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Effects of cadmium on the growth, muscle composition, digestion, gene expression of antioxidant and lipid metabolism in juvenile tilapia (*Oreochromis niloticus*)

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Cadmium could induce various degrees of harm to aquatic organisms. A 30-day feeding trial was conducted to investigate the effects of cadmium on growth, muscle composition, digestive enzyme activity, gene expression of antioxidants and lipid metabolism in juvenile genetic improvement of farmed tilapia (GIFT, *Oreochromis niloticus*, Initial weight: 21.36 ± 0.24 g). Four cadmium concentrations of aquaculture water were designed: 0, 0.2, 0.4, and 0.6 mg/L Cd^{2+}. The main results are as follows: Compared with the control group (0 mg/L Cd^{2+}), the weight gain rate (WGR), specific growth rate (SGR), daily growth index (DGI), and spleen index (SI) of juvenile GIFT under cadmium stress were significantly decreased ($p < 0.05$). The contents of crude protein and crude lipid in muscle were significantly decreased ($p < 0.05$), and the ash was significantly increased ($p < 0.05$). The activities of trypsin, lipase, and α-amylase in the intestinal were significantly decreased ($p < 0.05$). The relative expression levels of carnitine palmityl transferase 1 (*cpt-1*), peroxisome proliferator-activated receptor α (*pparα*), *pparγ*, hormone-sensitive lipase (*hsl*), lipoprotein lipase (*lpl*), malate dehydrogenase (*mdh*), leptin (*lep*), fatty acid synthetase (*fas*), cholesterol response element binding protein 1 (*srebp1*), squalene cyclooxygenase (*sqle*), and stearoyl-CoA desaturase (*scd*) genes in liver were significantly decreased ($p < 0.05$). The relative expression levels of catalase (*cat*), superoxide dismutase (*sod*), glutathione S-transferase (*gst*), and glutathione peroxidase (*gsh-px*) genes in the liver were significantly decreased ($p < 0.05$). In conclusion, exposure to cadmium stress could impact growth, muscle composition, digestive enzyme activity, gene expression of antioxidant and lipid metabolism in juvenile GIFT.

KEYWORDS

growth, antioxidant, lipid metabolism, cadmium, tilapia

1 Introduction

The heavy metal cadmium (Cd) is a prevalent environmental pollutant and a potential toxin with detrimental health effects on aquatic organisms (Johri et al., 2010). Cadmium has been identified as a prominent environmental contaminant and non-essential metal in China, exerting harmful impacts on organisms even at low concentrations (Chellaiah, 2018). According to the national standards of China, the concentration of cadmium in aquaculture water should not exceed 100 μg/L (Jiang et al., 2014). However, certain fishponds in specific regions of China have been found to have cadmium levels that surpass the regulations set by the China Food Administration (Cheung et al., 2008; Leung et al., 2014). Previous studies have demonstrated that cadmium could induce various degrees of harm to aquatic organisms, including hepatic impairment, reproductive dysfunction, lymphocyte proliferation, cardiovascular system lesions, and oxidative stress-induced damage to the antioxidant defense system (Lee et al., 2023).

The antioxidant system plays a crucial role in mitigating oxidative stress induced by environmental factors. Therefore, ecotoxicological studies commonly employ antioxidant parameters such as catalase (CAT), superoxide dismutase (SOD), glutathione-S-transferase (GST), and glutathione peroxidase (GSH-Px) (Atli et al., 2016). This system aids in detoxifying fish, establishing cellular REDOX potential, and regulating key cellular processes (Da Rocha et al., 2009; Ji and Yeo, 2021). The lipid metabolism of fish is commonly employed to investigate energy metabolism, providing direct insights into lipid deposition and energy balance in fish (Wang et al., 2018). Additionally, it serves as a vital source of essential fatty acids for growth, health, reproduction, and overall physiological functions (Turchini et al., 2009). Research has demonstrated that lipid metabolism plays a pivotal role in nutrient uptake, lipid transportation, and the β-oxidation pathway in fish (Lu et al., 2013; Saito et al., 2021). However, there is a paucity of relevant studies on the impact of cadmium on fish lipid metabolism, necessitating further research to ascertain whether cadmium can induce fish lipid metabolism dysfunction.

Genetic improvement of farmed tilapia (GIFT, *Oreochromis niloticus*) has emerged as a highly cultivated economic fish globally and is recognized as one of the dominant fish species endorsed by the Food and Agriculture Organization of the United Nations. These improvements include large size, rapid growth, high protein content, and strong stress resistance (Munguti et al., 2022; Zhang et al., 2024). With the continuous advancement of tilapia aquaculture, there has been a concurrent rise in the prevalence of heavy metal contamination among tilapia (Younis et al., 2020; Hamouda and Abd Alkareem, 2021). However, there is a scarcity of research investigating the effects of heavy metal pollution on the antioxidant and lipid metabolism of tilapia. Therefore, this study focused on GIFT as the research subject and utilized a laboratory simulation static method to introduce varying doses of cadmium into aquaculture water. The objective was to investigate the effects of cadmium stress on growth performance, muscle composition, digestive enzyme activity, gene expression of antioxidant and lipid metabolism in juvenile GIFT. This study aims to provide scientific references regarding the effects of heavy metal pollution during aquaculture processes.

2 Materials and methods

2.1 Experimental feed and fish

The experimental feed was procured from Nanning Haida Biotechnology Co., LTD., Nanning, China. The crude lipid content of the feed was 3.61 ± 0.15%. The crude protein content of the feed was 33.86 ± 0.68%. The ash content of the feed was 6.93 ± 0.17%, and the moisture content of the feed was 9.34 ± 0.26%.

The juvenile GIFT was purchased from Guangxi ANGUI Aquaculture Co., LTD, Nanning, China. The use of juvenile GIFT for this study was approved by the Ethics Committee of Guangxi Minzu University (Approval number: GXMZU-2022-001).

2.2 Acclimatization and culture

After a 15-minute disinfection period using a 10 mg/L potassium permanganate solution, the juvenile GIFT was acclimated in a laboratory aquaculture system for 14 days. The acclimatization conditions were water temperature with 26 ± 1 °C, pH 7-8, dissolved oxygen content ≥ 6 mg/L, and light-dark cycle of 12 hours each. The juvenile GIFT in each group were fed three times daily at 9:00, 14:00, and 19:00, ensuring that the daily feeding quantity was sufficient until satiation was observed.

After a 14-day acclimatization period, a total of 360 juvenile GIFT with an initial body weight of 21.21 ± 0.24 g were randomly allocated into four experimental groups in triplicate, making a total of 12 tanks with 30 fish in each tank. Each tank had a volume of 720 L (150 cm × 80 cm × 60 cm). The juvenile GIFT was reared under the same conditions as used during the 14-day acclimatization period.

According to the previous experimental findings, the cadmium levels (Cd^{2+}) in the water environment cannot exceed 5 μg/L (Joseph, 2009). The concentration of cadmium in the experimental water was determined to be 40-fold, 80-fold, and 120-fold. Then the concentrations of Cd^{2+} in the four groups were 0 mg/L (control group), 0.2 mg/L, 0.4 mg/L, and 0.6 mg/L, respectively. The cadmium chloride ($CdCl_2$) utilized in this study was of analytical purity and branded as Aldrich, which was procured from Sigma-Aldrich (Shanghai) Trading Co. Ltd., Shanghai, China. During the entire duration of the experiment, all tanks were still water-farmed at the aquaculture systems. Daily feces removal and one-third water exchange were conducted once a day in each tank while maintaining a constant concentration of Cd^{2+} venom by adding the same volume and concentration to each tank. The experimental period lasted for 30 days.

2.3 Sampling

After being starved for 24 h, 9 fish were randomly selected from each experimental group (3 fish per tank). The sampled fish were individually anesthetized using 0.2 g/L ethyl 3-aminobenzoate mesylate (MS-222, Adamas, Shanghai Adamas Reagent Co., Ltd., Shanghai, China). Then their body weight and length were measured individually. The sample fish was placed on ice and a pair of scissors was utilized to carefully remove one side of the fish's muscle, exposing its internal organs completely. The samples of the intestinal, dorsal muscle, and liver were subsequently extracted with meticulous precision using ophthalmic forceps placed in labeled sample bags and stored in liquid nitrogen. After all the samples were collected, they were meticulously preserved at a bone-chilling temperature of -80°C in an ultra-low temperature refrigerator for subsequent analysis.

2.4 Calculation of growth performance

The survival rate (SR), weight gain rate (WGR), specific growth rate (SGR), daily growth index (DGI), body length gain rate (BLG), feed conversion ratio (FCR), condition factor (CF), hepatosomatic index (HSI), and viscera index (VSI) of juvenile GIFT were calculated using the following formula:

$$SR\ (\%) = 100 \times \frac{\text{final amount of fish}}{\text{initial amount of fish}}$$

$$WGR\ (\%) = 100 \times \frac{\text{final body weight (g)} - \text{initial body weight (g)}}{\text{initial body weight (g)}}$$

$$SGR\ (\%/d) = 100 \times \frac{[\ln(\text{final body weight)} \text{(g)}] - [\ln(\text{initial body weight)} \text{(g)}]}{\text{days}}$$

$$DGI\ (\%/d) = 100 \times \frac{[(\text{final body weight)} \text{(g)}]1/3 - [(\text{initial body weight)} \text{(g)}]1/3}{\text{days}}$$

$$BLG\ (\%) = 100 \times \frac{\text{final body length (cm)} - \text{initial body length (cm)}}{\text{initial body length (cm)}}$$

$$FCR = \frac{\text{total feed intake (g)}}{\text{final body weight (g)} - \text{initial body weight (g)}}$$

$$CF\ (\%) = 100 \times \frac{\text{body weight (g)}}{[\text{body length (cm)}]^3}$$

$$HSI\ (\%) = 100 \times \frac{\text{liver weight (g)}}{\text{body weight (g)}}$$

$$VSI\ (\%) = 100 \times \frac{\text{viscera weight (g)}}{\text{body weight (g)}}$$

2.5 Determination of muscle composition

The crude protein, crude lipid, ash, and moisture content of dorsal muscle were determined according to the methods of the Association of Official Analytic Chemists (AOAC, 2005). Briefly, crude protein was determined by the Kjeldahl nitrogen determination method. Crude lipid was determined by the Soxhlet extraction method. Ash was determined by the burning method in a muffle furnace at 550 °C. Moisture was determined by the oven drying constant weight method at 105°C.

2.6 Determination of digestive enzyme activity

The method of Liu Y. et al. (2023) was applied to determine the α-amylase, trypsin, and lipase activity in the intestinal tract of juvenile GIFT. The α-amylase, trypsin, and lipase activity was determined using an ELISA analyzer (RT-6100, Rayto, Shenzhen, China) and assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). All the instruction manuals can be found and downloaded at http://www.njjcbio.com/ (accessed on 10 May 2024).

In simple terms: α-amylase activity was determined by starch-iodine colorimetry method. The unit of α-amylase was defined as the interaction between each milligram of tissue protein and the substrate starch at 37°C for 30 minutes, and every ten milligrams of hydrolyzed starch corresponded to one unit of enzyme activity (U/mgprot). Trypsin activity was determined by the ultraviolet colorimetry method. The unit of trypsin was defined as a change of 0.003 per minute in the absorbance value of the reaction substrate per milligram of tissue protein at pH 8.0 and 37°C, representing one unit of enzyme activity (U/mgprot). Lipase activity was determined by methylation-specific oligonucleotide ligation assay and microplate method. The unit of lipase was defined as per gram of tissue protein reacting with the substrate in the reaction system for 1 minute at 37°C, and every 1 μmol substrate consumed was one unit of enzyme activity (U/gprot).

2.7 Determination of gene expression

The gene expression of carnitine palmityl transferase 1 (*cpt-1*), peroxisome proliferator-activated receptor α (*pparα*), peroxisome proliferator-activated receptor γ (*pparγ*), hormone-sensitive lipase (*hsl*), lipoprotein lipase (*lpl*), malate dehydrogenase (*mdh*), leptin (*lep*), fatty acid synthetase (*fas*), cholesterol response element binding protein 1 (*srebp1*), squalene cyclooxygenase (*sqle*), stearoyl-CoA desaturase (*scd*), catalase (*cat*), superoxide dismutase (*sod*), glutathione S-transferase (*gst*), and glutathione peroxidase (*gsh-px*) in the liver of juvenile GIFT were determined by the real-time quantitative polymerase chain reaction (RT-qPCR) method. The RT-qPCR methods of Zhang et al. were applied (Zhang et al., 2023), and the brief steps were as follows:

First, total RNA was extracted from the liver of juvenile GIFT using the Steady Pure Universal RNA Extraction Kit (Accurate Biology Biotechnology Engineering Ltd., Changsha, China) and assessed for concentration and purity by measuring absorbance ratios at 260:280 nm with an ND-2000 spectrophotometer (Thermo, Waltham, MA, USA).

Second, the reverse transcription of 1000 ng total RNA into cDNA was performed using the Evo M-MLV reverse transcription kit (Accurate Biology Biotechnology Engineering Ltd., Changsha, China). The process involved incubation at 30 °C for 10 min, followed by incubation at 42 °C for 15 min, a final step at 95 °C for 5 min, and cooling to 5 °C for an additional 5 min.

Third, the forward and reverse primers of *cpt-1, pparα, pparγ, hsl, lpl, mdh, lep, fas, srebp1, sqle, scd, cat, sod, gst,* and *gsh-px* genes in the liver of juvenile GIFT were designed based on the mRNA sequences of tilapia available in the National Center for Biotechnology Information (NCBI) database, and synthesized by Shanghai Sangon Bioengineering Technology Service Co., LTD, Shanghai, China. *β-actin* was employed as the internal reference gene. Detailed primer information is provided in Table 1.

Fourth, the RT-qPCR assays were conducted using a LightCycler 96 RT-qPCR Detection System (Roche, Basel, Switzerland) and the TB Green Premix Ex TaqTM II (Tli RNaseH Plus) RT-qPCR kit produced by Takara Biomedical Technology (Beijing) Co., Ltd., Beijing, China. The thermal cycling conditions for the RT-qPCR reactions included an initial denaturation step at 95 °C for 10 s followed by amplification with 40 cycles consisting of denaturation at 95 °C for 60 s; annealing at 60 °C for 30 s; and extension at 72 °C for 90 s. The specificity of the reactions was confirmed by analyzing the melting curve obtained during heating to 95 °C.

The $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak, 2008) was applied to calculate the relative expression levels of *cpt-1, pparα, pparγ, hsl, lpl, mdh, lep, fas, srebp1, sqle, scd, cat, sod, gst,* and *gsh-px* genes in the liver of juvenile GIFT.

2.8 Data analyses

The data underwent initial processing in Microsoft Excel 2023 (Version number: 16.78 [23100802], Microsoft Corporation, Washington, WA, USA). Subsequently, a one-way analysis of variance (ANOVA) was performed using the IBM SPSS 26 software package (International Business Machines Corporation, Armonk, NY, USA). Normality and homogeneity of variances among the groups were evaluated before conducting the statistical tests. For comparisons between groups, Duncan's multiple-range test was utilized. Statistical significance was set at $p < 0.05$ level and results are reported as mean ± standard error (mean ± SE).

3 Results

3.1 Effects of cadmium on the growth performance of juvenile GIFT

Compared to the control group (0 mg/L Cd^{2+}), the weight gain rate (WGR), specific growth rate (SGR), and daily growth index (DGI) of juvenile GIFT significantly decreased ($p < 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the lowest WGR, SGR, and DGI, which were significantly lower

($p < 0.05$) than those in the 0.2 and 0.4 mg/L Cd^{2+} groups, as shown in Table 2.

Compared to the control group (0 mg/L Cd^{2+}), the feed conversion ratio (FCR), condition factor (CF), hepatosomatic index (HSI), and viscera index (VSI) of juvenile GIFT significantly increased ($p < 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.4 mg/L Cd^{2+} group exhibited the highest CF, but no significant difference ($p > 0.05$) was observed compared to the 0.2 and 0.6 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the highest HSI and FCR, which were significantly higher ($p < 0.05$) than those in the 0.2 mg/L Cd^{2+} group, but no significant difference ($p > 0.05$) was observed compared to the 0.4 mg/L Cd^{2+} group. The 0.6 mg/L Cd^{2+} group exhibited the highest VSI, but no significant difference ($p > 0.05$) was observed compared to the 0.2 and 0.4 mg/L Cd^{2+} groups, as shown in Table 2.

Compared to the control group (0 mg/L Cd^{2+}), the final weight and survival rate (SR) of juvenile GIFT significantly decreased ($p < 0.05$) in the 0.6 mg/L Cd^{2+} group. However, there was no significant difference ($p > 0.05$) between 0, 0.2, and 0.4 mg/L Cd^{2+} groups, as shown in Table 2.

There was no significant difference ($p > 0.05$) between 0, 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups on the body length gain rate (BLG), as shown in Table 2.

The relationship between cadmium levels and final weight, WGR, SGR, DGI, BLG, CF, HSI, VSI, FCR, and SR of juvenile GIFT was shown in Figures 1-10 based on a linear regression analysis, in which the order of coefficient of determination (R^2) from largest to smallest was: R^2 (FCR) > R^2 (SR) > R^2 (SGR) > R^2 (final weight) > R^2 (WGR) > R^2 (DGI) > R^2 (BLG) > R^2 (HSI) > R^2 (VSI) > R^2 (CF).

3.2 Effects of cadmium on the muscle composition of juvenile GIFT

Compared to the control group (0 mg/L Cd^{2+}), the content of crude protein and crude lipid in the muscle of juvenile GIFT significantly decreased ($p < 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.4 mg/L Cd^{2+} group exhibited the lowest crude protein content, which was significantly lower ($p < 0.05$) than that in the 0.2 mg/L Cd^{2+} group, but no significant difference ($p > 0.05$) was observed compared to 0.6 mg/L Cd^{2+} group. The 0.6 mg/L Cd^{2+} group exhibited the lowest crude lipid content, but no significant difference ($p > 0.05$) was observed compared to 0.2 and 0.4 mg/L Cd^{2+} groups, as shown in Table 3.

Compared to the control group (0 mg/L Cd^{2+}), the content of ash in the muscle of juvenile GIFT significantly decreased ($p < 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the highest ash content, but no significant difference ($p > 0.05$) was observed compared to 0.2 and 0.4 mg/L Cd^{2+} groups, as shown in Table 3.

However, there was no significant difference ($p > 0.05$) between 0, 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups on the moisture content in the muscle of juvenile GIFT, as shown in Table 3.

TABLE 1 The forward and reverse primers of genes for RT-qPCR.

Gene	Primer sequence (5′→3′)	Amplicon size (bp)	Gene Bank
β-actin[1]	F: TGACCCAGATCATGTTTGAGACC	146	XM_031811226.1
	R: CTCGTAGATGGGTACTGTGTGGG		
pparα[2]	F: GTGGCTGCTATTATCTGCTGTGGAG	140	XM_019346353.2
	R: CTGGGGAAAAGGAAGGTGTCATCTG		
mdh[3]	F: GGTGCTCGCTTCTTGTGGACAG	121	XM_005450070.4
	R: GACGGCCTCATTCTCATCTTCTTCC		
cpt-1[4]	F: ATTGGCAGGACAGCGACTACATTG	143	XM_019362661.2
	R: GGAAGGAGGTGAAGGGTCATCTAGG		
lpl[5]	F: CTTCAGCCAGAACCAGCAGAGC	142	NM_001279753.1
	R: GTCGGTGGTGATGAGGAAGGATTG		
lep[6]	F: GAAGTGGATCGCTGAGCATCTGG	129	XM_005449522.4
	R: CCATCCAAGCAGACCGTGACTATG		
hsl[7]	F: CAAGCGGCATCAGTCAGGAATAGG	80	XM_005463937.4
	R: CTCAACTCGGGGTCAATGGCATAC		
pparγ[8]	F: GTACACGGAGGCTACACGGAAAC	139	XM_019358463.2
	R: CTGCTTCTGCTGAACGAGACTGAC		
fas[9]	F: AAGCCTTGTGTGCCTTCATCCAG	133	XM_003454056.5
	R: TCCCTGTGAGCGGAGGTGATTAG		
srebp1[10]	F: GAACAGCAGCCGACAGATCACTC	116	XM_005473610.4
	R: TACAGCAGCCATTAACGAGCAAGTC		
sqle[11]	F: CTGACGGGAGGAGGGATGAGTG	82	XM_003453510.5
	R: CATACAGGTCGGGAATGCTCTTGAG		
scd[12]	F: ACAAGCTCTCCGTGCTGGTCAT	102	XM_005471382.2
	R: GCAGAGTTGGGACGAAGTAGGC		
cat[13]	F: TGAATGAGGAGGAGCGACAGAGAC	118	XM_003447521.5
	R: CCATAGTCTGGATGCACAGCCTTC		
sod[14]	F: TCCAGCCTGCCCTCAAGTTTAATG	121	XM_003449940.5

(Continued)

TABLE 1 Continued

Gene	Primer sequence (5'→3')	Amplicon size (bp)	Gene Bank
gst[15]	R: TCCCGTTTGATTGCCTCCATTAGC		
	F: CACCCCAGATCCCAAACCCAAAC	140	XM_025897213.1
	R: CAACAAGCAGCACATCAGCAAGG		
gsh-px[16]	F: AACTTCCATTCCCCTGCGATGATG	123	NM_001279711.1
	R: CGTCAGGACCAACCAGGAACTTC		

F, Forward primer; R, Reverse primer; β-actin, Internal reference gene; [2]pparα, Peroxisome proliferator-activated receptor α; [3]mdh, Malate dehydrogenase; [4]cpt-1, Carnitine palmityl transferase 1; [5]lpl, Lipoprotein lipase; [6]lep, Leptin; [7]hsl, Hormone-sensitive lipase; [8]pparγ, Peroxisome proliferator-activated receptor γ; [9]fas, Fatty acid synthetase; [10]srebp1, Cholesterol response element binding protein 1; [11]sqle, Squalene cyclooxygenase; [12]scd, Stearoyl-CoA desaturase; [13]cat, Catalase; [14]sod, Superoxide dismutase; [15]gst, Glutathione S-transferase; [16]gsh-px, Glutathione peroxidase.

3.3 Effects of cadmium on the digestive enzyme activity of juvenile GIFT

Compared to the control group (0 mg/L Cd^{2+}), the α-amylase and lipase activity in the intestinal of juvenile GIFT significantly decreased ($p < 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the lowest α-amylase activity, which was significantly lower ($p < 0.05$) than that in the 0.2 mg/L Cd^{2+} group, but no significant difference ($p > 0.05$) was observed compared to 0.4 mg/L Cd^{2+} group. The 0.6 mg/L Cd^{2+} group exhibited the lowest lipase activity, which was significantly lower ($p < 0.05$) than that in the 0.2 and 0.4 mg/L Cd^{2+} groups, as shown in Table 4.

Compared to the control group (0 mg/L Cd^{2+}), the trypsin activity in the intestinal of juvenile GIFT significantly increased ($p < 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the highest trypsin activity, which was significantly higher ($p < 0.05$) than that in the 0.2 mg/L Cd^{2+} group, but no significant difference ($p > 0.05$) was observed compared to 0.4 mg/L Cd^{2+} group, as shown in Table 4.

3.4 Effects of cadmium on the gene expression of lipid metabolism of juvenile GIFT

Compared to the control group (0 mg/L Cd^{2+}), the expression levels of peroxisome proliferator-activated receptor α (pparα), malate dehydrogenase (mdh), carnitine palmityl transferase 1 (cpt-1), lipoprotein lipase (lpl), leptin (lep), hormone-sensitive lipase (hsl), peroxisome proliferator-activated receptor γ (pparγ), fatty acid synthetase (fas), cholesterol response element binding protein 1 (srebp1), stearoyl-CoA desaturase (scd), and squalene cyclooxygenase (sqle) genes in the liver of juvenile GIFT significantly decreased ($p < 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the lowest expression levels of cpt-1, pparα, mdh, pparγ, hsl, lep, fas, srebp1, sqle, and scd, which were significantly lower ($p < 0.05$) than those in the 0.2 and 0.4 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the lowest expression levels of lpl, which was significantly lower ($p < 0.05$) than that in the 0.2 mg/L Cd^{2+} group, but no significant difference ($p > 0.05$) was observed compared to 0.4 mg/L Cd^{2+} group, as shown in Figure 11.

3.5 Effects of cadmium on the gene expression of antioxidant of juvenile GIFT

Compared to the control group (0 mg/L Cd^{2+}), the expression levels of catalase (cat) and superoxide dismutase (sod) genes in the liver and gills of juvenile GIFT significantly decreased ($p < 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the lowest expression levels of cat and sod, which were significantly lower ($p < 0.05$) than those in the 0.2 and 0.4 mg/L Cd^{2+} group, as shown in Figure 12.

TABLE 2 Effects of cadmium on the growth performance of juvenile GIFT.

Index	Cadmium levels (mg/L)				F-value	p-value
	0	0.2	0.4	0.6		
Initial weight (g)	21.36 ± 0.24	21.36 ± 0.24	21.36 ± 0.24	21.36 ± 0.24	/	/
Final weight (g)	60.43 ± 1.22[a]	56.63 ± 2.56[ab]	56.6 ± 3.31[ab]	52.55 ± 0.35[b]	2.159	0.171
WGR[1] (%)	183.99 ± 8.24[a]	164.35 ± 3.16[b]	164.67 ± 2.62[b]	145.41 ± 3.18[c]	31.369	0.000
SGR[2] (%/d)	3.62 ± 0.08[a]	3.25 ± 0.06[b]	3.15 ± 0.02[b]	3.02 ± 0.05[c]	62.099	0.000
DGI[3] (%/d)	3.90 ± 0.06[a]	3.51 ± 0.03[b]	3.50 ± 0.04[c]	3.26 ± 0.06[d]	87.274	0.000
BLG[4] (%)	40.26 ± 2.30	39.66 ± 0.82	39.05 ± 1.72	36.70 ± 2.75	1.773	0.303
CF[5] (%)	2.07 ± 0.51[b]	3.46 ± 0.21[a]	3.49 ± 0.10[a]	3.34 ± 0.23[a]	15.327	0.010
HSI[6] (%)	0.55 ± 0.07[c]	1.35 ± 0.18[b]	1.63 ± 0.07[a]	1.71 ± 0.06[a]	72.453	0.000
VSI[7] (%)	10.68 ± 0.27[b]	11.96 ± 0.17[a]	11.92 ± 0.35[a]	12.40 ± 1.10[a]	4.611	0.037
FCR[8]	1.52 ± 0.02[c]	1.7 ± 0.02[b]	1.92 ± 0.03[a]	1.97 ± 0.02[a]	75.293	0.000
SR[9] (%)	100 ± 0[a]	98.89 ± 1.11[a]	97.78 ± 1.11[a]	94.44 ± 1.11[b]	6.226	0.017

[1]WGR, Weight gain rate; [2]SGR, Specific growth rate; [3]DGI, Daily growth index; [4]BLG, Body length gain rate; [5]CF, Condition factor; [6]HSI, Hepatosomatic index; [7]VSI, Viscera index; [8]FCR, Feed conversion ratio; [9]SR, Survival rate. All the above data are mean ± SE (n = 3). Different superscript letters in the same row indicate significant differences among the data (p< 0.05).

Compared to the control group (0 mg/L Cd^{2+}), the expression levels of glutathione S-transferase (*gst*) and glutathione peroxidase (*gsh-px*) genes in the liver of juvenile GIFT significantly decreased ($p< 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the lowest expression levels of *cat* and *sod*, which were significantly lower ($p< 0.05$) than those in the 0.2 and 0.4 mg/L Cd^{2+} group, as shown in Figure 12.

Compared to the control group (0 mg/L Cd^{2+}), the expression levels of *gst* and *gsh-px* genes in the gills of juvenile GIFT significantly increased ($p< 0.05$) in the 0.2, 0.4, and 0.6 mg/L Cd^{2+} groups. The 0.6 mg/L Cd^{2+} group exhibited the highest expression levels of *gst*, which was significantly higher ($p< 0.05$) than that in the 0.2 and 0.4 mg/L Cd^{2+} group. The 0.6 mg/L Cd^{2+} group exhibited the highest expression levels of *gsh-px*, which was significantly higher ($p< 0.05$) than that in the 0.2 mg/L Cd^{2+} group, but no significant difference ($p > 0.05$) was observed compared to 0.4 mg/L Cd^{2+} group, as shown in Figure 12.

4 Discussion

The growth performance is a crucial indicator that reflects the health and growth status of fish (Zhang et al., 2023). The results of this study demonstrated that cadmium could reduce the growth performance of juvenile GIFT. This may be because: First, the cadmium in aquaculture water leads to a reduction in fish appetite and feed intake, resulting in a decrease in the final body weight, specific growth rate, and weight gain rate of fish (Khanh et al., 2022). Heydarnejad et al. conducted an experiment where rainbow trout (*Oncorhynchus mykiss*) were subjected to sub-lethal concentrations (1 and 3 μg/L) of cadmium, and it was observed that the rainbow trout exhibited a decrease in appetite and a significant decline in feeding activity as the concentration of cadmium increased (Heydarnejad et al., 2013). The study of Drąg-Kozak et al. revealed the presence of anorexia in Prussian carp (*Carassius gibelio* Bloch) when exposed to
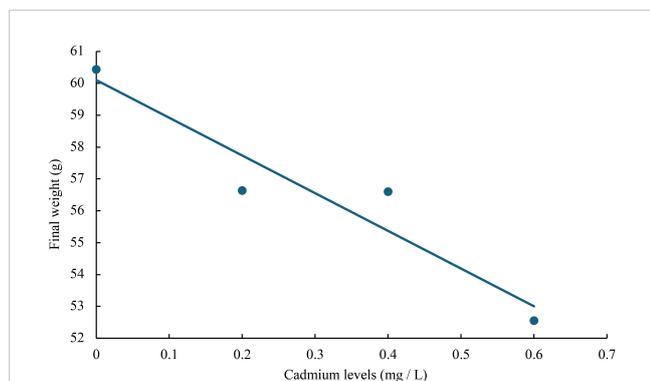

FIGURE 1
Relationship between cadmium levels and final weight of juvenile GIFT based on a linear regression analysis, in which Y = -11.835X + 60.103, R^2 = 0.9018.
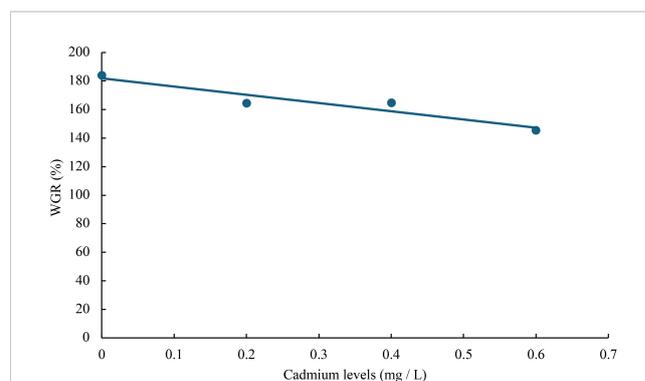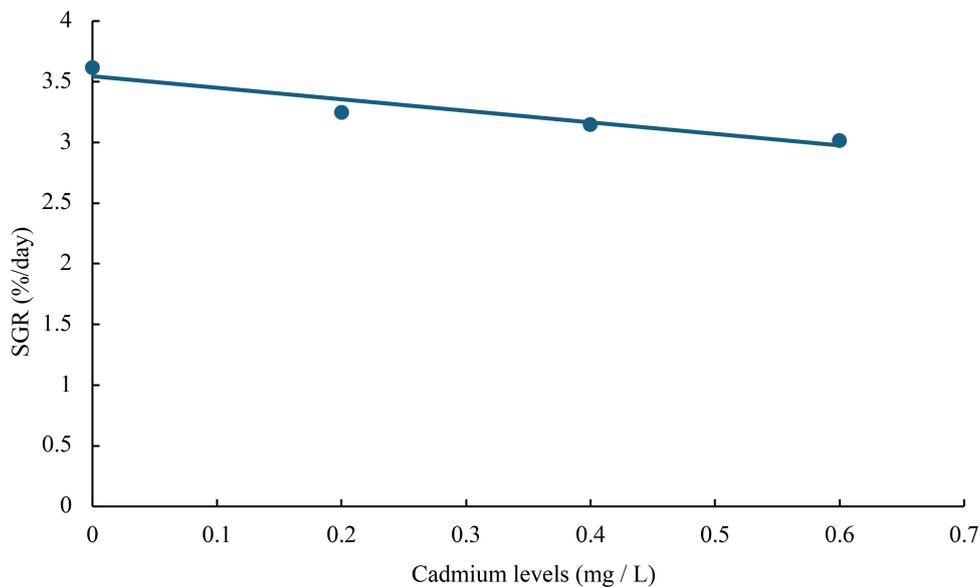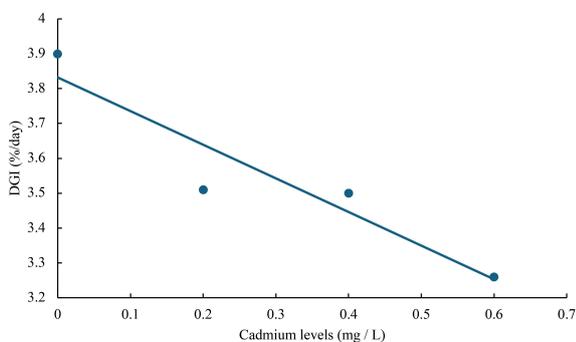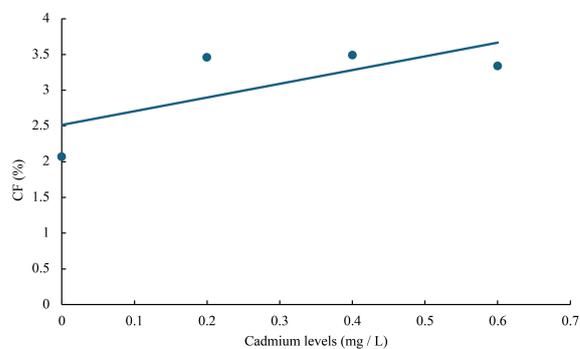

FIGURE 2
Relationship between cadmium levels and WGR of juvenile GIFT based on a linear regression analysis, in which Y = -57.71X + 181.92, R^2 = 0.8949.

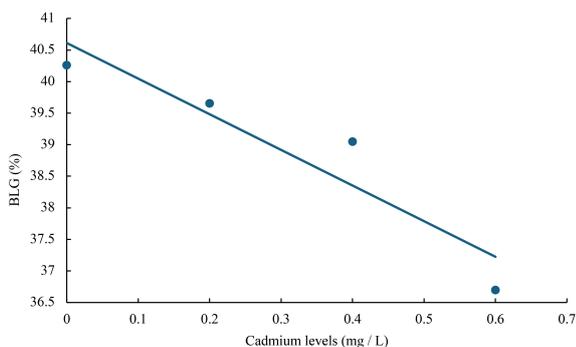FIGURE 3
Relationship between cadmium levels and SGR of juvenile GIFT based on a linear regression analysis, in which Y = -0.95X + 3.545, R^2 = 0.9052.
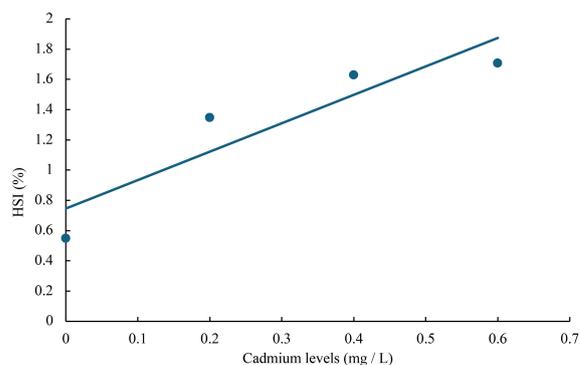

FIGURE 4
Relationship between cadmium levels and DGI of juvenile GIFT based on a linear regression analysis, in which Y = -0.965X + 3.832, R^2 = 0.8849.

FIGURE 6
Relationship between cadmium levels and CF of juvenile GIFT based on a linear regression analysis, in which Y = 1.92X + 2.514, R^2 = 0.5267.

FIGURE 5
Relationship between cadmium levels and BLG of juvenile GIFT based on a linear regression analysis, in which Y = -5.645X + 40.611, R^2 = 0.8744.

FIGURE 7
Relationship between cadmium levels and HSI of juvenile GIFT based on a linear regression analysis, in which Y = 1.88X + 0.746, R^2 = 0.8399.
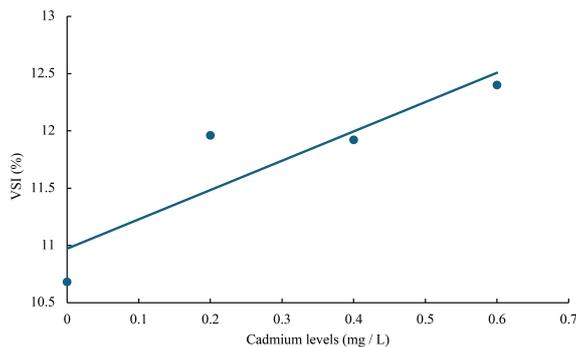
FIGURE 8
Relationship between cadmium levels and VSI of juvenile GIFT based on a linear regression analysis, in which Y = 2.56X + 10.972, R^2 = 0.7992.
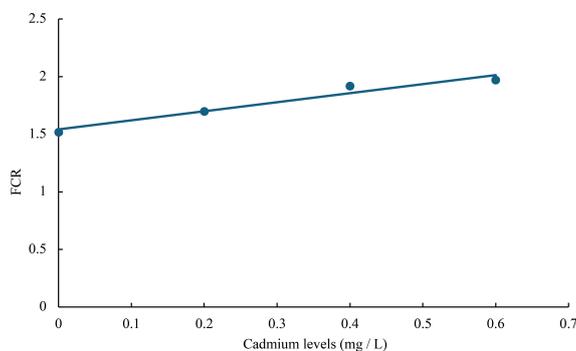

FIGURE 9
Relationship between cadmium levels and FCR of juvenile GIFT based on a linear regression analysis, in which Y = 0.785X + 1.542, R^2 = 0.9504.

cadmium (Drąg-Kozak et al., 2021). Second, cadmium stress could induce inflammation in the viscera of fish, lesions in liver tissue, enlargement of liver cells, and dilation of capillaries. As a result, there is a significant increase in the hepatosomatic index and viscera index of fish (Abdel-Tawwab et al., 2024). Ahmed et al. discovered that exposure to heavy metals, such as lead and cadmium, during their investigation of the histological lesions of freshwater climbing perch (*Anabas testudineus*), resulted in hepatomegaly, obesity, and a pronounced inflammatory response (Ahmed et al., 2014). Third, cadmium stress could induce severe intestinal inflammation and reduce the activity of digestive enzymes, thereby impairing the absorption and utilization of feed by fish (Heydarnejad et al., 2013; Ayyat et al., 2017). The study of Fazio et al. revealed that catfish (*Mystus seenghala*) exhibited a significant reduction in feed absorption and an increase in feed conversion ratio under cadmium stress conditions (Fazio et al., 2022). In addition, cadmium stress could affect the health of fish, resulting in decreased disease
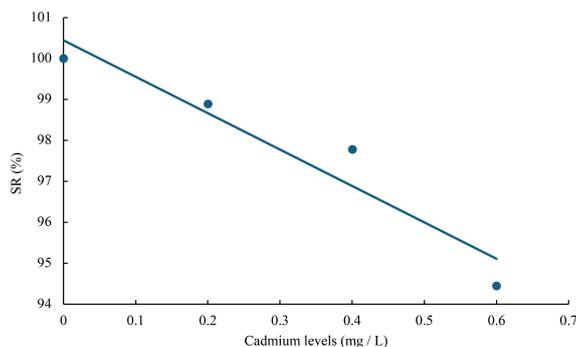

FIGURE 10
Relationship between cadmium levels and FCR of juvenile GIFT based on a linear regression analysis, in which Y = –8.895X + 100.45, R^2 = 0.9138.

TABLE 3 Effects of cadmium on the muscle composition of juvenile GIFT.

Index	Cadmium levels (mg/L)				F-value	p-value
	0	0.2	0.4	0.6		
Moisture (%)	71.17 ± 1.53	71.67 ± 1.31	72.02 ± 1.37	72.18 ± 1.24	1.285	0.344
Crude protein (%)	21.72 ± 0.15[a]	21.29 ± 0.20[b]	20.42 ± 0.27[c]	20.71 ± 0.33[c]	64.050	0.000
Crude lipid (%)	6.37 ± 0.11[a]	5.38 ± 0.19[b]	5.35 ± 0.13[b]	5.28 ± 0.11[b]	165.520	0.000
Ash (%)	1.47 ± 0.03[b]	1.88 ± 0.01[a]	1.79 ± 0.02[a]	1.96 ± 0.04[a]	1231.310	0.000

All above data are mean ± SE (n = 3). Different superscript letters in the same row indicate significant differences among the data (p< 0.05).

resistance and stress resistance, and significantly reduced the survival rate (Hansen et al., 2002).

In this study, cadmium stress significantly reduced the content of crude protein and crude lipid, and significantly increased the content of ash in the muscle of juvenile GIFT, indicating that cadmium stress could affect the muscle composition of fish. This may be because: First, under cadmium stress, juvenile tilapia could generate a substantial quantity of reactive oxygen species (ROS), including anions, hydroxyl radicals, and hydrogen peroxide (Hu et al., 2021). Cadmium could induce oxidative stress in the fish via ROS, leading to heightened physical energy expenditure, increased respiratory rate, and accelerated energy metabolism rate in the fish. Consequently, this diminished nutrient absorption and utilization by the fish (Abdel-Tawwab et al., 2012). The content of crude protein and crude lipid was significantly reduced. Similar results were observed in yellow catfish (*Pelteobagrus fulvidraco*) and Nile tilapia (*Oreochromis niloticus*), where exposure to cadmium significantly decreased the content of crude lipid and crude protein of the fish (Abdel Tawwab and Wafeek, 2010; Tan et al., 2010). Second, the increase in heavy metal content in water leads to the excessive absorption of heavy metal elements by fish bodies (Pandey and Madhuri, 2014). However, these heavy metal elements could not be effectively metabolized within the fish bodies, resulting in an elevation of inorganic salt content and subsequently leading to an increase in ash content (Tan et al., 2010). Similar studies have demonstrated that the presence of cadmium in aquatic environments leads to an increase in the ash content of tilapia (Abdel Tawwab and Wafeek, 2010). The ash content of juvenile tilapia exposed to heavy metals exhibited a significantly higher level compared to that of juvenile tilapia cultured in unpolluted aquatic environments (Abdel-Tawwab et al., 2017).

The activities of trypsin, α-amylase, and lipase in the intestine can serve as indicators of the digestive efficiency of fish (Zaki et al., 2023).

The findings of this study demonstrated a positive correlation between cadmium concentration in the water and trypsin activity in the intestinal of juvenile GIFT, while α-amylase and lipase activities exhibited a significant decrease. These results indicated that cadmium stress could significantly impact the digestive efficiency of the fish. This may be because: First, the binding of cadmium to thiol groups in the active center of digestive enzymes, specifically cysteine residues, leads to inhibition of lipase and amylase activities (Wu et al., 2014). Second, cadmium stress could induce oxidative stress within the intestinal of fish, leading to impairment of both the antioxidant and immune systems. This subsequently resulted in necrosis of the intestinal tissue structure, ultimately causing a downregulation in digestive enzyme activity (Ferain et al., 2021). Attia et al. investigated the barrier effect of cadmium on intestinal tissues and observed that cadmium disrupted the metabolic processes in these tissues, leading to structural damage within the intestines (Attia et al., 2022). Third, in cadmium-stressed environments, trypsin activity in fish was upregulated to facilitate food digestion and provide energy (Wu et al., 2013). Moreover, trypsin could activate protease-activated receptor 2 (PAR-2) through specific small interfering RNA (siRNA), thereby inducing the release of the inflammatory factor interleukin 8 (IL-8) and causing intestinal inflammation in fish (Larsen et al., 2008). Similar studies have demonstrated that the long-term exposure of three-spined stickleback (*Gasterosteus aculeatus*) to cadmium significantly decreased the activity of digestive enzymes in the intestine (Hani et al., 2018). When investigating the impact of dietary cadmium on the aquatic bird (*Cairina moschata*) lipid metabolism and storage, Lucia et al. discovered that cadmium effectively hindered fatty acid synthesis, leading to a reduction in lipase activity (Lucia et al., 2010). The study of Silvestre et al. investigated the differential protein expression of Chinese mitten crab (*Eriocheir sinensis*) in response to cadmium exposure, revealing

TABLE 4 Effects of cadmium on the digestive enzyme activity of juvenile GIFT.

Index	Cadmium levels (mg/L)				F-value	p-value
	0	0.2	0.4	0.6		
α-amylase (U/mgprot)	0.34 ± 0.02[a]	0.30 ± 0.02[b]	0.26 ± 0.02[c]	0.24 ± 0.02[c]	11.222	0.003
Trypsin (U/mgprot)	697.51 ± 23.36[c]	1592.97 ± 73.29[b]	2013.74 ± 16.41[a]	2043.31 ± 28.07[a]	149.416	0.000
Lipase (U/gprot)	7.86 ± 0.22[a]	5.88 ± 0.16[b]	5.28 ± 0.27[c]	4.85 ± 0.16[d]	123.125	0.000

All above data are mean ± SE (n = 3). Different superscript letters in the same row indicate significant differences among the data (p< 0.05).
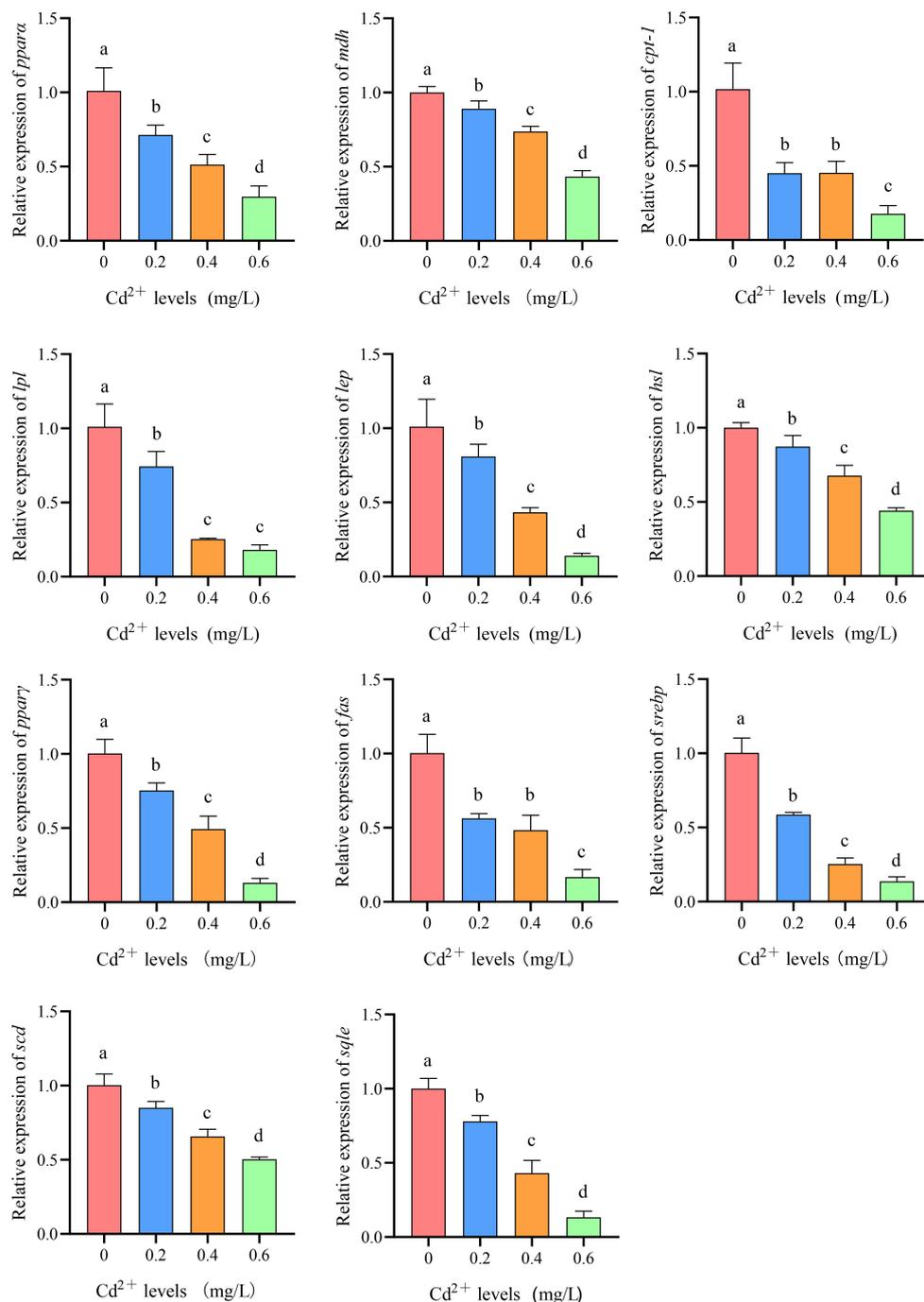
FIGURE 11
Effects of cadmium on the expression levels of peroxisome proliferator-activated receptor α (*pparα*), malate dehydrogenase (*mdh*), carnitine palmityl transferase 1 (*cpt-1*), lipoprotein lipase (*lpl*), leptin (*lep*), hormone-sensitive lipase (*hsl*), peroxisome proliferator-activated receptor γ (*pparγ*), fatty acid synthetase (*fas*), cholesterol response element binding protein 1 (*srebp1*), stearoyl-CoA desaturase (*scd*), and squalene cyclooxygenase (*sqle*) genes in the liver of juvenile GIFT. All the above data are mean ± SE (n = 3). Different superscript letters in the figure indicate significant differences among the data ($p < 0.05$).

an upregulation of trypsin levels in *Eriocheir sinensis* following prolonged cadmium exposure (Silvestre et al., 2006).

In this study, compared with the control group, the expression of lipid anabolism and catabolism-related genes in the liver of juvenile GIFT decreased significantly with the increase of cadmium concentration, indicating that cadmium could inhibit the nutrient

metabolism and energy metabolism of the fish. This may be because: First, the deposition of lipids was inhibited under cadmium stress due to the inhibition of both *de novo* synthesis and β-oxidation of fatty acids (Pan et al., 2018). Ferain et al. observed that the relative expression levels of genes involved in lipid metabolism in the liver of rainbow trout, including carnitine palmitoyl transferase 1 (*cpt-1*),
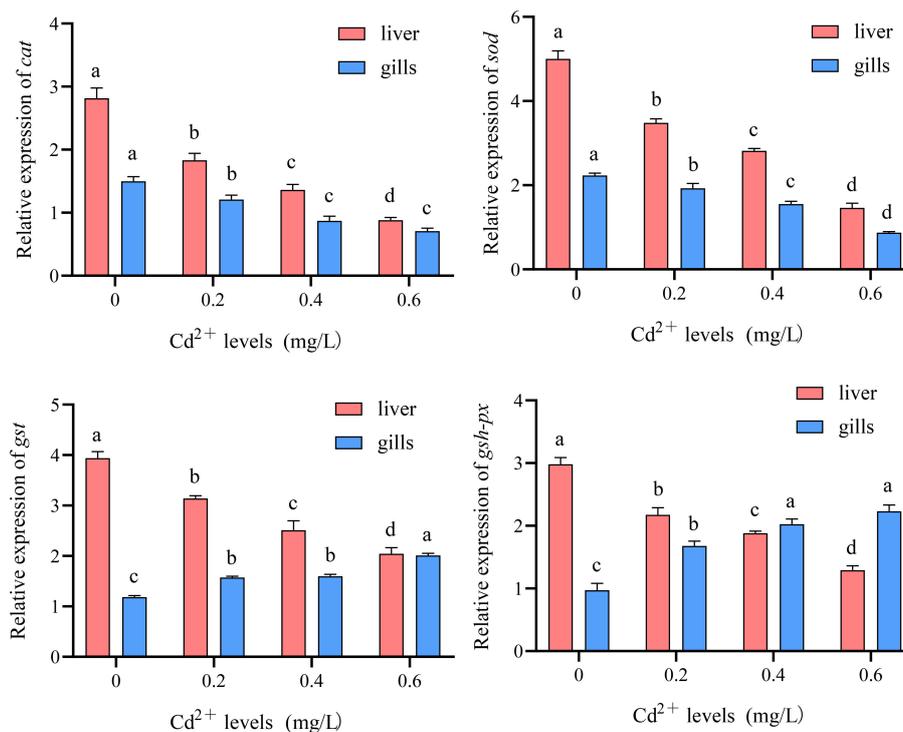
FIGURE 12

Effects of cadmium on the expression levels of catalase (*cat*), superoxide dismutase (*sod*), glutathione S-transferase (*gst*), and glutathione peroxidase (*gsh-px*) genes in the liver and gills of juvenile GIFT. All the above data are mean ± SE (n = 3). Different superscript letters in the figure indicate significant differences among the data (*p* < 0.05).

hormone-sensitive lipase (HSL), and lipoprotein lipase (*lpl*), significantly decreased under cadmium stress (Ferain et al., 2021). Guo et al. demonstrated that environmental cadmium stress had a significant impact on the triglyceride and lipid metabolism pathway of carp, specifically highlighting its disruptive effect on the β-oxidation pathway (Guo et al., 2022). Second, cadmium stress-induced oxidative stress in the liver of fish, resulting in the disruption of liver tissue structure and downregulation of genes associated with lipid metabolism (Shi et al., 2018). Wang et al. demonstrated that cadmium could cause oxidative damage and apoptosis in clam (*Meretrix meretrix*), and mitochondria were the key cell organelles affected by cadmium toxicity (Wang et al., 2021). Third, cadmium could cause oxidative stress in the liver, and then activate the AMPK/PPAR-γ/NF-κB pathway, resulting in the downregulation of peroxisome proliferator-activated receptor α (*pparα*) and *pparγ* gene expression, and finally induce autophagy and apoptosis (Wang et al., 2021). Zhu et al. investigated the effect of cadmium exposure on hepatic lipid metabolism in mice, and they observed that such exposure resulted in dysregulation of the transcriptome associated with lipid metabolism, particularly affecting the PPAR signaling pathway-mediated lipid metabolism pathway, thereby inducing disorders in lipid metabolism (Zhu et al., 2024). Fourth, the presence of Cd^{2+} could compete with Ca^{2+} or other bivalent metal cations, thereby inducing ion imbalances, which could alter oxidative stress levels and lead to mitochondrial dysfunction,

subsequently resulting in dysregulated disruption of lipid metabolic pathways and downregulation of lipid metabolism-related genes (Liu X. et al., 2023). The effects of low-level cadmium exposure on lipid metabolic pathways in mice were investigated by Go et al., who discovered that cadmium regulated lipid synthesis, oxidation, and mitochondrial oxidative phosphorylation in mice exposed to low-level cadmium (Go et al., 2015). Dai et al. discovered that cadmium exposure induced hepatic oxidative stress in carp (*Procypris merus*), resulting in excessive autophagy. Additionally, cadmium competed with bivalent metal ions, leading to mitochondrial dysfunction and downregulation of genes related to lipid metabolism (Dai et al., 2020).

In this study, there was a significant decrease in the relative expression levels of catalase (*cat*) and superoxide dismutase (*sod*) genes in the liver and gills of juvenile GIFT with increasing cadmium concentrations. However, there was a significant decrease in the relative expression levels of glutathione S transferase (*gst*) and glutathione peroxidase (*gsh-px*) genes in the liver, while a significant increase was observed in their relative expression levels in the gills. The results showed that different tissues of tilapia had different responses to cadmium stress. This may be because: First, after prolonged exposure to cadmium stress, fish had a significant increase in the production of ROS that surpassed the body's endogenous antioxidant defense system. Consequently, this leads to profound physiological distress, resulting in irreversible tissue damage and cellular necrosis while concurrently suppressing the expression of antioxidant genes

(Renu et al., 2021; Mukherjee et al., 2022). Second, cadmium stress induced Nrf2 binding and dissociated Nrf2 from Keap1, leading to the translocation of Nrf2 into the nucleus and subsequent suppression of antioxidant gene expression. Simultaneously, the levels of *gsh-px* and *gst* regulated by the Nrf2 signaling pathway were significantly diminished, indicating inhibition of Nrf2 and thereby demonstrating impairment in fish's antioxidant system (Al-sawafi et al., 2017; Ahmatjan et al., 2023). Similar studies showed that: Hu et al. demonstrated a significant down-regulation of the relative expressions of *sod* and *cat* genes in zebrafish (*Danio rerio*) exposed to cadmium stress (Hu et al., 2022). Woo et al. investigated the effect of heavy metals on the antioxidant system of Javanese medaka (*Oryzias javanicus*), and they observed a significant decline in the levels of SOD upon exposure to stressors such as Zn^{2+} and Cd^{2+} (Woo et al., 2009). Wang et al. observed that Cd could alter the molecular conformation of SOD (Wang et al., 2015). Third, the upregulation of *gsh-px* and *gst* in gills may be attributed to the toxic excitatory effect caused by cadmium stress, leading to the emergence of an overcompensation mechanism. This mechanism could trigger the upregulation of antioxidant gene expression to eliminate reactive oxygen species *in vivo* and mitigate oxidative damage (Boyes et al., 2012). A similar study has shown that cadmium stress could trigger an upregulation of *gsh-px* and *gst* levels in the gills of olive flounder (*Paralichthys olivaceus*), which facilitates the elimination of reactive oxygen species (ROS) generated under cadmium stress (Lee et al., 2022).

5 Conclusions

In conclusion, under the experimental conditions, the growth performance, body composition, digestive enzyme activity, expression of lipid metabolism, and antioxidant genes of juvenile GIFT were significantly affected by cadmium stress. Therefore, it is crucial to prioritize water quality protection efforts during this stage of juvenile fish development. Furthermore, the assessment of antioxidant responses and lipid metabolism in tilapia could potentially serve as a biomarker for heavy metal pollution such as cadmium. This finding offers valuable insights for water pollution monitoring.

Data availability statement

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

Ethics statement

The animal study was approved by Biomedical Ethics Committee of Guangxi Minzu University, Nanning, China. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

Funding

Conflict of interest

Publisher's note

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