RESEARCH ARTICLE

Effect of Probiotics Derived from *Lactobacillus* DH42 as an Alternative to Antibiotics on the Growth Performance, Gut Health, and Immune Response in Broiler Chickens

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Abstract

This study was aimed to assess the impact of dietary supplementation with a probiotic (*Lactobacillus* DH42) on the growth performance, carcass traits, blood biochemical profile, gut microbiome, gut morphology, and immune response of broiler chickens. A total of 162 day-old mixed sex Vencobb 400 broiler chicks were randomly assigned to three dietary groups, each with six replicated pens (n=6), containing nine broiler chickens: 1) basal diet without any growth promoter (CON), 2) basal diet with antibiotic (Bacitracin methylene disalicylate - BMD) at 500 g/ton feed (AGP), and 3) basal diet with probiotic (*Lactobacillus* DH42) at 1 mL/bird in drinking water (PRO). Body weight, feed intake, and FCR were monitored weekly for 35 days. Blood biochemistry and immune response were evaluated at day 28 and 35 of the trial. Carcass traits, gut microbiome, and gut morphology were assessed at the end of the trial. The results indicated that PRO significantly increased final body weight, average daily gain and improved FCR compared to the control group ($p<0.05$). There were no significant differences in average daily feed intake, antibody titres, carcass traits and most blood biochemistry parameters, except for cholesterol concentration, which was significantly lower in the PRO and AGP groups compared to the CON group. Both PRO and AGP supplementation led to a significant decrease in caecal *E. coli* and *Salmonella* counts and a significant increase in *Lactobacillus* counts compared to the CON group. Probiotic supplementation also enhanced gut morphology compared to the AGP and CON groups. In conclusion, probiotic supplementation shows promise as an alternative to antimicrobials in broiler production, with beneficial effects on broilers fed an antibiotic-free diet.

Key words: Broiler chicken, Growth performance, Gut microbiota, Immune response, *Lactobacillus.*

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Introduction

The poultry industry is experiencing rapid growth within the global livestock production sector. Various factors, such as breed, nutrition, and animal health, are crucial in optimizing production efficiencies (Mottet and Tempio, 2017). However, the high efficiency of meat and egg production necessitates a focus on specific nutrients and health management. The overall health and proper functioning of the gastrointestinal tract (GIT) are essential for optimal poultry performance (Aruwa *et al.*, 2021). Finding alternative feed additives that can control pathogens and promote growth is imperative.

Probiotics provide health benefits when consumed in sufficient quantities," play a crucial role in enhancing immunity, health, and growth in broilers. Lactic acid bacteria (LAB) are the primary probiotics used in animal feeds, offering benefits such as gut microbiota modulation, immunomodulation, and antimicrobial effects (Ashaolu, 2020). LAB species like *Enterococcus, Lactobacillus,* and *Streptococcus* are natural microflora in the GIT, producing lactic acid (Hill *et al.*, 2014).

Lactobacillus, a major genus of LAB with over 200 species, is a key candidate for probiotic use in poultry, enhancing feed digestion, nutrient absorption, growth performance, and immune responses (Al-Khalaifa *et al.*, 2019). Probiotics reduce the risk of gastrointestinal pathogen colonization, improving food safety (Kizerwetter-Świda and Binek, 2016). However, not all LABs are probiotics, and their characteristics and safety profiles must be evaluated. Probiotic strains must exhibit acid and bile tolerance, antimicrobial activity, adherence to intestinal mucosa, and modulation of intestinal

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barrier functions to qualify as probiotics. Native and species-specific probiotics, particularly LAB, are crucial for optimizing productive performance in poultry (Noohi *et al.*, 2021). In this study, *Lactobacillus* DH42, isolated from Deshi chicken GIT, was prepared as a probiotic to evaluate its effects on growth performance and gut health in broiler chickens.

Materials and Methods

Experimental Design, Diets and Bird Management

The study protocol was approved by Institutional Animal Ethics Committee of West Bengal University of Animal and Fishery Sciences, (Ethics Approval Number: 763/GO/Re-S/ReRc-L/03/CCSEA/76/2024-25), Kolkata, India. 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 probiotics (*Lactobacillus* DH42) at a rate of 1 mL/bird in drinking water (PRO). The basal diet 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. The birds were reared under deep litter system adopting standard procedures. 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.

Preparation of Probiotics

The active probiotic culture *Lactobacillus* DH42, sourced from Deshi chicken, was acquired from the Department of Dairy Microbiology, Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences, Mohanpur, Nadia, India. Sub-culturing was conducted in MRS broth at 37°C for 15-18 h for inoculation. To make it suitable for broiler chickens, the probiotics were prepared in liquid form using a skim milk-based medium. Skim milk powder (30 g) was dissolved in 1000 mL of distilled water, and dextrose (10 g) and nutrient mix (2.5 g) were added to the solution. The mixture was then divided into conical flasks and sterilized at 121°C for 5 min. After cooling the active MRS broth culture of *Lactobacillus* DH42 was inoculated at a rate of 1% and incubated at 37°C for 15-18 h to obtain a probiotic preparation. The probiotic preparation was stored at 5-7°C up to 7 days.

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, Average daily feed intake (ADFI) and FCR were calculated using standard formulae 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.

Carcass Characteristics and Blood Biochemical Analyses

On day 35, 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. Blood samples were collected from broiler chickens on day 35 after a 12-h fasting period from the wing vein. Twelve birds per treatment were randomly selected, with two birds chosen from each pen. Blood was collected without anticoagulant, and 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.

Enumeration of Pre-Caecal Bacterial Count

The caecal contents of chickens slaughtered on day 35 were aseptically collected and 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, 1 g of caecal content was diluted tenfold with sterile phosphate-buffered saline (PBS). Subsequently, 10 µL 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 h, and the characteristic colonies for each bacterial group were counted using a digital colony counter (HiMedia, India). The results were expressed as Log_{10} colony-forming units (cfu) per gram of the sample.

Morphological Study of Small Intestine

On day 35, 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.

Measurement of Antibody Titre against ND and IBD 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 Venkateswaara Hatcheries Private Limited Pune (India), were administered on day 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 day 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.

Statistical Analysis

The data on body weight gain, feed intake, and FCR were analyzed using one-way analysis of variance for completely randomized design with the statistical software SPSS (SPSS Inc, 1997) and mean differences were compared by Duncan's *post-hoc* test. 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 $p \leq 0.05$ was used to determine significance, while $p \leq 0.01$ was considered a trend.

Results and Discussion

Average Daily Gain, Feed Intake and Feed Efficiency

The average daily weight gain (ADG) of the birds significantly ($p<0.05$) increased in the PRO group compared to the CON and AGP groups during the grower phase (15-28 days) (Table 1). There were no significant differences among the treatment groups during the starter (1-14 days) and finisher (29-35 days) phases. However, the ADG of the birds significantly increased in the PRO group compared to the CON group over the entire 35-day experiment, while the AGP group showed no significant differences with either the PRO or CON group. The final body weight of the birds was significantly higher in the PRO group compared to the CON group. The average daily feed intake (ADFI) of the birds was significantly higher in the CON and AGP groups compared to the PRO group during the starter phase. There were no significant differences in ADFI among the treatment groups for the rest of the experimental period or over the entire 35-day experiment. The feed conversion ratio (FCR) was significantly improved in the PRO group compared to the CON and AGP groups during the starter phase. Additionally, a significantly improved FCR was observed in the PRO group compared to the CON group during the grower phase (15-28 days) and the overall experimental period, while the AGP group did not show a significant difference with either the PRO or CON groups. However, FCR was not different among the treatment groups during the finisher phase.

Table 1: Effect of probiotic (*Lactobacillus* DH42) on final body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and FCR of broiler chickens

Parameter	Attribute	Treatment			SEM (n=6)	P-Value
		CON	AGP	PRO		
ADG (g/d)	d1-14	37.42	38.11	39.17	0.484	0.352
	d15-28	67.22^{b}	68.61^{b}	72.41^{a}	0.747	0.005
	d29-35	91.50	102.29	110.46	4.224	0.190
	d1-35	60.16^{b}	63.15^{ab}	66.72^{a}	0.944	0.008
	Final BW	2153.13^{b}	2257.63^{ab}	2382.40^{a}	32.989	0.008
ADFI (g/d)	d1-14	43.95^{a}	43.47^{ab}	41.81^{b}	0.375	0.040
	d15-28	107.43	105.09	101.76	1.725	0.428
	d29-35	161.97	157.27	152.94	3.597	0.620
	d1-35	92.95	90.88	88.02	1.223	0.270
FCR (g intake/ g gain)	d1-14	1.18^{a}	1.14^{a}	1.07^{b}	0.016	0.021
	d15-28	1.60^{a}	1.54^{ab}	1.41^{b}	0.034	0.055
	d29-35	1.83	1.57	1.42	0.079	0.096
	d1-35	1.55^{a}	1.44^{ab}	1.32^{b}	0.031	0.006

^{ab}Means bearing different superscripts in the same row differ significantly ($p \leq 0.05$). CON- control diet, AGP- control diet supplemented with Antibiotic (BMD) at 200 mg/MT feed, PRO- control diet with probiotic at 1 mL/bird/day

The supplementation of *Lactobacillus* DH42 probiotics in the drinking water of broiler chickens in this study resulted in improvements in final body weight, overall ADG, and FCR. These findings aligned with previous research indicating that the use of probiotic *Lactobacillus* can enhance the growth performance of birds (Li *et al.*, 2018; Wu *et al.*, 2021). Additionally, the inclusion of *L. plantarum* B1 in the diet was found to enhance weight gain and FCR in broilers (Peng *et al.*, 2016), and feeding *B. licheniformis* was shown to increase body weight and ADG significantly (Chen and Yu, 2020). In contrast, studies conducted by Mohammed *et al.* (2022) demonstrated that dietary supplementation with probiotics had no significant effect on ADFI, ADG, or FCR among chicken flocks.

Immune Response

There were no significant differences ($p>0.05$) in antibody titers to infectious bursal disease (IBD) and Newcastle disease vaccines (NDV) between the dietary treatment groups on day 28 and day 35 (Table 2). These findings were consistent with previous studies (Toghyani *et al.,* 2015; Rehman *et al.,* (2020), who also reported that ND antibody titers were not statistically influenced by a probiotic-supplemented diet. In contrast, Manafi *et al.* (2017) reported higher antibody concentrations against Newcastle disease in broiler chickens supplemented with *B. subtilis*.

Serum Biochemical Profile

The concentrations of glucose, total protein, albumin, uric acid and triglyceride in serum did not vary significantly ($p>0.05$) between groups in this study. However, the PRO and AGP supplemented groups showed significantly lower cholesterol concentrations compared to the CON group (Table 3). This finding is consistent with a previous study by Reuben *et al.* (2021), which reported that probiotics of *Lactobacillus* spp. helped reduce total cholesterol concentrations with probiotic supplementation. Another study using probiotics *Lactobacillus salivarius* showed significant reductions in serum total cholesterol and triglyceride concentrations in broiler chickens (Shokryazdan *et al.*, 2017). Similarly, Shah *et al.* (2021) reported lower cholesterol levels with the use of *Lactobacillus* as a probiotic. Further, a linear increase in plasma total protein was also observed in the probiotic-supplemented group at 0.5% of basal feed (Astuti *et al.*, 2022). Abdel-Hafeez *et al.* (2017) however found no impact of probiotics on the blood biochemical profile.

Carcass Characteristics

No significant differences ($p>0.05$) were observed in slaughter body weight, eviscerated carcass weight, dressing percentage, and the weights of various carcass components across the different treatment groups (Table 4). These results were consistent with the findings of Shah *et al.* (2021), who also reported that a multistrain probiotic had no impact on carcass characteristics. Similar non-significant results were obtained by Sarangi *et al.* (2016). Reuben *et al. (*2022) used *Lactobacillus* spp. at 10^8 cfu/mL and did not observe significant changes in carcass traits. Likewise, Aristides *et al.* (2012), also reported no significant differences in different cuts between control and treatment groups.

Table 2: Effect of probiotic on antibody titre (log_{10}) against infectious bursal disease vaccine (IBDV) and New castle disease vaccine (NDV) of broiler chickens at day 28 and 35

Disease	Attribute	Treatment			SEM (n=6)	P-Value
		CON	AGP	PRO		
IBDV	d28	1.82	1.87	2.16	0.104	0.375
	d35	3.10	3.15	3.11	0.101	0.978
NDV	d28	3.93	3.96	4.03	0.082	0.897
	d35	2.23	2.48	2.33	0.081	0.467

CON- control diet, AGP- control diet supplemented with Antibiotic (BMD), PRO- control diet with probiotic

Table 3: Effect of probiotic (*Lactobacillus* DH42) on blood biochemical profile of broiler chickens at day 35

Attribute	Treatment			SEM (n=6)	P-Value
	CON	AGP	PRO		
Glucose (mg/dL)	257.60	253.80	265.60	9.04	0.880
Total protein (mg/dL)	4.64	6.00	7.06	0.478	0.111
Albumin (mg/dL)	1.98	1.30	1.62	0.139	0.131
Uric acid (mg/dL)	2.92	3.04	2.92	0.123	0.913
Triglyceride (mg/dL)	44.20	56040	42.40	4.880	0.477
Cholesterol (mg/dL)	142.40^a	99.00^b	99.60^b	6.962	0.004

CON- control diet, AGP- diet supplemented with Antibiotic (BMD), PRO- diet with probiotic

Gut Microbes

The *E. coli* count was significantly lower ($p<0.05$) and the *Lactobacillus* count was significantly higher ($p<0.05$) in the PRO and AGP groups compared to the CON group. The *Salmonella* count was significantly lower in the PRO group compared to the CON group, with no difference observed between the AGP and PRO groups (Table 5). These findings concurred with previous study by Kupryś-Caruk *et al.* (2019), which reported that the inclusion of *Lactobacillus* spp. as a probiotic significantly reduced the number of *E. coli* counts. Kazemi *et al.* (2019) also observed similar findings when using $3x10^9$ cfu/mL *Lactobacillus* spp., noting reduction in the number of pathogenic bacteria and an increase in beneficial bacterial counts.

Gut Morphology

There was no significant effect on villi height (VH) and crypt depth (CD) in the duodenum and jejunum, as well as the VH/CD ratio in the jejunum. However, the VH/CD ratio in the duodenum was significantly higher in the PRO and AGP groups compared to the CON group. Additionally, the VH in the ileum was significantly higher in the PRO group compared to the CON group, while the CD in the ileum was significantly lower in the PRO group compared to the AGP and CON groups. Furthermore, the VH/CD ratio in the ileum was significantly higher in the PRO group compared to the AGP and CON groups (Table 6). Soumeh *et al.* (2021) also demonstrated improved gut morphology with *Lactobacillus* species supplementation, leading to enhanced nutrient absorption. Khattab *et al.* (2021) also observed positive changes in gut morphology with LABs supplementation. Biswas *et al.* (2019) found improved villus height and other morphometric parameters with a multi-strain probiotic. Mangisah *et al.* (2021) reported increased ileal villus height with *L. casei* supplementation. However, Astuti *et al.* (2022) did not observe significant changes in gut morphology with specific probiotic supplementation.

Table 4: Effect of probiotic (*Lactobacillus* DH42) on carcass characteristics of broiler chickens at day 35

Attribute	Treatment			SEM (n=6)	P-Value
	CON	AGP	PRO		
Slaughter BW (g)	2162.67	2163.00	2225.50	21.295	0.406
Eviscerated BW (g)	1430.17	1401.17	1467.67	18.661	0.367
Dressing Percentage (%)	66.12	64.73	65.98	0.496	0.480
Breast (g)	542.83	549.17	575.67	9.928	0.381
Frame (g)	275.17	273.00	277.67	3.521	0.878
Thigh (g)	175.33	170.67	178.67	4.282	0.768
Drumstick (g)	207.67	199.00	211.67	4.358	0.507
Wing (g)	108.67	110.67	111.67	2.908	0.922
Neck (g)	54.83	53.67	55.83	1.646	0.879
Gizzard (g)	47.44	47.65	49.33	1.027	0.736
Liver (g)	41.16	42.90	42.91	0.924	0.696
Heart (g)	9.60	10.42	10.47	0.248	0.287
Spleen (g)	2.19	1.79	2.32	0.142	0.290
Bursa (g)	4.29	4.39	4.45	0.198	0.949
Abdominal Fat (g)	30.28	27.09	34.32	2.400	0.495

CON- control diet, AGP- diet supplemented with Antibiotic (BMD), PRO- diet with probiotic

Table 5: Effect of probiotic (*Lactobacillus* DH42) on viable bacteria numbers (log_{10}cfu/g) in caecal content of broiler chickens at day 35

Attribute	Treatment			SEM (n=6)	P-Value
	CON	AGP	PRO		
E. coli	5.65^a	5.04^b	4.95^b	0.082	0.000
Salmonella	5.43^a	5.19^{ab}	4.96^b	0.077	0.034
Lactobacillus	5.91^b	7.06^a	7.07^a	0.133	0.000

^{abc}Means bearing different superscripts in the same row differ significantly ($p \leq 0.05$). CON- control diet, AGP- diet supplemented with Antibiotic (BMD), PRO- diet with probiotic

Table 6: Effect of probiotic (*Lactobacillus* DH42) on gut morphology of chickens at day 35

Gut part	Attribute	Treatment			SEM (n=6)	P-Value
		CON	AGP	PRO		
Duodenum	Villi height (VH; µm)	1326.77	1479.05	1528.72	44.616	0.157
	Crypt depth (CD; µm)	174.98	164.22	162.75	12.966	0.382
	VH/CD ratio	7.57[b]	9.02[a]	9.44[a]	0.318	0.021
Jejunum	Villi height (VH; µm)	1001.88	1097.48	1220.85	41.704	0.089
	Crypt depth (CD; µm)	263.32	257.35	252.98	5.873	0.804
	VH/CD ratio	3.82	4.33	4.82	0.206	0.135
Ileum	Villi height (VH; µm)	639.59[b]	802.53[ab]	854.56[a]	39.534	0.049
	Crypt depth (CD; µm)	180.65[a]	174.50[a]	153.76[b]	4.806	0.037
	VH/CD ratio	3.54[c]	4.58[b]	5.57[a]	0.283	0.001

[ab]Means bearing different superscripts in the same row differ significantly ($p \leq 0.05$). CON- control diet, AGP- diet supplemented with Antibiotic (BMD), PRO- diet with probiotic

Conclusion

Birds supplemented with probiotics (derived from *Lactobacillus* DH42) showed improved growth performance, including higher final body weight, increased average daily gain and better FCR compared to the control group. Additionally, the probiotic group had lower blood cholesterol levels and reduced levels of harmful bacteria like *E. coli* and *Salmonella,* while increasing beneficial bacteria like *Lactobacillus* in the gut. The inclusion of probiotics in the broiler chicken diet also improved gut morphology. These results suggest that probiotics can be a viable alternative to antibiotics in poultry production, especially for broilers on an antibiotic-free diet, promoting overall bird health. This study highlights the potential of probiotics as a promising option for enhancing gut health in poultry without relying on antibiotics.

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References

Abdel-Hafeez, H. M., Saleh, E. S. E., Tawfeek, S. S., Youssef, I. M. I., & Abdel-Daim, A. S. A. (2017). Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. *Asian-Australasian Journal of Animal Sciences*, *30,* 672-682.

Al-Khalaifa, H., Al-Nasser, A., Al-Surayee, T., Al-Kandari, S., Al-Enzi, N., Al-Sharrah, T., Ragheb, G., Al-Qalaf, S., & Mohammed, A. (2019). Effect of dietary probiotics and prebiotics on the performance of broiler chickens. *Poultry Science*, *98,* 4465-4479.

Aristides, L. G. A., Paiao, F. G., Murate, L. S., Oba, A., & Shimokomak, M. (2012). The effects of biotic additives on growth performance and meat qualities in broiler chickens. *International Journal of Poultry Science*, *11,* 599-604.

Aruwa, C.E., Pillay, C., Nyaga, M.M., & Sabiu, S. (2021). Poultry gut health – microbiome functions, environmental impacts, microbiome engineering and advancements in characterization technologies. *Journal of Animal Science and Biotechnology*, *12,* 119.

Ashaolu, T.J. (2020). Immune boosting functional foods and their mechanisms: A critical evaluation of probiotics and prebiotics. *Biomedicine & Pharmacotherapy*, *130,* 110625.

Astuti, F.D., Sugiharto, S., Yudiarti, T., Widiastuti, E., Wahyuni, H.I., & Ayaşan, T. (2022). Growth performance, blood variables, intestinal bacterial content, and morphological measurements of broilers supplemented with Lactobacillus casei-fermented mixture of red rice and aromatic ginger. *Veterinary World, 15,* 818-826.

Biswas, A., Junaid, N., Kumawat, M., Qureshi, S., & Mandal, A.B. (2019). Influence of dietary supplementation of probiotics on intestinal histo-morphometry, blood chemistry and gut health status of broiler chickens. *South African Journal of Animal Science*, *48,* 968.

Chen, Y.C., & Yu, Y.H. (2020). Bacillus licheniformis–fermented products improve growth performance and the fecal microbiota community in broilers. *Poultry Science*, *99,* 1432-1443.

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., Calder, P.C., & Sanders, M.E. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, *11*, 506-514.

Kazemi, S.A., Ahmadi, H., & Karimi Torshizi, M.A. (2019). Evaluating two multistrain probiotics on growth performance, intestinal morphology, lipid oxidation and ileal microflora in chickens. *Journal of Animal Physiology and Animal Nutrition*, *103*, 1399-1407.

Khattab, A.A.A., El Basuini, M.F.M., El-Ratel, I.T., & Fouda, S.F. (2021). Dietary probiotics as a strategy for improving growth performance, intestinal efficacy, immunity, and antioxidant capacity of white Pekin ducks fed with different levels of CP. *Poultry Science*, *100,* 100898.

Kizerwetter-Świda, M., & Binek, M. (2016). Assessment of potentially probiotic properties of Lactobacillus strains isolated from chickens. *Polish Journal of Veterinary Sciences*, *19*, 15-20.

Kupryś-Caruk, M., Chabłowska, B., Michalczuk, M., & Stefańska, I. (2019). Comparison of the effect of lactic acid bacteria added to feed or water on growth performance, health status and gut microbiota of chickens broilers. *Annals of Warsaw University of Life Sciences - SGGW - Animal Science*, *58,* 55-67.

Li, Z., Wang, W., Liu, D., & Guo, Y. (2018). Effects of *Lactobacillus acidophilus* on the growth performance and intestinal health of broilers challenged with *Clostridium perfringens*. *Journal of Animal Science and Biotechnology*, *9*, 25.

Manafi, M., Khalaji, S., Hedayati, M., & Pirany, N. (2017). Efficacy of *Bacillus subtilis* and bacitracin methylene disalicylate on growth performance, digestibility, blood metabolites, immunity, and intestinal microbiota after intramuscular inoculation with *Escherichia coli* in broilers. *Poultry Science*, *96*, 1174-1183.

Mangisah, I., Yunianto, V.D., Sumarsih, S., & Sugiharto, S. (2021). Supplementation of garlic powder and *Lactobacillus casei* to improve nutrient digestibility, physiological conditions, and performance of broiler during starter phase. *Journal of the Indonesian Tropical Animal Agriculture*, *46*, 336-346.

Mohammed, A.A., Darwish, H.A.M., Negm, A.A.E., & Abdel- Maguid, S.A. (2022). Economic performance of different concentrations of probiotics on broiler chickens. *Research Journal for Veterinary Practitioners*, *10*, 7-23.

Mottet, A., & Tempio, G. (2017). Global poultry production: Current state and future outlook and challenges. *World's Poultry Science Journal*, *73,* 245-256.

Noohi, N., Papizadeh, M., Rohani, M., Talebi, M., & Pourshafie, M. R. (2021). Screening for probiotic characters in lactobacilli isolated from chickens revealed the intra-species diversity of *Lactobacillus brevis*. *Animal Nutrition*, *7*, 119-126.

Peng, Q., Zeng, X.F., Zhu, J.L., Wang, S., Liu, X.T., Hou, C.L., Thacker, P.A., & Qiao, S.Y. (2016). Effects of dietary *Lactobacillus plantarum* B1 on growth performance, intestinal microbiota, and short chain fatty acid profiles in broiler chickens. *Poultry Science*, *95*, 893-900.

Quinn, P.J., Quinn, Peter, J., Carter, M.E., Markey, B., & Carter, G.R. (1994). *Clinical Veterinary Microbiology*. Wolfe, London, UK.

Rehman, A., Arif, M., Sajjad, N., Al-Ghadi, M.Q., Alagawany, M., Abd El-Hack, M.E., Alhimaidi, A.R., Elnesr, S.S., Almutairi, B.O., Amran, R.A., Hussein, E.O.S., & Swelum, A.A. (2020). Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity. *Poultry Science*, *99*, 6946-6953.

Reuben, R.C., Sarkar, S.L., Ibnat, H., Roy, P.C., & Jahid, I.K. (2022). Novel mono- and multi-strain probiotics supplementation modulates growth, intestinal microflora composition and haemato-biochemical parameters in broiler chickens. *Veterinary Medicine and Science*, *8*, 668-680.

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.

Sarangi, N.R., Babu, L.K., Kumar, A., Pradhan, C.R., Pati, P.K., & Mishra, J.P. (2016). Effect of dietary supplementation of prebiotic, probiotic, and synbiotic on growth performance and carcass characteristics of broiler chickens. *Veterinary World*, *9*, 313-319.

Shah, S.M.T., Islam, Md.T., Zabin, R., Roy, P.C., Meghla, N.S., & Jahid, I.K. (2021). Assessment of novel probiotic strains on growth, haemato-biochemical parameters, and production costs of commercial broilers in Bangladesh. *Veterinary World*, *14*, 97-103.

Shokryazdan, P., Faseleh Jahromi, M., Liang, J. B., Ramasamy, K., Sieo, C. C., & Ho, Y. W. (2017). Effects of a Lactobacillus salivarius mixture on performance, intestinal health and serum lipids of broiler chickens. *Plos One*, *12*, e0175959.

Soumeh, E.A., Cedeno, A.D.R.C., Niknafs, S., Bromfield, J., & Hoffman, L.C. (2021). The Efficiency of probiotics administrated via different routes and doses in enhancing production performance, meat quality, gut morphology, and microbial profile of broiler chickens. *Animals*, *11*, 3607.

SPSS Inc (1997). *SPSS Base 7.5 for Windows User's Guide*. Prentice Hall.

Toghyani, M., Mosavi, S. Kazem, Modaresi, M., & Landy, N. (2015). Evaluation of kefir as a potential probiotic on growth performance, serum biochemistry and immune responses in broiler chicks. *Animal Nutrition*, *1*, 305-309.

Venkys (2017). *Vencobb-400 Broiler Management Guide*. Venkateshwara Hatcharies Pvt. Limited, Pune, India, http://tirumalagroup.com/.

Wu, Z., Yang, K., Zhang, A., Chang, W., Zheng, A., Chen, Z., Cai, H., & Liu, G. (2021). Effects of *Lactobacillus acidophilus* on the growth performance, immune response, and intestinal barrier function of broiler chickens challenged with *Escherichia coli* O157. *Poultry Science*, *100,* 101323.