Research Article

Ameliorative Influences of Prebiotic on Productive Performance, Intestinal Microbiota Load, and Immune Response of Broiler Chickens

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Abstract | Antibiotic overuse, which disturbs poultry's natural gut flora and fuels the growth of microbial resistance, is now raising concerns for human health. As a result, using antibiotics is swiftly overshadowed by alternatives in order to minimize any lasting effects, combat microbial resistance, and preserve productivity. The study was aimed to investigate the influence of a prebiotic (PB), mannan-oligosaccharides (MOS) enriched with β-glucans, and an antibiotic growth promoter (AGP) on growth performance, intestinal microbial load, and immune response in broiler chickens. A total of 450 Cobb-500 unsexed DOCs were divided in response to the treatment into broilers fed a control diet (no additives), control supplemented with PB (100 g/100 kg feed), or control supplemented with AGP (Lincomycin 2.2%, 15 g/100 kg feed), having six replications in each treatment (25 birds per replication). Growth parameters were recorded. On days 22 and 34, HI antibody titer was determined against Newcastle disease (ND) and ELISA was performed for Infectious Bursal Disease (IBD). On day 35, birds were sacrificed to determine carcass yield and harvest intestinal contents for microbial and histomorphological examination. Live weights and live weight gains were higher ($p = 0.05$) in PB and AGP groups with the lowest feed intake in AGP ($p = 0.05$). AGP-fed birds had better uniformity than the birds reared on the control diet and this result was similar to that of PB-fed birds. The treated groups had higher dressing yields ($p = 0.01$). Breast meat yield was increased in the PB-fed group ($p = 0.01$), with a reduction of abdominal fat ($p = 0.01$). The duodenal total viable count (TVC) was higher ($p = 0.05$) in AGP-treated birds. Total *E. coli* count (TEC) in the duodenum, ileum, and caecum decreased ($p = 0.01$) in the PB-fed birds. Antibody titer increased significantly ($p < 0.05$) with PB supplementation in both collections against ND and IBD. Villi length increased ($p = 0.05$) in the PB-fed group. In conclusion, dietary supplementation of MOS enriched with β-glucans may be considered as a performance enhancer, reducing the pathogenic bacteria load, and boosting the immune response. PB may be a suitable alternative to AGP.

Keywords | Antibiotic, Broiler, Immunity, Microbiology, Prebiotic.

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INTRODUCTION

Considerable reports have appeared recently stating that human consumption of meat and eggs based on indiscriminate feeding of sub-therapeutic levels of antibiotics to poultry builds up microbial resistance and poses a high risk of liver and kidney damage (Mehdi et al., 2018; Sweeney et al., 2018; Kim and Lillehoj, 2019) with deaths in extreme

cases (Heianza et al., 2020). Researchers quite agreed that microbial resistance develops bacterial infections that go beyond medication (Jampilek, 2022; Stracy et al., 2022). Antibiotics were used as medicinal agents and growth boosters in livestock and poultry during the few decades to enhance performance and reduce mortality. However, increased considerations regarding the development of microbial resistance and the disruption of indigenous gut flora have generated worries about antibiotic usage in farm animals (Yun et al., 2017; Teng and Kim, 2018). The reduction of antibiotic use as growth promoters has compelled researchers to look for sound alternative sources that could meet the desired goals of feed additives in animal production (WHO, 2018).

One such candidate may be prebiotics (PB), which, according to Gibson and Roberfroid (1995), may be defined as a non-digestible feed additive that is beneficially attributed to increasing the microbial balance, encouraging animal growth, and activating one or a few beneficial bacterial metabolisms in gut. The most important merit of prebiotics is leaving no undesirable residues in meat. Moreover, *Bifidobacteria* and *Lactobacilli* are stimulated by prebiotic feeding, which ultimately favors animal health. PB has an advantage over probiotics as it activates bacteria that are already adapted to the environment and are naturally present in the gastrointestinal tract (Snel et al., 2002). The prebiotic, mannan oligosaccharides (MOS), is extracted from the exterior coating of yeast cell walls that contains proteins, glucans, phosphate radicals, and mannose (Klis et al., 2002). The protein present in MOS has relatively high proportions of serine, threonine, aspartic, and glutamic acids but it has a paucity of methionine (Jianyi and Weifen, 2001), which is essential for improving performance. MOS improves performance via regulation of type-1 fimbriae (mannose sensitive lectin) producing pathogenic bacteria, immune system amplification, and activation of development and synthesis of mucin and brush boundary enzymes in the intestine (Ferket, 2004). The addition of prebiotics to the diet has been shown increase body weight and performance (Torres-Rodriguez et al., 2007; Walker et al., 2018) and exert better feed efficiency (Salianeh et al., 2011). Their inclusion in the diet results in better carcass yields (Moilwa et al., 2021). MOS (Spring et al., 2000; Jamroz et al., 2004) is efficient in reducing microbial contamination (Walker et al., 2018). MOS is effective in lowering infection by *Salmonella* and *coliform* in birds (Spring et al., 2000; Chee et al., 2010) by the inhibition of adhesion to epithelial tissues in the GI tract (Froebel et al., 2020). MOS can boost antibody production against the viruses that cause infectious bursal diseases, Newcastle disease, and avian influenza (Tohid et al., 2010; Salehimanesh et al., 2016). Intestinal health-promoting bacteria may ferment PB, enhancing intestinal microbial architecture, gut epithelial cell integrity, and ultimately the host's general health (Teng and Kim, 2018). The inclusion of MOS and β-glucan in the broilers' diet increases villi height (Teng et al., 2021). Data reported from Southeast Asian laboratories on the effects of MOS on growth, gut health, and an immune response is scanty, and some results are inconsistent. Moreover, unlike probiotics, the investigations into the efficacy of PB in comparison with antibiotic growth promoters were addressed to a limited extent, and a few results are also inconsistent, indicating environmental influence in addition to the nature of test materials.

This study was carried out for the first time in Bangladesh to our knowledge, to evaluate the effects of dietary supplementation of MOS that includes β-glucans originating from *Saccharomyces cerevisiae* as PB and antibiotic growth promoter (AGP) on performance, meat yield, intestinal microbial load, and immunity of broilers. The ultimate hypothesis had been tested was whether β-glucan enriched MOS could be a suitable alternative to AGP in the diet of broiler chickens.

MATERIALS AND METHODS

Animal welfare statement

The methods used in the experiment were approved by the Animal Welfare & Experimental Ethics Committee, Faculty of Veterinary Science, Bangladesh Agricultural University. (AWEEC/BAU/2021) (27).

Experimental birds, layout, and management

This research was conducted with 450 mixed sex broiler DOCs (Cobb 500), and the experiment was designed to last for 35 days. The broiler DOCs were divided into three groups, each of 150 chicks (six replicates of each of 25 birds), and fed a complete plant-based corn-soya-based diet. The experimental diets were: A. a corn-soya-based diet (control); A + a prebiotic (PB) containing MOS+β-glucans (100 g/100 kg feed); and A + an antibiotic growth promoter (AGP) (Lincomycin 2.2%, 15 g/100 kg feed). All the additions were commercially available powders that were mixed well with the diet. Before the arrival of chicks, the house was subjected to cleaning, washing, disinfection, and drying. Twenty equal-sized pens were made of wire net and bamboo materials. Each pen was 2.33 m^2 (1.525 m × 1.525 m). Feeders, waterers, adjacent buckets, and all other essential equipment were thoroughly cleaned, rinsed, and disinfected using a $TH4^+$ disinfectant (a solution including glutaraldehyde, dodecyl dimethyl ammonium chloride, dioctyl dimethyl, dimethyl ammonium chloride, and octyl decyl dimethyl ammonium chloride, Sogeval, France, Marketed by-Century Agro Ltd, Bangladesh). At a depth of roughly 2.5 cm, fresh and dry rice husks were used as bed-

Table 1: Ingredient and nutrient composition of starter and grower diet (%).

Ingredients	Starter (0-21d)	Grower (22-35d)
Corn	47.20	57.81
Soybean meal (44% CP)	44.45	33.94
Soybean oil	4.78	4.57
Di calcium phosphate	1.55	1.65
Limestone	0.86	0.87
Sodium chloride	0.31	0.31
Sodium bi-carbonate	0.23	0.24
DL-Methionine	0.29	0.24
CJL-Lysine-Monohydrochloride	0.07	0.10
L-Threonine	0.01	0.02
Vit-Min Premix[1]	0.25	0.25
Nutrients		
DM	86.31	86.79
ME, kcal/kg	3000	3000
CP	22.85	19.64
Lysine	1.46	1.07
Cystine	0.40	0.31
(Methionine	0.58	0.56
Met+Cys	0.98	0.87
Threonine	1.03	0.80
Histidine	0.66	0.51
Arginine	1.79	1.29
Ileusine	1.17	0.85
Leusine	2.12	1.65
Phenylalanine	1.33	0.97
Valine	1.23	0.92
Calcium	0.90	0.90
Available P	0.45	0.45

[1]Each kg vitamin mineral premix contained: vitamin A palmitate 6,600 IU; cholecalciferol 2,200 IU; menadione dimethylpyridine bisulfite 2.2mg; riboflavin, 4.4mg; pantothenic acid 13mg; niacin 40mg; choline chloride 500mg; biotin 1 mg; vitamin B_{12} 22 µg; ethoxyquin 125mg; iron 50mg; copper 6mg; zinc 40mg; manganese 60mg; selenium 0.2mg.

-ding materials. Throughout the trial, the birds were fed and watered *ad libitum*, and the lighting schedule was 16 h of light followed by 8 h of darkness. Diets based entirely on vegetable protein were formulated utilizing locally accessible basic ingredients. Raw feed materials were procured from the local market and chemical analyses were performed in Degussa Lab, Germany (Courtesy of Evonik Degussa GmbH). Table 1 shows the detailed composition of the experimental diets. The birds were fed a starter diet (ME = 3000 kcal/kg; CP = 22.85 %) for the first three weeks and a grower diet (ME = 3000 kcal/kg; CP = 19.64 %) for the remaining period of 14 days. Feed was given on newspaper during the early period of five days in a plastic tube feeder of 3kg capacity. One round drinker (2 L capacity) in each pen was supplied to facilitate watering. For the remaining weeks, two feeders (each of 3 kg capacity) and one drinker (8 L capacity) were allotted for the birds of each pen. An individual bucket was placed in each pen for feeding purposes. Weekly feed consumption was calculated as the difference between the feed supplied at the beginning of the week and the feed remaining at the end of each week. The spilled feed that was in good condition was carefully separated, weighted and then added to the new allocation of the feed to be supplied into the following week and the part of the wasted amount was just discarded. The temperature and humidity were measured four times daily using a digital thermo-hygrometer (HTC-2, Velveeta, Makkar Trading Company, India) suspended from the roof at chick level and adjusted as the birds were growing. Fresh and clean drinking water was supplied thrice a day (morning, noon, and evening). Feeders were cleaned when necessary. Birds were immunized against common viral diseases (Newcastle Disease, Infectious Bronchitis, and Infectious Bursal Disease) as a part of the disease prevention program. All vaccines were procured from Intervet International, BV, Boxmeer, and the Netherlands through their local representatives and administered as per manufacturers' instructions. The experimental buildings and the area around them were subject to a stringent biosecurity program.

Performance records

Initial body weight was recorded (46 g/bird) on the chicks' arrival. Then the birds were weighed weekly in the evening before feeding time, and the average was recorded at the end of each week. Based on body weight gain and feed intake, the feed conversion ratio (FCR) was calculated. Mortality was recorded and at the same time, the feed intake was adjusted to the number of birds that was died during the experimental period. During the trial period, the temperature and humidity of the house were noted using a digital thermo-hygrometer.

Processing of broilers

One male and one female from each replication were sacrificed by the end of the experiment. To facilitate processing, the feed was withdrawn 12 h before slaughtering but the water was supplied as usual. The birds were killed by cervical dislocation and allowed to bleed for 5 min and subsequently immersed in hot water (51-55°C) for 120 sec to facilitate de-feathering. The head, shank, viscera, giblet, and abdominal fat were separated. Dressed broilers were cut into different parts such as breast, thigh, drumstick, wing, and back. Then cut-up parts and giblet were weighed

and recorded.

Microbial study

On day 35, the intestinal contents of similar birds that were being evaluated for meat yield were collected. Three particular places in the intestine were selected for the collection of intestinal content: the duodenum, ileum, and caecum. The caecum samples were taken from the left cecum, which was 1.27 cm away from the ileocecal junction. Thus, there were three samples from each bird. The same procedure was followed for the remaining birds. Eventually, 108 samples were collected for microbial study. Immediately after killing, intestinal contents were collected separately in sterile Eppendorf tubes and refrigerated at 4°C. Digesta was pumped out and blended. Then the samples were transferred into test tubes containing 10 mL of phosphate buffer solution (PBS). One ml of diluted solution from each test tube was placed in another test tube containing 9 ml of PBS. Then 50 µl of the sample from the 2nd test tube was dropped onto the agar plate using a micropipette and spread using a sterile glass spreader. For each tray, a single sterile spreader was used. After that, the plates were incubated in an incubator for 48 h at 37°C. Plates containing 30–300 colonies were counted after incubation. Nutrient Agar (NA) was used for total bacteria, and Eosin-Methylene Blue (EMB) Agar was used for total *E. coli*.

Histomorphological investigation

The middles of the duodenum, ileum, and cecum were cut into two-centimeter segments, flushed with physiological saline, and immediately immersed in 10% neutral buffered formalin. After drying in a graded alcohol series, the formalin was then cleaned with methyl benzoate and embedded in paraffin wax. Haematoxylin and eosin were used to stain 5 µm sections. The samples were analyzed using a Zeiss Axiostar plus test microscope with transmitted light bright field examinations, upgradeable to a professional digital image analysis system. For each sample, 10 intact, well-oriented crypt-villus units were chosen at random. Villus height was defined as the distance between the tip of the villus and the villus-crypt junction, while crypt depth was defined as the distance between two villus invaginations.

Serological investigation

On days 22 and 34, three ml of blood was collected via the wing vein from each replicate (12 birds per treatment) for serological studies. The sera were tested for antibody titer against Newcastle disease using the haemagglutination inhibition (HI) test (Anon, 1971) following the administration of ND vaccines. The test was conducted by using the constant 4 HA unit antigen and decreasing serum method (Beta-procedure). An Enzyme-Linked Immunosorbent Assay (ELISA) kit was used for the determination of antibody titer against IBD.

Statistical analysis

Analysis of data was performed in a completely randomized design for ANOVA using the SAS (2009) statistical computer package software. Mean values were separated by Duncan's multiple range test where ANOVA showed a significant difference with a probability of <5% or higher.

RESULTS

Performance characteristics

Table 2 showed that PB and AGP-treated groups achieved higher final body weight and body weight gain (p = 0.05) than the control group. The AGP-fed birds had a lower feed intake followed by PB in comparison with the control group (p = 0.01). A tendency for improved FCR (p = 0.06) was observed in PB and AGP-fed groups. PB and AGP-fed birds showed statistically similar uniformity, however, the AGP group exhibited better uniformity as compared to the control group (p = 0.05). The survivability of the birds was statistically non-significant.

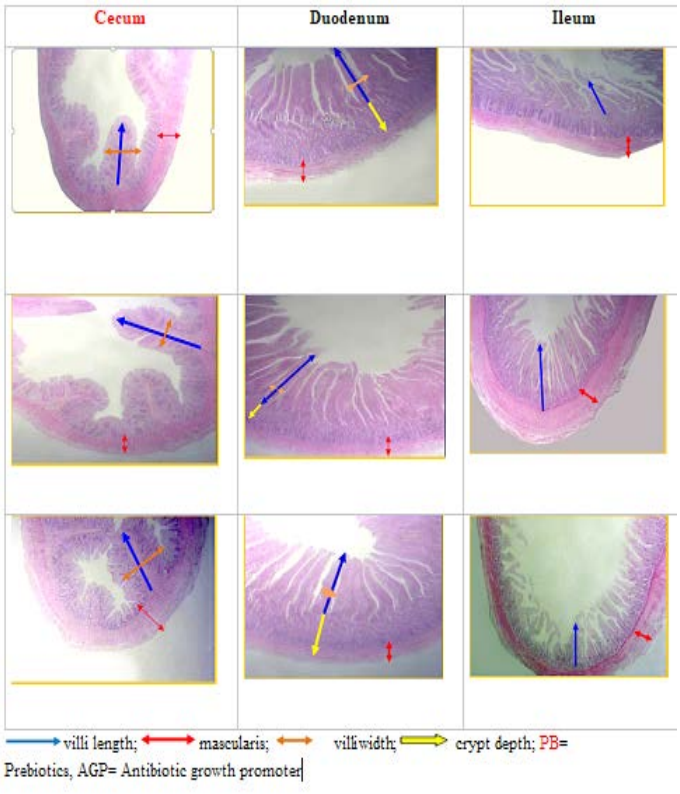

Figure 1: Histological changes (obj 1:100) of villi in different treated groups.

Edible meat yield characteristics

Dietary supplementation of AGP and PB had a significant influence on the edible meat yield characteristics in broilers (Table 3). Both PB and AGP when supplemented independently increased dressing yield (p = 0.01) as compared to the control. The PB-fed group yielded a higher (p = 0.02) amount of thigh meat as compared to the control.

Table 2: Effects of feeding prebiotic (PB) and antibiotic growth promoter (AGP) on the growth performance of broiler chickens (0-35 days) (Mean±SEM).

Variables	Dietary treatments			
	Control	PB	AGP	*p*-value
Initial live weight (g/b)	46.5±0.16	46.6±0.19	46.7±0.10	0.39
Final live weight (g/b)	1831.3^{b}±10.14	1946.9^{a}±11.48	1927.4^{a}±31.33	0.05
Weight gain (g/b)	1784.8^{b}±10.22	1900.3^{a}±11.49	1880.7^{a}±31.34	0.05
Feed intake (g/b)	3127.8^{a}±8.86	3049.3^{b}±25.96	2984.6^{c}±22.06	0.01
Feed conversion ratio	1.75±0.01	1.61±0.01	1.59±0.03	0.06
Uniformity (%)	92.3^{b}±1.21	95^{ab}±2.34	97.5^{a}±1.53	0.05
Survivability (%)	97.5±1.53	100±0.00	100±0.00	0.08

^{abc}Means sharing alphabets not common as superscripts in a row differ significantly at the stated level of probability; PB= Prebiotics, AGP= Antibiotic growth promoter; SEM= Standard error of mean

Table 3: Edible meat yield characteristics of broilers fed on prebiotic (PB) and antibiotic growth promoter (AGP) (Mean±SEM).

Variables	Control	PB	AGP	*p*-value
Live weight (g/b)	1975.0±9.62	1986.0±7.48	1980.0±6.12	0.07
Dressed weight (%)	69.6^{b}±0.16	71.5^{a}±0.17	70.9^{a}±0.33	0. 01
Thigh (g)	134.4^{b}±2.4	144.8^{a}±2.06	139.0^{ab}±2.90	0. 02
Drumstick (g)	95.4±1.72	100±2.98	94.6±3.41	0. 07
Breast meat (g)	404.0^{b}±18.67	523.0^{a}±13.56	421.0^{b}±11.34	0.01
Gizzard (g)	33.2^{b}±1.83	39.8^{a}±1.80	40.6^{a}±1.17	0. 03
Abdominal fat (g)	47.0^{a}±4.06	28.0^{b}±2	40.0^{a}±1.58	0. 01

^{abc}Means sharing not a common alphabet in a row differ significantly at the stated level of probability; PB= Prebiotics, AGP= Antibiotic growth promoter; SEM= Standard error of mean.

Table 4: Effects of feeding prebiotic (PB) and antibiotic growth promoter (AGP) on total viable count (TVC) and total *E. coli* count (TEC) of duodenum, ileum, and caecum (Mean±SEM).

	Dietary treatments			
	Control	PB	AGP	*p*-value
	TVC			
Duodenum	16000^{b}±1523.11	29508^{a}±1422.33	31640^{a}±2233.29	0.05
Ileum	16520^{b}±740.23	12040^{c}±1123.32	23560^{a}±1123.43	0.01
Caecum	44460^{a}±9748.12	27412^{b}±1426.21	44200^{a}±2342.84	0.01
	TEC			
Variables (CFU/g)×10^{5}	Control	PB	AGP	
Duodenum	4000.0^{a} ±1733.20	308.0^{b} ±81.60	384.0^{b} ±133.90	0.01
Ileum	3680.0^{a}±985.10	300.0^{b}±73.80	2280.0^{a}±344.10	0.01
Caecum	44600.0^{a}±8347.50	14252.0^{b}±967.30	31560.0^{a}±4873.40	0.01

^{abc}Means bearing dissimilar superscripts in a row differ significantly at the stated level of probability; PB= Prebiotics, AGP= Antibiotic growth promoter; SEM= Standard error of mean.

The drumstick yield did not differ significantly among the treatments. However, PB-supplemented birds produced higher breast meat (p = 0.01). The gizzard content was significantly higher in the treated groups (p = 0.03) and the abdominal fat was significantly reduced (p = 0.01) upon PB supplementation.

Total viable count (TVC) and Total *E. coli* count (TEC) of duodenum, ileum, and caecum

The feeding effect of PB and AGP on TVC is presented in Table 4. Higher TVC was found in the duodenum of PB and AGP-treated groups as compared to the control (p = 0.05). However, in ileum and caecum, TVC was found to be significantly lower (p = 0.01) in PB-fed birds.

Table 5: Effects of feeding prebiotic (PB) and antibiotic growth promoter (AGP) on antibody titer against ND and IBD (Mean±SEM).

Variables	Dietary treatments			
	Control	PB	AGP	*p*-value
Antibody titer against ND				
First blood collection (Day 22)	40.0^{b}±22.34	179.2^{a}±31.35	102.4^{ab}±38.4	0.01
Second blood collection (Day 34)	59.2^{b}±20.18	236.8^{a}±80.7	128.0^{ab}±35.05	0.05
Antibody titer against IBD				
First collection (Day 22)	5.8^{b}±0.49	7.4^{a}±0.24	6.6^{ab}±0.24	0.05
Second collection (Day 34)	5.4^{b}±1.21	7.8^{a}±0.2	7.4^{a}±0.4	0.05

^{abc}Means bearing not a common superscript in a row differ significantly at the stated level of probability; PB= Prebiotics, AGP= Antibiotic growth promoter; SEM= Standard error of mean

Table 6: Effect of prebiotic (PB) and antibiotic growth promoter (AGP) on broiler gut morphology (µm) on day 35 (Mean±SEM).

Variables	Control	PB	AGP	*p*-value
Duodenum				
Villus height	1547.6±122.45	1785.9±75.56	1642.6±157.85	0.29
Villus depth	101.9±4.79	110.40±6.82	100.7±4.48	0.64
Crypt depth	168.8±13.09	208.6±16.62	194.9±10.27	0.07
Villus height: Crypt depth	9.3±0.53	9.2±1.05	8.9±1.20	0.99
Ileum				
Villus height	536.8^{b}±18.95	652.4^{a}±7.94	597.8^{ab}±33.37	0.05
Villus depth	81.8±4.56	93.4±9.27	86.7±5.19	0.68
Crypt depth	127.4±15.32	121.5±15.34	119.9±18.13	0.99
Villus height: Crypt depth	4.9±0.72	6.3±0.78	6.2±0.95	0.63
Caecum				
Villus height	162.0±19.72	193.1±31.36	176.2±31.16	0.67
Villus depth	63.6±5.08	70.5±2.69	57.9±3.80	0.12
Crypt depth	36.4^{ab}±2.6	32.3^{b}±2.45	40.2^{a}±2.29	0.05
Villus height: Crypt depth	4.6±0.50	5.9±0.71	4.7±1.02	0.07

^{abc}Means bearing not a common superscript in a row differ significantly at the stated level of probability; PB= Prebiotics, AGP= Antibiotic growth promoter; SEM= Standard error of mean

Data in Table 4 reveals that feeding PB reduced TEC count significantly (p = 0.01) in all the segments of the small intestine. The birds fed on the control diets showed the highest TEC in all segments of the intestine.

Immune response against ND and IBD

Table 5 indicated that the PB group had the highest HI antibody titer against ND (p = 0.01) on day 22 (p = 0.01) and day 34 (p = 0.05). The value of PB was statistically similar to that of AGP. In the case of IBD, the values were higher (p = 0.05) for the PB group in both collections on days 22 and 34. The antibody titer in the AGP group did not vary significantly in the first collection but increased significantly in the second collection as compared to the control (p = 0.05).

Gut morphology

Histomorphological studies showed that the duodenal villus height, villus depth, crypt depth, and the ratio between villus height and crypt depth did not show any significant differences (p > 0.05) among treatment groups (Table 6). The ileal villus height showed significant differences (p = 0.05) among treatment groups, where the highest value was obtained for PB. The other illeal parameters were similar. In the caecum, only the crypt depth was reduced (p = 0.05) for the PB group than AGP. The histological changes (obj 1:100) of villi in different sections of the gut are displayed in Fig. 1. It is evident that the PB supplementation influenced villi height positively.

DISCUSSION

Performance characteristics

Despite lower feed consumption, broilers that received PB exerted significantly better body weight gain than those of the control. The results suggest that birds receiving prebiotics utilize nutrients more efficiently than their control counterpart. Sarangi et al. (2016) reported reduced feed intake upon prebiotic feeding. The reduced feed intake is also in agreement with the previous findings of Sohail et al. (2012) and Midilli et al. (2008). The findings from the experiment of Kairalla, (2022) revealed that broiler chicken fed with 0.4 and 0.6% Agrimos® (Mannan Oligosaccharide and β-glucans) feed powder significantly outperformed those fed with 0% and 0.2% Agrimos® in terms of ultimate body weight, body weight gain, and also feed conversion ratio.

Several explanations regarding the positive influence of dietary prebiotics on broiler chickens are available in the literature. Prebiotics are normally useful in increasing the beneficial microorganisms in the intestine (Khalesi et al., 2021; Spring et al., 2000) and in improving bird's immunity (Shashidhara and Devegowda 2003), with consequent influence on body weight gain (Parks 2001). Further, these are potential alimentary supplements that reduce the harmful effects of putrefactive factors and increase nutrition output (Fooks and Gibson 2002; Gunal et al., 2006). Moreover, when the bird's gut is infected by pathogenic bacteria, lymphocytes aggregate and the thickness of mucosa layer increases, thus reducing the nutrient absorbance (Gunal et al., 2006). In such a condition, prebiotics is useful in improving the situation. Prebiotic consumption has been reported to be effective in improving feed intake and production by reducing the pathogen population (Walker et al., 2018). Additionally, it has been suggested that using prebiotics lengthens the gut, which increases nutrient absorbance area and enhances bird efficiency (Santin et al., 2001). The increase in live weight gain in broilers following the inclusion of prebiotics in the diet as obtained in this study is also in agreement with the results of several authors (Kim et al., 2011; Toghyani et al., 2011; Sohail et al., 2012; Hosein et al., 2013). Changes in gut microbiota caused by prebiotic administration in broiler diet are predominantly responsible for improving the performance of broiler chickens (Ricke et al., 2020; Reuben et al., 2021).

The tendency of improved FCR is an indication of better nutrient utilization and such results of better feed efficiency in the prebiotic-fed group have been reported previously by several researchers (Midilli et al., 2008; Sohail et al., 2012; Li et al., 2014; Alizadeh et al., 2010). Evidence suggests that supplementing prebiotics in poultry feed at a rate of 200 g/100 kg improves the feed conversion ratio (Falaki et al., 2011).

Meat yield characteristics

Sojoudi et al. (2012) reported that the prebiotic-containing diet showed a higher ($p = 0.05$) carcass yield compared to that of the control group which supports our study. As the harmful bacteria are inhibited and nutrients are accumulated in greater amounts due to the addition of prebiotics in the diet, it results in a higher yield of the carcass (Toghyani et al., 2011). Greater breast meat, thigh, and gizzard yields were noticed in our experiment. Likewise, Santin et al. (2001) showed greater yields in the breast, gizzard, and thigh in the MOS-fed birds. Taking abdominal fat into consideration, Mahmud et al. (2008) found in synchronization with our results that birds fed the control diet had the greatest abdominal fat content, whereas birds fed the MOS-supplemented diet had the lowest value. This coincides with the results of our present study. One reason could be that probiotics increased the growth of helpful bacteria like *Lactobacillus*, which in turn decreased the activity of Acetyl-CoA carboxylase, the rate-limiting enzyme in the production of fatty acids (Abdel-Hafeez et al., 2017), thus causing lower fat deposition. On the contrary, MOS and inulin-supplemented diets when fed to broilers did not lower fat deposition in the abdomen (Samarasinghe et al., 2003).

Microbial count

The duodenum of the AGP group had the highest TVC. Moreover, a higher level of TVC was found in the prebiotic receiving group. This phenomenon might be an indication of increased health-promoting bacteria in the prebiotic-treated group. Such findings are in agreement with Abdel-Raheem et al. (2012), who studied the concentrations of bacteria belonging to *Lactobacillus spp.* in the duodenum and jejunum digesta at day 42 and found that they were significantly higher in prebiotic-supplemented broilers compared with the control. Concerning caecum, the results differed ($p = 0.01$) among the dietary treatments, where the prebiotic group comprised the lowest TVC over the other groups. The results from the duodenum, ileum, and caecum indicate that the use of AGP may increase the number of bacteria, which may be due to the emergence of antibiotic-resistant bacteria. This fact was supported by the findings of Ribeiro et al. (2007), who reported that the use of an antimicrobial agent produced higher colony counts in the caecum, while prebiotics yielded lower counts. The prebiotic-fed birds increased bacterial count over control in the duodenal region, which may be due to the higher growth of beneficial bacteria, and the results following those of Kim et al. (2011). They reported that the total bacteria and *lactobacilli* increased by 0.25% in MOS-treated groups and decreased in avilamycin treated groups. Similarly, a 25 folds' reduction of *Salmonella spp.* population in

chickens' intestine that was fed prebiotic (MOS) compared to control (Spring et al., 2000; Yaqoob et al., 2021). The improved beneficial bacterial count with the addition of prebiotics is also supported by Falaki et al. (2011), who observed that different levels of prebiotics relatively increased lactic acid bacteria in the intestines of broilers.

The principal mechanism of prebiotics in lowering harmful bacteria is immunomodulation, which means selective stimulation of lactic acid-producing bacteria, giving rise to the concentration of short-chain-fatty-acids (SCFA)s for example, acetate, propionate, and butyrate, in particular, are the chosen energy foundation of colonocytes and favor gut integrity. Lower pH is linked to higher SCFA content and fermentation activity, which is correlated with pathogen inhibition and increased nutrient bioavailability (Józefiak, 2004). Bacteria are less likely to penetrate the gut barrier as a result of the increased interaction of SCFA with immune cells, direct communication in the digestive tract, and altered mucin content (Lee and Salminen, 2009). Thus, it inhibits some harmful bacteria and lowers their colonization, like *Salmonella* and *Campylobacter* (Charalampopolus and Rastall, 2009; Reuben et al., 2021). The *E. coli* count in the duodenum was significantly lower ($p = 0.01$) in the treatment groups. Our findings of lower *E. coli* count in PB-fed birds were supported by Falaki et al. (2011). The lower TEC in the caecum of PB-fed groups is consistent with the results of some previous reports (Koc et al., 2010; Kim et al., 2011; Falaki et al., 2011; Gajewska et al., 2012). The results of TEC in the caecum and ileum showed that the PB group had lower *E. Coli* than AGP and control which is reinforced by Koc et al. (2010) and Kim et al. (2011) upon *S. cerevisiae* supplementation.

Immunity

Prebiotics boost immunity against infectious diseases (Al-Khalaifah, 2018). The results of increased primary HI antibody titer against Newcastle disease (ND) on day 22 are in agreement with Houshmand et al. (2012), who reported an increase in antibody tires against ND at 21 days of prebiotic supplementation. The secondary HI antibody titer was also increased in PB-fed birds. Such improvement in the serum antibody titer of broilers, fed prebiotics assists in the production of the immune response. Some earlier investigations (Alizadeh et al., 2010; Awaad et al., 2011; Hosein et al., 2013) yielded results similar to the current findings. A study by Awaad et al. (2011) showed that a specific combination of MOS and β-glucans extracted from yeast cell walls administered to chickens had a powerful immunomodulatory impact, elicited an immune reaction, and improved the efficacy of vaccination. Zakeri and Kashefi (2011) showed that birds fed MOS had superior serum antibody titers at the age of 25 days old. This finding is in close agreement with the findings of the present study. On the other hand, Sadeghi et al. (2013) showed that dietary MOS and β -glucan supplementation was successful in improving the immune responses and overall health of pathogen-infected chicks but had no discernible impact on immune parameters in the non-infected group of chicks. Kim et al. (2011) showed that plasma immunoglobulins were not affected by feeding prebiotics.

MOS is unique in immune regulation in that it boosts the defensive antibody response to increase disease tolerance while suppressing the acute phase (fever) response (Ferket et al., 2002). Results of the primary ELISA antibody titer on day 22 showed that the PB group comprised significantly higher ($P = 0.05$) antibody titre against IBD. This finding was strongly aligned with Houshmand et al. (2012), who expressed that 21 d old birds showed significantly increased antibody titer against IBD compared to the control group with the dietary addition of the prebiotics. The results of the secondary ELISA antibody titer on day 34 also revealed greater antibody titers. In agreement with the result, a recent study by Rehman et al. (2020) also showed that MOS helps in acquiring greater antibody titer for IBD. The higher antibody titers observed in birds treated with prebiotics might be attributed to the beneficial effect of microflora on the gut to maintain a sound balance of immunopotent cells.

Gut morphology

Dietary inclusion of MOS as a prebiotic influenced positively villus height and crypt depth. Longer villi suggest more mature epithelia and improved absorptive function due to higher villus absorptive area. Moreover, villus height and crypt depth are direct representations of gut function and health. In the intestinal mucosal crypts, new epithelial cells are generated and move upward with the villi (Schat et al., 1991) and the crypt may be thought of as a villus factory. MOS as a prebiotic has been shown to change the mucosal architecture in birds, resulting in longer villi and improved performance (Yang et al., 2007). Following our present findings, Salavati et al. (2020) stated that the villus length was increased in MOS-fed birds compared to the control diet as an intestinal morphometric parameter and the crypt depth. Pelicano et al. (2005) demonstrated that when prebiotics was added to the diet of broilers, villus length and breadth were increased. It is believed that the increase in the beneficial microbial population caused by dietary MOS supplementation may have an effect on intestinal morphology in broiler chickens (Salavati et al. 2020).

CONCLUSION

The use of prebiotic MOS enriched with β-glucans is

effective for enhancing growth performance, lowering the pathogenic bacterial load, improving gut health, and boosting the immunity of commercial broilers. The results also suggest that the prebiotic (MOS enriched with β-glucans) used in this study may be considered a suitable alternative to the antibiotic.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

STATEMENT OF NOVELTY

In order to reduce any long-term effects, combat microbial resistance, and maintain productivity in broiler chickens, this research demonstrated the effectiveness of prebiotic as an antibiotic substitute. As a consequence of this research findings, sustainable broiler farming can be achieved.

AUTHORS' CONTRIBUTIONS

The trial was conducted by Bibek Chandra Roy, the first author of this manuscript. Shubash Chandra Das, Bipul Chandra Ray and Sachchidananda Das Chowdhury all contributed to the planning, processing, and interpretation of the data. Sukumar Saha, Khan Md. Shaiful Islam and everyone else contributed to the review of the manuscript and provided required suggestions. The original draft was prepared by Bibek Chandra Roy and revision of the manuscript were carried by Tanvir Ahmed in collaboration with Shubash Chandra Das.

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