

Physicochemical properties of raw potato starch and its protective effect on bone quality in meat ducks with enteritis

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ABSTRACT

Compromised bone quality predisposes domesticated birds to fractures in meat ducks, leading to pain and bad welfare. As a fermentable type II resistant starch, raw potato starch (RPS) probably supports gut health and potentially mitigate this inflammatory bone loss. Accordingly, the physicochemical properties of RPS were characterized, and its protective effect on bone quality in meat ducks were evaluated in the present study. The outcomes from electron microscope and X-ray diffraction showed that RPS possessed a round or oval granules with a smooth surface and a B-type crystalline structure. Subsequently, a total of 60 1-day-old meat ducks were randomly assigned to two treatment groups and fed either a basal diet or a diet containing 12% RPS until 21 d (Animal trial 1). The results revealed that dietary supplementation of RPS increased ($P < 0.05$) tibial ash content of meat ducks, which negatively correlated gut leak and serum inflammatory status. To confirm the protective role of RPS in enteritis-induced bone loss in meat ducks. Ducklings were allocated to a phosphate-buffered saline control group, dextran sodium sulphate (DSS) oral administration group, and DSS treatment and fed a 12% RPS diet group with 8 repetitions of 10 birds each to 21 d (Animal trial 2). Duck challenged DSS exhibited increased bone resorption and compromised tibia density and mechanical properties ($P < 0.05$). DSS treatment downregulated the expressions of tight junction proteins and triggered intestinal inflammation in ileum, whereas failed to change the composition of caecal microbiota. In meat ducks with DSS-induced enteritis, feeding a diet of RPS reversed inflammation-induced bone loss. This effect was accompanied by increased serum phosphorus levels ($P < 0.05$) and reduced pro-inflammatory cytokines in the bone marrow microenvironment, leading to decreased osteoclastic bone resorption. These findings support that a diet with 12% RPS might be one of promising holistic approaches for mitigating systemic inflammation and consequent bone loss in meat ducks.

Introduction

Leg deformities in meat ducks are a significant welfare and economic concern in commercial poultry production, leading to reduced mobility and feed intake. It is estimated that leg problems account for approximately 2% of mortality, with up to 42% of ducks affected by conditions such as tibial dyschondroplasia (Zhang, et al., 2018). In addition to genetic factors, nutritional density, and feeding management, intestinal homeostasis is also closely linked to bone turnover in domesticated birds. Impairment of intestinal barrier function has been shown to compromise tibia mass in broilers, a defect that can be reversed by

improving intestinal integrity (Zhang, et al., 2021). In particular, meat ducks are highly sensitive to environmental stress, pathogens, and feed toxins, these factors known to trigger low-grade intestinal inflammation and damage barrier function (Bi, et al., 2018). By inflaming the intestinal epithelium, these pathogenic processes lead to villous blunting and a reduced surface area (Conrad, et al., 2019). These structural changes directly impair the absorption of critical bone-forming nutrients, including calcium (Ca) and vitamin D, thereby compromising bone quality and resulting in osteoporosis development. Meat ducks challenged with *Escherichia coli* showed decreased bone mass, which was accompanied by inflammation in both the ileum and bone marrow

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(Zhang, et al., 2022a). These findings suggest that the disturbance of intestinal homeostasis probably induces bone loss in domesticated birds.

In the pharmacologic management of intestinal inflammation, conventional treatment strategies aim to control symptoms through agents such as antibiotics and other biologics. However, a significant adverse effect of these therapies, especially antibiotics, is a twofold increase in the risk of osteoporosis in meat duck and mice (Rios-Arce, et al., 2020). Consequently, optimizing existing therapies through patient stratification, personalized medicine, and nutritional adjuvant therapy has become an increasing priority. Early intervention is therefore critical, as preventive strategies like targeted dietary modification during preclinical intestinal dysfunction can potentially halt the progression of enteritis and its associated bone loss. As a nutritional intervention, resistant starch acts as a fermentable substrate for the hindgut microbiota, which fosters a beneficial host-microbiome relationship that supports intestinal health in meat ducks, positioning it as a potential complementary therapy to prevent intestinal inflammation and related complications (Qin, et al., 2023). Based on a meta-analysis of 9 randomized controlled trials, resistant starch supplementation (22–45 g/d) significantly improved intestinal barrier function in healthy subjects (Shen, et al., 2017). Study on obese mice found that the supplementation of potato resistant starch could modulate the gut microbiota to reduce the releases of inflammatory factors in intestine (Liang, et al., 2021). Moreover, feeding meat ducks a diet supplemented with 12% raw potato starch (RPS), a type II resistant starch, was found to alleviate lipopolysaccharide-triggered inflammation and intestinal damage (Qin, et al., 2022). The benefits of resistant starch extend beyond intestinal health to bone metabolism. Rodent models have shown resistant starch attenuates bone loss in ovariectomized mice and reduces arthritis-induced bone erosion through microbiota-mediated modulation of bone-marrow inflammation (Bai, et al., 2021). Study on meat ducks found that the dietary graded level of RPS supplementation from 0 to 24% could enhance tibia strength and ash to varying degrees in Cherry Valley duck, which related with the increased contents of propionate and butyrate in the cecum digest (Zhang, et al., 2022b). Meat ducks consumed the diet with 12% RPS was noticed to mitigate *Escherichia coli*-induced bone loss by inhibiting NF- κ B inflammasome activation (Zhang, et al., 2022b), implying that dietary supplementation with resistant starch may be a potent nutritional adjunct for mitigating both intestinal inflammation and bone loss. Given that RPS resists digestion in the upper gastrointestinal tract and can be fermented by gut microbiota, changes in gut microbial composition may serve as one potential mechanism responsible for the beneficial effects of RPS on bone metabolism. Notably, gut microbiome dysbiosis has been detected in both patients with osteoporosis and ovariectomized rodent experimental models (Seely, et al., 2021). A comparison between germ-free and conventionally raised mice revealed that microbial colonization reduces trabecular and cortical bone mass (Schwarzer, et al., 2016). Consistently, gut microbiota manipulation via oral antibiotic treatment or microbiota transplantation from conventionally raised donors alters bone density and inflammatory status in mice (Yan and Charles, 2017).

Owing to the resistance of RPS to upper gastrointestinal digestion and its subsequent fermentation mediated by gut microbiota, waterfowl are endowed with prominent intestinal fermentative potential and efficient energy acquisition from indigestible carbohydrates, making feasible the practical use of RPS in duck farming (Józefiak, et al., 2004). However, though exploring the correlation between chronic gut inflammation and bone metabolism and verifying dietary regulation efficacy is vital, chronic enteritis models for meat ducks are lacking. For this purpose, oral gavage of dextran sodium sulphate (DSS) was performed to induce intestinal inflammation in meat ducks, enabling this study to investigate the interplay between intestinal homeostasis and bone metabolism in the presence of dietary RPS, which constitutes a major innovation of this work. In addition, It is well known that starch crystallinity, chain-length distribution and thermal properties govern its digestibility and physiological functions (Bajaj, et al., 2018). High

crystallinity suppresses enzymatic digestion in the upper gut, while chain-length distribution modulates hindgut fermentation and intestinal microbiota. Thermal stability, indicated by thermal properties, affects starch performance during processing and its biological effects *in vivo*. Therefore, the present study first undertook a comprehensive physico-chemical characterization of RPS, analysing its granule morphology, crystallinity pattern, chain-length distribution, pasting and thermal properties, to establish a foundational material profile. Subsequently, systematic research was conducted to evaluate the effect of RPS on tibia quality in both conventional and enteritis-induced duck models, combined with serum biochemistry analysis and caeca microbiome sequencing. This research will provide essential data to determine the therapeutic potential of RPS in preventing bone loss due to chronic intestinal inflammation.

Materials and methods

Determination of RPS properties

The RPS used in this study was obtained from AVEVE Co. Ltd (Leuven, Belgium) and subsequently characterized in terms of its microstructure, crystallinity pattern, chain length, pasting characteristics, and thermal properties.

- (1) Microstructure. The microstructure of RPS was examined using a scanning electron microscope (Hitachi Model S-3000H) at an acceleration voltage of 5 kV. Images were captured at various magnifications to assess granule morphology, size distribution, and surface features, following the standardized protocol as previously described (Polesi and Sarmento, 2011).
- (2) Crystallinity pattern. The crystallinity pattern of RPS was analysed by X-ray diffraction (XRD) using a diffractometer (Shimadzu XRD 7000, Beijing, China) equipped with a Cu K α radiation source ($\lambda = 1.5406 \text{ \AA}$). Scans were performed in continuous mode over a 2θ angular range of 4° to 60° with a step size of 0.02° and a dwelling time of 2 s per step. Diffraction patterns were collected and processed to assess the degree of crystallinity and identify crystalline phases present in the sample.
- (3) Chain-length distribution. The chain-length distribution of the RPS was determined by fluorophore-assisted carbohydrate electrophoresis according to a modified method based on previous description (Ma, et al., 2017). Approximately 10 mg of isolated and purified amylopectin was suspended in 0.9 mL of distilled water and heated in a 95°C water bath for 15 min. Subsequently, 2.5 mL of 0.1 mol/L sodium acetate buffer (pH 4.0) containing 5 μL of isoamylase (1,000 U/mL) was added. The reaction mixture was incubated at 40°C for 24 h. The enzymatic hydrolysis was terminated by boiling the sample for 20 min. The mixture was then centrifuged, and the supernatant was collected and filtered through a $0.45\text{-}\mu\text{m}$ membrane. The filtered hydrolysate was analysed by high-performance size-exclusion chromatography (HPSEC, Thermo Scientific, San Jose, CA, USA) using a refractive index detector. The mobile phase was a 0.1 mol/L sodium acetate solution, delivered at a constant flow rate of 0.6 mL/min. Chain-length distributions were derived from the resulting chromatogram by peak integration, which typically exhibited a bimodal profile.
- (4) Pasting characteristics. The pasting properties of 10% (w/v) aqueous suspensions of RPS were analysed using a Rapid Visco Analyser (RVA; Perten Instruments, Hägersten, Sweden). A standard heating and cooling profile were applied: the samples were equilibrated at 50°C for 1 min, heated to 95°C at a rate of $12^\circ\text{C}/\text{min}$, held at 95°C for 3 min, cooled to 50°C at $12^\circ\text{C}/\text{min}$, and finally maintained at 50°C for 2 min. Key pasting parameters, including peak viscosity (PV), final viscosity (FV), and

gelatinization temperature (GT), were derived from the resulting viscosity versus time curve using the proprietary software.

- (5) Thermal properties. The thermal properties of RPS were characterized using differential scanning calorimetry (DSC) on a DSC 4000 instrument (PerkinElmer, Waltham, MA, USA). Approximately 5 mg of this dispersion was sealed in an aluminium DSC pan and heated from 25 to 110°C at a constant rate of 10°C/min, using an empty pan as a reference. The temperatures of the onset (To), peak (Tp), and conclusion (Tc) were read based on the received thermograms.

Animals, diets, and management

The trial utilized Cherry Valley M3 medium-sized meat ducks (male), purchased from a local hatchery. All ducks were housed under a 23:1 light-dark cycle from d 1 to 21, with *ad libitum* access to feed and water. The house temperature was maintained at $32 \pm 2^\circ\text{C}$ from d 1 to 3, then linearly decreased to 22°C by d 21. Relative humidity was maintained between 50% and 60% in practice. The basal diet was formulated as a 2-mm-diameter pellet diet to meet the nutritional requirements of meat ducks (NY/T 2122-2012), with the dietary composition detailed in Table 1. The 12% RPS inclusion level used in this study was based on our recent research (Zhang, et al., 2022b). RPS used in the present study were obtained from AVEBE Ltd (Veendam, Netherlands) and contained 54.7 % resistant starch (dry matter basis). The resistant starch content in the diets was analyzed using a commercial kit (Megazyme Ltd, Wicklow, Ireland) following AACC Method 32-40.01 (McCleary, et al., 2002). The dry matter, crude protein, Ca, and total phosphorus of diets was determined according to the Association of Official Analytical Chemists (AOAC, 2012). Both confirmed the proper preparation of experimental diets. All animal procedures complied with the regulations of the Animal

Care and Use Committee of Henan Agricultural University (Approval No. HNND-2025-021), in which the ducks were euthanatized by sodium pentobarbital (30 mg/kg of body weight) injected intravenously via the brachial vein.

Study design

Trial 1. The effects of RPS on the intestinal integrity and bone mass of meat duck were first investigated in this study. A total of 60 1-day-old meat ducks were randomly assigned to two treatment groups and fed either a basal diet or a diet containing 12% RPS until 21 d. Each group included 30 ducks and housed in three separate cages ($2.2 \times 1.2 \times 0.9$ m). On d 21, all ducks were weighed after an 8-hour fast, blood samples were collected and centrifuged at $3000 \times g$ for 15 min at 4°C to gain serum for D-Lactic acid and inflammatory cytokines contents test. The left tibiae were collected for the determination of ash content.

Trial 2. To investigate the relationship between intestinal homeostasis and bone metabolism, a DSS-induced enteritis model was established in meat ducks. For this, a total of 240 ducklings were allocated to a phosphate-buffered saline (PBS) control group (Ctrl), DSS oral administration group (DSS), and DSS treatment and fed a 12% RPS diet group (DSS+RPS) with 8 pens of 10 birds each. Each individual pen was defined as the experimental unit. The DSS-treated birds were consecutively given 1.2 g/kg body weight DSS by oral gavage starting on d 14 to 21 of age, with minor modifications to the protocol described in a previous study (Chang, et al., 2024). During the trial period, body weights at 1, 14, and 21 d of age, feed intake, the number of death and culling birds were recorded for calculating the body gain, feed intake to body gain ration, and mortality during the periods of d 1-14 and d 15-21. On d 21, blood samples were collected to quantify serum levels of inflammatory cytokines and bone turnover biomarkers. Birds were then euthanized for tissue collection. The left tibia was harvested for bone property analysis, and the mid-ileum was collected for assessment of intestinal integrity. Bone marrow was aspirated from the right tibia for determining inflammatory profile. Additionally, cecal contents were collected, snap-frozen in liquid nitrogen, and stored at -80°C for subsequent analysis of microbial composition.

Ileum lesion score

The ileum was cut longitudinally, and the intestinal lesion score was determined following the previously described (Vidanarachchi, et al., 2013). Specifically, normal intestinal appearance without gross lesions (score = 0, normal); thin-walled or friable and few whitish plaques in the serosal surface (score = 1, mild); thin-walled, focal necrosis or ulceration, small amounts of gas production (score = 2, moderate); thin-walled, large patches of necrosis, gas-filled intestine, small flecks of blood (score = 3, marked or severe); severe extensive necrosis, marked haemorrhage, excessive amounts of gas in the intestine (score = 4, very severe).

Tibial growth, mechanical property, and bone mass

Following soft tissue removal, tibial length and perimeter were measured using tape measure. Bone mechanical properties were assessed via three-point bending test using a texture analyser (TA.XT. Plus, Stable Microsystems Ltd., UK) at a constant load of 50 kg until failure. Force-displacement data were acquired to determine yield load, ultimate load, energy to failure (area under the curve, AUC), and stiffness. Stiffness was calculated as the slope of the linear elastic region of the force-displacement curve. Subsequently, all fragments from each tibia were pooled, and bone volume was measured using the water displacement method. The samples were then degreased via extraction in ethyl ether for 48 h, followed by oven drying at 105°C for 24 h to obtain the fat-free weight. Bone density was calculated as the fat-free weight (g) divided by the tibial volume (cm^3). Finally, the dry,

Table 1
Dietary formulation and composition (as fed basis).

Item	Basal diet	Raw potato starch diet
Ingredients, %		
Corn	59.82	44.85
Soybean meal	33.22	35.91
Raw potato starch	0.00	12.00
Soybean oil	0.50	1.50
Calcium Carbonate	1.10	1.04
Dicalcium phosphate	1.75	1.82
L-Lysine-HCL	0.12	0.07
DL-Methionine	0.16	0.17
L-Threonine	0.02	0.02
Bentonite	2.33	1.64
Sodium chloride	0.30	0.30
Choline chloride	0.15	0.15
Vitamin premix ¹	0.03	0.03
Mineral premix ²	0.50	0.50
Total	100.0	100.0
Calculated analysis, %		
Apparent metabolism energy, MJ/kg	11.72	11.73
Crude protein	19.50	19.51
Calcium	0.90	0.89
Non-phytate phosphorus	0.42	0.41
Digestibility Lysine	1.00	0.98
Digestibility Methionine	0.42	0.43
Nutrient analysis (%)		
Resistant starch	3.73	7.66
Crude protein	19.37	19.63
Calcium	0.91	0.93
Total phosphorus	0.70	0.68

¹ Provided per kilogram of diet: Cu ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 8 mg; Fe ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 80 mg; Zn ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), 90 mg; Mn ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$), 70 mg; Se (NaSeO_3), 0.3 mg; I (KI), 0.4 mg.

² Provided per kilogram of diet: retinol, 2.06 mg; cholecalciferol, 0.04 mg; vitamin E, 30.01 mg; thiamine, 1 mg; riboflavin, 3.9 mg; pyridoxine, 3.375 mg; vitamin B₁₂, 0.01 mg; calcium pantothenate, 8.85 mg; folate, 0.5 mg; biotin, 0.1 mg; niacin, 49.25 mg.

defatted tibiae were ashed in a muffle furnace at 550°C for 24 h to determine the ash content, which was expressed as a percentage of the fat-free weight.

Serum biochemical determination

Ileal mucosal supernatants were prepared by homogenizing tissues in ice-cold saline (1:9, w/v) followed by centrifugation. The concentrations of interleukin (IL)-1 β , IL-6, IL-10, and tumour necrosis factor (TNF)- α in serum and mucosal supernatants were quantified using specific enzyme-linked immunosorbent assay (ELISA) kits (Jiangsu Meimian Industrial Co., Ltd., Jiangsu, China), according to the manufacturer's instructions. Total protein content in the supernatants was determined using a bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA). D-Lactic acid concentration was measured with a commercial enzymatic assay kit (Megazyme, Ireland). As the markers of bone resorption, serum C-terminal cross-linked telopeptide of type I collagen (CTX) and tartrate-resistant acid phosphatase (TRAP), were quantified using commercial assay kits. Similarly, serum markers of bone formation, procollagen type I N-terminal propeptide (PINP) and alkaline phosphatase (ALP), were measured with their respective commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Serum total Ca was determined using the ortho-cresolphthalein complexone method, and inorganic phosphorus (P) via the molybdenum blue method, with a biochemical analyser (Yellow Springs Instrument Co. Inc., Yellow Springs, OH). All samples were analysed in triplicate within each assay.

Gene expression by real-time PCR

Total RNA was extracted from ileal mucosa, proximal tibia metaphysis, and flushed bone marrow using TRIzol™ Reagent (Invitrogen, Carlsbad, CA, USA). For reverse transcription, 1 μ g of total RNA from each sample was used as template in a 20 μ L reaction volume with the PrimeScript™ RT Reagent Kit (Takara Bio, Dalian, China), employing a combination of oligo (dT) and random hexamer primers to ensure comprehensive cDNA representation. RT-PCR analyses were performed using the ABI 7900 Sequence Detection System (Applied Biosystems, CA, USA), with three replicates per sample. Specific primers for each gene were designed using the NCBI Primer-BLAST tool (Table 2). The relative mRNA abundance of the targeted gene was adjusted for the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β -actin.

16S rRNA gene sequencing

Total genomic DNA was extracted from cecal contents using the PSP Spin Stool DNA Plus Kit (Invitex, Westburg, Netherlands). After evaluation of DNA concentration and purity, the V3-V4 hypervariable region of the bacterial 16S rRNA gene was amplified with the universal primers 338F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR products were purified, quantified, and pooled in equimolar amounts, and paired-end sequencing (2 $\times$ 250 bp) was performed on an Illumina MiSeq platform (Illumina, San Diego, CA, USA). Raw sequence data were processed using QIIME 2 (v2023.5). The obtained sequences were processed using MOTHUR (software package v1.39.5) for alignment and clustering (Schloss, et al., 2009), while the VSEARCH algorithm was utilized for chimera detection (Rognes, et al., 2016). Sequences were demultiplexed, quality-filtered (q $\geq$ 20), trimmed, and merged. Chimeric sequences were identified and removed using DADA2. Taxonomy was assigned against the SILVA (v138) reference database at a 97% similarity threshold. In subsequent statistical analyses, beta-diversity was estimated by calculating Bray-Curtis distances and visualized with principal co-ordinates analysis (PCoA). Normality and homogeneity of variance were verified prior to group comparisons, and the differences among Ctrl, DSS, and DSS+RPS groups were analyzed via one-way analysis of variance (ANOVA)

Table 2
Primers for quantitative real-time PCR.

Gene	Gene ID	Primer	Sequence (5'-3')	Size (bp)
ZO-1	XM_013104939.1	Reverse	tacgcctgtgaagaatgcag	86
		Forward	ggagtggtgtgtgttgcctt	
Occludin	XM_013109403.1	Reverse	caggatgtggcagaggaataca	160
		Forward	cctgtctgtagtcgctcaccat	
Claudin-1	XM_013108556.1	Reverse	tcattgtatggcaacagagtg	179
		Forward	cgggtgggtggataggaagt	
Mucin-2	XM_038180256.1	Reverse	tcctctgcatgtgacgactg	110
		Forward	cagaggcagaaggtgtcctc	
IL-17a	NM_001310350.1	Reverse	tccaacccttcgtctcttc	95
		Forward	gccgtatcaccttcccgtag	
IL-22	XM_038167538.2	Reverse	gctgtgtgtctctccctct	145
		Forward	ttagccaagggttaggtg	
TNF- α	EU375296.1	Reverse	agatgggaagggaatgaacc	51
		Forward	gttggcatagctgtcctgt	
IL-1 β	DQ393268.1	Reverse	gcatacaagggtacaagctc	131
		Forward	caggcggtagaagatgaagc	
IL-6	AB191038.1	Reverse	atctggcaacgacgataagg	87
		Forward	ttgtgaggagggtattctgg	
IL-10	NM_001310368.1	Reverse	ctgacctctaccagcgaag	106
		Forward	gagctgagcagctgaatgc	
TGF- β	NM_011577.2	Reverse	gactctccactgcgaagacc	99
		Forward	gactggcgagccttagttg	
β -actin	NM_001310421.1	Reverse	atttcgacgagagatggcc	108
		Forward	aaagatggctggaagagggc	
GAPDH	XM_038180584.2	Reverse	aatgttctggcagctctc	94
		Forward	gaccactgtccatgccata	

ZO-1, zonula occludens 1; TNF- α , tumor necrosis factor alpha; TGF- β , transforming growth factor beta; IL, interleukin; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

followed by Tukey's post-hoc test or Kruskal-Wallis H test with multiple pairwise comparisons using R software.

SCFAs determination

The concentrations of SCFAs in cecal digesta were determined using gas chromatography (GC, Varian CP-3800 instrument). Briefly, approximately 0.5 g cecal sample was homogenized in 2 mL ultrapure water and centrifuged at 3,000 g for 15 min. The resulting supernatant was collected, and 1 mL supernatant was combined with 0.2 mL pre-cooled 25% metaphosphoric acid solution, followed by incubation at 4°C for 30 min. The mixture was re-centrifuged at 3,000 g for 15 min, and acetate, propionate and butyrate contents were quantified via GC analysis.

Statistical analysis

Statistical analysis was performed using JMP software (version 13.0, SAS Institute, Cary, NC, USA). The normality of residuals was assessed using the Shapiro-Wilk test, and homoscedasticity was verified with Levene's test. Differences between the Ctrl and RPS groups were analysed using an unpaired, two-tailed Student's *t*-test. Multiple group comparisons were conducted via one-way ANOVA, with Tukey's post-hoc test for pairwise comparisons. Pearson correlation coefficients were calculated to assess linear relationships among tibial ash content, serum D-lactic acid, and the TNF- α /IL-10 ratio. For microbial 16S rRNA amplicon sequencing data, community-level statistical analyses were conducted in R using the Microbiota Process package (version 3.17). Data are presented as mean $\pm$ standard deviation (SD). A *P* $\leq$ 0.05 was considered statistically significant, and 0.05 < *P* < 0.10 was regarded as a statistical tendency.

Results

Molecular structure and thermal properties of RPS

The outcomes from scanning electron microscopes showed that the RPS displayed a round or oval structure with a smooth surface (Fig. 1A) and a B-type crystal structure, with characteristic peaks of weak, intermediate, and strong intensity at $2\theta = 17.6^\circ$, 22.5° , and 24.2° , respectively (Fig. 1B). The chain-length distribution was identified according to different degrees of polymerization (DP). There were 27.1% short A chains (DP 6-12), 49.0% medium B₁ chains (DP 13-24), 14.8% long B₂ chains (DP 25-36), and 6.5% super-long B₃ chains (DP ≥ 37), which was presented in Fig. 1C. The viscosity of RPS was characterized by showed a PV of 4432.4 mPa·s, a FV of 1629.3 mPa·s, and a GT of 66.7°C (Fig. 1D, E). Moreover, thermal analysis (Fig. 1F) revealed gelatinization onset (To), peak (Tp), and conclusion (Tc) temperatures of 60.6°C , 63.4°C , and 84.8°C , respectively.

RPS improved the tibia ash and might linked to the enhanced intestinal barrier function

Following structural characterization of RPS, a large-scale trial was conducted to evaluate the effects of dietary RPS on body weight, intestinal integrity, and bone quality in meat ducks. Herein, the birds consummated 12% RPS diet possessed a comparable body weight and increased tibia ash content ($P = 0.035$) than those fed the basal diets (Fig. 2A, B). Intestinal integrity, assessed by serum D-lactate fluctuation, was significantly improved by RPS supplementation (Fig. 2C). This was accompanied by a reduction in pro-inflammatory cytokines, with a significant decrease in IL- 1β ($P = 0.047$) and a trend for lower TNF- α ($P = 0.063$, Fig. 2D). In contrast, there was an increasing trend in the anti-inflammatory cytokine IL-10 ($P = 0.075$; Fig. 2E) and thereby reducing the ration of TNF- α to IL-10 ratio, a marker of inflammatory status (Fig. 2F). Pearson correlation analysis revealed a significant negative correlation between tibia ash content and both serum D-lactate and the

TNF- α /IL-10 ratio (Fig. 2G, H).

Dietary RPS supplementation improved intestinal barrier function and microbial composition

To confirm whether the enhanced bone mass by dietary RPS intervention is associated with the alteration in intestinal barrier function, a DSS-induced enteritis model was established. The DSS-treated birds displayed thinner and friable walled relative to the ducks from the Ctrl or DSS+RS group, but the multiple hemorrhagic spots failed to be found in the serosal surface. Accordingly, the DSS administration induced an increase in the score of ileal lesions ($P = 0.044$; Fig. 3A). With no apparent changes in the growth performance, including body weight, weight gain, feed intake, and its ratio, as well as mortality (Table 3), the DSS treatment resulted in the disturbance of intestinal integrity, evidenced by downregulated ($P = 0.039$) expressions of zonula occludens-1 (ZO-1), *occludin*, and *mucin-2* (Fig. 3B). However, the administration of DSS failed to alter the composition of cecal microbiota but increased the relative abundance of Proteobacteria (Fig. 3C-F). In response to dietary RPS treatment, the mRNA levels of tight junction proteins and *mucin-2* were increased by RPS supplementation in the DSS-treated ducks. At the phylum level, 16S rRNA sequencing identified Firmicutes and Bacteroidetes as the dominant taxa. Supplementation with 12% RPS significantly modulated the microbiota community, increasing the relative abundance of Firmicutes and decreasing Bacteroidetes, thereby raising the Firmicutes/Bacteroidetes ratio ($P = 0.046$). In terms of SCFA concentrations, which was presented in Table 4, DSS challenge did not significantly alter cecal SCFA levels compared with the Ctrl group. Relative to the DSS-alone group, dietary inclusion of 12% RPS markedly elevated the concentrations of acetate ($P = 0.048$), butyrate ($P = 0.034$) and total SCFAs ($P = 0.022$) in cecal digesta. Moreover, these RPS-induced alterations in gut barrier and microbiota were associated with a reversal of the elevated pro-inflammatory cytokine levels in the ileum, especially reduced the mRNA expression of IL- 1β ($P = 0.037$, Fig. 3G).

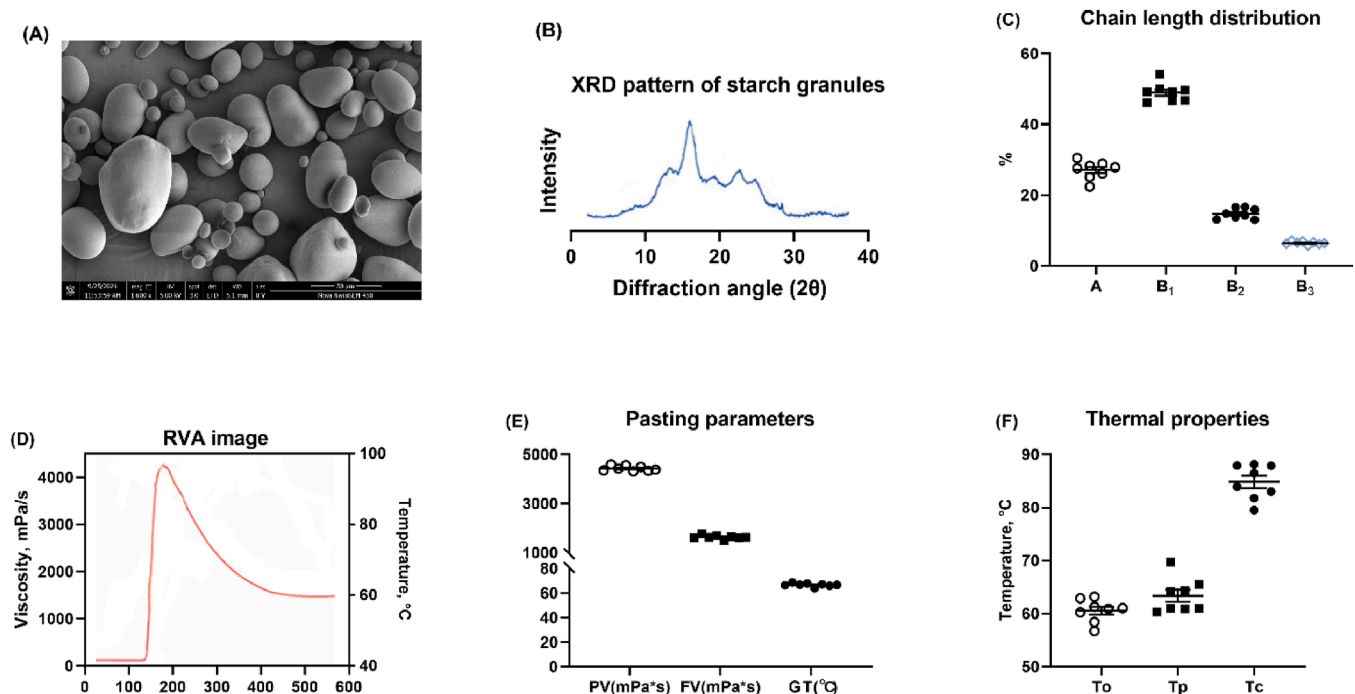


Fig. 1. Molecular structure and properties of raw potato starch (RPS). (A) Scanning diagram of the morphology of RPS; (B) X-ray diffraction pattern; (C) Chain length distribution of RPS; (D, E) Rapid Visco Analyser (RVA) image of starch and (E) pasting parameters, including pasting temperature (PT), peak viscosity (PV), and gelatinization temperature (GT); (F) The temperatures of the onset (To), peak (Tp), and conclusion (Tc) were read based on the received thermograms. Data is presented as the mean and standard deviation.

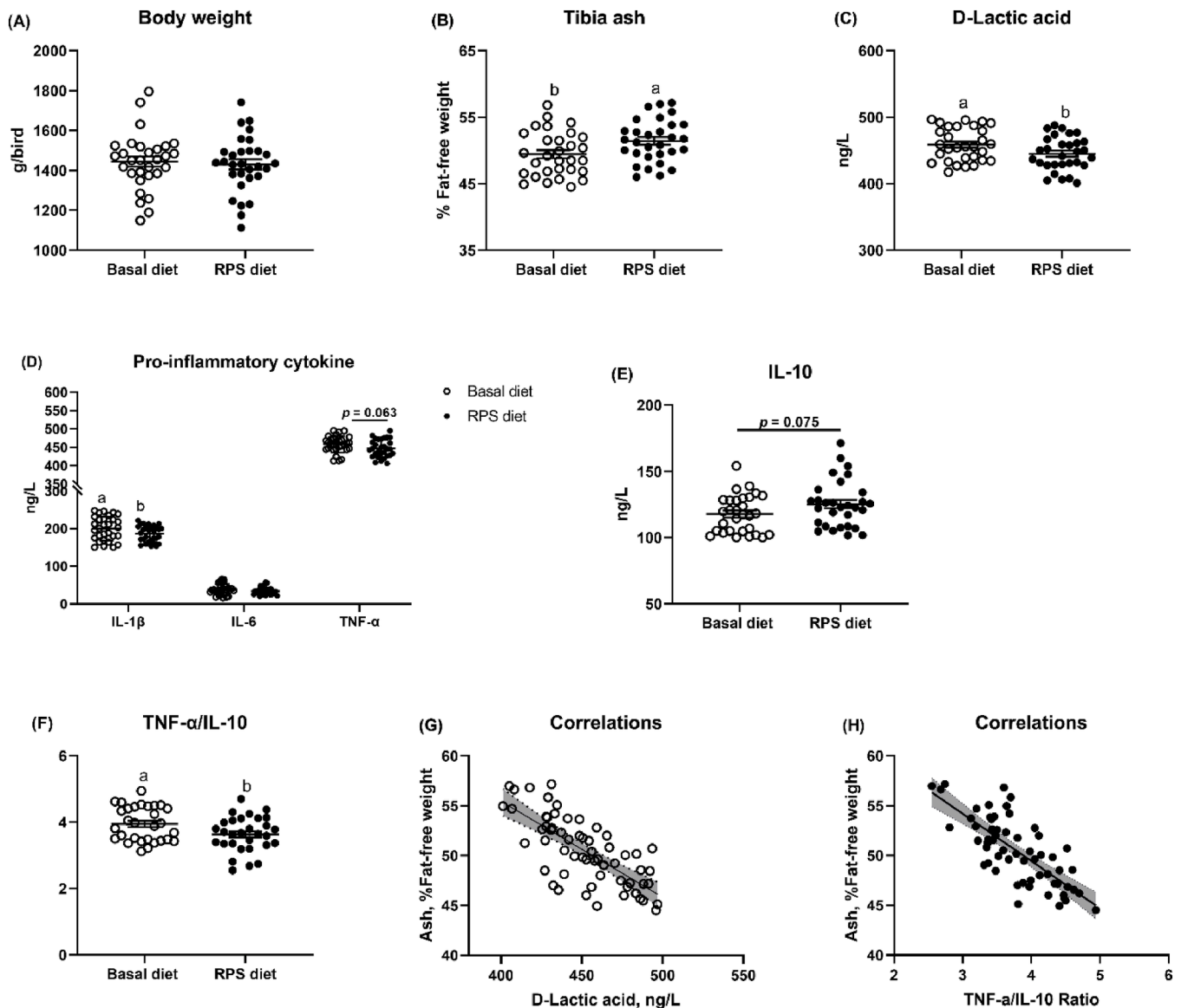


Fig. 2. The effects of dietary raw potato starch (RPS) on (A) body weight, (B) tibia ash, (C) serum D-lactic acid content, and (D-F) serum inflammatory cytokines in meat ducks. (G, H) Pearson's correlation between tibia ash content and serum D-lactic acid and TNF- α /IL-10. Data are expressed as mean and standard deviation. Statistical significance showed as different letters at $P < 0.05$. TNF- α , tumour necrosis factor alpha; IL, interleukin.

Dietary RPS intervention normalized enteritis-induced bone loss

Analysis of tibial characteristics revealed that neither DSS nor RPS affected bone growth, as tibia length and diameter were unchanged across groups (Table 5). While tibia density was significantly reduced by DSS treatment, which was further restored due to dietary RPS supplementation. In terms of mechanical properties, DSS decreased the ultimate load and slope, but did not alter the yield load or AUC. Dietary RPS supplementation in DSS-treated ducks notably reversed the DSS-induced reductions in tibia density, ultimate load, and slope. However, RPS did not alter tibia dimensions, ash content, yield load, or AUC compared to DSS-treated controls.

Inhibition of bone resorption and P absorption served as a potential mediator for PRS attenuating enteritis-induced bone loss

To further investigate the protective mechanism of dietary RPS, inframammary status, bone turnover, as well as serum Ca and P content were determined in this study. DSS challenge significantly increased

pro-inflammatory cytokines expression, including TNF- α , IL-1 β , and IL-17a ($P < 0.05$), which was reversed by dietary RPS. In contrast, anti-inflammatory factors such as IL-10 and TGF- β remained largely unchanged across groups (Fig. 4A). Meanwhile, the results of serum bone turnover markers revealed that DSS treatment elevated the levels of bone resorption markers, including TRAP activity ($P = 0.013$) and CTx level ($P = 0.028$), both of which were significantly attenuated by RPS supplementation (Fig. 4B). While the bone formation marker ALP activity was notably reduced by DSS treatment ($P = 0.047$), RPS supplementation showed a tendency to increase P1NP levels ($P = 0.057$), though not ALP activity, in DSS-treated ducks (Fig. 4C). There were no significant differences in serum Ca and P concentration between Ctrl in DSS groups (Fig. 4D, E). However, dietary supplementation of RPS increased ($P = 0.055$) serum P content in DSS-treated ducks (Fig. 4E).

Discussion

It is well known that RPS is characterized as a type of dietary fibre that remains undigested in the small intestine and undergoes

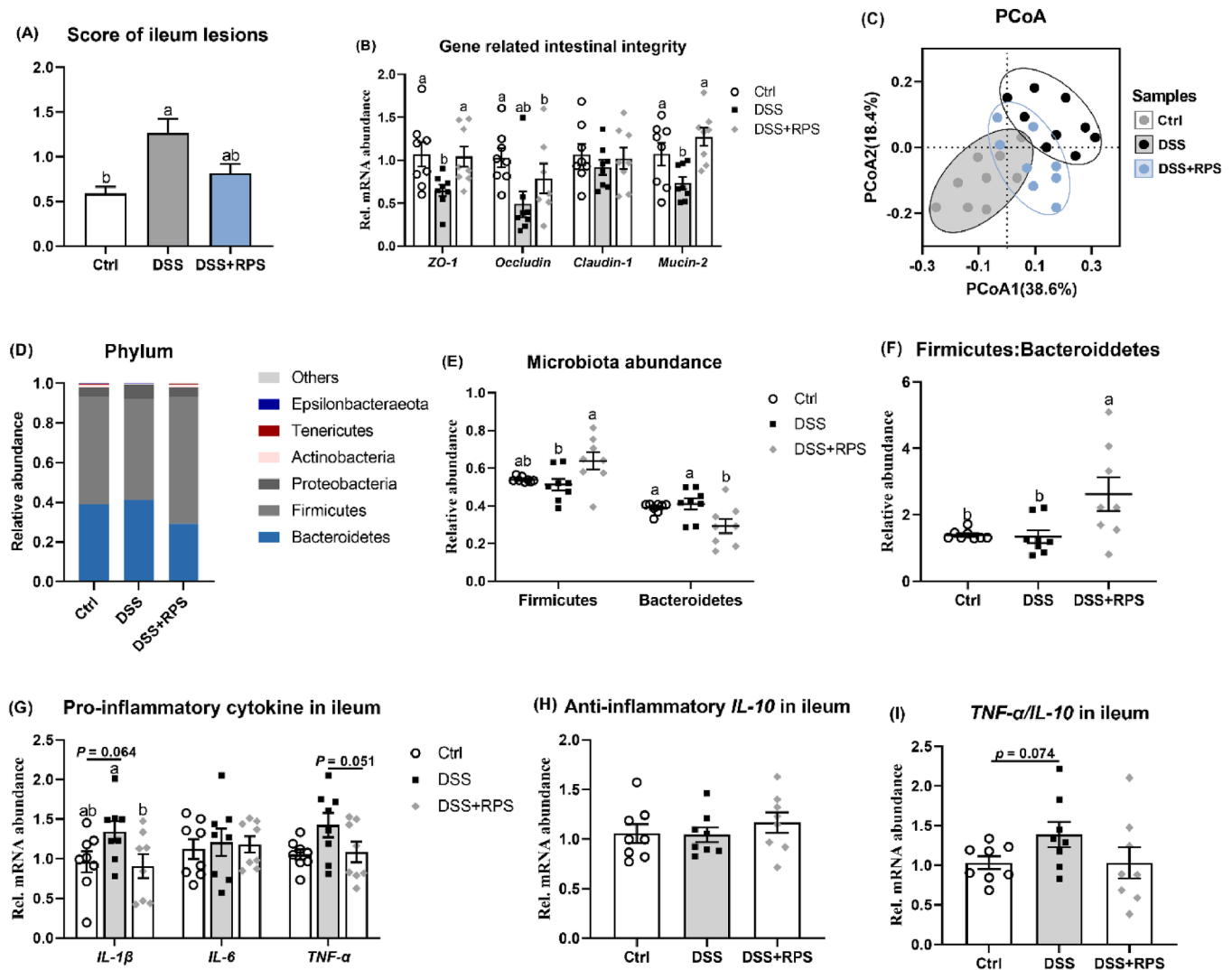


Fig. 3. Effects of dietary raw potato starch (RPS) supplementation on intestinal integrity, caecal microbiota composition, and systemic inflammatory status of dextran sodium sulphate (DSS)-induced enteritis ducks. (A) The ileum lesion score. (B) mRNA abundances of genes related intestinal integrity, including zonula occludens-1 (*ZO-1*), *occludin*, and *Claudin-1*, as well as *mucin-2* in ileum. (C) Principal coordinate analyses (PCoA) of beta diversity of bacterial operational taxonomic units. (D) Relative phylum level abundance of gut bacteria. The relative proportion of (E) Firmicutes and Bacteroidetes, and (F) the ratio of Firmicutes to Bacteroidetes. (G) the expression of pro-inflammatory cytokines including interleukin (*IL-1β*), *IL-6*, and tumour necrosis factor alpha (*TNF-α*). (H) mRNA levels of anti-inflammatory cytokine *IL-10*, and (I) its ratio to *TNF-α*. Data are expressed as mean and standard deviation. Statistical significance showed as different letters at $P < 0.05$.

fermentation in the large intestine, thereby altering the composition of gut microbiota and its metabolites. Emerging evidence in recent years suggests that dietary RPS supplementation can protect against gastrointestinal disorders, bone loss, and rheumatoid arthritis (Bai, et al., 2021; Qin, et al., 2019). This implies that dietary RPS intervention may be one of the nutritional strategies for intestinal dysfunction and its complications in ducks. Herein, data on RPS characteristics confirms that RPS exhibits an irregular or angular polygonal structure and adopts a B-type crystal structure, with the DP of RPS mainly ranging from 13 to 24. Subsequently, the roles of RPS in intestinal barrier function and bone metabolism were evaluated in both healthy and enteritis-affected meat ducks. The current results showed that dietary RPS inclusion enhanced tibia ash content and intestinal integrity, accompanied by a reduction in serum inflammatory status. Using a DSS-induced enteritis model in meat ducks, this study demonstrated that a 12% RPS diet alleviated DSS-induced intestinal disorders, including intestinal leakage, dysbiosis, and intestinal inflammation, which were closely correlated with improvements in tibia mass and mechanical properties by inhibiting bone resorption.

Research from epidemiological studies and experimental animal models supports the beneficial role of resistant starch in promoting intestinal health (Wen, et al., 2023). Specifically, a diet supplemented with 12% RPS was found to enhance intestinal integrity and alleviates enteritis (Qin, et al., 2023). Beyond the gut, 12% dietary RPS also exhibits a significant protective effect against bone loss in ovariectomized mice, a mechanism attributed to the regulation of gut microbiota and systemic inflammation (Tousen, et al., 2019). The results of this study indicate that dietary supplementation with 12% RPS enhanced intestinal barrier function, evidenced by reductions in serum D-lactic acid, pro-inflammatory cytokines (*IL-1β* and *TNF-α*), and the *TNF-α/IL-10* ratio, a key biomarker of the balance between a pro- versus anti-inflammatory state. These alterations were accompanied by a significant increase in tibia ash content in the present study. Moreover, the negative correlations observed between tibia ash content and both serum D-lactate levels and the *TNF-α/IL-10* ratio suggest that the RPS-enhanced bone mass in meat ducks may be mediated, at least in part, by improved intestinal integrity and mitigated enteritis. This finding aligns with the established connection between intestinal health

Table 3

Effects of dietary raw potato starch (RPS) and dextran sodium sulphate (DSS) on growth performance in meat ducks.

Items	Ctrl	DSS	DSS+RPS	p-value
Body weight, g/bird				
1d	51.83 ± 0.41	51.88 ± 0.72	51.62 ± 0.63	0.659
14d	686.66 ± 19.56	661.64 ± 40.08	685.48 ± 30.19	0.216
21d	1089.88 ± 80.17	1008.49 ± 51.8	1068.88 ± 103.57	0.140
Body gain, g/bird				
1-14d	634.83 ± 19.49	609.76 ± 40.15	633.86 ± 30.20	0.213
15-21d	403.22 ± 72.99	346.85 ± 51.92	383.40 ± 101.73	0.362
1-21d	1038.05 ± 80.03	956.61 ± 52.06	1017.26 ± 104.06	0.141
Feed intake, g/bird				
1-14d	805.96 ± 64.95	814.79 ± 47.88	812.46 ± 58.39	0.951
15-21d	728.51 ± 155.47	643.41 ± 51.01	635.51 ± 141.57	0.278
1-21d	1534.47 ± 196.7	1458.19 ± 75.27	1474.36 ± 138.22	0.552
Feed intake to body gain, g/g				
1-14d	1.27 ± 0.10	1.34 ± 0.08	1.28 ± 0.11	0.338
15-21d	1.82 ± 0.31	1.89 ± 0.35	1.78 ± 0.68	0.894
1-21d	1.48 ± 0.12	1.53 ± 0.11	1.46 ± 0.18	0.601
Mortality, %				
1-14d	1.04 ± 2.95	1.04 ± 2.95	1.67 ± 3.73	0.928
15-21d	1.04 ± 2.95	3.13 ± 4.31	1.04 ± 2.95	0.397
1-21d	2.08 ± 3.86	4.17 ± 4.45	2.08 ± 3.86	0.508

Data are expressed as mean ± standard deviation. Statistical significance showed as different letters at $P < 0.05$.**Table 4**

Effects of dietary raw potato starch (RPS) and dextran sodium sulphate (DSS) on short-chain fatty acids (SCFAs) in cecal content of meat ducks.

Item, μmol/g	Ctrl	DSS	DSS+RPS	P-value
Acetic acid	36.55 ± 3.43 ^{ab}	30.01 ± 5.32 ^b	37.04 ± 5.33 ^a	0.048
Propionic acid	12.53 ± 5.59	11.74 ± 1.62	12.99 ± 4.17	0.629
Butyric acid	7.57 ± 1.89 ^b	6.44 ± 1.92 ^b	10.33 ± 2.18 ^a	0.034
Total SCFAs	56.03 ± 5.72 ^{ab}	47.74 ± 8.13 ^b	60.48 ± 9.62 ^a	0.022

Data are expressed as mean and standard deviation. Statistical significance showed as different letters at $P < 0.05$.**Table 5**

Effects of dietary raw potato starch (RPS) and dextran sodium sulphate (DSS) on tibia characteristics in meat ducks.

Items	Ctrl	DSS	DSS+RPS	p-value
Length, mm	94.63 ± 4.38	95.23 ± 2.61	95.52 ± 2.25	0.854
Diameter, mm	5.80 ± 0.51	5.73 ± 0.49	5.63 ± 0.41	0.773
Density, g/cm ³	0.46 ± 0.10 ^a	0.35 ± 0.10 ^b	0.45 ± 0.10 ^a	0.049
Ultimate load, N	135.28 ± 22.08 ^a	106.62 ± 26.93 ^b	131.31 ± 16.89 ^a	0.73
Yield, N	93.78 ± 9.60	92.31 ± 8.26	95.37 ± 4.00	0.037
Slope, N/mm	381.03 ± 56.66 ^{ab}	309.35 ± 80.66 ^b	422.04 ± 68.77 ^a	0.013
AUC, N*mm	256.65 ± 57.42	288.60 ± 34.34	279.48 ± 52.48	0.423

Data are expressed as mean and standard deviation. Statistical significance showed as different letters at $P < 0.05$.

AUC, Area under the load-displacement curve; RPS, raw potato starch; DSS, dextran sodium sulphate.

and bone metabolism observed in other intestinal disorders (Matar, et al., 2025). To confirm the protective roles of RPS in bone metabolism mediated by intestinal homeostasis, an enteritis model was established in ducks using oral DSS treatment. This DSS challenge disrupted intestinal integrity, as indicated by reduced levels of tight junction proteins (ZO-1 and occludin) and mucin-2. Consequently, pro-inflammatory cytokine levels increased in both the ileum and serum, consistent with established links between gut-derived inflammation and bone loss (Zhang, et al., 2021). Presented research in broilers has shown that such barrier impairment and microbial dysbiosis

upregulate pro-inflammatory cytokines in the ileum and bone marrow, leading to inferior bone mass and material properties (Zhang, et al., 2021). This gut-bone axis is further supported by observations of poor bone health in IBD patients and by murine colitis models where DSS reduces bone mass and compromised microarchitecture. Similarly, in the present study, DSS-treated birds exhibited decreased bone density, tibial ultimate load and stiffness, indicators of impaired mechanical integrity previously associated with DSS (Ke, et al., 2019; Metzger, et al., 2019). The lack of significant change in dimensions was likely due to the relatively short challenge duration. In this case, dietary supplementation with 12% RPS reversed this DSS-induced bone loss and improved tibial strength in meat ducks. This beneficial role of RPS, previously confirmed in ovariectomized mice (Tousen, et al., 2019) and meat ducks (Zhang, et al., 2022b; Zhang, et al., 2022e) is correlated with enhanced gut barrier function, inhibited inflammation, and improved microbiota composition (Qin, et al., 2020; Qin, et al., 2019; Zhang, et al., 2022e). As expected, dietary RPS supplementation in this study counteracted the DSS-induced downregulation of tight junction proteins and suppressed the inflammatory response. These gut-protective effects of RPS may be attributed to its ability to alter gut microbiota composition and undergo hindgut fermentation, processes that are well known to promote intestinal health (Maier, et al., 2017; Warren, et al., 2018). In the context of poultry gut ecology, a higher Firmicutes abundance is associated with efficient energy metabolism (Dias, et al., 2025), while an expansion of Proteobacteria is a hallmark of dysbiosis (Shin, et al., 2015). A shift toward reduced Firmicutes and increased Proteobacteria and Bacteroidetes is characteristic of inflammatory bowel disease (Nishida, et al., 2018). In the current study, dietary RPS reversed the DSS-induced dysbiosis by increasing the proportion of Firmicutes and decreasing the abundance of Proteobacteria and Bacteroidetes. This restoration of a healthier microbial profile likely provided the foundation for the improved tibia quality observed in DSS-treated birds.

To elucidate the mechanism by which RPS reversed DSS-induced bone loss, inflammatory status and bone turnover was evaluated in this study. Emerging evidence supported that the chronic release of pro-inflammatory cytokines such as IL-6 and TNF- α could activate the nuclear factor-kappa B (NF- κ B) pathway, which suppresses bone formation and promotes bone resorption (Yin, et al., 2025). In particular, it was reported that TNF- α could exacerbate osteoclastogenesis (Guo, et al., 2023). In the current study, dietary RPS supplementation reversed the transcription of pro-inflammatory TNF- α , IL-1 β , and IL-17A, companying with comparable mRNA levels of anti-inflammatory IL-10 and TGF- β in bone marrow. Meanwhile, DSS-treated birds exhibited increased bone resorption, as indicated by elevated serum CTx concentrations and TRAP activity, which was normalized by dietary RPS treatment. These results suggested that dietary RPS inclusion probably suppressed inflammation-induced bone resorption. Moreover, the effects of DSS and RPS on bone formation also warrant consideration in the current study. Decreased bone formation is a documented consequence of colitis in both experimental models and IBD patients (Kreienbuehl, et al., 2024). The DSS-induced colitis model in mice directly hinders bone formation, reducing key histomorphometry parameters including trabecular double-labelled surface, mineralizing surface, and bone formation rate (Hamdani, et al., 2008). In this study, DSS treatment decreased serum ALP activity, implying intestinal inflammation might reduce bone formation. However, the similar levels of serum bone formation markers (P1NP and ALP) in DSS-treated ducks indicated that dietary RPS inclusion failed to stimulate osteogenesis. In addition, the closed connection between bone mass and hydroxyapatite also prompted us to evaluate the alternation in serum Ca and P response to DSS treatment and dietary RPS supplementation. In this study, experimental manipulations did not change the concentration of serum Ca in this study, whereas RPS supplementation increased serum P content in DSS-treated ducks, suggesting that the facilitation of the P absorption might present another plausible mechanism by which dietary RPS supplementation promotes bone mineralization in this study. Further evidence is required to

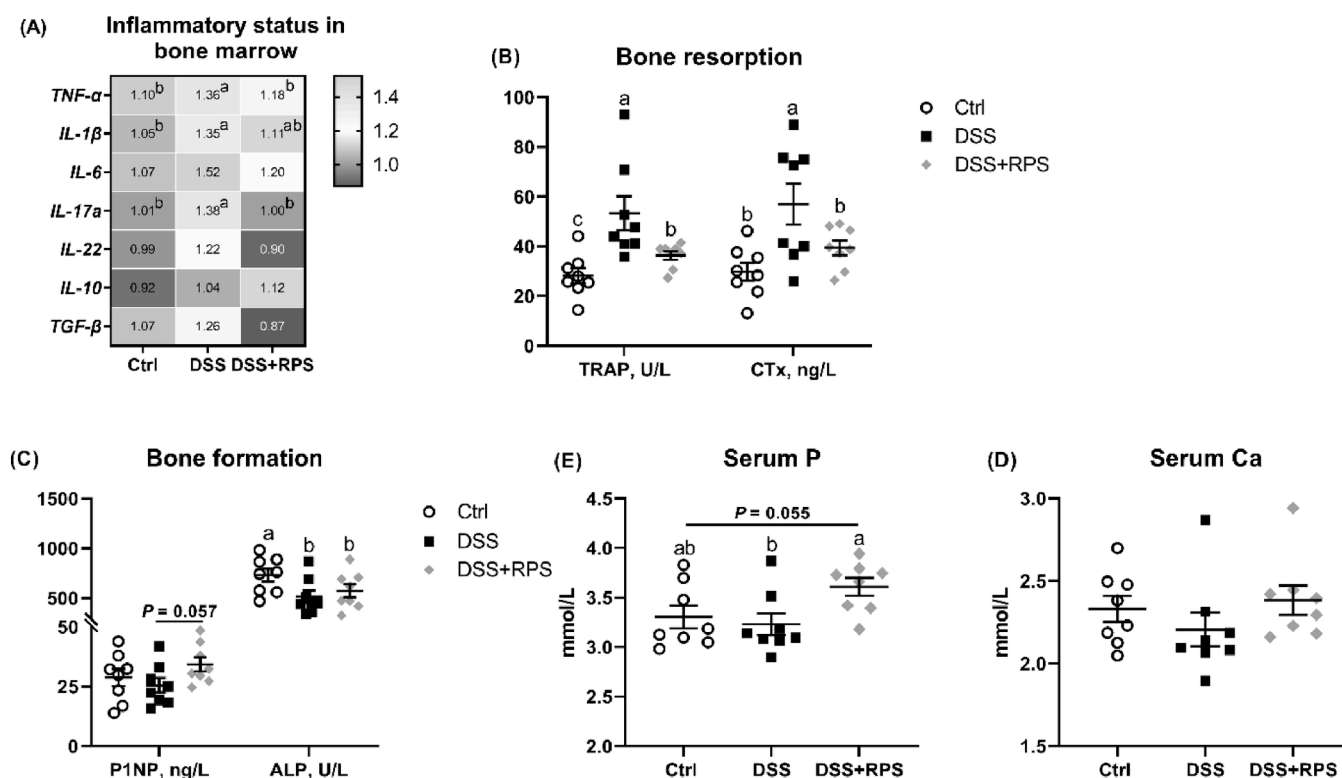


Fig. 4. The response of bone marrow inflammatory status (A), bone turnover markers, and serum calcium (Ca) and phosphorus (P) levels to dietary raw potato starch (RPS) and dextran sodium sulphate (DSS) was investigated in ducks. (B, C) Serum biomarkers of bone turnover, including C-terminal cross-linked telopeptide of type I collagen (CTx) and tartrate-resistant acid phosphatase (TRAP), procollagen type I N-terminal propeptide (P1NP), and alkaline phosphatase (ALP). (D, E) Serum content of Ca and P. Data are expressed as mean and standard deviation. Statistical significance showed as different letters at $P < 0.05$.

confirm the inference.

In the present study, alongside improved tibial bone density and mechanical properties, our analyses reveal that dietary supplementation with 12% RPS markedly alters the gut microbial community composition in DSS-challenged ducks. Specifically, this treatment elevated the relative abundance of Firmicutes while reducing that of Bacteroidetes. Previous research reported that a decreased Firmicutes/Bacteroidetes ratio alleviated bone loss in mice (Schepper, et al., 2019). Conversely, elevated Firmicutes abundance coupled with depleted Bacteroidetes levels was strongly correlated with exacerbated disease symptoms in collagen-induced arthritic mice fed a resistant starch-containing diet (Bai, et al., 2021). Such inconsistent findings suggest that gut microbiota-mediated bone regulation depends not merely on the Firmicutes/Bacteroidetes ratio, but also on microbial fermentation metabolites, particularly SCFAs. It is well established that members of the phylum Firmicutes generate substantial quantities of butyrate and propionate, whereas Bacteroidetes predominantly produce acetate and propionate (Louis and Flint, 2017). Consistently, the increased proportion of Firmicutes observed in our study raised caecal chyme propionate and butyrate concentrations, which potentially facilitated P absorption in DSS-treated ducks. Existing evidence demonstrates that SCFAs reduce intestinal luminal pH, improving the solubility and absorption efficiency of essential minerals (Nourmohammadi, et al., 2012; Zhang, et al., 2022a). Collectively, these results imply that SCFAs, rather than the Firmicutes/Bacteroidetes ratio, serve as the key intermediate regulators responsible for enhanced tibial bone characteristics in meat ducks.

Several limitations exist in the present study. The first is the enteritis model. DSS-induced enteritis is well established in mammals, yet its intestinal pathological effects in ducks have not been fully characterized, lacking histological observations, clinical symptom recording and toxicity evaluation. Second, bone quality was only assessed via bone density and mechanical parameters, micro-CT, bone histomorphometry

and osteoblast/osteoclast detection are still needed. Third, only inflammatory cytokines were quantified, with no direct evidence confirming the activation or inhibition of key pathways including NF-κB. Therefore, we admit the possibility that some of our conclusions may include overestimation or underestimation of the roles of RPS or DSS in tibia mass of meat ducks. Additional research will be essential to address these limitations in domesticated birds, including refining enteritis models and the effects of RPS and DSS challenge on bone metabolism and intestinal homeostasis.

In conclusion, data from this study demonstrated that a diet with 12% RPS significantly improved tibia mass of meat ducks and intestinal inflammatory status, which was characterized by reduced pro-inflammatory cytokine levels, and it showed a clear positive correlation between key markers of intestinal barrier homeostasis. Furthermore, moving beyond the gut, dietary RPS supplementation exerted a remarkable anti-inflammatory effect within the bone marrow microenvironment, thereby reducing consequent osteoclastic bone resorption and improving tibia quality in both conventional and DSS-treated meat ducks. Overall, this study indicates that gut microbiota-oriented nutritional intervention serves as an integrated and effective method to control systemic inflammation in meat ducks.

CRedit authorship contribution statement

Xin Yue: Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Jingyi Yu:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Yuhuang Hou:** Writing – original draft, Visualization, Validation, Formal analysis, Data curation. **Minh Tu Nguyen:** Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Xuemeng Si:** Visualization, Software, Resources, Methodology, Data curation. **Rui Zheng:** Writing – review & editing, Visualization,

Methodology, Formal analysis, Data curation. **Wen Chen:** Supervision, Resources, Project administration, Formal analysis, Conceptualization. **Yanqun Huang:** Writing – review & editing, Supervision, Project administration, Methodology, Data curation, Conceptualization. **Huaiyong Zhang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Disclosures

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper

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