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Effects of Astragalus–Ginseng Dietary Supplementation on the Growth and Stress Resistance of Yellow Catfish (*Pelteobagrus fulvidraco*)

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Abstract: Astragalus and ginseng, esteemed as traditional Chinese herbal medicines, have demonstrated the ability to bolster physical health and enhance the immune function of organisms. In this study, the effects of a dietary astragalus–ginseng mixture on the growth performance, intestinal health, and nonspecific immunity of yellow catfish (*Pelteobagrus fulvidraco*) were evaluated, by measuring growth performance indices, intestinal villus morphology, enzyme activities, and expression levels of immune-related genes. Yellow catfish (n = 120, initial weight: 5.07 ± 0.18 g) were randomly assigned to four dietary groups: a control group (CT, 0 mg/kg) and three astragalus–ginseng treatment groups (AG1, 500 mg/kg; AG2, 1000 mg/kg; AG3, 2000 mg/kg). Each group had three replicates and was fed for six weeks. The results demonstrate that the treatment significantly enhanced the growth performance, as evidenced by increases in FBW, WG, WGR, SGR, and HSI. These improvements may be related to an increase in intestinal villi length and increased LPS activity, both of which are associated with enhanced digestive function. Meanwhile, the activity of antioxidant enzymes in the liver, including CAT, SOD, and GSH, was increased, whereas the level of MDA was decreased. In the serum, GSH was up-regulated, while SOD activity was decreased. Immune-related enzyme activities, such as ALT and LZM, were up-regulated, while AST showed no significant difference. Moreover, the treatment also promoted the expression of the anti-inflammatory factor *IL-10*. The pro-inflammatory factors, such as *IL-1β*, *IL-6*, and *TNF-α*, were decreased with the addition of low concentrations but increased with high concentrations. In conclusion, supplementation with an astragalus–ginseng mixture could promote growth performance by increasing digestive enzyme activity and intestinal villi length, and improve disease and stress resistance traits by modulating immune genes and antioxidant enzyme activity. A dosage of 1000 mg/kg was found to be optimal.

Keywords: astragalus–ginseng; *Pelteobagrus fulvidraco*; growth; intestinal health; feed additive

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Key Contribution: (1) Dietary astragalus–ginseng mixture enhanced the growth performance and improved intestinal health in *Pelteobagrus fulvidraco*. (2) Dietary astragalus–ginseng mixture boosted antioxidant and immune functions in *Pelteobagrus fulvidraco*.

1. Introduction

As the largest aquaculture country, China's aquaculture industry has gradually become a vital growth driver for the rural economy [1]. Constrained by nutritional limitations, environmental challenges, and pathogen infections, intensive aquaculture faces the issues of disease outbreaks and high mortality rates, which significantly impede the development of the aquaculture industry [2,3]. Therefore, improving disease and stress resistance traits of cultured animals is crucial. Previously, antibiotics have been widely used in aquaculture for disease prevention and treatment. Recently, the accumulation of active antibiotic residues from aquaculture in the environment has raised public concerns [4,5]. Since the prohibition of antibiotics, research has increasingly focused on investigating alternatives, such as plant extracts and polysaccharides derived from medicinal plants. These products have been shown to enhance appetite and weight gain, with demonstrated antibacterial and anti-parasitic properties [6–8]. Hence, plant extracts can significantly enhance fish immunity and effectively control infectious diseases in aquaculture.

Astragalus membranaceus and *Panax ginseng* are two of the most widely utilized traditional herbal medicines worldwide, recognized for their diverse pharmacological properties. Astragalus polysaccharide (APS) is the main bioactive component of *A. membranaceus* and is primarily composed of glucose, along with varying amounts of rhamnose, galactose, arabinose, mannose, and other monosaccharides [9–11]. As a pleiotropic factor, APS has been reported to exhibit anti-tumor, antioxidant, anti-viral, and antimicrobial activities as well as beneficial impacts on intestinal health through the modulation of villus morphology and gut microbiota composition [8,12–15]. Ginsenosides are the major active components of ginseng and trigger an array of pharmacological responses, related to anti-hyperglycemia, anti-cancer, immunomodulatory, and antioxidant activities [16–22]. In recent years, ginseng polysaccharides have also attracted increasing attention as an important class of bioactive compounds. Polysaccharides isolated from *P. ginseng* are mainly composed of starch-like glucans and pectin [23,24]. The ginseng starch-like polysaccharide consists of α-D-(1,4)-glucans, 6-branched α-D-(1,4)-glucans, 3-branched α-D-(1,6)-glucans, and α-D-(1,6)-glucans without side chains, while ginseng pectin is mainly composed of homogalacturonan, rhamnogalacturonan I, and arabinogalactans [25–27]. Ginseng polysaccharides exhibit a range of biological activities, including immuno-modulatory, anti-tumor, antioxidant, and antidiabetic effects, primarily through the regulation of immune responses and oxidative stress pathways [28–30].

Building on their established pharmacological properties, both astragalus polysaccharides (APSs) and ginseng polysaccharides have increasingly been investigated for their potential to enhance antioxidant capacity, modulate immune function, and pro-mote disease resistance in aquatic species. Dietary supplementation with APSs has been shown to improve weight gain, growth rate, meat quality, immunity, and disease resistance in *Carassius auratus*, *Scophthalmus maximu*, and *Channa argus* [31–33]. In addition, APS has demonstrated adjuvant potential in aquaculture vaccines by enhancing antigen-specific immunity and protective efficacy in species such as *Epinephelus fuscoguttatus* and *Oncorhynchus mykiss* [34,35]. In contrast, ginseng polysaccharides are primarily used as immunostimulants rather than growth promoters in aquaculture, with studies in *Paralichthys olivaceus* and *Oncorhynchus mykiss* demonstrating enhanced immune responses following dietary

supplementation [36,37]. Due to their complementary immunomodulatory and physiological effects, the combination of APSs and ginseng polysaccharides may provide additive or synergistic benefits in aquaculture. However, supporting evidence in fish species remains limited. A relevant research is the combination of APS and *Eucommia ulmoides* leaf extract in *Larimichthys crocea*, which showed improved growth, antioxidant capacity, and immune regulation through MAPK and mTOR signaling pathways [38]. Given these promising findings, further studies are warranted to explore the potential synergistic effects of combining APSs and ginseng polysaccharides in fish, which could provide valuable insights for improving aquaculture practices.

Yellow catfish (*Pelteobagrus fulvidraco*) is an important economic species that is extensively distributed across China. With the intensification of aquaculture practices, infectious diseases caused by bacterial pathogens such as *Edwardsiella ictaluri* and *Aeromonas veronii* present a serious threat to the sustainable development of yellow catfish farming [39–41]. As feed additives, polysaccharides and plant extracts have been shown in the relevant literature to positively influence body composition and boost innate immunity in yellow catfish [42–44]. However, limited research has examined whether the mixture of APS and ginseng can enhance the stress resistance and immunity of yellow catfish to support its aquaculture. This study investigated the effects of APS and ginseng on the growth, intestinal health, enzyme activity, and non-specific immunity of yellow catfish, thereby providing a theoretical foundation for the development of feed additives, specifically for this species.

2. Materials and Methods

2.1. Experimental Design and Diets

The astragalus–ginseng mixture (crude polysaccharide content $\geq$ 40%) used in the study was provided by Shanghai Shangshang Biotechnology Co., Ltd. (Shanghai, China). Four experimental groups (CT, AG1, AG2, AG3) were fed diets supplemented with an astragalus–ginseng mixture at the following concentrations: 0 mg/kg (CT), 500 mg/kg (AG1), 1000 mg/kg (AG2), and 2000 mg/kg (AG3). The basal diet used in this study was purchased from Tongwei culture Co., Ltd. (Chengdu, China), with a proximate composition consisting of 41% crude protein, 10% crude lipid, 6% fibers, 16% crude ash, 1–4% calcium, and 2.6% lysine. The specific components are presented in Table 1. The basal diet was supplemented with an astragalus–ginseng mixture at four inclusion levels (0, 500, 1000, and 2000 mg/kg) to formulate the experimental diets. The astragalus–ginseng mixture was first dissolved in 500 mL of deionized water (25 $\pm$ 1 °C, pH 6.5) to prepare stock solutions of the respective concentrations. Subsequently, 50 g of the basal diet was thoroughly mixed with the corresponding solution to ensure uniform distribution. The diets were then oven-dried at 65 °C for 30 min and stored at 4 °C. To ensure freshness, new batches were prepared every two weeks.

Table 1. Ingredient composition of the experimental diets.

Ingredients	Experimental Feeds (Dry Matter %)			
	CT	AG1	AG2	AG3
Fish meal	44	44	44	44
Flour	19.55	19.55	19.55	19.55
Cottonseed meal	4	4	4	4
Soybean meal	15	15	15	15
Corn gluten meal	5	5	5	5
Fish oil	4.5	4.5	4.5	4.5
Brewer's yeast	6	6	6	6

Table 1. *Cont.*

Ingredients	Experimental Feeds (Dry Matter %)			
	CT	AG1	AG2	AG3
Minerals	1	1	1	1
Choline chloride	0.6	0.6	0.6	0.6
Multivitamin	0.2	0.2	0.2	0.2
Mold inhibitor	0.15	0.15	0.15	0.15
Total	100	100	100	100
Astragalus–ginseng mixture (mg/kg)	0	500	1000	2000

2.2. Experimental Animals

All animal procedures were strictly conducted in accordance with the Statute of the Experimental Animal Ethics Committee of Shanghai Ocean University. All operations were taken to minimize negative impacts on animals. The yellow catfish originated from wild yellow catfish that were induced for breeding through a commercial breeding program. After two weeks of acclimatization, 120 yellow catfish (5 ± 0.5 g) were randomly assigned to 12 glass tanks, with 10 fish per tank. A basal diet was provided during acclimatization period. Tanks were maintained at 25 ± 1 °C, with a pH range from 6.5 to 7.0, and continuous aeration was supplied to ensure adequate dissolved oxygen levels, which were maintained above 6.2 mg/L, with a circulating water volume of 8 L. Before the treatment, all fish were starved for 48 h. The fish were fed at a rate of 5% of their body weight on a daily basis. Every two weeks, the fish in each group were weighed to adjust the feed quantity as needed and were fed to satiation twice a day (9:00 and 18:00) for 45 days.

2.3. Growth Performance Assay

All test fish were fasted for 48 h prior sampling, and the final body weight (FBW) values were recorded for each group. At least three fish from each tank were sampled, anesthetized immediately with 100 mg/L MS-222 (Shanghai Reagent Corp., Shanghai, China) solution. The liver and other organs were removed and weighed accurately to evaluate the visceral somatic index (VSI) and hepato-somatic index (HSI). Growth performance index was calculated using the formula:

Weight gain (WG) = (FBW − IBW) × 100%
Weight gain rate (WGR) = (FBW − IBW)/IBW × 100%
Specific growth rate (SGR) = (lnFBW − lnIBW)/Days of test × 100%
Viscera-somatic index (VSI) = (Visceral Weight/FBW − Visceral Weight) × 100%
Hepato-somatic index (HSI) = (Liver Weight/FBW − Liver Weight) × 100%

2.4. Sample Collection

Blood samples were collected from the caudal vein into 1.5 mL tubes and were allowed to clot at room temperature for 2 h. Afterward, serum was obtained by centrifugation at 4000 rpm for 10 min, the supernatant was transferred to a new tube, and was stored at −80 °C.

The intestines were harvested for histological examination and analysis of digestive enzyme activity. One portion was immersed in paraformaldehyde (PFA), with the solution replaced six times at 10-min intervals, and was stored at 4 °C. The other portion was washed with pre-cooled phosphate-buffered saline (PBS) and homogenized (1:10 w/v) in freshly prepared ice-cold PBS, centrifuged at 4000 rpm for 10 min at 4 °C, and the resulting supernatant was collected for analysis.

The liver was separated into two parts: One part was washed with ice-cold phosphate-buffered saline and homogenized (1:10 w/v) in freshly prepared PBS, centrifuged at 4000

rpm for 10 min at 4 °C, and the resulting supernatant was collected for measurement. The other part was stored at −80 °C for subsequent immune-related index analysis.

The spleen and head kidney were collected for immune-related gene analysis, dissected on ice, and then stored at −80 °C.

2.5. Intestinal Microvillus Morphology

The intestine fixed in PFA was embedded in paraffin, using Automated Rotary Microtome (Leica, Berlin, Germany), and sectioned into 10 μm slices; the slices were stained by hematoxylin and eosin (H&E, Baton Rouge, LA, USA). A Ni-E microscope with a Nikon Ds-Ri2 camera (Nikon, Tokyo, Japan) was used to observe and photograph the slices.

2.6. Enzyme Activity Assay

Amylase (AMS) and lipase (LPS) were measured to evaluate intestinal absorptive capacity. Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH), and malondialdehyde (MDA) were measured to assess the liver's antioxidant enzyme activity, while alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lysozyme (LZM) were analyzed to determine the liver immune function. Serum was used to measure SOD and GSH levels. Enzyme activity was assessed using commercial kits (Nanjing Jiancheng Biotechnic Institute, Nanjing, China.

2.7. Quantitative Real-Time PCR Analysis

Total RNA was obtained using Trizol reagent (Sigma, Burlington, VT, USA), and the Evo M-MLV RT with gDNA Clean for qPCR II (Accurate biotechnology, Changsha, China) was used for cDNA synthesis and gDNA removal. The mRNA expression levels of immune factors *IL-1β*, *IL-6*, *IL-10*, and *TNF-α* were measured; qPCR primers (Table A1) were designed based on the sequences available in GenBank and synthesized by Sangon Biotechnology, Inc (Shanghai, China). β-actin was used as the housekeeping gene for the normalization of the data. Real-time PCR (qPCR) was performed on the Roche LightCycler®480 II Real-Time System (Roche, Switzerland) using the SYBR Green PCR Master Mix Kit (TaKaRa, Kusatsu, Japan). β-actin was used as the reference gene. The mRNA expression levels of the target genes were analyzed using the formula $R = 2^{-\Delta\Delta Ct}$, and each sample was replicated three times.

2.8. Statistical Analysis

A one-way ANOVA was performed using SPSS 26, after confirming data normality and homogeneity of variance with the Shapiro–Wilk and Levene's tests, respectively. Tukey's HSD test was used to compare differences between means. Data are presented as mean ± standard deviation (SD), with statistical significance set at $p < 0.05$.

3. Results

3.1. Growth Performance

After six weeks of feeding with experimental diets, no significant differences were observed in the VSI. However, FBW, WG, WGR, SGR, and HSI were significantly higher in both additive groups compared to the CT group ($p < 0.05$), with the group receiving the highest concentration exhibiting the highest values (Table 2).

Table 2. Effects of dietary astragalus–ginseng mixture levels on the growth performance of *Pelteobagrus fulvidraco*.

Parameters	0 mg/kg	500 mg/kg	1000 mg/kg	2000 mg/kg
IBW (g)	5.13 ± 0.12	5.04 ± 0.23	5.05 ± 0.22	5.06 ± 0.26
FBW (g)	9.75 ± 0.55 b	10.81 ± 0.12 ab	10.97 ± 0.27 ab	11.61 ± 0.76 a
WG (%)	46.35 ± 3.88 b	56.9 ± 1.41 ab	59.16 ± 0.58 a	65.53 ± 6.33 a
WGR (%)	90.54 ± 4.72 b	111.23 ± 8.43 a	117.20 ± 4.08 a	129.60 ± 12.36 a
SGR (%)	1.43 ± 0.05 b	1.66 ± 0.08 ab	1.72 ± 0.04 a	1.84 ± 0.12 a
VSI (%)	12.06 ± 1.80	13.18 ± 1.11	12.76 ± 1.15	13.24 ± 0.91
HSI (%)	1.01 ± 0.01 c	1.29 ± 0.06 b	1.63 ± 0.03 a	1.53 ± 0.02 a

Note: Data are express as mean ± SE. Data marked with different letters differ significantly ($p < 0.05$) among groups based on a one-way ANOVA. IBW: Initial body weight; FBW: Final body weight; WG: Weight gain; WGR: Weight gain rate; SGR: Specific growth rate; VSI: Visceral somatic index; HIS: Hepato-somatic index.

3.2. Intestinal Morphology

The H&E-stained intestinal sections showed that the length of intestinal villi was increased in all treatment groups compared to the control group, with the 1000 mg/kg group exhibiting the greatest length (Figure 1C), whereas the microvillus morphology of the 2000 mg/kg group showed signs of shrinkage (Figure 1D). To enhance clarity, intestinal muscular thickness (a) and intestinal villus length (b) were marked in the figures, highlighting the structural differences among groups. This result highlights the adverse effects of excessive intake of experimental diets and suggests that a moderate concentration is a more suitable choice for dietary purposes.

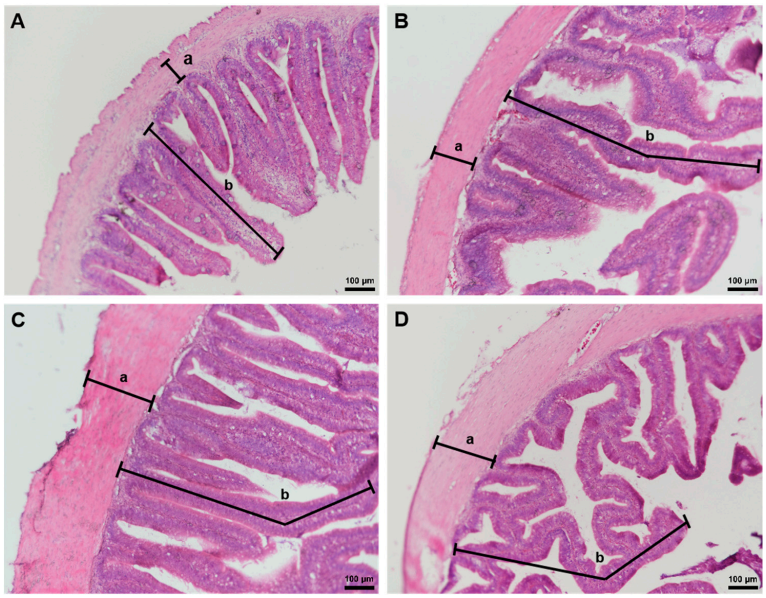

Figure 1. The intestinal morphology of yellow catfish fed diets with graded level of astragalus–ginseng mixture for 6 weeks (×100): 0 mg/kg (control) (**A**), 500 mg/kg (**B**), 1000 mg/kg (**C**), 2000 mg/kg (**D**); (a) intestinal muscular thickness, (b) intestinal villus length.

3.3. Digestive Enzyme Activity

The intestinal digestive enzyme activity results show that LPS activity was significantly increased following the administration of the astragalus–ginseng mixture (Figure 2B). At the same time, there were no significant differences in AMS activity among all groups (Figure 2A). Based on these results, we infer that the increase in LPS promotes food absorption, leading to improved growth performance. Meanwhile, the enhanced lipid absorption may contribute to the increase in the hepato-somatic index.

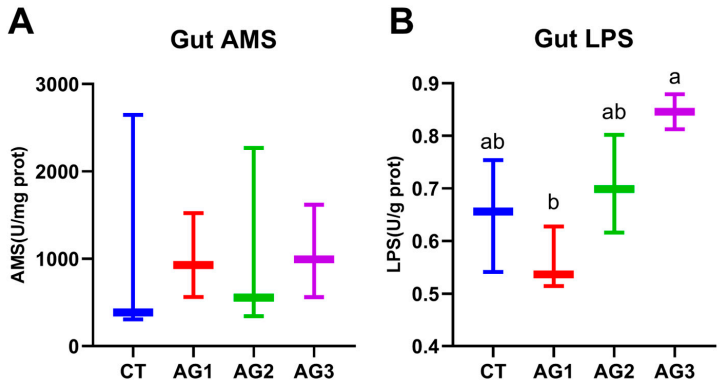

Figure 2. AMS (**A**) and LPS (**B**) activities in the gut of yellow catfish. Different letters indicate a significant difference among astragalus–ginseng mixture concentrations ($p < 0.05$).

3.4. Antioxidant Enzyme Activity

An antioxidant analysis was performed by measuring the MDA content and the activity of antioxidant enzymes in the liver and blood of yellow catfish. Liver SOD and GSH activities showed an increasing trend after feeding (Figure 3B,C), while MDA was significantly decreased (Figure 3D). Blood GSH showed the same trend as in the liver (Figure 3E). However, blood SOD activity decreased after feeding (Figure 3F). The liver CAT activity showed no significant differences among all groups (Figure 3A). In conclusion, feeding with experimental diets can upregulate the antioxidant capacity in the liver.

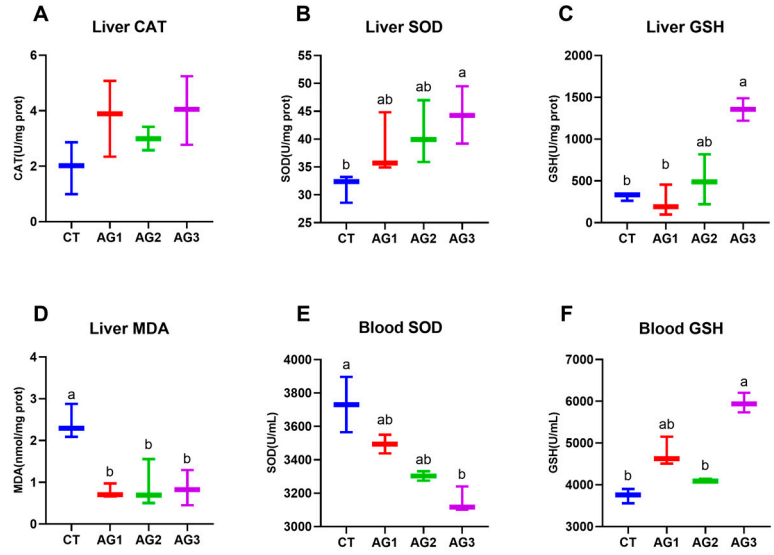

Figure 3. Antioxidant enzyme activity in yellow catfish. Liver CAT (**A**), liver SOD (**B**), liver GSH (**C**), liver MDA (**D**), blood SOD (**E**), and blood GSH (**F**). Different letters indicate a significant difference among astragalus–ginseng mixture concentrations ($p < 0.05$).

3.5. Immune-Related Enzyme Activity

The results of immune-related enzyme activity show that ALT and LZM activities were significantly elevated following the administration of the astragalus–ginseng mixture (Figure 4A,C). The activity of AST in all groups showed no significant differences (Figure 4B). These results indicate that experimental diets can enhance the production of the LZM. However, the increase in high concentration intake may burden liver function, contributing to the increase in AST activity.

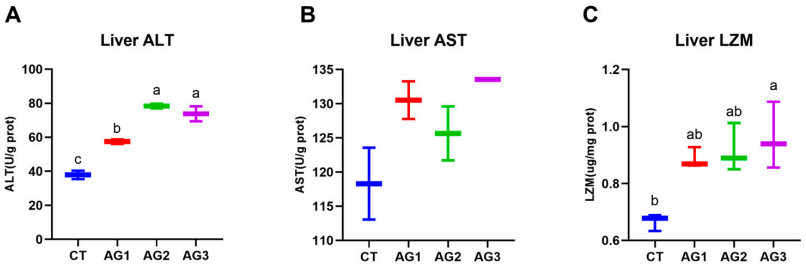

Figure 4. ALT (**A**), AST (**B**), and LZM (**C**) activities in the liver of yellow catfish. Different letters indicate a significant difference among astragalus–ginseng mixture concentrations. ($p < 0.05$).

3.6. Immune-Related Gene Expression

The expression levels of inflammatory genes in the liver of yellow catfish showed that *IL-1β* was significantly reduced in the fed group compared to the control group (Figure 5A). The expression levels of *IL-6* and *TNF-α* genes decreased at low concentrations but increased at high concentrations (Figure 5B,C). The relative expression of *IL-1β* was significantly reduced, while *IL-6* decreased initially and then increased in the spleen (Figure 5D,E). There were no significant differences in the relative expression level of *TNF-α* gene in the spleen among all groups (Figure 5F). In the kidney, the expression level of the *IL-1β* gene was significantly decreased with supplementation of the astragalus–ginseng mixture (Figure 5G), while the expression levels of *IL-6* and *TNF-α* genes showed no significant differences (Figure 5H,I). The relative expression of *IL-10* in all organs had no significant differences (Figure A1). In general, we found that all pro-inflammatory factors initially decreased first and then increased, suggesting that a low concentration of experimental diets can reduce the inflammatory response, while a high concentration may induce it.

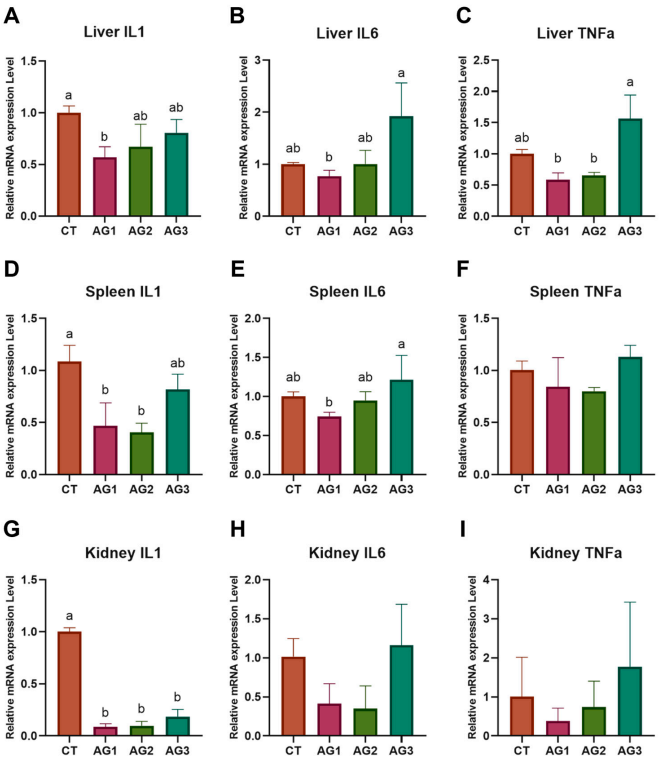

Figure 5. Immune-related genes expression levels of yellow catfish fed diets with graded levels of astragalus–ginseng mixture for 6 weeks. Liver *IL-1β* (**A**), liver *IL-6* (**B**), liver *TNF-α* (**C**), spleen *IL-1β* (**D**), spleen *IL-6* (**E**), spleen *TNF-α* (**F**), kidney *IL-1β* (**G**), kidney *IL-6* (**H**), kidney *TNF-α* (**I**). Different letters indicate a significant difference among astragalus–ginseng mixture concentrations ($p < 0.05$).

4. Discussion

Recently, astragalus and ginseng have attracted considerable attention as food additives to enhance resilience and immunity, primarily due to their ability to improve growth performance and immune function. It has been reported that 1000 mg/kg of astragalus is optimal for the growth of snakehead (*Channa argus*). A supplementation dosage of 1500 mg of astragalus is optimal for Nile tilapia (*Oreochromis niloticus*), while 688 mg/kg of astragalus is optimal for spotted sea bass (*Lateolabrax maculatus*) [15,32,45,46]. Ginseng has positive effects on growth; however, an optimal dosage has not yet been established [47]. Although the dietary supplementation of astragalus and ginseng has been studied in farmed fish, current supplementation strategies are still limited to single-component additions. Whether these two components exhibit synergistic effects that could further enhance the economic benefits of aquaculture remains unexplored. Addressing this gap, the present study investigates the physiological and immunological effects of varying concentrations of an astragalus–ginseng mixture in yellow catfish (*Pelteobagrus fulvidraco*).

In the present study, the effects of varying concentrations of an astragalus–ginseng mixture were evaluated in yellow catfish. The results indicate that 1000 mg/kg is the most effective concentration for enhancing growth performance, as reflected by improvements in FBW, WG, WGR, SGR, and HSI. These enhancements are likely associated with increased digestive enzyme activity and the elongation of intestinal villi. However, further increases in concentration resulted in adverse physiological responses. Notably, at 2000 mg/kg, signs of villus shrinkage were observed, along with changes in immune-related indicators, suggesting that excessive AG supplementation may disrupt intestinal health and trigger immune responses. To determine the optimal addition level of AG supplementation, we first evaluated the effects of different dosages on growth performance and digestive functions in yellow catfish.

Growth performance is typically influenced by two key factors: digestive enzyme activity and mucosal folds length [48]. The results show that LPS activity was lowest at 500 mg/kg, and with increasing concentrations, LPS activity gradually increased, reaching the highest level at 2000 mg/kg. No significant differences were observed in AMS activity across the treatment groups. Therefore, it can be hypothesized that the improvement in growth performance, particularly in HSI, may be attributed to the enhanced activity of LPS, which leads to an increase in liver fat mass induced by the astragalus–ginseng mixture. Similar results are observed in the present study, which investigates the effects of the APS diet on Nile tilapia (*Oreochromis niloticus*) and zebrafish [15,49]. On the other hand, the length of intestinal villi directly reflects nutrient absorption capacity [50]. The intestine is a vital digestive organ where digestion and absorption primarily occur, with absorption occurring mainly in the intestinal villi. Longer villi increase the surface area, enhancing nutrient absorption and ultimately improving growth performance. Studies have confirmed that APS can preserve gut structure by reducing immunological stress while enhancing intestinal barrier function [51,52]. Concurrently, multiple studies have demonstrated that APS can improve gut morphology and stimulate intestinal development [53,54]. Research on ginseng primarily emphasizes its role in modulating the intestinal microbiota by promoting beneficial probiotics and inhibiting harmful bacteria [55,56]. In this study, the dietary astragalus–ginseng mixture significantly increased intestinal villus length, particularly at a concentration of 1000 mg/kg. However, based on the histological results of intestine, as the concentration increase to 2000 mg/kg, gut villi showed signs of adhesion and even shrinkage, suggesting that higher concentrations may negatively impact intestinal health. However, this study did not investigate the effects of the dietary astragalus–ginseng mixture on the intestinal microbiota. Future studies should investigate

how different concentrations of the mixture impact gut microbiota, as it plays a key role in digestive health and immune function.

Given the significance of antioxidant capacity, the effects of the astragalus–ginseng mixture at different concentrations on antioxidant function were further explored. In many studies, the antioxidant capacity determines an animal's ability to withstand environmental stress. Under conditions of excessive stress, cells generate reactive oxygen species (ROS), which damage nucleic acids, oxidize proteins, and trigger lipid peroxidation, disrupting various cellular functions [57]. As ubiquitous antioxidant enzymes, SOD, CAT, and GSH collaboratively transform reactive oxygen species into harmless substances, protecting cells from oxidative damage [58,59]. Meanwhile, MDA level is commonly used as an indicator of lipid peroxidation and antioxidant capacity [60]. As effective immunopotentiators, the dietary intake of APS and ginseng has been shown to enhance antioxidant function [61,62]. CAT and SOD activity in the liver shows an increasing trend, which is consistent with findings observed in yellow perch (*Perca flavescens*). In the serum, SOD activity exhibits a significant decrease, similar to the trend in catla (*Catla catla*) [63,64]. GSH levels exhibited a significant increase in both the liver and serum, whereas the MDA level showed a significant decrease in the liver. These results indicate that the exogenous addition of astragalus–ginseng mixture in *Pelteobagrus fulvidraco* enhances the conversion of convert reactive oxygen species by increasing the level of antioxidant enzyme activity, thereby improving the antioxidant defense system. Notably, as the concentration of the mixture increased, antioxidant enzyme activities showed a corresponding increase, suggesting a potential dose-dependent effect, where higher concentrations may lead to more pronounced antioxidant effects.

Lysozyme is an immune and hydrolytic enzyme that plays a crucial role in non-specific immunity responses. Lysozyme accomplishes the bactericidal process by hydrolyzing the peptidoglycan component of the bacterial cell wall [65]. In addition to its bactericidal function, lysozyme exhibits anti-inflammatory and antiviral properties and modulates the secretion of other immune factors [66]. It has already been demonstrated that dietary APS can increase the activity of LZM [61,67]. ALT and AST are commonly recognized as reliable indicators of liver pathology [68]. Recent studies have demonstrated that following hepatocytic injury, both ALT and AST levels exhibit a significant upward trend [69]. Studies on piglets indicate that dietary APSs and ginseng polysaccharides can decrease ALT and AST levels, thereby alleviating liver dysfunction and reduced immunological stress [52]. In this research, the dietary astragalus–ginseng mixture significantly increases the level of LZM. However, following dietary intervention, no significant difference was observed in liver AST, whereas ALT levels increased significantly with higher concentrations of the astragalus–ginseng mixture, suggesting that a higher dose may impose greater stress on liver function. The effects of dietary APS on zebrafish yield consistent results [49].

The innate immune system serves as the first line of defense against invading pathogens and function through the pattern recognition receptors. In teleosts, the innate immunity system plays a more crucial role than in mammals due to the less diversified adaptive immune system of teleosts [70,71]. In vertebrates, cytokines serve as key regulators of the immune response and play a crucial role in linking the innate and adaptive immune systems [72]. IL-1β, IL-6, and TNF-α are key pro-inflammatory cytokines that function as pleiotropic molecules, exerting a wide range of biological effects in immune regulation [73–75]. The primary function of IL-10 is to limit and eventually terminate inflammatory responses, as well as regulate the proliferation of B cells and NK cells [76]. Recent research has confirmed that APSs and ginseng polysaccharides can regulate the levels of *IL-1β*, *IL-10*, and *TNF-a* during immune responses [52]. In this study, the relative mRNA expression of *IL-1β*, *IL-6*, and *TNF-α* in all three organs exhibited a trend of initial

decrease followed by an increase with higher concentrations of the astragalus–ginseng mixture; nevertheless, significant differences were observed only in certain cases. Meanwhile, the relative expression of *IL-10* showed an increasing trend in all groups. However, no significant differences were observed. This phenomenon suggests that a low concentration of the astragalus–ginseng mixture in the diet can alleviate the immune response and enhance the immune function. Additionally, a high concentration of the astragalus–ginseng mixture may elicit some immune responses, which could have adverse effects with long-term use.

Based on the preceding findings, the astragalus–ginseng mixture at 1000 mg/kg was the most effective in enhancing the growth and physiological performance of *Pelteobagrus fulvidraco*, while higher concentrations, particularly 2000 mg/kg, adversely affected intestinal health and triggered immune responses. Therefore, aside from determining the optimal dosage, the duration of administration is also an important factor that warrants further consideration. For instance, a study on Nile tilapia (*Oreochromis niloticus*) demonstrated that the inclusion of astragalus–ginseng mixture in their diet resulted in a time-dependent lysozyme activity and phagocytic capacity, which initially rose and then declined, with the peak activity being observed in the third week of a 1–4 week observation period [77]. Subsequently, a study on crucian carp (*Carassius auratus*) showed that pro-inflammatory factors such as *IL-1* and *IFN-α* increased progressively over the 4-week feeding period. Meanwhile, the anti-inflammatory factor *IL-10* exhibited a trend of initially decreasing and then increasing, with the lowest expression observed in the third week, suggesting that the third week might be the most optimal duration for supplementation [78]. Most studies currently focus on a single time point, with limited attention to changes over time. Future research should address this gap by exploring the effects of supplementation across different periods.

In recent years, the use of herbal mixtures in aquaculture, particularly for improving growth performance, antioxidant capacity, and immune function, has been widely studied, and these mixtures have played a significant role in replacing antibiotics. Additionally, novel findings have emerged regarding the use of herbal mixtures as vaccine adjuvants and their ability to enhance resistance against pathogens in fish. Recent research demonstrated the dietary astragalus enhancement in immune responses in carp (*Cyprinus carpio*), which also provides protection against *Aeromonas hydrophila*. Furthermore, ginseng has been shown to positively impact growth performance, immune response, and resistance to *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*), thereby further underscoring the potential of herbal extracts in aquaculture [36,79]. As a leading producer of traditional Chinese herbs, China's aquaculture industry faces challenges from diseases in intensive farming. The use of low-cost herbal mixtures as feed additives can reduce losses by providing antibacterial properties, offering significantly economic benefits. Building on the findings of this study, future research on herbal mixtures for yellow catfish could include pathogen challenge experiments, particularly with *Edwardsiella ictaluri* or *Aeromonas veronii*, to further assess and confirm the immune-enhancing effects of the astragalus–ginseng mixture.

5. Conclusions

In summary, the dietary astragalus–ginseng mixture in yellow catfish enhanced the activities of liver antioxidant enzymes, thereby further increasing the body's antioxidant capacity. Moreover, by promoting the activity of intestinal digestive enzymes and increasing the length of intestinal villi, the dietary astragalus–ginseng mixture improved intestinal health and enhance digestive capacity. Furthermore, the dietary astragalus–ginseng mixture also downregulated the expression level of pro-inflammatory genes, such as *IL-1β*, *IL-6*, and *TNF-α*, while upregulating the expression levels of *IL-10*, an anti-inflammatory gene. Based on these data, the excessive supplementation of the astragalus–ginseng mixture may

lead to immune responses and even toxicological effects, and the optimal concentration for the dietary supplementation of the astragalus–ginseng mixture is approximately 1000 mg/kg. While this study provides valuable insights, there are a few areas that could benefit from further exploration in future research. While this study provides a comprehensive characterization of the astragalus–ginseng mixture, it does not explore the optimal ratio of astragalus to ginseng, which warrants further investigation. Additionally, although the study focuses on a 6-week feeding trial, it lacks insights into the weekly progression, suggesting that future research should include a time-dependent analysis. Furthermore, although the effects on the immune system were assessed, the underlying mechanisms remain unexplored. Future studies could incorporate toxicity challenge experiments to further validate immune enhancement. These avenues will deepen our understanding and optimize the practical application of the astragalus–ginseng mixture in aquaculture.

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Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Real-time PCR primers.

Genes	Forward Primer	Reverse Primer	Accession No.
IL-1β	5-CCTGTGTGTTTGGGGATTG-3	5-CCTTGATGGTCTTTAGGCTCT-3	MF770571.1
IL-6	5-CCATCGGAGGAACACAGA-3	5-GTAGATAAGGCGCAGACATT-3	XM_027176013.2
IL-10	5-TCTGTAGGTTCCTCCTGCTT-3	5-AGGTCATCCTTGGATTCGT-3	XM_027144360.2
TNF-α	5-CAGGCAAACACACAAAGGC-3	5-GAGAAAGCTCCGAAAAACG-3	XM_027133763.2
β-actin	5-CCTAAAGCCAACAGGGAAAAG-3	5-GTCACGGCCAGCCAAATC-3	XM_027148463.2

Appendix B

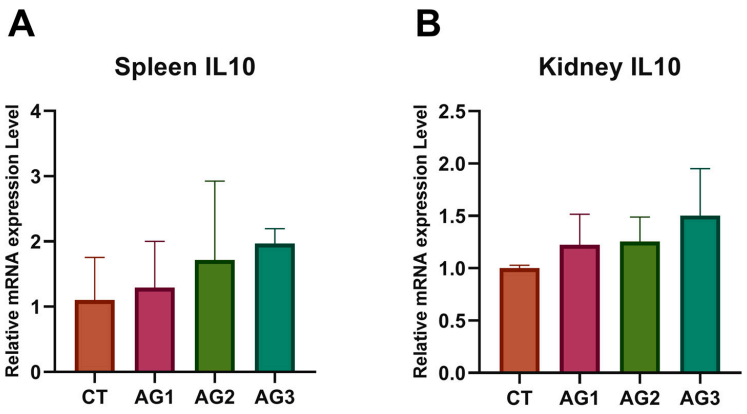

Figure A1. IL-10 gene expression levels of yellow catfish fed diets with graded levels of astragalus–ginseng mixture for 6 weeks. Spleen IL-10 (**A**), kidney IL-10 (**B**).

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