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EFFECT OF DIETARY *POLYGONATUM SIBIRICUM* POLYSACCHARIDE INCORPORATION ON GROWTH, BLOOD METABOLITES, TIBIA MINERALIZATION, AND NUTRIENT DIGESTIBILITY OF BROILERS

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Abstract

This study explored the impact of incorporating *Polygonatum sibiricum* polysaccharide (PSP) into broilers' diets on their growth, serum mineral levels, tibia characteristics, and nutrient digestibility. A total of 240 day-old male Ross-308 broiler chicks were randomly divided into three experimental groups, each consisting of 8 replicates of 10 birds. The birds were fed diets supplemented with PSP at three levels: 0, 400, and 800 mg·kg^{-1}, denoted as control, 400PSP, and 800PSP, respectively. Notably, the 800PSP group exhibited a linear improvement in feed conversion ratio and weight gain at 35 days of age. Crude protein and calcium digestibility were improved ($P>0.05$) by PSP inclusion. The PSP addition did not influence the carcass traits, except for the relative weight of pectoral muscle, which increased linearly ($P>0.05$) in the 800PSP group. Immune organ indexes exhibited a linear increase ($P>0.05$) in the PSP-fed groups. There were no notable changes in hepatic and renal function biomarkers at 21 and 35 days of age. However, at 35 days of age, the serum lipid profile was affected, as linear and quadratic reductions ($P>0.05$) in the concentrations of cholesterol, LDL and HDL were noticed. On day 21 of age, serum calcium, potassium, and iron levels exhibited linear increases ($P>0.05$) in the 800PSP group. Moreover, the tibia ash content at 21 and 35 days of age increased linearly and quadratically ($P>0.05$) in the 400PSP and 800PSP groups. In conclusion, incorporating PSP in broilers' diets improved their growth, tibia ash content, serum minerals and lipid profile, and nutrient digestibility, particularly at the 800 mg·kg^{-1} level.

Key words: polysaccharide, growth, digestibility, tibia characteristics, broiler

The intensive poultry production system has undergone significant changes to enhance productivity while complying with restrictions on antibiotic growth promoters (AGPs). Growing concerns about antimicrobial resistance, coupled with consumer demand for antibiotic-free products, have driven the industry to adopt practices that limit the overuse of AGPs (Abdel-Moneim et al., 2020 b, c). To address the ban on AGPs, the poultry industry has been exploring alternative strategies to maintain and enhance productivity. This transition has led to the exploration of various natural and safe additives, including prebiotics (Abd El-Hack et al., 2021; Shehata et al., 2022; Yang et al., 2023), probiotics (Abd El-Hack et al., 2020; Elbaz et al., 2023), essential oils (Brenes and Roura, 2010; Elbaz et al., 2022 b), herbal extracts (Ebeid et al., 2023; Elbaz et al., 2021; Mesalam et al., 2021), traditional Chinese medicine (TCM) (Dosoky et al., 2021), as well as improving the managerial and nutritional protocols (Abdel-Moneim et al., 2021, 2023; Elbaz et al., 2022 a; Siddiqui et al., 2022).

Plant-derived polysaccharides extracted from sources like herbs, fruits, and roots have gathered consideration due to their multifaceted benefits in poultry nutrition. These polysaccharides are known for their prebiotic, immunomodulatory, and antioxidant properties (Li et al., 2022; Wang et al., 2022 a). They stimulate the growth and activity of beneficial gut bacteria (Abdel-Moneim et al., 2020 a; Ashour et al., 2021). By fostering a balanced gut microbiota, polysaccharides contribute to improved nutrient absorption, enhanced immune responses, and overall gut condition in poultry (Saleh et al., 2021; Shan et al., 2019). Recent investigation has emphasized the advantages of polysaccharides derived from plants in improving various poultry production indices, including meat quality, growth performance, and carcass traits (Wang et al., 2022 b). Polysaccharides sourced from TCM have been extensively investigated and are gaining attention as alternatives to AGPs due to their low toxicity rate, diverse functionalities, and negligible adverse effects (Abdel-Moneim et al., 2020 a; Shan et al., 2019).

Polygonatum sibiricum polysaccharide (PSP) is a water-soluble compound extracted from *Polygonatum* rhizomes. It mainly comprises galactose and rhamnose, as outlined by Liu et al. (2018). PSP has demonstrated various medicinal properties, such as immune-boosting effects and antitumor, antiviral, anti-inflammatory, and antioxidant activities (Jiang et al., 2013; Lu et al., 2013; Peng et al., 2018). Furthermore, it has been documented that PSP can act as a neuroprotective agent, potentially addressing conditions such as diabetes, and osteoporosis, as well as alleviating inflammatory disorders (Wang et al., 2017; Zhang et al., 2015). Moreover, Shu et al. (2021) highlighted the importance of PSP in bolstering the immune system of immunosuppressed chickens treated with cyclophosphamide, suggesting its potential immunostimulant activity. Despite extensive research on the benefits of PSP in traditional human medicine, its application in poultry farming still needs to be explored. To fully exploit the benefits of PSP, additional investigation and fine-tuning are needed regarding factors including its optimal dosage, sourcing, specific mechanisms of action, and interaction with the bird's digestive system and microbiota.

As far as we know, the impacts of adding PSP to the broiler diets have yet to be extensively investigated. Therefore, this research aimed to examine the influence of PSP incorporation on growth performance, carcass traits, serum metabolites and minerals, nutrient digestibility, and tibia characteristics of broilers.

Material and methods

Ethics statement

The study's methodologies 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).

Experimental design and bird management

A total of 240 day-old male Ross-308 broiler chicks, with an average body weight of 44.98±0.17 g, were obtained from the hatchery of Bengbu Dacheng Food Co., Ltd. in Anhui, China. The chicks were randomly distributed into three experimental groups, each of 8 replications with ten birds per replication. PSP, a brown-yellow powder, sourced from Shaanxi Hannah Biotechnology Co., Ltd. (Xi'an, China), was incorporated into mashed corn-soybean meal basal diets. Starter and grower diets were formulated to meet the nutritional requirements of the strain (Table 1) (Aviagen, 2018). The chicks were provided with *ad libitum* access to the feed and fresh water throughout the study. Dietary supplementation of PSP was administered at 0, 400, and 800 $mg \cdot kg^{-1}$, representing the control, 400PSP, and 800PSP groups, respectively. The birds were housed in floor cages under a lighting program of 23L:1D for the first 7 days, followed by 18L:6D for the rest of the experimental period. Indoor temperature was kept at 35°C during the initial three days and gradually reduced to 20°C until 35 days of age. They were maintained under controlled environmental conditions throughout the study.

Table 1. Ingredients and composition of the basal diet

Items	Starter (1–21 d)	Grower (22–35 d)
Ingredients (%)		
soybean meal	38.65	34.10
maize	51.42	56.66
fish meal	3.50	2.00
ground limestone	0.90	1.05
maize starch	1.00	1.00
DL-methionine	0.09	0.10
iodine salt	0.25	0.25
dicalcium phosphate	0.95	1.10
soybean oil	3.00	3.50
vitamin-mineral premix[1]	0.24	0.24
Nutrient content[2]		
AME (MJ/kg)	11.35	12.46
crude protein (%)	23.12	20.47
lysine (%)	1.25	1.14
methionine (%)	0.48	0.43
methionine + cysteine (%)	0.84	0.76
calcium (%)	1.03	0.88
non-phytate phosphorus (%)	0.42	0.37

[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).

Growth performance

On 35 days of age and on a per-pen basis, feed intake (FI), body weight gain (BWG), initial and final body weight (FBW), and feed conversion ratio (FCR; g feed: g $gain^{-1}$) were measured.

Nutrient digestibility

At day 31 of age, a digestibility trial was performed to determine the digestibility coefficient of dry matter (DM), CP, EE, Ca, and P using the total collection method (Smeets et al., 2015). Eight birds were randomly selected, individually weighed and caged in metabolic cages for four consecutive days, after a 24-hour adaptation period. During these days, excreta were collected. The birds were provided with free access to diets and water during the collecting period. The dried excreta and diets underwent approximate analysis for DM (#934.01), CP (#968.06), EE (#934.01), and Ca and P (#990.08), following the AOAC guidelines (AOAC, 2006). Fecal nitrogen was determined using the procedure of trichloroacetic acid (Jacobsen et al., 1960).

Carcass characteristics

Carcass evaluation and sampling were conducted at the end of the experiment. From each group, eight chicks were randomly selected, fasted for 12 hours and then slaughtered by severing the jugular veins (Abdel-Moneim et al., 2022). The pectoral and leg muscles, hot carcass, thymus, gizzard, spleen, claw, wing, abdominal fat, proventriculus, bursa of Fabricius, and ingluvies were separated and weighed to estimate their relative weight to the LBW.

Serum biochemical analysis

Eight blood samples from each group were collected from the wing vein of the broilers at 21 days of age and post-slaughter at 35 days of age for biochemical assessment. The collected samples were centrifuged at 5°C and 3500 × g for 15 minutes, resulting in sera separation, which were subsequently stored at –80°C. Serum concentrations of glucose, albumin, alanine transaminase (ALT), total cholesterol, alkaline phosphatase (ALP), triglyceride, creatinine, urea, Ca, chlorine, iron, potassium, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were quantitatively measured using colorimetric methods in accordance with the guidelines provided by the commercial kits from the Nanjing Jiancheng Institute of Bioengineering (NJIB) in Jiangsu, China.

Tibia characteristics

On 21 and 35 days of age, the tibias (eight from each group) were extracted and cleared of the attached tissues to examine both their ash content and physical traits (e.g., the width, weight, and length). Following the procedure of Li et al. (2021), the ash content of the tibias was measured. Initially, after recording the left tibias' fresh weight, they were treated with petroleum ether for 96 hours to remove fat. Subsequently, they were dried at 105°C for 24 hours and then ashed in a muffle furnace for 12 hours at 550°C. The resulting tibias' ash contents were measured and calculated relative to their fresh weight.

Statistical analysis

The collected data underwent statistical analysis through the one-way ANOVA after confirming normality and homogeneity of variance with SPSS software (version 19.0; SPSS Inc., IL, USA). Tukey's multiple comparison test was used to determine statistically significant differences between means at $P<0.05$.

Results

Growth performance

The impacts of incorporating PSP into the diet on the growth performance of broilers are summarized in Table 2. Specifically, FBW and BWG exhibited linear and quadratic increases ($P<0.05$) in the 800PSP group. Moreover, the inclusion of PSP at 800 mg·kg^{-1} exhibited improvements ($P<0.05$) in the FCR and EPEI, while FI remained unaffected.

Nutrient digestibility

As illustrated in Table 3, the digestibility coefficients of CP and Ca were linearly increased ($P<0.05$) in PSP-treated birds compared with the unsupplemented ones. Digestibility of DM, EE, and P was not changed among the experimental groups.

Carcass characteristics

The impacts of feeding broilers with PSP-containing diets on their carcass characteristics are illustrated in Table 4. All studied carcass traits, including pectoral muscle, hot carcass, claw, wing, abdominal fat, gizzard, proventriculus, and ingluvies, were not influenced by dietary incorporation of PSP at the marketing age.

The weight of the bursa of Fabricius (%) was linearly elevated ($P<0.05$) in the 400PSP and 800PSP groups, while thymus weight (%) exhibited a linear raise ($P<0.05$) only in the 800PSP group (Table 4). The spleen index was not altered by PSP dietary supplementation.

Table 2. Impact of incorporating polysaccharides from *Polygonatum sibiricum* (PSP) in broilers' diet on their growth performance at the end of the experimental period

Items[1]	Levels of PSP, mg·kg^{-1}			SEM	P-values		
	0	400	800		PSP	L	Q
IBW (g)	45.33	45.06	44.54	0.169	0.151	0.610	0.717
FBW (g)	1652.2 b	1705.9 ab	1736.0 a	14.435	0.042	0.014	0.651
BWG (g.bird.period^{-1})	1606.9 b	1660.8 ab	1691.4 a	14.426	0.038	0.013	0.652
FI (g.bird.period^{-1})	2795.7	2788.1	2801.4	6.156	0.709	0.722	0.463
FCR (g feed.g gain^{-1})	1.740 a	1.680 ab	1.658 b	0.016	0.047	0.041	0.555

Means in the same row with different letters are significantly different at $P>0.05$, [1]IBW = initial body weight; FBW = final body weight; BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio; L = linear; Q = quadratic; SEM = standard error of means.

Table 3. Impact of incorporating polysaccharides from *Polygonatum sibiricum* (PSP) in broilers' diet on nutrient digestibility at the end of the experiment

Items[1]	Levels of PSP, mg·kg^{-1}			SEM	P-values		
	0	400	800		PSP	L	Q
DM (%)	71.33	71.47	71.90	0.482	0.909	0.688	0.902
CP (%)	77.53 b	78.77 b	80.30 a	0.443	0.006	0.002	0.760
EE (%)	87.40	88.50	88.40	0.323	0.350	0.240	0.401
Ca (%)	33.03 c	35.04 b	37.40 a	0.674	0.002	0.001	0.762
P (%)	67.10	68.03	68.53	0.329	0.209	0.093	0.739

Means in the same row with different letters are significantly different at P>0.05, [1]DM = dry matter; EE = ether extract; Ca = calcium; CP = crude protein; P = phosphorus; L = linear; Q = quadratic; SEM = standard error of means.

Table 4. Impact of incorporating polysaccharides from *Polygonatum sibiricum* (PSP) in broilers' diet on carcass traits (%) at the end of the experiment

Items	Levels of PSP, mg·kg^{-1}			SEM	P-values		
	0	400	800		PSP	L	Q
Dressing	72.96	71.82	71.91	1.458	0.947	0.788	0.857
Ingluvies	0.243	0.281	0.284	0.011	0.231	0.129	0.431
Proventriculus	0.412	0.405	0.417	0.006	0.714	0.743	0.460
Gizzard	1.669	1.565	1.600	0.031	0.409	0.385	0.311
Pectoral muscle	18.78 b	19.40 ab	20.29 a	0.250	0.031	0.010	0.758
Leg muscle	14.74	15.17	15.74	0.271	0.346	0.154	0.910
Wing	7.910	7.804	7.244	0.150	0.149	0.072	0.454
Claw	3.985	4.246	4.062	0.058	0.178	0.578	0.079
Abdominal fat	0.976	1.043	1.009	0.036	0.774	0.731	0.539
Immune organs index							
spleen	0.128	0.151	0.148	0.006	0.273	0.198	0.328
thymus	0.449 b	0.491 ab	0.525 a	0.013	0.037	0.012	0.864
bursa of Fabricius	0.175 b	0.201 a	0.200 a	0.005	0.047	0.040	0.177

Means in the same row with different letters are significantly different at P>0.05, L = linear; Q = quadratic; SEM = standard error of means.

Table 5. Impact of incorporating polysaccharides from *Polygonatum sibiricum* (PSP) in broilers' diet on serum biochemical constituents at 21 d of age

Items[1]	Levels of PSP (mg·kg^{-1})			SEM	P-values		
	0	400	800		PSP	L	Q
Glucose (mmol.l^{-1})	6.948	7.181	7.068	0.234	0.932	0.849	0.752
Liver function biomarkers							
albumin (g.l^{-1})	48.89	63.90	62.91	3.252	0.102	0.072	0.218
ALP (U.100ml^{-1})	9.505	10.15	9.947	0.303	0.705	0.581	0.540
ALT (U.100ml^{-1})	0.208	0.207	0.208	0.001	0.557	0.802	0.302
Renal function biomarkers							
urea (mg.l^{-1})	6.192	5.784	6.496	0.185	0.309	0.507	0.170
creatinine (mmol.l^{-1})	100.5	94.27	96.63	2.078	0.505	0.475	0.361
Lipid profile (mmol.l^{-1})							
total cholesterol	8.274	8.374	9.261	0.281	0.307	0.168	0.513
triglycerides	6.323	6.509	6.860	0.398	0.873	0.615	0.929
LDL-cholesterol	3.603	3.863	4.624	0.214	0.124	0.062	0.555
HDL-cholesterol	3.406	3.209	3.256	0.065	0.473	0.398	0.382

[1]ALT = alanine transaminase; ALP = alkaline phosphatase; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; L = linear; Q = quadratic; SEM = standard error of means.

Table 6. Impact of incorporating polysaccharides from *Polygonatum sibiricum* (PSP) in broilers' diet on serum biochemical constituents at 35 d of age

Items[1]	Levels of PSP, mg·kg^{-1}			SEM[2]	P-values		
	0	400	800		PSP	L	Q
Glucose (mmol.l^{-1})	72.18	75.37	75.61	1.606	0.657	0.419	0.685
Liver function biomarkers							
albumin (g.l^{-1})	7.393	7.454	7.111	0.255	0.861	0.679	0.732
ALP (U.100 ml^{-1})	9.691	10.01	10.37	0.273	0.630	0.347	0.962
ALT (U.100 ml^{-1})	0.208	0.211	0.205	0.211	0.069	0.213	0.155
Renal function biomarkers							
urea (mg.l^{-1})	6.509	6.478	6.446	0.191	0.992	0.901	0.999
creatinine (mmol.l^{-1})	99.52	90.32	91.46	2.412	0.251	0.182	0.314
Lipid profile (mmol.l^{-1})							
total cholesterol	13.18 a	9.828 b	9.801 b	0.499	>0.001	>0.001	0.017
triglycerides	10.76	9.803	9.761	0.248	0.179	0.103	0.369
LDL-cholesterol	5.852 a	3.905 b	3.679 b	0.302	>0.001	>0.001	0.029
HDL-cholesterol	5.178 a	3.963 b	4.174 b	0.180	0.003	0.005	0.015

Means in the same row with different letters are significantly different at P>0.05, [1]ALT = alanine transaminase; ALP = alkaline phosphatase; LDL = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; L = linear; Q = quadratic; SEM = standard error of means.

Table 7. Impact of incorporating polysaccharides from *Polygonatum sibiricum* (PSP) in broilers' diet on serum mineral concentrations at 21 and 35 d of age

Items	Levels of PSP, mg·kg^{-1}			SEM	P-values		
	0	400	800		PSP	L	Q
21 d							
calcium (mmol.l^{-1})	2.155 b	2.220 b	2.553 a	0.098	0.218	0.049	0.780
potassium (mmol.l^{-1})	1.801 b	1.886 b	1.962 a	0.037	0.222	0.048	0.950
chloride (mmol.l^{-1})	67.31	64.42	64.59	0.798	0.269	0.176	0.370
iron (mg.l^{-1})	64.79 b	66.50 ab	71.87 a	1.739	0.234	0.043	0.683
35 d							
calcium (mmol.l^{-1})	2.183	2.177	2.310	0.070	0.713	0.494	0.665
potassium (mmol.l^{-1})	1.838	1.829	1.901	0.043	0.848	0.855	0.594
chloride (mmol.l^{-1})	63.75	63.13	62.74	0.925	0.917	0.686	0.958
iron (mg.l^{-1})	65.35	64.42	65.43	1.599	0.965	0.986	0.795

Means in the same row with different letters are significantly different at P>0.05, L = linear; Q = quadratic; SEM = standard error of means.

Table 8. Impact of incorporating polysaccharides from *Polygonatum sibiricum* (PSP) in broilers' diet on tibia characteristics at 21 and 35 d of age

Items	Levels of PSP, mg·kg^{-1}			SEM	P-values		
	0	400	800		PSP	L	Q
21 d							
tibia weight (g)	4.157	3.368	4.648	0.256	0.079	0.721	0.059
tibia relative weight (%)	0.558	0.442	0.572	0.031	0.185	0.847	0.073
tibia length (cm)	6.793	6.253	6.557	0.130	0.247	0.454	0.136
tibia width (cm)	5.942	5.404	6.270	0.153	0.051	0.318	0.054
tibia ash content (%)	24.15 b	27.34 a	28.14 a	0.584	0.003	0.006	0.013
35 d							
tibia weight (g)	9.800	9.751	9.707	0.082	0.911	0.673	0.991
tibia relative weight (%)	0.558	0.548	0.556	0.008	0.885	0.921	0.635
tibia length (cm)	8.692	8.510	8.575	0.096	0.761	0.644	0.575
tibia width (cm)	8.011	7.743	8.063	0.142	0.648	0.474	0.564
tibia ash content (%)	35.27 b	45.69 a	47.15 a	1.785	0.003	0.004	0.020

Means in the same row with different letters are significantly different at P>0.05, L = linear; Q = quadratic; SEM = standard error of means

Serum biochemical indices

At day 21 of age, serum biomarkers for hepatic (albumin, ALT, and ALP) and renal (urea and creatinine) functions, lipid profile, and glucose level were not significantly affected by PSP inclusion in broiler diets (Table 5). Similarly, at 35 days of age, these parameters were not significantly influenced by PSP incorporation, except for lipid profile, which was reduced in PSP-treated groups (Table 6). Serum concentrations of total cholesterol, HDL, and LDL were reduced both linearly and quadratically in the 400PSP and 800PSP groups. The serum level of triglycerides was not altered among the experimental groups.

Serum minerals

The effects of feeding broilers diets containing PSP on their serum mineral concentrations are illustrated in Table 7. At day 21 of age, calcium, potassium, and iron levels were linearly elevated ($P<0.05$) in the 800PSP group. However, at day 35 of age, no changes were observed in the serum levels of calcium, potassium, chloride, and iron among the different experimental groups.

Tibia traits

As presented in Table 8, incorporating PSP into broilers' diets did not yield any notable impacts on the tibia's relative weight, width, or length at both 21 and 35 days of age. However, intriguingly, PSP inclusion at 400 and 800 $mg \cdot kg^{-1}$ showed a linear and quadratic increase ($P<0.05$) in the tibia ash content (%) in chickens at both ages.

Discussion

Plant-derived polysaccharides have gained attention due to their diverse biological properties, spanning antitumor and antiviral effects, hypoglycemic benefits, as well as immunostimulant, anti-inflammatory and antioxidant activities (Yang et al., 2024). These attributes have shown enhancements in broiler productivity and overall health. In this study, incorporating dietary PSP resulted in improved FBW and BWG. These findings align with earlier studies that noted enhanced juvenile broilers' growth when fed diets with 0.5, 1, and 2 $g \cdot kg^{-1}$ polysaccharides from *Astragalus membranaceus* for six weeks (Wu, 2018). Similarly, Long et al. (2020) and Yang et al. (2023) observed improvement in the growth of two strains of broilers (Arbor Acres and Cobb-500) with the treatment of polysaccharides from *Radix rehmanniae praeparata* (600 and 900 $mg \cdot kg^{-1}$) and *Lycium barbarum* (1000 and 2000 $mg \cdot kg^{-1}$). The enhanced broiler growth could be attributed to PSP's ability to improve gut permeability and nutrients uptake (Ren et al., 2017; Yang et al., 2024). Additionally, plant-derived polysaccharides have been reported to upregulate the digestive enzymes' expression. This upregulation increases the activity of lipase, protease, and amylase, eventually improving digestion function (Long et al., 2020). Moreover, Liu et al. (2021) emphasized that incorporating polysaccharides of *Yingshan yunwu* tea into the diet improved broilers' immunity and gut microbiota, offering an explanation for the noticed improvement in growth parameters.

The strong correlation between nutrient digestibility and animals' growth performance is a well-established fact. This study affirms that the inclusion of dietary PSP enhances the digestibility of CP and Ca in broilers. This could potentially account for the growth-promoting benefits of PSP. The same findings were noted by Xing et al. (2023), who documented that dietary supplementation of *Artemisia ordosica* polysaccharide at 750 $mg \cdot kg^{-1}$ elevated the digestibility coefficients of Ca and CP in normal Arbor Acres broilers and alleviated the reduction in CP digestibility in LPS-challenged ones compared to the control at 35 days of age. Previous research also indicates that incorporating plant extracts in broiler diets can improve nutrient digestibility by augmenting digestive enzyme activity, enhancing intestinal morphology, and influencing the composition of intestinal microflora (Park and Kim, 2020). Hence, the observed enhanced digestibility in our study may be attributed to these factors, contributing to the overall growth improvement in broilers.

The bursa of Fabricius, spleen, and thymus play pivotal roles as immune organs in poultry, fostering the development and growth of immune cells. The condition of these organs significantly influences the ability to resist diseases and the overall function of the immune system. In our study, we found that the PSP-treated groups exhibited an elevation in the relative weight of the bursa of Fabricius and thymus. The beneficial impact of PSP on broilers' immune organ performance could be ascribed to multiple pathways. Firstly, PSP protects the structure of immune organs from oxidative stress by elevating blood antioxidant levels. Secondly, it boosts the humoral immunity by enhancing immunoglobulin secretion and antibody production. Lastly, PSP shields immune organs by augmenting their relative weight, preserving their structural integrity, enhancing cell proliferation, reducing cell apoptosis, and regulating the expression of immune-related genes. Our findings align with those of Shu et al. (2021), who documented that polysaccharides derived from *Polygonatum sibiricum* at 800 $mg \cdot kg^{-1}$ preserved immune organ structure of broilers at 28 days of age and mitigated adverse effects caused by cyclophosphamide, as evidenced by histopathological examination.

The current results revealed that administering PSP did not impact renal and hepatic function biomarkers, indicating the absence of harmful impacts on the kidneys and liver due to the supplementary levels. This observation aligned with the enhanced growth performance as recorded in our study. Similar outcomes were documented by Shu et al. (2021), indicating that supplementation of PSP did not alter blood albumin and total protein levels. Additionally, serum albumin concentration serves as a marker reflecting protein metabolic and anabolism status in the liver, and the nutritional condition in broiler chicks (Gao et al., 2016).

The current study highlights the remarkable hypocholesterolemic effect of PSP, as evidenced by decreases in serum total cholesterol and LDL upon its dietary inclusion. Polysaccharides derived from plants exhibit various cholesterol-lowering actions, affected by many factors such as their fermentation by gut microbiota, chemical and physical properties, and sugar composition (Huang et al., 2018; Silva et al., 2021). These impacts involve inhibiting cholesterol esterase in the gut, and sequestering bile salts, thereby impacting the bioavailability of cholesterol (Hui and Howles, 2005). The presence of polysaccharides alters the viscosity of intestinal contents, impeding the diffusion of cholesterol-loaded micelles to the gastrointestinal lining, thereby limiting its absorption (Naumann et al., 2019). Moreover, polysaccharides bind to bile salts, reducing cholesterol emulsification and promoting its excretion in feces (Pengzhan et al., 2003). This reduction in recirculation of bile salts leads to increased conversion of endogenous cholesterol to primary bile salts (Garcia-Diez et al., 1996). Furthermore, short-chain fatty acids (SCFAs) generated from polysaccharide fermentation by gut microbiota inhibit cholesterol synthesis and participate in the formation of secondary bile salts critical for emulsification (Wahlström et al., 2016). The combined action and the potential synergetic impacts of these mechanisms elucidate the hypocholesterolemic effects of PSP observed in this study.

In this study, the incorporation of PSP resulted in elevated serum levels of calcium, iron, and potassium. Bone formation relies on essential minerals like Ca and K (Palacios, 2006). The elevated levels of Ca and K hint that PSP supplementation might stimulate bone growth and mineralization. The increased mineral levels in the birds' bloodstream indicate that PSP addition might improve the uptake of these minerals by the gut, resulting in enhanced utilization within the body. Although the precise mechanism behind increased mineral absorption by polysaccharides remains unclear, several potential mechanisms have been suggested (Ahmad et al., 2021). One possible effect contributing to increased absorption is the development of osmotically active sugars while fermenting polysaccharides after ingestion. These sugars promote the passive metal absorption, and fermentations generate weak organic acids facilitating mineral absorption (Nishito and Kambe, 2018). This is attributed to the fact that organic acids endogenously reduce the gut pH, facilitating the conversion of less absorbable forms of iron (Fe^{3+}) into the more readily usable ferrous form (Fe^{2+}) (Moustarah and Daley, 2024). Additionally, the fermentation of polysaccharides in the hindgut by intestinal microbiota leads to the formation of SCFAs, which include butyrate, propionate, and acetate. These SCFAs can stimulate epithelial cell proliferation, increasing the surface area for mineral absorption, and subsequently enhance mineral absorption (Alexander et al., 2019).

Leg ailments, a significant cause of setbacks in broiler farming, can result in bone malformations, disrupted coordination, and compromised bird welfare (Huang et al., 2019). One prominent example is tibia dyschondroplasia, a prevalent leg condition in broilers that detrimentally affects the quality of both pectoral muscles and legs (Cao et al., 2020). As presented in this study, the inclusion of PSP led to an increase in tibia ash content without influencing tibia relative weight, length, or width in broilers, suggesting that PSP primarily enhances bone mineralization rather than altering bone morphology or overall growth. These results align with a previous finding of Chen et al. (2015), which indicated that polysaccharides from *Trametes versicolor* enhanced bone health in diabetic rats. The heightened content of ash in the tibia reflects a greater mineral presence, encompassing Ca and K. Through PSP supplementation in the present study, enhanced mineral absorption was noticed, as evidenced by the elevation in serum potassium and calcium concentrations. This improved mineral absorption likely translates to the accumulation of these minerals in bone tissues, leading to an increased tibia ash content and fostering better bone mineralization (Yang et al., 2023). The intricate correlation between bone health, mineral uptake, and supplementing polysaccharides underscores the potential advantageous impacts of this feed supplement on the skeletal development of birds.

Conclusion

Based on our findings, this study presents new evidence suggesting that the inclusion of PSP in broiler feed could be a valuable addition, enhancing both their performance and well-being. The integration of PSP demonstrated strong hypocholesterolemic effects, enhanced nutrient digestibility, and elevated blood mineral levels, contributing to enhanced growth performance without adverse effects on tibia characteristics. The recommended level of supplementation was established to be 800 $mg \cdot kg^{-1}$. Nevertheless, more investigations are needed to determine the optimal incorporation level of PSP in broiler diets and fully grasp its mechanisms of action.

Conflict of interest

All authors declare that they have no conflicts of interest that could inappropriately influence this manuscript.

Data availability statement

The data supporting this study's findings are available on reasonable request from the corresponding author.

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