



Effect of a Synbiotic Diet on The Performance of Broiler Chickens

Soren et al.

Effect of Dietary Synbiotic as a Replacement for Antibiotics on the Growth Performance, Gut Health and Immune Response in Broiler Chickens

Stephen Soren*, Ranjita Ghosh, Joydip Mukherjee, Samiran Mandal, Surojit Mandal and Indranil Samanta and Guru Prasad Mandal

West Bengal University of Animal and Fishery Sciences, Kolkata-700037, India

* Correspondence: drsoren1507@gmail.com

ABSTRACT

This study examined the impact of dietary supplementation with a synbiotic 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 VenCobb-400 broiler chicks were randomly divided into three dietary groups, each with six replicated pens (n = 6), housing nine broiler chickens: 1) basal diet without any growth promoter (CON), 2) basal diet with antibiotic (Bacitracin methylene disalicylate - BMD) at 500g/ton feed (AGP), and 3) basal diet with synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructo oligosaccharide) at 1 ml/bird in drinking water (SYN). Body weight, feed intake, and feed conversion ratios (FCR) were monitored weekly for 42 days. Blood biochemistry was assessed on day 28, and immune response was evaluated on days 28 and 35. Carcass traits, gut microbiome, and gut morphology were examined at the end of the trial. The SYN group exhibited significantly higher final body weight and average daily gain compared to the CON and AGP groups. Feed conversion ratio was improved in the SYN and AGP groups compared to the CON group. Total *Escherichia coli* and *Salmonella* counts were significantly lower in the SYN group, while *Lactobacillus* counts were significantly higher. In conclusion, synbiotic supplementation could serve as a promising alternative to antimicrobials in broiler production, demonstrating beneficial effects in broilers fed an antibiotic-free diet.

KEYWORDS: Broiler chicken, Growth performance, Gut microbiota, Immune response, *Lactobacillus rhamnosus*.

Article received: 31 December 2024; Article accepted: 21 May 2025

INTRODUCTION

The non-therapeutic use of antibiotics in the poultry industry is a common practice to increase yield, promote growth, and protect birds from harmful bacteria. However, this practice has led to the development of antimicrobial-resistant microbes in birds and poultry products, leading to raising environmental concerns (Hayden et al., 2020). Some countries have banned antibiotic use, and consumer demand for antibiotic-free poultry products is increasing. Consequently, there is a growing need for alternatives to non-therapeutic antibiotic use in the poultry industry. Researchers are exploring alternative strategies, such as incorporating feed additives like essential oils, organic acids, enzymes, prebiotics, probiotics, synbiotics, and postbiotics, to replace antibiotics as growth promoters while maintaining poultry growth and well-being (Das et al., 2016; Reuben et al., 2021).

Synbiotics are the combination of probiotics and prebiotics. Probiotics rely on prebiotics as a substrate. The viability of probiotics is enhanced by supplementing them with appropriate non-digestible prebiotics (Sekhon and Jairath, 2010). Prebiotics improve gut health, increase beneficial bacterial count, lower pH and enhance the host animal's immunity (Reuben et al., 2021). Using synbiotics as a feed supplement could improve feed efficiency compared to using prebiotics and probiotics separately (Dakhil and Al-Shammari, 2023; Song et al., 2022). Therefore, safe and cost-effective strategies to reduce the microbial load are crucial for poultry producers (Eliżewska et al., 2020). With the increasing focus on safer and healthier chicken production, efforts have been ongoing to find AGP alternatives as feed supplements. While many studies have investigated the effects of probiotics and prebiotics separately on broilers, limited data is

available on the impact of synbiotics on broiler chickens' growth performance, gut health, and immune response. Most studies on synbiotics in broiler chickens have utilized powder form supplements, with limited research on liquid forms. This research intends to evaluate the impact of a synbiotic formulation featuring a distinctive blend (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on the growth efficiency, intestinal health, carcass traits, and immune reaction of broiler chickens as an antimicrobial substitute in poultry farming to address global health security concerns.

MATERIALS AND METHODS

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 with six replicated pens (n=6) containing nine broiler chickens per pen. The groups were: 1) basal diet without growth promoter (CON), 2) basal diet with antibiotic bacitracin methylene disalicylate (AGP) at 500g/ton feed, and 3) basal diet with synbiotics (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) at 1 ml/bird in drinking water (SYN). 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 recommendations for Vencobb 400 broiler chickens (Venkys, 2017). Experimental diets were prepared weekly, packed in high-density polyethylene bags with inner liners, and provided ad libitum along with water. The ingredient and chemical composition of the basal diet are detailed (Table 1).

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	Deoiled Soybean Meal (45%).	37.247	34.035	30.003
3	Vegetable oil	1.841	3.143	4.208
4	Dicalcium Phosphate	1.503	1.375	1.261
5	Limestone Powder	0.756	0.835	0.828
6	Salt	0.322	0.324	0.326
7	DL-methionine	0.314	0.260	0.231
8	L-Lysine HCL	0.226	0.154	0.131
9	L-Threonine	0.084	0.055	0.055
10	Toxin Binder	0.050	0.050	0.050
11	Sodium bi-carbonate	0.100	0.100	0.100
12	Choline Chloride, 60%	0.050	0.070	0.070
13	Trace mineral mixture	0.100	0.100	0.100
14	Vitamin premix	0.100	0.100	0.100
15	Antioxidant	0.010	0.010	0.010
16	Phytase 5000	0.010	0.010	0.010

Nutrient composition				
1	Metabolizable energy (kcal/kg) ⁺	3000.00	3100.00	3200.00
2	Crude protein (%) ⁺⁺	22.19	20.80	19.20
3	Ether extract (%) ⁺⁺	4.49	5.83	6.97
4	Crude fiber (%) ⁺⁺	4.00	4.24	5.36
5	Dry matter (%) ⁺⁺	90.32	91.29	90.28
6	Total Ash (%) ⁺⁺	11.48	13.51	11.54
7	Acid Insoluble Ash (%) ⁺⁺	0.82	0.82	0.66
8	Calcium (%) ⁺⁺	0.92	0.91	0.87
9	Total phosphorus (%) ⁺⁺	0.79	0.76	0.72
10	Available phosphorus (%) ⁺⁺	0.45	0.42	0.39
11	Lysine (%) ⁺	1.22	1.09	0.98
12	Methionine (%) ⁺	0.6	0.53	0.49
13	Methionine + cysteine (%) ⁺	0.88	0.80	0.74
14	Threonine (%) ⁺	0.77	0.70	0.65
15	Sodium (%) ⁺	0.16	0.16	0.16
16	Chloride (%) ⁺	0.18	0.18	0.18

⁺ Calculated values ⁺⁺ Analysed values

Preparation of synbiotics

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 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 synbiotic was prepared in liquid form. The synbiotic liquid base was prepared by dissolving skim milk powder (30 g), dextrose (10 g), fructooligosaccharide (50 g), and a nutrient mix (2.5 g) in 1000 ml of distilled water. 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 *Lactobacillus rhamnosus* NCDC 298 (10⁹ cfu/ml) culture was inoculated at a rate of 1% and incubated at 37°C for 15-18 hours to produce the synbiotic preparation. The synbiotic preparation was stored at 5-7°C for a maximum of 7 days.

Broiler Trial

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.

The initial body weight of each chicken was noted on the trial's first day. Average daily feed intake (ADFI) was determined by dividing the total feed eaten daily by the number of chickens present in each pen. The feed conversion ratio (FCR) was determined by evaluating the total feed consumption and weight increase for every replicate pen.

Blood samples for biochemical analysis were collected from broiler chickens on day 35 after a 12-hour fasting period. The samples were obtained from the wing vein. Twelve birds were randomly selected for each treatment, with two birds taken from each pen. Blood was collected without an anticoagulant, and the serum was stored at -20°C

before analysis. The serum levels of glucose, total protein, albumin, uric acid, triglycerides, and cholesterol were measured using commercially available kits from DiaSys Diagnostic India Pvt. Ltd., based in Mumbai, India.

Humoral Immune Response

The humoral immune response was assessed by measuring antibody levels after the administration of vaccines for Newcastle disease virus (NDV) and infectious bursal disease (IBDV). The B1 strain (0.2 mL) and LaSota strain (0.2 mL), both live lentogenic strains obtained from Venkateswara Hatcheries Private Limited in India, were administered via the ocular route on days 5 and 21. The intermediate plus vaccine for IBD (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 quickly transferred to centrifuge tubes without anticoagulant, and serum was collected through centrifugation. Antibody levels for NDV and IBDV were measured using an ELISA kit supplied by IDEXX Laboratories Inc. USA. The optical density (OD) for each sample was measured twice with a microplate reader from Meril Life, India, and the mean OD values were used for analysis.

Carcass characteristics

On day 42, two birds (one male and one female with body weights near the mean for that replicate) were randomly selected per replicate and euthanized by cervical dislocation for carcass trait assessment. Different body parts were precisely measured and documented using a digital scale.

Caecal Microbiota

The caecal contents of chickens slaughtered on day 42 were collected aseptically in a sterile sample collection bag (HiMedia, India). The samples were processed on the same day for bacteriological analysis to quantify *E. coli*, *Salmonella*, and *Lactobacillus* using a standard colony counting procedure (Quinn et al., 1994). One gram of caecal content was diluted tenfold with sterile phosphate-buffered saline (PBS). Subsequently, 10 μ L 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 (HiMedia, India). The results were expressed as Log₁₀ colony-forming units (cfu) per gram of the sample.

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 with Kelplus equipment from Pelican Equipments in Chennai, India, following method 968.06. 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 with Socsplus equipment from Pelican Equipments in Chennai, India, following method 920.39. Calcium content was determined as per 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 SPSS software (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, the Duncan test was used to detect differences among treatment means.

RESULT AND DISCUSSION

Average Daily Gain, Feed Intake and Feed Efficiency

The average daily gain (ADG) of the birds significantly increased ($p < 0.05$) in the synbiotic group compared to the control and antibiotic groups throughout the entire 42-day experiment period. The final body weight was significantly higher ($p < 0.05$) in the synbiotic group compared to the antibiotic and control groups. The average daily feed intake (ADFI) of the chickens did not vary significantly among the treatment groups during the different experimental phases or over the entire 42-day period. There were no significant differences in feed conversion ratio

(FCR) during the starter (1–14 days) and grower (15–28 days) phases. However, the FCR during the finisher phase (29–42 days) and overall period (1–42 days) was significantly improved ($p < 0.05$) in the synbiotic group compared to the control and antibiotic groups (Table 2). The study revealed that supplementing synbiotics led to a significant increase in final body weight and average daily gain, as well as an improvement in feed conversion ratio over a 42-day period. However, feed intake did not differ significantly among the treatment groups. Similar results were obtained by Dakhil and Al-Shammari (2023), showing significant improvements ($P < 0.05$) in body weight, cumulative weight gain, growth rate, and feed efficiency in the synbiotic group compared to the control group. Song et al. (2022) also observed a higher average daily gain in the synbiotic-supplemented group compared to the control group

($P < 0.05$). Aparna et al. (2022) reported that supplementation with *L. salivarius* at 10^{12} cfu/kg showed significant ($P < 0.05$) increase in weight gain and improvement in feed conversion ratio (FCR). These studies suggest that synbiotic supplementation positively impacts weight gain and feed conversion ratio. This improvement in weight gain may be attributed to probiotics' ability to secrete digestive enzymes like lipase, protease, and amylase, which help break down feed nutrients for better digestibility of starch, fat, and protein, resulting in increased availability of nutrients for the broilers and leading to higher live weight gain (Bedford, 2000). Additionally, improved FCR might be attributed to the combined effect of prebiotics and probiotics preserving normal microbial populations while simultaneously enhancing ileal digestibility (Nisar et al., 2021; Aziz Mousavi et al., 2018).

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

Attribute	Treatment ¹			SEM	P-Value
	CON	AGP	SYN		
ADG (g/d)					
d1-14	25.26	25.52	28.19	0.816	0.284
d15-28	73.61	76.43	76.27	1.299	0.636
d29-42	70.88	74.62	78.49	1.400	0.077
d1-42	56.58 ^c	58.86 ^b	60.98 ^a	0.567	0.001
Final BW	2419.01 ^c	2541.55 ^b	2603.81 ^a	23.810	0.001
ADFI (g/d)					
d1-14	29.09	28.99	28.58	0.070	0.796
d15-28	105.57	104.50	102.39	1.218	0.584
d29-42	133.13	129.97	129.85	1.107	0.409
d1-42	89.26	87.78	87.11	0.626	0.382
FCR (g intake/g gain)					
d1-14	1.16	1.14	1.06	0.025	0.222
d15-28	1.44	1.37	1.36	0.031	0.535
d29-42	1.89 ^a	1.74 ^b	1.67 ^b	0.036	0.029
d1-42	1.58 ^a	1.49 ^b	1.43 ^b	0.018	0.000

^{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 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). ² SEM, standard error of means (n=6)

Blood serum biochemical profile

The levels of glucose, total protein, albumin, uric acid, triglyceride, and cholesterol in serum showed no significant variation ($P > 0.05$) among the different dietary treatments in this study (Table 3). The study found that supplementing with synbiotics did not lead

to significant changes in serum glucose, total protein, albumin, uric acid, triglyceride, and cholesterol levels. This aligns with previous studies by Celińska et al. (2020), Abdel-Hafeez et al. (2017) and Khalil et al. (2021), which also reported no significant impact on the blood biochemical profile when synbiotics were added to the broiler diet.

Table 3. Effect of synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on blood biochemical profile of broiler chickens at day 35

Attribute	Treatment ¹			SEM ²	P-Value
	CON	AGP	SYN		
Glucose (mg/dl)	282.61	240.01	289.67	12.563	0.229
Total protein(mg/dl)	3.34	3.77	3.24	0.105	0.084
Albumin(mg/dl)	2.99	3.24	3.13	0.063	0.285
Uric acid (mg/dl)	3.26	3.11	3.34	0.083	0.543
Triglyceride (mg/dl)	173.50	170.43	159.90	8.607	0.680
Cholesterol (mg/dl)	171.12	177.08	157.89	9.001	0.83

¹ The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). ² SEM, standard error of means (n=6)

Immune Response

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

treatment groups on day 28 and day 35. This result is consistent with previous studies by Salehimanesh et al. (2016), Rehman et al. (2020) and Silva et al. (2009) which also reported no significant differences in antibody titers when synbiotic supplements were added to broiler chickens' diets. However, Pappula, (2021) reported that Humoral immune response (NDV titers) was higher ($P < 0.05$) in lauric acid+*Bacillus subtilis* group followed compared to control.

Table 4. Effect of synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on antibody titre (\log_{10}) against infectious bursal disease vaccine (IBDV) and New castle disease vaccine (NDV) of broiler chickens at day 28 and day 35.

Attribute	Treatment ¹			SEM ²	P-Value
	CON	AGP	SYN		
IBDV					
d28	3.07	3.10	3.18	0.046	0.621
d35	3.13	3.25	3.33	0.053	0.313
NDV					
d28	2.49	2.67	2.70	0.801	0.542
d35	2.79	2.81	2.89	0.478	0.682

¹ The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). ² SEM, standard error of means (n=6)

Carcass characteristics

There were no significant differences ($P > 0.05$) observed in slaughter body weight, eviscerated carcass weight, dressing percentage, and weights of various body parts (breast, frame, thigh, drumstick, wing, neck, gizzard, liver, heart, spleen, bursa, and abdominal fat) in grams among the treatment groups (Table 5). The current study found no significant differences in carcass characteristics when synbiotics were added to the diet of broiler chickens. These results are 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 synbiotics in broiler diets. Similarly, studies by Nisar et al. (2021), Sarangi et al. (2016), Ghasemi et al. (2014) and Ashayerizadeh et al. (2011) reported that supplementing synbiotics at different levels did not significantly affect carcass traits, such as thigh, breast, or wing weights. However, Hossain et al. (2024) reported that supplementation with MOS and yeast showed a positive effect ($P < 0.05$) on the breast, thigh, wing, back, and gizzard weight.

Table 5. Effect of synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on carcass characteristics of broiler chickens at day 42.

Attribute	Treatment ¹			SEM ²	P-Value
	CON	AGP	SYN		
Slaughter BW (g)	2443.75	2462.27	2509.26	20.898	0.479
Eviscerated BW (g)	1664.85	1684.91	1690.84	13.704	0.773
Dressing Percentage (%)	68.12	68.45	67.38	0.292	0.358
Breast (g)	598.67	606.67	585.00	11.199	0.779
Frame (g)	307.00	285.67	304.67	7.158	0.470
Thigh (g)	227.67	222.00	214.67	6.331	0.758
Drumstick (g)	217.00	228.33	224.00	3.624	0.498
Wing (g)	126.33	134.67	128.67	3.182	0.610
Neck (g)	58.17	67.33	62.00	2.107	0.218
Gizzard (g)	44.51	47.43	50.63	1.504	0.281
Liver (g)	33.07	39.51	38.03	1.889	0.395
Spleen (g)	1.94	2.26	2.31	0.227	0.820
Bursa (g)	1.43	1.43	0.69	0.234	0.622
Abdominal Fat (g)	38.04	35.93	42.84	1.919	0.367

¹ The control diet (CON), control diet was supplemented with Antibiotic (BMD) at 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). ² SEM, standard error of means (n=6)

Gut microbes

The count of *E. coli* and *Salmonella* were significantly lower ($p < 0.05$) in the synbiotic group compared to the control and antibiotic groups. Moreover, the count of *Lactobacillus* was significantly higher ($p < 0.05$) in the synbiotic group than in the control and antibiotic-supplemented groups (Table 6). This study showed that supplementing with synbiotics increased

Lactobacillus levels and a reduced in *E. coli* and *Salmonella* levels compared to the antibiotic and control groups. These results align with previous studies by Nopparatmaitree et al. (2022); Dibaji et al. (2014); Wein et al. (2020); Ayalew et al. (2022); Abdel-Wareth et al. (2019); Mookiah et al. (2014) and Erdođan et al. (2010), which also found that synbiotic supplementation boosted beneficial bacteria like *Lactobacilli* while decreasing harmful pathogens like *E. coli* and *Salmonella* in poultry.

Table 6. Effect of synbiotic (*Lactobacillus rhamnosus* NCDC 298 + fructooligosaccharide) on viable bacteria numbers (log₁₀ cfu/g) in caecal content of broiler chickens at day 42

Attribute	Treatment ¹			SEM ²	P-Value
	CON	AGP	SYN		
<i>E. coli</i>	6.92 ^a	6.66 ^b	6.39 ^c	0.068	0.002
<i>Salmonella</i>	6.77 ^a	6.57 ^a	6.18 ^b	0.071	0.000
<i>Lactobacillus</i>	6.97 ^c	7.23 ^b	7.54 ^a	0.064	0.000

^{abc} 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 200mg/MT feed (AGP), control diet with synbiotic at 1ml/bird/day (SYN). ² SEM, standard error of means (n=6)

CONCLUSION

Birds supplemented with synbiotics exhibited higher final body weight and average daily gain, along with an improved feed conversion ratio (FCR) throughout the experiment compared to the control group. Furthermore, synbiotic supplementation led to decreased caecal *E. coli* and *Salmonella* counts in comparison to other groups. *Lactobacillus* counts were notably elevated in birds receiving synbiotics compared to those in the control and antibiotic groups. These findings suggest that synbiotics could serve as effective alternatives to antibiotics in poultry production, particularly for broilers on an antibiotic-free diet, thereby promoting overall broiler health. Further research is necessary to fully comprehend and optimize the utilization of synbiotics in poultry production. This study underscores the potential of synbiotics as a promising option for improving gut health in poultry without the reliance on antibiotics.

REFERENCES

- Abdel-Hafeez, H.M., Saleh, E.S.E., Tawfeek, S.S., Youssef, I.M.I. and 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-Australas Journal of Animal Sciences*. 30:672–682.
- Abdel-Wareth, A.A.A., Hammad, S., Khalaphallah, R., Salem, W.M. and Lohakare, J. 2019. Synbiotic as eco-friendly feed additive in diets of chickens under hot climatic conditions. *Poultry Sciences*. 98: 4575–4583.
- AOAC, 1995. Official Methods of Analysis, 16th Edn. Association of Official Analytical Chemists, Arlington, VA.
- Aparna, N., Karunakaran, R., Parthiban, M., Rao, V. A. and Radhakrishnan, L. 2022. Effect of *Lactobacillus* in Broiler Chicken Fed Diets Enriched with 1%-3 Fatty Acids. *Indian Journal of Animal Nutrition*. 38(3): 317-326.
- Ashayerizadeh, A., Dabiri, N., Mirzadeh, K. and Ghorbani, M. 2011. Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. *Journal of Cell and Animal Biology*. 5(8): 152-156.
- Ayalew, H., Zhang, H., Wang, J., Wu, S., Qiu, K., Qi, G., Tekeste, A., Wassie, T. And Chanie, D. 2022. Potential feed additives as antibiotic alternatives in broiler production. *Frontiers in Veterinary Science*. 9:916473.
- Aziz Mousavi, S.M.A., Mahmoodzadeh Hosseini, H. and Mirhosseini, S.A. 2018. A Review of Dietary Probiotics in Poultry. *Journal of Applied Biotechnology Reports*. 5: 48–54.
- Bedford, M. 2000. Removal of antibiotic growth promoters from poultry diets: implications and strategies to minimise subsequent problems. *Worlds Poultry Science Journal*. 56: 347–365.
- Dakhil, A.J. and Al-Shammari, K.I.A. 2023. Potential influence of dietary synbiotic and fenugreek seed to improve the productive traits and economic cost in stressed broiler chickens. *AIP Conf. Proc.* 2776: 100008.

- Das, O., Patil, S.S., Savsani, H.H., Padodara, R.J., Garg, D.D., Marandi, S. and Barad, N. 2016. Effect of dietary prebiotics, probiotics and synbiotics as feed additives on blood profile and broiler performance. *International Journal of Science, Environment and Technology*. 5: 3546–3552.
- Dibaji, S.M., Seidavi, A., Asadpour, L. and Silva, F.M. 2014. Effect of a synbiotic on the intestinal microflora of chickens. *Journal of Applied Poultry Research*. 23: 1–6.
- Erdođan, Z., Erdođan, S., Aslantađ, Ö. and Çelik, S. 2010. Effects of dietary supplementation of synbiotics and phytobiotics on performance, caecal coliform population and some oxidant/antioxidant parameters of broilers. *Journal of Animal Physiology and Animal Nutrition*. 94: 40–48.
- Furuichi, Y. and Takahashi, T. 1981. Evaluation of Acid Insoluble Ash as a Marker in Digestion Studies. *Agriculture and Biology Chemistry*. 45: 2219–2224.
- Ghasemi, H.A., Kasani, N. and Taherpour, K. 2014. Effects of black cumin seed (*Nigella sativa* L.), a probiotic, a prebiotic and a synbiotic on growth performance, immune response and blood characteristics of male broilers. *Livestock Science*. 164: 128–134.
- Hayden, D., Karla, A. and Lixin, Z. 2020. A Review of Antimicrobial Resistance in Poultry Farming within Low-Resource Settings. *Animals*. 10: 1264.
- Hossain, M.I., Hossain, D.M.M. and Akhter, S. 2024. Enhancing Broiler Chicken Performance, Gut Microbiota, and Carcass Traits through Prebiotics (Mannan-oligosaccharides) and Probiotics (*Saccharomyces cerevisiae*). *Indian Journal of Animal Nutrition*. 41(2): 313–320.
- Khalil, K., Islam, M., Islam, M., Sujan, K., Islam, M. and Miah, M. 2021. Effect of selected probiotics and synbiotics on growth performance and blood-biochemical changes in broiler chickens. *Journal of the Bangladesh Agriculture University*. 19: 471–476. doi:10.5455/JBAU.120923
- Mookiah, S., Sieo, C.C., Ramasamy, K., Abdullah, N. and Ho, Y.W. 2014. Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. *Journal of the Science of Food and Agriculture*. 94: 341–348.
- Nisar, H., Sharif, M., Rahman, M., Rehman, S., Kamboh, A. and Saeed, M. 2021. Effects of Dietary Supplementations of Synbiotics on Growth Performance, Carcass Characteristics and Nutrient Digestibility of Broiler Chicken. *Brazilian Journal of Poultry Science*. 23(2): 1–10.
- Nopparatmaitree, M., Saenphoom, P., Bunlue, S., Washiraomornlert, S., Kitpipit, W. And Chotnipat, S. 2022. Dietary of Synbiotic Derived from Trimmed Asparagus by Products Combined with Probiotic Supplementation on Digestibility, Gut Ecology, Intestinal Morphology and Performance of Broilers. *Advances in Animal and Veterinary Sciences*. 10: 1371–1382.
- Oliveira, J.M., Marchi, D.F., Geronimo, B.C., Oba, A. and Soares, A.L. 2022. Effect of diets containing *Saccharomyces cerevisiae* fermentation products on broiler performance and meat quality. *Journal of Agricultural Sciences Research*. 2: 2–8.
- Pappula, R. 2021. Supplementation of Lauric Acid, Probiotic and their Combination on Performance and Immune Response of Commercial Broiler Chicken. *Indian Journal of Animal Nutrition*. 38(1): 55–60.
- Quinn, P.J., Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R. 1994. *Clinical Veterinary Microbiology*. Wolfe publishing, London. doi: 10.1016/S0007-1935(95)80184-7
- 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. and 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., Roy, P.C., Anwar, A., Hossain, M.A. and Jahid, I.K. 2021. Prebiotics, probiotics and postbiotics for sustainable poultry production. *Worlds Poultry Science Journal*. 77: 825–882.
- Salehimanesh, A., Mohammadi, M. and Roostaei-Ali Mehr, M. 2016. Effect of dietary probiotic, prebiotic and synbiotic supplementation on performance, immune responses, intestinal morphology and bacterial populations in broilers. *Journal Animal Physiology and Animal Nutrition*. 100: 694–700.
- Sarangi, N.R., Babu, L.K., Kumar, A., Pradhan, C.R., Pati, P.K. and 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.
- Sekhon, B. and Jairath, S. 2010. Prebiotics, probiotics and synbiotics: an overview. *Journal of Pharmaceutical Education and Research*. 1: 13-36.
- Silva, V.K., da Silva, J.D.T., Torres, K.A.A., de Faria Filho, D.E., Hada, F.H. and de Moraes, V.M.B. 2009. Humoral immune response of broilers fed diets containing yeast extract and prebiotics in the prestarter phase and raised at different temperatures. *Journal of Applied Poultry Research*. 18: 530–540.
- Ślizewska, K., Markowiak-Kopec, P., Żbikowski, A. and Szeleszczuk, P. 2020. The effect of synbiotic preparations on the intestinal microbiota and her metabolism in broiler chickens. *Scientific Reports*. 10: 4281.
- Song, D., Li, A., Wang, Y., Song, G., Cheng, J., Wang, L., Liu, K., Min, Y. and Wang, W. 2022. Effects of synbiotic on growth, digestibility, immune and antioxidant performance in broilers. *Animal*. 16: 100497.
- SPSS Inc, 1997. *SPSS Base 7.5 for Windows User's Guide*. Prentice Hall.
- Talapatra, S.K., Ray, S.C. and Sen, K.C. 1940. The analysis of mineral constituents in biological materials. 1. Estimation of phosphorus, chlorine, calcium, magnesium, sodium and potassium in food-stuffs. *Indian Journal of Veterinary Sciences*. 10: 243–258.
- Venkys, 2017. *Vencobb400 Broiler Management Guide*. Venkateshwara Hatcheries Pvt. Limited, Pune, India, <http://tirumalagroup.com/>.
- Wein, S., Ruangapanit, Y. and Syed, B. 2020. The Efficacy of Synbiotic Application in Broiler Chicken Diets, Alone or in Combination with Antibiotic Growth Promoters on Zootechnical Parameters. *Journal of World's Poultry Research*. 10: 469–479.