

Effects of a probiotic-fermented herbal medicines mixture on the growth performance, immune and antioxidative function of *Acanthopagrus schlegelii*

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Abstract

This study investigated the benefit of a probiotic-fermented herbals mixture on the growth performance, immune and antioxidative ability of *Acanthopagrus schlegelii*. The herbal medicines mixture of *Andrographis paniculata* *Sophora flavescens* *Salvia miltiorrhiza* *Hedyotis diffusa* *Gentiana scabra* and *Taraxacum mongolicum* were fermented with *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Enterococcus faecalis*. And then fed to a total of 500 *Acanthopagrus schlegelii* which were divided into 5 groups. The dietary level of each group was as follows: G0 (no additions), G1 (3‰ fermented herbals mixture), G2 (4‰ fermented herbals mixture), G3 (5‰ fermented herbals mixture), G4 (5‰ fermented herbals mixture), G5 (5‰ unfermented herbals mixture). The feeding experiment lasted for 56 days. The growth performance, immune and antioxidative ability of *Acanthopagrus schlegelii* were evaluated and results showed that fermented herbal medicines mixture(FHMM) could significantly increase the weight gain rate, acid phosphatase, and glutathione peroxidase activity of hepatic, superoxide dismutase, catalase and glutathione peroxidase activity of splenic; decrease malondialdehyde activity of hepatic, the lipase of the foregut. These results suggested that herbals being fermented with the probiotic could provide a new direction for feed supplements replace to antibiotics to enhance the immune and antioxidative ability of aquatic animal.

1. Introduction

Aquaculture plays a significant role in the global protein supply and relieves the catch pressure of aquatic natural resources(Lieke et al. 2019). However, factors such as high-density intensive fish farming, degraded water quality, bacterial infection and unreasonable management can lead to changes in the growth of fish, which have caused steep declines in production and huge economic losses in the aquaculture industry(Lee et al. 2020). Chemotherapeutic drugs such as antibiotics were normally used in the prevention and treatment of diseases in the past several years. Whereas, the use of chemotherapeutic drugs has led to various problems, including drug resistance, which may depress the immune system of the aquatic organism and residual of such substances in the final product that would be unsafe for human health(Heydari et al. 2019). Therefore, the use of antibiotics in aquaculture appears to be unsustainable. One of the best methods to counteract the disease is the use of functional feed additives with metabolism-promoting, stress-mediating, immunity-promoting, and antioxidant properties(Stratev et al. 2018).

Medicinal herbs are considered to be environmentally friendly, effective and safe additives for aquatic animals(Hai 2015). The sound effects of medicinal herbs as effective additives in aquaculture have been under evaluation in the last few years(A et al. 2016; Awad et al. 2013; Diler et al. 2017). Bioactive constituents of herbal medicines include volatile oils, phytoestrogens, triterpenoids, organic acids, saponins, phenols, flavonoids, alkaloids, waxes, glycosides and terpenoids(Shh et al. 2020). Herbal extracts can promote the fish growth by stimulating appetite, enhancing digestive enzyme activity, providing vitamins and minerals, bioactive components for animals or providing advantageous substrates for enteric microorganisms. Additionally, it has been proved that the use of herbal extract can

improve immune function in the aquatic animal by stimulating immune components and immune-related genes in humoral, cellular, gut-related and skin-mucosal(Srichaiyo et al.). Furthermore, phytochemicals of herbal extracts can restrain the oxidation system in the body by intensifying the antioxidant enzyme activities such as catalase, superoxide dismutase(Shh et al. 2020). That may help aquatic animals against oxidative stress when fighting pathogens(Atm et al. 2020).

Outside the plant cell, there is a layer of cytoderm composed of cellulose, pectin, lignin, hemicellulose and other components to protect the structural stability of cells(Burton et al. 2010). However, the existence of a cell wall makes it difficult to extract the effective components of medicinal herbs. The extraction rate of effective components of medicinal herbs will be efficiently improved by microbial fermentation. In the process of growth and reproduction of microorganisms, a variety of enzymes (such as protease, cellulase and ligninase) can be produced which can decompose cell walls. And the components of medicinal herbs will be transformed into new substances through various chemical reaction substances such as glucosylation, oxidant isomerization and esterification in process of microbial fermentation(Aisu et al. 2019). And microbial fermentation will decompose the toxic components in some medicinal herbs, then reduce the damage to edible animals. In addition, the antibacterial ability of medicinal herbs was improved after fermentation treatment(Li et al. 2020).

Andrographis paniculata is an officinal plant that was proved to have antipathogenic organisms and immunoregulatory activities(CHURIYAH et al. 2015). *Sophora flavescens* extract replacing feed antibiotics could improve the immune function and antibacterial ability of mutton sheep(Na RS et al. 2021). As the major constituent and a vital active matrix of *Hedyotis diffusa*, polysaccharides are valid immunomodulators with multifarious pharmacological activities, such as immunity-enhancing and anti-inflammatory(Rui et al.2021). Diets supplemented with *Taraxacum mongolicum* extract improved immunity responses ability, growth performance and antioxidant enzyme activity(Tan et al. 2019). *Salvia miltiorrhiza* and *Gentiana scabra* as medicinal plants have various functions, such as antioxidant, antibacterial and immunity enhancement(Han et al. 2018; Lu et al. 2021). So far, the effects of the above herbal extracts on growth and immunity in farmed aquatic animals have not been adequately scrutinized.

In this study, using *Andrographis paniculata*∥*Sophora flavescens*∥*Salvia miltiorrhiza*∥*Hedyotis diffusa*∥*Gentiana scabra*∥*Taraxacum mongolicum* as the raw material and *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Enterococcus faecalis* as the starter, herbal fermentation products used as feed additives were produced by solid fermentation. The goal of this research was to investigate the medical effects of the FHMM on antioxidant abilities, immune responses and growth performance in *Acanthopagrus schlegelii*.

2. Methods

2.1. Diet preparation and feeding trial

Juvenile *Acanthopagrus schlegelii* were purchased from a local fish farm in Nanao, Guangdong. Before the beginning of the experiment, For domestication and acclimatization of juveniles, the snapper were

given a diet with minced fillet during the first 10 d of the acclimation time, then with the diet for the control group during the second 10d of the acclimation time in the net cage(3.0m×3.0m×3.0m, L: W× H). Then, the snapper were starved for 24 h and anesthetized in diluted MS-222 at a concentration of 30 mg/L. The weighed fish were put into net cages(1m×1m×1.5m) at sea. Seawater conditions remained as follows: temperature, 21–28°C, salinity, 32‰. The experiment was performed with 500 *Acanthopagrus schlegelii*. snapper were divided into 5 groups (4 tanks per group, 25 fish per tank). All juvenile fish were fed two times per day to apparent satiation(7.30 AM and 5.30 PM). The feeding experiment lasted for eight weeks. The feed ingredients of each group are shown in Table 1. In our previous research, the production and fermentation methods of herbal medicines have been explored clearly. Briefly, *Andrographis paniculata*∥*Sophora flavescens*∥*Salvia miltiorrhiza*∥*Hedyotis diffusa*∥*Gentiana scabra*∥*Taraxacum mongolicum* were obtained from Kemu biological company in Guangzhou. The six herbs were separately dried, cut and fermented by the bacteria (*Bacillus subtilis*, *Enterococcus faecalis* and *Saccharomyces cerevisiae*)and mixed with cornmeal∥tikitiki∥honey and bran. The optimal fermentation conditions were as follows: the ratio of herbal medicines mixture to diet was 13:2, the inoculation amount was 4%, and the fermentation time was 72h.

Table 1
Formulation and nutritional ingredients of the five diets

Ingredient(dry matter %)	G0	G1	G2	G3	G4
Fish meal	53	53	53	53	53
Fermented bean flour	12	12	12	12	12
Fermented compound herbal medicines	0	0.3	0.4	0.5	0
Compound herbal medicines	0	0	0	0	0.5
astarch	3	3	3	3	3
Strong flour	18	18	18	18	18
Fish oil	6	6	6	6	6
Advice	3	2.7	2.6	2.5	2.5
Multivitamin and mineral premix ^a	3	3	3	3	3
Choline chloride	0.5	0.5	0.5	0.5	0.5
CaH ₂ PO ₄	1.5	1.5	1.5	1.5	1.5
Proximate composition (%)					
Moisture	11.23	10.98	10.79	11.17	11.10
Protein	41.13	41.11	41.20	41.12	41.11
Lipid	11.57	11.63	11.54	11.50	11.48
Ash	12.54	12.54	12.41	12.90	12.17
^a Multivitamin and mineral premix (lowest levels/kg): Fe: 8g, Mo:1g, Zn: 30g, Mg 2g, Co: 1g, I: 500mg, Se: 40mg, VA: 3×106IU, VD3: 2×106IU, Tocopherol: 59g, VK3: 6g, VB1: 6.5g, VB2: 15g, VB6: 13g, VB12: 99mg, Nicotinic acid: 90g, Pantothenic acid: 35g, Folic acid: 3g, VH: 99mg, Inositol: 99mg, Single phosphate: 200g.					

2.2. Sample collection

At the termination of the cultivation experiment, snapper were fasted for 24 h and then anesthetized in MS-222 (30 mg/L). Body length×width and weight of snapper in each net cage were determined promptly. Ten snapper were randomly selected from each cage and used for the following: two snapper for whole-body assay, three snapper viscera and liver assay, cut out the guts and liver for calculation of hepatosomatic index and viscerosomatic index. The remaining five snapper collected the venous blood from the caudal vasculature with a injector, then dissected to gather the splenic×liver and intestine samples. The blood samples were stored at room temperature for 1 h then centrifuged at 3000g, 10 min, and 4 °C to obtain the serum which was stored at ultralow temperature. Viscera were weighted

individually to calculate the HIS and VIS. The viscera samples were snap-frozen in the liquid nitrogen and finally transported to store at -80°C until enzyme analysis.

2.3. Calculations of biometric indices

Proximate composition of the diet and whole-body analysis were referred to standard methods (AOAC). Dry matter was analyzed via drying at 105°C at oven to a constant weight, crude fat analyzed by methanol: chloroform extraction, crude protein through the Kjeldahl method, and crude ash by incineration at 550°C in a muffle furnace for 12 h. Then, biometric indexes were calculated from the following formulae:

$$\text{Weight gain rate (WG)} = (W_t - W_0) / W_0$$

$$\text{Feeding rate (\%)} = 100 \times I / [t \times (W_0 + W_t) / 2]$$

$$\text{Survival rate (SUR, \%)} = \text{The last number} / \text{The initial number} \times 100$$

$$\text{Feed conversion rate (FCR)} = I / (W_t - W_0 + W_d)$$

$$\text{Specific growth rate (SGR, \%)} = [\ln(\text{The final average weight}) - \ln(\text{The initial average weight})] / t \times 100$$

$$\text{Hepatosomatic index (HSI)} = \{ \text{wet weight of liver (g)} / [\text{wet body weight (g)} - \text{wet weight of liver (g)}] \} \times 100$$

$$\text{Viscerosomatic index (VSI)} = \{ \text{wet weight of viscera and associated fat (g)} / [\text{wet body weight (g)} - \text{wet weight of viscera and associated fat (g)}] \} \times 100$$

In the above formula, W_t is the weight (g) of snapper at the end of the experiment, and W_0 is the Weight (g) of snapper at the beginning. T is the time of experiment (d), W_d is the weight (g) of the dead fish during the experiment, and I is the total feeding amount of dry matter.

2.4. Immunological and antioxidant enzymes analyses

In this study, the total cholesterol (T-CHO) content and activities of immune enzymes [alkaline phosphatase (AKP), Acid phosphatase (ACP), Lysozyme (LZM)] and antioxidant enzymes [Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), malondialdehyde (MDA)] in serum, spleen and liver were measured according to the method described by Wang et al. (Mlab et al. 2019; Wang et al. 2015) using a commercial assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

2.5. Statistical analysis

All data were conducted using the SPSS ver. 20.0 (Chicago, IL, USA), and performed with a one-way analysis of variance (ANOVA) to analysed the significant differences. Duncan's multiple range test was applied to indicate the mean values ($P < .05$). The results are expressed as mean $\pm$ S.D.

3. Results

According to Table 2, after the 56-day feeding period, compared to the other treatments, dietary supplementation of FHMM (G2) resulted in a significantly higher final weight, WG and SGR, but the VSI significantly decreased ($P < .05$). Compared with the control, the final weight, WG, SCR and VSI decreased ($P > .05$) in the *Acanthopagrus schlegelii* which fed the dietary supplementation of FHMM. There was no significant difference in FCR, SUR, HIS and body composition among all treatments.

Table 2
Growth performance in *Acanthopagrus schlegelii* fed experimental diets for 56 days.

	G0	G1	G2	G3	G4
Initial weight (g)	8.5 ± 0.06	8.5 ± 0.06	8.5 ± 0.06	8.5 ± 0.06	8.5 ± 0.06
Final weight (g)	35.24 ± 1.14 ^{ab}	38.86 ± 1.26 ^{bc}	39.98 ± 1.72 ^c	37.52 ± 0.97 ^{abc}	34.36 ± 1.49 ^a
WG(%)	3.12 ± 0.15 ^a	3.57 ± 0.15 ^{ab}	3.71 ± 0.20 ^b	3.41 ± 0.11 ^{ab}	3.07 ± 0.15 ^a
SGR(%)	2.54 ± 0.06 ^{ab}	2.71 ± 0.06 ^{bc}	2.76 ± 0.08 ^c	2.65 ± 0.05 ^{abc}	2.49 ± 0.08 ^a
FCR(%)	1.74 ± 0.24	1.89 ± 0.29	1.70 ± 0.07	1.94 ± 0.23	1.60 ± 0.18
SUR(%)	0.85 ± 0.06	0.87 ± 0.05	0.93 ± 0.03	0.86 ± 0.03	0.87 ± 0.08
HIS(%)	1.66 ± 0.07	1.46 ± 0.08	1.59 ± 0.08	1.51 ± 0.10	1.66 ± 0.16
VSI(%)	9.64 ± 0.46 ^b	8.80 ± 0.27 ^{ab}	8.29 ± 0.20 ^a	9.45 ± 0.42 ^b	8.87 ± 0.25 ^{ab}
Values are presented as the mean ± SE.(n = 4), and different superscripts are significantly ($P < 0.05$) as determined by Tukey's test.					

Serum immunological and antioxidant enzyme levels are presented in Fig. 1. As shown, dietary supplementation of FHMM significantly increased plasma SOD, GSH-Px, CAT activity in comparison to other treatments($P < .05$). There was no significant difference in ACP, AKP, MDA and LZM activity among the treatments(Table S2). SOD activity was improved with the increase in level, the highest level was related to the *Acanthopagrus schlegelii* fed a 5‰ FHMM diet. CAT and GSH-Px activity increased and then decreased with the content of FHMM, reaching the maximum at 4‰, and showed significantly higher by comparison with the control treatment($P < .05$). No difference was observed in T-CHO among control and fermented herbal treatments, supplement 5‰ unfermented herbal medicines mixture treatments resulted in significantly higher the other treatments($P < .05$).

Hepatic immunological and antioxidant enzyme levels are presented in Fig. 2. SOD, GSH-Px and ACP activity in the *Acanthopagrus schlegelii* fed the dietary supplementation of FHMM was significantly higher than the control treatment($P < .05$). There were no significant difference effects in T-CHO, AKP-CAT

and LZM activity($P > .05$) (Table S3). Hepatic MDA activity in the *Acanthopagrus schlegelii* fed the dietary supplementation of 5‰ FHMM was significantly lower than the control treatment($P < .05$), 5‰ unfermented herbal medicines mixture treatment resulted in lower the control treatment($P > .05$). ACP activity has shown significantly increased with 4‰ FHMM dietary($P < .05$), there was no difference in other treatments. All levels of the diet with FHMM significantly increased hepatic GSH-Px activity($P < .05$), control and unfermented herbal treatments were not significantly different.

Splenic immunological and antioxidant enzyme levels are presented in Fig. 1. As shown, dietary supplementation with FHMM resulted in significantly lower AKP and MDA activity and showed significantly higher GSH-Px activity compared to the control treatment($P < .05$). No significant difference in CAT activity was observed (Table S4). Dietary with unfermented herbal medicines mixture significantly increased splenic AKP activity, in comparison to the control treatment($P < .05$), there was no difference in GSH-Px, MDA and CAT activity.

As shown in Fig. 3, dietary supplementation with 3‰ and 4‰ FHMM resulted in significantly lower lipase in the foregut($P < .05$), and there was no difference in other treatments.

4. Discussion

4.1. Growth performance

To date, several researches were conducted to evaluate the positive effects of herbal medicines on the growth of fish. However, fermented herbal additives are rarely paid attention to. In this study, the final weight, WG and SGR of *Acanthopagrus schlegelii* fed the dietary with 4‰ FHMM was higher than in the control group. Similar to the present study, olive flounder showed significant weight gain after administration with *S. parauberis* and herbals mixture supplementation diet(Harikrishnan et al. 2011). Rui observed that *Hedyotis diffusa* polysaccharides showed the positive effects of improving growth performance in animals(Rui et al. 2021). The *Astragalus memranaceus* and *Glycyrrhiza uralensis* mixture fermented by *Bacillus subtilis* was used as feed additives, and the growth performance of carp was significantly improved(Zhao et al. 2017). But in the current study, no significant difference in the growth target among unfermented herbal treatment and the control group. According to the report, as probiotic bacteria, the bacillus was widely used in aquaculture for the enhancement of growth and the immune system(Kumar et al. 2010). Moreover, a previous study has shown that mixtures of herbals and probiotics can improve the growth performance of animals by enhancing digestibility(Symonds et al. 2012). Dietary supplementation with FHMM resulted in a lower HIS rate compared to the other treatments, and the emergence of herbal supplementation reduces the VSI rate. Similar results were observed by thyme, rosemary, and fenugreek supplementation in the feed of sea bass(Yilmaz et al. 2013), and the use of *Greek oregano Origanum heracleoticum* essential oil decreased the HIS and VSI in *Channel Catfish Ictalurus*(Zheng et al. 2009). The standpoint that an increase of dietary lipid levels makes for an enhanced liver and muscles deposited in carcass has been extensively studied(Jiang et al. 2015; Wang et al. 2005). And some researchers propose that VSI values were increased with the increase in dietary lipid

levels(Saoud 2011). Our study presumably that herbal supplementation improves lipid utilization in *Acanthopagrus schlegelii*. However, the specific mechanism needs to be further studied.

4.2. Effects of dietary FHMM on immune and antioxidant function

The addition of herb medicine in fish diets is a significant way to enhance the fish's antioxidant ability and immune systems(Dawood et al. 2018; Rufchaei et al. 2020). Nine enzymes related to immune and antioxidant capacity were analyzed in this experiment. AKP is a significant regulatory enzyme and is involved in many important functions of animals(Bo et al. 2010). They are often used as indicators of phagocytosis of macrophages(Li et al. 2018). As an important constituent of the nonspecific immune system, LZM is widely present in the blood serum and mucosa of fish that not only cytotoxic bacteria but also activates sediments and phagocytes(Yuan et al. 2007). In this study, no effects were observed among the group of fish feed supplement herbal medicines mixture in LZM activity. As a marker enzyme of macrophage phagocytosis, ACP involves in bacteriolysis by triggering the phagocytes at the infection site, enhancing the phagocytosis and response of immune cells(Bahi et al. 2017). Adding fermented or unfermented herbals to the diet increases the ACP activity of the liver owing to the *Hedyotis diffusa* as a component of herbal additive may have a protective effect on the liver. Components of *Hedyotis diffusa* including polysaccharides, flavonoids, anthraquinones and terpenes. As the major active substance, polysaccharides are effective immunomodulators(Rui et al. 2021).

Environmental stress circumstances can cause the immoderate improvement of reactive oxygen species (ROS) resulting in oxidative injuries. SOD, CAT, and GSH-Px as endogenous antioxidant enzymes played a critical role in removing harmful reactive oxygen derivatives, which were used to evaluate non-specific immune responses(A et al. 2013). Thus, improvements in the activity of these enzymes reveal stimulation of antioxidant defense. In this study, the presence of herbal in dietary increased SOD, GSH-Px ,CAT in serum and SOD in hepatic, GSH-Px activity in splenic. Should not be ignored that dietary administration of fermented herbals showed more significant effects. Moreover, FHMM additives improve GSH-Px activity in liver, but unfermented herbal medicines mixture additives showed the opposite effect. It has been proved that most herbal active ingredients are antioxidant substances that may help animals against oxidative stress during fighting pathogens(Atm et al. 2020). Many studies have proved that *Hedyotis diffusa* polysaccharides had an effective prohibitive influence on lipid peroxidation and improve the activities of antioxidant enzymes(Wang et al. 2018), such as significantly increasing the content of SOD, decreasing the concentration of MDA in the serum of mice(Ming 2014). *Andrographis paniculata* has antioxidant and stimulate the immune functions showed improvement of T-AOC, SOD, GSH-Px activity and reduction of MDA in chook, mice and ricefield eel(Deng et al. 2015; He et al. 2019; Shi et al. 2020). Moreover, dietary supplement of *Taraxacum mongolicum*(Yu et al. 2022) polysaccharide efficiency improved antioxidant and immune response of Jian carp. *Gentiana scabra* significantly accelerate lymphocyte proliferation, showed higher antioxidant resistance and free radical scavenging ability(Wang et al. 2014). Moreover, flavonoids from *Sophora flavescens*(Zhou et al. 2019) and polysaccharides from *Salvia miltiorrhiza*(Jiang et al. 2020) have been shown to have antioxidant effects.

In accordance with the obtained phenomena, previous studies indicated that probiotic use in aquaculture has been diminishing stress indices in fish(Gomes et al. 2008). *Clostridium butyricum* have been proved to improve antioxidant enzymes activities of *Marsupenaeus japonicas*(Duan et al. 2017) and *Yellow perch* in stressful condition(Elabd et al. 2017). Herbal-probiotic combinations could product a good balance of the synergies and improve the activity of antioxidant enzymes in Nile tilapia(D et al.). Flavonoids from *Sophora flavescens* and polysaccharides from *Salvia miltiorrhiza* have been reported to have antioxidant effects. We hypothesized that some antioxidant substances in herbal were absorbed easily by fish and stimulate antioxidant enzymes after probiotic fermentation.

MDA, the product of lipid peroxidation and a biomarker of oxidative damage(Li et al. 2018), has strong biotoxicity and can damage the structure and function of cells(Giri et al. 2017). A decreased MDA concentration usually indicates enhanced enzymatic antioxidant activities of the defence system. *Andrographis paniculata*(Wang et al. 2018) and *Hedyotis diffusa*(Ming 2014) polysaccharide have been shown to reduce the activity of MDA in animals. The current results show that dietary supplementation of FHMM was capable with diminishing oxidative conditions in hepatic and splenic of *Acanthopagrus schlegelii*, which suppressed oxidative stress(Abdel-Latif et al. 2020).In addition, lower MDA levels might by reason of higher antioxidant enzymes activity (such as SOD in this study) that neutralize pro-oxidant compounds(My et al. 2020).

Dietary administration of 3‰ and 4‰ FHMM decreased the lipase of foregut. Lipase is secreted from pancreas and play vital roles in fat digestion in the intestine. Some lipases could even adjust endocannabinoid, eicosanoid signalings and PPAR α in intestine(Cao et al. 2013; Obrowsky et al. 2013). So, the activity of lipase is one of the important parameters to affect systemic lipid metabolism in the whole body, and could be a breakpoint in research of adjusting systemic lipid metabolism in fish(Zhang et al. 2019). Increasing studies show that consumption of high-fat diet results in microbiota dysbiosis and inflammatory response in intestine of human and animals(Li et al. 2019). One report has it that inhibition of intestinal lipase could adjust lipid metabolism and sustain energy homoeostasis to a certain degree by decreasing lipid absorption. For example, inhibition of intestinal lipases could improve the immune function of serum and liver in Nile tilapia feeding high fat dietary(Zhang et al. 2019). Reports even claims that herbals represent a rich provenience of anti-lipase compounds(Zheng et al. 2010). Nevertheless, the inhibition of intestinal lipases would cause harmful effects in healthy fish. Therefore, further studies are needed to evaluate the mutual effect between intestinal lipases and health of aquatic animal under different nutritional or physiological situations.

4.3. Effects of herbal medicines mixture by probiotic fermentation

Mixtures of herbal formulas are not just an accumulation of the herbals, but with many synergies among the herbs which have better effect on immune stimulation or enhancement on disease resistance of fish(Choi et al. 2014). It has been proved by many studies that probiotics bacteria were improving the growth, immunological enzymes activity and inhibition of pathogenic bacteria. In addition, probiotics and

herbals mixture supplementation dietary may synergistic better impact for growth, disease resistance and immune system(Harikrishnan et al. 2011; Irianto and Austin 2010). Dietary supplementation of FHMM has a better improvement effect on the growth, immunity and antioxidant function, especially the addition of 4‰ concentration in this study. Herbal fermentation is a process of using microbial enzymes to transform the gratuitous substrates into compatible components, thereby enhancing the substrate properties through the production and accumulation of bioactive compounds(Hussain et al. 2016). Fermentation has been proved to improve bioactivity of many herbals via increasing the content of phenolic compounds and flavonoids(Hur et al. 2014). And fermented herbal also enhanced the host immunity by promoting the diversity of beneficial bacteria(Zhao et al. 2018). Thus, is not hard to understand that supplement of the fermented herbal to aged rats(Ramesh et al. 2012), *Litopenaeus vannamei* (Wang et al. 2022)and chicks(Wang et al. 2021) results in improved growth performance, immune response and activities of antioxidant which similar to currently results.

5. Conclusions

To sum up, after 56 d feeding experiment, fermented herbal is added to the dietary, significantly improve the growth performance and antioxidative ability of *Acanthopagrus schlegelii*, and decrease the lipase of foregut. The result provides direction for additive of fermented herbal additives, as to strengthen immune and antioxidative ability of aquatic animal.

Declarations

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Ethical Approval and Consent to participate Not applicable.

Human and Animal Ethics Not applicable.

Consent for publication All the authors have given the consent for publication of the study.

Availability of supporting data All the required data are available in the manuscript.

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Authors' contributions NIE and LIANG wrote the main manuscript text and work together on the experiment. Xie provided ideas, funding and a place for the research. All authors reviewed the manuscript.

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Figures

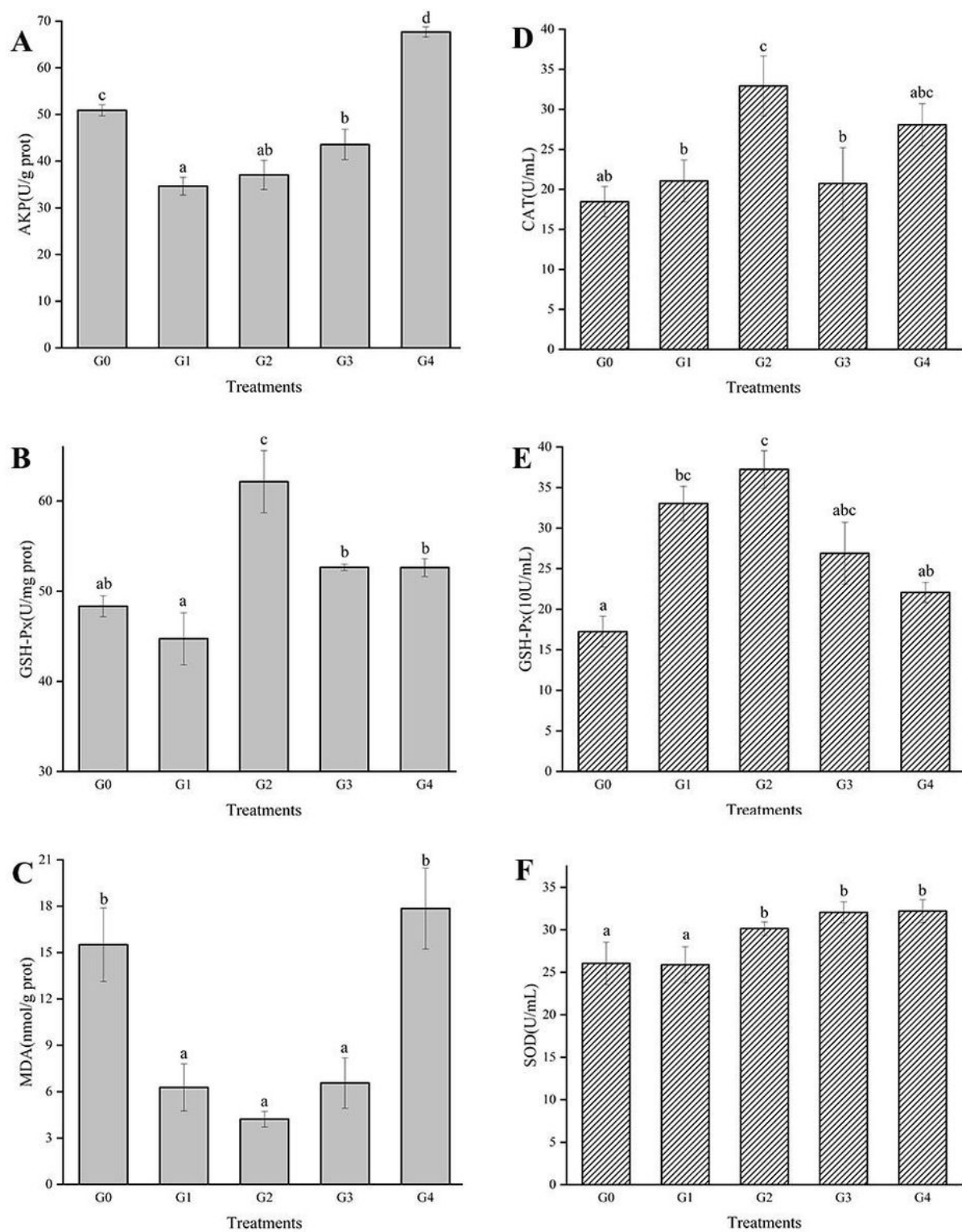


Figure 1

Parameters in serum(right) and spleen(left) (AKP (A), GSH-Px (B), MDA (C), CAT (D), GSH-Px (E), and SOD (F)) of *Acanthopagrus schlegelii* after dietary different concentrations of herbal mixtures. Data are shown as mean S.D. (n = 4). Different superscripts show significant differences among the groups (Duncan test).

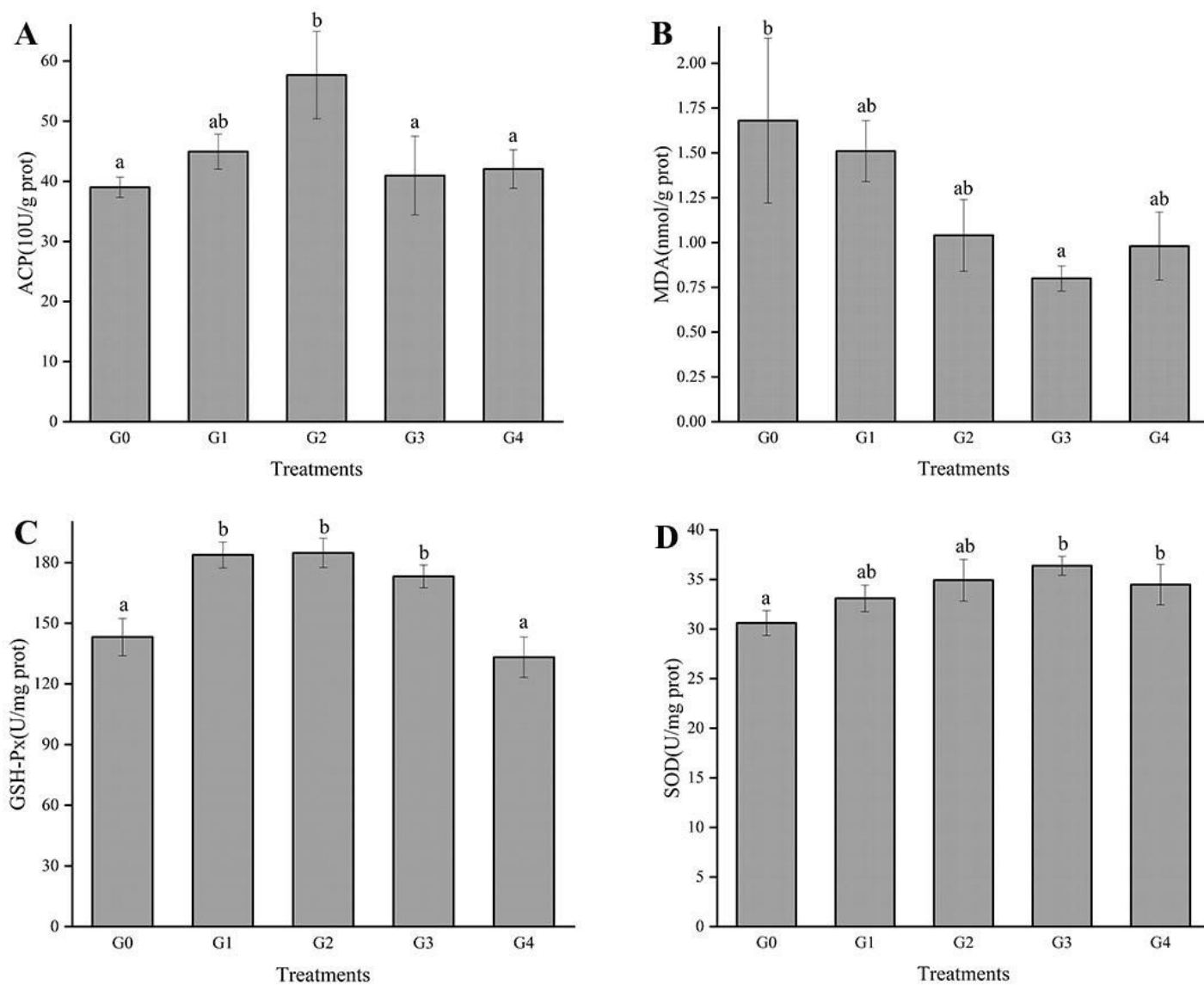


Figure 2

Parameters in the liver (ACP (A), MDA (B), GSH-Px (C), and SOD (D)) of *Acanthopagrus schlegelii* after dietary different concentrations of herbal mixtures. Data are shown as mean S.D. (n = 4). Different superscripts show significant differences among the groups (Duncan test).

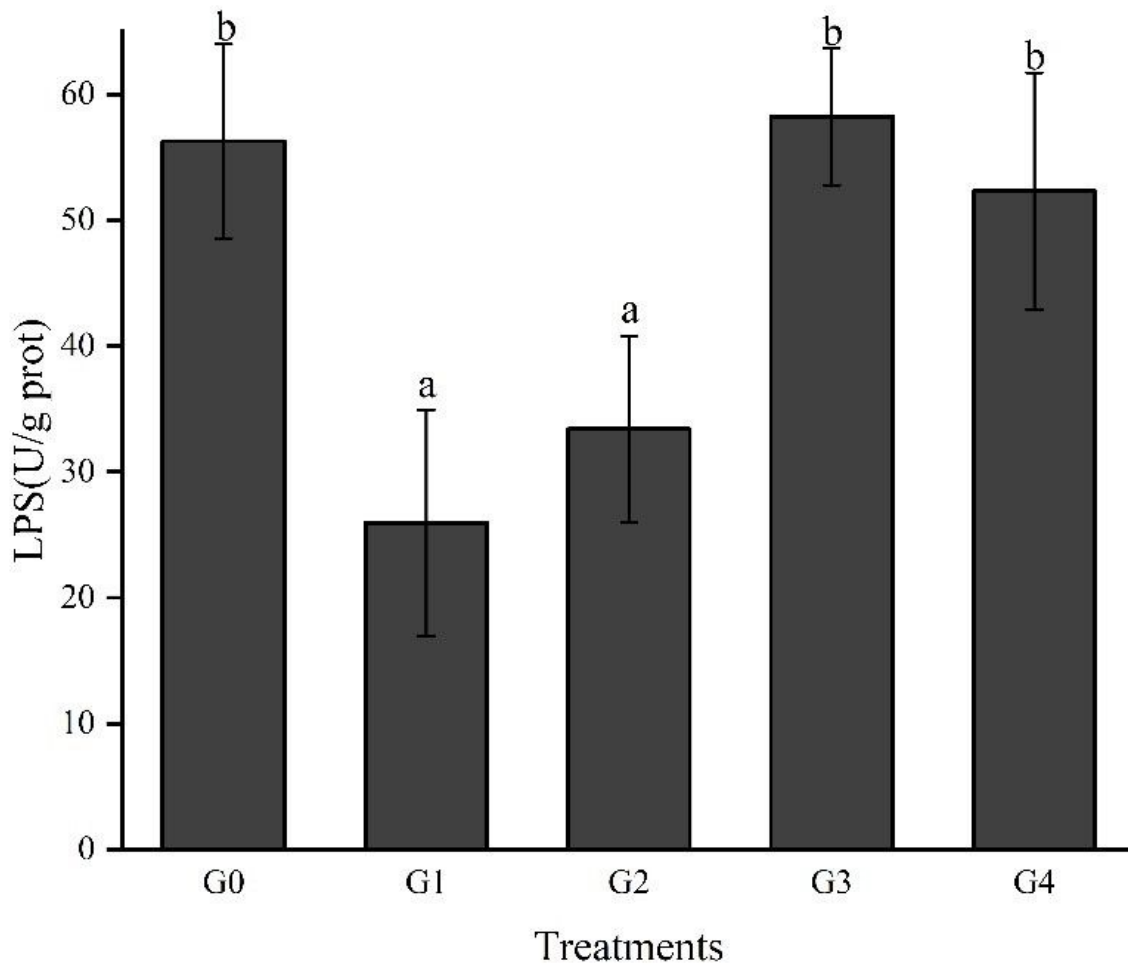


Figure 3

Effects of herbal mixtures on the activities of lipase in the foregut of *Acanthopagrus schlegelii*. Different superscripts show significant differences among the groups (Duncan test).

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