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

Authors: Wang Yuheng, Xu Xiaozhou, Wang Huicong, Chen Jun, Zhang Kun, Ni Xinyi

Date: 2018-12-24T00:00:00+00:00

Abstract

This study aimed to investigate the effects of Astragalus polysaccharides (APS) on growth performance, immune capacity, antioxidant capacity, and disease resistance of hybrid snakehead. Seven hundred twenty healthy hybrid snakehead with initial body weight of (24.5±0.5) g were randomly allocated into six groups (four replicates per group, 30 fish per replicate) and fed experimental diets containing 0 (APS0 group, as control), 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) APS supplementation for 60 days. The results showed that weight gain rate increased with APS supplementation, with significant differences observed in APS1.50 and APS2.00 groups compared to the control ($P<0.05$). Feed conversion ratio exhibited a decreasing-then-increasing trend with APS levels, reaching the minimum in APS1.50 group. Intestinal protease activity, lipase activity, and microvillus length increased progressively with APS supplementation, peaking at 1.50 g/kg, and declined thereafter. Plasma lysozyme (LSZ) activity, complement 3 (C3), complement 4 (C4), immunoglobulin M (IgM) contents, and whole blood respiratory burst activity increased initially and then decreased with APS supplementation, achieving maximum values in APS1.50 group, which were significantly higher than the control ($P<0.05$) but not significantly different from APS1.00 (except C3 content) and APS2.00 groups ($P>0.05$). Plasma acid phosphatase (ACP) activity followed a similar trend to LSZ activity, but peaked in APS1.00 group. APS1.50 group exhibited significantly higher superoxide dismutase (SOD) and catalase (CAT) activities in plasma and liver compared to the control ($P<0.05$), while malondialdehyde (MDA) content was significantly lower ($P<0.05$). Following Aeromonas hydrophila challenge, cumulative mortality within 96 h was lowest in APS1.50 group, significantly lower than the

control, APS0.25, and APS0.50 groups (P<0.05), but not significantly different from APS1.00 and APS2.00 groups (P>0.05). In conclusion, dietary APS supplementation at appropriate levels can enhance growth performance, digestive capacity, immune capacity, antioxidant capacity, and disease resistance in hybrid snakehead; considering all parameters, 1.50 g/kg APS supplementation is recommended.

Full Text

Effects of Astragalus Polysaccharide on Growth Performance, Immunity, Antioxidant Capability and Disease Resistance of Hybrid Snakehead

WANG Yuheng, XU Xiaozhou, WANG Huicong, CHEN Jun, ZHANG Kun, NI Xinyi
College of Animal Science and Veterinary Medicine, Jiangsu Polytechnic College of Agriculture and Forestry, Zhenjiang 212400, China

Abstract

This experiment aimed to investigate the effects of Astragalus polysaccharide (APS) on growth performance, immunity, antioxidant capability, and disease resistance of hybrid snakehead (*Channa maculata* × *Channa argus*). Seven hundred twenty healthy hybrid snakehead with an initial body weight of (24.5±0.5) g were randomly allocated into six groups, each with four replicates of 30 fish. The control group (APS0) was fed a basal diet, while the treatment groups were fed diets 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) of Astragalus polysaccharide for 60 days.

The results showed that dietary supplementation with Astragalus polysaccharide increased the weight gain rate (WGR) of fish, with significant differences observed in the APS1.50 and APS2.00 groups compared to the control (P<0.05). The feed conversion ratio (FCR) initially decreased and then increased with rising APS levels, reaching its lowest value in the APS1.50 group. Intestinal protease and lipase activities and microvilli length increased progressively with APS supplementation, peaking at 1.50 g/kg before declining at higher doses.

Plasma lysozyme (LSZ) activity and complement 3 (C3), complement 4 (C4), and immunoglobulin M (IgM) concentrations, along with whole blood respiratory burst activity, exhibited a similar trend—increasing initially and then decreasing with APS levels, with maximum values in the APS1.50 group that were significantly higher than the control (P<0.05). These parameters showed no significant differences compared to the APS1.00 (except C3) and APS2.00 groups (P>0.05). Plasma acid phosphatase (ACP) activity followed a comparable pattern, though its peak occurred in the APS1.00 group.

The APS1.50 group demonstrated significantly higher superoxide dismutase (SOD) and catalase (CAT) activities in both plasma and liver compared to the control ($P<0.05$), while malondialdehyde (MDA) content was significantly lower ($P<0.05$). Following challenge with *Aeromonas hydrophila*, the APS1.50 group exhibited the lowest cumulative mortality within 96 h, which was significantly lower than that of the control, 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 findings indicate that appropriate dietary supplementation of Astragalus polysaccharide can enhance growth performance, digestive capacity, immunity, antioxidant capability, and disease resistance in hybrid snakehead. Based on comprehensive consideration of all factors, the optimal dietary inclusion level of Astragalus polysaccharide for hybrid snakehead is 1.50 g/kg.

Keywords: Astragalus polysaccharide; hybrid snakehead; growth performance; immunity; antioxidant capability; disease resistance

Introduction

Hybrid snakehead (*Channa maculata* × *Channa argus*) is an F1 hybrid produced by crossing female blotched snakehead introduced from Guangdong with male northern snakehead from Zhejiang. This hybrid overcomes the limitations of traditional northern snakehead culture, which relies primarily on frozen trash fish and presents 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 expanded gradually in the Pearl River Delta and Yangtze River Delta regions, establishing it as an important aquaculture species in China [2].

However, high-density, intensive farming practices have led to deteriorating culture environments, resulting in slow growth and frequent disease outbreaks. The resulting antibiotic residues from disease treatment have severely impacted the healthy and sustainable development of the hybrid snakehead industry. Currently, some researchers have achieved progress in improving animal immunity and reducing disease incidence by supplementing feeds with Chinese herbal medicines, trace elements, and vitamins [3-4].

Astragalus polysaccharide (APS), extracted from the traditional Chinese herb *Astragalus membranaceus*, is composed of glucose, galactose, and other components. It possesses immunomodulatory functions that regulate 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 deeper understanding of its pharmacological effects, Astragalus polysaccharide has been widely applied in livestock and poultry production with demonstrated efficacy, and its application in some aquatic animals

has also yielded promising results. Xiang et al. [6] reported that dietary supplementation with 0.040%-0.074% Astragalus polysaccharide improved growth performance and immunity in *Schizothorax prenanti*, and other researchers have confirmed its role in enhancing non-specific immunity in common carp [8], tilapia [9], and gibel carp [10]. However, no reports have documented the effects of Astragalus polysaccharide on growth performance, digestive capacity, immunity, and antioxidant capability in carnivorous fish species.

This study investigated the effects of different dietary levels of Astragalus polysaccharide on growth performance and immunity in hybrid snakehead, exploring the mechanisms by which APS enhances immunity and reduces disease occurrence. The objective was to develop a green, safe, functional formulated feed for hybrid snakehead that enhances immunity without side effects, thereby reducing disease outbreaks while promoting growth and improving flesh quality.

Materials and Methods

1.1 Source of Astragalus Polysaccharide and Feed Preparation

Astragalus polysaccharide 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 presented 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 Astragalus polysaccharide. 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 pellets after adding appropriate water. The pellets were air-dried naturally 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	30.0
Soybean meal	20.0
Rapeseed meal	15.0
Cottonseed meal	10.0
Wheat bran	5.0
Wheat flour	15.0
Fish oil	2.0
Soybean oil	2.0
NaCl	0.5
Ca(H PO)	1.5
Premix	1.0

Item	Content
Total	**100.0**
Nutrient levels	
Crude protein	40.5
Crude lipid	8.2
Ash	10.3
Gross energy (MJ/kg)	18.6

Premix supplied 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 obtained 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 fresh fish mince formed into balls on the water surface, then gradually transitioned to the experimental diets by progressively adding them to the mince until the juveniles fully accepted the experimental feeds. After acclimation, 30 uniformly sized, healthy fish were selected for each cage (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 (4 m × 4 m inner diameter) placed in a 0.3 hm² pond without aquaculture activities 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) Astragalus polysaccharide. Fish were fed three times daily (07:00, 12:00, 17:00) at a feeding rate of 3%-5% body weight. The experiment 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. Total feed consumption per group was recorded to determine 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; a portion was immediately used for whole blood respiratory burst determination, while the

remainder was centrifuged at 3,500 r/min for 10 min at 4°C, and the plasma was stored at -80°C. Additionally, three fish per cage were dissected to obtain liver and intestinal tissues, which were rinsed with ice-cold physiological saline, blotted dry, and stored at -80°C. Two more 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, MDA content, plasma LSZ and ACP activities, and total antioxidant capacity (T-AOC) were measured using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions. Intestinal protease activity was determined using the Folin-phenol method [11]. Plasma C3, C4, and IgM concentrations were measured using ELISA kits from Nanjing Jiancheng Bioengineering Institute.

Whole blood respiratory burst activity was determined using the nitro-blue tetrazolium (NBT) method described by Anderson et al. [12]: (1) 100 L of anticoagulated blood was mixed with 100 L of 0.2% NBT solution (0.2 g NBT dissolved 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, centrifuged at 2,000 r/min for 5 min; (3) the supernatant was read at 540 nm using a spectrophotometer, with 1 mL N,N-dimethylformamide as blank. Respiratory burst intensity was expressed as OD values.

For transmission electron microscopy, mid-intestine tissues were cut into 0.5-1.0 mm^3 pieces, rinsed 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 microvilli length was measured using Image-Pro Plus software. Ten microvilli were randomly measured per sample, yielding 30 measurements 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

- Feed conversion ratio (FCR) = total 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, returned to cages, and fed normally. Cumulative mortality was recorded 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. Duncan' s multiple range test was used for inter-group comparisons, with significance set at $P<0.05$. Results are presented as mean ± standard error (SE).

Results

2.1 Effects of Astragalus Polysaccharide on Growth Performance

As shown in Table 2 , final weight and weight gain rate 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 weight gain rate than the APS0.25 group ($P<0.05$), while no significant differences were observed among APS0, APS0.50, and APS1.00 groups ($P>0.05$). Although feed conversion ratio did not differ significantly among groups ($P>0.05$), it decreased initially with increasing APS levels, reaching its minimum in the APS1.50 group, then increased slightly at higher supplementation levels. Survival rates were not significantly different 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

Groups	Initial weight (g)	Final weight (g)	WGR (%)	SR (%)	FCR
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 differ significantly (P<0.05). The same applies below.

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

As presented in Table 3 , dietary APS supplementation did not significantly affect intestinal amylase activity (P>0.05). However, intestinal protease and lipase activities increased initially and then decreased with rising APS levels. The APS1.50 group showed significantly higher activities than APS0 and APS0.25 groups (P<0.05), but no significant differences compared to APS1.00 and APS2.00 groups (P>0.05).

Figure 1 [Figure 1: see original paper] and Table 3 indicate that intestinal microvilli length increased with APS supplementation, reaching maximum length at 1.50 g/kg before declining at higher levels. The APS1.50 and APS2.00 groups exhibited significantly greater microvilli length than APS0, APS0.25, and APS0.50 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

2.3 Effects of Astragalus Polysaccharide on Blood Immune Indices

As shown in Table 4 , plasma LSZ activity and C3, C4, and IgM concentrations, along with whole blood respiratory burst activity, increased initially and then decreased with rising APS levels, reaching maximum values in the APS1.50 group. These values were significantly higher than those in the control group (P<0.05), with no significant differences compared to APS1.00 (except C3) and APS2.00 groups (P>0.05). Plasma ACP activity showed a similar trend, but peaked in the APS1.00 group, which was significantly higher than the control (P<0.05) but not significantly different from other groups (P>0.05).

Table 4 Effects of APS on blood immune indices 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 illustrated in Figure 2 [Figure 2: see original paper], plasma IgM concentration was lowest in the APS0 group (1.22 g/L), significantly lower than in APS0.50, APS1.00, APS1.50, and APS2.00 groups ($P<0.05$), but not significantly different from APS0.25 ($P>0.05$). The highest IgM concentration occurred in the APS1.50 group (1.75 g/L), which was significantly higher than 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 differ significantly (P<0.05).

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

As shown in Table 5 , plasma CAT activity increased initially and then decreased with rising APS levels, with APS0.50, APS1.00, APS1.50, and APS2.00 groups significantly higher than APS0 and APS0.25 groups ($P<0.05$). The APS1.00 and APS1.50 groups were also significantly higher than APS0.50 and APS2.00 groups ($P<0.05$). Plasma SOD activity and T-AOC showed similar trends, with APS1.00 and APS1.50 groups significantly higher than the other four groups ($P<0.05$), peaking in the APS1.50 group. Plasma MDA content decreased initially and then increased with APS supplementation, reaching its minimum in the APS1.50 group, which was significantly lower than APS0, APS0.25, and APS0.50 groups ($P<0.05$) but not significantly different from APS1.00 and APS2.00 groups ($P>0.05$).

Table 5 Effects of APS on plasma antioxidant indices 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

Groups	CAT (U/mL)	SOD (U/mL)	T-AOC (U/mL)	MDA (nmol/mL)
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 presented in Table 6 , hepatic CAT activity increased initially and then decreased with rising APS levels, reaching its maximum at 1.50 g/kg supplementation. The APS1.50 group was significantly higher than APS0, APS0.25, and APS0.50 groups ($P<0.05$) but only slightly higher than APS1.00 and APS2.00 groups ($P>0.05$). Hepatic SOD activity was highest in the APS2.00 group, significantly greater than all other groups ($P<0.05$). Hepatic MDA content decreased initially and then increased with APS supplementation, with APS1.50 and APS2.00 groups significantly lower than the other four groups ($P<0.05$).

Table 6 Effects of APS on liver antioxidant indices 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

As shown in Figure 3 [Figure 3: see original paper], cumulative mortality after *A. hydrophila* challenge was lowest in the APS1.50 group, significantly lower than in APS0, APS0.25, and APS0.50 groups ($P<0.05$), but not significantly different from 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 differ significantly ($P<0.05$).

Discussion

3.1 Effects of Astragalus Polysaccharide on Growth Performance

Published research demonstrates that Astragalus polysaccharide significantly promotes growth in various aquatic species, including *Schizothorax prenanti* [6], tilapia [13], red swamp crayfish [14], and Nile tilapia [15]. The present study indicates that dietary supplementation with 1.50 or 2.00 g/kg Astragalus polysaccharide significantly improved growth performance in hybrid snakehead compared to the unsupplemented control. This improvement may be attributed

to several mechanisms: (1) As a bioactive polysaccharide extracted from *Astragalus*, APS at appropriate levels promotes proliferation of beneficial intestinal bacteria such as *Lactobacillus*, *Yeast*, *Pseudomonas*, and *Bifidobacterium*, which produce various digestive enzymes and ultimately enhance digestive enzyme activity [16]; (2) APS promotes secretion of digestive juices, improving nutrient digestion and absorption [17]; (3) APS significantly increases intestinal villus length, crypt depth, and muscular layer thickness, while increasing numbers of goblet cells and intraepithelial lymphocytes [18], thereby enhancing digestive and absorptive capacity and promoting growth; (4) Certain active components in APS may promote protein synthesis, enhancing the ability to convert absorbed nutrients into body protein and thus accelerating growth [6,19].

The current results demonstrate that Astragalus polysaccharide promoted intestinal microvillus development, enhanced intestinal digestive enzyme activities, improved nutrient utilization, accelerated growth rate, and reduced feed conversion ratio in hybrid snakehead. For this carnivorous fish species, the optimal APS supplementation level for growth promotion was 1.50-2.00 g/kg, which differs from reported optimal levels of 0.040%-0.074% for *S. prenanti* [6], 100-200 mL/kg APS liposome for tilapia [13], 0.40%-0.80% for red swamp 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, suggesting that these factors should be considered in practical applications.

3.2 Effects of Astragalus Polysaccharide on Immunity

Fish possess a relatively underdeveloped specific immune system, relying primarily on non-specific immunity, which includes factors such as LSZ, ACP, complement, and antimicrobial peptides. LSZ hydrolyzes bacterial mucopolysaccharides, eliminating pathogenic bacteria and serving as a crucial non-specific immune factor [20], with its activity representing an important quantitative indicator of non-specific immunity [21]. ACP is a marker enzyme of macrophage lysosomes, released during phagocytic and encapsulation immune responses [22]. Complement constitutes an essential component of the fish immune system, functioning to combat microbial invasion and clear immune complexes [23].

The present results indicate that appropriate dietary APS supplementation enhanced non-specific immunity and disease resistance in hybrid snakehead, with optimal effects at 1.50 g/kg. These findings align with studies showing that 1,000-1,500 mg/kg APS 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] proposed that APS improves immunity by enhancing cellular metabolism, promoting immune organ development, and stimulating the function and activity of 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 upregulating mRNA expression of cytokines such as nuclear factor- B (NF- B), interleukin-1 (IL-1), and tumor

necrosis factor- (TNF-) that are closely associated with disease pathogenesis [27].

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

3.3 Effects of Astragalus Polysaccharide on Antioxidant Capability

During pathological or stress conditions, fish produce excessive reactive oxygen species (ROS) and free radicals that attack proteins, DNA, and cell membranes, causing metabolic disorders, slow growth, compromised immunity, increased feed conversion ratio, and reduced product quality [29-30]. When ROS and free radicals increase, SOD and CAT activities in blood rise to eliminate these harmful substances [31]. Zhang et al. [24] reported that appropriate dietary APS significantly increased plasma SOD and CAT activities in tilapia and enhanced antioxidant enzyme activities in liver and heart tissues while reducing MDA content [25]. Jia et al. [32] demonstrated that 1.5-3.0 g/kg APS significantly reduced SOD activity and T-AOC in CCl -damaged hepatocyte culture medium and serum of common carp, while markedly inhibiting hepatic MDA synthesis. 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 markedly inhibiting MDA synthesis. These findings suggest that APS enhances antioxidant enzyme activity, reduces oxidative damage from free radicals, and improves overall antioxidant capacity, consistent with conclusions reported 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 evaluate fish disease resistance by measuring short-term cumulative mortality. Hong et al. [37] reported that dietary supplementation with 0.8% APS increased survival by 26.67% in red swamp crayfish challenged with white spot syndrome virus (WSSV) compared to the positive control, demonstrating enhanced antiviral capability. Similar challenge tests in common carp [9] and catfish [38] have confirmed that APS strengthens disease resistance. The current study yielded comparable results, showing that dietary APS reduced cumulative mortality in hybrid snakehead challenged with *A. hydrophila*, further demonstrating that APS enhances disease resistance by improving immunity

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' s disease resistance enhancement in fish.

Conclusion

Dietary supplementation with Astragalus polysaccharide improves growth performance, immunity, antioxidant capability, and disease resistance in hybrid snakehead. Effects become pronounced at supplementation levels above 1.00 g/kg, but slightly decline at 2.00 g/kg. Therefore, the optimal dietary inclusion level of Astragalus polysaccharide for hybrid snakehead is 1.50 g/kg.

References

[1] Zhang XC, Chen KC, Zhu XP. Research status on aquaculture of *Channa argus*, *Channa maculata* and their hybrid [J]. Guangdong Agricultural Sciences, 2011, 32(22): 132-134.

[2] Zhang C, Liu NF, Yang XQ, et al. Comparison of karyotypes among blotched snakehead ( ), northern snakehead ( ) and their hybrid [J]. Journal of Shanghai Fisheries University, 2005, 14(2): 103-107.

[3] Ma HJ, Liu XY, Feng XY, et al. Effects of dietary organic selenium supplementation on growth performance and antioxidant capacity of hybrid snakehead [J]. Feed Research, 2013(4): 57-59.

[4] Yu HH, Xue M, Han F, et al. Comparative study of several immunomodulators on growth performance, immunity and survival after bacterial infection in Japanese seabass [J]. Chinese Journal of Animal Nutrition, 2014, 26(8): 2386-2396.

[5] Nejatian M, Hatami M, Mohammadifar MA. Effect of gum tragacanth exuded by three Iranian *Astragalus* on mixed milk protein system during acid gelation [J]. International Journal of Biological Macromolecules, 2013, 53: 168-176.

[6] Xiang X, Chen J, Zhou XH, et al. Effects of Astragalus polysaccharide on growth, body composition and immune indices of *Schizothorax prenanti* [J]. Acta Hydrobiologica Sinica, 2011, 35(2): 291-299.

[7] Kallon S, Li XR, Ji J, et al. Astragalus polysaccharide enhances immunity and inhibits H9N2 avian influenza virus in vitro and in vivo [J]. Journal of Animal Science and Biotechnology, 2013, 4(1): 22.

[8] Yin GJ, Jeney G, Racz T, et al. Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non-specific immune response of tilapia, *Oreochromis niloticus* [J]. Aquaculture, 2006, 253(1/2/3/4): 39-47.

[9] Yin GJ, Ardo L, Thompson KD, et al. Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and

protection against *Aeromonas hydrophila* [J]. Fish & Shellfish Immunology, 2009, 26(1): 140-145.

[10] Hu B, Liu J, Hou YQ, et al. Effects of Astragalus polysaccharide on non-specific immunity of gibel carp [J]. Water Resources and Hydropower Engineering, 2008, 28(3): 108-111.

[11] Biochemistry Teaching and Research Section, Department of Biology, Peking University. Biochemistry Experimental Guide [M]. Beijing: People' s Education Press, 1979: 73-74.

[12] Anderson DP, Siwicki AK. Basic hematology and serology for fish health programs [J]. Diseases in Asian Aquaculture, 1995, 2: 185-202.

[13] Peng T, Hu TJ, Lin Y, et al. Effects of Astragalus polysaccharide liposome on growth performance of GIFT tilapia [J]. Southwest China Journal of Agricultural Sciences, 2012, 25(6): 2368-2371.

[14] Hong XP, Xia SY, Tang JN, et al. Effects of Astragalus polysaccharide on growth and non-specific immune indices of red swamp crayfish [J]. Journal of Shanghai Ocean University, 2013, 22(4): 571-576.

[15] Zahran E, Risha E, Abdelhamid F, et al. Effects of dietary Astragalus polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*) [J]. Fish & Shellfish Immunology, 2014, 38(1): 149-157.

[16] Chen Y, Zhou HQ. Effects of three polysaccharides on protease and amylase activities in intestine and hepatopancreas of gibel carp [J]. Journal of Shanghai Fisheries University, 2005, 14(4): 468-471.

[17] Xiao L. Effects of *Bacillus subtilis* JS01 and Astragalus polysaccharide on growth and immune function of Jian carp [D]. Master' s thesis. Ya' an: Sichuan Agricultural University, 2012.

[18] Huang YZ. Effects of Astragalus polysaccharide on growth performance and immune function of tilapia [D]. Fuzhou: Fujian Agriculture and Forestry University, 2009.

[19] Li HQ, Zhao WG, Lu XH. Chemical composition and structural analysis of Astragalus polysaccharide as an animal immune enhancer [J]. Journal of Traditional Chinese Veterinary Medicine, 2008, 27(5): 5-9.

[20] Saurabh S, Sahoo PK. Lysozyme: an important defense molecule of fish innate immune system [J]. Aquaculture Research, 2008, 39(3): 223-239.

[21] Su HC, Xie GS, Bian HH, et al. Immunoprotective effects of two polysaccharides as adjuvants for inactivated *Edwardsiella tarda* vaccine in turbot (*Scophthalmus maximus*) [J]. Oceanologia et Limnologia Sinica, 2012, 43(5): 1001-1007.

[22] Liu Y, Jiang XL, Lu Q, et al. Effects of polymannuronic acid on immune-related enzyme activities and lysozyme/hemolysin activities in *Penaeus chinen-*

sis [J]. Journal of Fisheries of China, 2000, 24(6): 549-553.

[23] Merrifield DL, Dimitroglou A, Foey A, et al. The current status and future focus of probiotic and prebiotic applications for salmonids [J]. Aquaculture, 2010, 302(1/2): 1-18.

[24] Zhang WN, Lin X, Wang SK, et al. Effects of Astragalus polysaccharide on non-specific immunity and gastrointestinal endocrine function of tilapia [J]. Chinese Journal of Animal Nutrition, 2010, 22(2): 401-409.

[25] Bai DQ, Wu X, Guo YJ, et al. Effects of long-term Astragalus polysaccharide feeding on antioxidant and non-specific immune indices of yellow catfish [J]. Chinese Journal of Animal Nutrition, 2011, 23(9): 1622-1630.

[26] Bai DZ, Dong F, Tang WT, et al. Research progress on pharmacological effects of Astragalus polysaccharide [J]. Heilongjiang Medicine Journal, 2014, 27(1): 103-106.

[27] Li Q, Hu JH, Gao B, et al. Recent research progress on immunomodulatory effects of Astragalus polysaccharide [J]. Chinese Journal of Experimental Traditional Medical Formulae, 2017, 23(2): 199-206.

[28] Chen Q, Jiang LL, Xiao YX, et al. Effects of Astragalus polysaccharide on growth performance and serum immunoglobulin in broilers [J]. Feed Research, 2013(3): 50-52.

[29] Martínez-Álvarez RM, Morales ZAE, Sanz A. Antioxidant defenses in fish: biotic and abiotic factors [J]. Reviews in Fish Biology and Fisheries, 2005, 15(1/2): 75-88.

[30] Madeira D, Narciso L, Cabral HN, et al. Influence of temperature in thermal and oxidative stress responses in estuarine fish [J]. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2013, 166(2): 237-243.

[31] Wang YH, Wang YY, Mai KS, et al. Effects of dietary curcumin supplementation on growth, body composition and antioxidant enzyme activities of juvenile turbot [J]. Journal of Fisheries of China, 2016, 40(9): 1299-1308.

[32] Jia R, Cao LP, Xu P, et al. In vitro and in vivo hepatoprotective and antioxidant effects of Astragalus polysaccharides against carbon tetrachloride-induced hepatocyte damage in common carp (*Cyprinus carpio*) [J]. Fish Physiology and Biochemistry, 2012, 38(3): 871-881.

[33] Chen YJ, Li Y, Zhang RL, et al. Regulatory effects of Astragalus polysaccharide on non-specific immune function in immunosuppressed loach [J]. Cereal & Feed Industry, 2016(4): 60-62, 69.

[34] Yan H, Xie YP, Sun SG, et al. Chemical analysis of *Astragalus mongholicus* polysaccharides and antioxidant activity [J]. Carbohydrate Polymers, 2010, 82(3): 636-640.

[35] Li R, Chen WC, Wang WP, et al. Antioxidant activity of Astragalus polysaccharides and antitumour activity [J]. Carbohydrate Polymers, 2010, 82(2): 240-244.

[36] Elabd H, Wang HP, Shaheen A, et al. *Astragalus membranaceus* (AM) enhances growth performance and antioxidant stress profiles in bluegill sunfish (*Lepomis macrochirus*) [J]. Fish Physiology and Biochemistry, 2016, 42(3): 955-966.

[37] Hong XP, Lu HD, Zhang QH, et al. Effects of Astragalus polysaccharide on resistance to white spot syndrome virus (WSSV) infection in red swamp crayfish [J]. Journal of Shanghai Ocean University, 2014, 23(3): 423-428.

[38] Bai DQ, Wu X, Zhu GX, et al. Astragalus polysaccharides enhance cellular immune response and disease resistance in yellow catfish [J]. Israeli Journal of Aquaculture, 2012, 64(55): 688-695.

[39] Xu M, Ma GH. Study on prevention and treatment of grass carp hemorrhagic disease with Astragalus polysaccharide [J]. Journal of Anhui Agricultural Sciences, 2008, 36(30): 13202-13230.

Note: Figure translations are in progress. See original paper for figures.