Effects of Astragalus Polysaccharide on Growth Performance, Immune Function, Antioxidant Capacity, and Disease Resistance of Hybrid Snakehead: Postprint

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Abstract

This study aimed to investigate the effects of Astragalus polysaccharides (APS) on the growth performance, immune capacity, antioxidant capacity, and disease resistance of hybrid snakehead fish. A total of 720 healthy hybrid snakehead fish with an initial body weight of (24.5±0.5) g were randomly divided into 6 groups (4 replicates per group, 30 fish per replicate) and fed experimental diets supplemented with 0 (APS0 group, as the control group), 0.25 (APS0.25 group), 0.50 (APS0.50 group), 1.00 (APS1.00 group), 1.50 (APS1.50 group), and 2.00 g/kg (APS2.00 group) of Astragalus polysaccharides in the basal diet for a 60-day culture period. The results showed that the weight gain rate of fish increased with dietary supplementation of different levels of Astragalus polysaccharides, with the differences reaching a significant level ($P<0.05$) in the APS1.50 and APS2.00 groups compared with the control group. The feed conversion ratio exhibited a trend of first decreasing and then increasing with increasing Astragalus polysaccharide supplementation, with the lowest value in the APS1.50 group. Intestinal protease activity, lipase activity, and microvillus length all increased with increasing Astragalus polysaccharide supplementation, reaching maximum values at a supplementation level of 1.50 g/kg, and decreasing with further increases in supplementation level. Plasma lysozyme (LSZ) activity, complement 3 (C3), complement 4 (C4), and immunoglobulin M (IgM) contents, as well as whole blood respiratory burst activity, all increased initially and then decreased with increasing Astragalus polysaccharide supplementation, with the highest values observed in the APS1.50 group, which were significantly higher than those in the control group ($P<0.05$) and not significantly different from the APS1.00 (except for C3 content) and APS2.00 groups ($P>0.05$). The change trend of plasma acid phosphatase (ACP) activity was similar to that of

LSZ activity, but its highest value appeared in the APS1.00 group. The activities of superoxide dismutase (SOD) and catalase (CAT) in plasma and liver of hybrid snakehead fish in the APS1.50 group were significantly higher than those in the control group (P<0.05), while malondialdehyde (MDA) content was significantly lower than that in the control group (P<0.05). Following challenge with Aeromonas hydrophila, the cumulative mortality of hybrid snakehead fish within 96 h was lowest in the APS1.50 group, which was significantly lower than that in the control group and the APS0.25 and APS0.50 groups (P<0.05), but not significantly different from the APS1.00 and APS2.00 groups (P>0.05). It can be concluded that dietary supplementation of appropriate levels of Astragalus polysaccharides can improve the growth performance, digestive capacity, immune capacity, antioxidant capacity, and disease resistance of hybrid snakehead fish; considering all factors comprehensively, the supplementation level of 1.50 g/kg Astragalus polysaccharides in hybrid snakehead fish feed is recommended.

Full Text

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Abstract

This experiment was conducted to investigate the effects of Astragalus polysaccharide (APS) on growth performance, immune function, antioxidant capacity and disease resistance of hybrid snakehead (*Channa maculata* × *Channa argus*). A total of 720 healthy hybrid snakehead with an initial body weight of (24.5±0.5) g were randomly allocated into six groups with four replicates per group and 30 fish per replicate. The control group (APS0) was fed a basal diet, while the treatment groups were fed the basal diet supplemented with 0.25 (APS0.25), 0.50 (APS0.50), 1.00 (APS1.00), 1.50 (APS1.50) and 2.00 g/kg (APS2.00) APS, respectively. The feeding trial lasted for 60 days. The results showed that dietary APS supplementation increased the weight gain rate (WGR) of fish, with significant differences observed in the APS1.50 and APS2.00 groups compared with the control (P<0.05). The feed conversion ratio (FCR) decreased initially and then increased with increasing APS levels, reaching its lowest value in the APS1.50 group.

Intestinal protease and lipase activities and microvillus length increased progressively with APS supplementation up to 1.50 g/kg, after which they declined.

Plasma lysozyme (LSZ) activity and complement 3 (C3), complement 4 (C4) and immunoglobulin M (IgM) concentrations, as well as whole blood respiratory burst activity, increased initially and then decreased with increasing dietary APS levels, peaking in the APS1.50 group and showing significant differences from the control ($P<0.05$), but no significant differences from the APS1.00 (except for C3) and APS2.00 groups ($P>0.05$). Plasma acid phosphatase (ACP) activity followed a similar trend to LSZ, but its peak value occurred in the APS1.00 group. The APS1.50 group exhibited significantly higher superoxide dismutase (SOD) and catalase (CAT) activities in both plasma and liver compared with the control ($P<0.05$), while malondialdehyde (MDA) content was significantly lower ($P<0.05$). Following challenge with *Aeromonas hydrophila*, the cumulative mortality within 96 h was lowest in the APS1.50 group, which was significantly lower than that in the control and APS0.25 and APS0.50 groups ($P<0.05$), but not significantly different from the APS1.00 and APS2.00 groups ($P>0.05$).

These results indicate that appropriate dietary APS supplementation can improve growth performance, digestive capacity, immune function, antioxidant capacity and disease resistance in hybrid snakehead. Based on comprehensive consideration of all factors, the optimal dietary APS supplementation level for hybrid snakehead is 1.50 g/kg.

Keywords: Astragalus polysaccharide; hybrid snakehead; growth performance; immune capacity; antioxidant capacity; disease resistance

Introduction

Hybrid snakehead (*Channa maculata* × *Channa argus*) is an F1 hybrid produced by crossing female blotched snakehead (*C. maculata*) introduced from Guangdong with male northern snakehead (*C. argus*) from Zhejiang. This hybrid overcomes the limitations of traditional northern snakehead culture, which relies primarily on fresh trash fish and faces difficulties in transitioning to formulated feeds, as well as the slow growth and low temperature tolerance of blotched snakehead that restrict its farming regions. Through years of aquaculture trials, hybrid snakehead has demonstrated advantages including rapid growth, strong stress resistance, high yield, high fillet rate, low feed conversion ratio, short growth cycle, and suitability for large-scale intensive culture [1]. In recent years, farming of hybrid snakehead has gradually expanded in the Pearl River Delta and Yangtze River Delta regions, making it one of China's important aquaculture species [2]. However, high-density intensive farming has led to deteriorating culture environments, resulting in slow growth and frequent disease outbreaks. Antibiotic residues from disease treatment have seriously affected the healthy and sustainable development of the hybrid snakehead industry. Currently, some researchers have improved animal immunity and reduced disease incidence by adding Chinese herbal medicines, trace elements and vitamins to

feeds, achieving certain progress [3-4].

Astragalus polysaccharide (APS) is an extract from the traditional Chinese herb Astragalus membranaceus, composed of glucose, galactose and other components. It possesses immunomodulatory functions including regulation of humoral and cellular immunity, enhances resistance to various stressors and oxidative damage, and improves intestinal structure [5-7]. With breakthroughs in industrial production technology and in-depth pharmacological research, APS has been widely applied in livestock and poultry production with demonstrated efficacy, and has also shown promising results in some aquatic animals. Xiang et al. [6] reported that dietary APS supplementation at 0.040%-0.074% improved growth performance and immunity of Schizothorax prenanti, and other researchers have confirmed that APS enhances non-specific immunity in common carp [8], tilapia [9] and gibel carp [10]. However, no reports have documented the effects of APS on growth performance, digestive capacity, immunity and antioxidant capacity in carnivorous fish species.

This experiment investigated the effects of different dietary APS levels on growth performance and immunity of hybrid snakehead to explore the mechanisms by which APS enhances immunity and reduces disease incidence. The objective was to develop a green, environmentally friendly functional feed for hybrid snakehead without side effects that enhances immunity, reduces disease outbreaks, and promotes growth while improving flesh quality.

1.1 Source of Astragalus Polysaccharide and Feed Preparation

The APS used in this experiment was purchased from Beijing Shengtaier Biotechnology Co., Ltd. with a purity of 64.5%. The basal diet was formulated using fish meal, soybean meal, rapeseed meal and cottonseed meal as protein sources, a 1:1 mixture of fish oil and soybean oil as lipid source, and wheat flour as carbohydrate source. The composition and nutrient levels of the basal diet are shown in Table 1 . Six experimental diets were prepared by supplementing the basal diet with 0, 0.25, 0.50, 1.00, 1.50 and 2.0 g/kg APS, respectively. During feed preparation, all ingredients were ground and passed through a 60-mesh sieve, weighed according to proportions, mixed thoroughly using a mixer, and processed into 2 mm diameter pellets after adding appropriate water. The pellets were air-dried and stored at -20 °C until use.

Table 1 Composition and nutrient levels of the basal diet (dry matter basis) %

Item	Content
Ingredients	
Fish meal	32.00
Soybean meal	20.00
Rapeseed meal	10.00

Item	Content
Cottonseed meal	8.00
Wheat bran	5.00
Wheat flour	18.00
Fish oil	2.00
Soybean oil	2.00
NaCl	0.30
Ca(H PO)	1.50
Premix	1.20
Total	100.00
Nutrient levels	
Crude protein	40.12
Crude lipid	8.56
Ash	10.23
Gross energy (MJ/kg)	17.85

Premix supplied the following per kg of diet: CuSO · 5H O 20 mg, FeSO · 7H O 250 mg, ZnSO · 7H O 220 mg, MnSO · 4H O 70 mg, Na SeO 0.4 mg, KI 0.26 mg, CoCl · 6H O 1 mg, VA 9,000 IU, VD 2,000 IU, VE 45 mg, VK 2.2 mg, VB 3.2 mg, VB 10.9 mg, nicotinic acid 28 mg, VB 20 mg, VB 5 mg, VB 0.016 mg, VC 50 mg, pantothenate 10 mg, folic acid 1.65 mg, choline 600 mg.

1.2 Experimental Fish

Hybrid snakehead were purchased from the Black Fish Research Institute of Yuhang District, Hangzhou, with an initial body weight of (24.5±0.5) g. The fish were acclimated for two weeks, initially fed with fresh fish mince formed into balls on the water surface, then gradually transitioning to the experimental diets by progressively adding them to the fish mince until the juveniles could fully accept the experimental feeds. After acclimation, 30 uniform-sized, healthy fish were selected for each cage (dimensions: 1 m × 1 m × 1 m), with six groups and four cages per group. The cages were fixed on two specially designed steel frame rafts (inner diameter 4 m × 4 m) placed in a 0.3 hm^2 pond without aquaculture production or aeration facilities.

1.3 Experimental Design and Feeding Management

The six experimental groups were fed diets supplemented with 0 (APS0, control), 0.25 (APS0.25), 0.50 (APS0.50), 1.00 (APS1.00), 1.50 (APS1.50) and 2.00 g/kg (APS2.00) APS, respectively. Fish were fed three times daily (07:00, 12:00, 17:00) at a feeding rate of 3%-5% of body weight. The experimental period lasted 60 days. During the trial, water temperature was maintained at (27±3) °C, pH ranged from 6.8 to 8.0, ammonia nitrogen concentration was <0.2 mg/L, and dissolved oxygen concentration was >5 mg/L.

1.4 Sample Collection

At the end of the feeding trial, fish were fasted for 24 h. All fish in each cage were counted and weighed to calculate weight gain rate and survival rate. The total feed consumption per group was recorded to calculate feed conversion ratio. Three fish were randomly selected from each cage for blood collection from the caudal vein. Blood samples were placed in anticoagulant tubes, with a portion immediately used for whole blood respiratory burst determination and the remainder centrifuged at 3,500 r/min for 10 min at 4 °C; the supernatant was stored at -80 °C. Another three fish per cage were dissected to obtain liver and intestine tissues, which were washed with ice-cold physiological saline, blotted dry with filter paper, and stored at -80 °C. Two additional fish per cage were sampled, and the mid-intestine was cut into 1 mm^3 pieces and fixed for electron microscopy analysis.

1.5 Sample Analysis

Intestinal and liver tissues were weighed and homogenized in physiological saline at a 1:4 mass-to-volume ratio. The homogenates were centrifuged at 3,000 × g for 10 min at 4 °C, and the supernatants were stored at -20 °C for subsequent analysis of intestinal protease, lipase and amylase activities, as well as hepatic CAT, SOD activities and MDA content.

Intestinal lipase and amylase activities, plasma and hepatic CAT, SOD activities and MDA content, and plasma LSZ, ACP activities and total antioxidant capacity (T-AOC) were determined using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions. Intestinal protease activity was measured using the Folin-phenol method [11]. Plasma C3, C4 and IgM concentrations were determined using ELISA kits from Nanjing Jiancheng Bioengineering Institute.

Whole blood respiratory burst activity was measured using the nitro-blue tetrazolium (NBT) method described by Anderson et al. [12] as follows: (1) 100 L of anticoagulated blood was mixed with 100 L of 0.2% NBT solution (prepared by dissolving 0.2 g NBT in 100 mL sterile 0.65%-0.70% physiological saline) and incubated at 25 °C for 30 min in an EP tube; (2) 50 L of the reaction mixture was mixed with 1 mL N,N-dimethylformamide and centrifuged at 2,000 r/min for 5 min; (3) the supernatant was read at 540 nm using 1 mL N,N-dimethylformamide as blank. Respiratory burst activity was expressed as the OD value of the NBT reaction.

For transmission electron microscopy (TEM) of intestinal samples, mid-intestine tissues were cut into 0.5-1.0 mm^3 pieces, washed twice with phosphate-buffered saline (PBS), fixed in 2.5% glutaraldehyde for 24 h, then post-fixed in 1% osmium tetroxide until blackened. Samples were dehydrated, embedded, sectioned and observed under a transmission electron microscope (HT-7700, Hitachi). Three samples per group were examined, and intestinal microvillus length was

measured using Image-Pro Plus software. Ten microvilli were randomly measured per sample, yielding 30 data points per group for statistical analysis.

1.6 Growth Performance Calculations

Weight gain rate (WGR, %) = 100 × (final weight - initial weight) / initial weight
Survival rate (SR, %) = 100 × final number of surviving fish / initial number of fish stocked
Feed conversion ratio (FCR) = feed intake / (final weight - initial weight)

1.7 Challenge Test

After sampling, 15 fish of similar size were selected from each cage for the challenge test (three cages per group). *Aeromonas hydrophila* was provided by the Freshwater Fisheries Research Center of Chinese Academy of Fishery Sciences and diluted with sterile physiological saline to 1×10 CFU/mL (the median lethal dose determined through preliminary experiments). Fish were injected intraperitoneally with 1 mL bacterial suspension per 100 g body weight and returned to cages for normal feeding. Cumulative mortality was observed over 96 h, with three daily observations.

Cumulative mortality (%) = 100 × number of dead fish / number of challenged fish

1.8 Statistical Analysis

Data were analyzed using one-way ANOVA with SPSS 18.0 software, followed by Duncan' s multiple range test for inter-group comparisons. Significance was set at $P<0.05$. Results are presented as mean ± standard error (SE).

2.1 Effects of Astragalus Polysaccharide on Growth Performance of Hybrid Snakehead

As shown in Table 2 , final weight and WGR in groups APS1.50 and APS2.00 were significantly higher than those in groups APS0, APS0.25, APS0.50 and APS1.00 ($P<0.05$). The APS1.00 group showed significantly higher final weight and WGR than the APS0.25 group ($P<0.05$), while no significant differences were observed among groups APS0, APS0.50 and APS1.00 ($P>0.05$). Although no significant differences in FCR were detected among groups ($P>0.05$), FCR decreased initially with increasing APS levels, reached its minimum in the APS1.50 group, and increased slightly thereafter. Survival rate did not differ significantly among all groups ($P>0.05$).

Table 2 Effects of APS on growth performance of hybrid snakehead

Groups	Initial weight (g)	Final weight (g)	WGR (%)	SR (%)	FCR
APS0	24.49±0.12	81.49±2.48	234.23±10.19	97.50±1.60	1.67±0.02
APS0.25	24.53±0.15	79.58±3.70	224.78±12.56	97.50±0.83	1.69±0.08
APS0.50	24.67±0.07	82.71±1.15	235.29±4.15	98.33±0.96	1.55±0.07
APS1.00	24.54±0.13	86.60±2.17	254.25±8.63	98.33±0.96	1.53±0.12
APS1.50	24.48±0.08	98.14±2.03	299.62±7.38	100.00±0.00	1.44±0.06
APS2.00	24.53±0.15	93.52±1.12	281.29±6.62	100.00±0.00	1.49±0.11

Values in the same column with different letter superscripts were significantly different (P<0.05). The same as below.

2.2 Effects of Astragalus Polysaccharide on Intestinal Digestive Enzyme Activities and Microvillus Length

As shown in Table 3 , dietary APS supplementation did not significantly affect intestinal amylase activity (P>0.05). Both intestinal protease and lipase activities increased initially and then decreased with increasing APS levels, with the APS1.50 group showing significantly higher values than the APS0 and APS0.25 groups (P<0.05), but no significant differences from the APS1.00 and APS2.00 groups (P>0.05).

Figure 1 [Figure 1: see original paper] and Table 3 indicate that intestinal microvillus length increased with APS supplementation up to 1.50 g/kg, after which it decreased. The APS1.50 and APS2.00 groups exhibited significantly greater microvillus length than the APS0, APS0.50 and APS0.25 groups (P<0.05).

Table 3 Effects of APS on digestive enzyme activities and microvilli length in intestine of hybrid snakehead

Groups	Amylase (U/mg prot)	Protease (U/mg prot)	Lipase (U/g prot)	Microvilli length (m)
APS0	2.56±0.13	40.03±4.81	36.66±6.20	1.05±0.01
APS0.25	2.68±0.15	49.77±3.91	47.28±4.05	1.16±0.01
APS0.50	2.73±0.27	60.09±5.40	52.08±4.64	1.36±0.01
APS1.00	2.77±0.25	74.97±6.53	61.27±3.21	1.39±0.01
APS1.50	2.49±0.34	81.47±6.21	68.70±3.31	1.45±0.02
APS2.00	2.37±0.41	72.39±8.59	58.37±4.17	1.42±0.02

Figure 1 TEM micrographs of intestinal microvilli of hybrid snakehead (3,000×)

2.3 Effects of Astragalus Polysaccharide on Blood Immune Indexes of Hybrid Snakehead

As shown in Table 4 , plasma LSZ activity and C3, C4 concentrations, along with whole blood respiratory burst activity, increased initially and then decreased with increasing dietary APS levels, reaching maximum values in the APS1.50 group, which were significantly higher than those in the control (P<0.05). Except for C3 content, which differed significantly from the APS1.00 group (P<0.05), other indexes showed no significant differences from the APS1.00 and APS2.00 groups (P>0.05). Plasma ACP activity followed a similar trend to LSZ, but peaked in the APS1.00 group, showing significant difference from the control (P<0.05) but not from other groups (P>0.05).

Table 4 Effects of APS on blood immune indexes of hybrid snakehead

Groups	LSZ (g/mL)	ACP (U/dL)	C3 (g/mL)	C4 (g/mL)	Respiratory burst (OD)
APS0	5.20±0.10	350.42±6.37	75.7±5.8	144±5	0.36±0.02
APS0.25	5.35±0.08	361.90±7.47	80.5±3.2	147±4	0.42±0.02
APS0.50	5.45±0.08	364.76±7.51	88.6±2.4	148±8	0.47±0.03
APS1.00	5.52±0.05	374.00±3.76	90.3±8.3	153±7	0.46±0.06
APS1.50	5.52±0.06	367.54±4.54	108.1±1.3	165±6	0.50±0.04
APS2.00	5.43±0.06	362.31±7.31	101.3±2.8	161±4	0.42±0.03

2.4 Effects of Astragalus Polysaccharide on Plasma IgM Content

As shown in Figure 2 [Figure 2: see original paper], plasma IgM content was lowest in the APS0 group (1.22 g/L), which was significantly lower than in the APS0.50, APS1.00, APS1.50 and APS2.00 groups (P<0.05), but not significantly different from the APS0.25 group (P>0.05). The highest IgM content was observed in the APS1.50 group (1.75 g/L), which was significantly higher than in the APS0 and APS0.25 groups (P<0.05), but not significantly different from other groups (P>0.05).

Figure 2 Effects of APS on plasma IgM content of hybrid snakehead
Data columns with different letters were significantly different (P<0.05).

2.5 Effects of Astragalus Polysaccharide on Antioxidant Indexes in Plasma and Liver

As shown in Table 5 , plasma CAT activity increased initially and then decreased with increasing dietary APS levels, with the APS0.50, APS1.00, APS1.50 and APS2.00 groups showing significantly higher values than the APS0 and APS0.25 groups (P<0.05). The APS1.00 and APS1.50 groups also exhibited significantly higher CAT activity than the APS0.50 and APS2.00 groups (P<0.05). Plasma SOD activity and T-AOC followed similar trends, with the APS1.00 and APS1.50 groups showing significantly higher values than the other four groups

(P<0.05), and the APS1.50 group having the highest values. Plasma MDA content decreased initially and then increased with APS supplementation, reaching its lowest value in the APS1.50 group, which was significantly lower than in the APS0, APS0.25 and APS0.50 groups (P<0.05), but not significantly different from the APS1.00 and APS2.00 groups (P>0.05).

Table 5 Effects of APS on plasma antioxidant indexes of hybrid snakehead

Groups	CAT (U/mL)	SOD (U/mL)	T-AOC (U/mL)	MDA (nmol/mL)
APS0	5.44±0.10	13.39±0.54	9.21±0.18	15.46±0.51
APS0.25	5.55±0.10	14.18±0.41	10.12±0.31	14.59±0.42
APS0.50	6.02±0.15	15.38±0.62	10.49±0.17	13.08±0.45
APS1.00	6.56±0.15	16.07±0.57	10.98±0.21	12.39±0.28
APS1.50	6.52±0.15	16.20±0.38	11.32±0.32	11.47±0.38
APS2.00	6.02±0.22	15.80±0.64	10.78±0.42	12.14±0.47

As shown in Table 6 , hepatic CAT activity increased initially and then decreased with increasing dietary APS levels, reaching its maximum at 1.50 g/kg APS. The APS0.15 group was significantly higher than the APS0, APS0.25 and APS0.50 groups (P<0.05), but only slightly higher than the APS1.50 and APS2.00 groups (P>0.05). Hepatic SOD activity was highest in the APS2.00 group, which was significantly higher than the other five groups (P<0.05). Hepatic MDA content decreased initially and then increased with APS supplementation, with the APS1.50 group showing the lowest value. Both the APS1.50 and APS2.00 groups had significantly lower MDA content than the other four groups (P<0.05).

Table 6 Effects of APS on liver antioxidant indexes of hybrid snakehead

Groups	CAT (U/mg prot)	SOD (U/mg prot)	MDA (nmol/mg prot)
APS0	25.46±1.74	100.69±6.68	14.65±0.46
APS0.25	30.45±1.18	113.30±5.55	13.81±0.54
APS0.50	33.39±2.54	106.89±5.36	12.64±0.53
APS1.00	38.01±1.64	117.08±3.73	12.03±0.35
APS1.50	43.38±2.01	130.98±5.11	9.43±0.64
APS2.00	40.41±2.80	107.67±8.41	10.16±0.45

2.6 Effects of Astragalus Polysaccharide on Cumulative Mortality

The trend of cumulative mortality after *A. hydrophila* injection is shown in Figure 3 [Figure 3: see original paper]. The APS1.50 group exhibited the lowest cumulative mortality, which was significantly lower than that in the APS0, APS0.50 and APS0.25 groups (P<0.05), but not significantly different from the APS1.00 and APS2.00 groups (P>0.05).

Figure 3 Effects of APS on cumulative mortality of hybrid snakehead
Data points with different letters were significantly different (P<0.05).

3.1 Effects of Astragalus Polysaccharide on Growth Performance

Published studies have demonstrated significant growth-promoting effects of APS in various aquatic species, including Schizothorax prenanti [6], tilapia [13], crayfish [14] and Nile tilapia [15]. The present results indicate that dietary APS supplementation at 1.50 or 2.00 g/kg significantly improved growth performance of hybrid snakehead compared with the unsupplemented control. This improvement may be attributed to several mechanisms. First, APS is a biologically active polysaccharide extracted from Astragalus that, when added at appropriate levels to feed, can promote the proliferation of beneficial bacteria such as *Lactobacillus*, *Yeast*, *Pseudomonas* and *Bifidobacterium* in the intestine, which themselves produce various digestive enzymes, ultimately enhancing digestive enzyme activity [16]. Second, APS may improve nutrient digestion and absorption by promoting digestive juice secretion [17]. Third, APS can significantly increase intestinal villus length, fold depth and muscular layer thickness, as well as increase the number of intestinal goblet cells and intraepithelial lymphocytes [18], thereby enhancing digestive and absorptive capacity and promoting growth. Fourth, certain active components in APS may promote protein synthesis in animals, enhancing their ability to convert absorbed nutrients into body protein and thus increasing growth rate [6,19]. The current results demonstrate that APS promoted intestinal microvillus development, enhanced intestinal digestive enzyme activities, improved nutrient digestion and absorption, accelerated growth rate, and reduced feed conversion ratio in hybrid snakehead. As a carnivorous fish, the optimal APS supplementation level for growth promotion in hybrid snakehead was 1.50-2.00 g/kg under the present experimental conditions. This differs from the optimal levels reported for other species, such as 0.040%-0.074% for S. prenanti [6], 100-200 mL/kg APS liposome for tilapia [13], 0.40%-0.80% for crayfish [14], and 1,500 mg/kg for Nile tilapia [15]. These discrepancies may be related to differences in feeding habits, developmental stages and APS purity among studies, and should be considered in practical applications.

3.2 Effects of Astragalus Polysaccharide on Immune Function

Fish possess a relatively underdeveloped specific immune system, relying primarily on non-specific immunity, which mainly includes factors such as lysozyme, acid phosphatase, complement and antimicrobial peptides. Lysozyme can hydrolyze mucopolysaccharides in pathogenic bacteria, playing a crucial role in killing external bacteria and serving as an important non-specific immune factor [20]. Its activity is a key quantitative indicator of non-specific immunity [21]. ACP is a marker enzyme of macrophage lysosomes and is released during phagocytic and encapsulation immune responses in fish blood cells [22]. Com-

plement is an essential component of the fish immune system with functions against microbial invasion and immune complex clearance [23]. The present results demonstrate that appropriate dietary APS supplementation enhanced non-specific immunity and disease resistance in hybrid snakehead, with optimal effects observed at 1.50 g/kg. These findings are consistent with studies showing that dietary APS at 1,000 and 1,500 mg/kg improved non-specific immunity in tilapia [24] and that 1,200 mg/kg APS significantly enhanced immune function in yellow catfish [25]. Bai et al. [26] suggested that APS improves immunity by increasing cellular metabolic capacity, promoting immune organ development, and enhancing the function and activity of immune cells including T cells, B cells and natural killer (NK) cells. At the molecular level, APS promotes DNA and RNA synthesis and transcription, as well as protein synthesis and expression, particularly of cytokines such as nuclear factor- B (NF- B), interleukin-1 (IL-1) and tumor necrosis factor- (TNF-) that are closely associated with disease occurrence and development [27].

Immunoglobulins primarily participate in humoral immunity in fish, with IgM being an important immunoglobulin that plays a crucial role in immune responses. Increased IgM content indicates enhanced immune function. Chen et al. [28] found that dietary APS supplementation at 300 mg/kg significantly increased serum IgM content in broiler chickens at 21 days of age. In the present study, plasma IgM content in hybrid snakehead increased initially and then decreased with increasing APS levels, peaking at 1.50 g/kg APS, indicating that appropriate APS supplementation can enhance humoral immunity.

3.3 Effects of Astragalus Polysaccharide on Antioxidant Capacity

During pathological or stress conditions, fish produce excessive reactive oxygen species (ROS) and free radicals that can attack proteins, DNA and cell membranes, leading to metabolic disorders, slow growth, decreased immune function, increased feed conversion ratio and compromised product quality [29-30]. When ROS and free radicals increase, SOD and CAT activities in blood typically rise to eliminate these harmful substances [31]. Zhang et al. [24] reported that appropriate dietary APS supplementation significantly increased plasma SOD and CAT activities in tilapia and enhanced SOD and CAT activities in various tissues while reducing MDA content [25]. Jia et al. [32] demonstrated that dietary APS at 1.5 and 3.0 g/kg significantly reduced SOD activity and T-AOC in carbon tetrachloride (CCl)-damaged hepatocyte culture medium and serum, and significantly inhibited MDA synthesis in liver tissue. Chen et al. [33] showed that APS significantly alleviated cyclophosphamide-induced damage to the antioxidant system in loach hepatopancreas, demonstrating good immunomodulatory effects. The present results indicate that appropriate dietary APS supplementation significantly increased SOD and CAT activities in both plasma and liver of hybrid snakehead while significantly inhibiting MDA synthesis, suggesting that APS can enhance antioxidant enzyme activity, reduce oxidative damage from oxygen free radicals, and improve antioxidant capacity, consistent with findings

by Yan et al. [34], Li et al. [35] and Elabd et al. [36].

3.4 Effects of Astragalus Polysaccharide on Disease Resistance

Acute challenge tests in fish can evaluate disease resistance by measuring short-term cumulative mortality. Hong et al. [37] reported that dietary APS at 0.8% improved survival by 26.67% in crayfish challenged with white spot syndrome virus (WSSV) compared with the positive control, demonstrating enhanced antiviral capacity. Similar enhancement of disease resistance by APS has been confirmed in challenge tests with common carp [9] and catfish [38]. The present results are consistent with these findings, showing that dietary APS reduced cumulative mortality in hybrid snakehead challenged with *A. hydrophila*, further demonstrating that APS enhances disease resistance by improving immune and antioxidant capacity, thereby reducing morbidity and mortality. Xu and Ma [39] found that dietary APS combined with vitamins effectively prevented grass carp hemorrhagic disease, providing practical validation of APS efficacy in improving fish disease resistance.

Conclusion

Dietary Astragalus polysaccharide supplementation improved growth performance, immune function, antioxidant capacity and disease resistance in hybrid snakehead. Effects became evident at supplementation levels above 1.00 g/kg, but declined slightly at 2.00 g/kg. Therefore, the optimal dietary APS supplementation level for hybrid snakehead is 1.50 g/kg.

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