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The impact of dietary supplementation of polysaccharide derived from *Polygonatum sibiricum* on growth, antioxidant capacity, meat quality, digestive physiology, and gut microbiota in broiler chickens

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ABSTRACT *Polygonatum sibiricum* polysaccharide (**PSP**) has demonstrated diverse medicinal properties, extensively researched for human applications. Nonetheless, there is a lack of studies investigating the potential advantages of PSP in poultry farming. The present study investigated the impact of incorporating PSP into broiler diets on their growth performance, meat quality, blood metabolites, antioxidative status, and ileal histomorphology. Two hundred and forty-one-day-old male Ross-308 broiler chicks (44.98 ± 0.79 g) were randomly assigned to 3 experimental groups, with 8 replicates of 10 birds each. The birds were fed diets supplemented with PSP at 0, 400, and 800 mg/kg (control, PSP400, and PSP800, respectively). The results revealed a linear ($P > 0.05$) improvement in body weight gain, European production efficiency index, and feed conversion ratio during the grower (22−35 d) and overall periods (1−35 d). The pH levels in the ingluvies, ileum, and cecum exhibited a linear reduction ($P > 0.05$) in the PSP800 group at d 21 and d 35, respectively. Villus height and crypt depth were increased in the PSP400 and PSP800 groups compared to the control group. PSP400 and PSP800 groups exhibited decreased hydrogen peroxide ($\mathbf{H_2O_2}$) levels and increased total antioxidant capacity (**TAC**) at 21 d, while at 35 d, TAC and sulfhydryl concentrations were elevated, and H_2O_2 was reduced only in the PSP800 group compared to the untreated one. No significant variations between the groups at the phylum and genus levels were observed, with *Bacteroidetes* and *Firmicutes* being the dominant phyla. However, PSP supplementation notably augmented *Firmicutes* and *Verrucomicrobiota* while reducing *Euryarchaeota* and *Proteobacteria*. At the genus level, there was an increase in *Akkermansia, Alistipes, CHKCI001, Erysipelatoclostridium*, and a decrease in *Methanobrevibacter*. Conclusively, incorporating PSP into broiler diets, particularly at a dosage of 800 mg/kg, improved growth performance, antioxidant capacity, and intestinal architecture and resulted in alterations in cecal microbiota without discernible impacts on digestive function and meat quality criteria.

Key words: polysaccharide, growth, gut microbiota, histomorphometry, broiler

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INTRODUCTION

Eminent progress has been made in enhancing productivity in modern intensive poultry production. Nonetheless, the utilization of antibiotic-growth-promoters (**AGP**), encompassing antibiotics and chemotherapeutic substances in poultry diets to boost productivity and combat diseases, has raised concerns about food and human safety (Castanon, 2007). Consequences such as drug residues and the development of resistant bacteria have been identified as substantial outcomes of these practices (Abdel-Moneim et al., 2020c). Consequently, the poultry industry is undergoing a transformation towards more sustainable and responsible approaches to meet the growing customer preference for safe, nutritious, and eco-friendly products (Abd El-Moneim et al., 2020; Chen et al., 2023; Elbaz et al., 2023; Saleh et al.,

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2023; Chen et al., 2024b). The transition from AGPs to alternative solutions has been motivated by concerns regarding resistance to antibiotics, as well as the increasing consumers' preference for poultry products free from antibiotics. This shift is not limited to specific regions, as the European Union banned the use of AGPs in poultry feed in 2006, setting an example for other countries to follow (Castanon, 2007).

Alternative substances, including prebiotics (Abd El-Hack et al., 2021; Shehata et al., 2022), probiotics (Abd El-Hack et al., 2020; Abdel-Moneim et al., 2020a), essential oils (Abd El-Hack et al., 2020; Elbaz et al., 2022b), traditional Chinese medicine (**TCM**) (Dosoky et al., 2021; Yang et al., 2023), and herbal extracts (Elbaz et al., 2021; Mesalam et al., 2021; Ebeid et al., 2023; Chen et al., 2024a), as well as improved management and nutritional practices (Abdel-Moneim et al., 2021; Elbaz et al., 2022a; Siddiqui et al., 2022; Abdel-Moneim et al., 2023), offer avenues to improve bird health and productivity, obviating the necessity for AGPs. Traditional Chinese medicine and phytochemicals, in particular, have emerged as promising substitutes owing to their plant-derived compounds with immunomodulatory, antioxidant, and antimicrobial attributes. Integrating TCM in poultry production aligns with eco-friendly practices and disease prevention. Recent research underscores the advantageous effects of plant-derived bioactives, particularly polysaccharides, in enhancing poultry growth, meat quality, and carcass characteristics (Wang et al., 2022b; Yang et al., 2023). Extensive research focused on polysaccharides commonly found in TCM plants and their extracts, which are rich in polysaccharides, have gained attention as AGP alternatives because of their multifunctionality, low toxicity, and limited adverse effects (Shan et al., 2019; Abdel-Moneim et al., 2020b; Sun et al., 2022).

Polygonatum sibiricum polysaccharide (**PSP**) represents a recently identified water-soluble compound extracted from the rhizome of *Polygonatum*. It is primarily composed of galactose and rhamnose (Liu et al., 2018a). This compound has been shown to possess medicinal efficacy by enhancing immune function (Peng et al., 2018), as well as showcasing antitumor (Peng et al., 2018), antiviral and antiinflammatory (Lu et al., 2013), and antioxidant properties (Jiang et al., 2013). Studies indicated that PSP may have the potential in addressing conditions like diabetes, osteoporosis, acting as a neuroprotective agent, and alleviating inflammatory disorders (Zhang et al., 2015; Wang et al., 2017). In a study conducted by Shu et al. (2021), the authors underscored the significance of polysaccharides derived from *Polygonatum sibiricum* in mitigating cyclophosphamide-caused immunosuppression in chickens, suggesting its potential immunostimulant activity. However, while the beneficial effects of PSP have been extensively studied in traditional human medicine, limited research exists on its potential application in poultry farming.

Presently, there is a lack of research evaluating the impact of dietary supplementation of PSP in broiler chickens. Therefore, the objective of this study was to explore the influence of PSP dietary inclusion on the growth, meat quality, antioxidative status, digestive physiology, and cecal microbiota of broiler chickens.

MATERIAL AND METHODS

Ethics Statement

The procedures of the present study were reviewed and approved by the Institutional Animal Care and Use Committee of Anhui Science and Technology University, Fengyang, Anhui Province in China (ECASTU-2019-P03).

PSP Composition Analysis

The percentages of crude protein, crude ash, ether extract, neutral detergent fiber, acid detergent fiber, calcium, and phosphorus in PSP were measured as reported by da Teixeira et al. (2018). The phenol-sulfuric acid method was used to assess the polysaccharides content in PSP (Masuko et al., 2005), which was purchased from Shaanxi Hannah Biotechnology Co., Ltd. (Xi'an, China).

Experimental Design and Bird Management

Two hundred and forty-one-day-old male Ross-308 broiler chicks (44.98 ± 0.79 g) were randomly assigned into 3 experimental groups, with 8 replicates of 10 birds each. The chicks were supplied by Bengbu Dacheng Food Co., Ltd.'s hatchery (Anhui, China). They were

Table 1. Ingredient and composition of the basal diet.

Items	Starter (1−21 d)	Grower (22−35 d)
Ingredients (g/kg)		
Corn	514.2	566.6
Corn starch	10.0	10.0
Soybean meal	386.5	341.0
Fish meal	35.0	20.0
Soybean oil	30.0	35.0
Dicalcium phosphate	9.5	11.0
Ground limestone	9.0	10.5
Iodine salt	2.5	2.5
DL-methionine	0.9	1.0
Vitamin-mineral premix[1]	2.4	2.4
Nutrient content[2]		
Crude protein	231.2	204.7
AME (MJ/kg)	11.35	12.46
Methionine	4.8	4.3
Lysine	12.5	11.4
Methionine + cysteine	8.4	7.6
Non-phytate phosphorus	4.2	3.7
Calcium	10.3	8.8

[1]vitamin-mineral premix provided per kg diet: IU: vit. A 4,000,000, vit. D_3 500,000; g: vit. E 16.7, vit. K 0.67, vit. B_1 0.67, vit. B_2 2, vit. B_6 67, vit. B_{12} 0.004, nicotinic acid 16.7, pantothenic acid 6.67, biotin 0.07, folic acid 1.67, choline chloride 400, Zn 23.3, Mn 10, Fe 25, Cu 1.67, I 0.25, Se 0.033, Mg 133.4

[2]Calculated according to NRC (1994).

provided with a starter (1−21 d) and grower (22−35 d) mash corn-soybean meal basal diet (Table 1) with ad libitum access to feed and fresh water. PSP was added to the diets at levels of 0, 400, and 800 mg/kg, respectively (control, PSP400, and PSP800, respectively). The chicks were reared in floor cages with a 23L:1D lighting program under controlled environmental conditions. For the first 3 d of the experiment, the indoor temperature was maintained at 35°C and gradually decreased to 20°C with a relative humidity of 58% till the end of the trial.

Growth Performance

On a pen basis, body weight and feed intake (**FI**) were measured on d 21 and 35. Body weight gain (**BWG**), European production efficiency index (**EPEI**), and feed conversion ratio (**FCR**) were calculated from the obtained data for each experimental phase as mentioned by Abdel-Moneim et al. (2022).

The Intestinal Morphology

At the end of the experiment, eight chicks per group were randomly chosen for blood and meat sampling. Birds were fasted for 12 h and then manually slaughtered. Following slaughter, the intestinal segments (duodenum, jejunum, ileum, and cecum) were gathered and weighed, and their length was measured. The boundary between the jejunum and ileum was determined by the presence of the yolk pedicle.

Gastrointestinal pH

On d 21 and 35, gastrointestinal organs, including proventriculus, ingluvies, gizzard, jejunum, duodenum, cecum, and ileum, were dissected. Subsequently, a small incision was made at the midpoint of each organ, and an electrode was promptly inserted into the organ content to measure the pH value using a pH meter (Shenzhen Jige Electromechanical Equipment Co., Ltd., Shenzhen, China).

Histomorphometric Evaluation

Ileal samples were collected (1.5 cm from the mid-ileum), were flushed with 0.9% saline, and were fixed in a 10% formalin solution. Tissue sections, 4 μm thick, were obtained from paraffin-embedded tissue blocks and were stained with hematoxylin and eosin following the protocol of Bancroft and Gamble (2002). Stained tissues were observed using a light microscope (Leica DM300 with Leica FLEXACAMC1), and representative fields were photographed for morphometric analysis using Leica LAS X dedicated software. Measurements of villus height (VH), and crypt depth (CD) were obtained by averaging data from 10 randomly chosen sections in each sample, and VH/CD ratio was calculated.

Antioxidative Status

Blood samples were collected from the wing vein at 21 d of age and after slaughtering at 35 d of age. Samples were then centrifuged for 20 min at 5℃ at 3,500 × *g* and the sera were separated and stored at -80℃. The serum concentrations of total antioxidant capacity (TAC), sulfhydryl, and hydrogen peroxide (H_2O_2) were colorimetrically measured following the manufacturing instructions of the commercial kits (NJIB, Jiangsu, China).

Meat Quality

Following the procedure documented by Hou et al. (2020), meat pH and color were determined. In brief, immediately after slaughter, the physical characteristics of pectoral and leg muscle samples were evaluated. Meat pH was measured by totally embedding the electrodes in the samples to ensure entire contact with the tissue fluid. The pH values were measured using a pH meter (Shenzhen Jige Electromechanical Equipment Co., Ltd., Shenzhen, China) and were recorded after the readings had stabilized. The CR-10 Plus chromameter (Zibo Diye Instrument Equipment Co., Ltd., Zibo, China) was used to measure color lightness (L*), yellowness (b*), and redness (a*) of pectoral and leg muscle samples.

In order to calculate drip loss (%), in triplicate, pectoral, and leg muscle meat samples were suspended in sealed plastic bags and stored at 4℃ for 24 h. The differences between the initial and final weights of the samples were calculated and expressed as relative weights to the initial weight. The calculation of cooking loss (%) was conducted in accordance with the procedure proposed by Honikel (1998). In brief, fresh slices of muscle samples measuring 4 × 3 × 1 cm^3 were weighed, positioned within sealed plastic bags, and subjected to cooking until an internal temperature of 70°C was attained, which took approximately 15 min in a water bath set at 80°C. Subsequently, the slices were cooled in water, dried, and reweighed. The percentage of cooking loss was determined using the formula: (initial weight - final weight)/ initial weight × 100%.

Cecal Microbiota Analysis

On d 35, cecum samples from 5 randomly selected birds from both the control and PSP800 groups (the highest level of the supplement used in the present study) were quickly collected, tightly tied with a thin thread, and stored in a -80℃ freezer for sequencing. Microbial DNA was extracted from the cecum samples using the HiPure Soil DNA Kits (Magen, Guangzhou, China), following the manufacturer's guidelines. The 16S rDNA target regions of the ribosomal RNA gene were PCR-amplified using a 50 μL mixture comprising

10 μL of 5 × Q5@ Reaction Buffer, 10 μL of 5 × Q5@ High GC Enhancer, 1.5 μL of 2.5 mM dNTPs, 1.5 μL of each primer (10 μM), 0.2 μL of Q5@ High-Fidelity DNA Polymerase, and 50 ng of template DNA. PCR reagents were sourced from New England Biolabs, USA. The purified amplicons were pooled equimolarly and subjected to paired-end sequenced (PE250) on an Illumina platform following standardized protocols. The V3-V4 hypervariable region of the bacterial 16S rRNA gene was specifically PCR-amplified using the forward primer 341F: 5′-CCTACGGGNGGCWGCAG-3′ and the reverse primer 806R: 5′-GGACTACHVGGGTATCTAAT-3′.

Reads Filtering and Assembly and Raw Tag Filtering

To obtain high-quality clean reads, we applied filtering steps to the raw reads using FASTP (version 0.18.0) based on the following criteria: 1) Removal of reads containing more than 10% of unknown nucleotides (N); 2) Discarding reads with less than 50% of bases with quality (Q-value) above 20; 3) Elimination of adapter contamination. Paired-end clean reads were merged into raw tags using FLASH (version 1.2.11) with a minimum overlap of 10 bp and a mismatch error rate of 2%. Furthermore, for obtaining high-quality clean tags, we performed additional filtering on the raw tags using the following standards: 1) Trimming raw tags starting from the first low-quality base site (default quality threshold is ≤3) until the desired length (default length is 3 bp); 2) Filtering out tags with a continuous high-quality base length of less than 75% of the tag length.

Chimera Removal and Community Composition Analysis

The clean tags were then clustered into operational taxonomic units (**OTU**) with a similarity threshold of 97% using the UPARSE (version 9.2.64) pipeline. Chimeric tags were removed using the UCHIME algorithm while retaining the effective tags for subsequent analysis. Within each cluster, the tag sequence with the highest abundance was selected as the representative sequence. The abundance of each taxonomic group was visualized using Krona (version 2.6). Circular layout representations depicting species abundance were generated using Circus (version 0.69-3). Additionally, a heatmap illustrating species abundance was created within the R project using the heatmap package (version 1.0.12).

Function Prediction

To predict the function of the OTUs/ASVs, we conducted a KEGG pathway analysis using Tax4Fun (version 1.0). To classify the bacterial microbiome phenotypes, BugBase was employed. The ecological functional profiles of bacteria were generated using the FAPROTAX database. For the functional grouping of fungi, FUNGuild (version 1.0) was utilized. Differences in functionality between groups were evaluated using Welch's t-test within the R project Vegan package (version 2.5.3).

Table 2. The composition of *Polygonatum sibiricum* polysaccharide.

Ingredients	Content (%)
Polysaccharide	81.95
Crude protein	1.31
Ether extract	1.86
Neutral detergent fiber	3.44
Acid detergent fiber	3.12
Crude ash	1.78
Calcium	0.42
Phosphorus	0.24

Statistical Analysis

The gathered data were statistically analyzed by One-way ANOVA after conducting the tests of normality and the homogeneity of variance using SPSS software (version 19.0; SPSS Inc., IL). The statistical significance among mean differences was determined at $P < 0.05$ using Tukey's multiple comparison test.

RESULTS

Composition of PSP

Data in Table 2 represent the chemical composition of PSP. Crude protein, crude ash, acid detergent fiber, neutral detergent fiber, calcium, phosphorus, ether extract, and polysaccharide levels of PSP were 1.31,1.78, 3.12, 3.44, 0.42, 0.24, 1.86, and 81.95%, respectively.

Growth Performance

Table 3 presents the impact of dietary PSP incorporation on the growth performance of broiler chicks. BWG, FI, FCR, and EPEI were not affected by PSP treatment during the starter period. However, all the aforementioned parameters, except FI, were linearly improved ($P > 0.05$) in the PSP400 and PSP800 groups during the grower period (22−35 d). Throughout the overall period, EPEI, FCR, and BWG were linearly enhanced ($P > 0.05$) in the PSP800 group and numerically in the PSP400 group, while FI remained unaffected.

Gut Morphology

Table 4 presents the influence of adding PSP to the diet on the gut structure of broiler chickens at 21 and 35 d old. The incorporation of PSP did not affect the proportional length and weight of the jejunum, duodenum, ileum, or cecum at either age.

Table 3. Effect of dietary *Polygonatum sibiricum* polysaccharide (**PSP**) on growth performance of broiler chickens from 1 to 35 d of age.

	Dietary PSP level, mg/kg				*P-values*		
Parameter[1]	0	400	800	SEM[2]	PSP	Linear	Quadratic
BWG, g.bird/d							
1–21 d	32.88	32.36	34.09	0.353	0.116	0.146	0.122
22–35 d	65.46^{b}	70.09^{a}	69.68^{a}	0.715	0.003	0.004	0.029
1–35 d	45.91^{b}	47.45^{ab}	48.33^{a}	0.412	0.038	0.013	0.652
FI, g.bird/d							
1–21 d	46.89	47.20	47.05	0.118	0.599	0.592	0.399
22–35 d	129.4	128.4	129.5	0.346	0.357	0.848	0.163
1–35 d	79.88	79.66	80.04	0.176	0.709	0.722	0.463
FCR, g feed.g/gain							
1–21 d	1.427	1.460	1.382	0.016	0.134	0.234	0.090
22–35 d	1.976^{a}	1.833^{b}	1.861^{b}	0.022	0.008	0.013	0.027
1–35 d	1.740^{a}	1.680^{ab}	1.658^{b}	0.016	0.010	0.041	0.555
EPEI							
1–21 d	245.8	237.0	262.8	5.335	0.130	0.180	0.119
22–35 d	597.4^{b}	666.1^{a}	667.8^{a}	12.74	0.021	0.014	0.139
1–35 d	271.4^{b}	290.7^{ab}	299.8^{a}	5.281	0.049	0.026	0.610

Means in the same row with different superscripts are significantly different.
[1]BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio, EPEI: European production efficiency index.
[2]SEM: standard error of means. Values with different superscript letters are statistically different ($P < 0.05$).

pH of the Digestive Organs

As presented in Table 5, the introduction of PSP resulted in a linear decrease ($P < 0.05$) in the pH levels of the ingluvies at d 21 and in the ileum and cecum at d 35 within the PSP800 group compared to the control. However, there were no notable changes in the pH of the other digestive organs at either age.

Histomorphometric Evaluation

The impact of dietary PSP on the ileal histomorphometry of broilers at 35 d of age is depicted in Figure 1. VH and CD were linearly increased ($P < 0.05$) in the PSP400 and PSP800 groups compared to the control. The VH/CD ratio was not significantly affected.

Table 4. Effect of dietary *Polygonatum sibiricum* polysaccharide (**PSP**) on gut morphology of broiler chickens at 21 and 35 d of age.

	Dietary PSP level, mg/kg				*P-values*		
Parameter	0	400	800	SEM[1]	PSP	Linear	Quadratic
21 d							
Intestinal segments' relative weight, %							
Duodenum	17.81	19.79	19.47	0.511	0.249	0.192	0.291
Jejunum	39.32	38.81	39.15	0.872	0.975	0.941	0.836
Ileum	33.53	31.59	32.10	0.770	0.603	0.481	0.483
Cecum	9.346	9.807	9.277	0.343	0.814	0.940	0.533
Total, g	36.07	34.43	35.63	0.783	0.706	0.830	0.430
Intestinal segments' relative length, %							
Duodenum	14.94	14.75	15.26	0.280	0.779	0.665	0.585
Jejunum	36.27	36.34	36.65	0.360	0.915	0.700	0.885
Ileum	36.54	36.92	36.44	0.431	0.905	0.927	0.668
Cecum	12.25	12.00	11.66	0.370	0.831	0.553	0.959
Total, cm	161.4	161.5	157.7	1.731	0.625	0.412	0.623
Density	0.224	0.213	0.182	0.004	0.562	0.866	0.298
35 d							
Intestinal segments' relative weight, %							
Duodenum	16.61	16.34	16.23	0.246	0.830	0.561	0.890
Jejunum	37.53	35.89	36.64	0.417	0.297	0.391	0.193
Ileum	35.90	36.71	36.52	0.381	0.697	0.540	0.567
Cecum	9.963	11.06	10.62	0.301	0.347	0.386	0.243
Total, g	45.55	45.21	45.12	0.373	0.898	0.666	0.882
Intestinal segments' relative length, %							
Duodenum	13.13	13.32	13.61	0.197	0.646	0.364	0.904
Jejunum	35.16	35.23	35.71	0.207	0.521	0.301	0.655
Ileum	36.05	35.82	34.90	0.309	0.294	0.145	0.600
Cecum	15.66	15.63	15.78	0.255	0.976	0.868	0.887
Total, cm	182.2	181.5	182.0	1.282	0.977	0.956	0.837
Density	0.250	0.249	0.248	0.003	0.961	0.784	0.975

Means in the same row with different superscripts are significantly different
[1]SEM: standard error of means, Density = total weight/ total length ratio

Table 5. Effect of dietary *Polygonatum sibiricum* polysaccharide (**PSP**) on digestive organs' pH of broiler chickens at 21 and 35 d of age.

	Dietary PSP level, mg/kg				*P-values*		
Parameter	0	400	800	SEM[1]	PSP	Linear	Quadratic
21 d							
Ingluvies	6.292[a]	6.128[ab]	5.724[b]	0.155	0.030	0.049	0.718
Proventriculus	4.458	4.558	4.384	0.120	0.858	0.817	0.623
Gizzard	2.618	2.718	2.626	0.074	0.849	0.968	0.577
Duodenum	6.146	6.060	5.952	0.086	0.686	0.633	0.478
Jejunum	6.424	6.336	6.214	0.088	0.656	0.370	0.932
Ileum	6.242	6.196	6.064	0.078	0.657	0.518	0.526
Cecum	7.272	7.250	7.096	0.092	0.728	0.473	0.754
35 d							
Ingluvies	6.220	6.290	6.206	0.050	0.783	0.592	0.667
Proventriculus	4.310	4.204	4.148	0.115	0.863	0.731	0.684
Gizzard	3.042	3.106	3.052	0.051	0.878	0.642	0.853
Duodenum	5.986	6.038	5.960	0.053	0.849	0.713	0.671
Jejunum	6.252	6.422	6.282	0.059	0.488	0.270	0.673
Ileum	6.772[a]	6.364[ab]	6.100[b]	0.114	0.039	0.270	0.673
Cecum	6.668[a]	6.326[ab]	6.084[b]	0.121	0.037	0.050	0.834

Means in the same row with different superscripts are significantly different
[1]SEM: standard error of means.

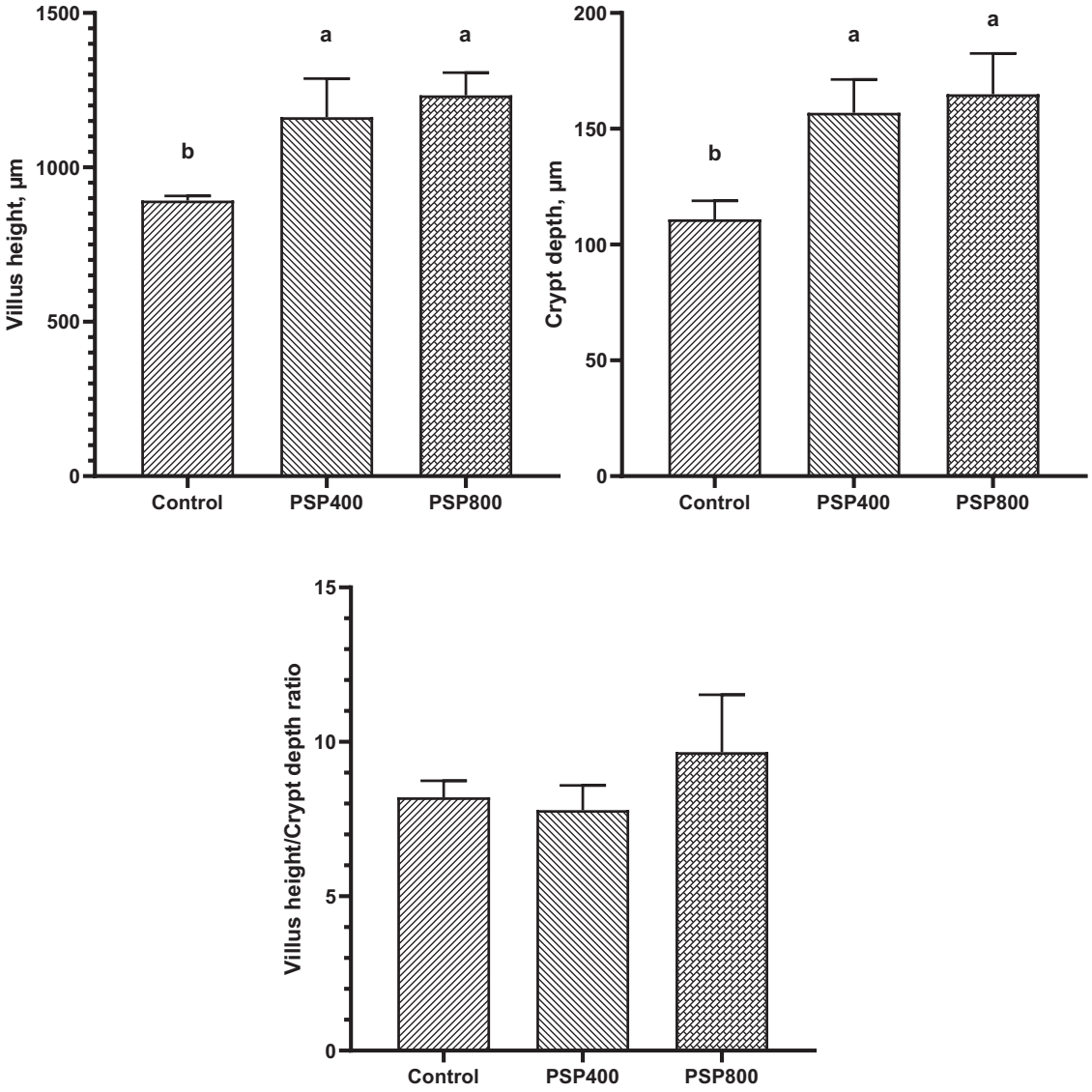

Figure 1. Effect of dietary *Polygonatum sibiricum* polysaccharide (**PSP**) on ileal histomorphometry of broiler chickens at 35 d of age. PSP400= 400 mg PSP/kg diet, PSP800 = 800 mg PSP/kg diet. Data presented as mean values with their standard errors. Values with different superscript letters are statistically different ($P < 0.05$).

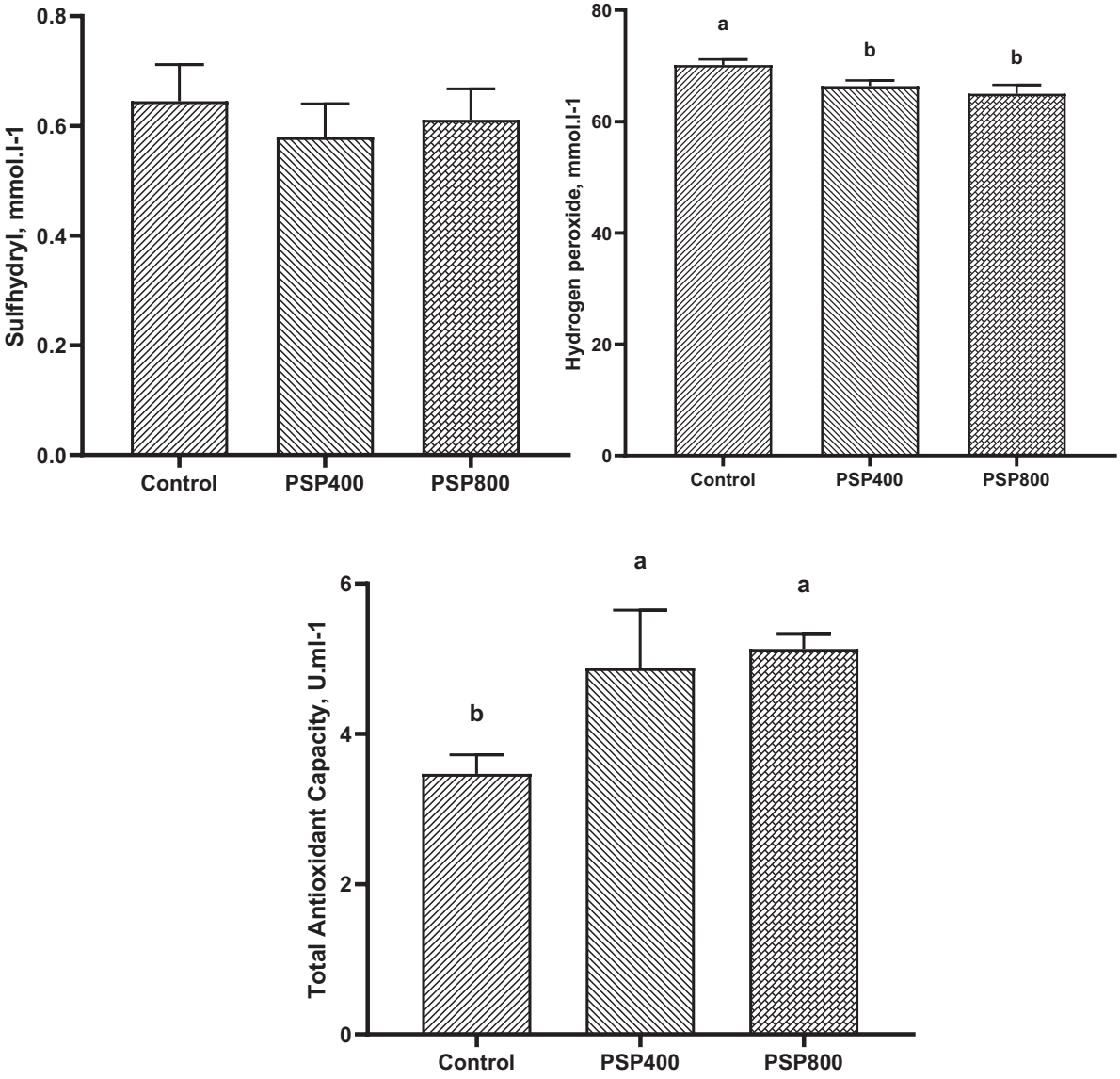

Figure 2. Effect of dietary *Polygonatum sibiricum* polysaccharide (**PSP**) on oxidative status in the serum of broiler chickens at 21 d of age. PSP400 = 400 mg PSP/kg diet, PSP800 = 800 mg PSP/kg diet. Data presented as mean values with their standard errors. Values with different superscript letters are statistically different ($P < 0.05$).

Antioxidative Status

Figures 2 and 3 illustrate the impacts of dietary PSP incorporation on the antioxidant status of broilers at 21 and 35 d of age. Dietary supplementation of PSP at 400 and 800 mg/kg reduced ($P < 0.05$) serum level of H_2O_2 and increased the concentration of TAC ($P < 0.05$) at 21 d of age, while sulfhydryl content was not affected. However, at 35 d of age, TAC and sulfhydryl levels were elevated ($P < 0.05$) and H_2O_2 content was reduced ($P < 0.05$) only in the PSP800 group compared to the control.

Meat Quality

Tables 6 and 7 show the impacts of the inclusion of PSP in broilers' diets on meat quality criteria of pectoral and leg muscles at 35 d of age. PSP supplementation did not exert a significant effect on the pH_{45}, pH_{24}, color, cooking, and drip losses of the collected samples compared to the unsupplemented group. However, there was a linear decrease ($P < 0.05$) observed in the yellowness (b*) of the meat from birds in the PSP800 group compared to the control.

Microbiota Dynamics

To assess the impact of PSP supplementation on caecal microbiota composition, 16S rDNA sequencing was performed on the cecal contents of both the control and PSP800 groups. The average number of raw reads was 109,518 and 121,169, while the average number of clean data was 109,440 and 121,079 for the PSP800 and control groups, respectively. Beta diversity indexes were calculated at the phylum and genus levels to assess differences in species diversity and richness differences between the treated and control groups. The results

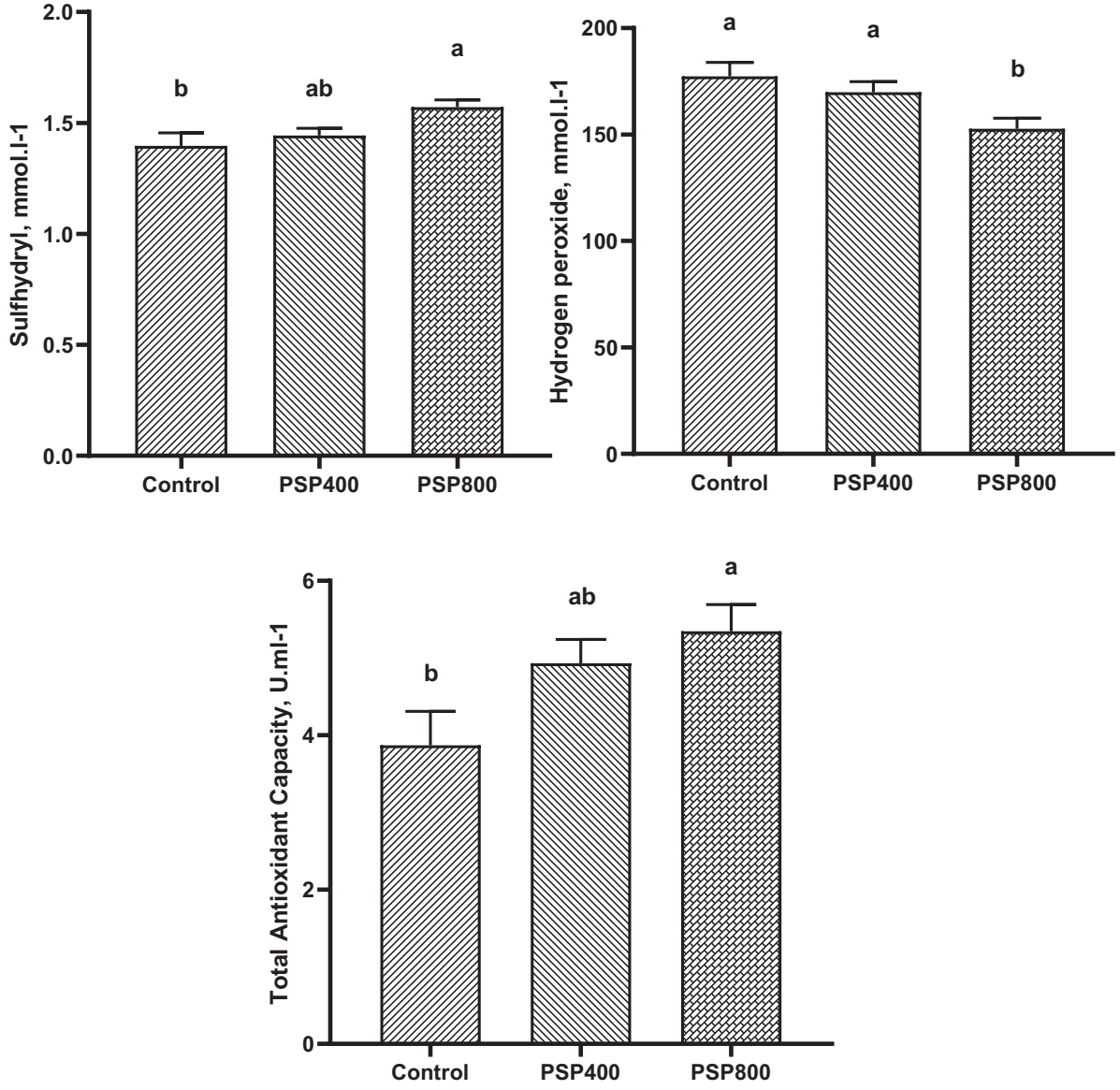

Figure 3. Effect of dietary *Polygonatum sibiricum* polysaccharide (**PSP**) on oxidative status in the serum of broiler chickens at 35 d of age. PSP400 = 400 mg PSP/kg diet, PSP800= 800 mg PSP/kg diet. Data presented as mean values with their standard errors. Values with different superscript letters are statistically different ($P < 0.05$).

revealed no significant variations in observed species between the groups (Figures 4A and 4B; $P > 0.05$). Analysis of microbial abundance at the phylum and genus levels revealed that *Bacteroidetes* and *Firmicutes* were the dominant phyla, accounting for 86.99% and 79.32% of detected microbes in the PSP800 and control groups, respectively (Figure 4C). Moreover, PSP supplementation notably augmented *Firmicutes* and *Verrucomicrobiota* while reduced *Euryarchaeota* and *Proteobacteria* microbes (Figure 4C). At the genus level, an increase in *Akkermansia, Alistipes, CHKCI001, Erysipelatoclostridium*, and a decrease in

Table 6. Effect of dietary *Polygonatum sibiricum* polysaccharide (**PSP**) on meat quality traits of the pectoral muscle of broiler chickens at 35 d of age.

	Dietary PSP level, mg/kg				*P-values*		
Parameter	0	400	800	SEM[1]	PSP	Linear	Quadratic
$L_{45\ min}$	52.48	52.22	51.72	0.139	0.062	0.053	0.650
$a_{45\ min}$	11.56	11.95	12.27	0.163	0.206	0.082	0.912
$b_{45\ min}$	7.553	7.429	7.175	0.162	0.658	0.379	0.860
$pH_{45\ min}$	7.126	7.036	6.808	0.119	0.564	0.309	0.794
$pH_{24\ h}$	6.306	6.158	6.172	0.062	0.597	0.411	0.564
Drip $loss_{24\ h}$/%	4.650	4.970	5.108	0.120	0.298	0.137	0.721
Cooking loss/%	23.01	22.56	22.53	0.253	0.719	0.479	0.713

Means in the same row with different superscripts are significantly different,
[1]SEM: standard error of means.

Table 7. Effect of dietary *Polygonatum sibiricum* polysaccharide (PSP) on meat quality traits of leg muscle of broiler chickens at 35 d of age.

Parameter	Dietary PSP level, mg/kg			SEM[1]	*P-values*		
	0	400	800		PSP	Linear	Quadratic
$L_{45\ min}$	52.83	52.46	52.83	0.170	0.626	0.993	0.343
$a_{45\ min}$	11.57	11.63	11.92	0.115	0.439	0.827	0.215
$b_{45\ min}$	7.310[a]	7.107[ab]	7.017[b]	0.171	0.049	0.047	0.267
$pH_{45\ min}$	7.108	7.001	7.082	0.130	0.948	0.936	0.757
$pH_{24\ h}$	6.646	6.332	6.208	0.148	0.492	0.257	0.771
Drip $loss_{24\ h}$/%	4.753	4.857	4.928	0.213	0.358	0.258	0.852
Cooking loss/%	22.87	22.69	22.84	0.189	0.846	0.587	0.729

Means in the same row with different superscripts are significantly different,
[1]SEM: standard error of means.

Methanobrevibacter were found (Figure 4D). Linear discriminant analysis (LDA) identified 41 high-dimensional biomarkers, with LDA scores >2.5 from phylum to species, illustrating distinct bacterial abundance between the 2 groups (Figure 5). Notably, *Bacteria, CHKCI001*, and *Rikenellaceae* were prominent in the PSP800 group, whereas *Methanobrevibacter, Euryarchaeota, Methanobacteriaceae, Methanobacteria*, and *Methanobacteriales* prevailed in the control group.

Functional and phenotypic abundance analyses were conducted to understand the pathways associated with microorganisms with varying abundance in the 2 groups. Tax4Fun functional abundance analysis (Figure 5A) revealed that these microorganisms were associated with various pathways, including ABC transporters, nitrogen metabolism, 2-component systems, methane metabolism, peptidoglycan biosynthesis, arginine and proline metabolism, cell cycle (caulobacter), cysteine, amino sugar, nucleotide sugar, and methionine metabolism, among others (Figure 6A). Additionally, PICRUSt2 functional abundance analysis revealed the involvement of these microorganisms in transcription, energy metabolism, amino acid metabolism, membrane transport, carbohydrate metabolism, signal transduction, lipid, cofactor, and vitamin metabolism, among others (Figure 6B). Furthermore, BugBase phenotypic abundance analysis demonstrated associations of these microorganisms with traits such as aerobic nature, negative biofilm formation, potentially pathogenic anaerobic features, stress tolerance, facultative anaerobic gram-positive characteristics, and the presence of mobile genetic elements (Figure 6C).

DISCUSSION

The utilization of plant-derived polysaccharides for enhancing the productivity and general health of broilers has gained traction due to their varied biological activities, including hypoglycemic benefits, spanning improvements in immunity, antioxidant capacity, antiviral, antitumor, and anti-inflammatory properties (Wang et al., 2022b; Yang et al., 2023). The bioactive potential of polysaccharides derived from *Yingshan yunwu, Radix rehmanniae praeparata, Lycium barbarum, Astragalus membranaceus, Camellia oleifera,* and *Ficus carica* has been investigated (Liu et al., 2021b; Shu et al., 2021; Yang et al., 2023). The findings of Liu et al. (2021b) highlighted that dietary incorporation of *Yingshan yunwu* tea-polysaccharides improved broilers' gut health and microbiota, meat quality, and immunity. Moreover, Shu et al. (2021) reported the immunostimulant potential of polysaccharides derived from *Polygonatum sibiricum* in safeguarding cyclophosphamide-immunosuppressed chickens. However, to the best of our knowledge, there is a dearth of research investigating the effects of dietary PSP incorporation as an AGP alternative on broiler chickens.

In the present study, dietary PSP incorporation improved the growth performance of broilers during the grower and overall period. The improvement in broiler growth might attributed to its capability to stimulate the expression of protease, amylase, and lipase (Long et al., 2020), thereby increasing the activities of these digestive enzymes and ultimately enhancing digestive function. Furthermore, it has been reported that plant-derived polysaccharides have the capacity to enhance intestinal permeability and improve nutrient absorption (Ren et al., 2017). These outcomes are consistent with Wu (2018) and Wang et al. (2015), who demonstrated improved growth performance in broilers treated with *Astragalus* polysaccharides. Additionally, Long et al. (2020) and Yang et al. (2023) observed that polysaccharides extracted from *Lycium barbarum* and *Radix rehmanniae praeparata* improved the growth performance of Arbor Acres and Cobb-500 broiler chickens.

The intestine plays a pivotal role in nutrient digestion, absorption, and immune function, significantly influencing animal health (Cui et al., 2023). It has been reported that plant-derived polysaccharides can enhance intestinal permeability and nutrient absorption (Ren et al., 2017) and stimulate the expression of digestive enzymes like protease, amylase, and lipase (Long et al., 2020). This upregulation increases the activities of these digestive enzymes, ultimately improving digestive function. Additionally, changes in small intestine length can directly affect nutrient uptake. In this study, we investigated the effect of PSP supplementation on the lengths of various intestinal segments and the pH levels of different digestive organs. The length and weight of the intestinal segment were not affected by PSP dietary supplementation. However, PSP use significantly

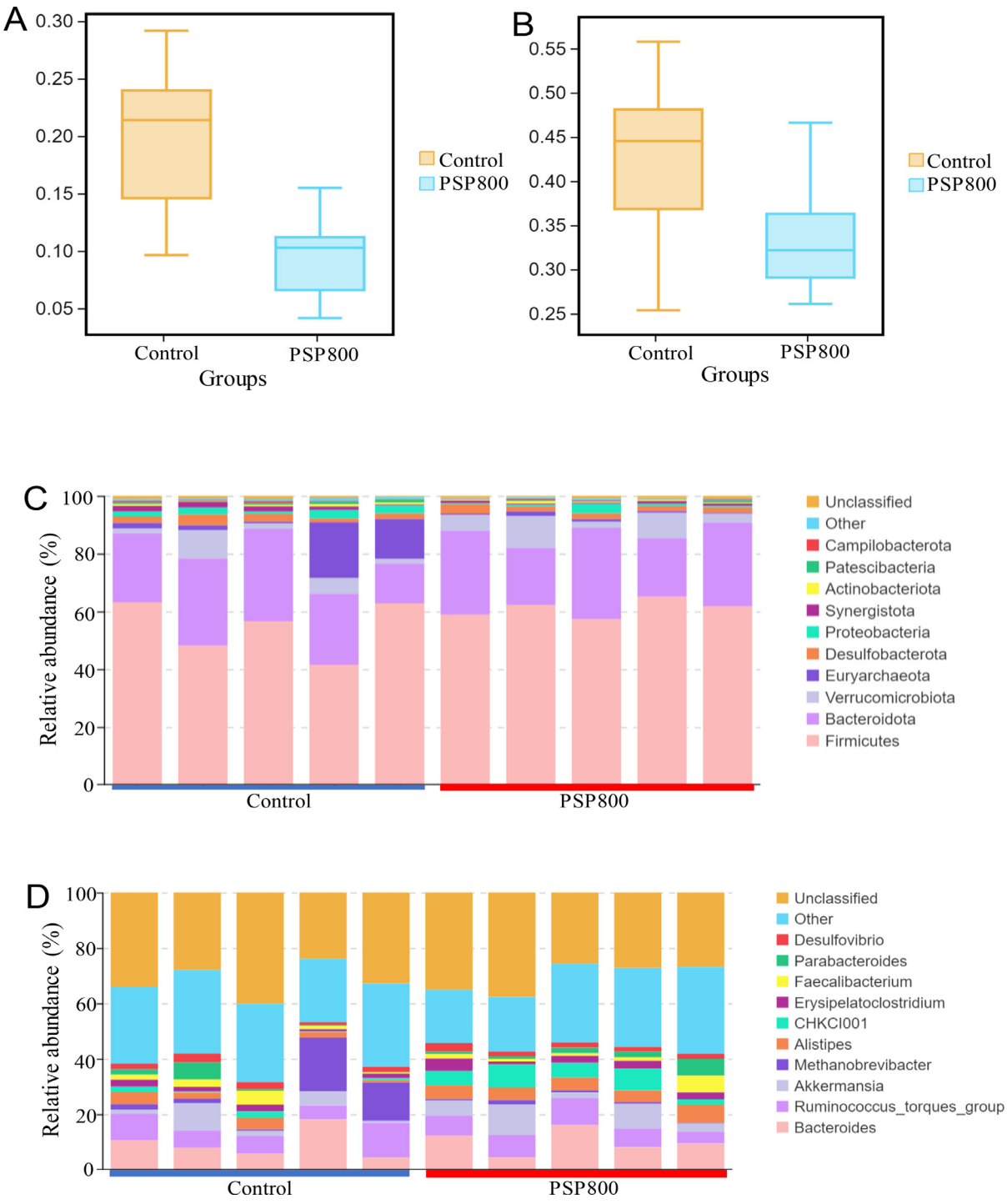

Figure 4. The differences in species diversity and richness in chicken cecal. Statistical tests for β diversity index at the phylum (A) and genus (B) levels. Relative microbial abundance at phylum (C) and genus (D) levels.

reduced pH, specifically in the cecum and ileum. Plant-derived polysaccharides can influence intestinal pH reduction in birds through various mechanisms. Typically, these polysaccharides undergo fermentation by gut microbiota in the cecum and ileum, producing SCFA (Wahlström et al., 2016). The metabolism of SCFA leads to an acidic environment, contributing to a lowered pH in the intestines (Nogal et al., 2021). Moreover, polysaccharides' microbial fermentation generates other secondary substances, such as succinate and lactate, further contributing to a lower intestinal pH (Wassie et al., 2021; Wang et al., 2022b). The decline in gut pH can significantly affect various physiological processes, including microbial populations, nutrient absorption, and overall gut health. The role of PSP in reducing ileal and cecal pH could additionally explain the improvement in growth traits in the present study.

Small intestine mucosa morphology is evaluated through indices like VH, CD, and the VH/CD ratio, which serve as key measures to assess small intestine nutrient absorption capacity. Higher values of these indices signify increased absorptive potential of the

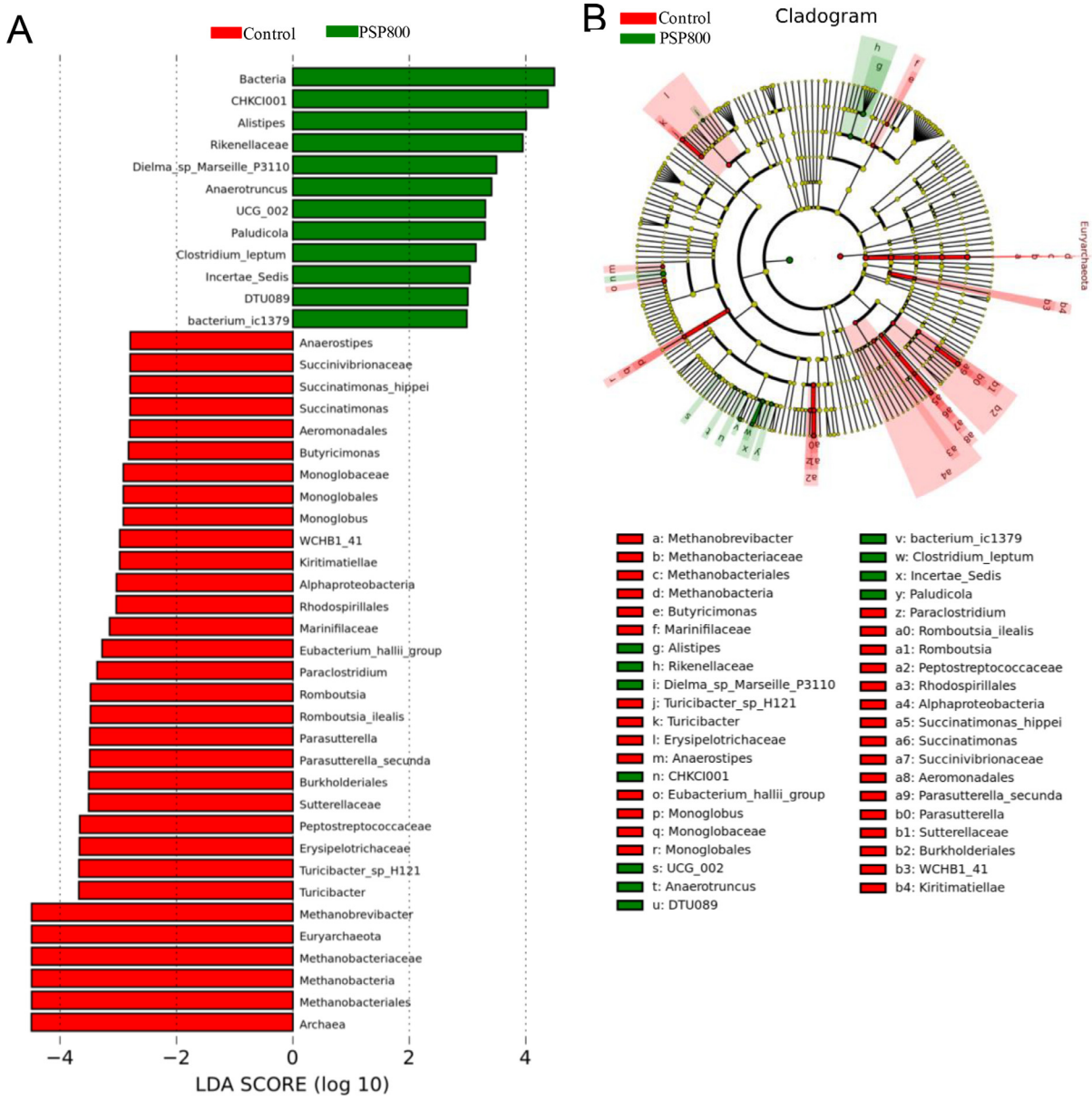

Figure 5. Linear discriminant analysis (**LDA**) effect size (**LEFSe**) analysis identified microbial taxa between PSP800 (green) and control (red) groups. (A) the histogram plot from LEfSe analysis displays the LDA scores of microbial taxa with significantly different abundance between the PSP800 supplemented and control groups (LDA score > 2.5). The length of the bar columns represents the LDA score. (B) the cladogram illustrates the variances in the relative abundance of microbial taxa from phylum to genus level between the PSP800 and control groups, with circles radiating from the inner to the outer side. The red and green points indicate a clear contrast in relative abundance between the PSP800 and control groups.

small intestine (Shehata et al., 2021; Li et al., 2022b). PSP exhibits the capacity to enhance intestinal architecture, contributing to its prebiotic effects by fostering beneficial intestinal bacteria (Li et al., 2009). Previous research noted significant increases in VH and VH/CD ratio in the jejunum of broilers treated with cyclophosphamide when supplemented with 600 or 900 mg/kg of gamma-irradiated *Astragalus* polysaccharides (Li et al., 2019). Similarly, Wang et al. (2021) reported that administering gamma-irradiated *Astragalus* polysaccharides at 600 mg/kg in broiler diets increased VH and VH/CD ratio in the duodenum, jejunum, and ileum. Consistent with these findings, our study observed a notable increase in VH and CD of the ileum following dietary PSP supplementation, indicating improved feed absorption. Hence, it's plausible that PSP could enhance broiler performance by positively impacting intestinal mucosal morphology and fostering intestinal health.

This study reveals the antioxidant properties of PSP, evident in the assessment of serum H_2O_2 and sulfhydryl levels. Hydrogen peroxide, produced by vascular and inflammatory cells, triggers oxidative stress by generating reactive oxygen species (**ROS**) (OH and O^{2-}) through activating NADPH oxidase (Coyle et al., 2006) and the Fenton's reaction involving Fe^{2+} (Ransy et al., 2020). Conversely, sulfhydryl groups found in thiols play a pivotal role in combating ROS during amplified oxidative stress (Erkus et al., 2015). The thiol pool in the plasma mainly includes low molecular weight thiols like glutathione and protein thiols such as albumin (Turell et al., 2013). Lately, thiol/disulfide balance and thiol levels have emerged as novel markers for oxidative stress

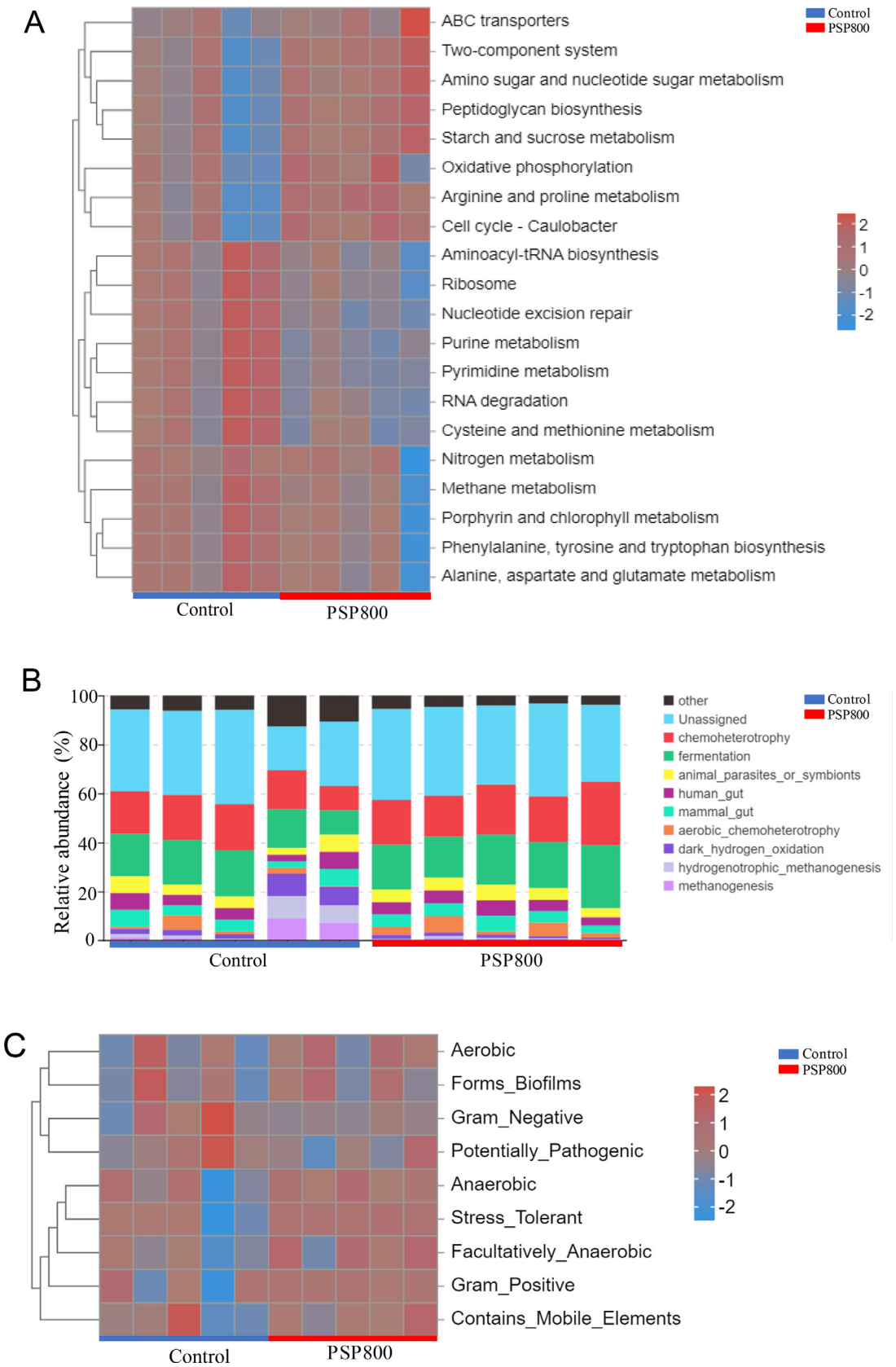

Figure 6. Functional and phenotypic abundance analyses for gut microbial with significant differences between control and PSP800 groups. Tax4Fun (A) and PICRUSt2 (B) functional abundance analyses. (C) represent BugBase phenotypic abundance analysis.

assessment (Kundi et al., 2015; Altıparmak et al., 2016; Shang et al., 2021). Notably, the present findings reveal that PSP incorporation increases serum TAC levels and reduces H_2O_2 concentration, indicating the potent ability of PSP to scavenge ROS effectively. Our results agree with those of Xing et al. (2023), who reported that *Artemisia ordosica* polysaccharide enhanced the antioxidant capacity of LPS-induced broiler chickens.

Similarly, the treatment with *Lycium barbarum* polysaccharide improved liver and serum antioxidant indices in Arbor Acres broilers (Long et al., 2020). Wang et al. (2022a) also documented the potent antioxidant activity of *Polygonatum sibiricum* polysaccharides.

Measuring the pH and color of meat is crucial for assessing chicken meat quality characteristics (Juncher et al., 2001). Improving raw meat quality has become a priority to meet evolving consumer demands. Notably, this study observed a linear decrease in the yellowness (b*) of leg meat in birds from the PSP800 group compared to the control. However, other meat color parameters, pH, drip, and cooking losses remained unaffected in both breast and leg muscles. These findings are consistent with a prior study (Wang et al., 2020). Conversely, Zhao et al. (2020) noted that polysaccharides from *Yingshan Yunwu* tea decreased the quality of the pectoral muscle of Chongren Chicks, altering its color and pH. However, Huang et al. (2021) reported that polysaccharides of *Morinda officinalis* improved the meat quality criteria of broilers with tibial dyschondroplasia. Additionally, chicken meat color serves as a crucial quality attribute, and a lower b* value indicates less pale meat (Fan et al., 2013). One possible mechanism could involve accelerated synthesis of myoglobin and fat deposition in muscles, leading to a lower b* value. Additionally, the antioxidant potential of TCM polyphenols may protect muscle cell membranes and reduce lipid peroxidation injuries (Yang et al., 2011).

This study explores the interactions between intestinal microflora and polysaccharides, an area that has yet to be minimally explored despite the extensive research on poultry gut microbe composition. *Bacteroidetes* and *Firmicutes* emerged as the primary phyla in the intestinal microflora in both PSP-treated and untreated groups, aligning with previous studies by Li et al. (2020) and Yadav et al. (2021). Li et al. (2020) illustrated that *Bacteroidetes* and *Firmicutes* dominated chicken intestinal microflora irrespective of polysaccharide treatment. Meanwhile, Yadav et al. (2021) reported that *Bacteroidetes, Firmicutes*, and *Proteobacteria* comprised around 90% of phyla in commercial chickens, with *Firmicutes* at 63.3% and *Bacteroidetes* at 24.4%. Additionally, our findings indicated a notable increase in the abundance of *Firmicutes* following PSP supplementation, consistent with the observations of Liu et al. (2018b) on dietary *Achyranthes bidentata* polysaccharides. Liu et al. (2021a) noted the potential of *Firmicutes* in enhancing the intestinal barrier function of broiler chickens, while Lin and Lee (2020) demonstrated how *Firmicutes* in the ileum and cecum digesta reduced pH values and ammonia nitrogen levels, potentially favoring the growth and health of the broilers.

In addition, we observed an increase in the abundance of *Verrucomicrobiota* in digesta following PSP supplementation, aligning with previous studies (Liu et al., 2023a; Liu et al., 2023b). Liu et al. (2023b) demonstrated that *Verrucomicrobiota* has the potential to enhance various intestinal aspects, such as VH and VH/CD ratio and the length of ileum and cecum. Another study by Liu et al. (2023a) indicated the potential of *Verrucomicrobiota* in increasing intestinal villus height. Concurrently, our findings indicated a reduction in the relative abundance of *Euryarchaeota* microbes in chicken cecum due to PSP supplementation. Barrera-Rojas et al. (2023) reported that *Euryarchaeota* might play a role in regulating methane production and assimilation in environmental settings. Consequently, our speculation stands that PSP supplementation might decrease methane production and assimilation, potentially enhancing the utilization of feed energy in birds. Moreover, our study indicated a decline in the relative abundance of *Proteobacteria* microbes due to PSP supplementation, which is consistent with the findings of Li et al. (2020), where *Yingshan Yunwu* tea polysaccharides were observed to reduce the relative abundance of *Proteobacteria* in chicken cecal contents.

Interestingly, our study also revealed that PSP treatment increased the relative abundance of *Akkermansia, Alistipes*, and *CHKCI001* while decreasing *Methanobrevibacter* in chicken cecum (Figure 1D). This aligns with previous studies suggesting the benefits of *Akkermansia* in protecting broiler chicken health by safeguarding the intestinal mucosa against injury induced by *S. pullorum* and promoting intestinal epithelium proliferation (Bortoluzzi et al., 2019; Zhu et al., 2020). Furthermore, Li et al. (2022a) found that the relative abundance of *Alistipes* in cecal contents might elevate intestinal mucosal factors such as mucin 2 while decreasing inflammatory cytokine concentrations, *Bax* gene expression, and the Bax/Bcl-2 ratio in the intestinal mucosa. Additionally, Deng et al. (2022) found that the relative abundance of *CHKCI001* in fresh greenish-yellow faeces from Xuefeng black-bone chickens had benefits in improving laying performance and feed conversion ratio. On the other hand, increasing the abundance of *Methanobrevibacter* has been linked to improving energy capture and fat accumulation. Recent studies have suggested that higher levels of *Methanobrevibacter* are correlated with increased abdominal fat in chickens, while lower levels are associated with reduced fat accumulation (Wen et al., 2019; Xiang et al., 2021).

To the best of our knowledge, this study introduces novel evidence indicating the potential of PSP as a beneficial feed supplement in enhancing broiler growth and health. The inclusion of PSP showed potent antimicrobial and antioxidant activities, positively influencing broilers' growth without adverse effects on meat quality and digestive physiology. The recommended inclusion levels of 800 mg/kg yielded promising results. However, further research is necessary to comprehensively understand the mechanisms involved and the optimal PSP inclusion levels in broiler production.

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DISCLOSURES

The authors declare no conflicts of interest.

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