFS/IAEC/32/23-24) Kolkata, India.

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