Original Research Articles

Effects of compound Chinese herbal medicine on growth performances, non-specific immunity and digestive enzyme activity of dongtingking crucian carp (*Carassius auratus indigentiaus*)

Cheng Ding[1,2a], Hu Xia[2,3b], Yanan Gong[2], Yunsheng Zhang[3], Yan Ning[3], Yixing Fang[3], Fuyan Chen[4], Jianchao Bu[3], Jia Yu[3], Guangqing Xiang[5], Jiezhen Huang[5], Pinhong Yang[2,3c]

[1] School of Life and Health Sciences, Hunan University of Science and Technology, Xiangtan, Hunan 411201, China, [2] Key Laboratory of special aquatic products, Hunan Applied Technology University, Changde, Hunan 415000, China, [3] Changde Key Innovation Team for wetland biology and Environmental Ecology, College of life and environmental sciences, Hunan University of Arts and Science, Changde, Hunan 415000, China, [4] Key Laboratory of Aquaculture genetic and breeding and Healthy Aquaculture of Guangxi; Guangxi Academy of Fishery Sciences, Nanning, Guangxi, 530021, China, [5] Longshan county animal husbandry aquatic affairs center,Tujia-miao Autonomous Prefecture of Xiangxi, Hunan, 416800, China

Keywords: Carassius auratus indigentiaus, compound Chinese herbal medicine, non-specific immunity, growth performances

https://doi.org/10.46989/001c.129714

Israeli Journal of Aquaculture - Bamidgeh

Vol. 77, Issue 1, 2025

This study investigates the effects of compound Chinese herbal medicine (CCHM) on growth performances, antioxidation activities, immune-related enzymes, and digestive enzymes of dongtingking crucian carp (*Carassius auratus indigentiaus)*. *Lycium barbarum* L., *Panax ginseng* C.A. Mey, *Astragalus membranaceus* and *Phragmites communis* Trin were ground and prepared into CCHM at the ratio of 4.5:3:1.5:1. The one-factor experiments were designed, in which different gradients of CCHM (0%, 1%, 2% and 4%) were added to basal feeds for *C. auratus indigentiaus*, which were fed for 90 d. The weight gain rate (WGR) and specific growth rate (SGR) in the experimental groups with CCHM addition were significantly higher than those of the control group (0%, without CCHM), the feed coefficient (FCR) was significantly lower in the experimental groups compared to the control group. In the experimental group with 4% of CCHM, WGR, and SGR of *C. auratus indigentiaus* were the highest (73.87±7.20 % and 0.63±0.15 %, respectively), while the FCR was the lowest (0.24±0.07 %). In the experimental group with 1% CCHM, the spleen index (SPI) increased significantly. However, the SPI of experimental groups, with 2% and 4% CCHM, showed no significant difference from that of the control group. The hepatosomatic index (HSI) of both experimental groups, with 2% and 4% of CCHM, was lower than that of the control group. However, there was no significant difference between the experimental group, which had 1% CCHM, and the control group. Activities of immune-related enzymes like lysozyme, acid phosphatase, and alkaline phosphatase (AKP) in the experimental groups with 1%, 2%, and 4% of CCHM were all higher than those of the control group. The lysozyme activity reached the highest in the experimental group with 1% of CCHM, the alkaline phosphatase (AKP) activity reached the peak in the experimental group with 2% of CCHM, and the acid phosphatase activity reached the highest in the experimental group with 2% of CCHM. Activities of antioxidant-related enzymes like catalase and glutathione peroxidase (GSH-PX) in *C. auratus indigentiaus* were all higher than those of the control group and reached the highest in the experimental group with 1% of CCHM. The total superoxide dismutase activity was highest in the experimental group with 4% of CCHM. Activities of intestinal digestive enzymes such as lipase, amylase, and trypsin in the experimental groups with 1%, 2%, and 4% of CCHM were higher than in the control group. Specifically, lipase and amylase activities were highest in the experimental group, with 1% of CCHM. In comparison,

a Co-first author. These authors contributed equally

b Co-first author. These authors contributed equally
Correspondence author: Hu Xia
E-mail: 867242290@qq.com

c Correspondence author: Pinhong Yang, E-mail: yph588@163.com

trypsin activity reaches the highest in the experimental group with 4% of CCHM. The results of this study show that adding CCHM into feed can increase the activity of immune-related enzymes and antioxidant-related enzymes in the serum of *C. auratus indigentiaus*, improve the activity of intestinal digestive enzymes, and promote growth. The appropriate CCHM content in basal feed for *C. auratus indigentiaus* is 1%.

INTRODUCTION

China has been in the leading position regarding aquaculture output, accounting for more than 60% of the global aquaculture output.[1] The demand for aquatic products expands the aquaculture scale continuously and makes disease issues more and more serious. Antibiotics are extensively used to assure aquatic animal health, threatening the quality of aquatic products and human health.[2] After the Ministry of Agriculture and Rural Affairs issued the No.119 announcement, China prohibited using feeds with growth promotion medical additives. Hence, research on feed-oriented antibiotic substitutes has become intensely scrutinized in healthy aquaculture.[3]

Chinese herbal medicine has a long research history in China. With various active ingredients, it is a natural immunopotentiator and an ideal antibiotic substitute.[4] The application of some ordinary Chinese herbal medicine to aquaculture has previously been reported. *Lycium barbarum* L. is a traditional Chinese medicine that can be used as both food and medicine. The main active ingredient, *Lycium barbarum* polysaccharide, is attractive to carnivorous freshwater fish, crabs, and other aquatic animals and can lower feed coefficients (FCR) after long-term supply.[5] *Astragalus membranaceus* can eliminate hydroxyl radicals and superoxide anion free radicals. Adding an appropriate amount of fermented *Astragalus membranaceus* into feed can improve the antioxidant index of *Epinephelus fuscoguttatus*.[6] As the active ingredient in *Astragalus membranaceus*, *Astragalus* polysaccharide can activate the immune responses of aquatic animals, while increasing phagocyte activity of hemocytes in *Penaeus vannamei* at 8 d.[7] Adding 0.01% of *Astragalus* polysaccharide into feed increases the survival rate of zebrafish infected with spring viremia of carp virus (SVCV).[8] *Panax ginseng* C.A.Mey is a rare medicinal herb with many beneficial active ingredients.[9] Ginseng polysaccharides and ginseng saponin are the most extensive components applied to aquaculture. Liu et al. found that ginseng polysaccharides can increase the immunologic functions and oxidation resistance of *Penaeus vannamei*.[10] Li et al. suggested that ginseng polysaccharides at 0.4 g/kg could significantly improve the weight gain rate (WGR) and liver-body ratio of black sea bream.[11] Ginseng saponin has been proven to prohibit the occurrence of inflammation. The traditional Chinese compound pulse-activating decoction prepared with ginseng saponin, and other ingredients might weaken proinflammatory cytokine expression levels.[12] *Phragmites communis* Trin. is the fresh or dry rhizome of reed, a type of gramineous plant.[13] There are few studies concerning using *Phragmites communis* Trin. in aquaculture, but many reports describe the protective effect on animal organs. Water extracts of *Phragmites communis* Trin. can mitigate fibrosis and lung tissue damage in mice with radiation-induced lung injury and inhibit cell apoptosis and inflammatory responses in the lungs.[14] It can improve hepatic pathological changes in rats caused by acute alcoholic damage through intragastric administration of *Phragmites communis* Trin. extracts.[15]

Compared to a single Chinese herb, the compound Chinese herbal medicine (CCHM) prepared by multiple Chinese herbs as an integral formulation has an improved therapeutic effect, highlighting the importance of Chinese herbal therapy utilizing synergistic effects.[16] It has been proved that using CCHM as feed additives in aquaculture can promote growth, enhance immunity, and so on.[17,18] Hence, *Lycium barbarum* L., *Astragalus membranaceus*, *Panax ginseng* C.A.Mey and *Phragmites communis* Trin. were mixed at variable ratios to prepare CCHM, which was added to the basal feed to explore its effect in aquaculture as a feed additive.

Carassius auratus indigentiaus, also called dongtingking crucian carp, is a natural carp species discovered in the Beimin Lake, Lishui, Changde City, Hunan Province. Consumers and culturists highly appreciate the species due to its flesh taste, strong adaptability, quick growth and other good characteristics.[19,20] In recent years, the aquaculture industry of *C. auratus indigentiaus* has achieved rapid development. Although it brings remarkable economic and other excellent traits, the high-density intensive culture and environmental worsening intensify stresses against *C. auratus indigentiaus*. Nevertheless, the influence of CCHM on intestinal digestive enzymes, growth indicators, antioxidants, and the immune system of *C. auratus indigentiaus* has not yet been reported. The current study investigated the potential of CCHM supplements in the basal feed for *C. auratus indigentiaus*. Growth index, activities of antioxidant and immunity-related enzymes in serum, as well as activities of intestinal digestive enzymes, were assessed. Effects of CCHM dosage on growth indicators, antioxidant and immune system, and activities of intestinal digestive enzymes of *C. auratus indigentiaus* were explored, aiming to provide experimental references for healthy cultivation and disease control of *C. auratus indigentiaus*.

MATERIALS AND METHODS

FISH FOR EXPERIMENT

Carassius auratus indigentiaus (38±5) g were collected from the fish breeding base of Hunan University of Arts and Science. Before sampling, the fish were kept in the laboratory cyclic cultivation system for two weeks, during which aeration was provided for 24h. The dissolved oxygen content was (6.0±0.2) mg/L, and the water temperature was maintained at (30±2) °C. Additionally, an appropriate amount of floating feed was offered to adult fish. The experiment was conducted with approval from the Institutional Animal

Table 1. Compound Chinese herb composition and nutrient levels

	Astragalus membranaceus	*Panax ginseng*	*Lycium barbarum*	*Phragmites communis*	Total
Additive amount /g	45	15	30	10	100
Crude protein /g	14.9	12.3	13.9	13	14.02
Crude fat /g	1.1	0.1	5	2.3	2.24
Crude ash /g	5	5	6	3	5.1
Carbohydrate /g	33.4	58.6	45	78	45.12
Cellulose /g	42.1	17.1	16.9	1.4	26.72
Total energy (KJ/g)	9.8	13.20	13.14	17.6	12.1

Care and Use Committees (JSDX-2022-00) of Hunan University of Arts and Science, Changde, China.

PREPARATION OF FEEDS WITH CCHM

After *Astragalus membranaceus*, *Lycium barbarumL.*, *Panax ginseng* C.A.Mey and *Phragmites communis* Trin. were ground into powder, they were mixed at the ratio of 4.5:3:1.5:1, and the CCHM powder was prepared. The powder was added to the prepared basal feed (control group) according to different concentration gradients. A total of four treatment groups were assessed, including the control group (without CCHM), low-dose group (1% of CCHM), middle-dose group (2% of CCHM) and high-dose group (4% of CCHM). The composition and nutritional levels of the CCHM additives are shown in **Table 1**. The composition and nutrients of feed are shown in **Table 2**.

FEEDING AND MANAGEMENT

Sixty *C. auratus indigentiaus* were stocked into each culture tank, including one control group and three test groups. After temporary culture for two weeks, fish were fed by forages with different dosages of CCHM at 8:30 and 17:00 (24-hour clock), respectively. The feeding amount was 2% of the body weight daily. The water in the tank was changed every 3d, and the water exchange volume was set to 1/3 of the total water volume. The culture tank was cleaned, supplied with oxygen, and exposed to air for 24 hours. The culture period was 90 d.

TEST OF GROWTH INDICATORS

Weight gain rate (WGR) =(W1-W0) / W0 × 100%

Specific growth rate (SGR) = (lnW1−lnW0)/ T × 100%

Feed coefficient (FCR) = feed consumption / (W1-W0) × 100%

Hepatosomatic index (HSI)=G1 / A × 100%;

Spleen index (SPI) =G2 / A × 100%;

W0 represents the initial weight of fish at the beginning of the experiment. W1 denotes the weight of the fish at the end of the experiment. T represents culture time (d). G1 represents the mean liver weight of fish, G2 represents the mean spleen weight of fish, and A is the mean weight of fish.

TEST OF SERUM ANTIOXIDASE AND IMMUNE-RELATED ENZYME ACTIVITY

Following 90 d of cultivation, five *C. auratus indigentiaus* samples were collected randomly from the test and control groups for euthanasia with MS-222. Later, 1 ml of blood was collected from the caudal vein with a sterile injection syringe and then centrifuged under 4°C for 10 min at the rate of 4,000 r/min. The serum was separated and stored in a 4°C refrigerator for later use. The kits were purchased from Nanjing Jiancheng Bioengineering Research Institute. According to operation steps in the kits' instruction, lysozyme content (Lys) and activities of total superoxide dismutase (T-SOD), catalase (CAT), acid phosphatase (ACP), alkaline phosphatase (AKP) and glutathione peroxidase (GSH-PX) in serum of *C. auratus indigentiaus* were measured by a multimode microplate reader (BioTek, USA).

TEST OF ACTIVITIES OF INTESTINAL DIGESTIVE ENZYMES

After 90 days of cultivation, five *C. auratus indigentiaus* samples were collected randomly from the test and control groups for euthanasia with MS-222 and blood sampling. Later, the middle intestinal tissues (2g) were collected from each *C. auratus indigentiaus*, to which pre-cooled normal saline (4°C) was added at the ratio of 1:9 (W/V). The mixture was mixed evenly in the ice-water bath for 30 minutes, and the homogenate was centrifuged at 4°C for 10 minutes at a rate of 3,000 r/min. The supernatant was separated. Test kits for amylase (AMS), lipase (LPS) and trypsin (TRS) were sourced from Nanjing Jiancheng Bioengineering Research Institute. According to the instruction operating steps, activities of AMS, LPS and TRS in the intestinal tract of *C. auratus indigentiaus* were measured by a multimode microplate reader (BioTek, USA).

DATA PROCESSING

Experimental data was expressed as means ± standard deviations. Since there are multiple independent groups for one factor， the one-way analysis of variance (ANOVA) was carried out using the SPSS15.0 statistical software. $P < 0.05$ indicates a significant difference. $P < 0.01$ expresses an extremely significant difference. $P > 0.05$ indicates no significant difference.

Table 3. Effects on of compound Chinese herbs growth performance of *C. auratus indigentiaus*

Item	CCHM additives			
	Control group	1%	2%	4%
Initial body weight (g)	38.00±5.00	38.00±5.00	38.00±5.00	38.00±5.00
Final body weight (g)	42.96±1.25	65.62±9.05	59.66±9.91	66.94±9.29
WGR (%)	11.58±1.37	70.44±8.76*	54.96±10.70**	73.87±7.20*
SGR (%)	0.14±0.02	0.61±0.16*	0.50±0.18*	0.63±0.15*
FCR (%)	1.38±0.02	0.25±0.06*	0.32±0.11*	0.24±0.07*

"*" and "**" indicate a significant difference at P<0.05 and P<0.01, respectively, between the treatment group and control groups.

Table 2. Composition and nutrient levels of experimental diet

Feed composition				
Material ratio (g/kg)	Control group	1%	2%	4%
Soybean meal	200	200	200	200
Rapeseed meal	400	400	400	400
Fish meal	110	110	110	110
Wheat	175	165	155	135
Soybean oil	60	60	60	60
Compound feed premix	30	30	30	30
bentonite	23	23	23	23
Choline chloride	1.7	1.7	1.7	1.7
Sodium diacetate	0.1	0.1	0.1	0.1
Ethoxyquinoline	0.2	0.2	0.2	0.2
Chinese herbal compound	0	10	20	40
Total	1000	1000	1000	1000
Nutrient level%				
Crude protein	33.97	33.98	33.99	34.01
Crude fat	8.85	8.86	8.86	8.88
Crude ash	5.56	5.58	5.59	5.68
Calcium	1.2	1.2	1.2	1.2
Carbohydrate	29.83	29.59	29.35	28.88
Total phosphorus	0.5	0.5	0.5	0.5
Crude fibre	6.24	6.48	6.73	7.22
Total energy (MJ/kg)	16.75	16.71	16.68	16.60

RESULTS

EFFECTS OF CCHM ON GROWTH PERFORMANCES

The CCHM was added into the basal feed of *C. auratus indigentiaus*. At 90 d, the WGR and SGR of the control group were 11.58±1.37 % and 0.14±0.02 %, respectively. WGR and SGR of test groups were all significantly higher than in the control group (P<0.05), while FCR was significantly lower (P<0.05). WGR and SGR reached the highest (73.87±7.20 % and 0.63±0.15 %, respectively) in the test group with 4% of CCHM, while FCR was the lowest (0.24±0.07 %) (**Table 3**).

SPI increased significantly in the test group with 1% of CCHM compared to the control group (P<0.05). However, the test groups with 2% and 4% CCHM showed no significant differences compared to the control group regarding SPI (P>0.05) (**Fig. 1**).

HSI of both test groups with 2% and 4% of CCHM was significantly lower than the control group (P<0.05). HSI of the test group with 1% of CCHM showed no significant difference from the control group (P>0.05) (**Fig. 2**).

EFFECTS OF CCHM ON ACTIVITIES OF SERUM IMMUNE-RELATED ENZYMES

LYS ACTIVITY

Serum Lys content was 1341.66 U/mL in the control group, 1419.75 U/mL in the low-dose group (1%), 1365.03 U/mL in the middle-dose group (2%), and 1372.83 U/mL in the high-dose group (4%). The serum Lys contents in test groups

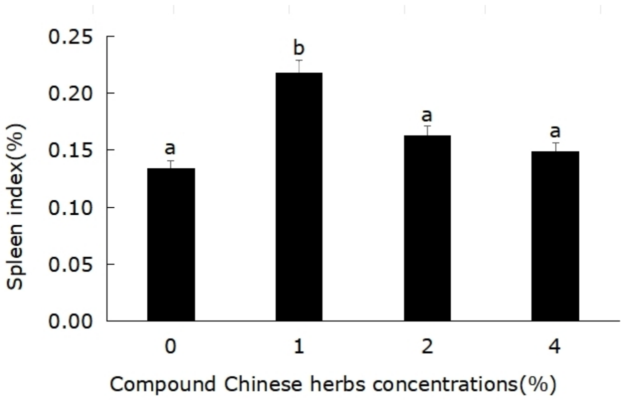

Fig. 1. Effect of CCHM on spleen index of *C. auratus indigentiaus*. Different letters indicate significant differences

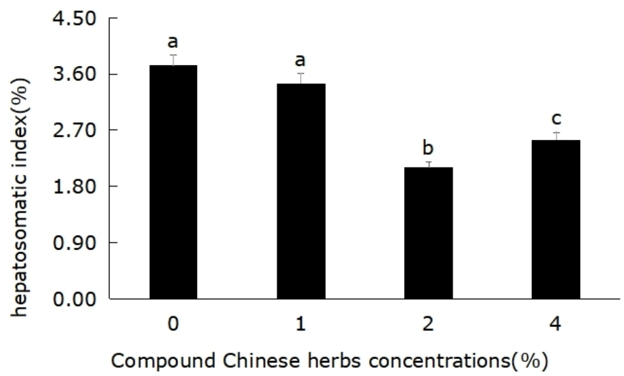

Fig. 2. Effect of CCHM on hepatosomatic index of *C. auratus indigentiaus*. Different letters indicate significant differences.

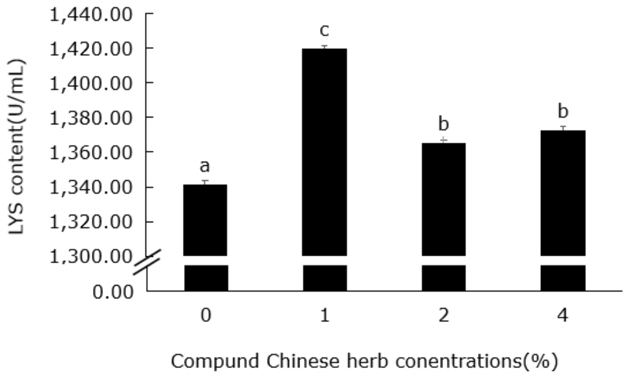

Fig. 3. Effect of CCHM on lysozyme content of *C. auratus indigentiaus*. Different letters indicate significant differences

were all significantly higher than in the control group (*P*<0.05). The serum Lys content reached the highest in the low-dose group (1%) (*P*<0.01) (**Fig. 3**).

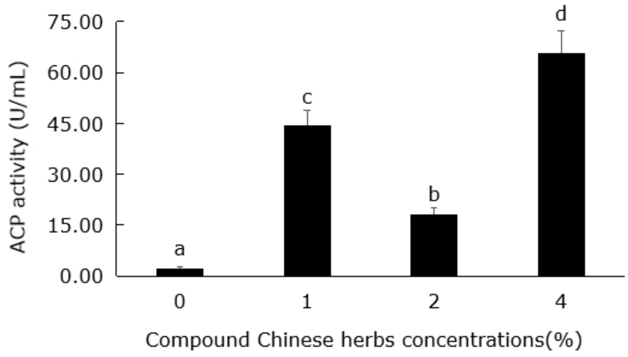

Fig. 4. Effect of CCHM on acid phosphatase of *C. auratus indigentiaus*. Different letters indicate significant differences

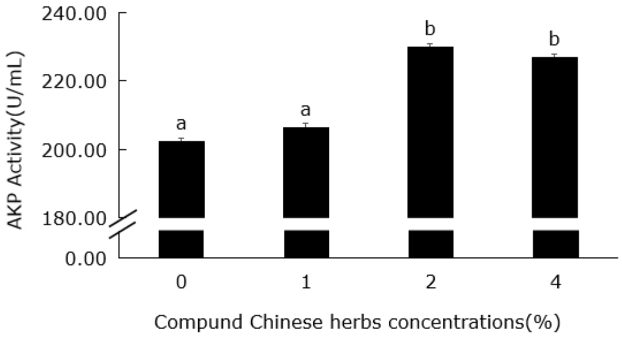

Fig. 5. Effect of CCHM on alkaline phosphatase of *C. auratus indigentiaus*. Different letters indicate significant differences.

ACP ACTIVITY

Serum ACP activity was 2.37 U/mL in the control group, 44.38 U/mL in the low-dose group (1%), 18.34 U/mL in the middle-dose group (2%), and 65.68 U/mL in the high-dose group (4%). Specifically, serum ACP activities of all test groups were significantly higher than the control group (*P*<0.05), reaching the peak in the high-dose group (4%) (*P*<0.01) (**Fig. 4**).

AKP ACTIVITY

Serum AKP activity was 202.32 U/mL in the control group, 206.62 U/mL in the low-dose group (1%), 229.84 U/mL in the middle-dose group (2%), and 226.83 U/mL in the high-dose group (4%). The serum AKP activities of the middle-dosage and high-dosage groups were significantly higher than the control group (*P*<0.05), reaching the peak in the middle-dosage group (2%) (*P*<0.01) (**Fig. 5**).

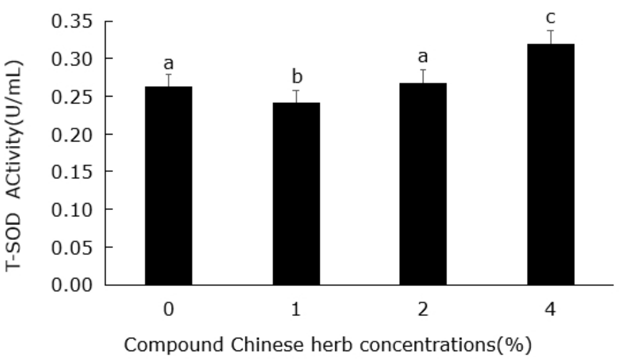

Fig. 6. Effect of CCHM on the activity of total superoxide dismutase in *C. auratus indigentiaus*. Different letters indicate significant differences.

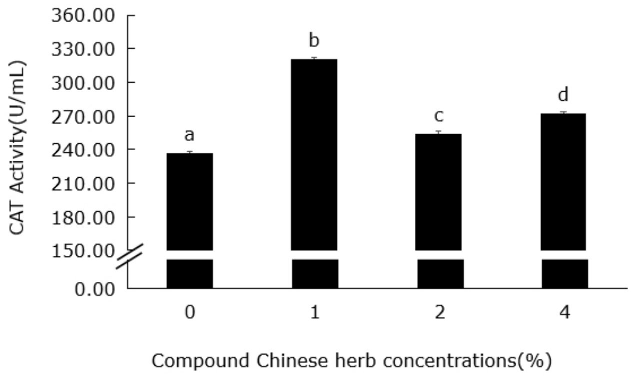

Fig. 7. Effect of CCHM on catalase activity of *C. auratus indigentiaus*. Different letters indicate significant differences.

EFFECTS OF CCHM ON ACTIVITIES OF SERUM ANTIOXIDANT ENZYMES

T-SOD ACTIVITY

Serum T-SOD activity was 0.26 U/mL in the control group, 0.24 U/mL in the low-dose group (1%), 0.27U/mL in the middle-dose group (2%), and 0.32U/mL in the high-dose group (4%). Specifically, the serum T-SOD activity of the low-dosage group decreased dramatically compared to the control group (*P*<0.05). However, the serum T-SOD activity of the middle-dosage group showed no significant difference from the control group (*P*>0.05). The serum T-SOD activity of the high-dosage group was significantly higher compared with the control group (*P*<0.05) (**Fig. 6**).

CAT ACTIVITY

Serum CAT activity was 236.87 U/mL in the control group, 320.43 U/mL in the low-dose group (1%), 254.19 U/mL in the middle-dose group (2%), and 272.25 U/mL in the high-dose group (4%). Specifically, the serum CAT activity of all test groups increased sharply compared with the control group (*P*<0.05), reaching a peak in the low-dosage group (1%) (**Fig. 7**).

GSH-PX ACTIVITY

Serum GSH-PX activity was 63.30 U/mL in the control group, 131.87 U/mL in the low-dose group (1%), 84.40 U/mL in the middle-dose group (2%), and 105.49 U/mL in the high-dose group (4%). Specifically, the serum CAT activity of all test groups was significantly higher than the control group (*P*<0.05), reaching a peak in the low-dosage group (1%) (**Fig. 8**).

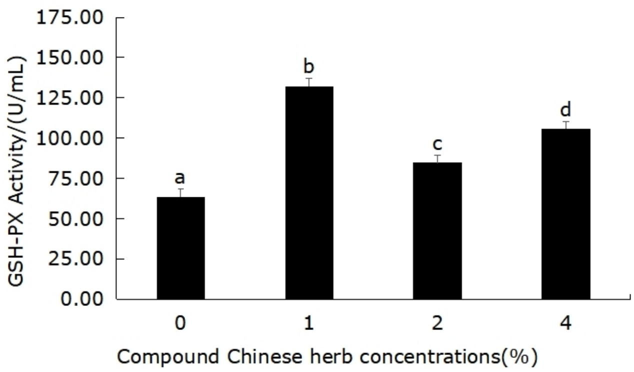

Fig. 8. Effect of CCHM on glutathione peroxidase activity of *C. auratus indigentiaus*. Different letters indicate significant differences.

EFFECTS OF CCHM ON ACTIVITIES OF INTESTINAL DIGESTIVE ENZYMES

TRS ACTIVITY

The intestinal TRS activity was 0.67 U/mg in the control group, 0.81 U/mg in the low-dose group (1%), 0.88 U/mg in the middle-dose group (2%), and 1.21 U/mg in the high-dose group (4%). Specifically, the intestinal TRS activity of all test groups was significantly higher compared to the control group (*P*<0.05) (**Fig. 9**).

AMS ACTIVITY

The intestinal AMS activity was 0.58 U/mg in the control group, 0.80 U/mg in the low-dose group (1%), 0.59 U/mg in the middle-dose group (2%), and 0.63 U/mg in the high-dose group (4%). Specifically, the intestinal AMS activity of the low-dosage group (1%) was the highest and was significantly higher than the control group (P<0.05). The intestinal AMS activities of the middle-dosage group (2%) and high-dosage group (4%) showed no significant difference from the control group (*P*>0.05) (**Fig. 10**).

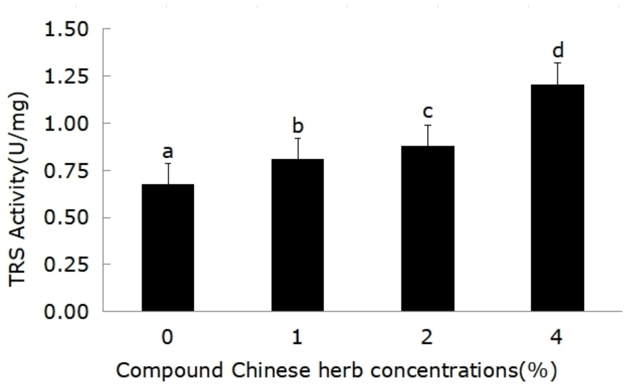

Fig. 9. Effect of CCHM on trypsin activity of *C. auratus indigentiaus*. Different letters indicate significant differences.

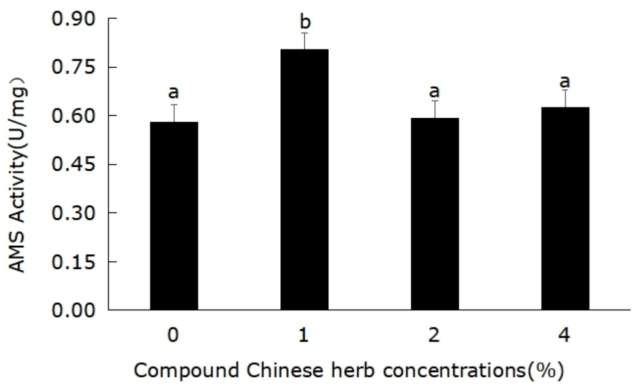

Fig. 10. Effect of CCHM on amylase activity of *C. auratus indigentiaus.* Different letters indicate significant differences.

LPS ACTIVITY

The intestinal LPS activity was 0.58 U/mg in the control group, 1.2 U/mg in the low-dose group (1%), 0.91 U/mg in the middle-dose group (2%), and 0.96 U/mg in the high-dose group (4%). The intestinal LPS activity of all test groups was significantly higher than the control group (P<0.05). The intestinal LPS activities of the middle and high-dosage groups presented no significant differences (P>0.05). However, the intestinal LPS activity of the low-dosage group (1%) revealed the highest value (P<0.01) (Fig. 11).

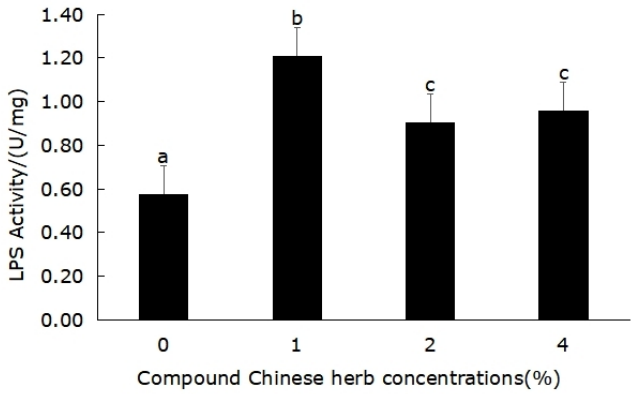

Fig. 11. Effect of CCHM on lipase activity of *C. auratus indigentiaus.* Different letters indicate significant differences

DISCUSSION

Compound Chinese herbal medicine (CCHM) has shown many beneficial effects on aquatic animals as a feed additive. *Carassius auratus indigentiaus* (*C. auratus indigentiaus*), a special strain of crucian carp, which has a variety of excellent characteristics and good breeding value. To our knowledge, there was no information up to now on the effects of CCHM on growth, immune response, and digestive capacity in the breeding industry of *C. auratus indigentiaus*. In the current work, we described the effects of feed containing three additional doses of CCHM on the growth performance, antioxidation activities and immune-related enzymes, digestive enzymes in *C. auratus indigentiaus*. As shown in the results, CCMH effectively improved the growth and enzyme activity.

The growth ability of aquatic animals is critical as it correlates with aquaculture production efficiency.[21] According to this study, WGR and SGR of *C. auratus indigentiaus* increased significantly using feeds containing CCHM, while FCR decreased sharply. Such outcomes demonstrate that CCHM can facilitate the growth and development of *C. auratus indigentiaus*, increase the feed conversion ratio, and promote digestion and absorption of basal feeds. It is reported that the formulation of CCHM containing *Astragalus membranaceus* and active substances in *Astragalus membranaceus* is beneficial for the growth of some aquatic fishes.[22] After feeding with the compounds derived from *Astragalus membranaceus*, *Angelica sinensis* and *Fructus crataegi* at the ratio of 1:1:1 for 4 weeks, WGR, SGR and FCR of Nile tilapia were increased significantly.[23] Feeds prepared for prawns by mixing *Astragalus membranaceus*, liquorice, *radix bupleuri* and *Angelica sinensis* at an equal ratio induced the WGR and SGR to reach the highest in the test group (containing 0.4%) and FCR being the lowest in the test group (containing 0.8%).[24] Li et al. added *Astragalus membranaceus*, garlic, *Chrysanthemum indicum L.*, *fructus crataegi*, and water extracts of *radix isatidis* into basal feeds of grass carp at an equal ratio. WGR and SGR of test groups (adding 1%, 2%, 4% and 8%) increased significantly compared to the control group.[25] The fish organ index is an important index that reflects fish body functions.[26] In the present study, the SPI of *C. auratus indigentiaus* showed no apparent changes compared to the control group. HSI declined considerably in the middle-dosage and high-dosage groups but showed no apparent differences between the low-dosage and control groups. Such results infer that CCHM can protect the liver and spleen of *C. auratus indigentiaus* to some extent.[27] SPI of the low-dosage group increased significantly, similar to reports by Wang et al.[28] These data reveals that Chinese herbal compounds can promote the growth and development of the spleen, while regulating the spleen and liver of *C. auratus indigentiaus* under low dosages.

The activity of serum immune-related enzymes is an essential indicator for evaluating the immunological health of fish. Hence, Chinese herbs' effects on aquatic animals' immunity are usually evaluated by assessing the activities of non-specific immune enzymes.[4,29] The metabolism of aquatic organisms is partly regulated by the phosphorylation and dephosphorylation of various compounds. This reaction requires catalysis by different phosphatases (e.g. ACP and AKP) and participates in the transfer and metabolism of phosphate groups.[30] The compound with 4% *Dendrobium officinale* could significantly increase the serum ACP activity of Gifu tilapia, and the compound with 8% *Dendrobium officinale* can significantly lower its serum AKP activity.[31] The serum AKP and ACP activities of *Epinephelus Lanceolatus* were significantly improved by adding CCHM into the basal feeds. Such outcomes prove that CCHM can activate AKP and ACP to participate in non-specific immune responses in fish bodies.[32] Lys is a kind of antibacterial peptide and the first defensive line of congenital immunity in fishes. It is often called a natural antibacterial agent in fish bodies and plays an essential role in the immune responses of fish.[33] The feeds containing *Glycyrrhiza glabra*, *Astragalus membranaceus*, honeysuckle, *Rheum officinale* and *semen cassia* were supplied to *Pelteobagrus fulvidraco* and found that serum Lys content in all test groups was significantly higher than that of the control group at 28 d.[29] In *Acipenser schrenckii*, Lu et documented that serum Lys content increased significantly at 76d after discontinuous supply with feeds containing 0.4g/kg of CCHM prepared by *Codonopsis pilosula*, *Schisandra chinensis*, *acanthopanax* and *Agastache rugosus*.[34] In summary, CCHM can promote abundant production of Lys in *Pelteobagrus fulvidraco* and *Acipenser schrenckii*. In our study, the LZM content, AKP activity, and ACP activity of the different test groups were far higher than those of the control group. LZM content reached the highest in the low-dosage group (1%), the AKP activity reached the highest in the middle-dosage group (2%), and ACP activity reached the peak in the high-dosage group (4%). These data support the hypothesis that CCHM can accelerate *C. auratus indigentiaus*' metabolism, promote immune responses, and enhance non-specific immunity.

During aquaculture operations, various physiological reactions and environmental changes quickly induce large-scale production of reactive oxygen species (ROS) in aquatic animals, thus producing oxidative stresses that inhibit growth and even cause abnormal deaths of aquatic animals.[35] The antioxidant defense system is conducive to eliminating excessive ROS and offset tissue and organ damage of farmed animals caused by oxidative stresses.[36] The fish antioxidant defense system comprises T-SOD, CAT, GSH-PX, and other antioxidants. It releases ROS by eliminating various metabolic activities of living bodies under typical situations to cope with oxidative stresses effectively and decompose them into low-toxic and non-toxic substances.[37] T-SOD reflects the ability to eliminate superoxide radicals and protect cells or tissues from ROS toxicity.[38] CAT catalyses hydrogen peroxide to form water and oxygen, playing a crucial role in the immunoregulation network of aquatic animals.[39] GSH-PX and CAT have the same effect, and CAT decomposition is accelerated with the assistance of GSH-PX.[40] Several herbal preparations have been tested for their promoting effect on antioxidant enzymes activity in aquatic animals. After a continuous supply of feeds containing CCHM (including *Astragalus membranaceus*) by 30d, the serum T-SOD and CAT activities of hybrid sturgeon (1% test group) was significantly higher than those at 0d.[41] Ghafarifarsani et al.[42] cultured common carp with different dosages of CCHM prepared from *Malvae sylvestris*, *Origanum vulgare,* and *Allium hirtifolium.* Within the dosage range from 0.5% to 5%, the T-SOD and GSH-PX activities under 1% reached the highest, and CAT activity under 5% was the highest.[42] The results in the present study show that CAT and GSH-PX activities of all test groups were significantly higher than those of the control group. They peaked in the low-dosage group, while T-SOD activity peaked in the high-dosage group, indicating that adding CCHMs improves antioxidant indicators of *C. auratus indigentiaus* and their responses to antioxidant stresses.

Fish digestive enzymes mainly originate from the pancreas and have a digestive effect on the intestinal tract. Hence, the digestive ability of fish can be reflected by the activity of intestinal digestive enzymes.[43] Among various digestive enzymes, AMS can facilitate the hydrolysis of starch and glycogen in fish bodies.[44] LPS facilitates the hydrolysis of triglycerides, producing free fatty acids and monoacylglycerols that can be absorbed, utilized, or stored by the body.[45] TRS hydrolyze proteins through pyrolysis of amino acid carboxyl protein chains and participates in metabolic reactions related to multiple proteins.[46] Hence, we chose to measure the activity of AMS, LPS and TRS in the intestine to evaluate the changes in the digestive ability of *C. auratus indigentiaus* after feeding the diet containing CCHM. After mixing CCHM composed of *Astragalus membranaceus*, honeysuckle, *Eucommia ulmoides,* and another nine Chinese herbs into feeds for *Acanthopagrus schlegelii*, it was reported that TRS and AMS activities in the test groups (concentration>3.5%) increased significantly. However, they showed no apparent difference in the low-dosage group. Among all dosage groups, CCHM increased LPS activity significantly.[47] CCHM feed containing similar proportions of *Ferula sinkiangensis*, *Medicago falcata L.* and garlic powder could increase TRS and LPS activities in the intestinal tract of *Lateolabrax japonicas* effectively. TRS and LPS activities reached the highest under the dosage of 2.0% and are significantly higher than those of the control group. However, AMS activity showed no noticeable difference.[48] Such outcomes demonstrate that CCHM can improve digestive enzyme activities in *Acanthopagrus schlegelii* and *Lateolabrax japonicas* to some extent, facilitate nutrient absorption, and increase feed utilization. In this experiment, the LPS activity of the test groups was significantly higher than that of the control group and reached a peak in the low-dosage group (1%). The AMS activity of the low-dosage group (1%) was far higher than that of the control group. However, the AMS activity of the low-dosage group (1%)

and middle-dosage group (2%) had no significant difference from the control group. After *Artemisia capillaris*, *rheum officinale*, and tulip were mixed at some ratios, the LPS activity and AMS activity of Gifu tilapia juvenile under the dosage of 10g/kg increased significantly,[49] corroborating results in the present work. TRS activity increased gradually with increasing CCHM dosages, inferring that the dosage of CCHM in basal feed had a linear relationship with TRS activity.

ACKNOWLEDGMENTS

This work was supported by the Hunan Natural Science Foundation (Grant No. 2025JJ50145), the National Natural Science Foundation of China (Grant No. 32360922), Guangxi Natural Science Foundation (Grant No. 2022JJA130338).

AUTHORS' CONTRIBUTION

Conceptualization: Cheng Ding (Equal), Hu Xia (Equal), Pinhong Yang (Equal). Methodology: Cheng Ding (Equal), Yunsheng Zhang (Equal), Yan Ning (Equal), Yixing Fang (Equal), Fuyan Chen (Equal), Jia Yu (Equal), Guangqing Xiang (Equal). Formal Analysis: Cheng Ding (Equal), Hu Xia (Equal), Jianchao Bu (Equal), Jiezhen Huang (Equal). Investigation: Cheng Ding (Equal), Hu Xia (Equal), Jianchao Bu (Equal), Jiezhen Huang (Equal). Writing – original draft: Cheng Ding (Equal), Hu Xia (Equal). Writing – review & editing: Hu Xia (Equal), Pinhong Yang (Equal). Funding acquisition: Pinhong Yang (Equal). Resources: Pinhong Yang (Equal). Supervision: Pinhong Yang (Equal).

COMPETING OF INTEREST – COPE

The authors of this study declare no conflict of interest.

ETHICAL CONDUCT APPROVAL – IACUC

The experiment was conducted with approval from the IACUC (Institutional Animal Care and Use Committees) of Hunan University of Arts and Science, Changde, China (NO. JSDX-2022-00).

INFORMED CONSENT STATEMENT

All of the authors have read and approved the paper for publication. It has not been published previously and is not being considered by any other peer-reviewed journal.

DATA AVAILABILITY STATEMENT

All are available upon reasonable request.

Submitted: November 28, 2024 CST. Accepted: December 16, 2024 CST. Published: March 10, 2025 CST.

REFERENCES

1. Hou TY, Tang DM, Zhang LP, Zhou QH, Wu ZB, Wu JM. Effects of antibiotics on aquaculture tailwater treated in constructed wetlands and their mitigation pathways. *Acta Hydrobiologica Sinica*. Published online 2024:1-11. doi:10.7541/2025.2024.0227

2. Li YN, Pan XN, Chen JY, Tong XH. Evaluation of the synergistic effect of traditional Chinese medicine black plum and antibiotics on the antibacterial effect of four fish-derived bacteria. *Chinese Journal of Fisheries*. Published online 2024:1-9. https://link.cnki.net/urlid/23.1363.S.20241011.1307.002

3. Ma XY, Li DG, Yu M, Zhou RZ. Research progress on the application of feed antibiotic substitutes in livestock and poultry production. *Chinese Journal of Animal Nutrition*. Published online 2024:1-11. https://link.cnki.net/urlid/11.5461.S.20240807.1412.002

4. Zhang HQ, Wang SY, Li JX, et al. Research progress on the non-specific immune effect of Chinese herbal medicine on aquatic animals. *Chinese Jourial of Fisheries*. 2024;37(02):107-115. doi:10.3969/j.issn.1005-3832.2024.02.015

5. Bi RR, Zhao Y, Sun YJ. Research progress on the regulation of intestinal microbiota and related physiological functions by phytochemical components of wolfberry. *Journal of Zhejiang University (Agriculture and Life Science)*. 2024;50(01):25-34. doi:10.3785/j.issn.1008-9209.2023.03.231

6. Xiao B, Zhou SJ, Wang YG, et al. Effects of fermented *Astragalus membranaceus* on the growth, digestion, immune function and resistance to ammonia nitrogen in brown-spotted grouper. *South China Fisheries Science*. 2023;19(02):161-169. doi:10.12131/20220197

7. Chang ZQ, Ge QQ, Sun M, Wang Q, Li J. Immune responses by dietary supplement with Astragalus polysaccharides in the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture Nutrition*. 2017;24(2). doi:10.1111/anu.12599

8. Li Y, Chao W, Kai XJ, et al. The effect of Astragalus polysaccharide on growth, gut and liver health, and anti-viral immunity of zebrafish. *Aquaculture*. 2021;540(1). doi:10.1016/j.a-quaculture.2021.736677

9. Rajput SA, Shaukat A, Rajput IR, et al. Ginsenoside Rb1 prevents deoxynivalenol-induced immune injury via alleviating oxidative stress and apoptosis in mice. *Ecotoxicology Environmental Safety*. 2020;220. doi:10.1016/j.ecoenv.2021.112333

10. Liu JT, Si YG, Wang Y, et al. Effects of *Astragalus* polysaccharides, *Panax ginseng* C. A. Mey polysaccharides and moringa powder on immune function and antioxidant capacity of *Penaeus vannamei* and its offspring. *Chinese Journal of Animal Nutrition*. 2023;35(06):3888-3901. doi:10.12418/CJAN2023.361

11. Li CZ, Hu K, Tang XL, Wu YX, Liang CJ, Yang XL. Effects of *Panax ginseng* C. A. Mey polysaccharides on growth performance and mRNA expression of antioxidant enzymes in *Acanthopagrus schlegelii*. *Journal of Huazhong Agricultural University*. 2015;34(06):94-100. doi:10.13300/j.cnki.hnlkxb.2015.06.059

12. Zheng YR, Tian CY, Fan CL, et al. Sheng-Mai Yin exerts anti-inflammatory effects on RAW 264.7 cells and zebrafish. *Journal of Ethnopharmacology*. 2021;267(1). doi:10.1016/j.jep.2020.113497

13. Li WB, Zhao WY, Pang MJ, Su YF. Study on the hydrophilic chemical composition of reed root (*Phragmites communis* Trin.). *Chinese Herbal Medicines*. 2024;55(01):51-56. doi:10.7501/j.issn.0253-2670.2024.01.007

14. Li JB, Zhang J, He X, Liu M, Yan K, Zhao WP. Protective effect of aqueous extract of reed root (*Phragmites communis* Trin.) on mice with radiation lung injury. *Chinese Journal of Pathophysiology*. 2023;39(07):1282-1288. doi:10.3969/j.issn.1000-4718.2023.07.015

15. Yao Z, Chen GY, Xie MZ, et al. Study on the protective effect of reed root extract on acute alcoholic liver injury in rats. *Lishizhen Medicine and Materia Medica Research*. 2022;33(01):95-98. doi:10.3969/j.issn.1008-0805.2022.01.23

16. Feng HW. *Based on Microbiomics, the Characteristics of Pathogenic Bacteria in Aquatic Economic Animals and the Application of Chinese Herbal Medicine for Immune Prevention and Control Were Analysed*. PhD dissertation. Liao Ning University; 2021. doi:10.27209/d.cnki.glniu.2021.002096

17. Bao YH, Zhang XY, Yin SJ, Zhang HQ, Xu JH. Effects of Compound Chinese Herbal Medicine on Intestinal Microbiota and Non-specific Immune Function of *Pelodiscus sinensis*. *Journal of Fishery Sciences of China*. 2023;05:86-94. doi:10.12131/20230069

18. Gao XZ, Zhang PY, Shi XY, Ben LZ, Guo QQ, Liu HY. Effects of compound Chinese herbal medicines on turbot growth, feed utilisation and non-specific immunity in feed. *Acta Hydrobiologica Sinica*. 2022;46(02):257-264. doi:10.7541/2021.2020.174

19. Liu LG, Yang PH, Xie CH, Wang XY, Xu LM. *Carassius auratus var. Dongtingking* and an ISSR analysis of the genetic diversity of three crucian carp strain populations. *Oceanologia et Limnologia Sinica*. 2015;46(02):426-431. doi:10.11693/hyhz20141000287

20. Xia H, Yang PH, Zhang YS, et al. Cloning of sIgZ heavy chain gene in *Carassius auratus indigentiaus* and immune response to *Aeromonas hydrophila*. *Journal of Fishery Sciences of China*. 2022;29(10):1449-1460. doi:10.12264/JFSC2022-0195

21. Wang JL, Meng XL, Lu RH, et al. Effects of *Rehmannia glutinosa* on growth performance, immunological parameters and disease resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio* L.). *Aquaculture*. 2015;435:293-300. doi:10.1016/j.aquaculture.2014.10.004

22. Yu W, Yang YK, Zhou QC, et al. Effects of dietary Astragalus polysaccharides on growth, health and resistance to *Vibrio harveyi* of *Lates calcarifer*. *International journal of biological macromolecules*. 2022;207:850-858. doi:10.1016/j.ijbiomac.2022.03.176

23. Abarike ED, Jian JC, Tang JF, Cai J, Yu H, Chen LH. Traditional Chinese Medicine Enhances Growth, Immune Response, and Resistance to *Streptococcus agalactiae* in Nile Tilapia. *Journal of Aquatic Animal Health*. 2019;31(1):46-55. doi:10.1002/aah.10049

24. Chen HH, Tu CL, Tang Y, Huang YC. Effects of Compound Chinese Herbal Medicine on the Growth, Digestive Enzyme and Immune Factor Activities and Anti-WSSV of *Penaeus vannamei*. *Journal of Fisheries of China*. 2017;41(11):1766-1778. doi:10.11964/jfc.20160910534

25. Li C, Zhang QZ, Yang YY, et al. Effects of different doses of compound Chinese herbal medicine immune enhancers on the growth performance and immune function of grass carp (*Ctenopharyngodon idella*). *Journal of Shanghai Ocean University*. 2011;20(04):534-540. https://kns.cnki.net/kcms2/article/abstract?v=-xbefZa1Cdu6MIgFEGR47iyQ7vo4dotQfJtjx02cmYMm33QtGQiJvy6-0n5RKmMkDBiqZRVygNgdF9bXPqThqQ0aa8vDt65CkBnRgaYd8TXNdNbBGcmDaLm9UmXGcotVDSBik-O5i6TXz2ztCLNdb7E9IGMY6pnX_Js2I3c8TBV5tvBURLJ0Oh3npaR5p3bz&uniplatform=NZKPT&language=CHS

26. Qu GJ, Zhang TY, Zhang XY, Meng HY, Dong XQ, Wang GQ. Study on the appropriate dosage of compound Chinese herbal medicine additives in the diet of golden carp. *Feed Industry*. Published online 2024:1-6. https://link.cnki.net/urlid/21.1169.S.20240808.1335.002

27. Bai CS, Wang H, Tian QF, et al. Study on the prevention and control effect of *Homotia pelliformis* HXS-M1 fermentation compound Chinese medicine on *E. coli* diarrhea mice. *China Animal Husbandry & Veterinary Medicine*. 2024;51(11):5014-5022. doi:10.16431/j.cnki.1671-7236.2024.11.036

28. Wang F, Li CG, Ma YW, et al. Effects of compound Chinese herbal medicines on Rainbow Trout growth and non-specific immune function. *Chinese Journal of Fisheries*. 2021;34(02):8-14. doi:10.3969/j.issn.1005-3832.2021.02.002

29. El BMF, Teiba II, Shahin SA, et al. Dietary Guduchi (*Tinospora cordifolia*) enhanced the growth performance, antioxidative capacity, immune response and ameliorated stress-related markers induced by hypoxia stress in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol*. 2022;120:337-344. doi:10.1016/j.fsi.2021.12.002

30. Pinoni SA, López, Mañanes AA. Alkaline phosphatase activity sensitive to environmental salinity and dopamine in muscle of the euryhaline crab *Cyrtograpsus angulatus*. *Journal of Experimental Marine Biology and Ecology*. 2004;307(1):35-46. doi:10.1016/j.je-mbe.2004.01.018

31. Shi WQ. *Effects of Dendrobium Officinale and Its Compound on Growth, Antioxidant Response Andintestinal Tract of Jifu Tilapia*. Master's Thesis. South China Agricultural University; 2018. https://kns.cnki.net/kcms2/article/abstract?v=-xbefZa1Cdt0j64rB-OqoK8hHVjHGGxcekS5Ss_GXAFHDx4Q2cUpDRX-0qj5sIFxOwHh6vzwuXkO3EsWWzJoSCJ0lBm2BOIarZM4GhBrkU3u9g79UbDLVB3hxN7yCUWteceu2jTSYq3tm332nSzZkPi4r4YJ-BzbnSkitTZ4Ix29_uB3zB7CesXrWS8DaFnbrftDU1H9fg=&uniplatform=NZKPT&language=CHS

32. Xu AL, Li ZB, Shang GJB, et al. Effects ofCompound Chinese Herbal Medicine on Growth, Non-specific Immunity and Digestive Enzyme Activities of Pearl Gentian Grouper. *Acta Oceanologica Sinica*. 2018;40(12):49-57. doi:10.3969/j.issn.0253-4193.2018.12.006

33. Tassanakajon A, Somboonwiwat K, Suoungul P, Tang S. Discovery of immune molecules and their crucial functions in shrimp immunity. *Fish Shellfish Immunol*. 2012;34(4):954-967. doi:10.1016/j.fsi.2012.09.021

34. Lu ZX, Luan XB, Wang D, Liu HB. Effects of intermittent feeding of three compound Chinese herbs on the growth and immune function of S. shi's sturgeon. *Chinese Journal of Fisheries*. 2020;33(01):13-18. doi:10.3969/j.issn.0253-4193.2018.12.006

35. Zhou Q, Zhang S, Geng X, et al. Antioxidant Effects of Roasted Licorice in a Zebrafish Model and Its Mechanisms. *Molecules*. 2022;27(22). doi:10.3390/molecules27227743

36. Chen X. *Effects of Hypoxic Stress on Antioxidant Status and Metabolic Function of Juvenile Chinesemitten Crabs (*Eriocheir Sinensis*)*. Master's Thesis. Shanghai Ocean University; 2023. doi:10.27314/d.cnki.gsscu.2023.000819

37. Gao JW, Wu H, Li SM, Xie M, Li WX, Song R. Effects of ammonia nitrogen and cadmium stress on antioxidant system and immune function of Furong crucian carp (*Carassius auratus* Furong carp♀× *Cyprinus carpio* red crucian carp♂). *Acta Hydrobiologica Sinica*. 2022;46(04):448-456. doi:10.7541/2021.2020.282

38. Cobbina SJ, Xu H, Zhao T, et al. A multivariate assessment of innate immune-related gene expressions due to exposure to low concentration individual and mixtures of four kinds of heavy metals on zebrafish (*Danio rerio*) embryos. *Fish Shellfish Immunol*. 2015;47(2):1032-1042. doi:10.1016/j.fsi.2015.11.003

39. Wang C, Li Z, Sun Y, Chen Q, Lu J. Effects of Chinese herbal medicines mixture on growth performance digestive enzyme activity immune response of juvenile Japanese seabass, *Lateolabrax japonicus*. *Aquaculture Nutrition*. 2018;(1). doi:10.1111/anu.12597

40. Atenclo L, Moreno I, Jos A, et al. Effects of dietary selenium on the oxidative stress and pathological changes in tilapia (*Oreochromis niloticus*) exposed to a microcystin-producing cyanobacterial water bloom. *Toxicon*. 2009;53(2):269-282. doi:10.1016/j.toxicon.2008.11.011

41. Fu TB, Lai QF, Gao BD, Xu L, You HZ, Zhou K. Effects of compound Chinese herbal medicine on antioxidant, non-specific immunity and digestive enzyme activities of hybrid sturgeon. *Feed Research*. 2022;45(05):58-61. doi:10.13557/j.cnki.issn1002-2813.2022.05.012

42. Ghafarifarsani H, Hoseinifar SH, Adorian TJ, Goulart FFR, Raissy M, Van DH. The effects of combined inclusion of *Malvae sylvestris*, *Origanum vulgare*, and *Allium hirtifolium boiss* for common carp (*Cyprinus carpio*) diet: Growth performance, antioxidant defence, and immunological parameters. *Fish Shellfish Immunol*. 2021;119:670-677. doi:10.1016/j.fsi.2021.10.014

43. Cahu C, Rnnestad I, Grangier V, Infante JLZ. Expression and activities of pancreatic enzymes in developing sea bass larvae (*Dicentrarchus labrax*) in relation to intact and hydrolysed dietary protein; involvement of cholecystokinin. *Aquaculture*. 2004;238(1-4):295-308. doi:10.1016/j.aquaculture.2004.04.013

44. Vöhringer ML, Becker TW, Krieger G, Jacobi H, Witte I. Synergistic DNA damaging effects of malondialdehyde/Cu(II) in PM2 DNA and in human fibroblasts. *Toxicol Lett*. 1998;94(3):159-166. doi:10.1016/s0378-4274(98)00002-2

45. Tingsen J, Hui L, Junwa H, et al. Mulberry leaf extract improves non-specific immunity and antioxidant capacity of largemouth bass (*Micropterus salmoides*) fed a high-starch diet. *Frontiers in Marine Science*. 2022;9. doi:10.3389/fmars.2022.1029360

46. Miao L, Charles O, Lin Y, et al. Interactive effects of mulberry leaf meal and bamboo charcoal additive on growth performa-nce, antioxidant capacity, and disease resistance of genetically improved farmed tilapia (Gift) juvenile (*Oreochromis niloticus*). *Aquaculture Reports*. 2020;18. doi:10.1016/j.aqrep.2020.100483

47. Hu XW, Liu LH, Chen Q, Li WJ, Li ZB. Effects of compound Chinese herbal medicines on the growth, non-specific immunity and intestinal digestive enzymes of black seabream (*Acanthopagrus schlegelii*). *Marine Science*. 2024;48(01):56-67. doi:10.11759/hykx20230714002

48. Xu AL. *Effects of Three Chinese Herbal Medicines on the Growth of Japanese Sea Bass (*Lateolabrax Japonicus*), Non-Specific Immunity and Digestive Enzyme Activities*. Master's Thesis. Jimei University; 2016. https://kns.cnki.net/kcms2/article/abstract?v=-xbefZa1CdtXRviUMYFb4l-98bhu1Oa4laRQXVYxLaqI4hrk_T_HJh8p3LLwgP8P5yb6HmXK8ggWe2W2-pF_XEgi6hJpgqbhZayWe__AafFF_LbxAV-B4tgU63Tjw8x2DjDunORIXAqqiMDc96Y3PUYeeW5Wy0sngWPXDNV0nz_vIcAr7i3kLtcnzaGcHqIepTpQ9-ianLA=&uniplatform=NZKPT&language=CHS

49. Zhu CZ, Sun LY, Xie WL, Yi GF. Effects of three kinds of feed additives on growth performance, digestive enzyme activity and liver and intestinal tissue structure of juvenile *Oreochromis niloticus*. *Freshwater Fisheries*. 2018;48(02):94-99. doi:10.13721/j.cnki.dsyy.2018.02.014