

# Intervention Strategies of *Magnolia officinalis* Extract on Sturgeon: Growth Performance, Intestinal Health and Resistance to *Aeromonas hydrophila* Infection

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## Research Article

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# Abstract

This study aims to investigate the effects of *Magnolia officinalis* extract (MOE) on the growth, immunity and disease resistance of sturgeon. 360 juvenile *Hybrid Sturgeon* were selected and divided into a four experimental groups (0, 100, 300 and 500 mg/kg MOE), with 3 replicates in each group. After 8 weeks of cultivation, growth performance was measured. Subsequently, 10 tail fish from each group were subjected to a 7-day pathogen challenge test by intraperitoneal injection (0.2 mL of  $3.30 \times 10^7$  CFU/mL) of the bacterial solution.

Specifically, MOE improved growth performance and body composition in sturgeon ( $P < 0.05$ ). Compared with the HP0 group, MOE supplementation significantly enhanced intestinal antioxidant enzyme and digestive enzyme activities while significantly reducing malondialdehyde (MDA) levels ( $P < 0.05$ ). Regarding intestinal immunity, MOE upregulated anti-inflammatory cytokines TGF- $\beta$ 1 and IL-10, as well as non-specific immune enzymes ACP, AKP, and LZM, while suppressing the expression of pro-inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$ , and IL-8 ( $P < 0.05$ ). Furthermore, MOE significantly increased villus height and width in the duodenum and spiral valve segments, upregulated the relative expression of tight junction proteins Claudin, Occludin, and ZO-1, and elevated the abundance of beneficial gut bacteria *Hafnia* ( $P < 0.05$ ). Infection experiments demonstrated that MOE effectively improved sturgeon survival rate, alleviated intestinal pathological damages such as congestion, hemorrhage, and inflammatory cell infiltration, and enhanced immune function and intestinal barrier integrity. In summary, MOE enhances growth performance, antioxidant capacity, and intestinal health in sturgeon, and improves resistance to *Aeromonas hydrophila* infection.

## 1. Introduction

Sturgeon is a high-end specialty aquatic product breeding variety in China and a core raw material for caviar. The domestic production of sturgeon ranks first globally, accounting for over 70% of the world's total output (Liu et al., 2020). As of 2023, the national sturgeon production was approximately 150,000 tons (Gu et al., 2025). However, with the rapid popularization of intensive and high-density breeding models, the load of the breeding water body has intensified, and fish stress occurs frequently, leading to large-scale outbreaks of opportunistic pathogenic bacteria such as *Aeromonas hydrophila*, causing intestinal lesions, weakened immunity and massive deaths in sturgeons, which has become a core bottleneck restricting the high-quality development of the sturgeon breeding industry (Shen et al., 2014). Currently, in aquatic farming, antibiotics are still relied upon for bacterial disease control (Sarkar and Rashid, 2012; Shao et al., 2021). However, long-term abuse can easily lead to the development of antibiotic resistance in pathogenic bacteria, drug residues in aquatic products and imbalance of the micro-ecology of water bodies, etc., and there is an urgent need to develop safe and efficient new green feed additives to replace traditional antibacterial drugs.

The main bioactive components of *Magnolia officinalis* extract (MOE) are magnolol and honokiol, which exhibit multiple biological activities including antioxidant, anti-inflammatory, antibacterial, and gut

microbiota-regulating effects, showing promising application prospects in livestock and poultry farming (Sarrica et al., 2018; Lv et al., 2024). Previous livestock studies have verified that dietary MOE supplementation effectively improves growth performance and feed utilization efficiency, as well as intestinal physiological functions in pigs and poultry (Oh et al., 2018; Lin et al., 2021; Zhang et al., 2025). In meat ducks, MOE also optimizes systemic antioxidant status and intestinal mucosal morphology to facilitate growth performance (Lin et al., 2017). Aquatic studies have demonstrated that MOE markedly enhances growth performance and feed efficiency, strengthens resistance against *Aeromonas hydrophila*, and elevates post-challenge survival rate in tilapia (Han et al., 2022). Appropriate dietary magnolol supplementation significantly boosts resistance to *Nocardia* infection and survival rate of largemouth bass (Xin et al., 2022). Mechanistic investigations reveal that MOE suppresses the expression of pro-inflammatory cytokines IL-6, TNF- $\alpha$  and IL-1 $\beta$  via regulating NF- $\kappa$ B and MAPK signaling pathways (Guo et al., 2018), and optimal doses of magnolia bioactive compounds remarkably enhance fish immune responses (Huang et al., 2022). In addition, magnolol participates in the metabolic regulation of intestinal microbiota, and there exists a bidirectional interaction between magnolol and intestinal microbial communities (Rempel et al., 2012).

The intestine serves as a vital organ for nutrient digestion, antioxidant defense and immune regulation in fish. Intestinal injury and microbiota dysbiosis are major contributors to retarded growth and weakened disease resistance of sturgeon under intensive culture conditions. Accordingly, improving intestinal health through nutritional manipulation is an effective strategy to promote sturgeon growth and reduce disease incidence. As a promising green feed additive, MOE possesses potent antioxidant, anti-inflammatory, antibacterial and gut flora-modulating properties. Nevertheless, systematic research regarding the regulatory effects and underlying mechanisms of MOE on sturgeon growth, intestinal homeostasis and resistance to *Aeromonas hydrophila* infection remains limited. Therefore, this study systematically explores the ameliorative effects of MOE on growth and disease resistance of sturgeon, so as to provide theoretical support for the popularization and application of MOE in eco-friendly sturgeon aquaculture.

## 2. Materials and methods

### 2.1 Experimental animals and drugs

The experiment selected *Hybrid Sturgeon* with a weight of  $6 \pm 1.5$ g, which was purchased from Beichuan Qiangjiang Family Sturgeon Fish Farm Co., Ltd. (Mianyang, China). The Magnolia extract was purchased from Sichuan Hengrui Tongda Biotechnology Co., Ltd. (China) (purity  $\geq 90\%$ , with magnolol for  $\geq 57.13\%$  and Magnolyl  $\geq 38.3\%$ ).

### 2.2 Growth trial design

The experiment consisted of a control group (HP0) fed a basal diet, and three treatment groups fed the basal diet supplemented with 100, 300 and 500 mg/kg of MOE, designated as HP100, HP300, and HP500, respectively. Prior to the formal trial, juvenile fish were acclimated for two weeks to eliminate

stress and adapt to the rearing conditions. A total of 360 healthy juvenile sturgeon were randomly divided into four groups, each with three replicates of 30 fish per replicate. The feeding trial lasted for 8 weeks. During the experiment, fish were fed twice daily (8:30 and 18:00), and feces were removed before each feeding. The daily feeding rate was 3% of body weight, and any uneaten feed was removed 30 min after feeding. One-third of the water was replaced every two days. Water temperature was maintained at  $18 \pm 1^\circ\text{C}$ , dissolved oxygen at  $\geq 6.0$  mg/L, ammonia nitrogen at  $\leq 0.02$  mg/L, and nitrite at  $\leq 0.03$  mg/L. The rearing tanks measured 1 m  $\times$  0.5 m  $\times$  1 m. The composition of the basal diet is presented in Supplementary Table 1. At the end of the trial, all fish were fasted for 24h, then intestinal contents, intestinal tissue, liver, and blood samples were collected for subsequent analyses.

## 2.3 Disease resistance test

The bacterial solution was diluted by a 2-fold gradient 5 times (mixing 5 mL of bacterial solution with 5 mL of physiological saline), and then 100  $\mu\text{L}$  of each concentration of bacterial solution was taken and spread on the plates. The plates were cultured at a constant temperature of  $28^\circ\text{C}$  for 48 hours, and the number of colonies was counted. Meanwhile, the  $\text{OD}_{600}$  value was measured (Supplementary Table 2). The relationship between the  $\text{OD}_{600}$  value and the number of colonies is shown in Fig. 1 in the attachment. The experiment was set up with 5 concentrations ( $9.97 \times 10^7$ ,  $5.84 \times 10^7$ ,  $3.30 \times 10^7$ ,  $1.23 \times 10^7$ ,  $5.45 \times 10^6$  CFU/mL) based on the measured number of colonies, and a 96h acute challenge test was conducted. The mortality situation was recorded. Based on the test results, the half-lethal concentration of *Aeromonas hydrophila* challenge to sturgeon was calculated as  $3.30 \times 10^7$  (CFU/mL). After the 8-week growth test, an *Aeromonas hydrophila* infection test was conducted for each group. According to the test method of Huang (Huang et al., 2022), 10 fish were randomly selected from each group (three replicates), a total of 120 fish and 0.2 mL of the *Aeromonas hydrophila* bacterial solution with a concentration of  $3.30 \times 10^7$  (CFU/mL) was intraperitoneally injected. The infection lasted for 7 days. After the test, intestinal, liver and blood samples were collected for subsequent tests.

## 2.4 Growth performance measurement

During the experiment, the feeding volume and leftover food volume of the fish were recorded every day. After the 8th week, the fish were fasted for 24h, and their body weight and body length were measured. The weight of internal organs was also measured and recorded. Based on the feeding volume, the following indicators were calculated (Huang et al., 2022).

Growth Rate (WGR) (%) = (Final Weight - Initial Weight) / Initial Weight  $\times$  100

Specific Growth Rate (SGR) (%) = (ln Final Weight - ln Initial Weight) / Number of Cultivation Days

Feed Conversion Ratio (FCR) (%) = Feed Consumption (g) / (Final Weight - Initial Weight)

Fat Content (CF) (g/cm<sup>3</sup>) = Final Weight / Body Length<sup>3</sup>

Liver-Body Ratio (HSI) (%) = (Liver Weight / Final Weight)  $\times$  100

Viscera-Body Ratio (VSI) (%) = (Viscera Weight / Final Weight) × 100

## 2.5 Body composition measurement

The direct drying method (GB5009.3-2016) was used to dry the samples in a 105°C oven until constant weight was achieved, and the moisture content of the samples was determined. Using petroleum ether as the extractant, according to the provisions of "Food Safety National Standard - Determination of Fat in Food" (GB 5009.6–2016), the crude fat content of the fish body was determined using the Soxhlet extraction method. Crude protein was determined according to the method specified in "Food Safety National Standard - Determination of Protein in Food" (GB 5009.5–2016). Crude ash was determined according to (GB 5009.4–2016), and the crude ash content was determined by heating the sample in a muffle furnace at 550°C for 6 hours.

## 2.6 Biochemical index measurement

The serum and intestinal tissue weights of *Hybrid Sturgeon* were weighed. 1:9 mass-volume ratio was added with normal saline, and the tissues were homogenized. The supernatant was obtained after centrifugation at 2500 rpm for 10 minutes at 4°C in a refrigerated centrifuge. The activities of pancreatic protease, amylase, lipase, lysozyme (LZM), acid phosphatase (ACP), alkaline phosphatase (AKP), total antioxidant capacity (T-AOC), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) in the intestinal tissue were determined according to the instructions of the biological reagent kits provided by Nanjing Jiancheng Biological Company.

## 2.7 Intestinal tissue sections

The collected liver and intestinal tissues were immersed in 4% paraformaldehyde fixative for preservation. The intestinal and liver tissue sections were prepared using the paraffin embedding technique. First, gradient dehydration was performed using ethanol concentrations (75%, 85%, 95%, 100%, 100%) and then transparented with xylene (100%). The samples were then immersed in paraffin and embedded. After cooling and demolding, the paraffin-embedded tissues were placed in a 4°C refrigerator for storage. The paraffin-embedded tissues were cut into 5 µm thick sections using a microtome and placed in a water bath at 42°C for spreading. The tissue was picked up from the water bath using a carrier slide, slightly dried to remove moisture, and then placed in a 60°C oven for 1 hour. The sections were deparaffinized with xylene, rehydrated with ethanol concentrations (75%, 85%, 95%, 100%), and then transparented with xylene. Finally, they were sealed with neutral gum. The intestinal villus height (VH), intestinal villus width (VW), and intestinal mucosa thickness (MT) were measured using ImageJ software.

## 2.8 Clinical symptom observation and cumulative mortality rate in the infection test

After the infection test, the clinical symptoms of each group of *Hybrid Sturgeon* were observed, the clinical symptoms of *Hybrid Sturgeon* were recorded, and the death situations of each group were statistically analyzed. When the color of *Hybrid Sturgeon* became significantly lighter or turned pale gray,

swam slowly and closely attached to the bottom of the water, and had no obvious movement of the gills, etc., typical symptoms, it was determined as a dying fish and selected out. While *Hybrid Sturgeon* with stable swimming and normal body color were defined as surviving fish. The entire observation process lasted for 7 days, during which the established operation procedures and observation standards were strictly followed to ensure the accuracy and reliability of the data. After the observation, the cumulative mortality rate of each group was calculated based on the recorded death numbers. This was used as an important indicator to evaluate the impact of different treatment factors on the survival status of *Hybrid Sturgeon*.

Cumulative mortality rate (%) = (cumulative death number / initial number) × 100%

## 2.9 Gut microbiome sequencing

The intestinal contents were sent to Shanghai Baiqu Technology Co., Ltd. for sequencing. Using 16S rDNA high-throughput sequencing technology, the total microbial DNA of colonic chyme was extracted using the QIAamp Fast DNA Stool Micro Kit (Qiagen, Germany), and the operation was carried out strictly in accordance with the instructions of the kit. The amplification reaction of the 16S rDNA V3-V4 hypervariable region was performed on the ABI Gene Amp 9700 PCR thermal cycler (Applied Biosystems, USA), and the universal primers used were 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). After DNA amplification, the purity and quality of the extracted DNA were detected using 1% agarose gel electrophoresis (Pang et al., 2022). The purified PCR products were concentrated and normalized, and then sequenced in both ends using the Illumina Miseq sequencing platform (Illumina, USA). The original sequencing sequences were analyzed using the microbial ecology quantitative analysis software (version 1.9) (Pang et al., 2021). Statistical and visualization analysis were performed using RStudio software and Graphpad Prism 9.5 software.

## 2.10 Quantitative real-time PCR (qRT-PCR) analysis

The intestinal barrier genes (ZO-1, Occludin, Claudin) and immune genes (IL-1 $\beta$ , IL-8, IL-10, TNF- $\alpha$ , TGF- $\beta$ 1) in the tissues were examined using PCR technology. 100 mg of intestinal tissue samples were taken and placed in enzyme-free EP tubes. Total RNA was extracted using RNA Trizol reagent (TaKaRa, Japan). The quality and concentration of total RNA were determined using a spectrophotometer, with the A260/A280 ratio ranging from 1.8 to 2.0. The reverse transcription experiment was carried out according to the manufacturer's instructions, and the cDNA template obtained after reverse transcription was detected using a fluorescent quantitative kit. The primers were from the NCBI database and synthesized by Chengdu Qingke Biotechnology Co., Ltd. The primers are shown in Supplementary Table 3 of the information attachment.  $\beta$ -actin was used as the internal reference gene, and the mRNA relative level was calculated using the  $2^{-\Delta\Delta C_t}$  method (Rao et al., 2013).

## 2.11 Statistical methods

The data were tested for normality and homogeneity of variance using Excel. Statistical differences were evaluated using SPSS 27.0 software through one-way ANOVA and t-tests. Each group of experiments

was repeated three times, and the data results were expressed as the mean  $\pm$  standard error. GraphPad Prism 9.5 was used for plotting. The same letters indicated no significant difference ( $P > 0.05$ ), while different letters indicated significant differences ( $P < 0.05$ ).

## 3. Results

### 3.1 MOE improves the growth conditions of *Hybrid Sturgeon*

The experimental design is shown in Fig. 1A. Regarding the growth performance, MOE significantly increased the WGR and SGR in the HP300 and HP500 groups, and decreased the FCR ( $p < 0.05$ ). Compared with the HP0 group, no significant differences were observed in HSI, CF and VSI among all groups ( $p < 0.05$ ) (Supplementary Table 4). In terms of body composition, there were no significant differences in the crude ash content among the groups ( $p > 0.05$ ) (Fig. 1B). Compared with the HP0 group, the crude fat content of the HP100 and HP500 groups significantly increased ( $p < 0.05$ ) (Fig. 1C). The crude protein content significantly increased in the HP500 group ( $p < 0.05$ ) (Fig. 1D). The moisture content in the HP100 group significantly increased ( $p < 0.05$ ) (Fig. 1E). In addition, it was found that the trypsin, lipase, and amylase activities of the experimental groups all significantly increased ( $p < 0.05$ ) (Fig. 1F-H). The trypsin activity reached the highest level in the HP300 group, followed by the HP500 group and the HP0 group. The lipase activity was significantly higher in the HP300 and HP500 groups than in the HP0 group, and the amylase activity was the highest in the HP300 group. PCA and correlation analysis revealed that MOE significantly altered the body composition of sturgeon (Fig. 1I-J).

### 3.2 MOE enhances the intestinal function of *Hybrid Sturgeon*

As shown in Supplementary Fig. 2, MOE reduced intestinal MDA content and increased the levels of T-AOC, SOD, CAT, and GSH-PX ( $p < 0.05$ ) (Supplementary Fig. 2A-E). Regarding intestinal barrier function, MOE was found to upregulate the expression of intestinal *ZO-1*, *Claudin* and *Occludin* ( $p < 0.05$ ) (Fig. 2A-C). In terms of duodenal intestinal structure, MOE significantly increased VH, MT, and VW in the HP300 and HP500 groups ( $p < 0.05$ ) (Fig. 2D and Supplementary Table 5). Concerning intestinal non-specific immunity, MOE enhanced the activities of the non-specific immune enzymes ACP, AKP, and LZM in the intestinal tract of the experimental groups ( $p < 0.05$ ) (Fig. 2E-G). With respect to immune and inflammatory factors, MOE inhibited the expression of the intestinal inflammatory genes *TNF- $\alpha$* , *IL-1 $\beta$*  and *IL-8* ( $p < 0.05$ ) (Fig. 2H-J). Meanwhile, MOE increased the relative gene expression of *TGF- $\beta$ 1* and *IL-10* in the HP300 and HP500 groups ( $p < 0.05$ ) (Fig. 2K-L).

### 3.3 MOE regulates the dominant gut microbiota

In this study, the HP0 and the HP500 group were selected for gut microbiota analysis based on growth performance results. The HP500 group exhibited the best WGR and SGR, representing the maximum response level to the growth-promoting effect of MOE. Therefore, 16S rDNA sequencing was performed

on the intestinal contents of the HP0 and HP500 groups. As shown in Supplementary Fig. 3, a total of 967 ASVs were detected across the two groups, including 750 unique ASVs in the HP0 group, 148 unique ASVs in the HP500 group, and 69 core ASVs shared by both groups, indicating that the number of bacterial taxa was higher in the HP0 group than in the HP500 group (Supplementary Fig. 3A). The Chao1 and Shannon indices decreased in the HP500 group, with no significant difference between the HP0 and HP500 groups (Supplementary Fig. 3B–C). PCoA analysis showed that the contribution of the PCoA1 axis was 21.46%, that of the PCoA2 axis was 11.70%, and the difference between the two principal coordinates was 9.76% (Fig. 3A), indicating that MOE induced alterations in the gut microbiota composition. Furthermore, MOE resulted in significant changes in seven bacterial phyla/genera in the intestine (Fig. 3B). Among these phyla and genera, MOE increased the relative abundance of p\_Firmicutes\_A by 5.1% and decreased that of p\_Firmicutes\_D by 4.8%. Firmicutes and Proteobacteria were the dominant phyla at this level (Supplementary Fig. 3D). Meanwhile, MOE feeding increased the relative abundances of *g\_Clostridium\_T* and *g\_Hafnia* by 17.8% and 12.4%, respectively (Supplementary Fig. 3E). Moreover, after MOE feeding, *g\_Clostridium\_T* and *g\_Hafnia* became the dominant genera at this level, indicating that MOE increased the abundance of dominant bacterial taxa in the gut of *Hybrid sturgeon*.

LEfse analysis revealed that, after MOE feeding, *g\_Hafnia* was the biomarker genus in the intestine of hybrid sturgeon (Fig. 3C). Correlation analysis showed a negative correlation between *g\_Hafnia* and *g\_Bifidobacterium\_388775* (Fig. 3D). Subsequently, KEGG metabolic function prediction analysis of the gut microbiota indicated that MOE primarily affected the metabolic functions of the gut microbiota in hybrid sturgeon, with carbohydrate metabolism, amino acid metabolism, metabolism of cofactors and vitamins, energy metabolism, nucleotide metabolism, lipid metabolism, and glycan biosynthesis and metabolism being the major pathways involved (Fig. 3E). These results suggest that dietary MOE enhances the intestinal digestion and absorption capacity of *Hybrid sturgeon*.

### **3.4 MOE construction and evaluation of the *Hybrid Sturgeon* model for resistance to *Aeromonas hydrophila* infection**

The experimental design is shown in Fig. 5A. After being infected with *Aeromonas hydrophila*, the cumulative mortality rate of the final control group was 53.33%, that of the HP100 group was 43.33%, that of the HP300 group was 23.33%, and that of the HP500 group was 26.67% (Fig. 4B). After intraperitoneal injection of *Aeromonas hydrophila* into *Hybrid Sturgeon*, the clinical symptoms that appeared within one week are shown in Fig. 5C. The diseased *Hybrid Sturgeon* showed decreased vitality, rapid surface movement accompanied by head and body surfacing; externally, there was bruising around the mouth, redness of the anus; dissection revealed congestion and redness of the intestines, and small bleeding points scattered on the liver. In addition, the spiral valve intestines of *Hybrid Sturgeon* infected with *Aeromonas hydrophila* showed obvious damage (Fig. 4D). Deeply stained small round cell nuclei were observed in the intestinal mucosa layer and lamina propria, with obvious inflammatory cell infiltration, and bleeding points appeared; the cell arrangement was loose, the intercellular space was

enlarged, and the cell boundaries were not obvious; some villi were broken, atrophied, and epithelial cells fell off. With the increase in the addition level of MOE, the degree of intestinal villus damage in the infection group gradually decreased, and the intestinal damage and inflammatory symptoms were less in the HP300 and HP500 infection groups (Fig. 4D). Meanwhile, after being infected with *Aeromonas hydrophila*, the liver cells of the HP100 infection group showed liver parenchymal hemorrhage, a large number of red blood cells infiltration, and an enlarged cell gap (Fig. 4E). The liver cells of the HP300 infection group had unclear boundaries, and there was a small amount of inflammatory infiltration phenomenon. The central vein of the liver of the HP500 infection group had a large number of red blood cells, and the liver parenchyma was infiltrated with red blood cells.

### **3.5 MOE improves intestinal function following *Aeromonas hydrophila* infection.**

The intestinal immune changes following *Aeromonas hydrophila* infection are shown in Fig. 5. After *Aeromonas hydrophila* infection, the relative expression level of the anti-inflammatory factor TGF- $\beta$ 1 was significantly increased ( $p < 0.05$ ), with the highest level observed in the HP300 group (Fig. 5A). The relative expression levels of the pro-inflammatory factors TNF- $\alpha$ , IL-1 $\beta$ , and IL-8 were significantly upregulated after *Aeromonas hydrophila* infection ( $p < 0.05$ ) (Fig. 5B-D). The relative expression level of the anti-inflammatory factor IL-10 was also significantly elevated after *Aeromonas hydrophila* infection ( $p < 0.05$ ), with the HP300 group showing the highest level (Fig. 5E). The regulatory mechanism of MOE on inflammation is illustrated in Fig. 5F. Regarding non-specific immunity, compared with the HP0 group after *Aeromonas hydrophila* infection, the activities of ACP, AKP and LZM in the HP300 and HP500 groups were significantly increased after *Aeromonas hydrophila* infection ( $p < 0.05$ ) (Fig. 5G-I). Dietary MOE significantly enhanced the inflammatory response of *Hybrid sturgeon* against *Aeromonas hydrophila* infection, indicating a clear anti-inflammatory effect.

Furthermore, compared with the HP0 group after *Aeromonas hydrophila* infection, the relative expression levels of the ZO-1 gene in the HP100, HP300 and HP500 groups were significantly increased after *Aeromonas hydrophila* infection ( $p < 0.05$ ) (Fig. 5J). Compared with the HP0 group after *Aeromonas hydrophila* infection, the relative expression levels of the *Claudin* gene in the HP300 and HP500 groups were significantly increased after *Aeromonas hydrophila* infection ( $p < 0.05$ ) (Fig. 5K). Compared with the HP0 group after *Aeromonas hydrophila* infection, the relative expression levels of the *Occludin* gene in the HP300 and HP500 groups were significantly increased after *Aeromonas hydrophila* infection ( $p < 0.05$ ), with no significant difference between these two groups ( $p < 0.05$ ) (Fig. 5L).

### **3.6 MOE enhances the serum antioxidant capacity after infection with *Aeromonas hydrophila***

Before infection with *Aeromonas hydrophila*, the MDA content in *Hybrid Sturgeon* serum decreased significantly with the increase in the amount of MOE added to the feed ( $p < 0.05$ ) (Fig. 6A); after infection with *Aeromonas hydrophila*, the addition of MOE to the feed could significantly inhibit the increase in serum MDA levels caused by this bacterium ( $p < 0.05$ ) (Fig. 6A). Regarding the antioxidant enzymes, it

was found that in the case of no infection with *Aeromonas hydrophila*, the contents of SOD, CAT, GSH-PX and T-AOC in the serum of *Hybrid Sturgeon* significantly increased with the increase in the addition amount of MOE in the feed ( $p < 0.05$ ) (Figs. 6B-E); while after infection with *Aeromonas hydrophila*, the contents of SOD, CAT, GSH-PX and T-AOC in the serum of each experimental group after infection were significantly lower than those before infection ( $p < 0.05$ ) (Figs. 6B-E). The mechanism of MOE's antioxidant effect in the serum is shown in Fig. 6F. Thus, it can be seen that adding MOE to the feed can significantly enhance the disease resistance of *Hybrid Sturgeon* against *Aeromonas hydrophila*.

### 3.7 Comprehensive analysis

The results of this chapter indicate that adding MOE to the feed can significantly improve the growth performance, body composition, digestive enzyme activity, and intestinal health of *Hybrid Sturgeon*, and enhance their resistance to *Aeromonas hydrophila* infection. In the 56-day growth experiment, it was found that the addition of MOE increased the weight, WGR, and SGR of *Hybrid Sturgeon* and decreased the FCR. Meanwhile, MOE significantly increased the activities of intestinal trypsin, lipase, and amylase, enhanced the non-specific immune enzymes (ACP, AKP, LZM) activity and antioxidant enzymes (SOD, CAT, GSH-Px) levels in the intestine, reduced the MDA content, and enhanced the immune defense and antioxidant capacity of the organism. In addition, MOE also improved the intestinal flora (g-Hafnia) and tissue morphology structure (increased villus height and width), upregulated the expression of tight junction proteins (*Claudin*, *Occludin*, *ZO-1*), enhanced the mechanical barrier function, and regulated the expression of inflammatory factors (inhibiting *TNF- $\alpha$* , *IL-1 $\beta$* , *IL-8*, promoting *TGF- $\beta$ 1*, *IL-10*), alleviated intestinal inflammatory responses. In the infection test, it was found that MOE could improve intestinal immunity, intestinal functional structure, and serum antioxidant capacity, reduce the mortality rate caused by *Aeromonas hydrophila*, and thereby enhance the disease resistance of *Hybrid Sturgeon*. In summary, MOE may improve the resistance of *Hybrid Sturgeon* to *Aeromonas hydrophila* infection by synergistically regulating growth metabolism, immune response, antioxidant defense, and intestinal barrier function, and has good application potential in the prevention and control of bacterial diseases and growth promotion in aquaculture.

## 4. Discussion

This study aims to develop natural functional feed additives and apply them to the aquaculture of sturgeon to enhance their growth performance, immunity, and disease resistance. Previous studies have shown that the active components of MOE have the functions of regulating intestinal health, improving feed conversion rate, and alleviating oxidative stress, thereby promoting growth (Huang et al., 2022; Lin et al., 2021). However, there are limited systematic studies on MOE in sturgeon, especially *Hybrid Sturgeon*, regarding their growth performance. In this study, adding MOE to the feed significantly increased the final weight, WGR, and SGR. Among them, the HP300 and HP500 groups had the best growth performance. Meanwhile, MOE significantly increased the content of crude fat and crude protein in the fish body. Moreover, MOE significantly increased the activities of intestinal amylase, trypsin, and lipase.

This indicates that MOE can improve the activity of digestive enzymes in the intestine, promote the digestion and absorption of nutrients, and thereby enhance the growth performance of sturgeon. Similarly, adding 0.02–0.03% Magnolol to the diet of Linwu ducks can significantly improve growth performance (Lin et al., 2017). Adding 0.02–0.08% Magnolol to the tilapia feed can increase the content of crude protein and crude fat in the body and increase the final weight (Huang et al., 2022). Adding 0.05% MOE to the diet can increase the average daily gain and average daily feed intake of pigs and reduce the incidence of diarrhea (Zhang et al., 2025). This is consistent with the results of this study. This result may occur because the active components in MOE improve the digestion and absorption of feed by enhancing digestive enzymes, promoting the expression of genes related to lipid and protein transport in the intestine, increasing the protein and crude fat content in the body, and thereby improving growth performance (Lv et al., 2024).

Intestinal antioxidant enzyme activity and intestinal barrier are important functions for intestinal protection. Antioxidant enzymes such as SOD, CAT, and GSH-Px can alleviate oxidative damage (Aliko et al., 2018). Tight junction proteins (Claudin, Occludin, ZO-1) are important components of the intestinal mechanical barrier and also an important barrier against pathogen invasion, playing a key role in maintaining intestinal integrity and permeability (Feldman et al., 2005; Van Itallie et al., 2008). In this experiment, after feeding with MOE for 56 days in the diet, the contents of SOD, CAT, GSH-Px, and T-AOC in the intestine significantly increased, and the MDA content significantly decreased. Meanwhile, after feeding with MOE, the relative expression levels of *Claudin*, *Occludin* and *ZO-1* genes in the intestine of *Hybrid Sturgeon* also increased. Moreover, MOE increased the height, width, and muscle layer thickness of the intestinal villi of *Hybrid Sturgeon*. Relevant studies have found that dietary supplementation of 0.04–0.08% Magnolol can enhance the activity of CAT and SOD in plasma and intestinal tissues, while reducing the MDA level (Huang et al., 2022). Subcutaneous injection of  $10^{-5}$  g/kg Magnolol can also reduce lipid peroxidation reactions in serum, liver, and lung tissues (Kong et al., 2020). Additionally, adding 0.05% MOE to the diet increases the height and width of intestinal villi and the thickness of the muscle layer in pigs (Zhang et al., 2025). The thickwood alcohol can also alleviate the intestinal villus structure damage induced by *Streptococcus suis* by regulating the intestinal flora (Chen et al., 2021). Meanwhile, the magnolol can increase the expression levels of intestinal barrier factors such as Claudin, Occludin and ZO-1 (Cheng et al., 2026; Xu et al., 2024). This indicates that MOE can improve the intestinal biochemical function, barrier function and intestinal structure to enhance the intestinal health of fish and promote their growth and development. The emergence of this result may be due to the phenolic hydroxyl groups and allyl groups of the magnolol, which are easily oxidized by phenolic hydroxyl groups and prone to addition reactions (Zhang et al., 2019), thereby reducing the damage to tissue structure caused by oxidative stress.

In aquatic animals, the immune system is considered the main immune defense line for resisting pathogen infections and maintaining a healthy state. LZM, ACP and AKP as typical non-specific immune factors (Bao et al., 2020), are important indicators of the physiological defense level of fish. Pro-inflammatory factors (TNF- $\alpha$ , IL-1 $\beta$  and IL-8) and anti-inflammatory factors (IL-10 and TGF- $\beta$ 1) inhibit excessive inflammatory responses through negative feedback mechanisms and maintain immune

homeostasis (Guimaraes et al., 2018). In this study, MOE may effectively enhance the activity of non-specific immune enzymes (ACP, AKP, LZM) in *Hybrid Sturgeon*, thereby improving the immune defense ability of the body. Meanwhile, the relative expression levels of *TGF-β1* and *IL-10* in the intestinal tissues of the MOE-added group were significantly increased, while the relative expression levels of *TNF-α*, *IL-1β* and *IL-8* were significantly decreased. This indicates that MOE may enhance the non-specific and specific immunity in the body, thereby improving the immune defense ability. Relevant studies have found that adding 0.05% MOE to the diet of weaned piglets can increase the levels of *TGF-β1* and white *IL-10* in serum, small intestine and ileum, and reduce the inflammatory response of the body (Zhang et al., 2025). The magnolol can also alleviate liver inflammation and immune response by down-regulating the gene expression levels of pro-*IL-1β*, *TNF-α* and *IL-6* in the liver of mice (Lv et al., 2024). *Magnolia officinalis* can effectively improve intestinal pathological damage and barrier gene expression, and inhibit intestinal damage by reducing the expression levels of inflammatory cytokines *IL-1β*, *IL-6* and *TNF-α*, and activating the PI3K/AKT/NF-κB pathway (Liu et al., 2024). The magnolol can also increase the resistance of grouper to *Vibrio harveyi* infection by up-regulating LZM in serum (Zhang et al., 2025). Similar studies have found that the fermentation products can down-regulate the expression of pro-inflammatory factors by regulating the NF-κB signaling pathway (Chen et al., 2025). These research results are consistent with this study.

Microbial regulation of the intestine can prevent intestinal damage and play a key role in regulating intestinal immune regulation and internal environmental homeostasis (Duerkop et al., 2009). Previous studies have found that in the intestinal microbiota of marine and freshwater fish, the Firmicutes phylum, Proteobacteria phylum, Bacteroidetes phylum, and Actinobacteria phylum are all major dominant bacterial groups, with their relative abundances summing up to 90% of the microbial community (Liu et al., 2018; Simona et al., 2018; Genetics et al., 2015). Moreover, they have significant impacts on nutrient absorption and immune function regulation in the host's intestine. Supplementing Magnolol in the diet can significantly increase the relative abundance of beneficial bacteria in the intestinal tract (Fan et al., 2023). In this study, the Firmicutes phylum and Proteobacteria phylum are the dominant bacterial groups at the phylum level. After feeding with MOE, the relative abundance of Firmicutes phylum-A increased, while that of Firmicutes phylum-D decreased. The Firmicutes phylum, as an important bacterial group in the fish's intestine, plays a key role in nutrient metabolism and immune regulation. This differential regulatory effect may reflect the selective influence of MOE on different subgroups of Firmicutes phylum. Additionally, the Firmicutes phylum contributes to the synthesis of short-chain fatty acids and glycolipid metabolism, and the increase in its abundance may lead to increased fat accumulation in the fish's liver (Aron et al., 2013). Relevant studies have shown that an increase in the relative abundance of Proteobacteria phylum can cause imbalance in the intestinal microbiota, thereby affecting the health of the fish (Javed et al., 2019). At the genus level, after feeding with MOE, the *Clostridium* genus emerged as the most dominant bacterial group, showing significant changes, with the greatest increase in relative abundance. The *Faecalibacterium* genus also showed a significant increase in relative abundance and became one of the dominant genera in the HP500 group. Studies have shown that certain members of the Proteobacteria phylum have positive effects in maintaining intestinal barrier function and competing

for exclusion of pathogenic bacteria (Binek et al., 2016). The *Faecalibacterium* genus, as an important member of the Proteobacteria phylum, its increase in the fish's intestine may be related to the immune regulatory effect of MOE. LEfse analysis further confirmed the selective regulatory effect of MOE. In the experimental group, the *Faecalibacterium* genus and unclassified *Hafnia* were identified as marker species. This indicates that MOE may optimize the intestinal microbiota structure by inhibiting certain potential pathogenic or opportunistic pathogens. We speculate that MOE may optimize the microecological balance of the *Hybrid Sturgeon*'s intestine through selective regulation.

Experimental bacterial challenge tests are widely used to assess the health status of fish (Maita, 2007). *Aeromonas hydrophila* is ubiquitous in global aquaculture and can cause high mortality in fish (Harikrishnan and Balasundaram, 2005). The infection test results of this study show that adding MOE to the feed can significantly increase the survival rate of *Hybrid Sturgeon* after being infected with *Aeromonas hydrophila*, and enhance the activities of antioxidant enzymes (SOD, CAT, T-AOC, GSH-PX) in the serum after infection. Meanwhile, MOE effectively inhibits the expression of pro-inflammatory factors (*TNF- $\alpha$* , *IL-1 $\beta$* , *IL-8*) in the intestinal tract after infection, promotes the expression of anti-inflammatory factors (*TGF- $\beta$ 1*, *IL-10*), enhances the activity of non-specific immune enzymes (ACP, AKP, LZM), and upregulates the expression of intestinal tight junction proteins (*Claudin*, *Occludin*, *ZO-1*). Similar studies have found that supplementing magnolol or magnolol phenols can alleviate the adverse reactions of infected meat chickens with *Salmonella enterica* by regulating mucosal gene expression and the intestinal microbiota (Chen et al., 2021). Adding 20 mg/kg magnolol to the feed of spotted catfish can protect them from death caused by infection with *Aeromonas hydrophila*, and magnolol reduces the hemolytic activity of the supernatant of *Aeromonas hydrophila* by inhibiting the expression of aeromonas toxin (Dong et al., 2017). Adding 0.04–0.08% magnolol to the feed can reduce the mortality rate of tilapia to *Aeromonas hydrophila* and increase the relative survival rate (Huang et al., 2022). Meanwhile, low concentrations of magnolol can reduce the hemolytic enzyme activity of *Aeromonas hydrophila* by inhibiting the expression of aerolysin (Dong et al., 2017). Further research has found that adding magnolol to the feed of tilapia can increase the activities of SOD, CAT and GSH-Px after infection and enhance the resistance to pathogenic bacteria (Huang et al., 2022). Another study has shown that adding magnolol to tilapia can increase the activities of SOD, CAT and GSH-Px after infection and enhance the resistance to pathogenic bacteria (Huang et al., 2022). This is consistent with the results of this study. It indicates that adding an appropriate amount of MOE to the feed can improve the disease resistance of *Hybrid Sturgeon* to *Aeromonas hydrophila* and improve intestinal function, but even adding MOE cannot completely eliminate the damage caused by *Aeromonas hydrophila* infection to the body. This result may occur because magnolol can induce bacterial death through multiple pathways and inhibit bacteria by inhibiting cell division, gene transcription, protein translation or the generation of cellular components (Feng et al., 2018), thereby improving intestinal health and growth performance.

## 5. Conclusion

Based on the overall results of the present trial, MOE as a high-quality plant-derived feed additive, exerts multiple beneficial effects on sturgeon, including promoting growth performance, improving intestinal

health, and regulating intestinal microbial communities. Mechanistically, MOE simultaneously activates the systemic antioxidant defense system, boosts intestinal immune responses, and preserves the integrity of the intestinal mucosal barrier, thereby elevating the disease resistance of sturgeon. These findings lay a solid scientific foundation for the extensive application of MOE in commercial sturgeon aquaculture.

## Declarations

### Author contributions: CRediT

Guang Fan: Writing – original draft, Writing – review and editing, Data curation, Formal analysis, Software.

Xiaoping Li: Writing – review and editing, Supervision, Validation

Yilei Liang: Writing – original draft, Data curation, Formal analysis, Software, Methodology.

QiuHong Wu: Methodology, Supervision.

Zhiwen Yang: Project administration.

Pan Zhou: Validation.

Li Jiang: Project administration.

Yachao Wang: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Visualization

### Ethical Declaration

This animal research plan has been approved by the Ethics Committee of Southwest University of Science and Technology.

### Declaration of competing interests

All the authors declare that there are no conflicts of interest in this study.

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### Supplementary material

All supporting materials are presented in the attachment.

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## Clinical trial number

not applicable

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## Figures

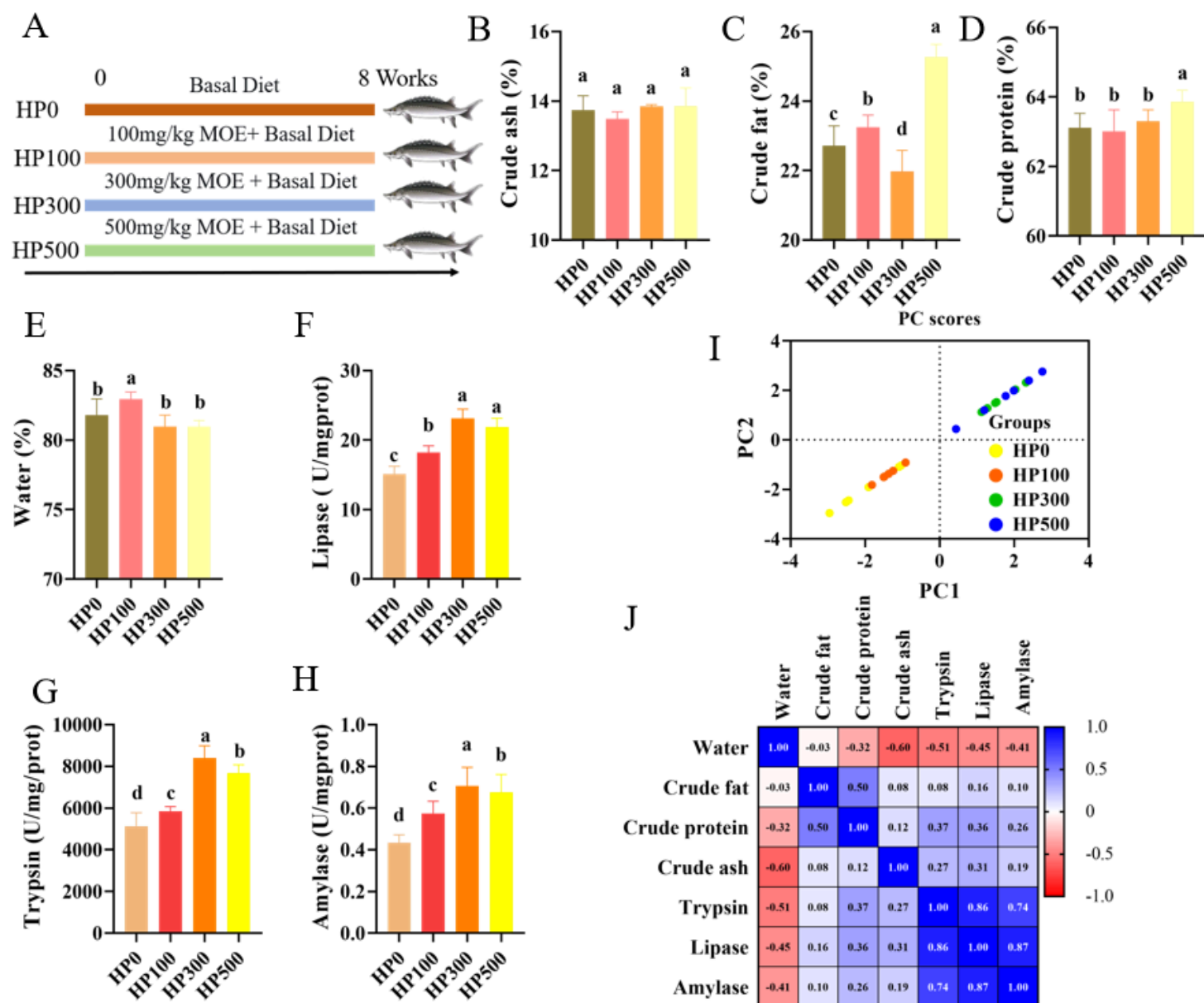


**Figure 1**

Body composition and intestinal digestive enzymes

(A) Design of the feeding experiment. (B) Crude ash. (C) Crude fat. (D) Crude protein. (E) Water. (F) Lipase. (G) Trypsin. (H) Amylase. (I) PCA analysis. (J) Correlation analysis. Data are presented as means  $\pm$  SEM (n=6). Different lowercase letters indicate significant differences among groups ( $p < 0.05$ )

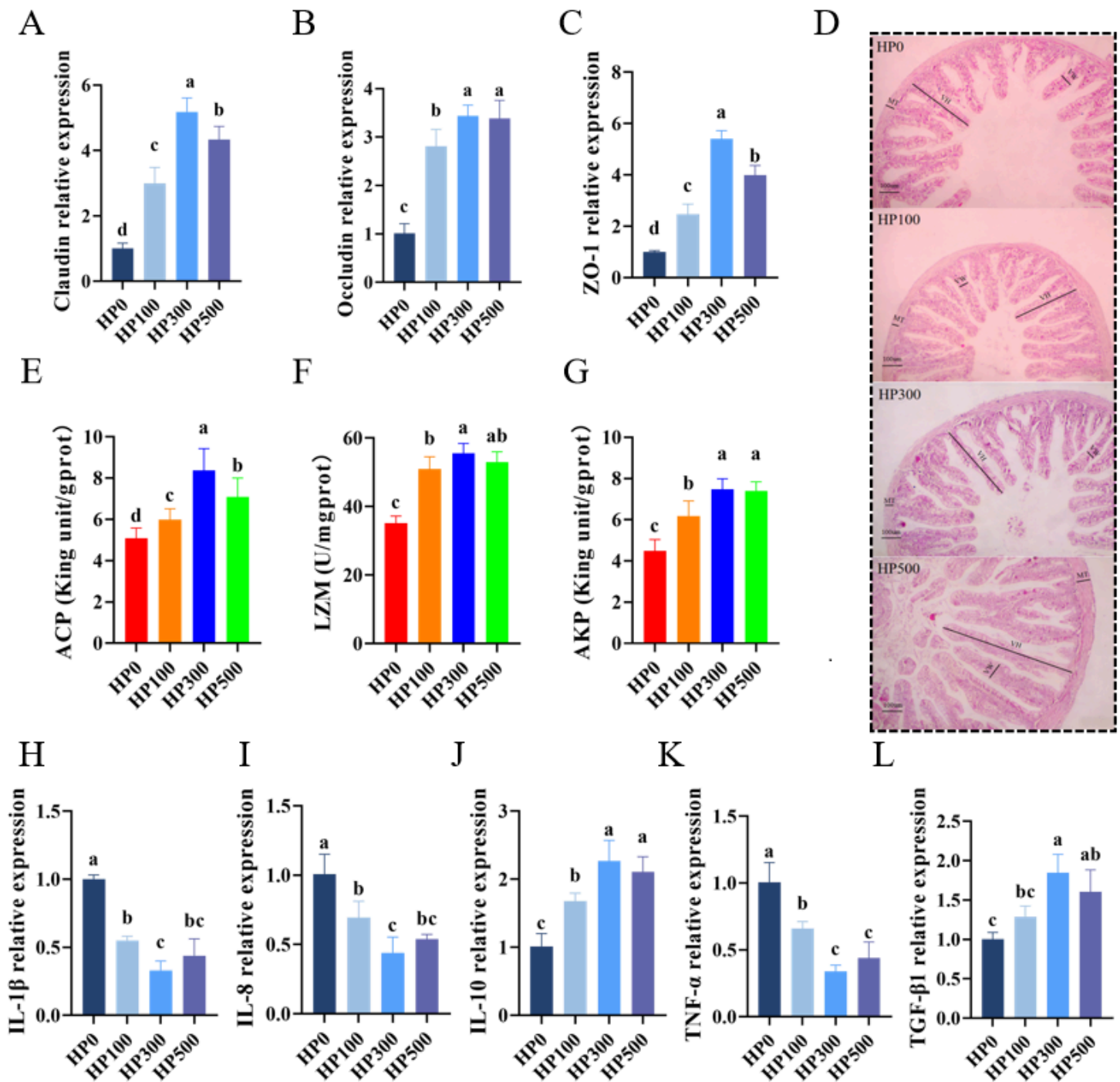


**Figure 2**

MOE improves intestinal health of *hybrid sturgeon*.

(A) *Claudin* expression level. (B) *Occludin* expression level. (C) *ZO-1* expression level. (D) Intestinal structure. (E) ACP content. (F) LZM content. (G) AKP content. (H) Relative expression level of *IL-1 $\beta$* . (I) Relative expression level of *IL-8*. (J) Relative expression level of *IL-10*. (K) Relative expression level of *TNF- $\alpha$* . (L) Relative expression level of *TGF- $\beta$ 1*. Data are presented as means  $\pm$  SEM (n=6). Different lowercase letters indicate significant differences among groups ( $p < 0.05$ )

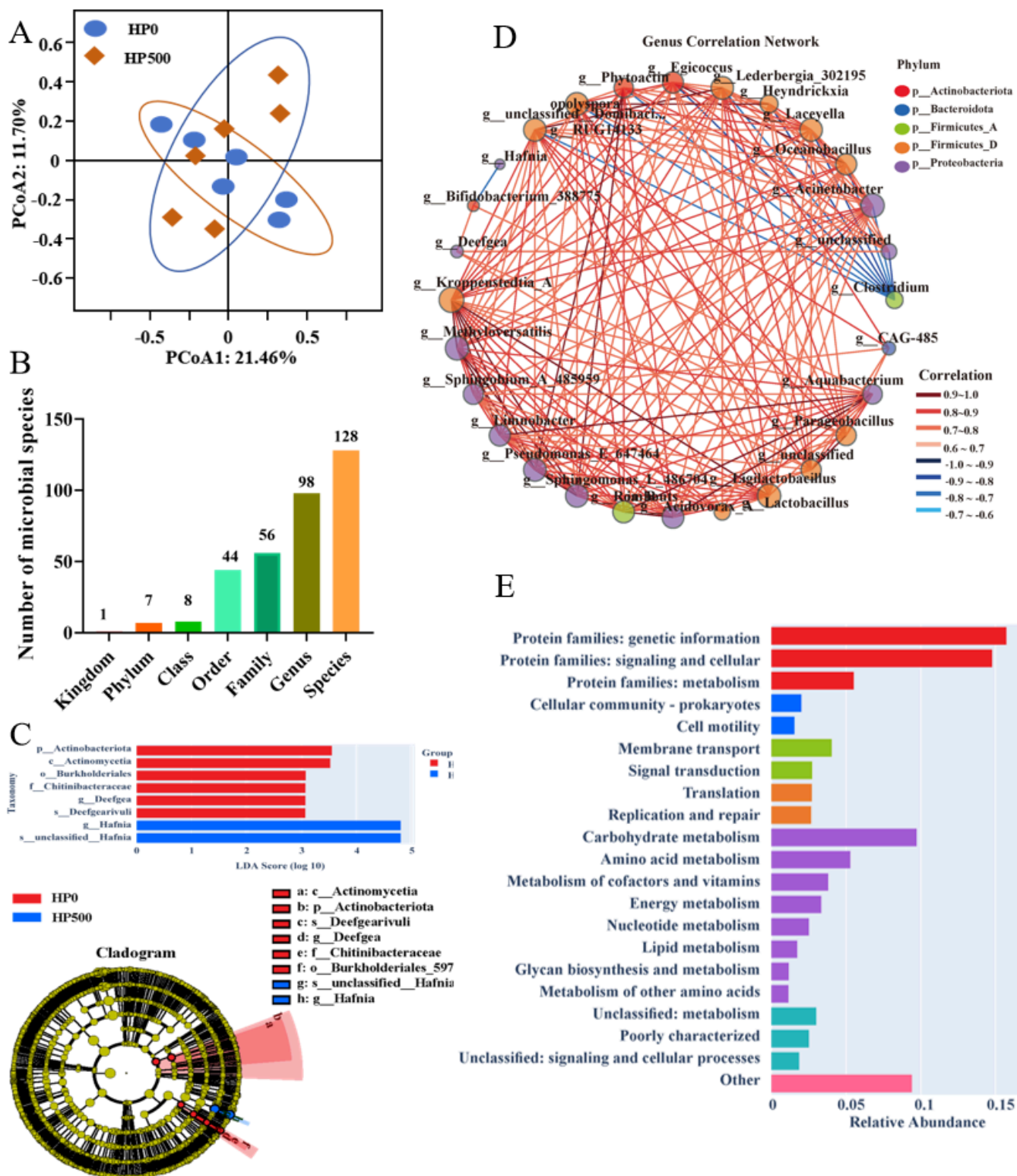


**Figure 3**

Effect of MOE on the gut microbiota of hybrid sturgeon.

(A) PCoA analysis. (B) Number of microbial taxa. (C) LEfSe analysis of the gut microbiota. (D) LEfSe analysis of the gut microbiota. (E) KEGG analysis of the gut microbiota. Data are presented as means  $\pm$  SEM (n=6). Different lowercase letters indicate significant differences among groups ( $p < 0.05$ )

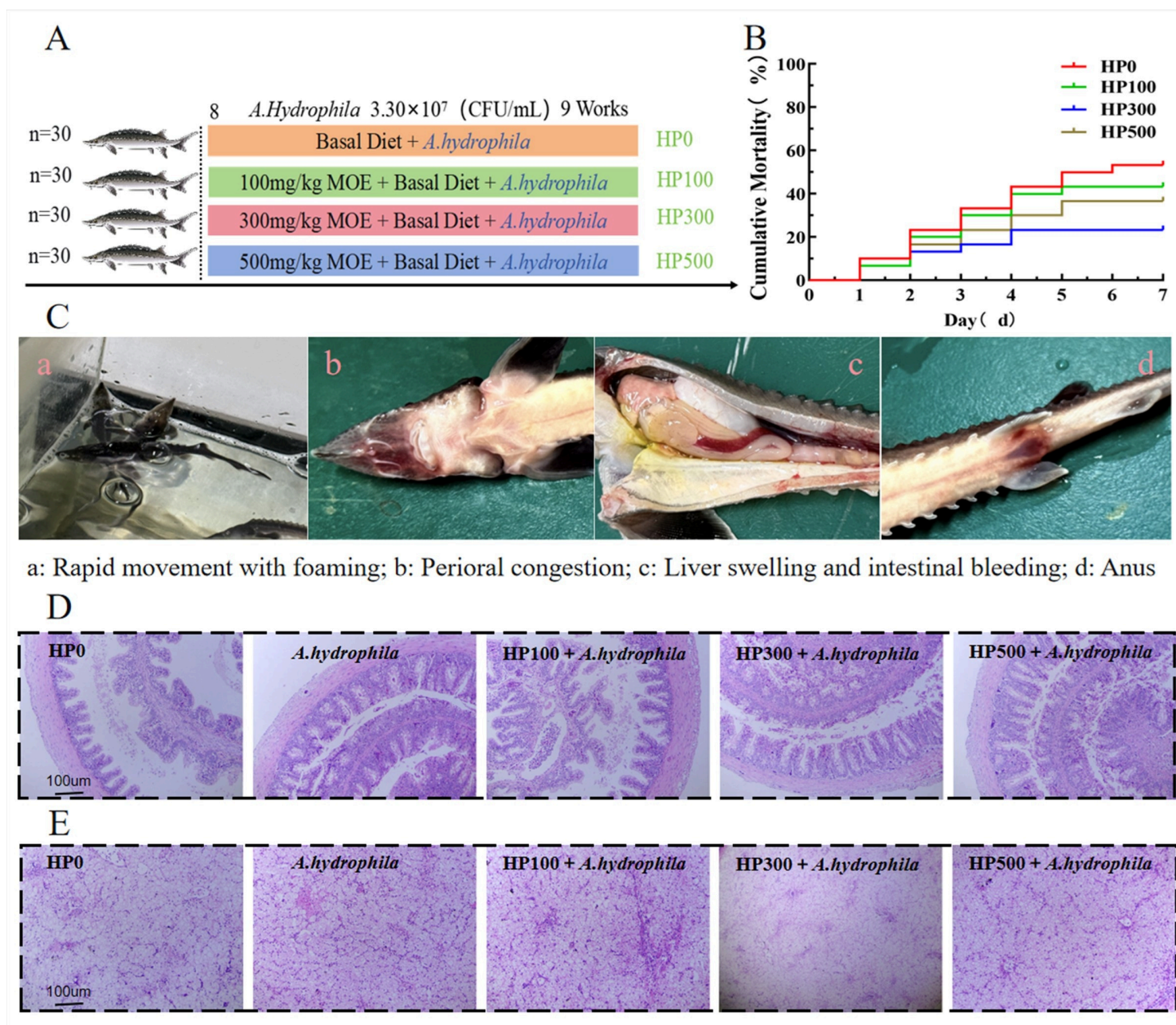


**Figure 4**

Improvement of clinical symptoms in *Hybrid Sturgeon* fish after infection with *Aeromonas hydrophila* by MOE

(A) Design of the *Aeromonas hydrophila* infection experiment. (B) Changes in mortality rate after *Aeromonas hydrophila* infection. (C) Symptom diagram of *Hybrid Sturgeon*. (D) Intestinal section. (E) Liver section.

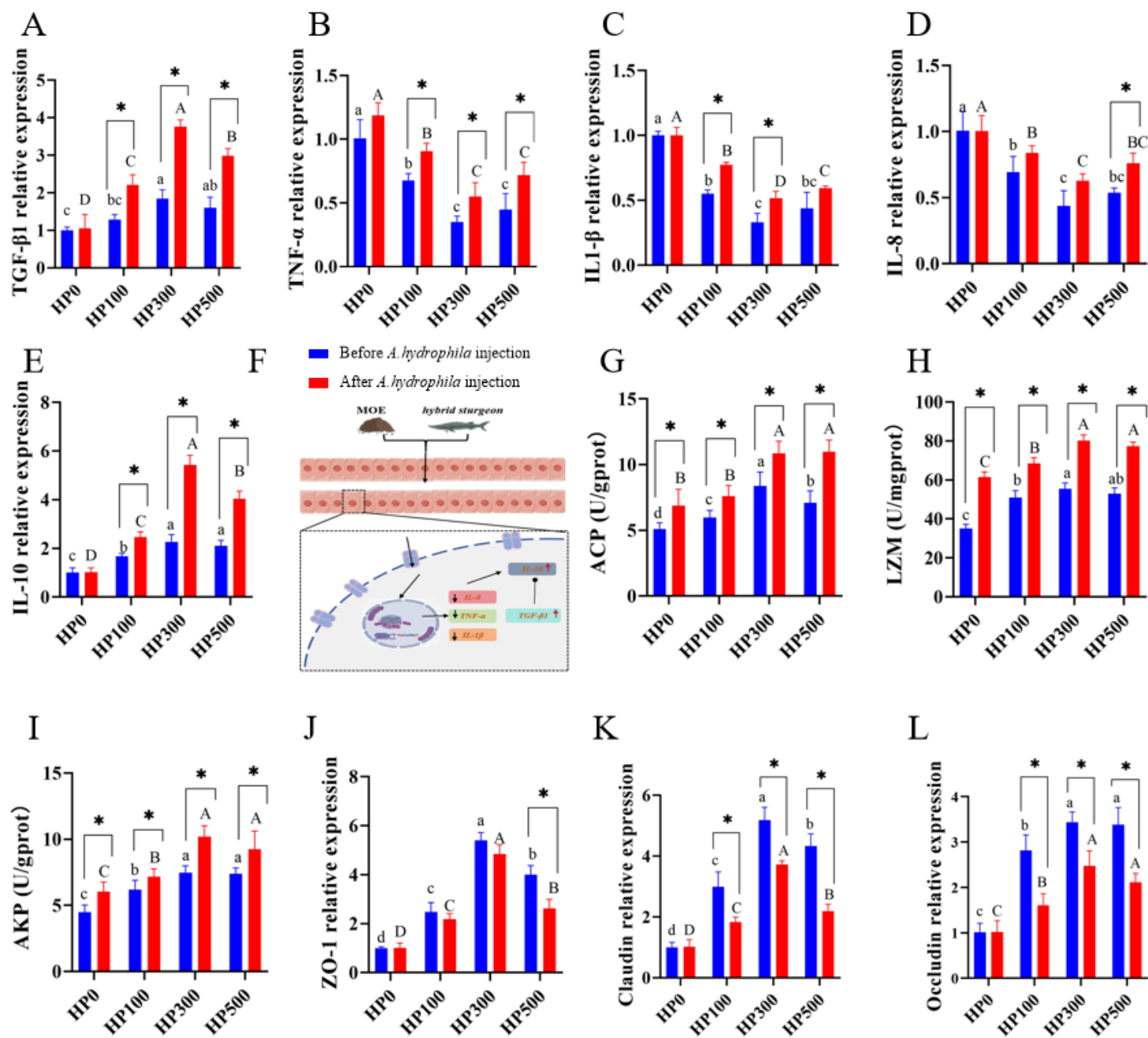


**Figure 5**

Effect of MOE on intestinal function following *Aeromonas hydrophila*

(A) *TGF-β1* expression level. (B) *TNF-α* expression level. (C) *IL-1β* expression level. (D) *IL-8* expression level. (E) *IL-10* expression level. (F) MOE regulatory mechanism of inflammation. (G) ACP content. (H) LZM content. (I) AKP content. (J) *ZO-1* expression level. (K) *Claudin* expression level. (L) *Occludin* expression level. Data are presented as means ± SEM (n=6). Different lowercase letters indicate significant differences among groups ( $p < 0.05$ )

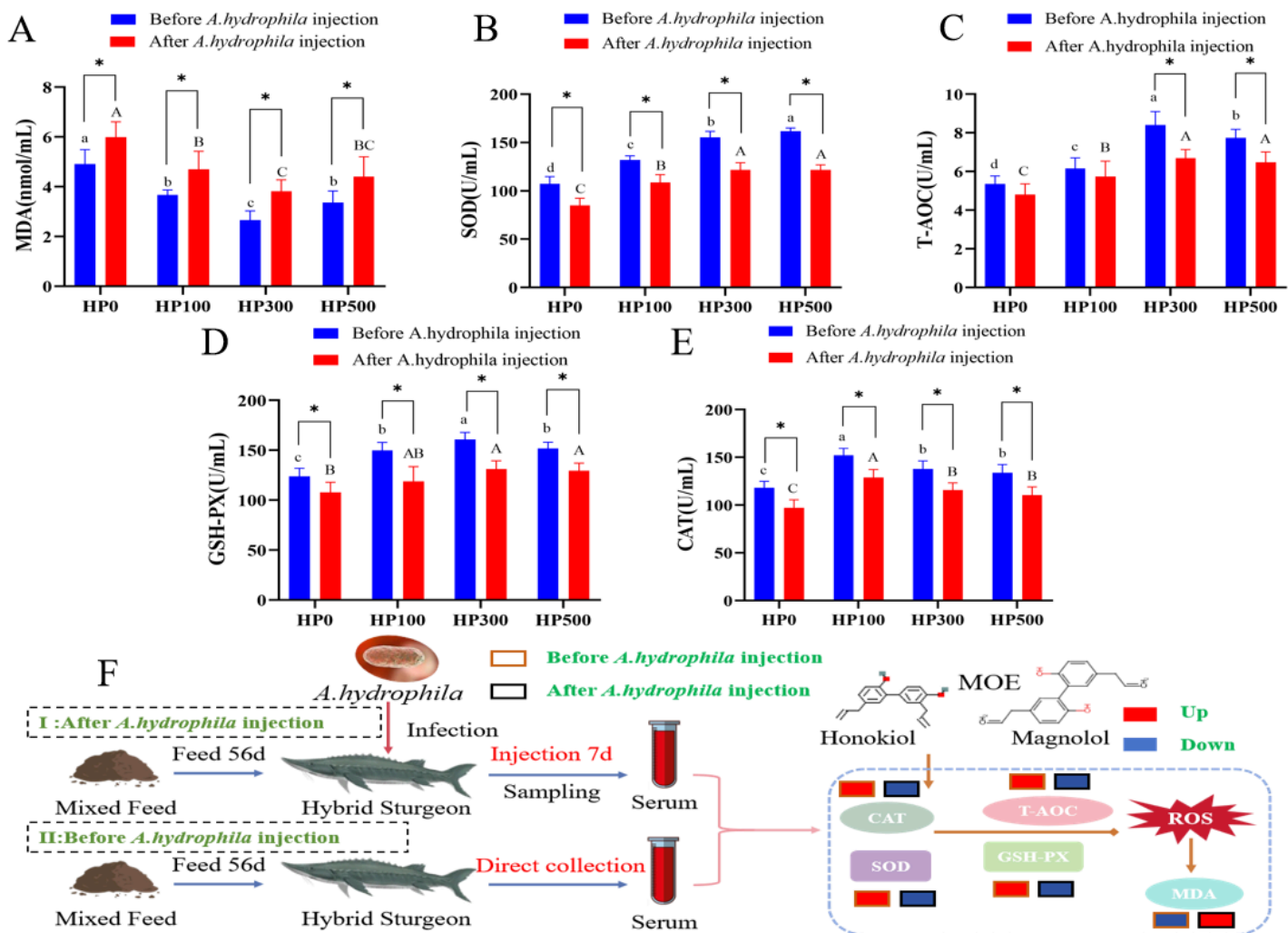


**Figure 6**

The effect of MOE on the blood antioxidant capacity after infection with *Aeromonas hydrophila*.

(A) MDA content. (B) SOD content. (C) T-AOC content. (D). GSH-PX content. (E) CAT content. (F) Schematic diagram of MOE antioxidant in serum. Data are presented as means  $\pm$  SEM (n=6). Different lowercase letters indicate significant differences among groups ( $p < 0.05$ )

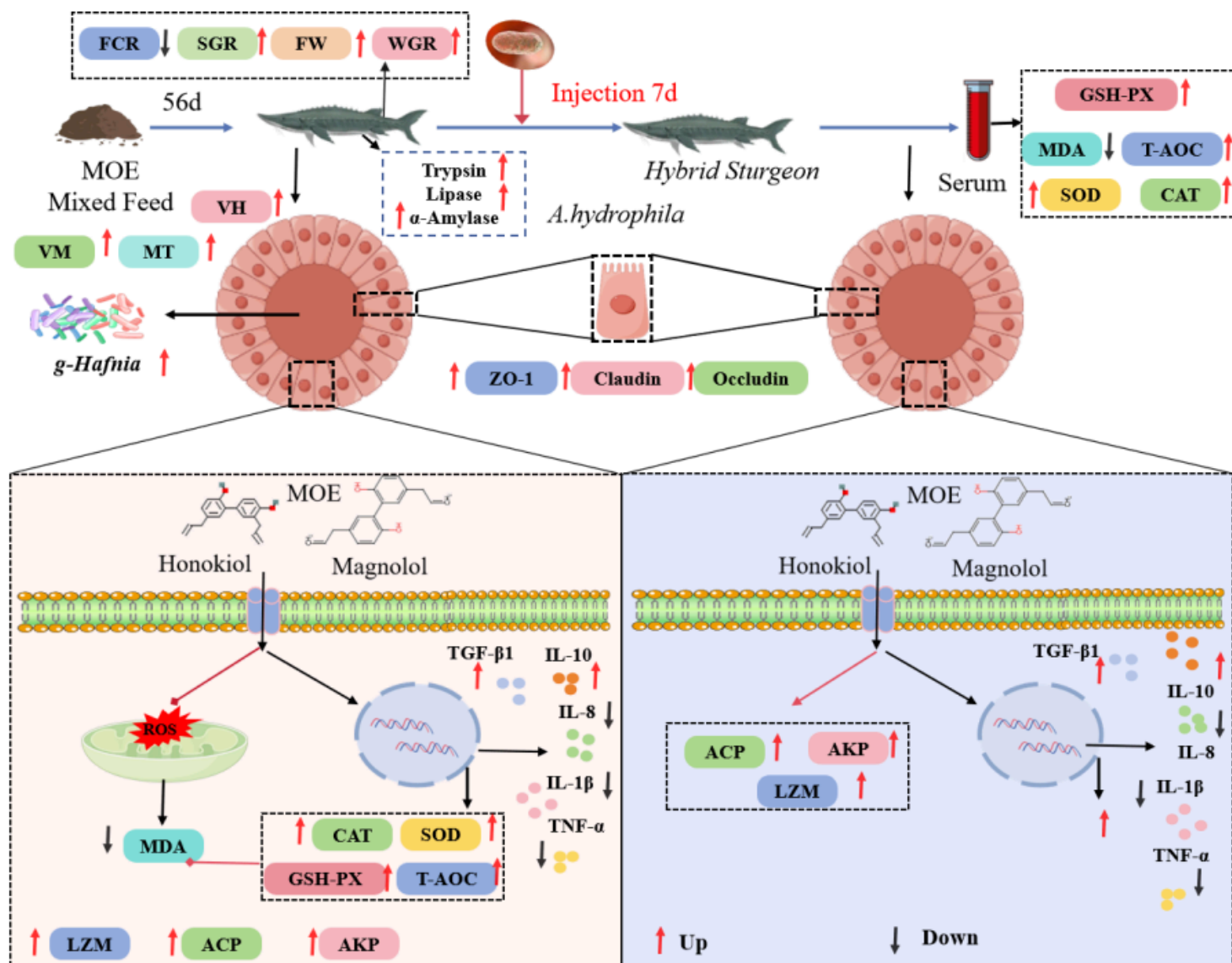


Figure 7

Mechanism Diagram of MOE's Improvement on the Intestinal Immunity, Intestinal Antioxidation and Growth Performance of *Hybrid Sturgeon*

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Appendices2.docx](#)