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Fermented tea residue improved growth performance, liver antioxidant capacity, intestinal morphology and resistance to *Aeromonas hydrophila* infection in juvenile largemouth bass (*Micropterus salmoides*)

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The study aimed to evaluate the effects of fermented tea residue (FT) on growth performance, intestinal morphology, liver antioxidant capacity and Aeromonas hydrophila infection in juvenile Largemouth bass. A total of 240 fish were randomly distributed in 12 tanks with 20 fish per tank (4 treatments with 3 replications) and fed with diets FT at the rate of 0 (control), 2, 4 and 6%. The weight gain rate (WGR), specific growth rate (SGR) and intestinal villi height (VH) of juvenile largemouth bass were significantly higher than those of the control group after feeding FT ($P< 0.05$); meanwhile, the liver superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and catalase (CAT) activities of juvenile largemouth bass were significantly higher and the malondialdehyde (MDA) levels were significantly lower than those of the control group after feeding FT ($P< 0.05$). Mortality occurred in all groups of largemouth bass after the injection of *A.hydrophila*, but feeding FT reduced the cumulative mortality compared with the control group ($P< 0.05$). In juvenile largemouth bass infected with *A.hydrophila*, the relative mRNA expression of the intestinal anti-inflammatory factors IL-10 and TGF-α was significantly higher and that of the pro-inflammatory factors IL-1, IL-15, IL-8, and TNF-α was significantly lower ($P< 0.05$). In summary, it can be seen that a 2% FT addition can improve the liver antioxidant capacity of juvenile largemouth bass, enhance the resistance to *A.hydrophila* and increase the growth of largemouth bass.

KEYWORDS

tea residue, largemouth bass, growth performance, immune capacity, *A. hydrophila*

1 Introduction

Largemouth bass (*Micropterus salmoides*) is a carnivorous warm-water fish from California, U.S.A. It has become one of the main species of freshwater aquaculture in China because of its strong adaptability, fast growth rate and tender flesh (Bureau 2021). In 2020, the farmed production reached 619,519 tons, an increase of 29.66% compared with 2019; (Bureau, 2021). However, under intensive farming, disease outbreaks are more frequent in largemouth bass, which are mainly caused by parasites, bacteria and fungi, The producers need to use a lot of antibiotics and disinfectants to treat these diseases, which leads to economic losses (ValladãO et al., 2015). However, the misuse of antibiotics or chemicals can cause many negative effects on the environment, animals and humans (Cabello, 2010; Rico and Brink, 2014). Therefore, the use of antibiotics is slowly being restricted. It has become a trend in the global aquaculture industry to use positive immune stimulants to enhance the innate immune mechanism of fish to increase their disease resistance (Fuchs et al., 2015; ValladãO et al., 2015).

Studies have shown that preventive treatments promote innate immune responses in fish and reduce disease outbreaks in aquaculture (Ma et al., 2020; Tadese et al., 2020). The use of micro-ecological preparations, such as fermented feed, and plant-based additives can better enhance the immunity of aquaculture animals (Burr et al., 2010; Newaj-Fyzul and Austin, 2015; Dawood et al., 2018; Niu et al., 2020). The addition of Aqualase® (a yeast-based commercial probiotic composed of Saccharomyces cerevisiae and Saccharomyces ellipsoidal) in the feed can moderate the intestinal microbiota of rainbow trout and improve immunity and growth (Adel et al., 2017). The inclusion of 2.5 - 2.61*10^7 CFU/kg of two probiotics (*Lactococcus lactis* and *weissella confuse*) in the diet can improve the growth performance of fingerling great sturgeon, improve the immune index, and increase the height of intestinal villi (Yeganeh Rastekenari et al., 2021). The fermented dragon fruit in the diet improves the growth performance and feed utilization of *Platax pinnatus* and increases antioxidant enzyme activity to some extent (Chu et al., 2021). Feeding fermented feed by *Bacillus subtilis* to Penaeus monodon is shown to improve the growth performance, feed digestibility, survival rate and immunity of spot prawns (De et al., 2018).

After soaking goldfish infected with *A. hydrophila* for a certain period of days with 1% compound herbal water, the damaged primary gill flaps, liver, heart and muscle tissue structure of goldfish are restored (Ramasamy et al., 2010). Immunostimulant (mixture of Chinese herbs and Bacillus), could significantly upregulate the expression of NADPH oxidase genes and antioxidant genes in tilapia spleen neutrophils, thus improving the immunity of tilapia (Abarike et al., 2019).

Tea residue is a by-product of tea refining, which is rich in tea polyphenols, theanine and other active ingredients, with natural antioxidants, immune promotion and other functions (Rietveld and Wiseman, 2003; Hamer, 2007). There are more types of fermented feed, but the result of synergistic fermentation of bacteria and enzymes is better than the result of bacteria and enzymes alone (Xie et al., 2015). Because the combination of bacteria and enzymes can make the degradation of macromolecules more complete, the fermentation efficiency is higher (Sun et al., 2021). The addition of green tea powder to the diet could increase the serum TP content and SOD activity of rainbow trout, upregulate the mRNA expression levels of anti-inflammatory factors and downregulate the mRNA expression levels of pro-inflammatory factors in the spleen and kidney (Nootash et al., 2013). Green tea could improve the growth performance and feed utilization of *Paralichthys olivaceus*, and effectively reduce serum glutathione transaminase (GPT) and low-density lipoprotein (LDL) in fish (Cho et al., 2007). And it improves the growth performance and health of Nile Tilapia against infection by *A.hydrophila* (Abdel et al., 2010). Tea and fermented feed have positive effects on growth performance, immune system, pathogen protection and immunity in different fish species. The application of fermented tea residue on largemouth bass is limited. In this experiment, we investigated the effects of FT with different addition ratios on the growth performance, intestinal tissue structure, antioxidant capacity and infection of *A.hydrophila* of largemouth bass.

2 Materials and methods

2.1 Ethical statement

This study was conducted in strict accordance with the Experimental Animal Management Regulations of Southwest University of Science and Technology. All of the procedures were performed following the Declaration of Helsinki and relevant policies in China.

2.2 Experimental diets and design

Four test diets were prepared. Basal diets and rations with 2%, 4% and 6% fermented tea residue (FT) were added to the basal diet. The composition of the base diet is shown in Table 1, and the nutritional composition of the diets is shown in Table 2. After the base diet was prepared, we added different proportions of FT to the base diet and then mixed and kneaded them into soft pellets (1.2-mm diameter) for feeding. FT is made by crushing tea residue and adding 4% corn flour, 0.8% glucose, 35% water, 0.1% probiotics and enzyme preparations to ferment thoroughly at 36°C for 3 days. FT is the tea residue produced

after Fuxuan 9 processing, from Sichuan, China. Probiotics and enzyme preparations were purchased from a biological company; the product label number is Q/12JX 4450-2019. The fermentation bacteria include *Lactobacillus Plantarum* ≥ 1.0×10^9 CFU/g, *Bacillus subtilis* ≥ 1.5×10^9 CFU/g, *Saccharomyces cerevisiae* ≥ 1.0×10^9 CFU/g; the enzyme preparation contains cellulase ≥ 1000 U/g, xylanase ≥ 500 U/g, β-glucanase ≥ 3000 U/g, β-mannanase ≥ 50 U/g.

2.3 Experimental fish and breeding management

Healthy juvenile largemouth bass (average weight of about 5 g) were obtained from Meishan City, Sichuan Province, China. Upon arrival, all fish were tamed in a test environment for one week. In the formal trial, after all fish were starved for 24 hours, 240 largemouth bass were randomly divided into 4 treatments (groups T0 - T3). T0 was the control group receiving the basal diet, and T1-T3 were fed with FT added to the basal diet at 2%, 4% and 6%, respectively. Each treatment was stocked with three replicates stocked with 60 fish (20 fish/tank) in the tank (1m * 50cm * 1m). During the experiment, fish were hand-fed with experimental diets twice a day at a rate of 3% of body weight (8:30 am and 4:30 pm). We changed 1/5 - 3/5 of the water every 2 - 3 days and used the pump to remove the bottom feces. The water temperature was natural and the dissolved oxygen content was greater than or equal to 6.0 mg/L, pH 7.0 ± 0.2, ammonia nitrogen ≤ 0.02 mg/L. The rearing experiment lasted for 56 days.

TABLE 1 Composition and nutrient levels of the basal diet (air-dry basis).

Items	Content(%)
Ingredients	
fish meal	44
chicken powder	10
cassava starch	8
flour	11
gluten	2
soybean meal	12
soybean oil	6
squid ointment	4
$Ca(H2PO4)_2$	1.5
premix	1.5
Total	100

1) Premix (per kilogram of premix): VA 8000000 IU, VD 2000000 IU, VE 5000 UI, VK 1000 mg, VB_1 1500 mg, VB_2 1500 mg, VB_6 800 mg, VB_{12} 20 mg, nicotinamide 400 mg, calcium pantothenate 25 mg, folic acid 25 mg, biotin 8 mg, inositol 100 mg; $MnSO_4{\cdot}H_2O$ 50 mg, KI 100 mg, $CoCl_2$ (1%) 100 mg, $CuSO_4{\cdot}5H_2O$ 20 mg, $FeSO_4{\cdot}H_2O$ 260 mg, $ZnSO_4{\cdot}H_2O$ 150 mg, Na_2SeO_3 (1%) 50 mg.

TABLE 2 Nutrient composition of the basal and experimental diets.

Groups	EE	CP	Ash	Moisture
T0	8.8	49.7	15.71	11.1
T1	8.6	48.2	15.8	39.1
T2	8.6	47.5	15.7	37.1
T3	8.5	48.5	15.7	37.5

1) Nutrient levels were measured in values.

2.4 *A.hydrophila* challenge test

At the end of the feeding trial, 30 fish of each group (10 fish/tank) were selected to be injected with *A.hydrophila* for infection, and previously we derived from pre-experiment that the LC_{50} of *A.hydrophila* on juvenile largemouth bass was 1.65×10^6 CFU/ml at an injection dose of 0.2 ml/tail. The trial fish were anesthetized with an appropriate amount of 50 ppm MS-222 for 3-5 min, and then 0.2 ml of *A.hydrophila* liquid with a concentration of 1.65×10^6 CFU/ml was slowly injected from the abdominal cavity using a 1 ml injector, and the breeding environment was kept unchanged.

2.5 Sample collection

For statistical analysis of growth performance, we fasted for 24 h at the end of the breeding test. We weighed the total weight of each test tank and counted the number of surviving fish for statistical analysis. Nine fish were randomly selected from each replicate, anesthetized with 100 ppm MS-222, and their body weight and length were measured. Subsequently, the midgut of three fish was randomly selected from each group of nine fish and fixed with 4% paraformaldehyde for intestinal histological observation. Take the remaining fish viscera to measure the body index. collected livers and intestines were snap frozen in liquid nitrogen (-196°C) and then transferred to -80°C refrigerator for storage. On the third day after the *A.hydrophila* infection treatment, all fish were fasted for 24 h, anesthetized with 100 ppm MS-222, and the whole intestines were taken into sterile tubes, snap-frozen in liquid nitrogen (-196°C), and then transferred to -80°C refrigerator storage for intestinal inflammatory factor expression assay. Growth indicators and cumulative mortality were calculated as follows:

$$\text{WGR (weight gain rate, \%)} = 100 \times (W_t - W_0)/W_0$$

$$\text{SR (survival rate, \%)} = 100 \times (N_t/N_0)$$

$$\text{SGR(specific growth rate, \%/}d\text{)} = 100 \times (lnW_t - lnW_0)/t$$

$$\text{HSI (Hepatosomatic index, \%)} = 100 \times W_h/W$$

$$\text{CF}\left(\text{Condition factor g/cm}^3\right) = W/L^3$$

$$\text{Cumulative mortality rate}\ (\%) = (N_d/N_a) \times 100$$

Where N_t and N_o represent the total number of fish samples at the beginning and end of the experiment, respectively; W_t and W_0 are the initial and final weight data at the beginning and end of the experiment (g); W is the body weight per fish, W_h is the liver weight of per fish. t represents the number of experimental days (day); L is the length of the fish (cm). N_d is the cumulative number of fish dead; N_a is the initial number of fish after injection of *A.hydrophila*.

2.6 Sample measurement

2.6.1 Histomorphology of the intestine

The midgut of juvenile largemouth bass was rinsed with saline and fixed with 4% paraformaldehyde. After processing, sections (5 μm) were sectioned using a paraffin slicer, followed by hematoxylin-eosin (HE) staining and image acquisition by light microscopy, with the observed sections first observed under low magnification and the appropriate areas selected for image acquisition under high magnification. The data for villi height, width, and thickness of the muscular layer were measured by Image-Pro Plus software.

2.6.2 Analysis of antioxidant enzyme activities and immune enzyme activities in liver

The liver tissues stored at -80 °C were thawed on ice, and the tissues were homogenized with saline 1:9 according to the kit instructions, centrifuged at 4 °C and 2,500 r/min for 10 min, and then the supernatant was taken as the tissue homogenate. Total protein (TP), superoxide dismutase (SOD), total antioxidant capacity(T-AOC), glutathione peroxidase (GSH-Px), malondialdehyde (MDA) and catalase (CAT) were measured in the liver using a spectrophotometer or enzyme marker according to the steps of the kit (Nanjing Jiancheng Institute of Biological Engineering) instructions.

2.6.3 Measurement of intestinal inflammatory factor expression after *A.hydrophila* infection

Intestinal tissues stored at -80°C were placed in RNAase-free centrifuge tubes and ground using a microtissue homogenizer. Total RNA was extracted from the intestinal tissues by the Trizol method (TaKaRa, Japan). The concentration of RNA was measured using a micro ultraviolet spectrophotometer. The first-strand cDNA was synthesized using the kit (product number RR047A, TaKaRa) according to the instructions. Protocol for reverse transcription: 37°C for 15 minutes; 85°C for 5 seconds. The primers were used to refer to the study of Xv (Xv et al., 2021). Table 3 shows the PCR primers used in this study for the coding sequences of *IL-1β*, *IL-8*, *IL-10*, *IL-15*, *TNF-α* and *TGF-β* genes in the largemouth bass genome. And *β-actin* was used as an internal reference gene, the specific primers for *β-actin* and target genes were synthesized by Tsingke Biotechnology Co., Ltd. Quantitative real-time PCR (qPCR) was performed using NovoStart SYBR qPCR SuperMix Plus (Novoprotein) on Bio-Rad CFX96 (Bio-Rad) in a total volume of 20μL.

2.7 Calculations and statistical methods

All data are expressed as mean± SD. Significance levels were determined by one-way analysis of variance (ANOVA) with

TABLE 3 Primer sequences for real-time PCR.

Genes	Primers	Sequence 5′–3′	TM (°C)	Accession number
IL–8	F	CGTTGAACAGACTGGGAGAGATG	64.9	RNA-seq by (Xv et al., 2021)
	R	AGTGGGATGGCTTCATTATCTTGT		
IL–10	F	CGGCACAGAAATCCCAGAGC	62.1	RNA-seq by (Xv et al., 2021)
	R	CAGCAGGCTCACAAAATAAACATCT		
IL–15	F	GTATGCTGCTTCTGTGCCTGG	62	RNA-seq by (Xv et al., 2021)
	R	AGCGTCAGATTTCTCAATGGTGT		
IL–1β	F	CGTGACTGACAGCAAAAAGAGG	59.4	RNA-seq by (Xv et al., 2021)
	R	GATGCCCAGAGCCACAGTTC		
TGF-β	F	GCTCAAAGAGAGCGAGGATG	59	RNA-seq by (Xv et al., 2021)
	R	TCCTCTACCATTCGCAATCC		
TNF-α	F	CTTCGTCTACAGCCAGGCATCG	63	RNA-seq by (Xv et al., 2021)
	R	TTTGGCACACCGACCTCACC		
β-actin	F	AAAGGGAAATCGTGCGTGAC	60	RNA-seq by (Xv et al., 2021)
	R	AAGGAAGGCTGGAAGAGGG		

IL-1β, interleukin-1β; IL-8, interleukin-8; IL-10, interleukin-10; IL-15, interleukin-15; TNF-α, tumor necrosis factor-α; TGF-β1, transforming growth factor-β.

IBM SPSS Statistics 23. Multiple comparisons were performed using the Tukey multiple range test. The statistical significance level was set at p<0.05. Graphs were drawn using GraphPad Prism6 (GraphPad Software, Inc., USA).

3 Results

3.1 Growth performance and morphometric parameters

As the growth performance and morphological indices are shown in Table 4, FT can significantly improve the weight gain rate and specific growth rate of juvenile largemouth bass compared with T0 ($P< 0.05$). Compared with T3, T1 and T2 also significantly increased the weight gain and specific growth rate of juvenile largemouth bass ($P< 0.05$). The condition factor of T1 was significantly higher than the rest of the other groups, and FT could significantly reduce the HSI index of juvenile largemouth bass ($P< 0.05$). However, there was no significant difference in the mortality rate among all groups ($P > 0.05$).

3.2 Cumulative mortality of juvenile largemouth bass after injection of *A.hydrophila*

In this trial, largemouth bass were infected with *A.hydrophila* and mortality was counted for 3 consecutive days, as shown in Figure 1. After the injection of *A.hydrophila*, all groups showed mortality on the first day, but the cumulative mortality rate of the control group was higher than that of the other groups ($P< 0.05$), and the cumulative mortality rate rapidly increased with time. The cumulative mortality rate for T0 was 43.33%, while T1 was 23.33%, T2 was 20.00%, and T3 was 26.67%, respectively.

3.3 Morphological observation of the intestinal tract of juvenile largemouth bass

The tissue structure of the midgut of juvenile largemouth bass is shown in Figure 2, and the characteristics of the midgut villi are shown in Table 5. The intestinal villi of juvenile largemouth bass in the control group were shorter and sparser, with fewer villi and goblet cells ($P< 0.05$). The height of intestinal villi was significantly higher in the test group than in the control group ($P< 0.05$).

3.4 Largemouth bass liver antioxidant index

The antioxidant indices in the livers of juvenile largemouth bass are shown in Table 6. Compared with the control group, the activities of SOD, GSH-PX, CAT and the level of T-AOC in the livers showed a significant increase as well as a significant decrease in MDA content in juvenile largemouth bass after 8 weeks of FT feeding ($P< 0.05$).

3.5 Expression of intestinal inflammatory factors in juvenile largemouth bass after *A.hydrophila* infection

Infection with *A.hydrophila* caused an inflammatory response in the intestine characterized by increased expression of pro-inflammatory factors and decreased expression of anti-inflammatory factors. In this experiment, we measured the expression of intestinal inflammatory factors in largemouth bass after infected by *A.hydrophila*. And the results are shown in Figure 3. In juvenile largemouth bass fed FT for 8 weeks, the relative mRNA expressions of intestinal pro-inflammatory factors IL-1β, IL-15, IL-8 and TNF-α decreased significantly, while the relative mRNA expressions of anti-inflammatory factors IL-10 and TGF-β increased significantly ($P< 0.05$). The relative mRNA expression of anti-inflammatory factors IL-10 and TGF-β1 was significantly higher in T2 compared with those of T1 and T3. In contrast, the relative mRNA expression of pro-inflammatory factors IL-1β and IL-15 was significantly lower in T2 compared with T1 and T3 ($P<0.05$).

4 Discussion

Tea residue is rich in tea polyphenols, theanine, tea saponin and other active substances, which can improve the production

TABLE 4 Growth performance and morphological indicators.

Items	T0	T1	T2	T3
WGR	137.73 ± 11.16^{C}	219.27 ± 7.77^{A}	204.83 ± 18.71^{A}	175.93 ± 2.84^{B}
SGR	1.53 ± 0.06^{C}	2.07 ± 0.06^{A}	2.00 ± 0.10^{A}	1.80 ± 0.00^{B}
SR	98.33 ± 2.89	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
CF	1.73 ± 0.21^{B}	2.03 ± 0.06^{A}	1.70 ± 0.10^{B}	1.80 ± 0.00^{B}
HSI	3.23 ± 0.50^{A}	2.00 ± 0.2^{B}	1.97 ± 0.45^{B}	$2.20 \pm 0.0.36^{B}$

Different letters indicate significant differences (P< 0.05); Values are presented as mean ± SD (n = 3); WGR, weight gain rate; SR, survival rate; SGR, specific growth rate; HSI, Hepatosomatic index; CF, Condition factor.

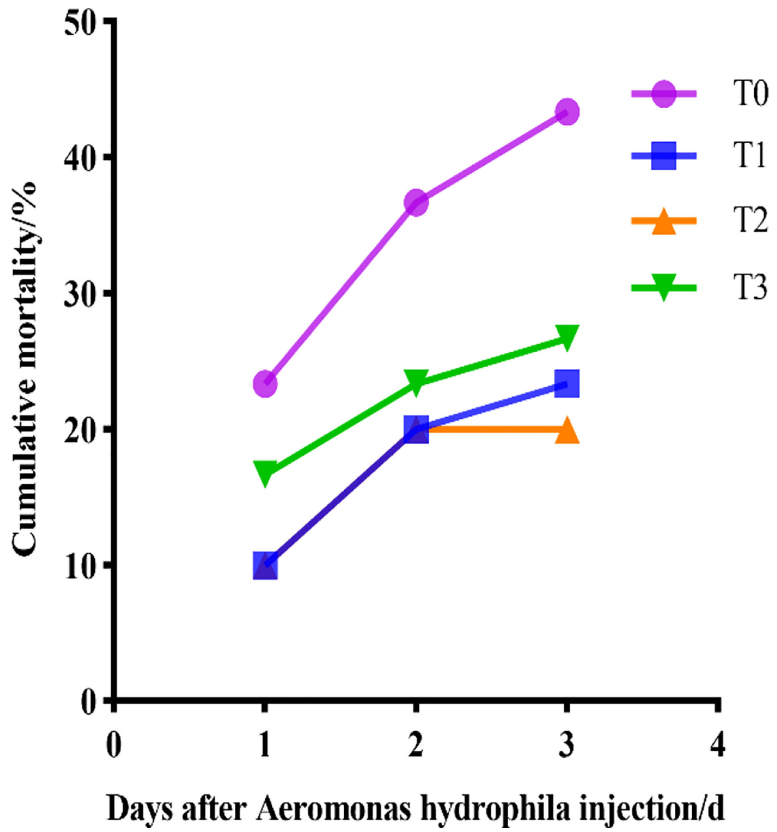

FIGURE 1
Cumulative mortality of juvenile largemouth bass after injected with *A.hydrophila*.

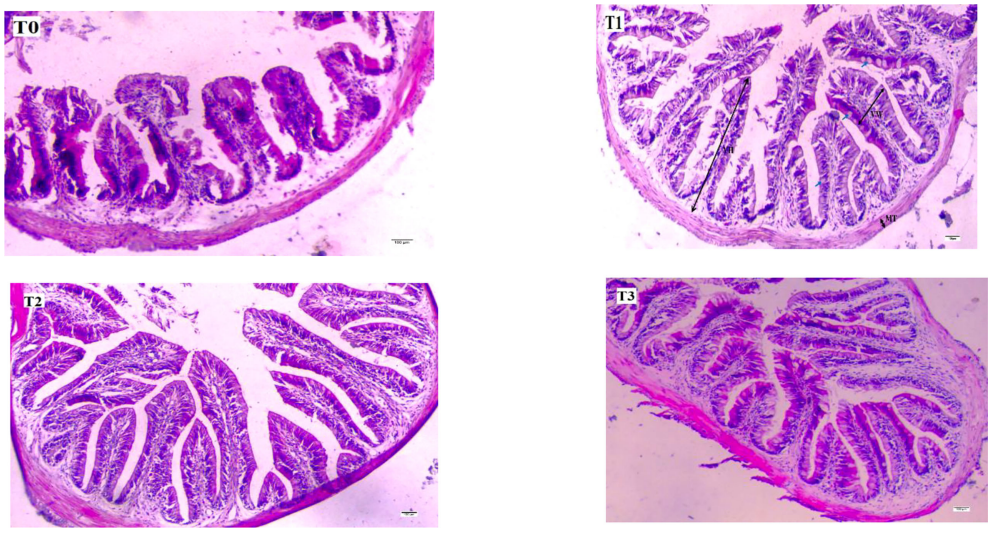

FIGURE 2
Effect of different scales of FT on the morphology of the mid-gut of the juvenile largemouth bass (X100, H&E staining, scale bar = 100(μm). Villi height (VH), villi width (VW), muscular layer thickness (MT), and the blue arrows indicated are goblet cells.

TABLE 5 Characteristics of midgut villi of largemouth bass.

Items	VH/μm	VW/μm	MT/μm
T0	582.47 ± 30.15^{C}	216.14 ± 14.10^{A}	74.59 ± 3.85
T1	793.92 ± 24.30^{A}	199.57 ± 11.64^{B}	71.72 ± 4.05
T2	725.50 ± 23.74^{B}	216.16 ± 15.11^{A}	76.37 ± 3.95
T3	720.64 ± 56.81^{B}	181.69 ± 14.05^{C}	73.75 ± 4.56

Different letters indicate significant differences (P< 0.05); Values are presented as mean ± SD (n = 5); VH, Villi height; VW, villi width; MT, muscular layer thickness.

performance of livestock and meat quality (Hamer, 2007). It has been shown that the addition of appropriate amounts of tea to diets can improve the activity of digestive enzymes, reduce anti-nutritional factors, and improve the use of nutrients, thus promoting the growth of fish (Zhang et al., 2015; Zheng et al., 2017). Similar to this study, plant extracts such as Common Sage (*Salvia officinalis*), Coneflower (*Echinacea angustifolia*), Cornelian cherry (*Cornus mas L.*), Rose hip and Safflower can stimulate the innate immune response and feed intake, thus improving fish growth performance (Dadras et al., 2016; Dadras et al., 2019; Ahmadifar et al., 2022). Fermented feeds can reduce the anti-nutritional factors in feeds and increase the digestion and absorption capacity of feeds, thus promoting the growth of the organism (Ilha et al., 2017; Wang et al., 2017). The addition of fermented tea residue to the diet can improve the fattening performance and digestive performance of fattening pigs (Ding et al., 2020). In this study, FT was able to improve the weight gain rate and specific growth rate of juvenile largemouth bass, but the weight gain rate and specific growth rate were lower than the actual production values, which was probably because the experiment was conducted during the seasonal change in autumn and winter, the temperature may have affected the growth performance of largemouth bass.

The intestine is an important site for digestion and absorption of nutrients as well as protection against pathogens in fish. Intestinal histological assessment is an effective method to assess the effect of dietary components on the intestinal health of fish (Chauhan and Singh, 2018; Ding et al., 2020). Some studies found that tea polyphenols and fermented feed could increase the height of intestinal villi and thickness of the muscle layer in fish, and improve the intestinal histology of fish (Mamauag et al, 2019; Ma et al., 2021; Zhuo et al., 2021). It was found that the height and width of intestinal villi of juvenile largemouth bass were higher than those of the control group after feeding FT, which indicated that the integrity and stability of the intestinal tract were enhanced, thus strengthening the digestion and absorption of nutrients by juvenile largemouth bass and promoting their growth, this might be one of the reasons for their better growth performance compared to the control group.

Oxidative stress is a state in which there is an imbalance between oxidation and antioxidant action in the body, a negative effect produced by free radicals in the body, which predisposes the body to age and disease (Bai et al., 2017). The most common enzymatic antioxidants present in animals are CAT, SOD and GSH-Px, which mainly serve to scavenge peroxides in the body to protect it from damage caused by oxidative stress (Yuan et al., 2019; Chen et al., 2020). The antioxidant enzyme activity in animals determines the antioxidant capacity of their bodies, which can be used to assess the health of fish (Tovar-Ramírez et al., 2010). The level of T-AOC is one of the indicators of antioxidant capacity in animals, which is an important indicator of the antioxidant capacity of fish directly (Cui et al., 2014; Yu et al, 2021). This study found that FT could enhance the activity of SOD, GSH-Px and CAT as well as the level of T-AOC in the liver of juvenile largemouth bass, which indicated that FT could enhance the antioxidant capacity of juvenile largemouth bass and slow down the oxidative damage to the organism. MDA is a peroxidation metabolite generated by lipids in the body under the influence of free radicals. The level of MDA content can directly reflect the damage to the body by free radicals, and higher levels of MDA reflect higher peroxidation reactions in the

TABLE 6 Analysis of antioxidant enzyme activities and immune enzyme activities in liver.

Items	T0	T1	T2	T3
TP (mgprot/mL)	3.09 ± 0.19	3.55 ± 0.17	3.53 ± 0.22	3.50 ± 0.33
SOD (U/mgprot)	115.22 ± 1.79^{C}	176.41 ± 4.35^{A}	152.72 ± 9.70^{B}	148.64 ± 3.63^{B}
GSH-PX (U/mgprot)	18.04 ± 1.18^{D}	51.96 ± 2.01^{A}	40.68 ± 2.67^{C}	46.76 ± 0.86^{B}
T-AOC (U/mgprot)	2.64 ± 0.30^{C}	5.65 ± 0.35^{A}	3.58 ± 0.26^{B}	3.57 ± 0.39^{B}
MDA (nmol/mgprot)	5.65 ± 0.31^{A}	4.07 ± 0.01^{B}	3.55 ± 0.12^{C}	3.57 ± 0.14^{C}
CAT (U/mgprot)	144.51 ± 3.30^{C}	229.84 ± 7.10^{B}	241.40 ± 14.16^{B}	273.59 ± 9.61^{A}

Different letters indicate significant differences (P< 0.05); Values are presented as mean ± SD (n =5); TP, total protein; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; T-AOC, total antioxidant capacity; GSH-PX, glutathione peroxidase.

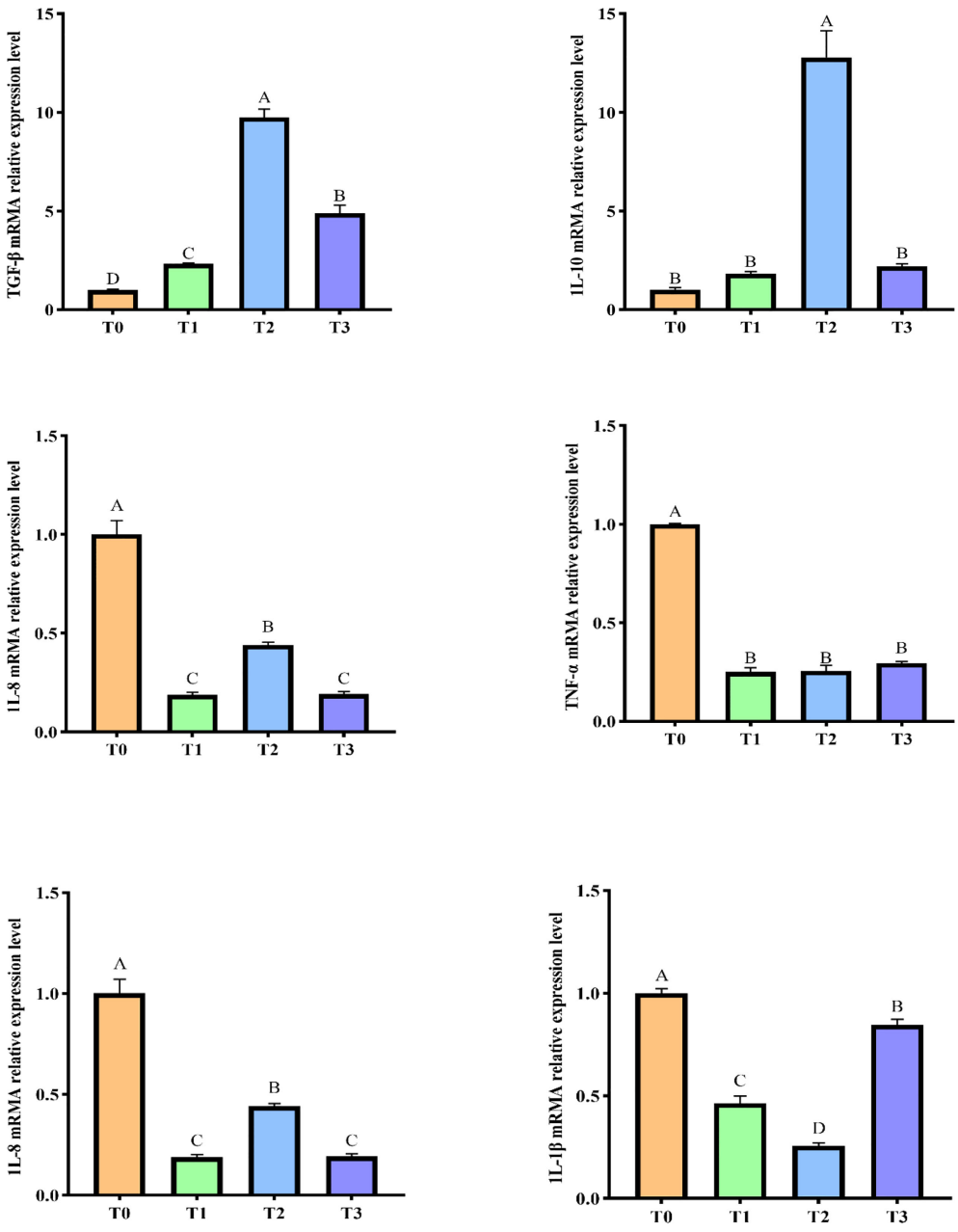

FIGURE 3
Effect of feeding FT on the expression of inflammatory factors in the intestine of largemouth bass after infection with *A.hydrophila*. Different letters indicate significant differences ($P < 0.05$); Values are presented as mean ± SD (n =5). IL-1β: interleukin-1β; IL-8: interleukin-8; IL-10: interleukin-10; IL-15: interleukin-15; TNF-α: tumor necrosis factor-α; TG F-β1: transforming growth factor-β.

body (Janero, 1990; Yu et al., 2019). In this study, we found that feeding FT could reduce the liver MDA content of juvenile largemouth bass. The results were generally consistent with other studies that dietary medical plants or fermented tea residue could enhance the activity of SOD, GSH-Px and CAT as well as the level of T-AOC and reduce the content of MDA in rainbow trout (Ghafarifarsani et al., 2022), Holstein heifers (Xie et al., 2020), common carp (Ahmadifar et al., 2022), Siberian sturgeon (Hasanpour et al., 2019), Tilapia (Qian et al., 2021), Sea bream (Pérez-Jiménez et al., 2012) and juvenile Wuchang bream (Guo et al., 2020).

Bacterial infection is often used as a final indicator of fish health status after nutrient analysis (Wang et al., 2015; Li et al., 2020). Bacterial enteritis is the most common intestinal disease

of freshwater fish. Among many pathogenic bacteria, *A.hydrophila* is usually considered one of the main pathogens causing intestinal inflammation in fish (Cascón et al., 2000; Macpherson et al., 2012). In our study, the test fish fed FT showed a higher survival rate after infected with *A.hydrophila*, which may be due to the probiotic bacteria used in tea residue with fermentation to promote the immune response of largemouth bass. This is in agreement with previous studies that found green tea or probiotics can enhance the resistance of Nile tilapia (Abdel et al., 2010; Cavalcante et al., 2020), Cyprinus carpio (Chandravanshi et al., 2020), Lates calcarifer (Lin et al., 2017) and Labeo rohita (Rai et al., 2015) to *A.hydrophila*. It showed that feeding FT improved the survival of juvenile largemouth bass and protected the intestine from damage by *A.hydrophila*.

Intestinal immune regulatory molecules (cytokines) expression is positively correlated with the immune status of fish (Gil, 2002; Reda et al., 2018). Inflammation occurs as an important component of the innate immune responses. Therefore, inflammatory cytokines are often used as biomarkers of immune regulation (Safari et al., 2016). Fish cytokines can be classified into anti-inflammatory factors (such as IL-10 and TGF-β1) and pro-inflammatory factors (such as TNF-α, IL-1β, IL-15 and IL-8), which have important functions in the immune response. Enterocolitis decreases the expression of anti-inflammatory factors and increases the expression of pro-inflammatory factors, so they can indicate inflammatory damage at the molecular level (Song et al., 2014; Fcab et al., 2019). Dietary medical plants and probiotics can regulate fish intestinal innate immunity by promoting anti-inflammatory factors and reducing the expression of pro-inflammatory factors, thus strengthening the resistance of fish to disease-causing agents and thus slowing down inflammation (Panigrahi et al., 2007; Nootash et al., 2013; Feng et al., 2019; Vazirzadeh et al., 2019; Ahmadifar et al., 2022). In this study, it was found that feeding FT can reduce the mRNA relative expression levels of pro-inflammatory factors IL-1β, IL-15, TNF-α and IL-8, and increase the mRNA relative expression levels of anti-inflammatory factors IL-10 and TGF-β1 in juvenile largemouth bass. Therefore, the appropriate amount of FT can regulate the inflammatory state in the intestine of juvenile Largemouth bass after infection with *A.hydrophila*, thereby reducing the intestinal damage caused by *A.hydrophila*.

5 Conclusion

In this research, we found that the addition of a certain amount of FT could improve the growth performance and antioxidant capacity of juvenile largemouth bass, improve intestinal health, and increase resistance to *A.hydrophila*. A comprehensive analysis of this experiment showed that 2% FT addition was more effective.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

This trial was approved by the Southwest University of Science and Technology in China, Institutional Animal Care and Use Committee. All of the procedures were performed by the Declaration of Helsinki and relevant policies in China.

Author contributions

LJ: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. XZ: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. JY: Conceptualization, Methodology, Investigation, Writing – review and editing. SB: Formal analysis, Data curation, Investigation. JL: Data curation, Investigation. QW: Data curation, Investigation. MW: Investigation. YW: Investigation, Supervision, Funding acquisition. BL: Investigation, Writing – review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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