



# Impact of Postbiotic Produced from *Lactobacillus rhamnosus* NCDC 298 as a Replacement for Antibiotics on the Growth Performance, Gut Health and Immune Response in Broiler Chickens

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## ABSTRACT

The experiment was conducted during the month of June–July 2025 at the Department of Animal Nutrition, WBUAFS, Kolkata, West Bengal, to study the effect of postbiotics on broiler chicken performance. This study aimed to assess the impact of dietary supplementation with a postbiotic (inactivated *Lactobacillus rhamnosus* NCDC 298) on the performance of broiler chickens. A total of 162 day-old broiler chicks were randomly assigned to three dietary groups: 1) basal diet (CON), 2) basal diet with antibiotic (Bacitracin methylene disalicylate-BMD) at 500 gton<sup>-1</sup> feed (AGP), and 3) basal diet with postbiotic at 1 ml/bird in drinking water (POS). Body weight, feed intake, and feed conversion ratios (FCR) were monitored weekly for 42 days. Blood biochemistry and immune response were assessed on days 28 and 35 of the trial. Carcass traits, gut microbiome, and gut morphology were evaluated at the end of the trial. The results showed that the postbiotic supplementation (POS) significantly improved FCR compared to the CON and AGP groups ( $p<0.05$ ). Antibody titers for infectious bursal disease virus (IBDV) and Newcastle disease virus (NDV) did not differ significantly among the groups, except for a higher NDV titer on day 28 in the POS group compared to CON and AGP ( $p<0.05$ ). Total *E. coli* counts were significantly lower in the POS group compared to CON and AGP ( $p<0.05$ ), while total *Salmonella* counts were lower in the POS group compared to CON ( $p<0.05$ ). In conclusion, postbiotic supplementation shows promise as an alternative to antimicrobials in broiler production.

**KEYWORDS:** Chicken, growth performance, *Lactobacillus rhamnosus*, postbiotic

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**Data Availability Statement:** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## 1. INTRODUCTION

In order to boost productivity, encourage growth, and shield birds from pathogens, the poultry industry has long used non-therapeutic antibiotics. Nevertheless, this practice has recently been linked to the development of bacteria resistant to antibiotics in poultry and bird products, which can have an adverse effect on the environment (Hayat et al., 2020, Letlhogonolo et al., 2020; Paintsil et al., 2021, Manyi-Loh et al., 2018; Oniciuc et al., 2018). The use of antibiotics is prohibited in some nations, and consumer demand for poultry products free of antibiotics is rising. As a result, the poultry industry has a high demand for alternatives to the use of non-therapeutic antibiotics (Cuevas-González et al., 2020). In order to replace antibiotics as growth promoters without compromising the growth and welfare of poultry, researchers are looking into alternate strategies, such as adding feed additives like essential oils, organic acids, enzymes, prebiotics, probiotics, synbiotics, and postbiotics (Reuben et al., 2021, Abd El-Hack et al., 2022; Oliveira et al., 2022; Morgan, 2023). The scientific community is very interested in and investigating the use of prebiotics, probiotics, synbiotics, and postbiotics in poultry production. These dietary supplements have the potential to replace antibiotics by enhancing poultry health and productivity while lowering reliance on antimicrobials. Although probiotics have numerous health advantages, there is disagreement regarding their efficacy and functionality. According to recent research, probiotics should be more precisely formulated to optimize their positive effects on different animal species. Furthermore, it has been discovered that some probiotic bacterial strains contain genes resistant to antibiotics, which can be passed on to the environment and gut microbiota (Gueimonde et al., 2013; Imperial and Ibana, 2016). Additionally, research has indicated that certain probiotics may have adverse effects on the host by aggravating tissue inflammation in patients with inflammatory bowel disease and causing local inflammation in healthy hosts (Ahmad et al., 2022). The term "postbiotic" has been coined, broadening the definition of probiotics beyond their intrinsic viability (Abd El-Ghany et al., 2022, Martín and Langella, 2019). Postbiotics are soluble substances (stabilized bacteria, cellular products, or metabolic by-products) obtained primarily from *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, fecal bacteria, and *Saccharomyces cerevisiae* yeast that are secreted by living microorganisms or released following microbial lysis (Moradi et al., 2020, Abd El-Ghany et al., 2022, Cicenia et al., 2016; Tiptiri-Kourpeti et al., 2016; Johnson et al., 2019). According to recent studies, postbiotics may improve intestinal cell adhesion, change the immune system, and secrete different metabolites, among

other health benefits (Akter et al., 2020, Aguilar-Toalá et al., 2018). Compared to probiotic-supplemented feed preparations, non-viable microorganisms or microbial cell extracts have an advantage because probiotic viability can vary and dead cells may outnumber live cells (Terpou et al., 2019, Danladi et al., 2022, Chang et al., 2022, Human et al., 2019). Additionally, the shelf life of poultry products can be considerably shortened by non-viable microbes and extracts (Taverniti and Guglielmetti, 2011). This study aimed to investigate the effects of postbiotics (inactivated *Lactobacillus rhamnosus* NCDC 298) on the growth performance, carcass characteristics, gut microflora, and immunity of broiler chickens as an alternative to antimicrobials in the poultry production system to minimize the impact on global health security.

## 2. MATERIALS AND METHODS

The experiment was conducted during the month of June–July, 2025, at the Department of Animal Nutrition, WBUAFS, Kolkata, West Bengal, to study the effect of postbiotics on broiler chicken performance.

### 2.1. Broiler chickens, experimental design and diets

A total of 162 one-day-old mixed-sex commercial broiler chickens (Vencobb 400, Venkys, Pune, India) were randomly divided into three 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 (AGP) at a rate of 500 g/ton feed, and 3) basal diet with postbiotics-inactivated *Lactobacillus rhamnosus* NCDC 298 at a rate of 1 ml/bird in drinking water (POS). 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.

1. Evonik Methinine SEA Pte. Ltd. Singapore.
2. Qiqihar LongjiangFufeng 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 Specialties. Ltd., Solan, Himachal Pradesh.
6. Contains zinc 4.0%, manganese 4.0%, iron 1.5%, copper 0.8%, iodine 0.4%, selenium 300ppm, chromium 200ppm (Zenex Animal Health India Pvt. Ltd, Patiya, Ahmedabad,

India).

7. Contains vitamin E100 g, vitamin A 40000000 IU, vitamin D<sub>3</sub> 12000000IU, pantothenic acid 60 g, vitamin K 8 g, vitamin B<sub>1</sub> 120 g, vitamin B<sub>2</sub> 24 g, vitamin B<sub>6</sub> 10 g, vitamin B<sub>12</sub> 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 Pte Ltd, Singapore).

11. Analyzed values (average of triplicate values).

## 2.2. Preparation of postbiotics

The active probiotic culture *Lactobacillus rhamnosus* NCDC 298 was obtained from the Department of Dairy Microbiology, Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences, Mohanpur, Nadia, West Bengal, India. Sub-culturing was carried out in MRS broth at 37°C for 15–18 hours for inoculation. To facilitate feeding to broiler chickens, the postbiotics were prepared in liquid form using a skim milk-based medium. Skim milk powder (30 g) was reconstituted in 1000 ml of distilled water, and dextrose (10 g) and nutrient mix (2.5 g) were added to the skim milk solution. The mixture was then distributed into conical flasks and heated at 121°C for 5 minutes. After cooling to room temperature, the active MRS broth culture of *Lactobacillus rhamnosus* NCDC 298 was inoculated at a rate of 1% and incubated at 37°C for 15–18 hours to obtain a probiotic preparation. This preparation was further heated in a boiling water bath for 10 minutes to ensure complete inactivation of the live probiotic culture, resulting in a postbiotic preparation. The postbiotic preparation was stored at 5–7°C for up to 7 days.

## 2.3. Management and rearing of birds

Before the chickens arrived, the experimental house, feeding, and watering troughs were cleaned and disinfected. The chickens were housed in floor pens (1.22×0.76 m<sup>2</sup>) separated by plastic wire netting. Rice husk and chopped paddy straw served as litter, and each pen had sterile plastic feeders and water troughs. For the first two days, the chicks had continuous lighting from compressed fluorescent lamps, followed by a lighting schedule of 23 hours of light and one hour of darkness each night. The temperature in the poultry house was controlled using heating elements, starting at 32°C on day 1 and gradually decreasing to 24°C by day 22. Proper ventilation was maintained with exhaust fans throughout the trial. All birds were vaccinated against Newcastle Disease virus (NDV) at 5 and 21 days of age and infectious bursal disease virus (IBDV) at 12 days of age.

## 2.4. 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.

## 2.5. 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.

## 2.6. Collection of blood samples for serum biochemical analyses

Biochemical blood samples were collected from broiler chickens on day 35 after a 12-hour fasting period. The samples were drawn from the wing vein. Twelve birds per treatment were randomly selected, with two birds chosen from each pen. Blood was collected without an 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.

## 2.7. Enumeration of pre-caecal bacterial count

The caecal contents of chickens slaughtered on day 42 were aseptically collected in a sterile sample collection bag (HiMedia, India). The samples were processed on the same day for bacteriological analysis using a standard colony counting procedure to quantify *E. coli*, *Salmonella*, and *Lactobacillus* in the caecal content (Quinn et al., 1994). For analysis, one gram of caecal content was diluted tenfold with sterile phosphate-buffered saline (PBS). Subsequently, 10µL 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 then incubated at 37°C for 24 to 48 hours, and the characteristic colonies for each bacterial group were counted using a digital colony counter

Table 1: Composition of basal diets

SL. No.	Ingredients	Starter (Day 1-14)	Grower (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 <sup>1</sup>	0.314	0.260	0.231
8.	L-Lysine HCL <sup>2</sup>	0.226	0.154	0.131
9.	L-Threonine <sup>3</sup>	0.084	0.055	0.055
10.	Toxin Binder <sup>4</sup>	0.050	0.050	0.050
11.	Sodium bi-carbonate	0.100	0.100	0.100
12.	Choline Chloride, 60% <sup>5</sup>	0.050	0.070	0.070
13.	Trace mineral mixture <sup>6</sup>	0.100	0.100	0.100
14.	Vitamin premix <sup>7</sup>	0.100	0.100	0.100
15.	Antioxidant <sup>8</sup>	0.010	0.010	0.010
16.	Phytase 5000 <sup>9</sup>	0.010	0.010	0.010
<u>Nutrient composition</u>				
1.	Metabolizable energy (kcal/kg) <sup>10</sup>	3000.00	3100.00	3200.00
2.	Crude protein (%) <sup>11</sup>	22.19	20.80	19.20
3.	Ether extract (%) <sup>11</sup>	4.49	5.83	6.97
4.	Crude fiber (%) <sup>11</sup>	4.00	4.24	5.36
5.	Dry matter (%) <sup>11</sup>	90.32	91.29	90.28
6.	Total Ash (%) <sup>11</sup>	11.48	13.51	11.54
7.	Acid Insoluble Ash (%) <sup>11</sup>	0.82	0.82	0.66
8.	Calcium (%) <sup>11</sup>	0.92	0.91	0.87
9.	Total phosphorus (%) <sup>10</sup>	0.79	0.76	0.72
10.	Available phosphorus (%) <sup>10</sup>	0.45	0.42	0.39
11.	Lysine (%) <sup>10</sup>	1.22	1.09	0.98
12.	Methionine (%) <sup>10</sup>	0.6	0.53	0.49
13.	Methionine+cysteine (%) <sup>10</sup>	0.88	0.80	0.74
14.	Threonine (%) <sup>10</sup>	0.77	0.70	0.65
15.	Sodium (%) <sup>10</sup>	0.16	0.16	0.16
16.	Chloride (%) <sup>10</sup>	0.18	0.18	0.18

(HiMedia, India). The results were expressed as Log10 colony-forming units (cfu) per gram of the sample.

#### 2.8. Morphological study of the small intestine

On day 42, 12 chickens from each dietary group were slaughtered, and small intestinal tissue samples were collected to measure the 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 villi per section were selected based on intact lamina propria. Each sample was observed in at least three sections with ten measurements each, and

the values were averaged for analysis.

#### 2.9. Measurement of antibody titer against Newcastle disease virus and infectious bursal disease virus

The humoral immune response was evaluated by measuring antibody levels after administering vaccines for Newcastle disease virus (NDV) and infectious bursal disease (IBDV). The B1 strain (0.2 ml) and LaSota strain (0.2 ml), live lentogenic strains from Venkateswara Hatcheries Private Limited in India, were administered on days 5 and 21 via eye drops. The IBD intermediate plus type vaccine (0.2 mL) was given on day 14. Blood samples (2 ml) were collected from the wing vein of two randomly selected birds from each replicate pen on days 28 and 35. The samples were immediately transferred to centrifuge tubes without anticoagulant, and serum was extracted through centrifugation. 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.

#### 2.10. Chemical analysis of feed samples

The feed samples were analyzed using the methods outlined by Anonymous (1995). Dry matter (DM) was determined using method 934.01. Crude protein (CP) was analyzed using method 968.06 with Kelplus equipment from Pelican Equipment in Chennai, India. Crude fiber (CF) was measured using the Foss Fiber Cap 2021 Fiber Analysis System from Foss Analytical in Hilleroed, Denmark. Ether extract (EE) was determined using method 920.39 with Socplus equipment from Pelican Equipment in 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).

#### 2.11. Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) with the statistical software SPSS (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 the experimental units for other parameters. Mortality data met homogeneity criteria and did not require transformation for statistical analysis. A significance level of  $p \leq 0.05$  was used to determine significance, while  $p \leq 0.01$  was considered a trend. When treatment had a significant effect, the Duncan test was used to detect differences among treatment means.

### 3. RESULTS AND DISCUSSION

#### 3.1. Average daily gain, feed intake and feed efficiency

The average daily gain (ADG) of the birds increased

more significantly ( $p < 0.05$ ) in the postbiotic group than the control group from 15 to 28 days of age, while the postbiotic group was not different from the antibiotic-treated group (Table 2). No significant differences among treatment groups were found in the rest of the experimental period or over the entire experiment period (1–42 d). The average daily feed intake (ADFI) of chickens was not varied significantly among treatment groups were found in the different phases of the experimental period or over the entire experiment period (1–42 d). Consistent with earlier research, the present study found that postbiotic supplementation had no significant effect on average daily gain, average daily feed intake, or final body weight (Chuang et al., 2021; Nelson et al., 2018; Oliveira et al., 2022). In contrast to our results, Zeinali and Mohammadi (2022) found that adding postbiotics to the feeds at varying concentrations greatly increased daily weight gain when compared to the control group ( $p < 0.05$ ). Chaney et al. (2023) showed that supplementing poultry diets with postbiotics increased average daily gain. Likewise, research conducted by Linh et al. (2021), Liza et al. (2022) Ismael et al. (2022), Soren et al. (2024), and Khan et al. (2025) demonstrated that postbiotic supplementation improved feed utilization and growth. The biological variability in broiler chicken populations, insufficient dosage or duration of supplement administration, the limited impact of supplements on gut microbiota composition, and possible confounding environmental factors could all be contributing factors to the lack of significant effects in our study and prior research.

No significant differences in FCR were noted during the starter (1–14 days), grower phase (15–28 days) and finisher (29–42 days) phase. The FCR during the overall period (1–42 days) for the postbiotic group was improved ( $p < 0.05$ ) in comparison to the control and antibiotic group. Over the course of the study, postbiotics significantly improved the feed conversion ratio (FCR) when compared to the control and antibiotic groups over the period of 1–42 days ( $p < 0.05$ ). This finding aligns with previous studies that have also demonstrated improved FCR with postbiotic supplementation. Humam et al. (2019), Zeinali and Mohammadi, (2022), Soren et al. (2024) and Khan et al. (2025) reported similar results, showing improved FCR with postbiotic bacteria supplementation. Conversely, Oliveira et al. (2022) and Nelson et al. (2018) did not observe changes in FCR with postbiotic supplementation. A possible improvement in gut health and nutrient utilization in broiler chickens is suggested by the higher FCR linked to postbiotics. Postbiotics may influence gut microbiota, promote beneficial microbial fermentation of feed, and enhance nutrient absorption. These results are consistent with previous research, although variations in experimental designs, bird populations, and supplementation protocols

Table 2: Effect of postbiotic (*Lactobacillus rhamnosus* NCDC 298) on final body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) of broiler chickens

Attribute	Treatment <sup>1</sup>			SEm $\pm$	$p=0.05$
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>		
<b>ADG (g/d)</b>					
d1-14	25.26	25.52	25.67	0.193	0.712
d15-28	74.80 <sup>b</sup>	76.43 <sup>ab</sup>	78.55 <sup>a</sup>	0.655	0.053
d29-42	71.36	71.48	72.30	1.156	0.944
d1-42	57.14	57.81	58.84	0.406	0.237
Final BW	2442.35	2470.72	2513.70	17.043	0.238
<b>ADFI (g/d)</b>					
d1-14	29.07	29.34	28.82	0.311	0.811
d15-28	105.58	104.62	104.71	1.043	0.928
d29-42	133.13	131.02	126.88	1.512	0.240
d1-42	89.26	88.34	86.81	0.699	0.373
<b>FCR (g intake g<sup>-1</sup> gain)</b>					
d1-14	1.15	1.15	1.13	0.015	0.717
d15-28	1.41	1.37	1.33	0.019	0.218
d29-42	1.87	1.84	1.76	0.025	0.209
d1-42	1.56 <sup>a</sup>	1.55 <sup>a</sup>	1.48 <sup>b</sup>	0.012	0.004

<sup>ab</sup>Means bearing different superscripts in the same row differ significantly; <sup>1</sup>The control diet (CON) was supplemented with Antibiotic (BMD) at 500 g t<sup>-1</sup> feed (AGP), control diet with postbiotic at 1 ml bird<sup>-1</sup> day<sup>-1</sup> (POS)

may account for differences in outcomes.

### 3.2. Immune response

There were no significant differences ( $p>0.05$ ) in antibody titers to the infectious bursal disease (IBD) vaccine between the dietary treatment groups on day 28 and day 35, as well as for the Newcastle disease (ND) vaccine on day 35. However, the titers of the Newcastle disease (ND) vaccine were significantly ( $p<0.05$ ) higher in the postbiotic group compared to the control and antibiotic-supplemented

groups on day 28 (Table 3). Antibody titers to the Newcastle disease (ND) vaccine on day 35 and to the infectious bursal disease (IBD) vaccine on days 28 and 35 did not differ significantly ( $p>0.05$ ) between the dietary treatment groups in this investigatory study. However, the titers of the Newcastle disease (ND) vaccine were significantly ( $p<0.05$ ) higher in the postbiotic group compared to the control and antibiotic-supplemented groups. This finding aligns with previous research by Danladi et al. (2022), which also

Table 3: Effect of postbiotic (*Lactobacillus rhamnosus* NCDC 298) on antibody titer (log10) against infectious bursal disease vaccine (IBDV) and Newcastle disease vaccine (NDV) of broiler chickens at day 28 and day 35.

Attribute	Treatment <sup>1</sup>			SEm $\pm$	$p=0.05$
	CON	AGP	POS		
<b>IBDV</b>					
d28	3.09	3.12	3.17	0.038	0.677
d35	3.14	3.27	3.31	0.050	0.401
<b>NDV</b>					
d28	2.50b	2.68b	3.08a	0.075	0.001
d35	2.80	2.82	2.90	0.050	0.735

<sup>ab</sup>Means bearing different superscripts in the same row differ significantly; <sup>1</sup>The control diet (CON) was supplemented with Antibiotic (BMD) at 500 g/ton feed (AGP), control diet with postbiotic at 1 ml/bird/day (POS)

found no notable differences in IBDV antibody titers when postbiotic supplements were included in broiler chickens' diets. Ismael et al. (2022) demonstrated that supplementing broiler chickens' diets with postbiotics significantly increased antibody titers in response to NDV vaccines compared to the control group, consistent with earlier findings (Hand,

2020 and Abd El-Ghany et al., 2022).

### 3.3. 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

Table 4: Effect of postbiotic (*Lactobacillus rhamnosus* NCDC 298) on blood biochemical profile of broiler chickens at day 35

Attribute	Treatment <sup>1</sup>			SEm $\pm$	$p=0.05$
	CON	AGP	POS		
Glucose (mg dl <sup>-1</sup> )	272.34	241.67	254.62	14.247	0.704
Total protein(mg dl <sup>-1</sup> )	3.20	3.71	3.44	0.097	0.093
Albumin(mg dl <sup>-1</sup> )	2.97	3.18	3.14	0.060	0.347
Uric acid (mg dl <sup>-1</sup> )	173.43	164.91	139.44	7.845	0.185
Triglyceride (mg dl <sup>-1</sup> )	167.92	174.52	174.05	7.078	0.923
Cholesterol (mg dl <sup>-1</sup> )	3.20	3.11	3.20	0.080	0.875

<sup>1</sup>The control diet (CON) was supplemented with Antibiotic (BMD) at 500 g t<sup>-1</sup> feed (AGP), control diet with postbiotic at 1 ml bird<sup>-1</sup> day<sup>-1</sup> (POS)

study (Table 4). The study found that supplementing with postbiotics did not lead to significant changes in serum total protein, glucose, albumin, uric acid, triglyceride, and cholesterol levels, which is consistent with previous studies (Chuang et al., 2021 and Liza et al., 2022).

### 3.4. 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). The current study did not show any statistically significant differences in carcass characteristics when postbiotics were added to the diet of broiler chickens. These results are

Table 5: Effect of postbiotic (*Lactobacillus rhamnosus* NCDC 298) on carcass characteristics of broiler chickens at day 42

Attribute	Treatment <sup>1</sup>			SEm $\pm$	$p=0.05$
	CON	AGP	POS		
Slaughter BW (g)	2453.01	2470.22	2497.17	17.340	0.606
Eviscerated BW (g)	1663.58	1674.87	1693.22	13.144	0.676
Dressing Percentage (%)	68.28	67.33	67.81	0.217	0.213
Breast (g)	602.67	608.83	591.83	10.467	0.818
Frame (g)	296.33	299.50	312.83	4.627	0.321
Thigh (g)	224.83	208.00	218.67	4.447	0.312
Drumstick (g)	222.67	219.17	225.33	2.501	0.629
Wing (g)	130.50	129.83	124.67	2.175	0.516
Neck (g)	82.75	58.92	64.33	1.423	0.295
Gizzard (g)	45.97	46.77	47.87	0.978	0.752
Liver (g)	36.29	39.51	37.65	1.151	0.547
Spleen (g)	2.10	2.40	2.08	0.150	0.648
Bursa (g)	1.43	0.79	1.20	0.139	0.164
Abdominal Fat (g)	36.99	38.71	42.82	1.639	0.348

<sup>1</sup>The control diet (CON) was supplemented with Antibiotic (BMD) at 500 g t<sup>-1</sup> feed (AGP), control diet with postbiotic at 1 ml bird<sup>-1</sup> day<sup>-1</sup> (POS)

consistent with previous studies by Oliveira et al. (2022), which also found no significant differences in carcass yield or breast yields with varying levels of postbiotics in broiler diets. Similarly, studies by Linh et al. (2021), Zeinali and Mohammadi (2022) and Liza et al. (2022) reported that supplementing postbiotics at different levels did not significantly affect carcass traits, such as thigh, breast, or wing weights.

### 3.5. Gut microbes

The count of *E. coli* was significantly lower ( $p<0.05$ ) in the postbiotic group compared to the control and antibiotic groups. The count of *Salmonella* was significantly lower ( $p<0.05$ ) in the postbiotic group compared to the control group, while the postbiotic group was not different from the antibiotic-treated group. Additionally, the count of *Lactobacillus* was significantly higher ( $p<0.05$ ) in the

Table 6: Effect of postbiotic (*Lactobacillus rhamnosus* NCDC 298) on viable bacteria numbers ( $\log_{10}$  cfu g $^{-1}$ ) in caecal content of broiler chickens at day 42

Attribute	Treatment <sup>1</sup>			SEm $\pm$	$p=0.05$
	CON	AGP	POS		
<i>E. coli</i>	6.81 <sup>a</sup>	6.66 <sup>a</sup>	6.44 <sup>b</sup>	0.533	0.009
<i>Salmonella</i>	6.70 <sup>a</sup>	6.57 <sup>ab</sup>	6.35 <sup>b</sup>	0.060	0.043
<i>Lactobacillus</i>	7.12 <sup>b</sup>	7.16 <sup>b</sup>	7.48 <sup>a</sup>	0.042	0.000

<sup>ab</sup>Means bearing different superscripts in the same row differ significantly; <sup>1</sup>The control diet (CON) was supplemented with Antibiotic (BMD) at 500 g t $^{-1}$  feed (AGP), control diet with postbiotic at 1 ml bird $^{-1}$  day $^{-1}$  (POS)

postbiotic group compared to the control and antibiotic-supplemented groups (Table 6). This study demonstrated that supplementing with postbiotics led to an increase in *Lactobacillus* count and a decrease in *E. coli* and *Salmonella* count compared to the antibiotic and control groups, consistent with previous studies (Roto et al., 2017, Gingerich et al., 2021). Chuang et al. (2021) showed higher *Lactobacillus* levels with 5% and 10% *Saccharomyces cerevisiae* fermented wheat bran. In contrast, Kang et al. (2015) did not find significant differences in *Lactobacillus* counts with various fermented rice bran treatments. Furthermore,

Chuang et al. (2019) observed no significant increase in *Lactobacillus* spp. with postbiotic supplementation in broiler diets.

### 3.6. Gut morphology

There were no significant differences ( $p>0.05$ ) in the height (VH), width (VW), crypt depth (CD), and the ratio of villi height to crypt depth in the duodenum, jejunum, and ileum among the treatment groups (Table 7). The present study did not show any significant effect on gut morphology in the treatment groups. This is in line with previous studies

Table 7: Effect of postbiotic (*Lactobacillus rhamnosus* NCDC 298) on gut morphology of broiler chickens at day 42

Attribute	Treatment <sup>1</sup>			SEm $\pm$	$p=0.05$
	CON	AGP	POS		
<b>Duodenum</b>					
Villi height (VH; $\mu$ m)	1552.83	1547.19	1621.50	45.300	0.779
Crypt depth (CD; $\mu$ m)	189.83	178.50	97.17	19.673	0.106
VH/CD ratio	8.24	14.23	19.46	2.041	0.71
<b>Jejunum</b>					
Villi height (VH; $\mu$ m)	955.67	964.33	1029.33	40.443	0.744
Crypt depth (CD; $\mu$ m)	123.33	117.17	99.50	5.875	0.239
VH/CD ratio	8.78	8.36	9.69	0.435	0.472
<b>Ileum</b>					
Villi height (VH; $\mu$ m)	661.33	670.60	692.50	23.605	0.871
Crypt depth (CD; $\mu$ m)	134.33	128.50	123.67	7.614	0.864
VH/CD ratio	5.00	5.36	6.61	0.504	0.417

<sup>1</sup>The control diet (CON) was supplemented with Antibiotic (BMD) at 500 g t $^{-1}$  feed (AGP), control diet with postbiotic at 1 ml bird $^{-1}$  day $^{-1}$  (POS)

by Firman et al. (2013) and Chuang et al. (2021), which also found no significant difference in gut morphology with postbiotic supplementation. However, Chuang et al. (2019) and Lin et al. (2023) reported a positive effect on gut morphology when postbiotics were added to the diet of broiler chickens.

#### 4. CONCLUSION

Adding postbiotics improved feed conversion rate (FCR) and reduced harmful bacteria in birds compared to the control group. Postbiotics enhanced bird nutrition and reduced pathogenic bacteria. *Lactobacillus* levels were higher in birds with postbiotic supplementation. Newcastle disease vaccine titers were also higher in the postbiotic group on day 28. This research highlights the importance of postbiotics in poultry health and production.

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#### 6. REFERENCES

Abd El-Ghany, W.A., Fouad, H., Quesnell, R., Sakai, L., 2022. The effect of a postbiotic produced by stabilized non-viable *Lactobacilli* on the health, growth performance, immunity, and gut status of colisepticaemic broiler chickens. *Tropical Animal Health and Production* 54, 286. <https://doi.org/10.1007/s11250-022-03300-w>.

Abd El-Hack, M.E., El-Saadony, M.T., Salem, H.M., El-Tahan, A.M., Soliman, M.M., Youssef, G.B.A., Taha, A.E., Soliman, S.M., Ahmed, A.E., El-kott, A.F., Al Syaad, K.M., Swelum, A.A., 2022. Alternatives to antibiotics for organic poultry production: types, modes of action and impacts on bird's health and production. *Poultry Science* 101, 101696. <https://doi.org/10.1016/j.psj.2022.101696>.

Abd El-Hack, M.E., El-Saadony, M.T., Shafi, M.E., Qattan, S.Y.A., Batiha, G.E., Khafaga, A.F., Abdel-Moneim, A.E., Alagawany, M., 2020. Probiotics in poultry feed: A comprehensive review. *Journal of Animal Physiology and Animal Nutrition* 104, 1835–1850. <https://doi.org/10.1111/jpn.13454>.

Aguilar-Toala, J.E., Garcia-Varela, R., Garcia, H.S., Mata-Haro, V., Gonzalez-Cordoba, A.F., Vallejo-Cordoba, B., Hernández-Mendoza, A., 2018. Postbiotics: An evolving term within the functional foods field. *Trends in Food Science & Technology* 75, 105–114. <https://doi.org/10.1016/j.tifs.2018.03.009>.

Ahmad, R., Yu, Y.H., Hsiao, F.S.H., Dybus, A., Ali, I., Hsu, H.C., Cheng, Y.H., 2022. Probiotics as a friendly antibiotic alternative: assessment of their effects on the health and productive performance of poultry. *Fermentation* 8, 672. <https://doi.org/10.3390/fermentation8120672>.

Akter, S., Park, J.H., Jung, H.K., 2020. Potential health-promoting benefits of paraprobiotics, inactivated probiotic cells. *Journal of Microbiology and Biotechnology* 30, 477–481. <https://doi.org/10.4014/jmb.1911.11019>.

Anonymous, 1995. AOAC, 1995. Official Methods of Analysis, 16th ed. Association of Official Analytical Chemists, Arlington, VA. [https://usgs.primo.exlibrisgroup.com/discovery/fulldisplay/alm a991003768599703886/01USGSL\\_INST:01USGSL\\_INST](https://usgs.primo.exlibrisgroup.com/discovery/fulldisplay/alm a991003768599703886/01USGSL_INST:01USGSL_INST). Access date xxxx.

Chaney, E., Miller, E.A., Firman, J., Binnebose, A., Kuttappan, V., Johnson, T.J., 2023. Effects of a postbiotic, with and without a saponin-based product, on turkey performance. *Poultry Science* 102, 102607. <https://doi.org/10.1016/j.psj.2023.102607>.

Chang, Y., Saeed Omer, S.H., Li, G., Lian, H., Liu, Y., 2022. Research advance on application of microbial fermented fodder in broilers production: A short review. *Open Journal of Animal Sciences* 12, 200–209. <https://doi.org/10.4236/ojas.2022.122015>.

Chuang, W.Y., Lin, L.J., Hsieh, Y.C., Chang, S.C., Lee, T.T., 2021. Effects of *Saccharomyces cerevisiae* and phytase co-fermentation of wheat bran on growth, antioxidation, immunity and intestinal morphology in broilers. *Animal Bioscience* 34, 1157–1168. <https://doi.org/10.5713/ajas.20.0399>.

Chuang, W.Y., Lin, W.C., Hsieh, Y.C., Huang, C.M., Chang, S., Lee, T.T., 2019. Evaluation of the combined use of *Saccharomyces Cerevisiae* and *Aspergillus Oryzae* with phytase fermentation products on growth, inflammatory, and intestinal morphology in broilers. *Animals* 9, 1051. <https://doi.org/10.3390/ani9121051>.

Cicenia, A., Santangelo, F., Gambardella, L., Pallotta, L., Iebba, V., Scirocco, A., Marignani, M., Tellan, G., Carabotti, M., Corazziari, E.S., Schiappa, S., Severi, C., 2016. Protective role of postbiotic mediators secreted by *Lactobacillus rhamnosus* GG versus lipopolysaccharide-induced damage in human colonic smooth muscle cells. *Journal of Clinical Gastroenterology* 50, S140–S144. <https://doi.org/10.1097/mcg.0000000000000681>.

Cuevas-González, P.F., Liceaga, A.M., Aguilar-Toala, J.E., 2020. Postbiotics and paraprobiotics: From concepts to applications. *Food Research International* 136, 109502. <https://doi.org/10.1016/j.foodres.2020.109502>.

Danladi, Y., Loh, T.C., Foo, H.L., Akit, H., Md Tamrin, N.A., Naeem Azizi, M., 2022. Effects of postbiotics and paraprobiotics as replacements for antibiotics on growth performance, carcass characteristics, small intestine histomorphology, immune status and hepatic growth gene expression in broiler chickens. *Animals* 12, 917. <https://doi.org/10.3390/ani12070917>.

Firman, J.D., Moore, D., Broomhead, J., McIntyre, D., 2013. Effects of dietary inclusion of a *Saccharomyces cerevisiae* fermentation product on performance and gut characteristics of male turkeys to market weight. *International Journal of Poultry Science* 12, 141–143. <https://doi.org/10.3923/ijps.2013.141.143>.

Furuichi, Y., Takahashi, T., 1981. Evaluation of acid-insoluble asha as a marker in digestion studies. *Agricultural and Biological Chemistry* 45, 2219–2224. <https://doi.org/10.1080/00021369.1981.10864873>.

Gingerich, E., Frana, T., Logue, C.M., Smith, D.P., Pavlidis, H.O., Chaney, W.E., 2021. Effect of feeding a postbiotic derived from *Saccharomyces cerevisiae* fermentation as a preharvest food safety hurdle for reducing *Salmonella enteritidis* in the ceca of layer pullets. *Journal of Food Protection* 84, 275–280. <https://doi.org/10.4315/JFP-20-330>.

Gueimonde, M., Sánchez, B.G., de los Reyes-Gavilán, C., Margolles, A., 2013. Antibiotic resistance in probiotic bacteria. *Frontiers in Microbiology* 4, 202. <https://doi.org/10.3389/fmicb.2013.00202>.

Hand, T.W., 2020. All bacteroides are equal but some are more equal than others for the induction of IgA. *Cell Host Microbe* 27, 319–321. <https://doi.org/10.1016/j.chom.2020.02.011>.

Hayat, K., Zhou, Y., Menhas, S., Bundschuh, J., Hayat, S., Ullah, A., Wang, J., Chen, X., Zhang, D., Zhou, P., 2020. *Pennisetum giganteum*: An emerging salt-accumulating/tolerant non-conventional crop for sustainable saline agriculture and simultaneous phytoremediation. *Environmental Pollution* (Barking, Essex) 1987 265, 114876. <https://doi.org/10.1016/j.envpol.2020.114876>.

Humam, A.M., Loh, T.C., Foo, H.L., Samsudin, A., Mustapha, N., Zulkifli, I., Izuddin, W., 2019. Effects of feeding different postbiotics produced by *Lactobacillus plantarum* on growth performance, carcass yield, intestinal morphology, gut microbiota composition, immune status, and growth gene expression in broilers under heat stress. *Animals* 9, 644. <https://doi.org/10.3390/ani9090644>.

Imperial, I.C.V.J., Ibana, J.A., 2016. Addressing the antibiotic resistance problem with probiotics: reducing the risk of its double-edged sword effect. *Frontiers in Microbiology* 7, 1983. <https://doi.org/10.3389/fmicb.2016.01983>.

Ismael, E., Ismail, E., Khalefa, S., Elleithy, E., Elmosalamy, S., Marouf, S., El-din Fahmy, K., 2022. Evaluation of *Saccharomyces cerevisiae* yeast fermentate and xylanase in a reduced-energy diet fed to broiler chicken. *International Journal of Veterinary Science* 11, 141–150. <https://doi.org/10.47278/journal.ijvs/2021.096>.

Johnson, C.N., Kogut, M.H., Genovese, K., He, H., Kazemi, S., Arsenault, R.J., 2019. Administration of a postbiotic causes immunomodulatory responses in broiler gut and reduces disease pathogenesis following challenge. *Microorganisms* 7, 268. <https://doi.org/10.3390/microorganisms7080268>.

Kang, H.K., J.H. Kim, C.H., Kim., 2015. Effect of dietary supplementation with fermented rice bran on the growth performance, blood parameters and intestinal microflora of broiler chickens. *European Poultry Science. EPS* 79, 112. <https://doi.org/10.1399/eps.2015.112>.

Khan, M.H., Mukherjee, J., Soren, S., Jas, R., Pakhira, M.C., Mondal, S., Paul, A., Samanta, I., Mondal, A., Nsereko, V., 2025. Effects of *Saccharomyces cerevisiae* fermentation product (SCFP) and phytogenic feed additive as alternatives to antibiotic growth promoters on pathogen mitigation, immunomodulation and production performance in commercial broiler chickens. *Poultry Science* 104, 105743. <https://doi.org/10.1016/j.psj.2025.105743>.

Letlhogonolo, A., Zahra, M., Tlou, G., Monnye, M., 2020. The current status of the alternative use to antibiotics in poultry production: an african perspective. *Antibiotics* 9, 594. <https://doi.org/10.3390/antibiotics9090594>.

Lin, J., Comi, M., Vera, P., Alessandro, A., Qiu, K., Wang, J., Wu, S., Qi, G., Zhang, H., 2023. Effects of *Saccharomyces cerevisiae* hydrolysate on growth performance, immunity function, and intestinal health in broilers. *Poultry Science* 102, 102237. <https://doi.org/10.1016/j.psj.2022.102237>.

Linh, N., Preston, T., Qui, N., Van, L., Thu, V., Vui, N., 2021. The effect of an aqueous extract of yeast-fermented rice to growth performance and carcass traits of chickens. *Livestock Research for Rural Development* 33, 109.

Liza, R., Ismita, J., Islam, K., Chowdhury, R., Debi, M., Joy, N., 2022. Effects of yeast (*Saccharomyces cerevisiae*) fermented rice bran with urea on the production performance of broiler. *Journal of the Bangladesh Agricultural University* 20, 57–63. <https://doi.org/10.5455/JBAU.130284>.

Manyi-Loh, C., Sampson, M., Meyer, M., Anthony, O., 2018. Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules* 23,

795. <https://doi.org/10.3390/molecules23040795>.

Martín, R., Langella, P., 2019. Emerging health concepts in the probiotics field: Streamlining the definitions. *Frontiers in Microbiology* 10, 1047. <https://doi.org/10.3389/fmicb.2019.01047>.

Moradi, M., Kousheh, S.A., Almasi, H., Alizadeh, A., Guimaraes, J.T., Yilmaz, N., Lotfi, A., 2020. Postbiotics produced by lactic acid bacteria: The next frontier in food safety. *Comprehensive Reviews in Food Science and Food Safety* 19, 3390–3415. <https://doi.org/10.1111/1541-4337.12613>.

Morgan, N.K., 2023. Advances in prebiotics for poultry: Role of the caeca and oligosaccharides. *Animal Production Science* 63, 1911–1925. <https://doi.org/10.1071/AN23011>.

Nelson, J.R., McIntyre, D.R., Pavlidis, H.O., Archer, G.S., 2018. Reducing stress susceptibility of broiler chickens by supplementing a yeast fermentation product in the feed or drinking water. *Animals (Open Access Journal) MDPI* 8, 173. <https://doi.org/10.3390/ani8100173>.

Oliveira, J.M., Marchi, D.F., Geronimo, B.C., Oba, A., Soares, A.L., 2022. Effect of diets containing *Saccharomyces cerevisiae* fermentation products on broiler performance and meat quality. *Journal of Agricultural Science Research* 2764–0973 2, 2–8. <https://doi.org/10.22533/at.ed.973222201032>.

Oniciuc, E., Likotrafiti, E., Alvarez-Molina, A., Prieto, M., Santos, J., Alvarez-Ordóñez, A., 2018. The present and future of whole genome sequencing (WGS) and whole metagenome sequencing (WMS) for surveillance of antimicrobial resistant microorganisms and antimicrobial resistance genes across the food chain. *Genes* 9, 268. <https://doi.org/10.3390/genes9050268>.

Paintsil, E., Ofori, L., Akenten, C., Fosu, D., Ofori, S., Lamshoft, M., May, J., Danso, K., Krumkamp, R., Dekker, D., 2021. Antimicrobial usage in commercial and domestic poultry farming in two communities in the Ashanti Region of Ghana. *Antibiotics* 10, 800. <https://doi.org/10.3390/antibiotics10070800>.

Quinn, P.J., Quinn, Peter, J., Carter, M.E., Markey, B., Carter, G.R., 1994. Clinical veterinary microbiology. London: Wolfe Publishing. <https://www.vet-ebooks.com/clinical-veterinary-microbiology/>.

Reuben, R.C., Sarkar, S.L., Roy, P.C., Anwar, A., Hossain, M.A., Jahid, I.K., 2021. Prebiotics, probiotics and postbiotics for sustainable poultry production. *World's Poultry Science Journal* 77, 825–882. <https://doi.org/10.1080/00439339.2021.1960234>.

Roto, S.M., Park, S.H., Lee, S.I., Kaldhone, P., Pavlidis, H.O., Frankenbach, S.B., McIntyre, D.R., Striplin, K., Brammer, L., Ricke, S.C., 2017. Effects of feeding original xpctm to broilers with a live coccidiosis vaccine under industry conditions: part 1. growth performance and salmonella inhibition. *Poultry Science* 96, 1831–1837. <https://doi.org/10.3382/ps/pew445>.

Soren, S., Mandal, G.P., Mondal, S., Pradhan, S., Mukherjee, J., Banerjee, D., Pakhira, M.C., Amla, Mondal, A., Nsereko, V., Samanta, I., 2024. Efficacy of *Saccharomyces cerevisiae* fermentation product and probiotic supplementation on growth performance, gut microflora and immunity of broiler chickens. *Animals* 14, 866. DOI: <https://doi.org/10.3390/ani14060866>

SPSS Inc., 1997. SPSS Base 7.5 for Windows User's Guide. Prentice Hall.

Talapatra, S.K., Ray, S.C., Sen, K.C., 1940. The analysis of mineral constituents in biological materials. 1. Estimation of phosphorus, chlorine, calcium, magnesium, sodium and potassium in foodstuffs. *Indian Journal of Veterinary Science* 10, 243–258.

Taverniti, V., Guglielmetti, S., 2011. The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). *Genes and Nutrition* 6, 261–274. <https://doi.org/10.1007/s12263-011-0218-x>.

Tiptiri-Kourpeti, A., Spyridopoulou, K., Santarmaki, V., Aindelis, G., Tompouliou, E., Lamprianidou, E.E., Saxami, G., Ypsilantis, P., Lamprini, E.S., Simopoulos, C., Kotsianidis, I., Galanis, A., Kourkoutas, Y., Dimitrellou, D., Chlichlia, K., 2016. *Lactobacillus casei* exerts anti-proliferative effects accompanied by apoptotic cell death and up-regulation of TRAIL in colon carcinoma cells. *PLoS One* 11, e0147960. <https://doi.org/10.1371/journal.pone.0147960>.

Terpou, A., Papadaki, A., Lappa, I.K., Kachrimanidou, V., Bosnea, L.A., Kopsahelis, N., 2019. Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients* 11, 1591. <https://doi.org/10.3390/nu11071591>.

Venkys, 2017. Vencobb400 broiler management guide. Venkateshwara Hatcheries Pvt. Limited, Pune, India, <http://tirumalagroup.com/>.

Zeinali, S., Mohammadi, M., 2022. Effect of supplementing different levels of fermented *Saccharomyces cerevisiae* on performance and immune responses of broilers. *Journal of Animal Science Research* 32, 15–29. <https://dx.doi.org/10.22034/as.2022.36929.1532>.