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Starvation and refeeding influence the growth, biochemical index, intestinal microbiota, and transcriptomic profiles of golden pompano *Trachinotus ovatus* (Linnaeus 1758)

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Starvation is a common stress in fish that is caused by environmental changes, and refeeding after starvation is believed to cause compensatory growth. Here, we evaluated the impacts of starvation for 7 d, followed by refeeding for 7 d on growth, gut microbiome, biochemical indices, liver transcriptome, and immune response in golden pompanos (*Trachinotus ovatus*). Starvation induced hypoglycemia, reduced triglyceride concentration, and considerably affected the activities of glycolysis related enzymes, including glucokinase (GK), pyruvate kinase (PK), and fructokinase 6-phosphate (PFK). Additionally, starvation for 7 d increased the concentrations of oxidative stress indicators, including cortisol (COR), superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT) and non-specific immunity parameters, including alkaline phosphatase (ALP), acid phosphatase (ACP), and lysozyme (LYZ). parameters to normal levels. Moreover, starvation affected the diversity and composition of the intestinal microbiota of *T. ovatus*. At the phylum level, the dominant phyla were Proteobacteria, Spirochaetes, and Tenericutes, while the dominant genera were *Brevinema*, *Haematospirillum*, and *Mycoplasma*. Transcriptome analysis of liver tissues showed that the mRNA expression of *GK*, *PK*, and *PFK*, were altered by starvation, and the trends were consistent with the activity levels of the enzymes. A total of 2,287 DEGs were identified among the control, starvation, and refeeding groups. DEGs in starvation (ST7) vs. control (CK) groups were mainly involved in cell cycle, DNA replication, and mitosis, whereas those in the refeeding (RT7) vs. ST7 groups were associated with stimulus responses and carbohydrate metabolism. Overall, most starvation-induced changes in enzyme activity, intestinal microbiome, immune response,

and liver transcriptome were gradually restored to normal after refeeding for 7 d. These data provide a theoretical reference for the farming of *T. ovatus* during periods of feed scarcity.

KEYWORDS

Trachinotus ovatus, starvation, refeeding, transcriptome profiles, intestinal microbiome

Introduction

Environmental changes, including temperature and seasonal variations, can cause food deprivation among animals. Animals respond to starvation stress by lowering their metabolic rate and increasing energy metabolism, including lipid and glycogen metabolism (Navarro and Gutiérrez, 1995) to maintain normal body functions and regulate enzyme activities (Su et al., 2022). Compensatory growth is observed when animals are returned to normal feeding after a period of starvation (Wilson and Osbourn, 1960). However, there is considerable variation in the degree of compensatory growth in aquatic animals depending on the species and starvation duration (Wang et al., 2009). Previous findings suggest that compensatory growth can increase the body weight of fish and increase feed utilization (Maclean and Metcalfe, 2001; Huang et al., 2008; Blanquet and Oliva-Teles, 2010). Therefore, it is important to evaluate the effects of starvation and refeeding on compensatory growth in fish.

Fish can survive during starvation and other harsh conditions by regulating biochemical and physiological mechanisms (Sakyi et al., 2020). Starvation activates the antioxidant defense system (Sakyi et al., 2020), of which superoxide dismutase (SOD) and catalase (CAT) are two key enzymes in the antioxidant defense system of aquati7c animals (Su et al., 2022). Moreover, studies have reported increased SOD and CAT activities in animals during starvation (Sipra et al., 2017; Showkat et al., 2019; Su et al., 2022). Malondialdehyde (MDA), which is produced by lipid peroxidation, can lead to oxidative damage to cell membranes, and is considered a biomarker of oxidative stress (Liu et al., 2011). These indicators can be used to assess the impact of starvation and refeeding on fish.

The composition of the intestinal microflora plays an important role in maintaining homeostasis, health status and growth in fish (Flint et al., 2012). Therefore, several studies have investigated effects of starvation on gut microbiome composition in fish (Sakyi et al., 2020; Yang et al., 2019; Jawahar et al., 2022). There are trillions of intestinal microflorae in the intestine (Zhang et al., 2014; Zhang et al., 2016). The intestinal microbiota of aquatic animals (Li et al., 2012; Li et al., 2014) is affected by feeding habits (Ghanbari et al., 2015), physiological conditions (Yan et al., 2012), living environment (Sullam et al., 2012; Dehler et al., 2016), and feed type (Ringø et al., 2016). Starvation is a common phenomenon in fish farming (Namrata et al., 2011; Xia et al., 2014). During the period of starvation, a series of physiological changes occur in fish (Guderley et al., 2003), which not only affects the growth of animals, but also cause changes in intestinal microbiota (Namrata et al., 2011; Okada et al., 2013). Starvation has been shown to alter intestinal microbiota in *Lates calcarifer* (Xia et al., 2014), *Ctenopharyngodon idellus* (Tran et al., 2018), and *Oreochromis niloticus* (Sakyi et al., 2021). Starvation-induced changes in gut microbial composition and function can affect growth, metabolism, and inflammatory responses in fish (Xu et al., 2018; Sakyi et al., 2021). However, the existential correlation between starvation and changes in gut microbiota remains unknown in some fish species.

Another mechanism through which organisms adapt during starvation is by regulating metabolic activities to maintain homeostasis (Keogh et al., 2016). Recently, transcriptome analysis using next-generation sequencing (NGS) technologies have provided new insights into the response of various aquaculture animals to starvation and refeeding (Yang et al., 2019; Jawahar et al., 2022). For example, the muscle transcriptomes of *Larimichthys crocea* (Qian et al., 2016), *Oncorhynchus mykiss* (Rescan et al., 2007), and *Lctalurus punctatus* (Liu et al., 2013) have been analyzed during starvation and refeeding. Starvation can promote the metabolism of energy materials, such as body fat and protein, and significantly increase the expression of hormone synthesis-related genes to resist stress damage (Chen et al., 2022; Elbialy et al., 2022). Liver tissue is an important site for nutrient metabolism, and plays an important regulatory role in animals during starvation (Pilkis and Granner, 1992).

Golden pompano (*Trachinotus ovatus*) is one of the most important farmed fish in the South China Sea (Liu et al., 2020). Similar to other species, the growth and survival of golden pompano is affected considerable by environmental variations; however, the effect of starvation on gut microbial composition and metabolic pathways in golden pompano have not been thoroughly elucidated. Here, we examined the impacts of

starvation on growth, gut microbiome, biochemical indices, liver transcriptome, and immune response in golden pompanos. It is anticipated that the findings of this study would serve as theoretical references for sustainable aquaculture of golden pompano.

Materials and methods

Fish and challenge experiments

A total of 360 healthy golden pompano (mean weight, 108.65 ± 1.36 g) independently bred by our research group were randomly assigned to nine floating net cages (1.0 m × 1.0 m × 1.0 m), with 40 fish per cage. The control (CK), starvation (ST), and refeeding (RT) groups were set in parallel, with three floating net cages per group. The CK group were fed to visual satiety twice daily (7:00 am and 5:00 pm), the ST group were starved for 7 d (ST7), while fish in the RT group were refed for 7 d after 7 d of starvation (RT7). At the end of the experimental period, the fish were anesthetized using 100 mg/L eugenol (Shanghai Medical Instruments Co., Ltd., Shanghai, China). The CK group was sampled on d-0, 7, and 14 (CK0, CK7, CK14); the ST group was sampled on d-7 (ST7); and the RT group was sampled on d-7 of refeeding after 7 d of starvation (RT7). The water conditions during the experimental period were as follows: salinity, 32%; temperature; 27-31°C; and pH, 7.5-8.2. Additionally, nine fishes were randomly sampled from each group, weighed for growth analysis, and blood samples were then collected for serum biochemical analysis. Liver and intestines tissues were rapidly isolated, frozen in liquid nitrogen, and stored for further analysis. Moreover, intestinal tissue was sampled for intestinal microflora analysis, while liver tissue was used for transcriptome analysis. All animal experimentations were performed in accordance with the regulations and guidelines of the Animal Care and Use Committee of the South China Sea Fisheries Research Institute, Chinese Academy of Fisheries Sciences (No. SCSFRI96-253).

Serum biochemical indices, glycolytic activity, non-specific immunity, and oxidative stress parameters

The concentrations of serum biochemical parameters, including triglyceride (TG), total protein (TP), glucose (GLU), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were determined using Mindray BS-420 automatic biochemical instrument (Shenzhen Mindray Biological Medical Electronics Co., Ltd., China). The activities of glycolysis enzymes, including glucokinase (GK), pyruvate kinase (PK), and 6-phosphofructokinase (PFK) were determined using standard kits supplied by Nanjing Jian-Cheng Institute of Biological Engineering. Non-specific immunity parameters, including alkaline phosphatase (ALP), acid phosphatase (ACP), and lysozyme (LZM) activities, and oxidative stress parameters, including total antioxidant capacity (T-AOC), malondialdehyde (MDA) concentration, and catalase (CAT), superoxide dismutase (SOD), and cortisol (COR) activities, were analyzed according to previously described procedures (Liu et al., 2019; Yang et al., 2020; Ma et al., 2021), using enzyme kits (Beijing Sin-Uk Institute of Biological Technology, Beijing, China).

Intestinal microflora

Total bacterial DNA was isolated using the TIANamp Micro DNA Purification Kit (Tiangen, Beijing, China). DNA yield was measured in NanoDrop (Thermo Fisher Scientific, Waltham, MA, US), and quality was assessed by PCR amplification of the bacterial 16S rRNA genes. Bacterial DNA was used as the template for the 16S rRNA V3+V4 region amplification. All PCR reactions were performed using 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 μM of forward and reverse primers, and approximately 10 ng template DNA. The PCR products were purified using Qiagen Gel Extraction Kit (Qiagen, Germany), and sequence analysis was performed using Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/) (Edgar, 2013). Intestinal microbes were clustered into operational taxonomic units (OTUsusing the Silva database (http://www.arb-silva.de/) as reference genome (Quast et al., 2013). Multiple sequence alignments were performed using MUSCLE software (Version 3.8.31, http://www.drive5.com/muscle/) (Edgar, 2004).

Transcriptome analysis

Total RNA was isolated from liver tissues using Trizol reagent (Invitrogen), according to the manufacturer's protocol. RNA purity and integrity were assessed using NanoPhotometer® spectrophotometer (IMPLEN, CA, USA) and RNA Nano 6000 Assay Kit (Agilent Technologies, CA, USA), respectively. Sequencing libraries were generated using NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, USA) according to the manufacturer's instructions, and index codes were added to attribute sequences to each sample. Library sequencing was performed on the Illumina Novaseq platform, according to the manufacturer's instructions. Quality screening, including the calculation of Q20, Q30 and GC content were performed. Differential expression analysis was performed using the DESeq2 R package (1.16.1). Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes enrichment analysis of differentially expressed genes (DEGs) were performed using the cluster Profiler R package.

Real-time quantitative PCR

The expression profiles of *PK*, *GK*, and *PFK* were determined by RT-qPCR to validate the transcriptome data. Gene-specific (*PK*, *GK*, *PFK*) primers were designed using Primer Premier 6.0 software (Table 1). The qPCR reaction was performed using according to the manufacturer's instructions (TaKaRa). All PCR reactions were performed using 12.5 μL reaction volume containing 6.25 μL 2 × TB Green Premix Ex Taq II (Tli RNaseH Plus) (TaKaRa), 1 μL cDNA template, 0.5 μL each primer of forward and reverse and 4.25 μL of Milli-Q water. The relative expression of the genes was determined using the CT method ($2^{-\Delta\Delta CT}$) (Livak and Schmittgen, 2001).

Statistical analysis

The experimental data were analyzed using SPSS 22.0 software (IBM Corporation, New York, USA). Experimental results are expressed as mean ± standard error (SE). Significant differences were determined using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test, and means were considered statistically significant at $p < 0.05$.

Results

Changes of weights under starvation and refeeding

The body weights of golden pompano in the control (CK0, CK7 and CK14) and experimental (ST7 and RT7) groups are shown in Figure 1. There was a significant decrease in the body weight of fish in the ST7 group (101.88 g) after 7 d of starvation compared with the CK (108.65 g) and RT7 groups (130.61 g). However, there was no significant difference in body weight between RT7 and CK groups.

Serum biochemical index and glycolytic activity

There was a significant decrease ($p < 0.05$) in the concentrations of GLU, TG, and TP in the ST7 group compared with the CK group (Table 2), but there was no significant difference in LDL content between the groups. Additionally, starvation significantly increased ($p < 0.05$). GK activity, but inhibited the activities of PK and PFK. However, refeeding after starvation significantly decreased GK activity ($p < 0.05$) and increased PK and PFK activities, restoring them to normal levels.

Non-specific immunity and oxidative stress parameters

Starvation for 7 d increased the activities of oxidative stress indicators (COR, SOD, MDA, and CAT) and non-specific immunity parameters, including ALP, ACP, and LYZ (Table 3) compared with the control group. However, refeeding for 7 d restored (decreased) the activities of these parameters back to normal.

Intestine microbiota analysis

Richness and diversity

A total of 563,928 sequences were obtained from the samples of golden pompano, which were clustered into 3,295 OTUs (sequence similarity ≥ 97%). The OTUs were annotated using the Silva132 database, of which 1,393 (42.28%) OTUs were obtained at the genus level. α-diversity analysis showed that starvation caused a decrease ($p < 0.05$) in intestinal microbiota richness and diversity in golden pompano, including Ace, Chao1, Shannon's, and Simpson's indices, compared with the CK group (Table 4). Moreover, refeeding did not significantly reverse starvation-induced decrease in intestinal microbial diversity.

TABLE 1 Effects of starvation and refeeding on serum biochemical indices and glycolysis-related enzymes in golden pompano.

Parameters	CK0	ST7	RT7
GLU	10.11 ± 0.02^{a}	2.86 ± 0.04^{c}	7.50 ± 0.13^{b}
TG	1.21 ± 0.02^{a}	0.92 ± 0.03^{c}	1.02 ± 0.01^{b}
TP	33.71 ± 0.82^{a}	26.43 ± 1.21^{c}	31.50 ± 0.72^{b}
LDL	0.49 ± 0.10	0.40 ± 0.01	0.60 ± 0.06
HDL	1.84 ± 0.02^{a}	1.57 ± 0.02^{b}	1.64 ± 0.03^{b}
GK	38.93 ± 1.43^{c}	158.87 ± 2.83^{a}	54.21 ± 2.18^{b}
PK	123.34 ± 2.70^{a}	54.73 ± 1.15^{c}	96.52 ± 1.65^{b}
PFK	119.68 ± 4.20^{ab}	110.66 ± 2.50^{b}	127.64 ± 3.42^{a}

Triglyceride (TG, mmol/L), total protein (TP g/L), glucose (GLU, mmol/L), low-density lipoprotein (LDL, mmol/L) and high-density lipoprotein (HDL, mmol/L), Glucokinase (GK, U/L), pyruvate kinase (PK, U/L), 6-phosphofructokinase (PFK, U/L). Data are expressed as mean ± SE (N = 9). Different letters in the same line indicate significant different mean values among all treatments ($P < 0.05$).

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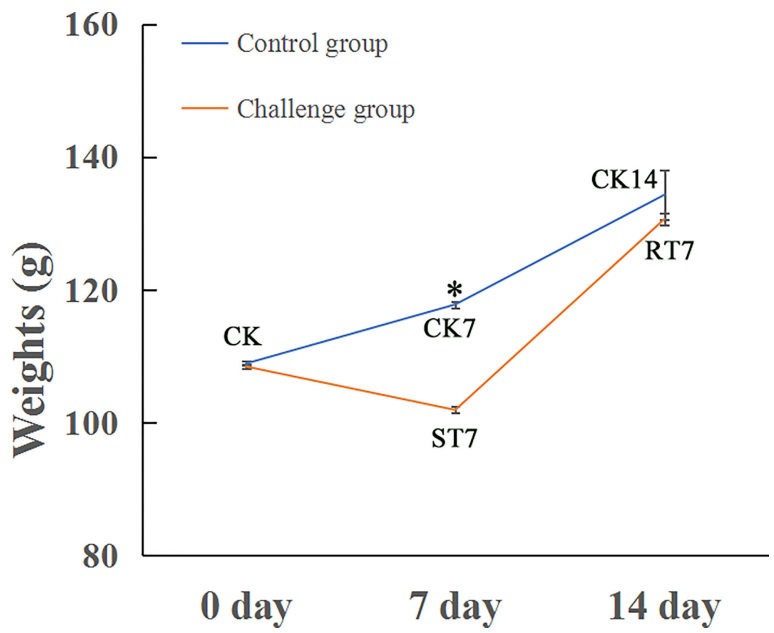

FIGURE 1
Growth curves of golden pompano under normal feeding, starvation, and refeeding. Asterisk indicated significant difference between two groups ($p < 0.05$); blue line represents the growth curve for control group (CK); red line indicates the growth curve for the challenge groups (starvation and refeeding groups).

Changes in the intestine bacterial composition

At the phylum level, the most dominant phyla were Proteobacteria, Spirochaetes, and Tenericutes (Figure 2A). Starvation increased the abundance of Spirochaetes, but decreased the abundance of Tenericutes; however, refeeding for 7 d significantly increased the abundance of Proteobacteria in the intestinal microbiota. At the genus level, the most dominant genera were *Brevinema*, *Haematospirillum*, and *Mycoplasma* (Figure 2B). There were some differences between groups, indicating that starvation and refeeding had some influence on intestinal microbial composition in golden pompano. Moreover, β-diversity analysis showed some differences among the sample groups (Figure 2C). Differences were also observed at the genus level: certain genera, such as Brevinema, Haematospirillum, and Mycoplasma (Figure 2D). Principal component analysis (PCA) and principal coordinate analysis (PCoA) confirmed that there were considerable differences in bacterial microbiota structure between the groups (Figures 3A, B). Linear discriminate analysis effect size (LEfSe) revealed that there were differential bacteria in

TABLE 2 Effect of starvation and refeeding on non-specific immunity and oxidative stress parameters in golden pompano.

Parameters	CK0	ST7	RT7
COR	26.51 ± 1.35	31.24 ± 2.04	28.01 ± 1.13
SOD	27.74 ± 2.31	29.78 ± 2.73	28.01 ± 2.01
T-AOC	1.23 ± 0.12^{a}	0.54 ± 2.03^{b}	31.50 ± 0.72^{a}
MDA	0.98 ± 0.17 ^{b}	1.65 ± 0.17 ^{a}	1.01 ± 0.16 ^{b}
CAT	8.24 ± 1.02 ^{b}	15.84 ± 0.68 ^{a}	10.25 ± 0.42^{b}
ALP	18.48 ± 1.48	23.14 ± 2.23	21.21 ± 1.08
ACP	12.35 ± 1.56	18.48 ± 0.96	16.12 ± 0.92
LYZ	11.61 ± 1.02	14.35 ± 1.42	13.96 ± 1.05

Cortisol (COR, ng/mL), Total antioxidant capacity (T-AOC, U/mL), Malondialdehyde (MDA, mmol/mL), Catalase (CAT, U/mL), Superoxide dismutase (SOD, U/mL), Alkaline phosphatase (ALP, mmol/L), Acid phosphatase (ACP, mmol/L), Lysozyme (LZM, mmol/L). Data are expressed as mean ± SE (N = 9). Different letters in the same line indicate significant different mean values among all treatments ($P < 0.05$).

TABLE 3 Illumina high-throughput bacterial diversity richness, diversity index (Shannon and Simpson), and estimated OTU richness (ACE and Chao1) of the intestinal bacterial of golden pompano.

Parameters	CK0	ST7	RT7
Richness estimators			
ACE	1234.08 ± 23.74	1379.55 ± 110.48	1109.48 ± 120.69
Chao1	1233.75 ± 33.69	1403.89 ± 129.09	1069.88 ± 121.01
Diversity estimators			
Shannon	5.72 ± 0.35	6.85 ± 0.47	5.74 ± 0.47
Simpson	0.88 ± 0.07	0.96 ± 0.02	0.93 ± 0.03

Data are expressed as mean ± SE (N = 9). Different letters in the same line indicate significant different mean values among all treatments ($P < 0.05$).

the intestinal microbiota of golden pompano in the refeeding group, including Proteobacteria (Figures 3C, D).

Liver transcriptome analysis

Sequencing, *de novo* assembly, and gene annotation

A total of 496,000,000 raw reads were obtained from nine cDNA libraries, among which 398,000,000 were successfully annotated on the reference genome (Table 5). Differential gene expression levels were estimated using the fragments per kilobase per million map reads (FPKM) method, with red and green colors indicating highly- and lowly-expressed genes, respectively. Genes from the control and experimental groups clustered separately, while gene expression was more consistent between each parallel group of the control and experimental groups.

Functional annotation of liver unigenes

Differential analysis identified 1,495 DEGs (693 upregulated and 802 downregulated) in the ST7 vs. CK groups, and 2,366 DEGs (1092 upregulated and 1276 downregulated) in the ST7 vs. RT7 groups. GO analysis showed that DEGs in the ST7 vs. CK groups were mainly enriched in organonitrogen compound metabolic process, cellular amide metabolic process, organonitrogen compound biosynthetic process, and peptide metabolic process, in the "Biological Process" category; in ribosome, intracellular ribonucleoprotein complex, ribonucleoprotein complex, and cytoplasmic part, in the "Cell Component" category; and in structural constituent of ribosome, structural molecule activity, oxidoreductase activity, and serine-type endopeptidase activity, in the "Molecular Function" category (Figures 4A–D). The top 10 GO enriched terms are shown in Figure 4. Additionally, DEGs in the ST7 vs. RT7 groups were mainly enriched in oxidation-reduction process, small molecule metabolic process, lipid localization, and lipid transport in the "Biological Process" category; in structural constituent of oxidoreductase activity and peptidase activity in the "Molecular Function" category (Figures 5A, B). There top 10 GO enriched terms are shown in Figure 5.

KEGG pathway analysis showed that the 1,495 DEGs in the ST7 vs. CK groups were enriched in 142 pathways (Figure 6A), most of which were related to energy substance and hormone metabolism. The 2,366 DEGs in the ST7 vs. RT7 groups were enriched in 142 KEGG pathways (Figure 6B), most of which were related to energy substance metabolism, hormone metabolism, and signal transduction.

TABLE 4 Statistics of transcriptome data.

Group	Library	Raw reads	Clean reads	Clean bases	Q20 (%)	Q30 (%)	G+C (content)
CK_1	FRAS190116784-1a	58326364	57254560	8.59G	98.02	94.25	49.87
CK_2	FRAS190116785-1a	43538260	42759494	6.41G	97.73	93.58	49.91
CK_3	FRAS190116786-1a	53949664	52929008	7.94G	97.85	93.92	50.08
ST_1	FRAS190116787-1a	45158456	44278984	6.64G	97.94	94.06	50.13
ST_2	FRAS190116788-1a	62533954	61060676	9.16G	97.98	94.18	50.19
ST_3	FRAS190116789-1a	43676400	42698058	6.40G	97.87	93.97	50.13
RT_1	FRAS190116790-1a	54625310	53299732	7.99G	97.91	94.02	50.55
RT_2	FRAS190116791-1a	43456296	42499742	6.37G	97.80	93.84	50.18
RT_3	FRAS190116792-1a	50780436	49609700	7.44G	97.98	94.25	50.91

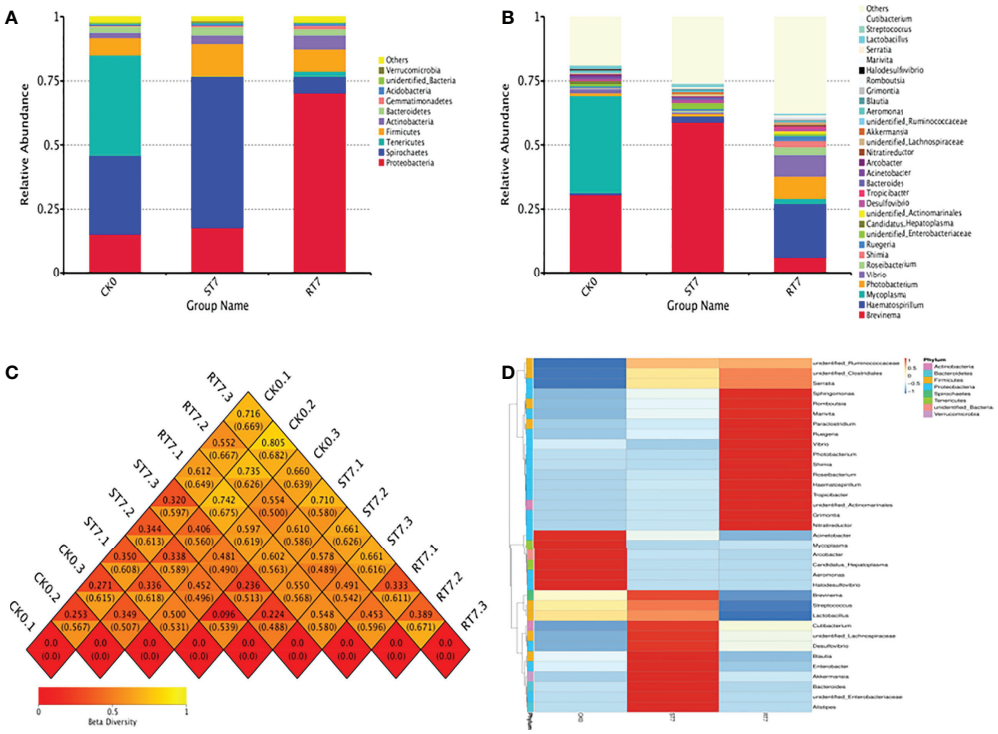

FIGURE 2
Alteration of intestine microbial structure. **(A)** Relative abundance of intestine microbial at phylum level. **(B)** Relative abundance of intestine microbial at genus level. **(C)** Heatmap of β-diversity. **(D)** Heatmap analysis of intestine microbial.

RNA-seq data validation

The expression profiles of three randomly selected genes (*PK*, *GK*, and *PFK*) were determined using qRT-PCR to validate the RNA-seq transcriptome data. The expression patterns of the three genes based on qRT-PCR were consistent with the RNA-seq data (Figure 7), indicating the specificity and accuracy of the transcriptome data. By starvation test, PK and PFK were up-regulated, while GK was down-regulated. After refeeding, PK was up-regulated, PFK and GK were down-regulated.

Discussion

During starvation, fish increase energy metabolism (glycogen, fat, and protein) to maintain normal body activities, resulting in weight lost (Navarro and Gutiérrez, 1995). Previous findings suggest that refeeding after short-term starvation could restore physiological functions to normal levels in fish (Metón et al., 2003; Rios et al., 2006). In the present study, the GLU level of the experimental fish decreased significantly under starvation, which was consistent with previous findings (Viegas et al., 2011; Pérez-Jiménez et al., 2012). However, fish in the CK group had higher GLU levels than those in the RT7 group, which could be attributed to the rapid utilization of glucose by the fish for compensatory growth after exposure to starvation for 7 d. Fish can use several strategies to cope with starvation, and variation have been observed in the GLU levels of fish species in response to starvation. For example, there was no significant change in glucose level in *Anguilla rostrata* (Suarez and Mommsen, 1987) and *Clarias lazera* (Navarro and Gutiérrez, 1995) after several months of starvation, whereas glucose level reduced by half in *Rhamdia hilarii* after 1 month of starvation (Machado et al., 1988).

TG are the most abundant lipids in the human body, and the decomposition products of TG are utilized for energy metabolism by several tissues (Grigorakis and Alexis, 2005). Under long-term starvation, TG can be decomposed to generate glycerol as a substrate in the gluconeogenesis pathway to provide energy for normal physiological activities (Grigorakis and Alexis, 2005). Consistent with previous findings (Hung et al., 1997; Pérez-Jiménez et al., 2005), fish in the CK group had significantly higher TG and lipoprotein concentrations than those in the ST group, indicating that the lipid material plays an important role in energy metabolism in fish during starvation. Fish regulate glucose metabolism during starvation by inhibiting glycolysis- and pentose phosphate-related enzymes

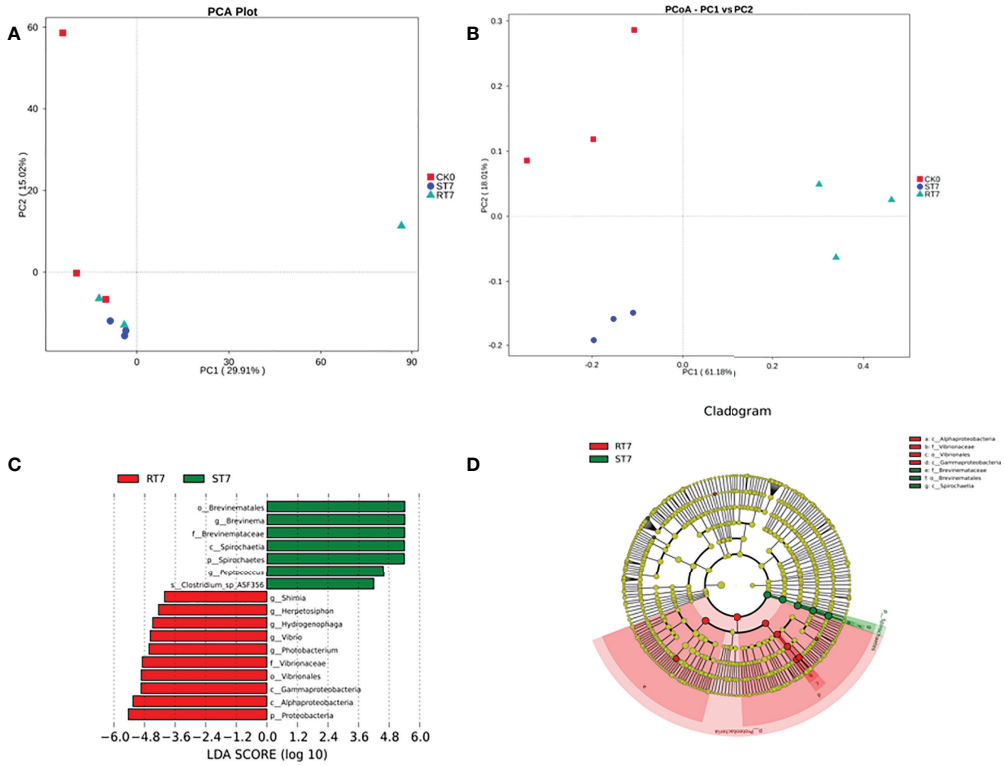

FIGURE 3
Inter-group variation and principal coordinates analysis (PCoA) in the relative abundance of intestine microbial communities. **(A)** PCoA plots based on weighted UniFrac metrics. **(B)** PCA plots based on unweighted UniFrac metric. **(C)** LDA score of Lefse-PICRUSt. The length of the column represents the effect size of bacterial lineages. **(D)** Lefse cladogram. The bacterial groups from family to class level are listed from center to outside. Each circle's diameter is proportional to the bacterial taxa's abundance. Only the taxa that linear discriminate analysis (LDA) value above 2.5 are shown.

and activating the activities of gluconeogenesis-related enzymes to maintain blood glucose balance (Pilkis and Granner, 1992; Nordlie et al., 1999). In the present study, starvation decreased the activities of glycolysis-related enzymes, including PK and PFK; however, both PFK and PK activities returned to normal after refeeding for 7 d, with PK activity significantly higher in the RT group compared with the CK group. These results indicated that starvation can inhibit the activities of glycolysis-related enzymes, and the change trends were positively correlated with blood GLU levels. However, GK activity showed a significant increase under starvation, but returned to normal after refeeding for 7 d, which was contrary to findings in *Oncorhynchus mykiss* (Kirchner et al., 2005), *Sparus aurata* (Metón et al., 2004), and hybrid grouper (Chen et al., 2022). The increase in GK activity during starvation may be due to the fact that it is the first rate-limiting enzyme in glycolytic pathway.

TABLE 5 Primers used in this study.

Primer name	primer sequences (5'-3')	Size(bp)
GK-F	GGGTTTTACCTTCTCCTTTCCC	191
GK-R	TGGTGGCTACTGTGTXATTCA	
PK-F	GCTGAAAAGGAAACACCAAAG	152
PK-R	AGATAACCCAGATGCCGACA	
PFK-F	ACAACACACCAACTGACACCT	175
PFK-R	AGTACAAGACGAGCTACGACG	
EF-1α-F	CCCCTTGGTCGTTTTGCC	170
EF-1α-R	GCCTTGGTTGTCTTTCCGCTA	

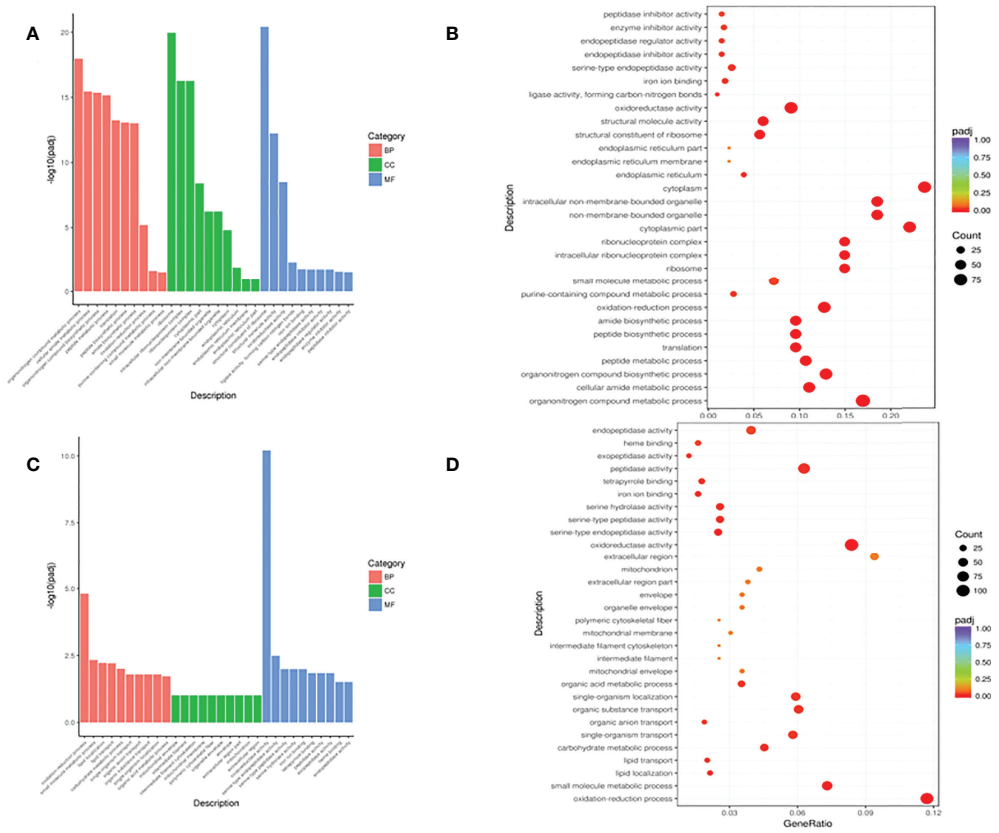

FIGURE 4
Gene ontology (GO) function annotation of DEGs. **(A)** GO annotation of DEGs in the control versus starvation groups; **(B)** Scatter plot of GO enrichment analysis of DEGs in the control versus starvation groups; **(C)** GO analysis of DEGs in the re-feeding versus starvation groups; **(D)** Scatter plot of GO enrichment analysis of DEGs in the re-feeding and starvation group.

As a substrate for glycogen synthesis, glucose plays an important role in maintaining normal physiological functions. Glycolysis promotes the decomposition and utilization of glucose, while gluconeogenesis promotes the synthesis of glucose and increases the body's blood glucose concentration, indicating that glucose is not completely consumed under short-term starvation.

COR is an important hormone involved in maintaining normal physiological function of the body, and the concentration of COR is often used as an indicator of the stress level of fish (Fatima et al., 2018). In the present study, COR level increased after 7 d of starvation, which was consistent with previous findings in several fish species (Martinez-Porchas et al., 2009), including *Garra gotyla* (Sharma et al., 2017) and *Salmo salar* (Waagbo et al., 2017). However, refeeding for 7 d restored COR levels back to normal. These results showed that the fish responded to starvation stress by increasing cortisol levels. Starvation has a direct impact on fish health and immunity, depending on the duration of feed scarcity (Kiron, 2012). Starvation can cause an imbalance between reactive oxygen species and antioxidant defense systems, resulting in oxidative stress (Jia et al., 2016). SOD and CAT are important antioxidants in the non-specific immune system (Peter et al., 2020), and MDA is an indicator of the rate of oxidative stress-induced lipid peroxidation (Zhao et al., 2014). In the present study, starvation increased SOD and CAT activities in the fish, which was consistent with previous findings (Morales et al., 2004). Moreover, MDA levels were higher in the ST7 group compared with the RT7 group, which was consistent with findings in *Pseudosciaena crocea* (Zhang et al., 2008). These results showed that starvation-induced stress affected MDA activity in fish (Han et al., 2018). Proper nutrition plays an important role in the immune status of fish (Cotter et al., 2019). In the present study, there was an increase in the activities of LYZ, ACP, and ALP during starvation, which was similar to findings in *Hippocampus erectus* (Su et al., 2022) and *Rutilus rutilus* (Abolfathi et al., 2012). LYZ, ACP, and ALP play important roles in nonspecific immunity and immune regulation (Rojtinnakorn et al., 2002; Foss et al., 2009; Lalles, 2019). However, the activities of LYZ, ACP, and ALP decreased after refeeding for 7 d.

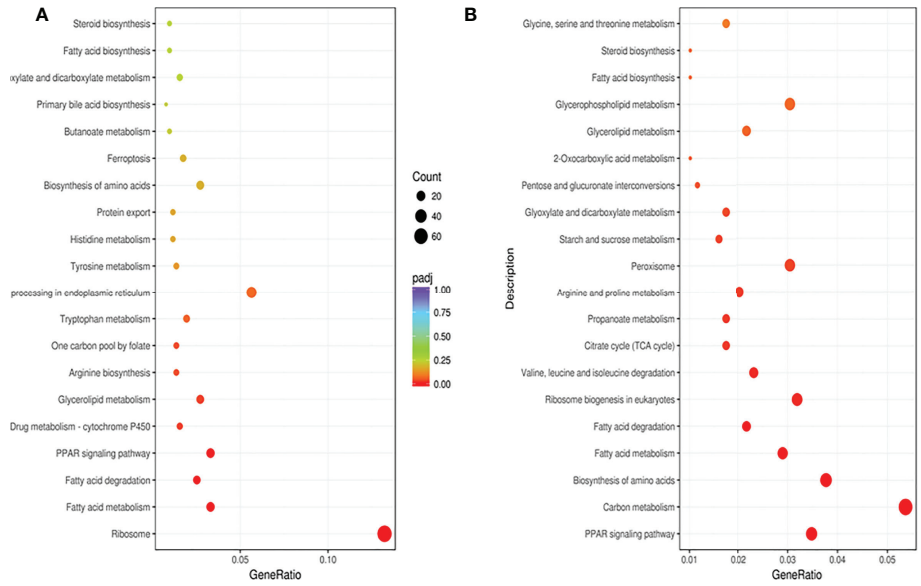

FIGURE 5
Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DEGs. **(A)** Pathway enrichment of DEGs in the control versus starvation groups; **(B)** Pathway enrichment of DEGs in the refeeding versus starvation groups.

The role of the intestinal microbiota in nutrient allocation and physiological regulation in animals cannot be overemphasized (Zhang et al., 2016). Studies have shown that the restoration of feed after short-term feed deprivation increases the energy source and the relative abundance of intestinal microorganisms. Moreover, feed availability causes increased competition between intestinal microbial species, which can affect species abundance, leading to the emergence of dominant species (Li et al., 2014). In the present study, the intestinal microbial diversity of golden pompano was in the

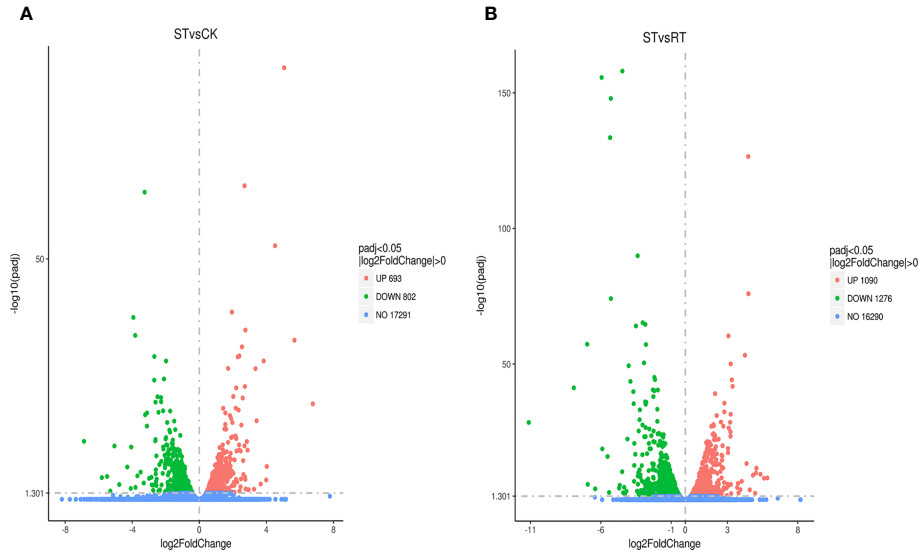

FIGURE 6
Differential gene statistics. **(A)** Volcano map of differential genes between the control group and starvation group; **(B)** Volcano map of differential genes between the refeeding and starvation group.

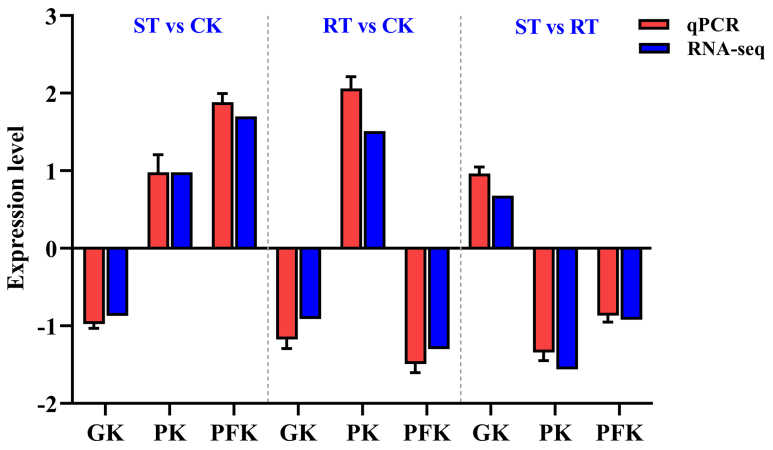

FIGURE 7
Comparison of gene expression between RNA-seq and quantitative real-time PCR (qRT-PCR) data.

order of RT7 > CK0 > ST7, which was consistent with findings in *Carassius auratus* (Li et al., 2014). During starvation, the body and intestinal microbiota is faced with energy crisis, inducing a decrease in the size of the intestine (Starck, 2003; Karasov et al., 2004; Zaldúa and Naya, 2014). Moreover, the composition and diversity of the intestinal microbiota is also affected by starvation. For example, the intestinal microbiota of zebrafish showed a downward trend under starvation stress (Semova et al., 2012). The intestinal microbiota diversity is closely related to the host's health (Flint et al., 2008; Pérez et al., 2010; Clemente et al., 2012). Studies have shown that human diarrhea and obesity caused by Clostridium are related to intestinal microbiota community diversity, and increased intestinal microbiota diversity can improve huma health (Ott et al., 2004; Clarke et al., 2014). In aquatic animals, red-operculum disease has been shown to reduce the intestinal microbiota diversity of fish (Li et al., 2017). Additionally, shrimps with white stool syndrome had lower intestinal microbiota diversity than healthy individuals (Hou et al., 2018). Since the host's intestinal environment is closely related to the surrounding environment, the intestinal microbiota diversity and composition of aquatic animals is highly susceptible to changes in the water environment, food, and other factors. There were differences in the dominant phyla between the ST7 and RT7 groups, indicating that the resumption of feeding after starvation affected the abundance of the dominant phyla, but not the types of dominant bacteria. Starvation and refeeding may have some effects on the abundance and species diversity of the intestinal microbiota of fish (Dhanasiri et al., 2011; Claesson et al., 2012).

Starvation and compensatory growth are common phenomena in the natural environment. In the present study, the compensatory growth phenomenon was observed in the fish after refeeding for 7 d, which was consistent with findings in *S. salar* (Martin et al., 2010), *C. Idella* (He et al., 2015), *O. mykiss* (Rescan et al., 2017), *Megalobrama amblycephala* (Zhu et al., 2014), and *Spinibarbus hollandi* (Yang et al., 2019). After short-term starvation, refeeding can promote rapid weight gain (compensatory growth) in fish; however, this might be affected by feed intake and food conversion rate (Jobling and Johansen, 1999). Starvation has considerable effects on animal physiology, altering cellular processes in several tissues. The liver is an important organ of metabolism in aquatic animals, and it is involved in energy metabolism, substance synthesis and secretion, and immune response. In the present study, the mRNA level of *GK* was downregulated in the ST7 and RT7 groups, but *PK* expression was upregulated, which was consistent with the respective levels of the enzymes in the fish. The upregulated DEGs in the ST7 vs. CK groups were mainly involved in lipid and glucose metabolism and protein degradation and metabolism. The upregulated DEGs in the ST7 vs. RT7 groups were mainly involved in ribosomal proteins, stimulus responses, and carbohydrate metabolism, which was similar to findings in *Danio rerio* (Jawahar et al., 2022) and trout (Rescan et al., 2017). These results suggested that the adaptive physiology exhibited during starvation is reversible after refeeding in golden pompano.

KEGG pathway analysis showed the DEGs were significantly enriched in complement and coagulation cascade, fat digestion and absorption, steroid biosynthesis, and peroxidase proliferator activated receptor signaling pathway, which was consistent with findings in *Larimichthys crocea* (Qian et al., 2016). These results confirmed the positive regulatory role of the above pathways in hormone synthesis and energy metabolism in golden pompano during starvation and refeeding. Starvation decreases nutrient metabolism in fish. However, the lower metabolic level is maintained for a while when refeeding is resumed, which could increase the proportion of energy used for growth,

thereby increasing food conversion rate and inducing compensatory growth (Dobson and Holmes, 1984; Miglavs and Jobling, 1989). Moreover, the increased appetite and feed intake after starvation period could also contribute to compensatory growth (Myung and Richard, 1995). During short-term starvation, fish metabolize body fat to generate energy, resulting in a decrease in fish body fat, which can trigger compensatory growth (Jobling et al., 1993). For example, *Acanthopagrus schlegelii* uses both fat and glycogen during starvation (Lou et al., 2006) and *Sciaenops ocellatus* uses mainly fat as energy source (Jiang et al., 2002). However, some fish species, including *Lutjanus sebae*, have been reported to mainly metabolize proteins during starvation to meet energy demand (Ou and Liu, 2007), indicating that different species utilize different energy storage materials during starvation. The liver is the main organ for energy metabolism and synthesis. During immune response, the liver rapidly produces acute phase response proteins to assist in the degradation of invading pathogens (Houlihan et al., 1986). The liver suppresses protein synthesis during starvation mainly through the reduction of multiple hormone signals (Gabillard et al., 2006). Moreover, protein synthesis is inhibited following reduced protein intake in fish, which might be a conserved response (Martin et al., 2010). Although protein synthesis and degradation reaches a certain balance during starvation, the overall shift is usually towards degradation, as evidenced by low serum protein levels during starvation (Peragon et al., 1999; Martin et al., 2002; Wang et al., 2006).

Conclusions

In summary, the impacts of starvation and refeeding on the growth, immune response, and gut microbiota of *T. ovatus* was examined. Starvation significant affected the activities of carbohydrate metabolism enzymes and increased the concentrations of COR, SOD, MDA, CAT, ALP ACP and LYZ. However, refeeding for 7 d restored most of the indices to their normal levels. Additionally, starvation increased the α-diversity of the gut microbial community, which was not affected by refeeding. Liver transcriptome analysis revealed that starvation and refeeding of affected protein, lipid, and steroid metabolism. Overall, these findings contribute to our understanding of how to fish respond to short starvation and refeeding, providing useful information for improving aquaculture management.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/- PRJNA863414, PRJNA863419.

Ethics statement

All experiments in this study were approved by the Animal Care and Use Committee of South China Sea fisheries Research Institute, Chinese Academy of fishery Sciences (no. SCSFRI96-253) and performed according to the regulations and guidelines established by this committee. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

BL, HG and D-CZ designed the research and wrote the paper. BL, HG and B-SL performed the experiments. BL, HG, B-SL, NZ, LG, and J-WY contributed to sample collection. BL analyzed the data and wrote the paper. SJ and D-CZ assisted with writing and proofreading. 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.

Publisher's note

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