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Research Article

Different Responses of Histology, Antioxidant, and Inflammation in Gill and Kidney of Yellow Catfish *Pelteobagrus fulvidraco* under Three Dietary Fat Levels

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This experiment investigated the influences of different dietary fat levels on histology, oxidative status, and immune response in gill and kidney of yellow catfish (*Pelteobagrus fulvidraco*). Three diets with different fat levels of 63.1 g/kg (low-fat, LF), 93.3 g/kg (medium-fat, MF), and 153.2 g/kg (high-fat, HF) were prepared to feed yellow catfish. The experiment continued for 56 days, and at the end of the experiment, gill and kidney tissues were sampled. As a result, both gill and kidney showed different degrees of tissue damage in HF group in terms of histology observation. HF increased the malondialdehyde content in gill but showed no effect on kidney, indicating that gill is more susceptible to injury than kidney under high-energy intake conditions. Additionally, HF diet significantly increased the activities of total-superoxide dismutase and catalase to eliminate excess peroxides both in gill and kidney. Moreover, HF diet significantly upregulated the expressions of pro-inflammatory cytokines (*il6* and *tnfα*) and down-regulated the expression of anti-inflammatory cytokines (*il10*), indicating that HF-diet-induced inflammatory response both in gill and kidney. These findings reveal the potential regulatory approach for fish gill and kidney health by dietary fat level, which will help to understand the adverse impacts of dietary lipid imbalance on the health of fish.

1. Introduction

Lipid is a necessary nutrient for fish, which plays an important source of energy and supports various physiological, developmental, and reproductive processes in animals [1]. Numerous studies have suggested that the nutritional conditions are closely related to the immunity of fish [2, 3]. Lipids not only provide energy for fish growth but also stimulate the immune system [4]. Dietary lipid imbalances will greatly influence on the disease resistance of fish, especially during the larval and juvenile stages [3]. So far, the effects of dietary lipid levels are mainly concentrated on growth, liver, and intestinal lipid metabolism [5–7]. However, the relationships between the dietary lipid levels and the health of gill and kidney have received little attention in fish.

Fish gill is considered an important immune organ to resist invasion by pathogenic microorganisms [8]. Kidney is not only an important osmotic regulation organ but also an important immune organ for fish, which plays a critical role in nutrient elements and water absorption, maintaining body fluid concentrations and hematopoiesis [9]. The health of fish gill and kidney mainly depends on their antioxidant, immune function, and structural integrity [10]. Studies reported that dietary lipid deficiency or excess can impair gill and kidney health through decreasing antioxidant function, immune function and structural integrity of grass carp [11, 12]. The antioxidant capacity of fish is mainly regulated by antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD), which act as free radical scavengers associated with oxidative stress [13]. Nrf2/Keap1 pathway plays a key role in maintaining intracellular peroxidation and antioxidant balance [14]. The inflammatory response is regulated by some cytokines like interleukin 8 (IL8), interleukin 6 (IL6), interleukin 10 (IL10), and tumor necrosis factor-alpha (TNFα) [15, 16]. Thus, it is very important to detect these molecular biomarkers to evaluate the health of gill and kidney. So far, the effects of dietary lipid levels on oxidative stress,

Table 1: Feed formulation and proximate analysis of experimental diets.

Ingredients (g/kg)	Low-fat diet	Middle-fat diet	High-fat diet
Casein	350	350	350
Gelatin	20	20	20
White fish meal	100	100	100
Wheat flour	200	200	200
Fish oil	15	30	60
Corn oil	15	30	60
Ascorbyl-2-polyphosphate	10	10	10
NaCl	10	10	10
$CaH_2PO_4{\cdot}2H_2O$	10	10	10
Vitamin mix	5	5	5
Mineral mix	5	5	5
Betaine	10	10	10
Cellulose	250	220	160
Proximate analysis (g/kg of dry matter basis)			
Crude ash	21.9	25.1	25.1
Crude protein	405.4	400.9	393.4
Crude lipid	63.1	93.3	153.2

Vitamin premix (mg or IU/kg diet): retinyl acetate 10,000 IU; cholecalciferol 1,000 IU; all-rac-a-tocopheryl acetate 30 IU; menadione nicotinamide bisulfite 7; thiamine hydrochloride 6; riboflavin 3; pyridoxine hydrochloride 12; D-calcium pantothenate 30; niacin 50; biotin1; folic acid 6; cyanocobalamine 0.03. Mineral mixture (mg/kg diet): $Ca(H_2PO_3)_2{\cdot}H_2O$ 1,000; $FeSO_4{\cdot}7H_2O$ 40; $ZnSO_4{\cdot}7H_2O$ 100; $MnSO_4{\cdot}H_2O$ 40: $CuSO_4{\cdot}5H_2O$ 2; $CaIO_3{\cdot}6H_2O$ 3; Na_2SeO_3 0.05; $CoSO_4$ 0.05.

immune response, and histology of gill and kidney in yellow catfish (*Pelteobagrus fulvidraco*) remained unknown.

Yellow catfish belongs to omnivorous freshwater fish, and it is popularly farmed in China due to its delicious taste and high market value [17]. A total of 0.6 million tons of yellow catfish were produced in China in 2022 [18]. Studies have suggested that the optimal lipid for juvenile yellow catfish is range from 90 to 120 g/kg [5, 19]. When diet lipid contents are over 120 g/kg, the liver will undergo steatosis in yellow catfish [19]. However, with the introduction of intensive aquaculture, a high-fat (HF) diet has been widely applied in aquaculture on account of its low-cost and protein-sparing action [20]. As a result, many negative effects on fish, like metabolic disorders and immune injury, frequently occurred [7, 21], which in turn decreased the disease resistance of fish to pathogens and elevated mortality during fish farming [21]. Therefore, the health status of fish has been regarded as one of the main aspects in aquaculture due to both the increasing importance of the aquaculture industry and the social awareness of animal health [20]. In this study, we explored the effects of dietary lipid levels on antioxidants, inflammation response, and histology of gill and kidney in yellow catfish, which will help to understand the impacts of dietary lipid imbalance on the health status of fish.

2. Materials and Methods

2.1. Feed Preparation. We formulated three diets with 63.1, 93.3, and 153.2 g/kg lipid levels, respectively. Feed formula and nutritional composition are shown in Table 1. The preparation method of feed is based on our previous study in Ling et al. [5]. Fish oil and corn oil (1 : 1, w/w) were used as the lipid sources. First, all feed ingredients were crushed and sieved. They were weighed and mixed according to the formula. Then, slowly added fish oil and corn oil while mixing. Finally, added about 40% of distilled water to mix well and then granulation by a pelleter. Dry the grained feed until the moisture is less than 10%, and store it at −20°C in a freezer.

2.2. Experimental Procedures. Yellow catfish was purchased from a local company in Wuhan (Hubei, China). Before the experiment, all fish were cultured in the indoor storage pond for 2 weeks to acclimation. At the beginning of the experiment, 270 healthy fish with approximate sizes (mean weight: 2.33 ± 0.2 g, mean ± SEM) were randomly placed into nine tanks (300 L), with 30 fish in each tank. Then, each experimental diet was randomly assigned to three tanks. The fish were fed to apparent satiation twice a day (08:00 and 16:00 hr, respectively). The experiment was conducted in a static aquarium system. Continue to inflate the aquarium tank to maintain sufficient dissolved oxygen. The experiment was carried out under a natural photoperiod (light-to-darkness ratio of 12/12 hr). Water temperature was 27.8 ± 0.5°C. Dissolved oxygen, pH, and NH4-N values were 6.01 ± 0.12, 7.81 ± 0.22, and 0.10 ± 0.04 mg/L, respectively.

2.3. Sampling. After 8 weeks of feeding experiment, fish were starved for 24 hr before sampling. All fish were anesthetized with MS-222 in 100 mg/L water. A total of nine fish were randomly selected from each tank, and the gill and trunk kidney (TK) were removed immediately; three fish were sampled for histological analysis, three fish were sampled for the test of enzyme activities, and another three fish were sampled for the analysis of the mRNA level of genes.

2.4. Histological Observation. Histological (hematoxylin/eosin (H&E) staining) analyses were carried out according to the

Table 2: Primers used for quantitative real-time PCR analysis.

Genes	Forward primer (5′–3′)	Reverse primer (5′–3′)	Accession no.
cat	TGCTGGTGAGTCTGGTTCAG	GGATTCCGCTTCTGAGAGTG	XM_027163801
sod1	CCTCAAAGGCACTGGAGAAG	AATCGGCAGTCACATTACCC	XM_027171881
nrf2	GATCTGCAACACTGCCTGAA	TGAAAGCAGCATCGTAGGTG	KX455917
keap1	CCTCACACTGCACAAGCCTA	CCTCCCTCCAACAGCATAAA	XM_027133478
il6	ATGCCTCACCTAGAGCAGGA	GTGAAGCTGTGCAGAATGGA	XM_027176013
il8	ACTGACTGCGATGCTTTGTG	TGTCCTTGGTTTCCTTCTGG	KY218792
tnfa	GAGGCAGATTTCCGAGTCAG	GCCATCGTTGTCCTCGTTAT	XM_027133763
il10	ACCTGGACACCGCACTATTC	ATGGTGTCGATGGGTGTTTT	XM_027144360
β-actin	GGACTCTGGTGATGGTGTGA	CTGTAGCCTCTCTCGGTCAG	EU161066
rpl7	GGCAAATGTACAGGAGCGAG	GCCTTGTTGAGCTTGACGAA	KP938522
hprt	ATGCTTCTGACCTGGAACGT	TTGCGGTTCAGTGCTTTGAT	KP938523
tuba	TCAAAGCTGGAGTTCTCGGT	AATGGCCTCGTTATCCACCA	KP938526
b2m	GCTGATCTGCCATGTGAGTG	TGTCTGACACTGCAGCTGTA	KP938520
ubce	TCAAGAAGAGCCAGTGGAGG	TAGGGGTAGTCGATGGGGAA	KP938524
tbp	AGCAAAGAGTGAGGAGCAGT	ACTGCTGATGGGTGAGAACA	KP938525
gapdh	TTTCAGCGAGAGAGACCCAG	ATGACTCTCTTGGCACCTCC	KP938521
18s rrna	AGCTCGTAGTTGGATCTCGG	CGGGTATTCAGGCGAGTTTG	KP938527
elfa	GTCTGGAGATGCTGCCATTG	AGCCTTCTTCTCAACGCTCT	KU886307

method described in the previous study in our lab [22]. Samples of gill and kidney were fixed for 24 hr in 4% polyformaldehyde. After alcohol gradient dehydration, xylene transparency, paraffin embedding, section, and staining with H&E, they were prepared for light microscopy.

2.5. Antioxidant Enzyme Activity and Malondialdehyde (MDA) Level Analysis. In order to assay the activities of several antioxidant enzymes, 0.2 g gill and kidney samples were homogenized in an ice-cold buffer and centrifuged at 20,000 x *g* at 4°C for 30 min, respectively. The supernatant was collected immediately to test the enzymatic activities. The concentration of soluble protein in the supernatant was determined by the Coomassie brilliant blue method using a Bradford Protein Assay Kit (A045-2-2) from Nanjing Jian Cheng Bioengineering Institute (Nanjing, China). T-SOD activity was determined strictly following the instructions of a commercial kit (S0101S) from Beyotime Biotechnology. CAT activity was measured with a commercial CAT assay kit (A007-2-1) from Nanjing Jian Cheng Bioengineering Institute (Nanjing, China). MDA level was determined using the MDA assay kit (S0131S) from Beyotime Biotechnology.

2.6. mRNA Expression Analysis by (Real-Time Quantitative) PCR. The mRNA levels of (*cat*, *sod1*, *nrf2*, *keap1*, *il6*, *il8*, *tnfα*, and *il10*) were examined by RT-qPCR methods in agreement with our previous study [23]. Total RNA was extracted from the gill and kidney by TRIzol RNA reagent (TaKaRa, Japan). The primers of each gene used are listed in Table 2. They were designed based on the genomic sequences from yellow catfish genome data [24]. A set of 10 housekeeping genes (*β-actin*, *18s rRNA*, *hprt*, *ubce*, *gapdh*, *tuba*, *b2m*, *rpl7*, *tbp*, *and elfa*) were selected in order to test gene transcription stability. Two control genes (*18srrna and b2m*) were regarded as housekeeping genes in gill and (*18srrna and elfa*) were regarded as housekeeping genes in kidney, respectively. The relative expression levels were calculated with the "delta–delta Ct" method [25].

2.7. Statistical Analysis. The experimental data were processed by SPSS 19.0 software, and the results were represented as mean ± SEM. Univariate analysis of variance and Tukey multiple tests were used, and the significant difference level was set as $P < 0.05$.

3. Results

3.1. Effects of Dietary Fat Level on the Histology of Gill and Kidney in Yellow Catfish. For the gill tissue, compared with the medium-fat (MF) diet group, the gill filaments of yellow catfish showed epithelial desquamation (ed), epithelial hyperplasia (eh), epithelial swelling (es), necrosis, and collapse of lamella (ncl), and shortened secondary lamellae (ssl) in HF diet group (Figure 1). For the kidney tissue, compared with the medium-lipid group, fish-fed HF caused epithelial cell swelling in the collecting tubule (CT) and proximal tubule (PT). The lumen of CT and PT also enlarged in the HF diet group (Figure 1).

3.2. Effects of Dietary Fat Level on the Activities of Antioxidant Enzymes and the MDA Level in the Gill and Kidney of Yellow Catfish. For the gill tissue, compared with the low-fat (LF) and MF diet group, the enzyme activities of T-SOD and CAT significantly increased in the HF group. The MDA level increased with the increasing of diet lipid level in the gill of yellow catfish (Figure 2). For the kidney tissue, the activity of T-SOD significantly increased both in the MF and HF groups as compared to the LF group; the activity of CAT significantly increased with the rising of diet lipid level. However, diet lipid level had no remarkable effect on the MDA content in the kidney of yellow catfish (Figure 2).

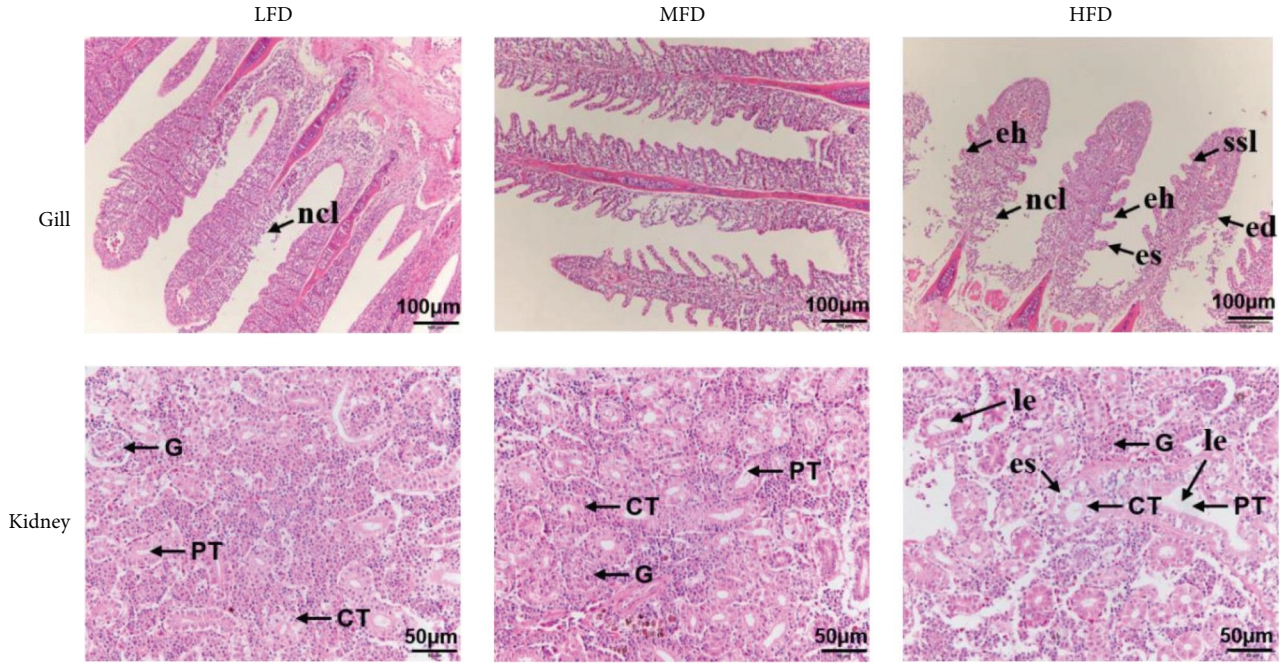

FIGURE 1: Effects of dietary fat level on the histology of gill (up row, hematoxylin and eosin staining, original magnification 100x) and kidney (down row, hematoxylin and eosin staining, original magnification 200x) in yellow catfish. ed, epithelial desquamation; eh, epithelial hyperplasia; es, epithelial swelling; ncl, necrosis and collapse of lamella; ssl, shortened secondary lamellae; le, lumen enlargement; CT, collecting tubule; PT, proximal tubule; G, glomerulus. LFD, low-fat diet; MFD, medium-fat diet; HFD, high-fat diet.

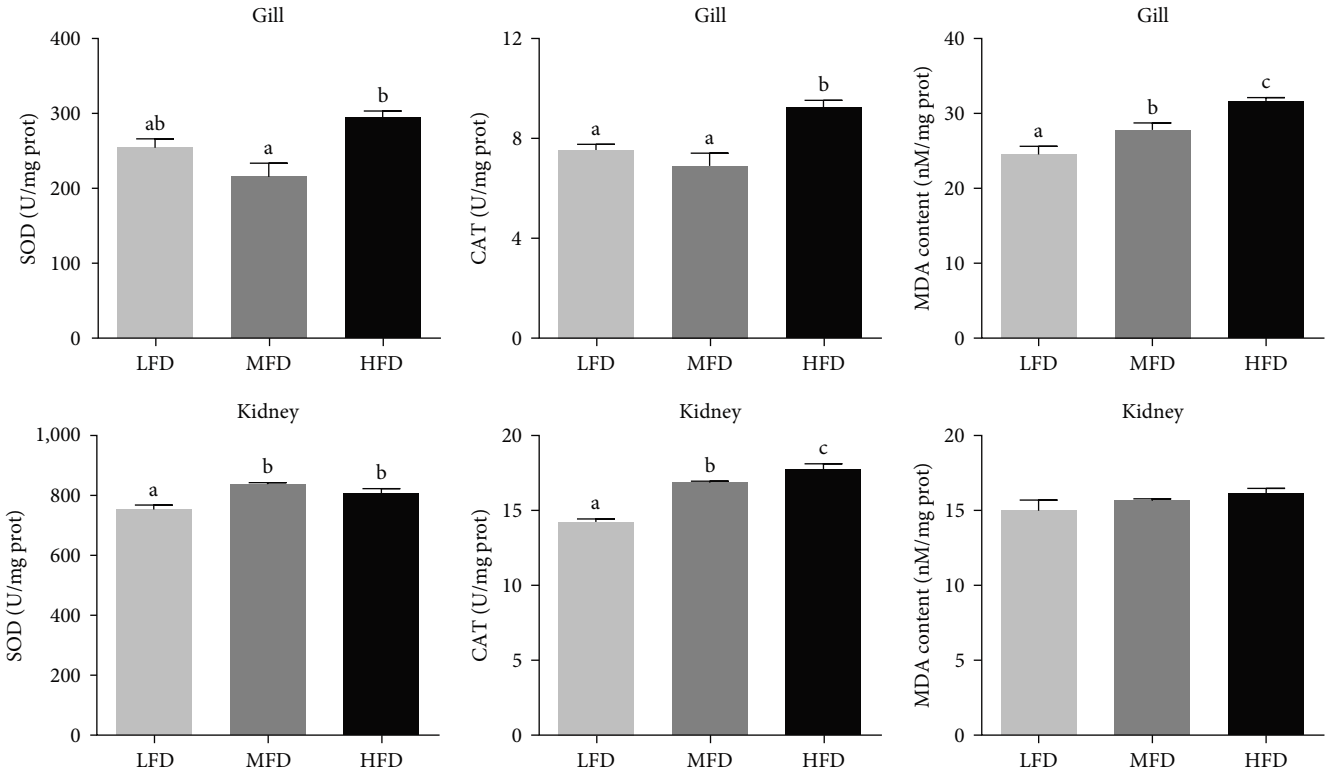

FIGURE 2: Effects of dietary fat level on the activities of enzymes involved in antioxidants and the MDA level in the gill and kidney of yellow catfish. Values are means ± SE ($n = 3$), and different letters denote the significant difference ($P < 0.05$). LFD, low-fat diet; MFD, medium-fat diet; HFD, high-fat diet.

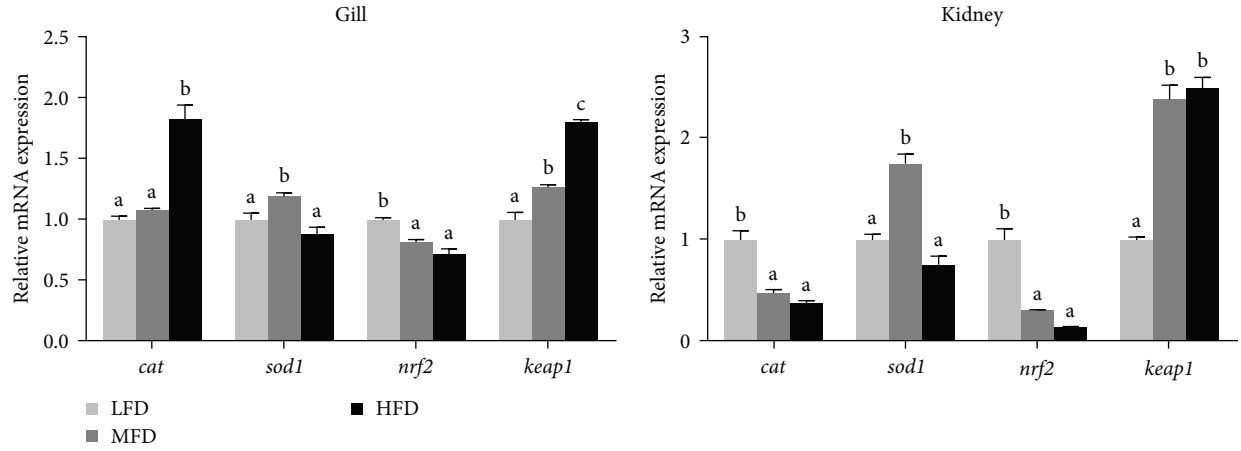

FIGURE 3: Effects of dietary fat level on the mRNA levels of genes involved in antioxidants. Values are means ± SE ($n = 3$), and different letters denote the significant difference ($P < 0.05$). LFD, low-fat diet; MFD, medium-fat diet; HFD, high-fat diet.

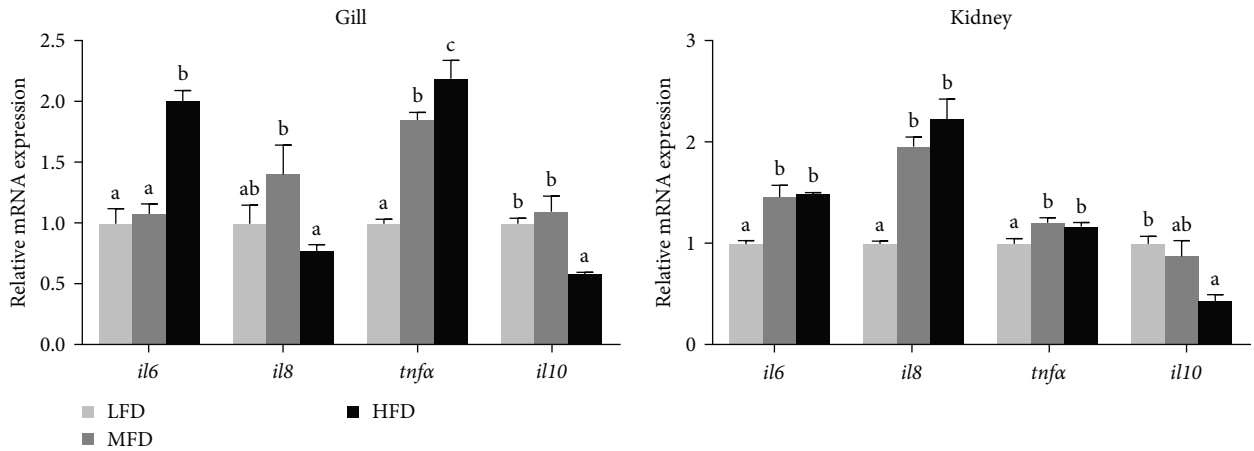

FIGURE 4: Effects of dietary fat level on the mRNA levels of genes involved in inflammation. Values are means ± SE ($n = 3$), and different letters denote the significant difference ($P < 0.05$). LFD, low-fat diet; MFD, medium-fat diet; HFD, high-fat diet.

3.3. Effects of Dietary Fat Level on the mRNA Levels of Genes Related to Antioxidants in the Gill and Kidney of Yellow Catfish. In gill, compared with the LF and MF groups, the mRNA level of *cat* significantly increased in the HF group. The expression of *sod1* was the highest in the MF group and showed no difference in the other two groups. The mRNA level of *nrf2* markly decreased both in MF and HF group as compared to the LF group. The mRNA level of *keap1* upregulated with the increasing of diet fat level (Figure 3). In kidney, the gene expression level of *sod1* was the highest in the MF group, but no significant difference in the other two groups. The expressions of *cat* and *nrf2* significantly decreased in the MF and HF groups as compared to the LF group. The expression level of *keap1* was the lowest in the LF group, whereas no significant difference in the other two groups (Figure 3).

3.4. Effects of Dietary Fat Level on the mRNA Levels of Genes Involved in Inflammation in the Gill and Kidney of Yellow Catfish. For the gill tissue, HF significantly increased *il6* and *tnfα* expression levels and significantly decreased *il8* and *il10* expression levels as compared to the MF group (Figure 4). For the kidney tissue, compared with the LF group, the mRNA level of *il6*, *il8*, and *tnfα* significantly increased in the MF and HF groups; *il10* gene expression was lowest in the HF group but showed no significant influence in the other two groups (Figure 4).

4. Discussion

At present, the effects of dietary fat levels are mainly concentrated on growth, liver and intestinal lipid metabolism, whereas little information was available on the effects of dietary lipid imbalance on the health of gill and kidney in fish. In this study, we investigated the effects of different dietary lipid levels on gill and kidney histology, and their oxidative status and immune response in yellow catfish, which will help to understand the impacts of dietary lipid imbalance on the health of fish.

The healthy growth of fish greatly depends on the health status of the gills, which is closely related to the structural integrity of the gills [26]. The structural integrity of gill is required for better absorption of nutrients. In the current

Table 3: The summaries of the effect of dietary fat levels on the changes of the antioxidant marks in different fish.

Fish species	Initial body weight (g)	Antioxidant marks	Tissues	Effect of dietary lipid level on the level of antioxidant marks (%)	Duration (day)	Reference
Ctenopharyngodon idella	261.41	MDA	Head kidney	0.59 > 2.14 = 3.6 = 5.02 < 6.66 < 8.01	56	[11]
Ctenopharyngodon idella	261.41	MDA	Spleen	0.59 > 2.14 = 3.6 = 5.02 = 6.66 = 8.01	56	[11]
Ctenopharyngodon idella	261.41	MDA	Skin	0.59 = 2.14 > 3.6 < 5.02 < 6.66 < 8.01	56	[11]
Ctenopharyngodon idella	261.41	MDA	Gill	0.59 > 2.14 = 3.6 = 5.02 = 6.66 < 8.01	56	[12]
Trachinotus ovatus	28.42	MDA	Muscle	9.13 = 10.59 > 12.12 = 14.98 = 16.25 < 19.26	56	[32]
Carassius auratus	31.58	MDA	Liver	5 = 8 > 11	56	[33]
Cichlasoma	1.2	MDA	Liver	4.77 = 8.75 < 11.68 = 16.25 > 23.7	42	[34]
Carassius auratus	2.04	MDA	Liver	2.5 = 4.39 = 6.61 = 8.42 = 10.81	60	[35]
Onychostoma macrolepis	2.96	MDA	Liver	3 > 6 = 9 < 12 = 15	56	[36]
Ctenopharyngodon idella	261.41	MDA	Anterior intestine	0.59 = 2.14 = 3.6 = 5.02 < 6.66 < 8.01	56	[37]
Ctenopharyngodon idella	261.41	MDA	Middle intestine	0.59 = 2.14 = 3.6 = 5.02 = 6.66 = 8.01	56	[37]
Ctenopharyngodon idella	261.41	MDA	Posterior intestine	0.59 > 2.14 > 3.6 <5.02 = 6.66 = 8.01	56	[37]
Pelteobagrus fulvidraco	2.33	MDA	Gill	6.31 < 9.33 < 15.32	56	This study
Pelteobagrus fulvidraco	2.33	MDA	Kidney	6.31 < 9.33 = 15.32	56	This study
Ctenopharyngodon idella	261.41	CAT	Head kidney	0.59 < 2.14 = 3.6 = 5.02 > 6.66 > 8.01	56	[11]
Ctenopharyngodon idella	261.41	CAT	Spleen	0.59 < 2.14 < 3.6 > 5.02 > 6.66 > 8.01	56	[11]
Ctenopharyngodon idella	261.41	CAT	Skin	0.59 = 2.14 = 3.6 < 5.02 > 6.66 > 8.01	56	[11]
Ctenopharyngodon idella	261.41	CAT	Gill	0.59 = 2.14 = 3.6 > 5.02 = 6.66 = 8.01	56	[12]
Carassius auratus	31.58	CAT	Liver	5 > 11	56	[33]
Cichlasoma	1.2	CAT	Liver	4.77 = 8.75 < 11.68 < 16.25 > 23.7	42	[34]
Carassius auratus	2.04	CAT	Liver	2.5 = 4.39 = 6.61 = 8.42 = 10.81	60	[35]
Onychostoma macrolepis	2.96	CAT	Liver	3 < 6 = 9 > 12 > 15	56	[36]
Ctenopharyngodon idella	261.41	CAT	Anterior intestine	0.59 = 2.14 < 5.02 = 6.66 = 8.01	56	[37]
Ctenopharyngodon idella	261.41	CAT	Middle intestine	0.59 < 2.14 < 3.6 > 5.02 > 6.66 > 8.01	56	[37]
Ctenopharyngodon idella	261.41	CAT	Posterior intestine	0.59 < 2.14 = 3.6 = 5.02 < 6.66 = 8.01	56	[37]
Labeo rohita	2.23	CAT	Liver	2 < 4 < 6 < 8 < 10 > 12	56	[38]
Pelteobagrus fulvidraco	2.33	CAT	Gill	6.31 = 9.33 < 15.32	56	This study
Pelteobagrus fulvidraco	2.33	CAT	Kidney	6.31 < 9.33 < 15.32	56	This study
Ctenopharyngodon idella	261.41	T-SOD	Head kidney	0.59 < 2.14 < 3.6 < 5.02 = 6.66 = 8.01	56	[11]
Ctenopharyngodon idella	261.41	T-SOD	Spleen	0.59 < 2.14 < 3.6 = 5.02 > 6.66 > 8.01	56	[11]
Ctenopharyngodon idella	261.41	T-SOD	Skin	0.59 < 2.14 < 3.6 < 5.02 > 6.66 > 8.01	56	[11]
Trachinotus ovatus	28.42	T-SOD	Muscle	9.13 = 10.59 = 12.12 < 14.98 = 16.25 = 19.26	56	[32]
Carassius auratus	31.58	T-SOD	Liver	5 = 8 < 11	56	[33]
Cichlasoma	1.2	T-SOD	Liver	4.77 = 8.75 < 11.68 = 16.25 > 23.7	42	[34]
Carassius auratus	2.04	T-SOD	Liver	2.5 < 6.61 > 8.42 = 10.81	60	[35]
Ctenopharyngodon idella	6.57	T-SOD	Liver	0 = 2.5 = 5 = 7.5 = 10	70	[2]
Onychostoma macrolepis	2.96	T-SOD	Liver	3 < 6 < 9 > 12 = 15	56	[36]
Ctenopharyngodon idella	261.41	T-SOD	Anterior intestine	0.59 < 2.14 < 3.6 < 5.02 > 6.66 = 8.01	56	[37]
Ctenopharyngodon idella	261.41	T-SOD	Middle intestine	0.59 < 2.14 < 3.6 = 5.02 > 6.66 > 8.01	56	[37]
Ctenopharyngodon idella	261.41	T-SOD	Posterior intestine	0.59 < 2.14 < 3.6 = 5.02 = 6.66 > 8.01	56	[37]

Table 3: Continued.

Fish species	Initial body weight (g)	Antioxidant marks	Tissues	Effect of dietary lipid level on the level of antioxidant marks (%)	Duration (day)	Reference
Labeo rohita	2.23	T-SOD	Liver	2 < 4 < 6 < 8 < 10 > 12	56	[38]
Pelteobagrus fulvidraco	2.33	T-SOD	Gill	6.31 = 9.33 < 15.32	56	This study
Pelteobagrus fulvidraco	2.33	T-SOD	Kidney	6.31 < 9.33 = 15.32	56	This study
Ctenopharyngodon idella	261.41	*sod1*	Head kidney	0.59 < 3.6 = 5.02 = 6.66 = 8.01	56	[11]
Ctenopharyngodon idella	261.41	*sod1*	Spleen	0.59 < 5.02 = 6.66 = 8.01	56	[11]
Ctenopharyngodon idella	261.41	*sod1*	Skin	0.59 < 2.14 < 3.6 = 5.02 > 6.66 = 8.01	56	[11]
Ctenopharyngodon idella	261.41	*sod1*	Gill	0.59 < 3.6 = 5.02 = 6.66 = 8.01	56	[12]
Ctenopharyngodon idella	261.41	*sod1*	Anterior intestine	0.59 < 2.14 < 5.02 > 6.66 = 8.01	56	[37]
Ctenopharyngodon idella	261.41	*sod1*	Middle intestine	0.59 < 2.14 = 3.6 = 5.02 = 6.66 = 8.01	56	[37]
Ctenopharyngodon idella	261.41	*sod1*	Posterior intestine	0.59 < 2.14 = 3.6 = 5.02 > 8.01	56	[37]
Pelteobagrus fulvidraco	2.33	*sod1*	Gill	6.31 < 9.33 > 15.32	56	This study
Pelteobagrus fulvidraco	2.33	*sod1*	Kidney	6.31 < 9.33 > 15.32	56	This study
Ctenopharyngodon idella	261.41	*cat*	Head kidney	0.59 < 2.14 = 3.6 = 5.02 > 6.66 > 8.01	56	[11]
Ctenopharyngodon idella	261.41	*cat*	Spleen	0.59 < 2.14 = 3.6 = 5.02 = 6.66 > 8.01	56	[11]
Ctenopharyngodon idella	261.41	*cat*	Skin	0.59 < 3.6 = 5.02 > 8.01	56	[11]
Ctenopharyngodon idella	261.41	*cat*	Gill	0.59 < 3.6 = 5.02 = 6.66	56	[12]
Ctenopharyngodon idella	261.41	*cat*	Anterior intestine	0.59 < 5.02 = 6.66 = 8.01	56	[37]
Ctenopharyngodon idella	261.41	*cat*	Middle intestine	0.59 < 3.6 = 5.02	56	[37]
Ctenopharyngodon idella	261.41	*cat*	Posterior intestine	0.59 < 3.6 > 8.01	56	[37]
Trachinotus ovatus	27.65	*cat*	Intestine	9.13 = 10.59 = 12.12 = 14.98 = 16.25 = 19.26	56	[39]
Trachinotus ovatus	27.65	*cat*	Liver	9.13 = 10.59 = 12.12 = 14.98 = 16.25 = 19.26	56	[39]
Pelteobagrus fulvidraco	2.33	*cat*	Gill	6.31 < 9.33 < 15.32	56	This study
Pelteobagrus fulvidraco	2.33	*cat*	Kidney	6.31 > 9.33 = 15.35	56	This study
Ctenopharyngodon idella	261.41	*keap1*	Head kidney	0.59 > 2.14 = 3.6 = 5.02 = 6.66 < 8.01	56	[11]
Ctenopharyngodon idella	261.41	*keap1*	Spleen	0.59 > 3.6 = 5.02 = 6.66 = 8.01	56	[11]
Ctenopharyngodon idella	261.41	*keap1*	Skin	0.59 > 2.14 > 5.02 = 6.66 < 8.01	56	[11]
Ctenopharyngodon idella	261.41	*keap1*	Gll	0.59 > 2.14 > 5.02 = 6.66 = 8.01	56	[12]
Ctenopharyngodon idella	261.41	*keap1*	Anterior intestine	0.59 > 2.14 = 3.6 = 5.02 < 6.66 = 8.01	56	[39]
Ctenopharyngodon idella	261.41	*keap1*	Middle intestine	0.59 = 2.14 > 3.6 = 5.02 < 8.01	56	[37]
Ctenopharyngodon idella	261.41	*keap1*	Posterior intestine	0.59 > 2.14 = 3.6 = 5.02 < 8.01	56	[37]
Trachinotus ovatus	27.65	*keap1*	intestine	9.13 = 10.59 = 12.12 = 14.98 = 16.25 = 19.26	56	[39]
Trachinotus ovatus	27.65	*keap1*	Liver	12.12 > 19.26	56	[39]
Pelteobagrus fulvidraco	2.33	*keap1*	Gill	6.31 < 9.33 < 15.35	56	This study
Pelteobagrus fulvidraco	2.33	*keap1*	Kidney	6.31 < 9.33 = 15.35	56	This study
Ctenopharyngodon idella	261.41	*nrf2*	Head kidney	0.59 < 2.14 = 3.6 = 5.02 = 6.66 = 8.01	56	[11]
Ctenopharyngodon idella	261.41	*nrf2*	Spleen	0.59 < 3.6 = 5.02 > 8.01	56	[11]
Ctenopharyngodon idella	261.41	*nrf2*	Skin	0.59 < 2.14 = 3.6 > 6.66 = 8.01	56	[11]
Ctenopharyngodon idella	261.41	*nrf2*	Gill	0.59 < 5.02 = 6.66 = 8.01	56	[12]
Ctenopharyngodon idella	261.41	*nrf2*	Anterior intestine	0.59 < 3.6 = 5.02 = 6.66 = 8.01	56	[37]
Ctenopharyngodon idella	261.41	*nrf2*	Middle intestine	0.59 < 2.14 < 5.02 > 8.01	56	[37]

Table 3: Continued.

Fish species	Initial body weight (g)	Antioxidant marks	Tissues	Effect of dietary lipid level on the level of antioxidant marks (%)	Duration (day)	Reference
Ctenopharyngodon idella	261.41	*nrf2*	Posterior intestine	0.59 < 3.6 = 5.02 = 6.66 = 8.01	56	[37]
Trachinotus ovatus	27.65	*nrf2*	Intestine	9.13 = 10.59 > 12.12 = 14.98 = 16.25 = 19.26	56	[39]
Trachinotus ovatus	27.65	*nrf2*	Liver	9.13 > 12.12 = 14.98 = 16.25	56	[39]
Pelteobagrus fulvidraco	2.33	*nrf2*	Gill	6.31 > 9.33 = 15.35	56	This study
Pelteobagrus fulvidraco	2.33	*nrf2*	Kidney	6.31 > 9.33 = 15.35	56	This study

Note. ">" represents the level of antioxidant marks was higher in the former group than the latter group; "=" represents the level of antioxidant marks was not significantly different between two groups; "<" represents the level of antioxidant marks was lower in the former group than the latter group. The capital letter of the antioxidant marks represents the enzyme activities, and the lowercase letter of the antioxidant marks represents gene expression.

study, the gill filaments showed epithelial desquamation (ed), epithelial hyperplasia (eh), epithelial swelling (es), necrosis and collapse of lamella (ncl), and shortened secondary lamellae (ssl) in HF diet group, indicating that the integrity of gill was destroyed by the excess level of dietary fat. Similar results were also reported in grass carp [12]. The TK not only regulates fluid and ion balance through the nephrons but is also considered to be an important immune organ for fish [8, 9, 27]. In the present study, compared with the MF group, fish-fed HF caused epithelial cell swelling in the CT and PT. The lumen of CT and PT also enlarged in the HF diet group, indicating that HF caused a great burden on the kidney function of yellow catfish, which is in agreement with the study in grass carp [11].

Oxidative stress occurs when the antioxidant enzyme system is interrupted or the reactive oxygen species over accumulated in the body [28]. MDA is widely used as an indicator of oxidative damage [29]. In the current study, the MDA level of gill significantly increased in HF group, which also supported the structural damage of gill we found above. Similarly, other studies also reported that a fish-fed HF diet significantly increased the MDA level in gill [12]. However, dietary lipid levels showed no significant influence on the MDA level in kidney of yellow catfish. The different results found between gill and kidney are probably due to the direct contact of the gill not only from the dietary fat but also from the dissolved dietary fat in the water column. Previous studies suggested that gill seem to be a better choice for monitoring oxidative stress because of their continuous and direct exposure to the water column [30]. Generally, the protective effects of antioxidant damage may be related to the increase in free radical scavenging ability [13]. SOD and CAT are two key enzymes that fight oxidative damage [22]. SOD catalyzes the conversion of O^{2-} to H_2O_2, whereas CAT decomposes H_2O_2 into H_2O and O_2 [13]. Therefore, we next investigated the antioxidant enzyme activities such as T-SOD and CAT in the gills and kidneys of yellow catfish. In this study, HF significantly increased the activities of T-SOD and CAT both in gill and kidney. The increased activities of these antioxidant enzymes may help to getting rid of excessive reactive oxygen under HF burden. Similarly, other studies also reported that HF diet significantly elevated the activities of T-SOD and CAT in fish [23, 31]. However, some studies suggested that HF downregulated the activities and mRNA levels of T-SOD and CAT in grass carp [11, 12]. Interestingly, we also found that HF reduced the mRNA level of *sod1* in both gill and kidney. However, HF significantly increased the mRNA level of *cat* in gill, whereas it significantly decreased the mRNA level of *cat* in kidney. Nrf2/Keap1 is an important signaling pathway that maintains the balance between peroxide and antioxidants [14]. In the present study, dietary lipid additive results in the decreased expression of *nrf2*, whereas the increased expression of *keap1* both in gill and kidney. Conversely, Ni et al. [11, 12] reported that fish fed with HF diet increased the expression of *nrf2* and decreased the expression of *keap1* both in gill and kidney of grass carp. Overall, the different changes of these antioxidant enzymes and genes between our studies and other studies are probably due to a large number of variables (such as level of fat, fish species, and tissue-specific). Here, we have summarized the effects of different dietary fat levels on these antioxidant marks in different tissues and species of fish in Table 3.

On the other hand, dietary lipid imbalances also will affect the immune status of fish. IL6, IL8, TNFα, and IL10 belonged to cytokines which regulated intracellular inflammatory responses [12]. IL6, TNFα, and IL8 are pro-inflammatory cytokines, whereas IL10 belongs to the anti-inflammatory cytokine [15, 16]. In this study, we pointed out that HF diet significantly increased the mRNA expression of *il6* and *tnfα* and reduced the mRNA expression of *il10*, indicating that HF diet promoted inflammation response both in gill and kidney. Similarly, Cortez et al. [40] also reported that HF diet increased the expression of *il6* and *tnfα* and promoted inflammation. Interestingly, we also found that HF reduced the expression of *il8* in gill but increased the expression of *il8* in kidney. However, Ni et al. [11, 12] also reported that dietary lipid level showed no effect on *il8* expression in gill, but HF diet increased *il8* expression in head kidney, indicating that the expression of *il8* by dietary lipid level is also species and tissue specificity.

5. Conclusion

In summary, excessive fat added to the feed can damage the immune organs of yellow catfish. Gills are more susceptible to injury than kidneys under high-energy intake conditions. HF diet increased antioxidant enzyme T-SOD and CAT activities to eliminate oxidative damage both in gill and kidney of yellow catfish. Moreover, HF accelerates inflammation response by regulating the expression of *il6*, *tnfα*, and *il10* both in gill and kidney. Overall, the impairment of gill and kidney in HF group results from the combination of oxidative damage and inflammatory responses.

Abbreviations

CAT: Catalase
il10: Interleukin 10
il6: Interleukin 6
il8: Interleukin 8
keap1: Kelch-like-ECH-associated protein 1
nrf2: NF-E2-related factor 2
sod1: Cu/Zn superoxide dismutase
tgfβ: Transforming growth factor-beta
tnfα: Tumor necrosis factor-alpha
T-SOD: Total SOD
LF: Low-fat
MF: Medium-fat
HF: High-fat.

Data Availability

Data will be made available upon request.

Ethical Approval

All the animal experiment procedures followed the guideline of the Animal Experimentation Ethics Committee of Wuhan Polytechnic University (WHPU).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Meiqin Zhuo designed the experiments; Jiali Jiang and Yuanhang Lian performed experiments and analyzed the samples with the help of Jun Chen and Yu Zhu; Jiali Jiang and Yu Zhu analyzed the data; Jiali Jiang and Meiqin Zhuo wrote the manuscript. Meiqin Zhuo revised the manuscript. All the authors approved the manuscript.

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