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Full Paper

Effect of plant polysaccharides (*Poria cocos* and *Astragalus* polysaccharides) on immune responses and intestinal microbiota of Dabry's sturgeons

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Searching for non-toxic and harmless feed ingredients that can improve growth performance and host immunity has always been the focus of attention in the protected areas for artificially bred Dabry's sturgeons. The present study explored the effect of dietary *Poria cocos* and *Astragalus* polysaccharides on the antioxidant status, expression of immune related genes, and composition and putative functions of gut bacterial communities in Dabry's sturgeons for the first time. In this study, Dabry's sturgeons were randomly divided into 3 groups and fed diets of normal, *P. cocos* polysaccharide-added (200 mg/kg), and *Astragalus* polysaccharide-added (200 mg/kg) food for 14 days. The results indicated that dietary *Astragalus* polysaccharide can increase the final body weight of Dabry's sturgeon. Compared with normal breeding individuals, feeding diets containing the *P. cocos* and *Astragalus* polysaccharides up-regulated the activity of superoxide dismutase, lysozyme, catalase, and glutathione peroxidase while also decreasing the levels of malondialdehyde. In addition, the *Astragalus* polysaccharide group had higher gene expression of two inflammatory cytokines, tumor necrosis factor alpha and immunoglobulin M, than the control group. Analysis of intestinal microbiota revealed that the dietary *Astragalus* polysaccharide improved the richness and diversity of major gut microbiota in Dabry's sturgeons, while the structure in the *P. cocos* polysaccharide group was clearly distinguished from that of the control group. Our results preliminarily indicated that dietary supplementation of *P. cocos* and *Astragalus* polysaccharides may contribute to better performance in growth, development, and inflammatory response for Dabry's sturgeons, and they provide basic guidance for plant polysaccharide additives in artificial breeding of sturgeons.

Key words: Dabry's sturgeon, *Poria cocos*, *Astragalus*, polysaccharide, gut microbiota

INTRODUCTION

Dabry's sturgeon (*Acipenser dabryanus*) is a typical endemic fish in the upper reaches of the Yangtze River in China, and the resource of natural Dabry's sturgeons sharply decreased in the late 20th century due to a series of human activities, including pollution, overfishing, and shipping [1, 2]. In order to protect the wild resource of Dabry's sturgeons, artificial captive breeding has been performed in recent years. However, increasing susceptibility to pathogens was observed under intensive breeding conditions, leading to a great loss in the culture process for the conservation of Dabry's sturgeons. Therefore, the improvement of growth performance and host immunity are areas of sustained focus the protected areas for artificially bred Dabry's sturgeons. Previous studies have proved that the gut microbiota has a significant effect on host health and can be modulated by dietary changes [3–5]. Meanwhile, Chinese medicinal herbs containing abundant natural components with broad-spectrum resistance to infections have been widely used as dietary additives in aquaculture [6–8].

Plant polysaccharides are natural and nontoxic macromolecular substances that widely exist in plant organs such as leaves, roots, flowers, and fruits [9]. In recent years, many polysaccharides, including *Astragalus* polysaccharide (AP), have been extensively used as immunostimulants in the aquaculture industry to promote growth performance and host defense in fish [10]. There is evidence indicating that AP has obvious growth-promoting and

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immunostimulatory activities in various fish species, such as the common carp [7], crucian carp [11, 12], grass carp [13], Nile tilapia [14, 15], largemouth bass [16], large yellow croaker [17], yellow catfish [18], turbot [8], zebrafish [19], and Asian seabass (*Lates calcarifer*) [6]. *Poria cocos* polysaccharide (PP), one major class of secondary metabolites isolated from *P. cocos*, is reported to be responsible for the immune-stimulating properties. β-glucan is the major PP component with β-(1→3)-linked glucose backbone and β-(1→6)-linked glucose side chains and possesses moderate anticancer activity [20]. However, the functions of AP and PP have been poorly investigated in fish to date.

In the present study, the influences of the addition of PP and AP to diet on the modulation of the antioxidant status, expression of immune-related genes, and composition and putative functions of gut bacterial communities were evaluated in artificially bred Dabry's sturgeons. This study offers the first insight into dietary supplementation of *A. dabryanus* with PP and AP, which should be helpful for the rational formulation of feeding strategies to improve host defense in *A. dabryanus* aquaculture operations.

MATERIALS AND METHODS

Experimental animal cultivation and preparation

Juvenile Dabry's sturgeons that were ten months of age were collected from the Chinese Sturgeon Research Institute, Yichang, China. PP and AP were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China) and Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China), respectively. The sturgeons were born in April 2020, and individuals with similar sizes (average body length of 45.7 ± 7.0 cm and average weight of 1,100 ± 68 g) were selected and maintained under aquaculture conditions (water temperature of 17 ± 1°C, ammonia-N <0.2 mg/L, nitrite-N <0.05 mg/L, and oxygen concentration >6.0 mg/L) with fully aerated water in February 2021. Natural day lengths (about 11 hr) were maintained for all treatment groups.

The selected sturgeons were randomly divided into three 3,000-L tanks (30 fish per group): the normal control (CT) group, AP diet group, and PP diet group. Sturgeons in the CT group were fed commercial forage (Jinjia Aquatic Feed, Zhejiang Jinjia Aquatic Feed Co., Ltd., Hangzhou, China) without any additives, while sturgeons in the AP and PP groups were fed diets prepared using the same commercial forage (basal diet) mixed with either AP or PP at a concentration of 200 mg/kg. The composition of the basal diet was as follows: crude protein ≥43%, crude fat ≥5.0%, crude fiber ≤5%, crude ash ≤18%, calcium 2.0%–5.0%, phosphorus 1.0–3.0%, and lysine ≥2.0%. After a 7-day adaption period, the sturgeons in all the groups were subjected to a feeding trial that lasted 14 days, with all the sturgeons being fed at 0.5% of body weight twice a day [21]. Water quality parameters were monitored regularly and maintained as follows: amino nitrogen level <0.2 mg/L, nitrite nitrogen level <0.05 mg/L, and dissolved oxygen concentration >6.0 mg/L.

Sample collection

All the experimental sturgeons were fasted for 24 hr and then anesthetized with tricaine methanesulfonate (MS-222) before sampling after the feeding trail. Six randomly selected sturgeons in each group were subjected to blood sampling to determine enzyme activity and intestine collection. The blood samples were extracted from the caudal fin with disposable sterile syringes and centrifuged at 3,000 rpm for 15 min at 4°C to obtain plasma samples. The intestines of the six fish were cleaned and rinsed with PBS. Some of them were collected for the examination of enzyme activity. Those of three fish were collected for histological observations and the examination of gene expression. The intestinal contents of the six fish were gently scraped with sterile forceps and collected into sterile tubes. The serum and tissue samples were frozen in liquid nitrogen and then transferred into a −80°C refrigerator for subsequent experiments.

Determination of the activities of immune parameters

Activities of superoxide dismutase (SOD), glutathione peroxidase (GSHpx), malondialdehyde (MDA), lysozyme (LZM), and catalase (CAT) were measured according to kit instructions (Nanjing Institute of Bioengineering Institute, China).

Expression of immune-related genes

RNA was extracted from intestines using TRIzol reagent (Invitrogen, Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer's instructions. RNase-free DNase I (Takara, Dalian, China) was added to the reaction mixture for 1 hr at 37°C to remove the genomic DNA. Total RNA of each sample was quantified and qualified by NanoDrop (Thermo Fisher Scientific Inc.) and 1% agarose gel before first-strand cDNA synthesis, and reverse transcription was performed with a FastQuant RT Kit (TIANGEN, Beijing, China). Quantitative real-time polymerase chain reaction (PCR) (qRT-PCR) was used to validate the expression pattern of the immune-related genes, including interleukin-1β (*IL-1β*), toll-like receptor 2 (*TLR2*), transforming growth factor beta (*TGF-β*), tumor necrosis factor alpha (*TNF-α*), *IgM*, and polymeric immunoglobulin receptor (*pIgR*). Primer information is shown in Supplementary Table 1. The PCR reaction was performed in triplicate wells using SYBR Premix Ex Taq™ II (Takara) on an Applied Biosystems StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, NJ, USA) with the following program: 95°C for 30 sec, followed by 40 cycles of 5 sec at 95°C, 30 sec at 60°C, and 45 sec at 72°C. The β-actin gene from the Dabry's sturgeon was used as the internal control gene. The relative gene expression values of each sample were calculated using the $2^{-\Delta\Delta Ct}$ method.

Composition and diversity of intestinal microbiota

Microbial DNA was extracted using the CTAB/SDS method. DNA concentration and purity were monitored on 1% agarose gels. According to the concentration, DNA was diluted to l ug/μL using sterile water. Phusion® High-Fidelity PCR Master Mix (New England Biolabs) was used to amplify 16S rRNA genes of V3–V4 hypervariable regions with universal primer pair 341F (5′-CCTAYGGGRBGCASCAG-3′) and 806R (5′-GGACTACNNGGGTATCTAAT-3′). Sequencing libraries were generated using a TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturer's recommendations and sequenced by Novogene Biotech Co., Ltd. (Beijing, China) on an Illumina NovaSeq platform with paired-end reads of 250 bp each.

The overlapping paired-end reads were merged using the FLASH V1.2.11 software (http://ccb.jhu.edu/software/FLASH/) and spliced to raw tags [22]. Quality filtering on the raw tags was performed under specific filtering conditions to obtain high-

quality clean tags according to the QIIME 2 (https://qiime2.org) quality control process (http://qiime.org/scripts/split libraries fastq.html) [23]. DADA2 in QIIME 2 (https://docs.qiime2.org/2023.5/plugins/available/dada2/) was used for analyzing the effective reads to generate an amplicon sequence variant (ASV) abundance file [24]. A representative sequence for each ASV was screened for further annotation and aligned against the SILVA database (https://www.arb-silva.de/) using the DADA2 pipeline to determine taxonomic information. TBtools (v1.116) [25] was applied to draw the heatmap pattern presenting the correlations between the mRNA levels of immune-related genes and relative abundances of intestinal bacteria. Alpha diversity indices, including the Chao1 index, Simpson index, and Shannon index, were determined using QIIME 2 to estimate the microbial diversity. Beta diversity, both weighted and unweighted UniFrac distances, was calculated using QIIME 2 and visualized by two-dimensional principal coordinate analysis (PCoA). Phylogenetic investigation of communities by reconstruction of unobserved stats 2 (PICRUSt2) was performed to analyze the predicted metagenomic functional content of each group at KEGG taxonomy level 3.

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM) values. All data were analyzed for significance by one-way analysis of variance (ANOVA) followed by a Duncan multiple comparison test using IBM SPSS Statistics v22.0. A value of $p<0.05$ was regarded as statistically significant.

Ethics statement

All sampling procedures involving handling and treatment during this study were approved by the Chinese Sturgeon Research Institute of the China Three Gorges Corporation and the Hubei Key Laboratory of the Three Gorges Project for Conservation of Fishes.

RESULTS

Growth performance

The results for growth performance are presented in Table 1. No significant differences in growth were observed in terms of body length between day 0 and day 14 in any group ($p>0.05$, Table 1). However, body weights were all significantly increased after 14 days of feeding ($p<0.05$). Furthermore, sturgeons in the AP group showed a significant increase in body weight compared with individuals in the CT group on day 14 (Fig. 1).

Biochemical parameters in the intestine and serum

In the intestine, the activities of SOD, GSHpx, LZM, and CAT, but not MDA, were increased in the PP and AP groups (Fig. 1a). Compared with the CT group, PP significantly up-regulated the activities of SOD (63.71%) and LZM (28.17%), while AP significantly increased the activities of SOD (59.75%), LZM (33.33%), and CAT (47.52%). Both PP and AP induced significant decreases in MDA activity (24.68% and 28.15%, respectively; Supplementary Table 2).

The expressed pattern of the five biochemical parameters in serum was similar to the expression levels in the intestine (Fig. 1b). The activities of GSHpx and LZM were significantly increased in the AP group (24.34% and 40.04%, respectively), whereas that of MDA was significantly decreased (24.10%). GSHpx activity was also significantly up-regulated by PP, increasing by 21.78% (Supplementary Table 2). Compared with the CT group, no significant change in SOD or CAT activity was observed in the PP and AP groups.

Immune-related gene expression

The mRNA expressions of six immune-related genes (*IL-1β, TLR2, TGF-β, TNF-α, IgM,* and *pIgR*) in the intestine were evaluated to examine the effects of PP and AP on the immune responses of *Acipenser dabryanus*. As shown in Fig. 2, compared with the CT group, there were no significant changes in the expression levels of *IL-1β, TGF-β*, and *pIgR* in the PP and AP groups. AP showed significantly increased expression levels of *TNF-α* and *IgM* compared with the CT group. However, there were no significant differences in the expression levels of *IL-1β, TLR2, TGF-β, TNF-α, IgM,* and *pIgR* between the PP and CT groups ($p>0.05$). Furthermore, there were no significant differences in the expression levels of *IL-1β, TGF-β, TNF-α*, and *pIgR* between the PP and AP groups.

Sequencing and diversity indices of intestinal microbiota

In total, 610,565, 561,962, and 517,766 effective reads were generated in the CT, PP, and AP groups after low-quality sequence removal and chimera filtering, with average lengths of 413.17, 420.83, and 418.50 bp, respectively (Supplementary Table 3). All the effective reads were distributed among 3,076 ASVs. For all the experimental samples together, 28 phyla, 79 classes, 174 orders, 282 families, and 568 genera were identified in the intestinal contents. According to the ASV data, the two dominant bacterial phyla for the CT group were Fusobacteriota (68.88%) and Proteobacteria (24.82%), while Proteobacteria (58.97%) and Firmicutes (37.78%) were dominant in the PP group. For the AP group, three bacterial phyla (Firmicutes 36.77%, Fusobacteriota 28.95%, and Proteobacteria 24.65%) accounted for over 90% of

Table 1. Effects of dietary PP and AP on growth performance in Dabry's sturgeons after 14 days (mean ± SEM)

Groups	Body length (cm)		Body weight (g)	
	0 day	14 day	0 day	14 day
CT	45.40 ± 3.86	45.50 ± 3.91	1,115.20 ± 47.54	1,179.00 ± 43.37[a]
PP	45.20 ± 3.06	46.06 ± 2.76	1,071.40 ± 19.51	1,190.20 ± 10.34[a]
AP	46.40 ± 3.52	47.60 ± 3.67	1,114.20 ± 21.61	1,287.80 ± 20.94[b]

"a" and "b" were significantly different within the same column ($p<0.05$).
SEM: standard error; CT: control group; PP: *Poria cocos*-supplemented group; AP: *Astragalus* polysaccharide-supplemented group.

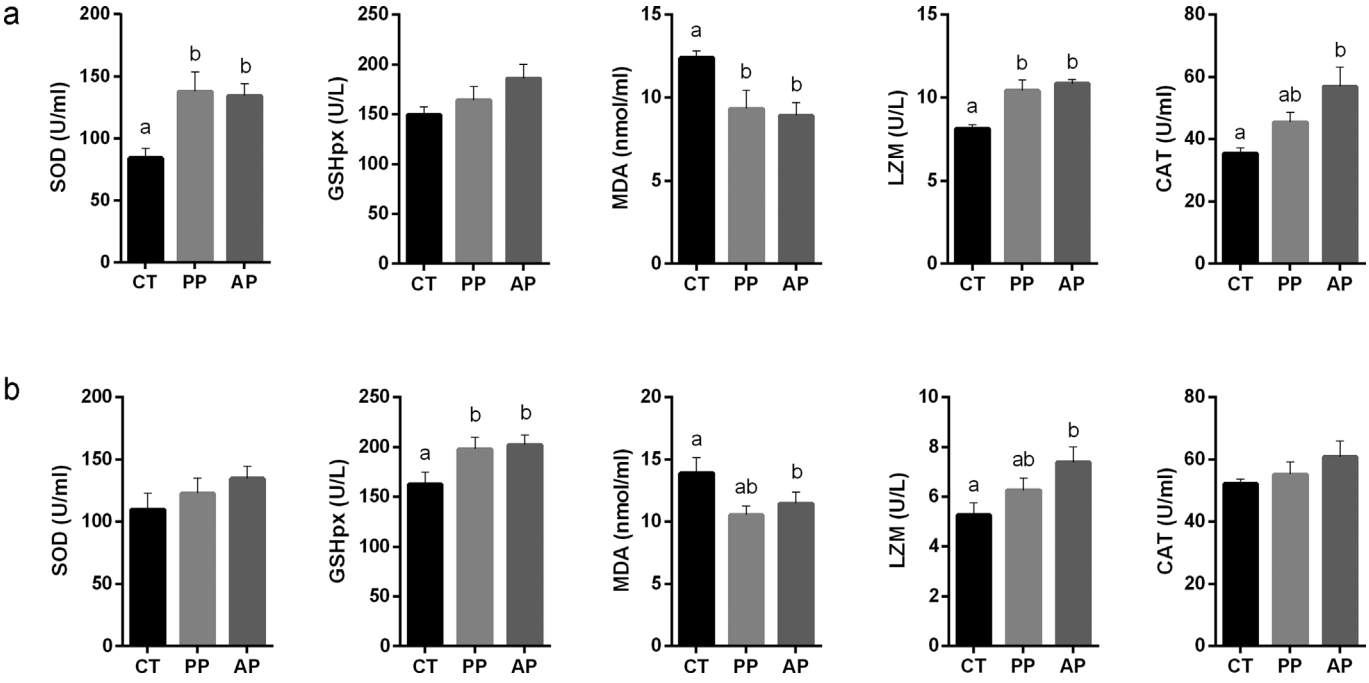

Fig. 1. Biochemical parameters in the CT, PP and AP groups. (a) SOD, GSHpx, MDA, LZM, and CAT in the intestine. (b) SOD, GSHpx, MDA, LZM, and CAT in serum. Error bars with different letters (a, b) indicate statistically significant differences based on Duncan's multiple comparison method ($p<0.05$, one-way ANOVA). CT: control group; PP: *Poria cocos*-supplemented group; AP: *Astragalus* polysaccharide-supplemented group; SOD: superoxide dismutase; GSHpx: glutathione peroxidase; MDA: malondialdehyde; LZM: lysozyme; CAT: catalase.

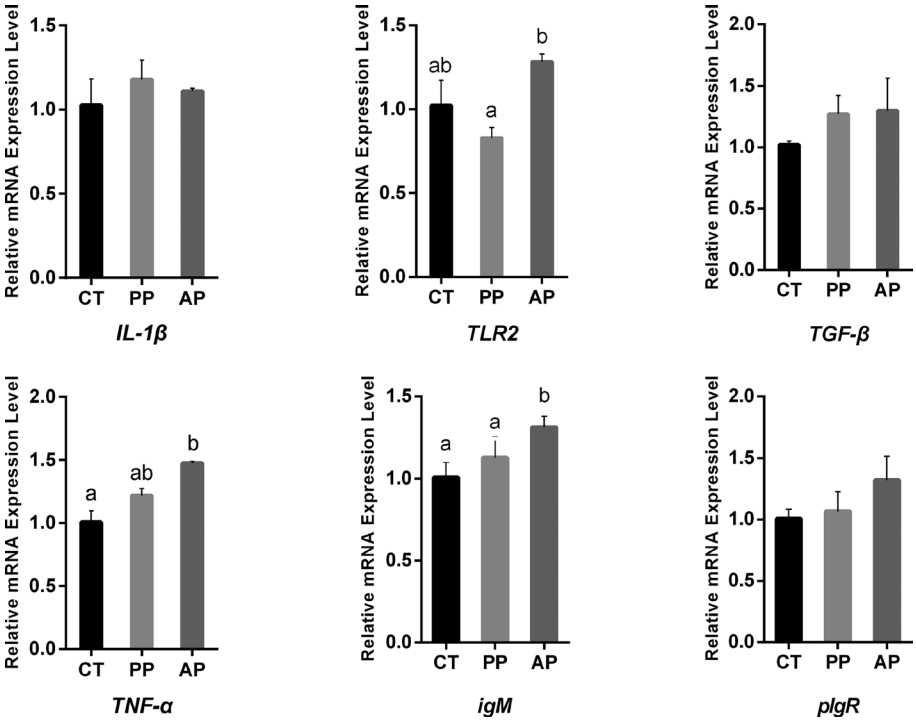

Fig. 2. The mRNA expressions of four immune-related genes (*IL-1β*, *TLR2*, *TGF-β*, *TNF-α, IgM,* and *pIgR)* in the intestine of Dabry's sturgeon. Error bars with different letters (a, b) indicate statistically significant differences based on Duncan's multiple comparison method ($p<0.05$, one-way ANOVA). CT: control group; PP: *Poria cocos*-supplemented group; AP: *Astragalus* polysaccharide-supplemented group; IL-1β: interleukin-1β; TLR2: toll-like receptor 2; TGF-β: transforming growth factor β; TNF-α: tumor necrosis factor-α; IgM: immunoglobulin M; pIgR: polymetric immunoglobulin receptor.

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all ASVs. Compared with the CT group, adding PP or AP to the diet enlarged the microbiome percentage of Firmicutes. On the other hand, the relative abundance of Fusobacteria was decreased remarkably after the supplementation of diet with PP or AP (Fig. 3a).

In order to explore the discriminatory bacterial taxa in the intestine for the PP- and AP-added diets, the predominant bacteria and phylogeny of the gut microbiota at the genus level were examined and clustered in all experimental groups. The phylogenetic composition of the intestinal bacterial community was conspicuously different among the CT, PP, and AP groups. *Cetobacterium* was the dominant bacterial group in both the CT and AP groups, while *Clostridium_sensu_stricto* was dominant in the PP group. Adding PP to the diet increased the relative abundances of *Clostridium_sensu_stricto, Acinetobacter, Aeromonas,* and *Exiguobacterium,* while the addition of AP to the diet up-regulated the activity of *Enterococcus* (Supplementary Fig. 1).

A rarefaction analysis was performed at the verge of approaching the saturation plateau across different gut samples, and it confirmed that a sufficient sampling depth was achieved for each group (Fig. 3b).

Structural modulation of the intestinal microbiota

Alpha diversities were examined using the Kruskal–Wallis H test. The Chao1 and Shannon indices showed significant differences between the AP and CT groups (Fig. 4a, 4b; Kruskal–Wallis pairwise analysis, $p<0.01$). Compared with the CT group, alpha diversity indices were significantly increased in the AP group. No significant difference in alpha diversity was detected between the PP and CT groups. The weighted UniFrac distance algorithm was adopted in the beta diversity analysis, and the results of PCoA and nonmetric multidimensional scaling analysis (NMDS) are shown in Fig. 4c, 4d. Based on the PCoA and NMDS plots, samples from each survey were grouped separately (Adonis PERMANOVA, $F=8.0236$, $p=0.001$). Additionally,

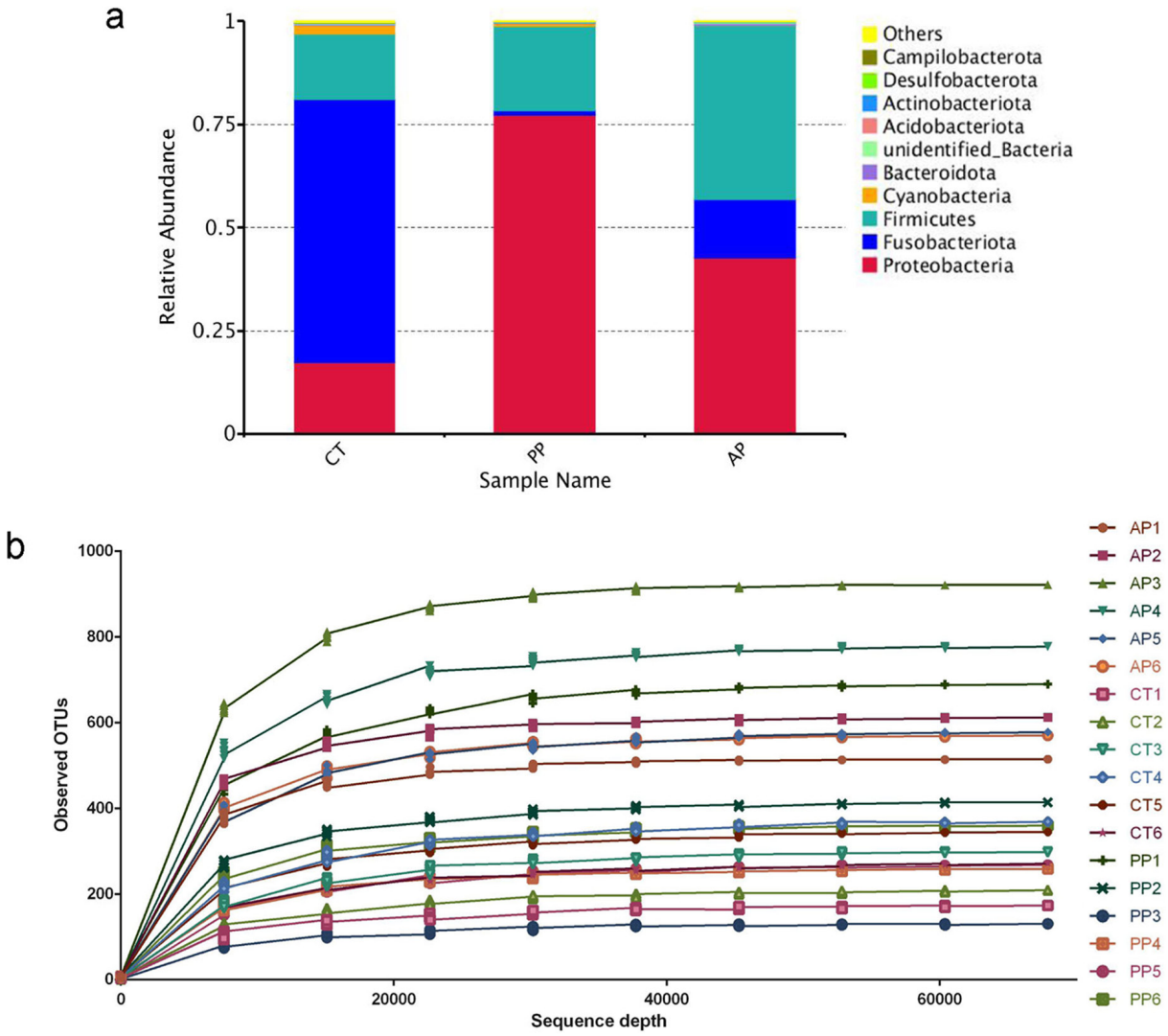

Fig. 3. Estimation of the intestinal bacterial community composition for the two plant polysaccharide-supplemented diets. (a) Taxonomy identification of microbial groups at the phylum taxonomic level. (b) Rarefaction curves of observed species numbers for intestinal microbiome groups.

CT: control group; PP: *Poria cocos*-supplemented group; AP: *Astragalus* polysaccharide-supplemented group.

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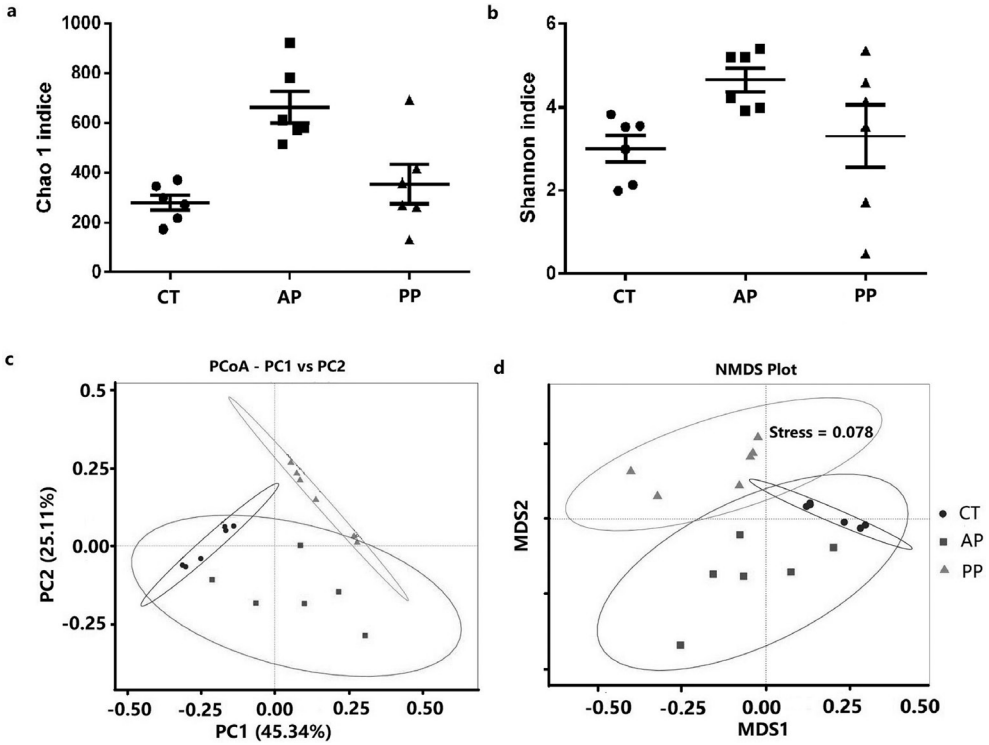

Fig. 4. Structural modulation of the intestinal microbiota in *A. dabryanus*. (a) Alpha diversity measured by Chao 1 and (b) Shannon indices. Data are shown as the mean ± SEM and were compared by Kruskal–Wallis H test. (c) Principal coordinate analysis (PCoA) plot and (d) non-metric multidimensional scaling (NMDS) plot revealing variations in the bacterial community structure among the experimental groups.

CT: control group; PP: *Poria cocos*-supplemented group; AP: *Astragalus* polysaccharide-supplemented group.

pairwise analysis of similarities (ANOSIM) comparisons found that there were substantial similarities between groups in terms of bacterial communities ($p<0.01$). The intestinal microbiota of the AP group exhibited a heterogeneous pattern and was distributed in a substantially larger ellipse in both PCoA and NMDS plots.

In order to identify the key members of the gut microbiota that responded to AP and PP in the AP and PP groups, both the t-test and linear discriminant analysis effect size (LEfSe) were used to analyze the species composition and community structure of the grouped samples. The abundances of Fusobacteriota in the AP and PP groups were lower than in the CT group (t-test; AP vs. CT, $p=0.016$; PP vs. CT, $p<0.001$; Fig. 5a, 5b). Furthermore, Firmicutes and Bacteroidota were significantly increased in the AP group compared with the CT group ($p<0.01$; Fig. 5b). Similar results were found in the LEfSe analysis, suggesting that gut microbe composition significantly differs after plant-polysaccharide feeding (Fig. 5c).

Metabolic functional changes in gut microbiota

Metabolic functional changes were analyzed based on the significant differences in bacteria compositions. Compared with the CT group, no obvious difference was observed in the PP group, whereas the AP group was clustered differently according to the results of the PCoA analysis on the KEGG Orthology (KO) pathway abundances (Fig. 6a). The heatmap showed the distributions of top 35 significantly different functional gene factors among the three groups (Fig. 6b). Bacterial gene functions related to KO pathways such as the phosphotransferase system, glycolysis, starch and sucrose metabolism, and peptidases and inhibitors were increased, while pathways such as quorum sensing, selenocompound metabolism, porphyrin metabolism, pentose phosphate pathway, and cationic antimicrobial peptide (CAMP) resistance were decreased in both the AP and PP groups compared with the CT group (Supplementary Table 4).

Correlation between intestinal microbiota composition and immune-related genes

The Pearson correlation coefficients shown in Fig. 7 illustrate the relationships between the expressed mRNA levels of the six immune-related genes and the intestinal bacterial abundances at the phylum level. The relative abundance of Desulfobacterota showed significantly positive interaction with the mRNA levels of *TNF* ($p<0.01$) and *IgM* ($p<0.05$). Expression of the *TGF* gene was positively correlated ($p<0.05$) with Firmicutes and Chloroflexi, while *pIgR* expression was positively associated ($p<0.05$) with Synergistota, Caldatribacteriota, and Patescibacteria. Moreover, the abundance of Thermoplasmatota showed positive interactions ($p<0.05$) with the mRNA level of *IL*.

DISCUSSION

Based on their low side effects and effective enhancement of immune activity, polysaccharides have been extensively used as dietary additives in aquaculture, especially for economic fish species. There is well-known evidence indicating that natural polysaccharides exhibit diverse biological functions, especially antioxidant and immunoregulatory activities. *P. cocos* and *Astragalus* polysaccharides have been extensively used as

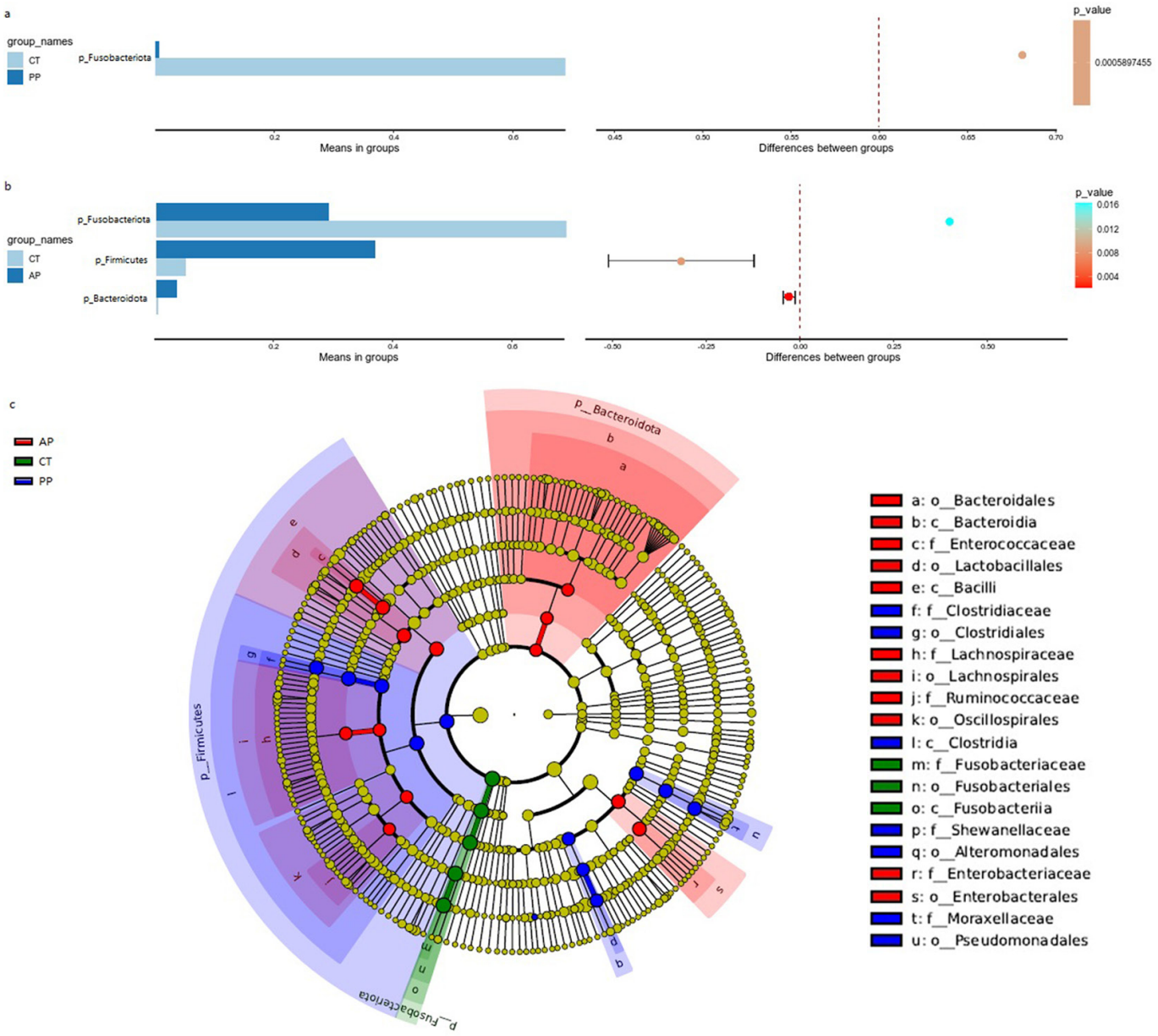

Fig. 5. Analysis of species composition and community structure between the grouped samples. (a, b) T-test bar plot of significantly different gut microbial species in sows at the phylum level. (c) Cladogram obtained from the LEfSe analysis, displaying the phylogenetic distribution of microbiota.

CT: control group; PP: *Poria cocos*-supplemented group; AP: *Astragalus* polysaccharide-supplemented group.

immunostimulants in the aquaculture industry to promote growth performance and host defense in several fish species other than sturgeons [10, 26]. Previous aquatic animal studies have reported that polysaccharides in diets could increase body weight gain [27, 28]. In the present study, the addition of *Astragalus* polysaccharide to diets at 200 mg/kg increased the body weights of Dabry's sturgeons, indicating the potential of *Astragalus* polysaccharide to enhance nutrient digestibility.

CAT, SOD, and GSHpx are important transformers of hydrogen peroxide and superoxide ion, while MDA is a representative end-product of lipid peroxidation. Consequently, the above four endogenous antioxidant enzymes (CAT, SOD, GSHpx, and MDA) are generally considered important evaluation metrics of biological antioxidant status [28]. In a previous study, purified *Astragalus* polysaccharide contained a high content of uronic acid and exhibited antioxidant activity [29]. Similarly, a galactoglucan with a main linkage type of 1,6-α-D-Galp has been purified from *P. cocos*, and it can significantly enhance the activity of antioxidant enzymes and attenuate the production of lipid peroxide [30]. Dietary PP and AP can also exert positive effects on the serum biochemical parameters of vertebrate species. Dietary AP can significantly increase the antioxidant enzymes activities of SOD, CAT, and/or LZM and significantly decrease that of MDA in the large yellow croaker (*Larimichthys crocea*) [31], common carp (*Cyprinus carpio*) [32], snakehead (*Channa argus*) [28], and Nile tilapia (*Oreochromis niloticus*) [14]. In

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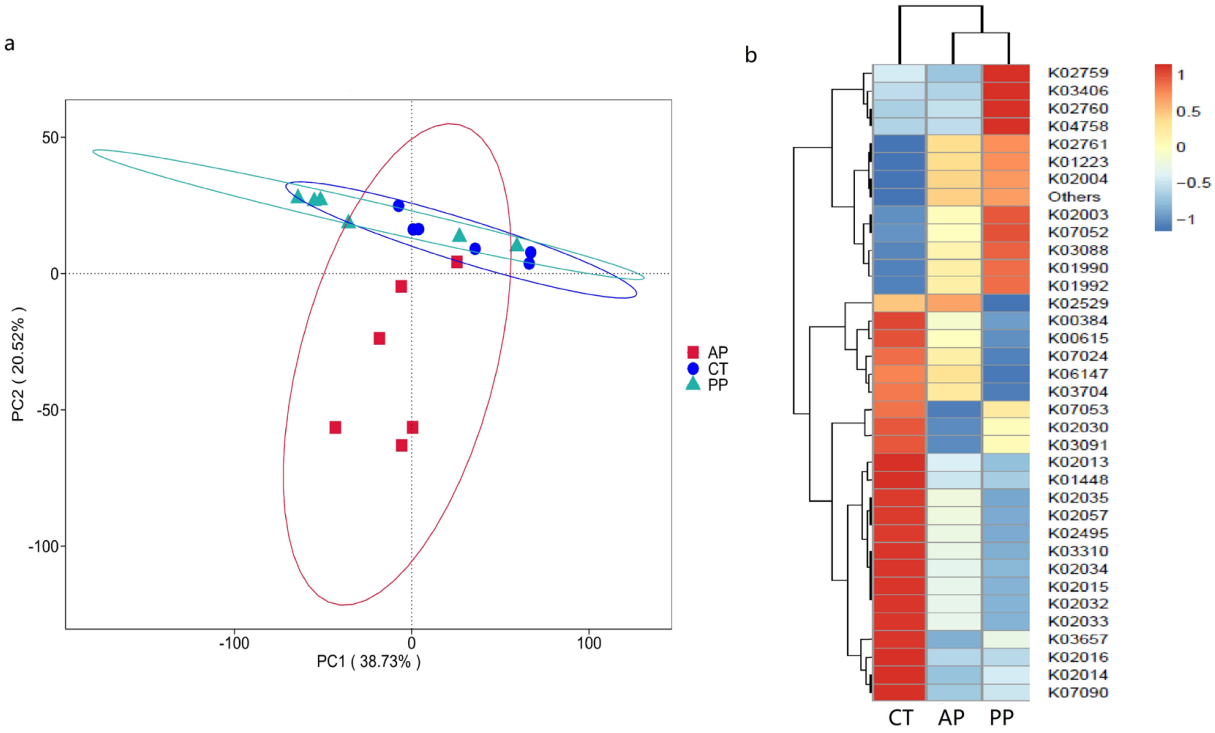

Fig. 6. Differences in metabolic functions of gut microbiota. (a) Principalcoordinate analysis (PCoA) plot of functional profiles among groups. (b) Heatmap showing significantly different functional pathways.

CT: control group; PP: *Poria cocos*-supplemented group; AP: *Astragalus* polysaccharide-supplemented group.

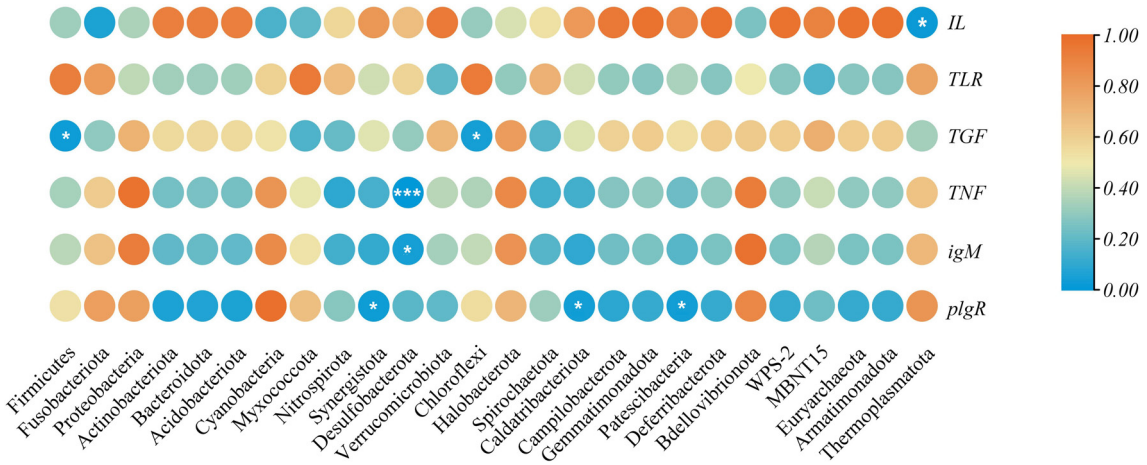

Fig. 7. Correlation between intestinal microbiota composition (at the phylum level) and immune-related genes by Pearson correlation analysis. *Significant difference. ***Extremely significant difference.

this study, both the addition of AP and PP to diet could increase the levels of antioxidant activity of CAT, SOD and GSHpx and effectively decrease that of MDA in the intestine and serum of Dabry's sturgeons, indicating that they did scavenge radicals and had obvious antioxidant effects that protected the fish from oxidative damage.

Cytokines are important immune parameters and are commonly used as reference genes for immune regulation studies [33]. TNF-α and IL-1β are key pro-inflammatory cytokines, and TGF-β is a major anti-inflammatory cytokine [34]. TLR2 is a kind of pattern recognition receptor (PRR) which can recognize the conserved pathogen-associated molecular patterns (PAMPs) and can initiate responses to various dangerous alterations of the gut microenvironment, such as inducing inflammatory cytokines and immune responses [35]. It has been reported that polysaccharides could promote potential immune responses by increasing the secretion of IL-1β and/or TNF-α [36–38]. TGF-β plays an important role in maintaining tissue homeostasis of immune processes, including wound repair and cell proliferation and differentiation, and overexpression of TGF-β could impair

the immune clearance of pathogens and protect the host from bacterial infection [39, 40]. The addition of AP to diet has been proven to enhance the expression of IL-1β and TNF-α in yellow catfish [41], common carp [7], and turbot [8]. Furthermore, PP could increase the levels of TNF in mice with cancer [42]. Similar results were observed in this study. The levels of the inflammatory cytokines TNF-α and IgM in the intestine were upregulated after dietary addition of AP in Dabry's sturgeons, indicating that polysaccharides may strengthen cellular immune function by improving the concentrations of major cytokines and further activating immune-related signal pathways, such as the MAPK signaling cascade [43] or TLR/NF-κB signaling pathway [44]. IgM plays a critical role in innate immunity by providing a first line of defense against microbial infections in fish species [45]. The mRNA level of IgM was significantly improved after AP feeding for 14 days, suggesting that the addition of AP to diets may enhance the immune response of Dabry's sturgeon. This speculation is reasonable given that AP has been shown to improve organic cellular immune functions by promoting lymphocyte proliferation and differentiation, presenting antigens, increasing the numbers and functions of macrophages, and stimulating natural killer cells and enhancing their killing efficiency [46].

In addition to the innate and adaptive immune systems, the gut microbiota can form a symbiotic relationship with its host by regulating digestion, gut development, nutrient absorption, and metabolism [47]. As far as we know, this study is the first to describe the gut microbiota composition of Dabry's sturgeons in the normal and plant polysaccharide-supplemented growth states. In the present study, dietary AP improved the richness and diversity of major gut microbiota in Dabry's sturgeon based on the Chao 1 and Shannon indices, while the beta diversity analysis revealed that the structure in the PP group was clearly distinguished from that in the CT group. The communities in the guts of Dabry's sturgeons were comprised of Fusobacteriota, Proteobacteria, and Firmicutes, which were generally described as common bacterial phyla in other carnivorous fish including sturgeons, such as the Asian sea bass [48] and Siberian sturgeon [49]. In agreement with a previous study reporting that AP can modulate gut microbiota structural and functional changes [50], dietary supplementation of AP significantly increased the ratio of Firmicutes and Bacteroidetes in the present study. Firmicutes and Bacteroidetes have been proven to be associated with host energy harvesting [51]; thus, the addition of AP to diet may promote the developmental growth of Dabry's sturgeons. However, further study of the potential functional influence of dietary AP supplementation in sturgeons is still required.

Desulfobacterota was found to be positively related to the serum level of the inflammatory cytokine TNF-α [52]. Besides, a positive correlation has been reported between the relative abundance of Fusobacteria and the expression levels of IL-8, which is involved in pathogen disease resistance, in grass carp [53]. In this study, the abundance of *Desulfovibrio* in the intestinal microbiota showed a positive correlation with the expression levels of TNF-α and IgM. However, we did not find any significant Pearson correlation between the intestinal microbiota composition and *IL-1β* in this study. The correlation between the relative abundances of gut microbiota and immune-related genes expression levels remains poorly explored in sturgeon species. Neither the functions of intestinal microbiota nor the causal relationships between changes in intestinal microbiota and immune-related genes are well understood, and further research is needed to understand how the functions and changes occur.

In conclusion, our study was the first to report the assessment of *P. cocos* and *Astragalus* polysaccharides as dietary supplements in Dabry's sturgeon aquaculture. The results showed that Dabry's sturgeons fed PP or AP show better performances in antioxidant capacity and immunity than normal breeding individuals. Furthermore, this study presents the effects of PP and AP on the gut microbiota composition of Dabry's sturgeon for the first time, and it showed that AP supplementation remarkably increased the ratio of Firmicutes and Bacteroidetes. Although the actual effect and mechanism require further experimental research, our research preliminarily proved that feeding PP or AP to Dabry's sturgeons may contribute to better performance in terms of growth and development, as well as inflammatory response, which would provide an important basis for efficient artificial breeding of Dabry's sturgeons.

ETHICS APPROVAL

All sampling procedures involving handling and treatment during this study were approved by the Chinese Sturgeon Research Institute of the China Three Gorges Corporation and the Hubei Key Laboratory of the Three Gorges Project for Conservation of Fishes.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article.

AUTHOR CONTRIBUTIONS

J.Z., D.Z., and J.Y. conceived this study. J.Z., D.Z., and T.T. carried out the sampling. J.Z. and D.S. performed the examination of antioxidant status. T.T., X.C., K.X., and J.Y. analyzed the expression of immune-related genes and the compositions and putative functions of gut bacterial communities. J.Z., X.C., and J.Y. contributed to the data collection and statistical analysis. J.Z. wrote the original manuscript, and J.Y. finalized the manuscript. All authors reviewed the manuscript.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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