Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.233

RESEARCH ARTICLE

β-1,3-Glucan, Precision Biotic, and *Bacillus subtilis* as Antibiotic Growth Promoter Alternatives: Effects on Broiler Performance, Gut Morphology, and Serum Biochemistry

Hassaan Munawar[1†], Asfa Fatima[1†], Muhammad Saif ur Rehman[1], Abdul Basit[2], Muhammad Aziz ur Rahman[1]* and Muhammad Qamar Bilal[1]

[1]Institute of Animal and Dairy Sciences, University of Agriculture Faisalabad, Faisalabad 38000, Pakistan; [2]Remount Veterinary and Farms Corps, Rawalpindi 46000, Pakistan. [†]Contributed equally
*Corresponding author: drazizurrahman@uaf.edu.pk

ARTICLE HISTORY (26-729)

Received: June 13, 2026
Revised: August 21, 2026
Accepted: August 24, 2026
Published online: October 12, 2026

Key words:
Blood metabolites
Gut health
Ileal digestibility
Immunomodulator
Precision biotic

ABSTRACT

The objective of the current study was to compare β-1,3-glucan, glycan-based precision biotic (PB), and *Bacillus subtilis* as alternatives to antibiotic growth promoters (AGPs) on gut morphology, nutrient utilization, blood biochemical parameters, and performance of broilers. For this purpose, a total of 500 one-day-old broiler chicks were randomly assigned to five experimental treatments, with 10 replicates of 10 birds each. Experimental birds were reared on a basal diet without AGP (CON) or the basal diet supplemented with AGP (100 g/ton), β-1,3-glucan (300 g/ton), a glycan-based PB (250 g/ton), and *Bacillus subtilis* (500 g/ton) in the diet. In the grower, finisher and overall phases, higher body weight, body weight gain and an improved (lower) FCR were observed in birds fed diet supplemented with PB ($P<0.05$). Similarly, the nutrient digestibility results showed that the highest digestibility was observed in birds fed a diet supplemented with PB ($P<0.05$). Moreover, the intestinal histology results showed that PB supplementation in diet increased villus height and goblet cell count in the jejunum and ileum ($P<0.05$). Similarly, PB dietary supplementation increased villus height, villus width, and crypt depth in duodenum ($P<0.05$). The results showed that supplementation with AGPs, and AGP alternatives positively influenced blood metabolites; total protein and albumin were highest in the PB group, while AST was lowest in the PB and β-1,3-glucan groups ($P<0.05$). Based on the results, it is concluded that AGP generally, while PB specifically, exhibited consistent positive responses on performance, nutrient digestibility, intestinal histology and blood biochemical indices in broilers.

To Cite This Article: Munawar H, Fatima A, Rehman MS, Basit A, Rahman MA and Bilal MQ, 2026. β-1,3-Glucan, precision biotic, and *Bacillus subtilis* as antibiotic growth promoter alternatives: effects on broiler performance, gut morphology, and serum biochemistry. Pak Vet J, 46(10): 2456-2465. http://dx.doi.org/10.29261/pakvetj/2026.233

INTRODUCTION

In developing countries, antibiotics are being regularly added to animal feed to reduce the occurrence of certain diseases and to enhance growth rate in poultry. However, growing evidence suggests that nonspecific and prolonged use of antibiotics as growth promoters contributes to bacterial resistance, which has considerable consequences on the future effectiveness of antibiotics (Mahmood *et al.*, 2021; Abdullah *et al.*, 2024). Therefore, there is an immediate need to explore alternatives to antibiotic growth promoters (AGPs) for the poultry industry to maintain broiler performance and mitigate the risks of disease and antibiotic resistance, especially in developing countries (Clement *et al.*, 2019).

Various kinds of alternatives to AGPs are being used in the poultry industry (Khallaf *et al.*, 2025), and one viable approach is the application of immunomodulatory substances, particularly β-1,3-glucan, to enhance the innate immune response and performance of animals (Chan *et al.*, 2009; Barsanti *et al.*, 2011). It has been reported that β-1,3-glucan is a glucose polymer that serves as a structural component of fungal and yeast cell walls and is abundant in yeast probiotics such as *Saccharomyces cerevisiae*. It has further been reported that β-1,3-glucan is being widely used in the medical field due to its properties that enhance immunity and exhibit anti-infection effects. Previous studies have reported that inclusion of β-1,3-glucan in the feed of poultry has been shown to improve resistance against bacterial infections (; Lowry *et al.*, 2005; Huff *et al.*, 2006;

Chen *et al.*, 2008), enhancing gut health (Cox *et al.*, 2010) and improving overall growth rate and feed conversion ratio (FCR) (Cox *et al.*, 2010; Moon *et al.*, 2016).

Another strategy to improve immunity and growth performance of broilers is the use of precision biotic (PB) in the diet. It has been reported that PBs are novel nutritional supplements that are also known as synthesized glycan feed additives (Yan *et al.*, 2023) and these PBs can selectively modulate the gut microbiome, improve growth performance, FCR, and litter quality in broilers (Jacquier *et al.*, 2022; Bortoluzzi *et al.*, 2025). A recent study demonstrated that PB, acting as a metabolic modulator, influences specific pathways that result in improved productivity, sustainability and welfare of broiler birds (Walsh *et al.*, 2021). Similarly, another study reported that feeding broilers with glycan-based PBs not only optimizes broiler productivity but also reduces footpad lesions due to decreased litter ammonia and pH and enhanced litter hygiene (Jacquier *et al.*, 2022). Recent research reported that the growth rate of birds and intestinal health were improved by the influence of precision glycan supplements in the diet (Blokker *et al.*, 2022).

In the last decade, multiple investigations were carried out on broilers to check the impact of probiotic as an alternative to AGPs (Raza *et al.*, 2024; Berberoğlu *et al.*, 2025). Probiotics are considered living microbial dietary supplements and impose a supportive influence on the host by promoting a balanced gut microbiome (Awad *et al.*, 2009) and recent study reported that *Bacillus subtilis* as a probiotic can enhance and sustain the beneficial bacterial population in the intestine (Cai *et al.*, 2024) and improve growth performance in broilers (Chen *et al.*, 2025; Jeong *et al.*, 2026). Although *Bacillus subtilis* is known to be used in poultry diets as an alternative to AGPs, studies on the use of β-1,3-glucan and PBs as alternatives to AGPs are limited. Previous studies used β-1,3-glucan and PBs as novel supplements to enhance broiler performance and health (Amer *et al.*, 2023; Leone and Ferrante, 2023), however, to the best of our knowledge, no study has explored the comparative influence of β-1,3-glucan, PBs and probiotics as alternatives to AGPs in broiler diets. Hence, this study aimed to evaluate the effect of replacing AGPs with β-1,3-glucan, PBs and *Bacillus subtilis* on growth parameters, nutrient digestibility, intestinal histology, blood biochemical parameters, and carcass characteristics of broilers.

MATERIALS AND METHODS

Ethical considerations: The experimental trial was performed at IFFCO Pakistan Feed Mills R&D Farms, FIEDMC Area, Tehsil Chak Jhumra, District Faisalabad. The current study plan, design, and procedures were approved by the Director, Graduate Studies, University of Agriculture Faisalabad, under approval code DGS-02-01-2024-23950.

Birds, management and experimental design: In the current study, a total of 500 one-day-old Ross 308 male broiler chicks were purchased from a local hatchery. Chicks were randomly assigned to five experimental treatments, each comprising 10 replicates of 10 birds. The duration of the experiment was 35 days. Experimental birds were reared on a basal diet without AGP (CON) or the basal diet supplemented with AGP (100 g/ton), β-1,3-glucan (300 g/ton), a glycan-based PB (250 g/ton), and *Bacillus subtilis* (500 g/ton) in the diet. The CON diets for the starter, grower and finisher phases were prepared according to the respective formulations. Each phase-specific basal diet was subsequently split into five sub-batches, one of which was retained as the CON diet, while AGP or AGP alternatives were added to the remaining batches according to the treatment plans. The final diets were mixed thoroughly to ensure a homogeneous distribution of ingredients and AGP or AGP alternatives.

The broiler birds were reared in separate pens measuring 1.5 m × 0.7 m × 1.21 m (L × W × H) per replicate. Rice hulls were used in a 2-inch layer as a bedding material. On arrival of the chicks, a 5% glucose solution was provided through the drinking water, and the weight of the chicks was recorded as an average of 39 ± 2 g. Birds had *ad libitum* access to water and mash feed. At chick placement, the initial brooding temperature was set at approximately 35°C and then gradually reduced according to the bird age and comfort, with a target temperature of 24°C during the final days of the experiment. Continuous lighting (24 h/day) was maintained throughout the trial following Pervaiz *et al.* (2025). Thermometers and hygrometers were positioned appropriately for regular monitoring of temperature and humidity.

The tested feed additives included β-1,3-glucan, a purified immunomodulatory compound derived from algae, included at 300 g/ton of feed as per Amer *et al.* (2023) and based on manufacturer recommendations (Aleta™, KEMIN Industries, Des Moines, IA, USA). Precision biotic, derived from functional glycans, was included at 250 g per ton of feed according to Jacquier *et al.* (2022) (Symphiome™, dsm-firmenich, Kaiseraugst, Switzerland) and *Bacillus subtilis* (probiotic strain) was included at 500 g per ton of feed to attain a desired concentration of 8×10^8 CFU/kg as supported by Opalinski *et al.* (2007) and according to the commercial product (GalliPro®, DSM). The additives were obtained from commercial sources and thoroughly mixed into the basal diet for uniform distribution. The AGP used in this study was a commercially available broad-spectrum antibiotic, Enramycin 8%, included at the manufacturer's recommended dose of 100 g/ton, as reported by Wang *et al.* (2016) in broiler studies. The diet formulations were prepared to fulfill the nutritional standards for broilers. The dietary nutrient specifications for the birds throughout the starter (1–7 days), grower (8–21 days), and finisher (22–35 days) phases of the diet were formulated to meet NRC (1994) recommendations, as shown in Table 1.

Data collection

Growth performance: The FCR is a key indicator of feed utilization efficiency and is among the primary metrics for evaluating growth performance. In the current study, FCR was calculated using body weight gain (BWG) and FI of the birds. Feed intake of birds was calculated by using the formula demonstrated by Rahman and Arifuzzaman (2021) given below:

$$\text{Feed intake (g)} = \text{Feed offered (g)} - \text{orts (g)}$$

To estimate BWG, BW was calculated once at the arrival of the chick on day 1 and at weekly intervals to get the average weight gain.

Using BWG and FI, FCR was also calculated every week following Rahman and Arifuzzaman (2021) using the formula:

FCR = FI (g) / BWG (g)

Table 1: Feed formulation for starter, grower and finisher stages of broiler birds

Ingredient (%)	Starter Diet	Grower Diet	Finisher Diet
Corn	58	60	61
Soybean Meal	23	21	17
Sunflower Meal	6.2	6.3	7.0
Canola Meal	5.2	5.2	7.0
Vegetable Oil	2.5	2.3	2.8
Limestone	1.6	1.7	1.7
[1]MCP	1.3	1.3	1.3
Common Salt	0.2	0.2	0.2
[2]Premix	0.1	0.1	0.1
Cellulose	0.2	0.2	0.2
Celite	1	1	1
Lysine Sulphate	0.4	0.4	0.4
L-Threonine	0.09	0.09	0.09
DL-Methionine	0.21	0.21	0.21
Total	100	100	100
Calculated Nutritional Content			
Crude Protein (%)	19.98	18.94	18.02
Metabolizable Energy (kcal/kg)	3050	3150	3230
Dry Matter (%)	89.31	89.29	89.36
Ether Extract (%)	5.29	5.63	5.41
Crude Fiber (%)	4.10	4.17	4.33
Calcium (%)	0.96	0.96	0.90
Available Phosphorus (%)	0.42	0.45	0.45
Ash (%)	5.02	4.91	4.98
Lysine (%)	1.27	1.30	1.28
Methionine (%)	0.50	0.45	0.49
Methionine + Cysteine (%)	0.85	0.81	0.88
Threonine (%)	0.76	0.70	0.81
Tryptophan (%)	0.23	0.22	0.22

[1]MCP: monocalcium phosphate, [2]Each kilogram of premix supplied per kilogram of diet contains the following: Vitamin A (15,000 IU), Vitamin D_3 (30,000 IU), Vitamin E (80 IU), Vitamin K_3 (3 mg), Vitamin B_1 (3 mg), Vitamin B_2 (8 mg), Vitamin B_3 (60 mg), Vitamin B_5 (15 mg), Vitamin B_6 (4 mg), Vitamin B_9 (2 mg), Vitamin B_{12} (0.02 mg), Choline chloride (60%) (700 mg), Zinc ($ZnSO_4.H_2O$) (80 mg), Copper ($CuSO_4.H_2O$) (10 mg), Iron ($FeSO_4.H_2O$) (60 mg), Manganese ($MnSO_4.H_2O$) (80 mg), Iodine (KI) (1 mg), and Selenium (0.2 mg).

Furthermore, FCR was corrected for mortality: Corrected FCR = [Total FI for a period]/{[Total days of birds alive at the end of the week] + [Total days of birds culled/dead]}/{[Total pen weight gained in the given week] + [Total weight of culled/dead birds in the given week]}/{[Total days of birds alive at the end of the week] + [Total days of birds culled/dead]}.

Ileal nutrient digestibility: To determine ileal nutrient digestibility, the ileal nutrient digestibility method was used. For this purpose, Celite® (1%), an acid-insoluble ash marker, was included in the starter, grower and finisher diets during batching and mixing.

On day 35 of the experiment, ten birds per treatment group (one bird per replicate) were randomly selected and slaughtered. The ileum was defined as the section extending from Meckel's diverticulum to approximately 2 cm anterior to the ileo-cecal junction. Ileal digesta were collected separately from each selected bird and stored at −20°C until analysis. Feed and ileal digesta samples were analyzed for DM, CP and ether extract (EE) using standard proximate analysis methods. Ileal nutrient digestibility was calculated as described by Thacker and Widyaratne (2012):

Digestibility Coefficient (%) = 100 - [100 × (% Marker in feed / % Marker in ileal digest) × (% Nutrient in ileal digest / % Nutrient in feed)]

Intestinal histology: At the end of the trial, ten birds/treatment (one bird per replicate) were randomly chosen on day 35 and slaughtered according to halal procedure, and distal segments (~2 cm) of the duodenum, jejunum and ileum were collected from each bird. Tissue segments were stored in 10% neutral buffered formaldehyde for 72 h, dehydrated in a graded series of ethanol, and cleared in xylene. Tissues were then infiltrated with paraffin in two stages (2 h in paraffin I, followed by overnight infiltration in paraffin II, both at 60°C) and embedded in wax to prepare tissue blocks. The blocks were sectioned using a microtome into 4 µm transverse and longitudinal sections, floated onto a water bath, mounted on albumin-coated glass slides, and stained with hematoxylin and eosin, following standard histological procedures (Bancroft and Gamble, 2008). For each bird, four sections were examined per intestinal segment, and ten well-oriented, intact villi and their corresponding crypts were measured in each section. Villus height was measured from the tip of the villus to the villus-crypt junction, villus width at the midpoint of the villus, and crypt depth from the base of the crypt to the villus-crypt junction. Slides were examined under a light microscope at 40× objective magnification, and measurements were performed using ToupView 3.7 image analysis software. Goblet cells were counted on the same hematoxylin and eosin-stained sections along the full length of each measured villus and expressed as the number of goblet cells per villus. The morphometric measures were determined as mentioned in an earlier study (Amer *et al.,* 2022).

Blood biochemical parameters: Ten birds per treatment were chosen randomly and slaughtered according to halal procedure. Clotted blood samples were centrifuged at 3000 × g for 15 minutes to isolate the serum, which was stored at −80°C until subsequent analysis. Serum levels of total protein (TP), albumin, glucose and globulin were analyzed using commercially available reagent kits (QCA Diagnostics, USA). Globulin concentration was calculated by subtracting albumin concentration from total protein concentration following Adegoke *et al.* (2018).

Kidney function markers, including creatinine and uric acid, as well as liver enzymes (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), were measured by ELISA kits specific to poultry following Sugiharto *et al.* (2024). All kit procedures were adhered to as per the manufacturer's guidelines.

Carcass characteristics: On the 35th day of the trial, the selection of ten birds per treatment was done on a random basis. The birds were weighed individually and slaughtered for the analysis of carcass characteristics. Birds were then eviscerated to obtain dressed carcass weight excluding the giblets, while the individual weights of breast, thigh, and giblet organs (heart, liver, gizzard) were recorded separately. For calculating percentages of dressing, breast and thigh meat, the weight of carcass, breast, and thigh meat was divided by the live weight of the birds and then multiplied by the gained value with 100. The weight of the

different internal organs was also divided by the live weight of the birds and then multiplied by 100 to get the relative weight percentages of these organs (Grigore *et al.*, 2023).

Dressing percentage: To determine the dressing percentage, the dressed carcass weight excluding the giblets was divided by the live weight of the birds in grams. The percentage for dressing was calculated by the formula below:

$$\text{Dressing (\%)} = \frac{\text{Dressed carcass weight excluding giblets (g)}}{\text{Live weight (g)}} \times 100$$

To calculate breast meat percentage on day 35, ten birds per treatment were weighed separately and then slaughtered. Breast meat % was calculated by using the following formula:

$$\text{Breast meat yield (\%)} = \frac{\text{Breast meat yield in grams}}{\text{Dressed carcass weight excluding giblets (g)}} \times 100$$

Similarly, thigh meat yield percentage was calculated by using the formula as given below:

$$\text{Thigh meat yield (\%)} = \frac{\text{Thigh meat yield in grams}}{\text{Dressed carcass weight excluding giblets (g)}} \times 100$$

Chemical analysis: Samples of experimental diets and ileal digesta were analyzed following Mutayoba *et al.* (2011). In brief, DM, CP, EE and moisture contents were measured using the methods used by Ghosh *et al.* (2019) and Ofori *et al.* (2019).

Statistical analysis: Data were analyzed using a one-way ANOVA to determine the effects of treatments. Duncan's Multiple Range Test was applied for mean comparisons at a significance level of $P<0.05$. The statistical model considered sources of variation, and the experimental unit was defined appropriately. Data were checked for normality and homogeneity of variances before analysis. Statistical analysis was conducted using STATGRAPHICS PLUS 5.0 (Statpoint Technologies, Inc., USA) according to Montgomery and Runger (2010).

RESULTS

Growth performance: Effects of dietary treatments on broiler growth performance are presented in Table 2. The results indicated that FI was not significantly affected ($P>0.05$) in the starter and grower phases but differed in the finisher and overall phases ($P<0.05$). The highest FI was observed in birds fed a PB-supplemented diet ($P<0.05$). During the grower phase, BWG was highest in the PB-supplemented group, while the BWG in *Bacillus subtilis* group did not differ significantly from that of the CON group. During the finisher and overall phases, supplementation resulted in higher BWG compared to the CON group. Birds fed β-1,3-glucan and PB also exhibited significantly greater BW during the grower, finisher and overall phases, with PB showing the highest values ($P<0.05$). The FCR varied significantly in all phases, with PB showing the best FCR in the grower phase ($P<0.05$), whereas in the finisher and overall phases, FCR was improved in supplemented groups compared to the control group ($P<0.05$). Overall, growth performance was significantly influenced by dietary treatments ($P<0.05$).

Nutrient digestibility: The impact of feeding diets supplemented with AGP, β-1,3-glucan and PB on nutrient digestibility is presented in Table 3. Results of nutrient digestibility showed that supplementation of AGP, β-1,3-glucan and PB in the diet influenced DM, EE and CP digestibility ($P<0.05$). Dry matter digestibility was higher in the PB and *Bacillus subtilis* groups as compared to other groups ($P<0.05$). Results of CP digestibility showed that CP digestibility was significantly higher in the PB group and was statistically comparable with *Bacillus subtilis* ($P<0.05$). Ether extract digestibility varied among treatments, with the highest values observed in PB and *Bacillus subtilis* groups ($P<0.05$).

Table 2: Impact of β-1,3-glucan, precision biotic and *Bacillus subtilis* on growth performance of broilers

	Control	AGP	β-1,3- glucan	Precision biotic	*Bacillus subtilis*	SEM	P-value
Starter phase							
Feed Intake (g)	106.73	107.35	111.21	109.25	112.34	0.297	0.059
Body weight (g)	138.83	139.32	130.92	132.1	135.98	0.226	0.084
[1]BWG (g)	98.83	99.32	90.92	92.10	95.98	0.183	0.083
[2]ADFI (g/d)	15.25	15.33	15.89	15.61	16.05	0.038	0.072
[3]ADG (g/d)	14.12	14.19	12.99	13.16	13.71	0.035	0.084
[4]FCR	1.08^{c}	1.08^{c}	1.22^{a}	1.19^{ab}	1.17^{b}	0.011	0.001
Grower phase							
Feed Intake (g)	1155.52	1144.60	1186.91	1183.07	1170.68	5.317	0.213
Body weight (g)	717.62^{c}	697.37^{bc}	720.34^{b}	726.88^{a}	699.84^{bc}	1.484	0.008
BWG (g)	578.79^{b}	558.05^{c}	589.42^{ab}	594.78^{a}	563.86^{bc}	2.376	0.008
ADFI (g/d)	82.54	81.76	84.78	84.50	83.62	0.134	0.213
ADG (g/d)	41.34^{ab}	39.86^{b}	42.10^{a}	42.48^{a}	40.28^{ab}	0.189	0.007
FCR	2.00^{b}	2.05^{ab}	2.01^{ab}	1.99^{c}	2.08^{a}	0.004	0.001
Finisher phase							
Feed Intake (g)	3322.34^{bc}	3181.04^{bc}	3332.18^{bc}	3365.63^{a}	3313.85^{b}	9.982	0.009
Body weight (g)	2620.82^{c}	2629.30^{bc}	2712.62^{b}	2725.88^{a}	2689.34^{bc}	4.150	0.008
BWG (g)	1903.20^{c}	1931.93^{b}	1992.28^{ab}	1999.00^{a}	1989.50^{ab}	3.338	0.008
ADFI (g/d)	237.31^{bc}	227.22^{c}	238.01^{b}	240.40^{a}	236.70^{ab}	0.322	0.009
ADG (g/d)	135.94^{ab}	137.99^{b}	142.31^{a}	142.79^{a}	142.11^{a}	0.099	0.006
FCR	1.75^{a}	1.65^{ab}	1.67^{ab}	1.68^{b}	1.67^{ab}	0.004	0.004
Overall							
Feed Intake (g)	4584.59^{bc}	4432.99^{c}	4630.30^{b}	4657.95^{a}	4596.87^{bc}	8.952	0.021
Body weight (g)	2620.82^{c}	2629.30^{bc}	2712.62^{b}	2725.88^{a}	2689.34^{bc}	4.236	0.036
BWG (g)	2580.82^{c}	2589.30^{b}	2672.62^{a}	2685.88^{a}	2649.34^{ab}	4.797	0.036
ADFI (g/d)	130.99^{ab}	126.66^{b}	132.29^{a}	133.08^{a}	131.34^{ab}	0.285	0.020
ADG (g/d)	73.74^{b}	73.98^{b}	76.36^{a}	76.74^{a}	75.69^{a}	0.108	0.033
FCR	1.78^{a}	1.71^{b}	1.73^{b}	1.73^{b}	1.74^{b}	0.003	0.028

[1]BWG: body weight gain, [2]ADFI: average daily feed intake, [3]ADG: average daily gain, [4]FCR: feed conversion ratio, SEM: Standard error of the mean; [a,b] values within the same row bearing different superscripts ([a, ab, b, bc]) indicate significant differences ($P<0.05$).

Intestinal histology: Results of intestinal histology are presented in Table 4. Results of intestinal histology of broilers showed that dietary treatments had an impact on selected intestinal histological parameters in broilers compared to the control (P<0.05). In the duodenum, villus height, villus width, crypt depth and villus height-to-crypt depth ratio (VH:CD) were significantly affected by experimental treatments (P<0.05). Villus height, villus width and crypt depth were significantly greater in the PB group, while the lowest villus height and villus width were observed in the control group and lowest crypt depth in *Bacillus subtilis* group (P<0.05). The VH:CD ratio was highest in the *Bacillus subtilis* group as compared to other treatments (P<0.05). Moreover, the goblet cell count was numerically highest in the PB group, but no statistically significant difference was observed (P>0.05). In the jejunum section, villus height, VH:CD ratio and goblet cell count were significantly changed due to experimental treatments (P<0.05), while villus width and crypt depth were not affected by supplementation of AGPs, PB, β-1,3-glucan and *Bacillus subtilis* (P>0.05). The VH:CD ratio was highest in the PB groups, while goblet cell count was highest in the group supplemented with β-1,3-glucan (P<0.05). In the ileum, villus height and goblet cell count were highest in the PB group, followed by β-1,3-glucan groups (P<0.05), while villus width, crypt depth and VH:CD remained unaffected statistically due to supplementation of AGPs, PB, β-1,3-glucan and *Bacillus subtilis* in the diet (P>0.05).

Blood biochemical parameters: Table 5 represents the results of blood biochemical parameters. The results regarding blood biochemical parameters revealed that experimental treatments had a significant effect on TP, albumin, globulin, AST, and ALT levels in broilers (P<0.05). Results of blood biochemical parameters explored that supplementation of AGP alternatives in the diet significantly elevated TP levels, while the highest blood TP level was observed in PB-supplemented groups followed by *Bacillus subtilis* and β-1,3-glucan (P<0.05). Similarly, albumin concentration was highest in the PB group as compared to other experimental treatments (P<0.05), while the level of globulin was significantly higher in AGP, PB and *Bacillus subtilis* groups (P<0.05).

Liver function markers results showed that AST and ALT concentrations were significantly altered with supplementation of AGP or AGP alternatives in the diet (P<0.05). Results revealed that AST levels were lowest in the PB group, followed by the β-1,3-glucan group, while the AGP group had higher AST levels (P<0.05). Furthermore, ALT levels were significantly higher in AGP, whereas PB, *Bacillus subtilis*, and control showed lower values (P<0.05). Moreover, glucose levels, uric acid and creatinine levels were not significantly affected by dietary treatments (P>0.05).

Table 3: Impact of β-1,3-glucan, precision biotic and *Bacillus subtilis* on nutrient digestibility of broilers

Digestibility (%)	Control	AGP	β-1,3- glucan	Precision biotic	*Bacillus subtilis*	SEM	P-value
Dry matter	80.44^{b}	86.12^{ab}	88.21^{ab}	91.51^{a}	89.56^{a}	2.406	0.008
Crude protein	42.78^{d}	49.79^{c}	52.23^{b}	59.81^{a}	56.75^{ab}	1.793	0.001
Ether extract	74.21^{b}	81.42^{ab}	83.8^{ab}	85.1^{a}	86.8^{a}	2.272	0.004

SEM: Standard error of the mean; ^{a,b} values within the same row bearing different superscripts ($^{a, ab, b, bc}$) indicate significant differences (P<0.05).

Table 4: Impact of β-1,3-glucan, precision biotic and *Bacillus subtilis* on intestinal histology of broilers

Parameters	Control	AGP	β-1,3- glucan	Precision biotic	*Bacillus subtilis*	SEM	P-value
Duodenum							
Villus height (µm)	470.90^{c}	530.23^{bc}	580.54^{b}	604.88^{a}	498.23^{bc}	10.09	0.001
Villus width (µm)	98.41^{d}	105.77^{c}	113.47^{ab}	117.21^{a}	110.09^{b}	3.915	0.014
Crypt depth (µm)	87.21^{bc}	99.34^{bc}	102.89^{b}	105.33^{a}	85.82^{c}	6.694	0.007
*VH: CD	5.39^{c}	5.33^{d}	5.64^{bc}	5.74^{b}	5.80^{a}	0.073	0.028
Goblet cell count	102.76	127.32	132.88	148.32	113.33	12.76	0.062
Jejunum							
Villus height (µm)	910.72^{d}	986.09^{c}	1021.22^{b}	1077.22^{a}	993.78^{bc}	21.77	0.003
Villus width (µm)	117.92	105.76	114.98	101.33	115.53	4.222	0.057
Crypt depth (µm)	129.92	136.77	132.56	137.78	141.36	7.475	0.071
VH: CD	7.00^{c}	7.20^{bc}	7.70^{b}	7.81^{a}	7.02^{bc}	0.136	0.009
Goblet cell count	214.73^{d}	276.91^{c}	311.98^{a}	302.87^{ab}	287.43^{b}	11.94	0.039
Ileum							
Villus height (µm)	509.82^{d}	683.55^{bc}	705.76^{b}	749.09^{a}	603.66^{c}	19.82	0.006
Villus width (µm)	157.92	131.56	122.84	112.87	143.72	3.866	0.061
Crypt depth (µm)	75.33	103.27	109.33	113.98	97.22	10.03	0.089
VH: CD	6.76	6.61	6.45	6.57	6.20	0.092	0.083
Goblet cell count	99.88^{d}	122.23^{c}	130.87^{b}	149.45^{a}	129.26^{bc}	14.20	0.006

*VH:CD = Villus Height to Crypt Depth Ratio; SEM: Standard error of the mean; ^{a,b} values within the same row bearing different superscripts ($^{a, ab, b, bc}$) indicate significant differences (P<0.05).

Table 5: Impact of β-1,3-glucan, precision biotic and *Bacillus subtilis* on blood biochemical parameters of broilers

Parameters	Control	AGP	β-1,3- glucan	Precision biotic	*Bacillus subtilis*	SEM	P-value
Total protein (g/dl)	3.59^{c}	3.78^{bc}	3.83^{b}	4.29^{a}	4.09^{ab}	0.114	0.002
Albumin (g/dl)	1.97^{c}	1.99^{c}	2.18^{b}	2.61^{a}	2.31^{ab}	0.190	0.027
Globulin (g/dl)	1.62^{b}	1.79^{a}	1.65^{ab}	1.68^{a}	1.78^{a}	0.038	0.008
AST (IU/l)	310.80^{b}	318.39^{a}	306.98^{c}	306.01^{c}	312.33^{bc}	1.802	0.020
ALT (IU/l)	20.99^{b}	22.92^{a}	21.01^{ab}	20.80^{b}	20.72^{b}	0.519	0.025
Glucose (mg/dL)	240.10	241.12	239.09	238.11	240.23	8.990	0.103
Creatinine (mg/dL)	0.30	0.31	0.29	0.20	0.23	0.034	0.066
Uric acid (mg/dL)	3.09	3.49	3.26	3.41	3.49	0.155	0.271

SEM: Standard error of the mean; ^{a,b} values within the same row bearing different superscripts ($^{a, ab, b, bc}$) indicate significant differences (P<0.05).

Carcass characteristics of broilers: The carcass characteristics results (Table 6) indicated that dressing percentage did not differ significantly among experimental treatments (P>0.05). Breast yield remained similar across treatments with no statistical differences observed (P>0.05). Moreover, thigh yield was highest in the β-1,3-glucan and *Bacillus subtilis* groups (P<0.05). Heart percentage was numerically higher in the PB and *Bacillus subtilis* groups, with no statistical differences observed (P>0.05). Gizzard percentage was highest in the PB group, yet the variation among treatments was non-significant (P>0.05). Finally, the liver percentage was highest in the β-1,3-glucan group and lower in the PB and *Bacillus subtilis* groups, with the differences being non-significant (P>0.05).

Table 6: Impact of β-1,3-glucan, precision biotic and *Bacillus subtilis* on carcass characteristics of broilers

Parameter %	Control	AGP	β-1,3-glucan	Precision biotic	*Bacillus subtilis*	SEM	P-value
Dressing	62.92	63.75	64.32	64.91	65.85	0.255	0.074
Breast	45.01	45.42	44.54	45.57	45.72	0.183	0.109
Thigh	27.01^{b}	27.13^{b}	28.63^{a}	27.96^{ab}	28.74^{a}	0.102	0.003
Heart	0.573	0.567	0.598	0.632	0.640	0.055	0.092
Gizzard	1.581	1.592	1.591	1.710	1.639	0.063	0.078
Liver	2.487	2.763	2.982	2.387	2.370	0.194	0.135

SEM: Standard error of the mean; ^{a,b} values within the same row bearing different superscripts indicate significant differences (P<0.05).

DISCUSSION

In poultry production, over the past few decades, antibiotics have been used in the feed at sub-therapeutic doses not only to control pathogenic bacteria but also to improve growth performance (Cox *et al.*, 2003; Kumar *et al.*, 2018; Yaseen *et al.*, 2025). Nevertheless, awareness regarding antibiotic-resistant bacteria and antibiotic residues in poultry products has encouraged many countries to ban the use of antibiotics as AGPs in poultry production (Van den Bogaard *et al.*, 2002; Cox *et al.*, 2003; Roth *et al.*, 2019). However, antibiotics as feed additives are still being used in many countries, especially in developing countries (Dutta *et al.*, 2019; Hosain *et al.*, 2021). Therefore, researchers all around the world, and in developing countries specifically, conduct a wide range of experiments to find safe, effective, and economical alternatives to AGPs for broiler production (Diarra and Malouin, 2014; Lone *et al.*, 2022; Yaqoob *et al.*, 2022; Azizi *et al.*, 2024). Keeping in view the global efforts to find alternatives to AGPs for poultry production, the current experiment compared the effectiveness of β-1,3-glucan, PB and *Bacillus subtilis* as alternatives to AGPs in broiler chickens.

It has been reported that parameters like average daily feed intake (ADFI), BW, and BWG and average daily gain (ADG) are inclusive metrics that could be used to explore growth performance and overall health of the birds (Torrey *et al.*, 2021). In the current study, decreased BW and BWG during grower, finisher and overall phases were observed in birds fed the CON diet, indicating the importance of AGPs as growth promoters in broiler diets. In the current study, supplementation of β-1,3-glucan, PB and *Bacillus subtilis* as an alternative to AGPs did not influence ADFI, BW, BWG and ADG during the starter period, while a reduction in FCR was observed in the control and AGP supplemented groups. Although similar to AGPs, the precise mechanisms through which many AGP alternatives exert their effects have not been fully elucidated, however, the mode of action of AGP alternatives is generally believed to occur in the gastrointestinal tract through the modulation of the gut-microbiota, epithelial integrity and local immune responses, which collectively influence nutrient utilization and growth efficiency (Nkukwana *et al.*, 2015).

Results of growth performance during the starter phase of the current study represent an absence of significant differences in key growth performance parameters, except FCR, suggesting that the experimental treatments had a limited measurable impact during 0–7 days. Starter growth performance results, except FCR, represent the importance of trial duration in detecting performance responses to experimental treatments (Anugom and Ofongo, 2019; Nduku and Nkukwana, 2026). Based on the results of the starter phase, it could be speculated that a short starter period (0–7 days) may be insufficient for subtle improvements in gut health to translate the impact of feeding β-1,3-glucan, PB and *Bacillus subtilis* into significant changes in BWG, particularly given the rapid physiological adjustments occurring during early post-hatch development. However, the higher FCR observed in birds fed β-1,3-glucan, PB and *Bacillus subtilis* represents a less favorable feed-efficiency outcome compared with CON and AGP groups, indicating that these AGP alternatives did not provide an immediate improvement in feed efficiency during the starter phase. A similar phase-dependent response was reported by Jeong *et al.* (2026), who observed no significant effects of *Bacillus subtilis* on growth performance at 7 days but reported improved final BW, ADG and FCR. It is also possible that the inclusion levels used in the current study produced responses during the later phases but did not elicit a measurable improvement in feed efficiency during the starter phase. However, these possibilities remain speculative because adaptation, gut microbiota, digestive enzyme activity and dose-response effects were not directly evaluated in the current trial. Better FCR during the finisher and overall phases of the current study indicates that the effects of AGP alternatives may become more evident over time. This finding indicates that the effects of the AGP alternatives (β-1,3-glucan, PB and *Bacillus subtilis*) may become more pronounced with the passage of considerable time, maturation of internal organs and cumulative improvement in gut function.

Improved growth performance, BWG, average weight gain and FCR during the growing, finisher and overall phases were observed in birds fed a PB-supplemented diet. It has been reported that PB supplementation improves BWG and FCR in growing broilers (Bortoluzzi *et al.*, 2025). The positive impact of PB supplementation in the diet on the performance parameters of birds observed in the current study may be explained by better nutrient digestibility and a positive impact on intestinal health. A recent study by Bortoluzzi *et al.* (2025) reported that the supplementation of PB in the diet has a positive impact on the growth performance of birds through favorable modulation of the cecal microbiota, including an increased abundance of bacteria

and genes responsible for short chain fatty acid (SCFA) production. However, cecal microbiota composition, genes related to SCFA production and SCFA concentrations were not directly measured in the current study. Therefore, this possible mechanism is based on the findings of Bortoluzzi *et al.* (2025) and cannot be confirmed by the current results. Similarly, other researchers reported that glycan-based PB supplementation has the capacity to enhance the performance of broilers by selectively altering the metabolic activities of microorganisms and nutrient-utilization pathways (Walsh *et al.*, 2021; Jacquier *et al.*, 2022; Blokker *et al.*, 2022; Bortoluzzi *et al.*, 2025). Walsh *et al.* (2021) indicated that a microbiome metabolic modulator enhanced growth in broilers and affected cecal metabolism of energy and amino acids. Equally, Jacquier *et al.* (2022) and Yan *et al.* (2023) found that the growth performance and microbiome responses of broilers fed PB showed an improvement. In the current study, better growth performance could be further justified by the better DM, CP and EE digestibility by supplementation of PB in the diet.

Similar to PB, β-1,3-glucan also had a positive impact on growth during the grower, finisher and overall phases as compared to the control. Guo *et al.* (2023) reported that dietary β-1,3-glucan enhanced BW, daily gain, FCR, and intestinal health in broilers even after *Clostridium perfringens* challenge. A study by Amer *et al.* (2022) also reported that β-glucan supplementation positively influences intestinal histology and parameters related to immunity, and hence increases nutrient utilization. It is speculated that enhanced growth in the current study may have resulted from improved immunity, intestinal morphology, nutrient absorption and nutrient utilization. Furthermore, better nutrient digestion in the current study by supplementing β-1,3-glucan in the diet further supports the results of enhanced BW, daily gain, and FCR. Similar to the current study findings, Amer *et al.* (2023) also indicated that dietary 1,3-β-glucans enhanced growth-related and physiological responses in broilers.

Similar to AGP, PB and β-1,3-glucan, a positive effect of *Bacillus subtilis* on performance parameters, CP, EE and DM digestibility and ileum, duodenum and jejunum morphology was also observed in the current study. Findings of *Bacillus subtilis'* positive impact on performance parameters, CP, EE and DM digestibility and ileum, duodenum and jejunum morphology are similar with the findings of previous studies that demonstrate the positive effect of probiotics on growth, nutrient digestibility, immune functions and intestinal morphology. It is well known that *Bacillus subtilis* increases BWG, ADG, and improves feed efficiency by reducing FCR in birds, potentially through promoting eubiosis and modulating the immune system. Results of the current study were further supported by recent research that indicates *Bacillus subtilis* supplementation has the potential to enhance growth performance, small intestine morphology, digestive enzyme activity, and nutrient utilization in broilers (Mohamed *et al.*, 2022; Cai *et al.*, 2024; Chen *et al.*, 2025). Chen *et al.* (2025) have indicated that *Bacillus subtilis* increased BW, decreased FCR, enhanced villus morphology, and enhanced alpha-amylase activity in low-protein-diet-fed broilers. The same results were also reported by Mohamed *et al.* (2022) and Cai *et al.* (2024), who noted improved intestinal morphology and performance responses in broilers fed with *Bacillus subtilis*. These biological effects are plausible since *Bacillus subtilis* spores can survive in the gastrointestinal tract, promoting beneficial microbial balance, and releasing digestive enzymes including amylase, protease and lipase (Cartman *et al.*, 2008; Latorre *et al.*, 2014; Ma *et al.*, 2018). Furthermore, Su *et al.* (2020) also reported that probiotics have potential to alter gut microbiome diversity and it could be speculated that improved gut microbial diversity and increase in level of digestive enzymes such as lipase, proteases and amylase by supplementation of probiotic in diet as observed in previous studies (Gao *et al.*, 2017) may increase nutrient utilization that cumulative enhance weight gain, and BW gain in finisher and throughout the study as expected. Nevertheless, in the current trial, the activity of digestive enzymes and gut microbiota was not directly quantified; however, better nutrient digestibility results could support this theory.

In the current study, digestibility values of DM, CP and EE were improved by supplementing AGP or an alternative, which directly translated to improved growth performance in grower, finisher and overall phases as discussed above. Enhanced nutrient digestibility by supplementing PBs could be attributed to their capacity to regulate microbial protein and nitrogen metabolism (Bortoluzzi *et al.*, 2023; Bortoluzzi *et al.*, 2024), while *Bacillus subtilis* could release enzymes to enhance nutrient digestibility (Mohamed *et al.*, 2022; Cai *et al.*, 2024; Chen *et al.*, 2025). Intestinal histology data demonstrated that villus height, villus width, crypt depth and VH:CD ratio in the duodenum were significantly influenced by experimental treatments. Results showed that PB supplementation in the diet generated the maximum villus height, villus width and depth of the crypt, and *Bacillus subtilis* gave the maximum VH:CD ratio that resulted in better nutrient utilization and hence better performance in the birds. It has been reported that an increase in the height and width of the villus suggests an increase in the absorptive surface area, which could further support PB-fed birds' higher digestibility and growth response. Nonetheless, greater crypt depth should be interpreted with caution because it may indicate active mucosal exfoliation or a higher rate of turnover (Laudadio *et al.*, 2012; Lei *et al.*, 2015). An increase in VH:CD ratio in the *Bacillus subtilis* group indicates greater absorptive efficiency since the index indicates the correlation between the development of the villus and the turnover of the crypt cell (Laudadio *et al.*, 2012; Gaweł *et al.*, 2025).

Villus height, VH:CD ratio and the number of goblet cells in the jejunum were also significantly altered, but there was no change in villus width or crypt depth. The PB gave the best villus height and VH:CD ratio, and β-1,3-glucan gave the best goblet cell count, which was statistically similar. Results of the current study further reported that PB had the greatest villus height and the number of goblet cells in the ileum, then β-1,3-glucan, but did not have a significant effect on villus width, crypt depth, and VH:CD ratio. These segment-specific reactions indicate that the AGPs and alternatives to AGPs had

varied effects on intestinal morphology among the intestinal regions. Greater enhancements in villus morphology and goblet cell density have been observed in broilers fed β-glucan, products of yeast and *Bacillus*-based probiotics in previous studies (Amer *et al.*, 2022; Mohamed *et al.*, 2022; Alagbe *et al.*, 2023; Cai *et al.*, 2024;). The fact that the number of goblet cells in the current study has gone up is that it could be a sign of greater mucosal protective potential, but the present study did not measure mucin levels or immune markers. Biochemical analysis of serum reflected that total protein and albumin were the highest in PB-fed birds, and that *Bacillus subtilis* and β-1,3-glucan also yielded greater results than the control. Furthermore, AGP and AGP alternatives in the current study that were PB, β-1,3-glucan and *Bacillus subtilis* showed a statistically significant increase in globulin. The increased total protein and albumin of PB-fed birds is consistent with the increased CP digestibility of this group and could be an indicator of better dietary protein availability or utilization in the current study.

Previous studies also supported the theory of correlation between nutrient utilization and serum protein fractions in broilers fed β-glucans and *Bacillus*-based probiotics (Amer *et al.*, 2022; Amer *et al.*, 2023; Cai *et al.*, 2024). However, serum protein fractions per se do not testify to improved immunity or hepatic protein production and thus, these results must be viewed as a sign of change in protein metabolic status. In the current study, dietary treatments had a significant effect on AST and ALT activities. Results of the current study indicated that AST was highest in the AGP group and lowest in the PB and β-1,3-glucan groups, while the level of ALT was highest in the AGP group and lowest in the control, PB and *Bacillus subtilis* groups. These findings suggest that there are alterations in serum transaminase activity due to experimental treatments. The highest ALT and AST activities observed in the AGP group may indicate possible hepatic stress associated with enramycin supplementation at the tested dose. This finding may have implications for animal welfare and food safety and therefore deserves further investigation. Nevertheless, these results alone cannot confirm hepatic damage because liver histology, oxidative stress biomarkers, and inflammatory biomarkers were not determined in the current study. The levels of glucose, creatinine, and uric acid were not significantly different, which indicated that the additives tested did not significantly change the measured levels of glucose or kidney-related serum indicators under the current conditions. Findings of uric acid results in the current study are in contrast to the study of Bortoluzzi *et al.* (2024), who reported low blood uric acid when PB was supplemented, which could be attributed to different diet composition and level of PB used in the diet. Findings of the current study show that the impacts of PB on nitrogen metabolism could be influenced by diet composition and the level of PB used in the diet. In the current study, AGP and AGP alternatives improved the growth performance that should be reflected in carcass response in birds fed AGP and AGP alternative diets. However, in the current study, carcass responses were less pronounced than growth, digestibility and intestinal histological responses. Results of the current study revealed that percentages of dressing, breast yield, heart, gizzard, and liver were not different in experimental treatments, but the percentages of thigh yield were higher in β-1,3-glucan and *Bacillus subtilis* groups. Previous studies also reported higher thigh yield in birds fed β-1,3-glucan and *Bacillus subtilis-supplemented* diets and linked this response to improved nutrient utilization and overall growth (Moon *et al.*, 2016; Ahiwe *et al.*, 2019; Ahammad and Kim, 2025). The thigh-specific response may be associated with differences in muscle composition and nutrient metabolism between the thigh and breast muscles; however, this possible mechanism requires further investigation.

Conclusions: Collectively, PB exhibited the most consistent positive effects on growth performance, nutrient digestibility, intestinal histology and serum protein fractions, while β-1,3-glucan and *Bacillus subtilis* also improved growth, digestibility, selected intestinal parameters and thigh yield. These benefits did not consistently extend to carcass traits, as only thigh yield differed significantly among treatments. Overall, the tested AGP alternatives appear safe for use in broiler diets. To the best of our knowledge, this is the first study to compare PB, β-1,3-glucan and *Bacillus subtilis* as AGP alternatives; as microbiota composition, digestive enzyme activity, short-chain fatty acids, immune markers, oxidative stress indices, and inflammatory cytokines were not evaluated, future studies should address the underlying mechanisms of these alternatives.

Acknowledgements: We acknowledge the support of IFFCO Pakistan Feed Mills R&D Farms FIEDMC Area, Tehsil Chak Jhumra, District Faisalabad, for providing experimental space. We also acknowledge the hard work of workers, lab staff, and technicians at the farm.

Authors contributions: Conceptualization, M.Q.B. and M.A.U.R; software, A.F., and H.M.; validation, M.Q.B., M.A.U.R., and M.S.U.R; methodology, H.M., A.B., and A.F.; formal analysis, M.H. and A.F.; investigation, H.M; data curation, A.B., M.H., and M.A.U.R.; writing-original draft preparation, A.F. and M.A.U.R.; resources, M.Q.B., M.A.U.R., and A.B.; writing-review and editing, M.A.U.R., M.S.U.R., and A.F.; visualization, M.A.U.R., M.S.U.R; supervision, M.A.U.R.; re-writing-approval and statistical analysis, M.A.U.R.; funding acquisition, M.H. and M.A.U.R.; project administration, M.A.U.R. All authors have reviewed and finalized the version of the manuscript for publication.

Institutional review board statement: All procedures carried out in the current experiment were approved by the Director, Graduate Studies, University of Agriculture Faisalabad, under approval code DGS-02-01-2024-23950.

Conflicts of interest: No conflict of interest is declared.

REFERENCES

Abdullah RM, Ali S, Aslam B and Arshad MI, 2024. Molecular characterization and drug resistance pattern of *Pseudomonas aeruginosa* isolated from poultry meat and meat products. Pakistan Veterinary Journal 44(3):812-818.

Adegoke AV, Abimbola MA, Sanwo KA, *et al*., 2018. Performance and blood biochemistry profile of broiler chickens fed dietary turmeric (*Curcuma longa*) powder and cayenne pepper (*Capsicum frutescens*) powders as antioxidants. Veterinary and Animal Science 6:95-102.

Ahammad GS and Kim IH, 2025. Dietary β-1,3-glucan supplementation in broilers with dextran sulfate sodium-induced colitis: impact on growth performance, organ weight, meat quality, and intestinal health. Canadian Journal of Animal Science 105:1-8.

Ahiwe EU, Omede AA, Abdallh ME, *et al*., 2019. Response of broiler chickens to dietary supplementation of enzymatically hydrolyzed glucan or mannan yeast products. Journal of Applied Poultry Research 28:892-901.

Alagbe EO, Schulze H and Adeola O, 2023. Growth performance, nutrient digestibility, intestinal morphology, cecal mucosal cytokines and serum antioxidant responses of broiler chickens to dietary enzymatically treated yeast and coccidia challenge. Journal of Animal Science and Biotechnology 14:1-13.

Amer SA, Attia GA, Aljahmany AA, *et al*., 2022. Effect of 1,3-β glucans dietary addition on the growth, intestinal histology, blood biochemical parameters, immune response, and immune expression of CD3 and CD20 in broiler chickens. Animals 12:3197.

Amer SA, Behairy A, Gouda A, *et al*., 2023. Dietary 1,3-β-glucans affect growth, breast muscle composition, antioxidant activity, inflammatory response, and economic efficiency in broiler chickens. Life 13:751.

Anugom Y and Ofongo RTS, 2019. Impact of aqueous *Ocimum gratissimum* leaf extract on growth performance, gut pH and bacterial counts in broiler chickens. International Journal of Poultry Science 18:309-316.

Awad WA, Ghareeb K, Abdel-Raheem S, *et al*., 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Science 88:49-56.

Azizi MN, Zahir A, Mahaq O, *et al*., 2024. The alternatives of antibiotics in poultry production for reducing antimicrobial resistance. World's Veterinary Journal 14:270-283.

Bancroft JD and Gamble M, 2008. Theory and practice of histological techniques. 6th Ed, Churchill Livingstone Elsevier, Philadelphia, USA, pp:527-537.

Barsanti L, Passarelli V, Evangelista V, *et al*., 2011. Chemistry, physico-chemistry and applications linked to biological activities of β-glucans. Natural Product Reports 28:457-466.

Berberoğlu TM, Riaz R, Gezer A, *et al*., 2025. Effects of probiotics (*Bacillus subtilis*), prebiotics (MOS + β-glucans) and their combination on growth performance, duodenal histomorphology and meat quality in broiler chickens. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 31:731-739.

Blokker B, Bortoluzzi C, Iaconis C, *et al*., 2022. Evaluation of a novel precision biotic on enterohepatic health markers and growth performance of broiler chickens under enteric challenge. Animals 12:2502.

Bortoluzzi C, Segura-Wang M, Aureli R, *et al*., 2024. Supplementation of precision biotic leads to improved growth performance by modulating the microbiome of broiler chickens fed corn- or wheat-based diets. Poultry Science 103:104451.

Bortoluzzi C, Tamburini I and Geremia J, 2023. Microbiome modulation, microbiome protein metabolism index, and growth performance of broilers supplemented with a precision biotic. Poultry Science 102:102595.

Bortoluzzi C, Watson M, Iuspa MA, *et al*., 2025. Precision biotics enhance growth performance in broiler chickens by selectively modifying their intestinal microbiome to better respond to enteric challenges. Poultry Science 104:105454.

Cai Y, Xiao C, Tian B, *et al*., 2024. Dietary probiotic based on a dual-strain *Bacillus subtilis* improves immunity, intestinal health, and growth performance of broiler chickens. Journal of Animal Science 102:skae183.

Cartman ST, La Ragione RM and Woodward MJ, 2008. *Bacillus subtilis* spores germinate in the chicken gastrointestinal tract. Applied and Environmental Microbiology 74:5254-5258.

Chan GCF, Chan WK and Sze DMY, 2009. The effects of β-glucan on human immune and cancer cells. Journal of Hematology and Oncology 2:1-11.

Chen KL, Weng BC, Chang MT, *et al*., 2008. Direct enhancement of the phagocytic and bactericidal capability of abdominal macrophage of chicks by β-1,3–1,6-glucan. Poultry Science 87:2242-2249.

Chen X, Yue W, Li Z, *et al*., 2025. Influence of *Bacillus subtilis* supplementation on growth performance and gut microbial composition in broiler chickens based on low-protein diets. Poultry Science 104:106002.

Clement M, Olabisi M, David E, *et al*., 2019. Veterinary pharmaceuticals and antimicrobial resistance in developing countries. In: Veterinary Medicine and Pharmaceuticals (Bekoe SO, Saravanan M, Adosraku RK and Ramkumar PK, eds): IntechOpen, London, UK, pp:1-20.

Cox CM, Stuard LH, Kim S, *et al*., 2010. Performance and immune responses to dietary β-glucan in broiler chicks. Poultry Science 89:1924-1933.

Cox NA, Craven SE, Musgrove MT, *et al*., 2003. Effect of sub-therapeutic levels of antimicrobials in feed on the intestinal carriage of *Campylobacter* and *Salmonella* in turkeys. Journal of Applied Poultry Research 12:32-36.

Diarra MS and Malouin F, 2014. Antibiotics in Canadian poultry productions and anticipated alternatives. Frontiers in Microbiology 5:1-15.

Dutta T, Yadav S and Chatterjee A, 2019. Antibiotics as feed additives for livestock: human health concerns. Indian Journal of Animal Health 58:121-136.

Gao Z, Wu H, Shi L, *et al*., 2017. Study of *Bacillus subtilis* on growth performance, nutrition metabolism and intestinal microflora of 1 to 42 d broiler chickens. Animal Nutrition 3:109-113.

Gaweł A, Bobrek K and Madej JP, 2025. Early posthatch feeding influences small intestine development in broiler chickens. Poultry Science 104:105861.

Ghosh S, Jahan I, Hossain ME, *et al*., 2019. The quality appraisal of broiler feed manufactured in different feed mills of Bangladesh. Indian Journal of Poultry Science 54:111-116.

Grigore DM, Mironeasa S, Ciurescu G, *et al*., 2023. Carcass yield and meat quality of broiler chicks supplemented with yeasts bioproducts. Applied Sciences 13:1607.

Guo W, Han D, Zhang F, *et al*., 2023. Effects of dietary β-1,3-glucan addition on the growth performance, mRNA expression in jejunal barrier, and cecal microflora of broilers challenged with *Clostridium perfringens*. Poultry Science 102:102349.

Hosain MZ, Kabir SL and Kamal MM, 2021. Antimicrobial uses for livestock production in developing countries. Veterinary World 14:210-221.

Huff GR, Huff WE, Rath NC, *et al*., 2006. Limited treatment with β-1,3/1,6-glucan improves production values of broiler chickens challenged with *Escherichia coli*. Poultry Science 85(4):613-618.

Jacquier V, Walsh MC, Schyns G, *et al*., 2022. Evaluation of a precision biotic on the growth performance, welfare indicators, ammonia output, and litter quality of broiler chickens. Animals 12:231.

Jeong JY, Kim J, Kim M, *et al*., 2026. Effect of probiotics on growth performance, cytokine levels, and gut microbiome composition of broiler chickens for 7 and 35 days. Journal of Animal Science and Technology 68:586-602.

Khallaf RM, Abd El-Hamied SS, Refaie AZ, *et al*., 2025. A study on the effect of rutin in the antibiotics enhancement controlling *E. coli* infection in broiler chicken. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 31:791-800.

Kumar A, Patyal A and Panda A, 2018. Sub-therapeutic use of antibiotics in animal feed and their potential impact on environmental and human health: a comprehensive review. Journal of Animal Feed Science and Technology 6:15-25.

Latorre JD, Hernandez-Velasco X, Kallapura G, *et al*., 2014. Evaluation of germination, distribution, and persistence of *Bacillus subtilis* spores through the gastrointestinal tract of chickens. Poultry Science 93:1793-1800.

Laudadio V, Passantino L, Perillo A, *et al*., 2012. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poultry Science 91:265-270.

Lei X, Piao X, Ru Y, *et al*., 2015. Effect of *Bacillus amyloliquefaciens*-based direct-fed microbial on performance, nutrient utilization, intestinal morphology and cecal microflora in broiler chickens. Asian-Australasian Journal of Animal Sciences 28:239.

Leone F and Ferrante V, 2023. Effects of prebiotics and precision biotics on performance, animal welfare and environmental impact: a review. Science of the Total Environment 901:165951.

Lone A, Mottawea W, Mehdi Y, *et al*., 2022. Bacteriocinogenic probiotics as an integrated alternative to antibiotics in chicken production: why and how? Critical Reviews in Food Science and Nutrition 62:8744-8760.

Lowry VK, Farnell MB, Ferro PJ, *et al*., 2005. Purified β-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar Enteritidis. International Journal of Food Microbiology 98:309-318.

Ma Y, Wang W, Zhang H, *et al*., 2018. Supplemental *Bacillus subtilis* DSM

32315 manipulates intestinal structure and microbial composition in broiler chickens. Scientific Reports 8:15358.

Mahmood MS, Ashraf A, Ali S, *et al*., 2021. Portrayal of *Punica granatum* L. peel extract through High Performance Liquid Chromatography and antimicrobial activity evaluation. Brazilian Journal of Biology. 83:e244435.

Mohamed TM, Sun W, Bumbie GZ, *et al*., 2022. Feeding *Bacillus subtilis* ATCC19659 to broiler chickens enhances growth performance and immune function by modulating intestinal morphology and cecum microbiota. Frontiers in Microbiology 12:798350.

Montgomery DC and Runger GC, 2010. Applied Statistics and Probability for Engineers. 5th Ed, John Wiley & Sons, Hoboken, USA, pp:1-17.

Moon SH, Lee I, Feng X, *et al*., 2016. Effect of dietary β-glucan on the performance of broilers and the quality of broiler breast meat. Asian-Australasian Journal of Animal Sciences 29:384-389.

Mutayoba SK, Dierenfeld E, Mercedes VA, *et al*., 2011. Determination of chemical composition and anti-nutritive components for Tanzanian locally available poultry feed ingredients. International Journal of Poultry Science 10:350-357.

Nduku X and Nkukwana T, 2026. Starter phase broiler response to *Moringa oleifera* leaf meal, a three-strain *Bacillus* probiotic, or butyric organic acid as alternatives to antibiotic growth promoters. Journal of Applied Animal Research 54:2651853.

Nkukwana T, Muchenje V, Masika P, *et al*., 2015. Intestinal morphology, digestive organ size and digesta pH of broiler chickens fed diets supplemented with or without *Moringa oleifera* leaf meal. South African Journal of Animal Science 45:362-370.

NRC, 1994. Nutrient requirements of poultry. 9th Rev Ed, National Academies Press, Washington DC, USA, pp:35-47.

Ofori H, Amoah F, Arah IK, *et al*., 2019. Proximate analysis and metabolizable energy of poultry feeds. ARPN Journal of Engineering and Applied Sciences 14:1026-1032.

Opalinski M, Maiorka A, Dahlke F, *et al*., 2007. On the use of a probiotic (*Bacillus subtilis* strain DSM 17299) as growth promoter in broiler diets. Brazilian Journal of Poultry Science 9:99-103.

Pervaiz R, Mustafa R, Farooq U, *et al*., 2025. Effect of dietary supplementation of biochars on growth performance, bone mineralization, footpad health, lymphoid organs weight, antibody titers of Newcastle disease and infectious bronchitis disease in broiler chicks. Veterinary Sciences 12:1-15.

Rahman M and Arifuzzaman M, 2021. An experiment on growth performance, specific growth rate and feed conversion ratio of rohu (*Labeo rohita*) and tilapia (*Oreochromis niloticus*) in tank-based intensive aquaculture system. International Journal of Aquaculture and Fishery Sciences 7:35-41.

Raza A, Abbas RZ, Karadağoğlu Ö, *et al*., 2024. Role of probiotics in increasing meat and egg production in poultry. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 30:753-760.

Roth N, Käsbohrer A, Mayrhofer S, *et al*., 2019. The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: a global overview. Poultry Science 98:1791-1804.

Su Y, Liu C, Fang H, *et al*., 2020. *Bacillus subtilis*: a universal cell factory for industry, agriculture, biomaterials and medicine. Microbial Cell Factories 19:173.

Sugiharto S, Zulpa Y, Agusetyaningsih I, *et al*., 2024. Physiological responses and intestinal conditions of broiler chickens treated with encapsulated *Acalypha australis* L. leaf extract and chitosan. Veterinary World 17:994-1000.

Thacker P and Widyaratne G, 2012. Effects of expeller-pressed camelina meal and/or canola meal on digestibility, performance and fatty acid composition of broiler chickens fed wheat–soybean meal-based diets. Archives of Animal Nutrition 66:402-415.

Torrey S, Mohammadigheisar M, Santos MN, *et al*., 2021. In pursuit of a better broiler: growth, efficiency, and mortality of 16 strains of broiler chickens. Poultry Science 100:100955.

Van den Bogaard AE, Willems R, London N, *et al*., 2002. Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers. Journal of Antimicrobial Chemotherapy 49:497-505.

Walsh MC, Jacquier V, Schyns G, *et al*., 2021. A novel microbiome metabolic modulator improves the growth performance of broiler chickens in multiple trials and modulates targeted energy and amino acid metabolic pathways in the cecal metagenome. Poultry Science 100:100800.

Wang HL, Ni XQ, Qing XD, *et al*., 2016. Effects of flavomycin, *Bacillus licheniformis* and enramycin on performance, nutrient digestibility, gut morphology and the intestinal microflora of broilers. Journal of Poultry Science 53:128-135.

Yan L, Chu T, Zhang Q, *et al*., 2023. Microbiome modulation by a precision biotic in broiler chickens: a commercial study validation. Poultry Science 102:102596.

Yaqoob MU, Wang G and Wang M, 2022. An updated review on probiotics as an alternative of antibiotics in poultry: a review. Animal Bioscience 35:1109-1120.

Yaseen K, Ali S, Rahman SU, *et al*., 2025. Comparative molecular virulence typing and antibiotic resistance of *Campylobacter* species at the human–animal–environment interface. Foodborne Pathogens and Disease 22(2):109-117.