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Selenium Protects Yellow Catfish (*Tachysurus fulvidraco*) from Low-Temperature Damage via the Perspective Analysis of Metabolomics and Intestinal Microbes

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Abstract: The effects of selenium supplementation in *Tachysurus fulvidraco (T. fulvidraco)* on low-temperature stress are not known. In this study, 280 healthy *T. fulvidraco* were divided into two groups, the G0 group (a control group) and the T0 group (a selenium treatment group on a 0.22 mg/kg diet), for a 6-week feeding time. Then, low-temperature stress (water temperature dropped from 26 to 13 °C, with a rate of 1 °C/h) was administered after that. The feeding results showed that selenium increased the percent weight gain (PWG), specific growth rate (SGR), and survival rate (SR) of *T. fulvidraco* and decreased the feed conversion rate (FCR), but these differences were not significant ($p > 0.05$). Under low temperatures, selenium still has no significant effects on antioxidant indexes such as glutathione peroxidase (GSH-Px) activity, superoxide dismutase (SOD) activity, and malondialdehyde (MDA) content in serum ($p > 0.05$). However, metabolomic analysis revealed that selenium caused changes in lipids and lipid-like molles, organic acids and their derivatives, and fatty acyls. Choline, linoleic acid, and glycerophospholipid metabolism pathways; d-arginine and d-ornithine metabolism; valine, leucine, and isoleucine degradation; and biosynthesis pathways, as well as pyrimidine metabolism pathways, were activated to produce these metabolites to combat against this stress. In addition, selenium increased the diversity of intestinal microbes in *T. fulvidraco* and decreased the relative abundance of *Plesiomonas*. However, the combined analysis showed the intestinal microbe changes did not affect metabolite production. In summary, selenium activated lipid, carbohydrate, and amino acid metabolism for energy substance provision, reduced the oxidation and production of other harmful substances, and increased the intestinal microbe diversity of *T. fulvidraco* to improve resistance to low-temperature stress.

Keywords: selenium; *Tachysurus fulvidraco*; low-temperature stress; metabolomics; intestinal microbes

Key Contribution: This study used metabolomics and 16S rRNA technology to investigate the effects of selenium supplementation on metabolites and intestinal microbes in *T. fulvidraco* under low-temperature stress. Thus, it provided valuable insights for a better understanding of the underlying mechanism of selenium supplementation to improve the resistance of *T. fulvidraco* under low-temperature stress.

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1. Introduction

Tachysurus fulvidraco, commonly known as Huangguding in China, is widely favored by consumers because of its delicious taste, tender meat, and few intramuscular spines.

T. fulvidraco is farmed in Hubei, Zhejiang, Jiangxi, Guangdong, and Sichuan provinces on a large scale. As a high-quality well-known breeding species, the production of *T. fulvidraco* in China reached 565,500 tons in 2020. *T. fulvidraco* is a warm-water fish that thrives in a suitable environment and relatively constant temperature [1]. Temperature stress alters the physiological and behavioral state of fish, including suppressing immunity, activeness, and growth, leading to death in severe cases and causing serious economic losses to aquaculture [2]. The resistance of fish to cold is affected by multiple factors, such as genetics, developmental stage, nutrition, and thermal history [3,4].

Fish, as poikilotherms, elicit a complex series of physiological responses to low-temperature stress, including physiological responses to nutrient metabolism and immunity and changes in intestinal microbes [5]. Metabolomics is a technique that views the living organism (cell, tissue, or organism) as a complete system and reveals the dynamic changes in the end products of gene expression (all metabolites with small relative molecular masses such as sugars, lipids, amino acids, and nucleotides) [6,7]. The qualitative and quantitative analyses of these metabolites can directly characterize the biochemical status and metabolic characteristics of living organisms and elucidate the relationship between metabolites and internal and external factors such as genetics, nutrition, and environment [8]. Fish intestinal microbes play a crucial role in improving nutrient utilization, regulating lipid catabolism, transport, and storage, and preventing polyunsaturated fatty acid peroxidation [9,10]. 16S rRNA gene sequencing technology can intuitively reflect the dynamic changes in microflora and has been widely used in the study of fish intestinal microbes [11,12].

Selenium, an essential trace element, plays a vital role in improving growth, enhancing immunity, and alleviating oxidative stress. Nano-selenium effectively mitigated the metabolism of glycerophospholipids and fatty acids, the damage of hepatocyte membrane, and the disruption of lipid metabolism in rainbow trout interfered by heat stress [13]. We previously established that selenium supplementation in feed increased the serum triglyceride contents and the expressions of genes involved in multiple pathways, such as pancreatic secretion, protein digestion and absorption, and fat digestion and absorption, thereby improving the resistance of *T. fulvidraco* to low-temperature stress [14,15]. However, how selenium affects the metabolites and intestinal microorganisms of yellow catfish under low-temperature stress has not been reported.

In this study, we used metabolomics and 16S rRNA technology to investigate the effects of selenium supplementation on metabolites and intestinal microbes in *T. fulvidraco* under low-temperature stress. Our findings provide a better understanding of the underlying mechanism of selenium supplementation to improve the resistance of *T. fulvidraco* to low-temperature stress.

2. Materials and Methods

2.1. Experimental Diets

Casein, corn starch, fish oil, lecithin oil, vitamins, and trace elements (without selenium) were used as the main ingredients to formulate a basal feed comprising 41.29% crude protein, 9.78% crude lipid, 4.73% ash, and 8.42% moisture. The specific composition of the basal feed is shown in Table 1. Sodium selenite used in the T0 group was purchased from Sigma with purity ≥98%, and the selenium supplementation level (0.22 mg/kg) was determined based on a previous study [16]. The ingredients were crushed and passed through a 60-mesh sieve. Each ingredient was mixed using the stepwise enlargement method and then mixed again in an NH-10 kneader by adding fish oil and lecithin oil. Subsequently, the mixture was blended using a B20 Powerful blender until dough was formed. The pelleted feed diets were made using an SLX-80 twin-screw extruder with a particle size of 2.0 mm and then granulated in a G-500 granulator. The feed diets were then dried in an oven at 55–60 °C. After cooling, the feed diets were sieved, subpackaged in sealing bags, and preserved at −20 °C for subsequent use.

Table 1. Feed ingredients and nutrient levels (dry matter basis, %).

Items	Content
Ingredients	
Casein	46.00
Corn starch	26.50
Fish oil	8.00
Lecithin oil	2.00
Vitamin premix [(1)]	0.50
Mineral premix [(2)]	1.50
Choline chloride	0.50
Vitamin C phosphate	0.10
NaCl	0.40
$Ca(H_2PO_4)_2$	2.00
Betaine	0.50
Carboxymethyl cellulose	3.00
Microcrystalline cellulose	9.00
Total	100.00
Nutrient levels [(3)]	
Crude protein	41.29
Crude lipid	9.78
Ash	4.73
Moisture	8.42

[(1)] Vitamin premix provided the following for per kg of the diet: Vitamin (V)A (2000 IU), VD_3 (700 IU), VE (10 mg), VK_3 (2.5 mg), VB_1 (2.5 mg), VB_2 (5 mg), VB_6 (3 mg), VB_{12} (0.01 mg), niacinamide (17.5 mg), *D*-calcium pantothenate (10 mg), folic acid (0.8 mg), biotin (0.045 mg), and inositol (25 mg); [(2)] Mineral premix provided the following per kg of the diet: $MgSO_4 \cdot H_2O_2$ (80 mg), $CuSO_4 \cdot 5H_2O$ (6 mg), $FeSO_4 \cdot H_2O$ (30 mg), $MnSO_4 \cdot H_2O$ (4.9 mg), $ZnSO_4 \cdot H_2O$ (86.95 mg), feed grade calcium iodate (5%) (15 mg), and feed grade cobalt sulfate (5%) (3 mg); [(3)] Measured value.

2.2. Fish and Feeding Management

The feeding experiment was conducted in an indoor recirculating aquaculture system at the Aquatic Research Laboratory of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences. A total of 280 *T. fulvidraco* (approximately 5.7 g/fish) were randomly divided into two groups (4 replicates per group, 35 fish per replicate) and stocked in a tank containing 300 L of water. *T. fulvidraco* in the two test groups were fed test diets without (G0 group) and with (T0 group) sodium selenite twice a day (at 9:00 and 18:00) for 6 weeks. Daily feeding rate was approximately 4% of total body weight. Indeed, few feeds remained during the experiment, and we adjusted the daily ration according to prior feeding responses. If the diet remained, it would be collected by siphoning and then be dried and weighed. During the feeding period, the sewage was discharged once a day, and water quality parameters were as follows: temperature of 26–30 °C, pH 7.0–7.5, ammonia nitrogen content $\leq$0.2 mg/L, nitrite content $\leq$0.02 mg/L, natural light, 24 h uninterrupted aeration. In addition, the feeding consumed and mortality were recorded daily. At the end of the feeding experiment, 20 fish from each tank were transferred to the temperature-controllable ecological tank system, about 150 L water per tank. The water temperature dropped from 26 to 13 °C, with a rate of 1 °C/h, and was sampled when the water temperature dropped to 13 °C. Under low-temperature stress, the fish were not fed, and their swimming ability weakened. However, they did not die.

2.3. Sample Collection and Preparation

After 6 weeks of feeding trial, the fish were fasted for 24 h, individually weighed, and counted for each tank as a unit. After the low-temperature stress, blood samples were collected from the caudal vein of four fish randomly selected from each replicate tank. The blood samples of two fish were mixed in a centrifuge tube, placed at room temperature for 30 min, and then centrifuged at 4000 rpm for 10 min at 4 °C. Subsequently, the serum samples were separated and preserved at −80 °C for antioxidant ability analysis and metabolomic analysis. The intestine was isolated from four fish (per tank) under aseptic

operating conditions, put into an enzyme-free tube, frozen with liquid nitrogen, and stored at −80 °C until intestinal microbe diversity analysis.

2.4. Growth Analysis

Percent weight gain (PWG, %) = 100 × (final body weight − initial body weight)/initial body weight;

Specific growth rate (SGR, %/d) = 100 × [ln (finial average weight) − ln (initial average weight)]/feeding day;

Survival rate (%) = 100 × (final number of fish)/(initial number of fish);

Feed conversion rate (FCR) = feed intake/wet weight gain.

2.5. Antioxidant Ability Analysis

The glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) content in serum were analyzed using kits from Nanjing Jiancheng Bioengineering Institute (China) in accordance with the instruction in each kit.

2.6. Metabolomics Analysis

Serum samples were pretreated according to the requirements of liquid chromatography–mass spectrometry (LC-MS/MS). Quality control samples were prepared by mixing all sample metabolites in equal volumes. The detection platform was an ultra-performance liquid chromatography–tandem time-of-flight mass spectrometry UPLC-TripletOF system from AB SCIEX. The chromatographic conditions, separation gradients, and mass spectrometry conditions were set according to the manufacturer's instructions. Identification for the differentially expressed metabolites (DEMs) was performed using the KEGG database (https://www.kegg.jp/kegg/pathway.html) accessed on 30 December 2019 and Python package scipy version 1.0.0. Statistical metabolic pathway enrichment of annotation and analysis was determined using Fisher's exact test and tested the related biological pathways. Metabolomics was commissioned from Shanghai Magi Biomedical Technology Co., LTD (Shanghai, China).

2.7. Analysis of Intestinal Microbe Composition

We tested microbial genomic DNA from the intestine using the FastDNA® Spin Kit for Soil DNA extraction (MP Biomedicals, Irvine, CA, USA). The following primers were designed for amplifying the V3 + V4 region of the bacterial 16S rRNA gene: 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR was conducted in an ABI GeneAmp® 9700 PCR thermocycler (ABI, Carlsbad, CA, USA), and the products were recovered and purified. Then, a small fragment library was constructed based on the characteristics of the amplified 16S rDNA region. The library was then subjected to paired-end sequencing on the Illumina Nova sequencing platform. High-quality tags were obtained after splicing and filtering of reads. Finally, the differences in microbe community structure among different groups were determined using principal coordinate analysis (PCoA) and a sample clustering tree.

2.8. Correlation Analysis of the Metabolomics and the Intestinal Microbes' Composition

SPSS software was used to calculate correlation coefficients and *p*-values. The Spearman correlation coefficients and *p*-values were used to elucidate the correlation between the metabolomics and the intestinal microbe composition in *T. fulvidraco*. A significant correlation was defined as a correlation coefficient >0.6 and $p < 0.05$.

2.9. Data and Statistical Analysis

Growth data were expressed as mean ± standard error ($n = 4$), and the *t*-test of SPSS software was used to compare differences between the two groups. Differences were considered significant at $p < 0.05$. Metabolomics raw data were preprocessed using the Progenesis QI (Waters Corporation, Milford, MA, USA) software, and the data matrix was

obtained by summation and normalization. Subsequently, the data matrix was processed using $\log_2$ logarithmization. Principal component analysis (PCA) and orthogonal partial least squares–discriminant analysis (OPLS–DA) were performed using the R package ropls (Version 1.6.2, manufacture, city, state, Country). Differences were considered significant at variable importance in projection (VIP) >1 and Student's *t*-test $p < 0.05$. The sequences of 16S rRNA were clustered into operational taxonomic units (OTUs) based on 97% consistency and aligned using the SILVA database (version 138) for species annotation analysis. Based on the results of OTU analysis, species annotation was performed using the representative sequences of each OTU to obtain the corresponding species information and distribution patterns. Subsequently, alpha-diversity analysis was performed on the OTUs to compare the composition of the microbial community structure. Beta-diversity analysis was also performed.

3. Results

3.1. Effect of Selenium on the Growth Performance and Antioxidant Ability under Low-Temperature Stress of T. fulvidraco

Selenium increased the PWG, SGR, and SR and decreased the FCR of *T. fulvidraco*. Selenium also enhanced the GSH-Px activity and reduced the serum MDA content of *T. fulvidraco* under low-temperature stress. However, these differences were not significantly compared with the G0 group ($p > 0.05$) (Table 2).

Table 2. Growth performance at normal feeding and antioxidant ability under low-temperature stress of *T. fulvidraco* fed with two different diets.

Groups	Weight Gain Rate (%)	Specific Growth Rate (%/d)	Survival Rate (%)	Feed Conversion Rate	GSH-Px (U/mL)	SOD (U/mL)	MDA (nmol/mL)
G0 group	116.48 ± 7.12	1.84 ± 0.08	95.00 ± 2.95	1.45 ± 0.08	196.81 ± 3.72	0.93 ± 0.01	6.71 ± 0.08
T0 group	133.31 ± 13.43	2.01 ± 0.14	97.86 ± 2.14	1.42 ± 0.09	228.98 ± 25.77	0.81 ± 0.06	5.85 ± 0.15

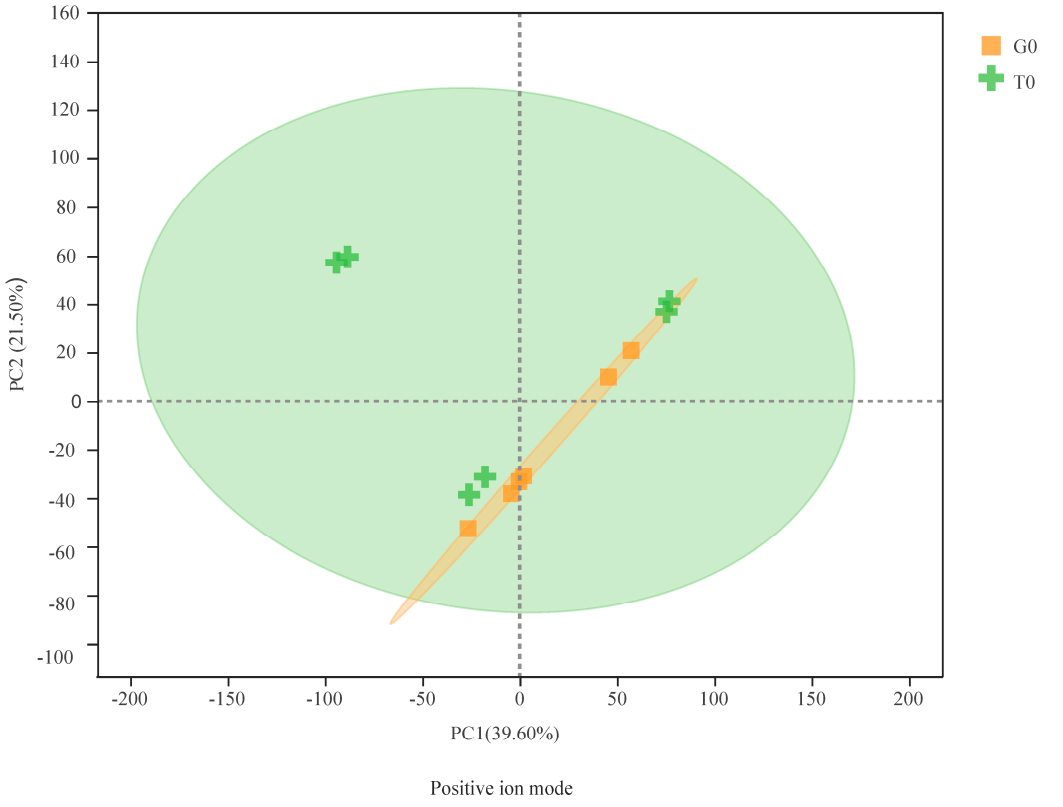
Note: In the same column, values with no letter or the same letter superscripts mean no significant difference ($p > 0.05$), while different small letter superscripts mean significant differences ($p < 0.05$).

3.2. Multivariate Statistical Analysis

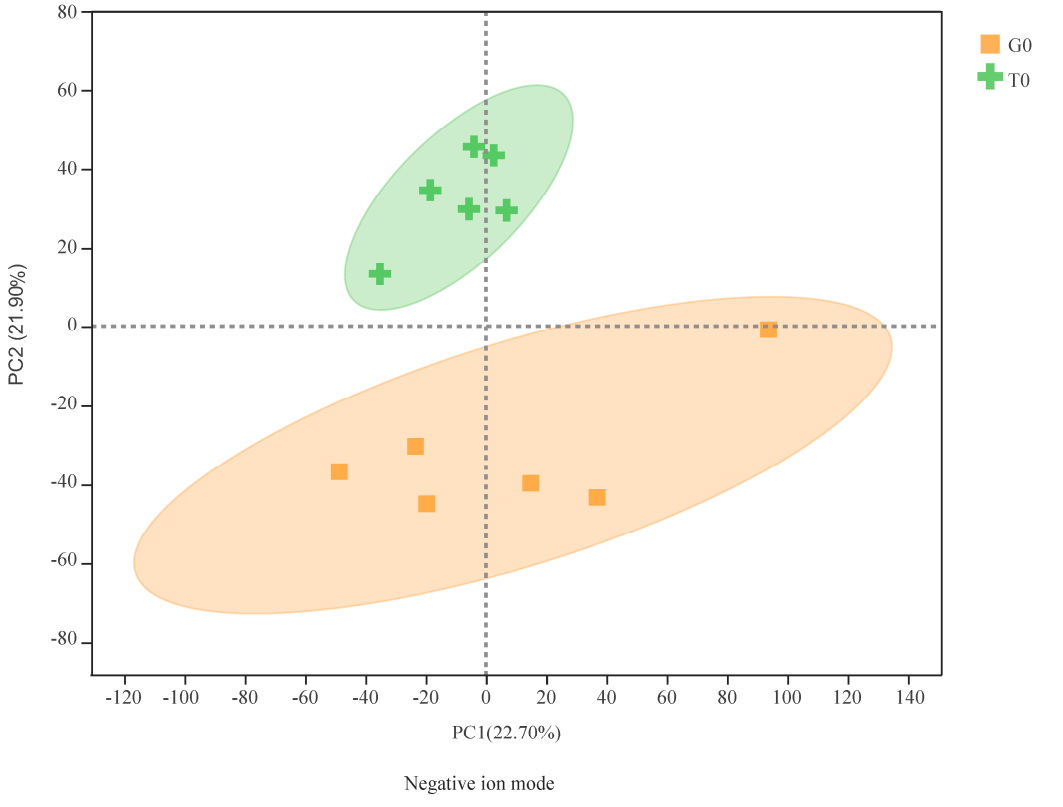
The PCA results suggested that the cumulative explanatory rates (R^2X) of the model were 0.611 and 0.56 in the positive and negative ion modes, respectively (Figure 1). OPLS–DA revealed $R^2X = 0.669$, $R^2Y = 0.997$, and $Q^2 = 0.823$ in the positive ion mode, and $R^2X = 0.41$, $R^2Y = 0.997$, and $Q^2 = 0.914$ in the negative ion mode (Figure 2). Generally, $R^2X > 0.5$ and $Q^2 > 0.5$ indicate the reliability of the model. Therefore, the two models fit well in both ion modes.

3.3. Identification of DEMs

Based on the thresholds of VIP > 1 and $p < 0.05$ (Student's *t*-test), compared with the G0 group, a total of 125 DEMs, including 53 significantly upregulated and 72 significantly downregulated DEMs, were identified in the T0 group (Figure 3). The Human Metabolome Database (HMDB) revealed that the 125 DEMs were mainly lipid and lipid-like molecules, organic acids and their derivatives, organoheterocyclic compounds, phenylpropanoids, polyketides, nucleosides, nucleotides, and analogs (Figure 4).

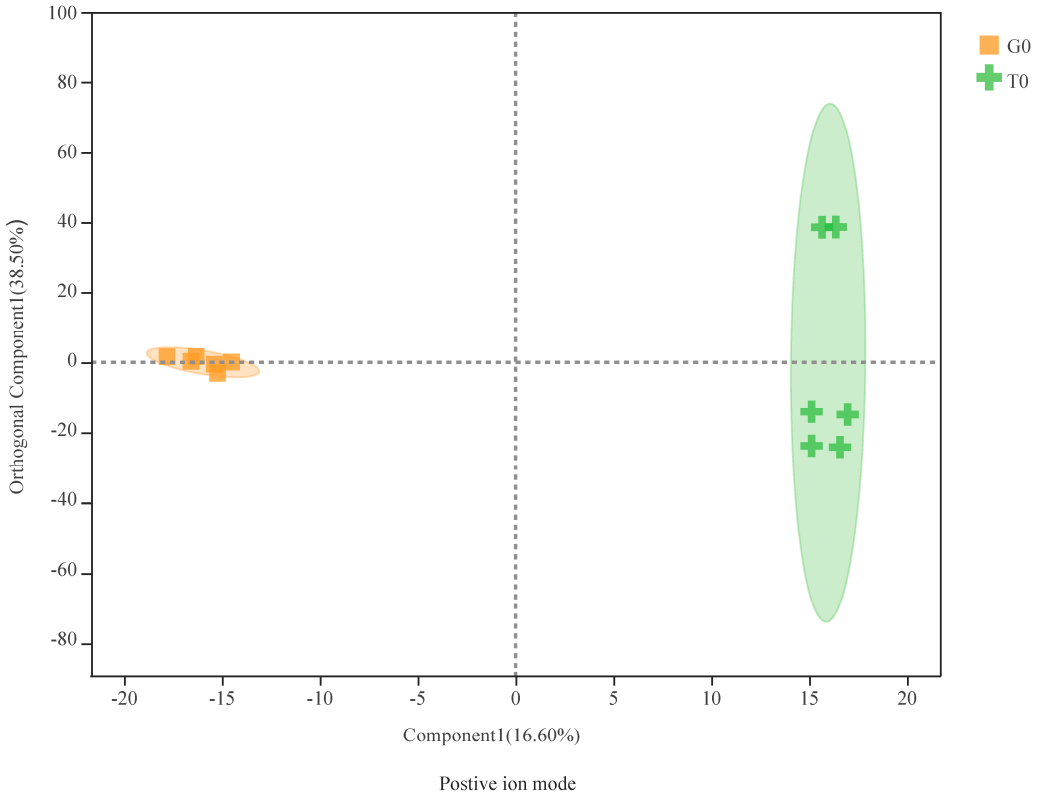


(**A**)

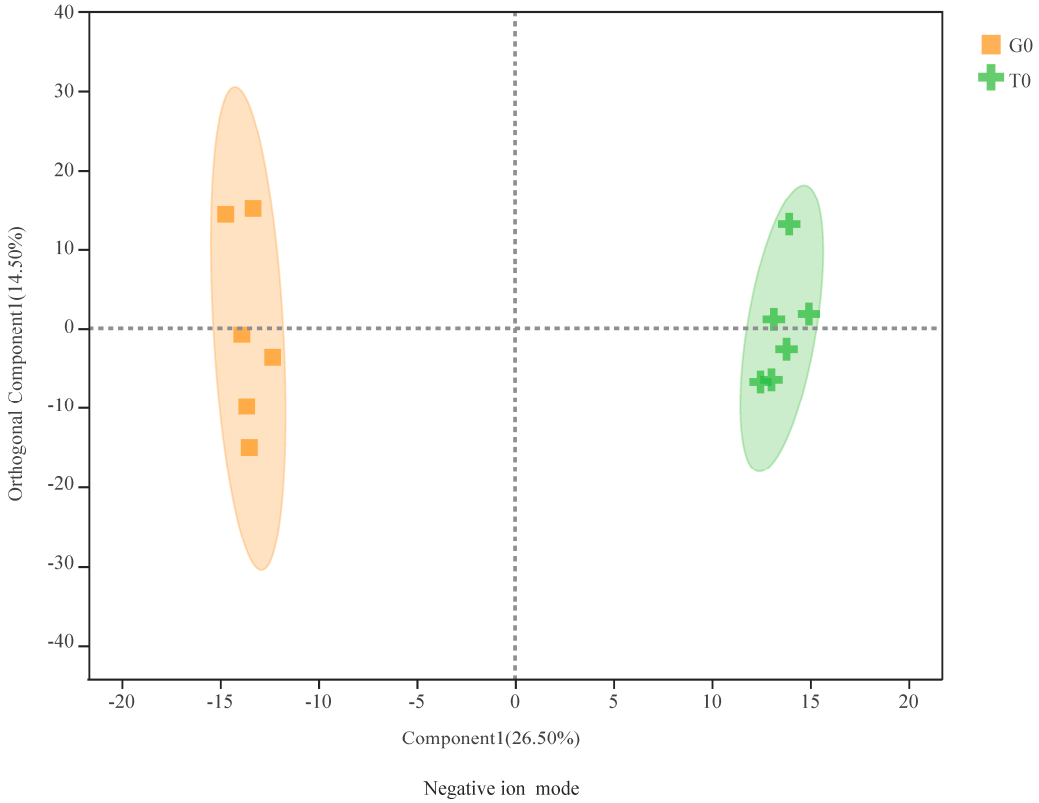


(**B**)

Figure 1. PCA score plot of serum samples from the G0 and T0 groups. Positive ion mode (**A**). Negative ion mode (**B**).



(A)



(B)

Figure 2. Score scatter plot of OPLS-DA mode of serum samples from the G0 and T0 groups. Positive ion mode (**A**). Negative ion mode (**B**).

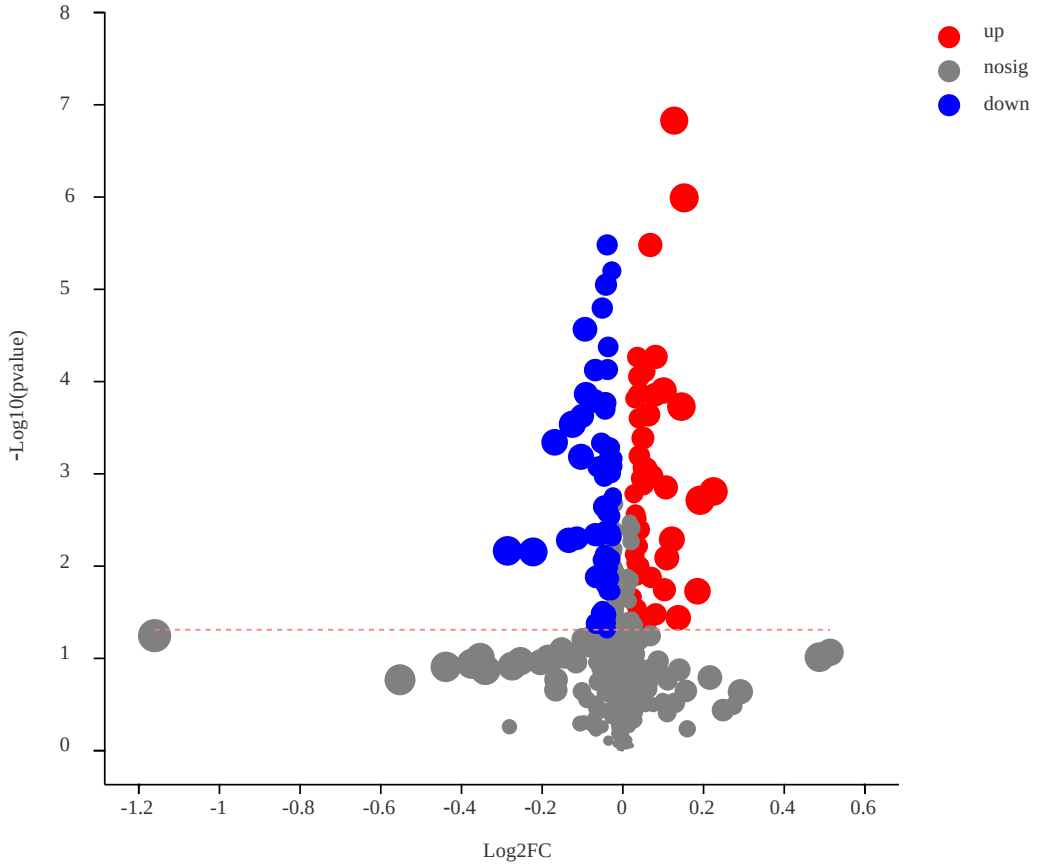


Figure 3. Volcano map of differentially expressed metabolites.

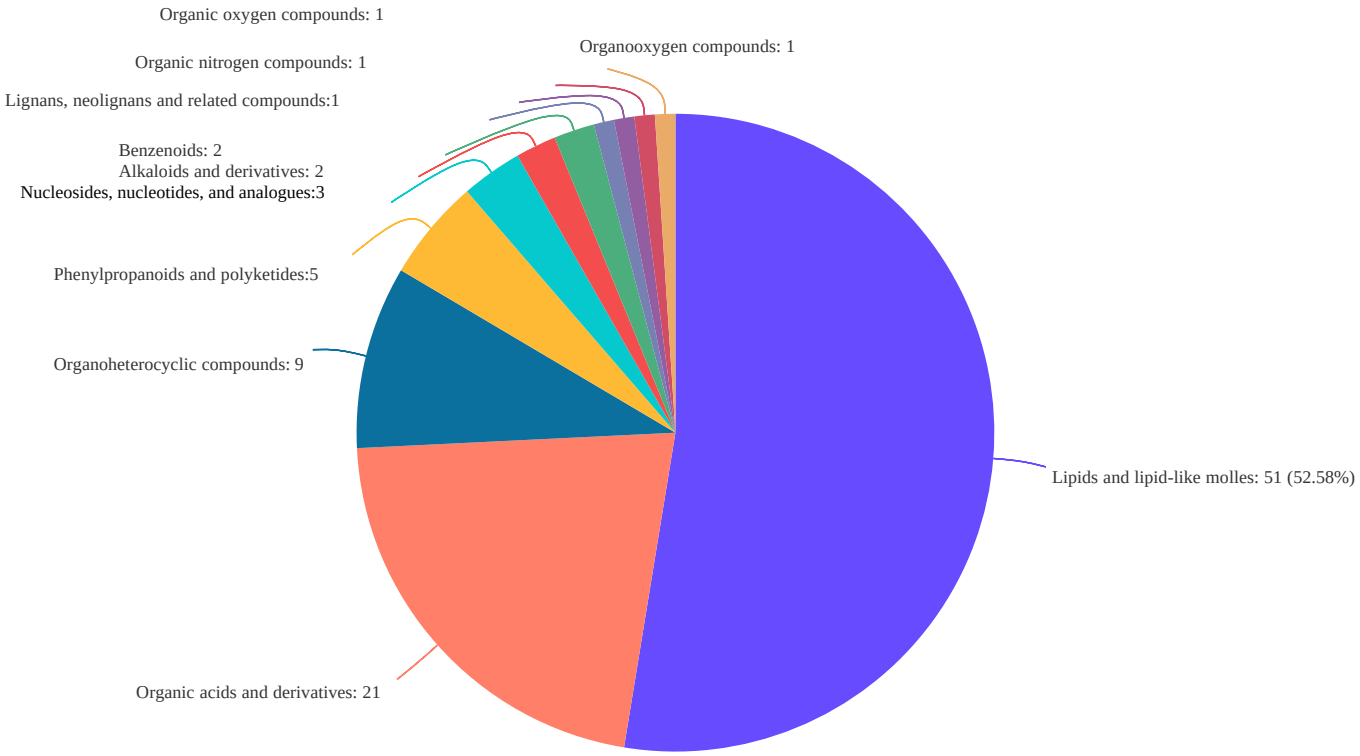


Figure 4. Classification of the identified differentially expressed metabolites according to the HMDB.

3.4. Analysis of DEMs

The HMDB compound classification (class hierarchy) suggested that glycerophospholipids made the largest proportion of both upregulated and downregulated DEMs; fatty acyls, indoles and their derivatives, prenol lipids, sphingolipids, sphingolipids, and pyrimidine nucleosides were among the upregulated DEMs. In contrast, carboxylic acids and their derivatives, organic nitrogen compounds, organic oxygen compounds, organic sulfuric acids and their derivatives, and purine nucleosides were among the downregulated DEMs (Figure 5). In particular, lysoPA (22:5 (4Z,7Z,10Z,13Z,16Z)/0:0) (DPA), lysoPC (20:5(5Z,8Z,11Z,14Z,17Z)) (EPA), ornithine, L-valine, and cytidine were significantly upregulated, whereas PC (22:6 (4Z,7Z,10Z,13Z,16Z,19Z) (DHA), lotaustralin, L-isoleucine, and deoxyinosine were significantly downregulated (Table 3).

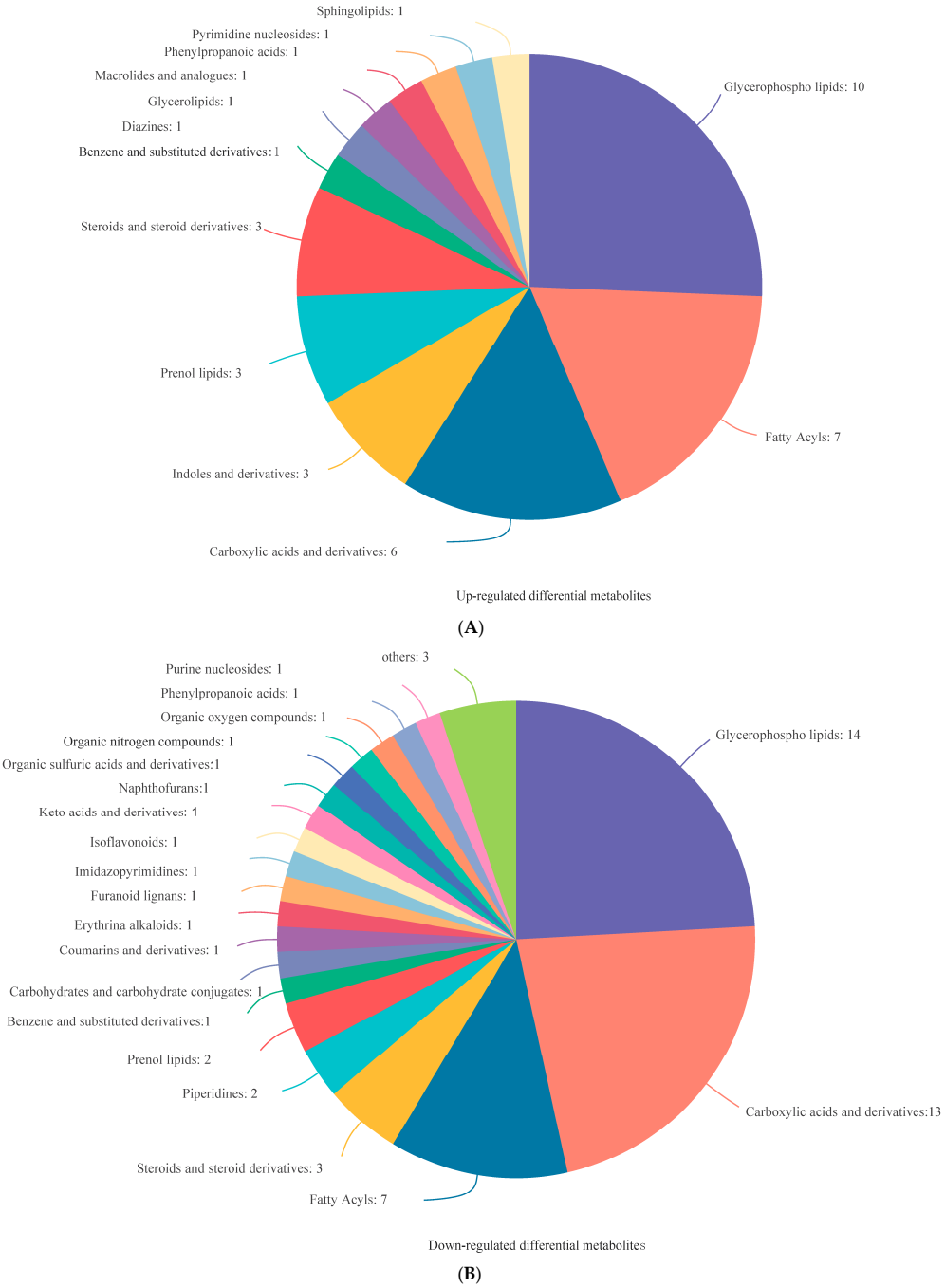


Figure 5. Analysis of upregulated (**A**) and downregulated (**B**) differentially expressed metabolites.

Table 3. Details of several specific differentially expressed metabolites.

Metab ID	Metabolite	Formula	Upregulated/Downregulated
metab_16358	LysoPA (22:5(4Z,7Z,10Z,13Z,16Z)/0:0)	$C_{25}H_{41}O_7P$	Upregulated
metab_11598	LysoPC (20:5(5Z,8Z,11Z,14Z,17Z))	$C_{28}H_{48}NO_7P$	Upregulated
metab_1757	Ornithine	$C_5H_{12}N_2O_2$	Upregulated
metab_10830	Valine	$C_5H_{11}NO_2$	Upregulated
metab_292	Cytidine	$C_9H_{13}N_3O_5$	Upregulated
metab_15415	PC (22:6(4Z,7Z,10Z,13Z,16Z,19Z)	$C_{48}H_{84}NO_7P$	Downregulated
metab_1538	Lotaustralin	$C_{11}H_{19}NO_6$	Downregulated
metab_10678	L-isoleucine	$C_6H_{13}NO_2$	Downregulated
metab_1066	Deoxyinosine	$C_{10}H_{12}N_4O_4$	Downregulated

3.5. Metabolic Pathway Analysis

The KEGG pathway enrichment results revealed that DEMs were primarily enriched in 11 metabolic pathways, including glycerophospholipid metabolism, linoleic acid metabolism, choline metabolism in cancer, protein digestion and absorption, pyrimidine metabolism, mineral absorption, D-arginine and D-ornithine metabolism, and valine, leucine, and isoleucine degradation and biosynthesis (Figure 6).

3.6. Intestinal Microbes Composition

Compared with the G0 group, the T0 group demonstrated an increase in intestinal microbe diversity, as well as an increase in the abundance of *Ralstonia*, *Acinetobacter*, *Brevundimonas*, *Chryseobacterium*, *Sphingobium*, *Sphingomonas*, and *Flavobacterium* and a decrease in the abundance of *Plesiomonas* at the genus level (Figure 7).

3.7. Correlation between Metabolites and Intestinal Microbes

The correlation analysis showed no significant consistency between the intestinal microbes and metabolites, indicating that the changes in metabolism caused by dietary selenium were not significantly correlated with intestinal microbes (Figure 8).

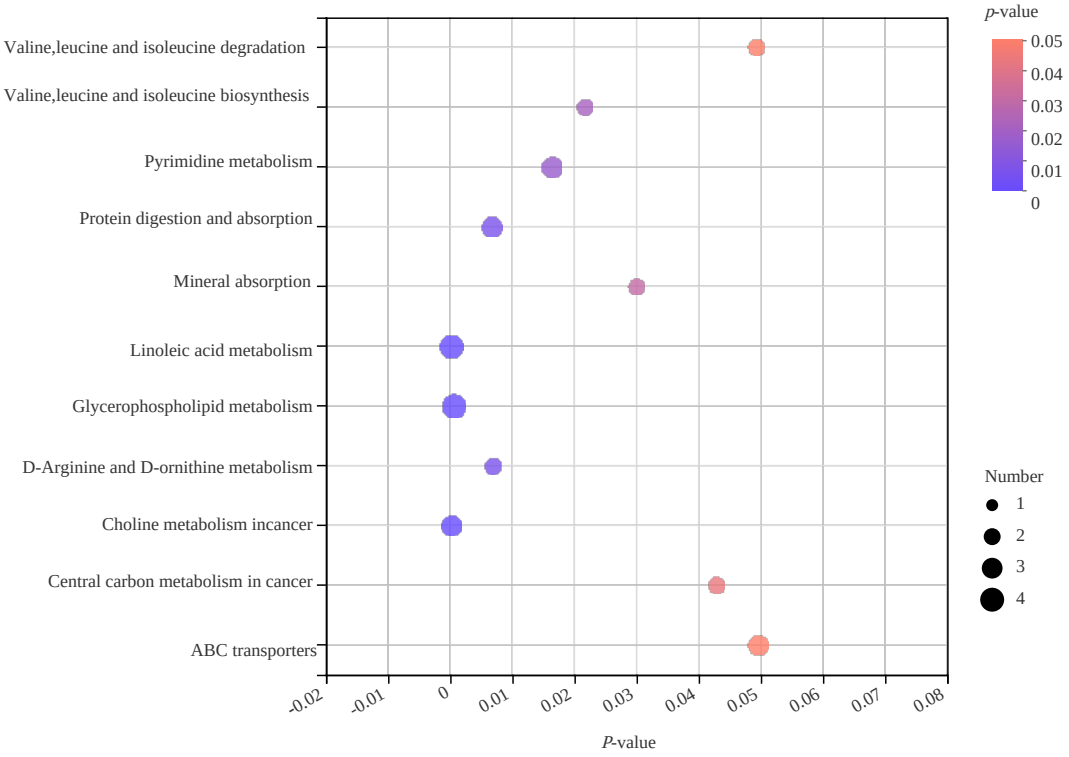


Figure 6. KEGG pathway enrichment of differential metabolites.

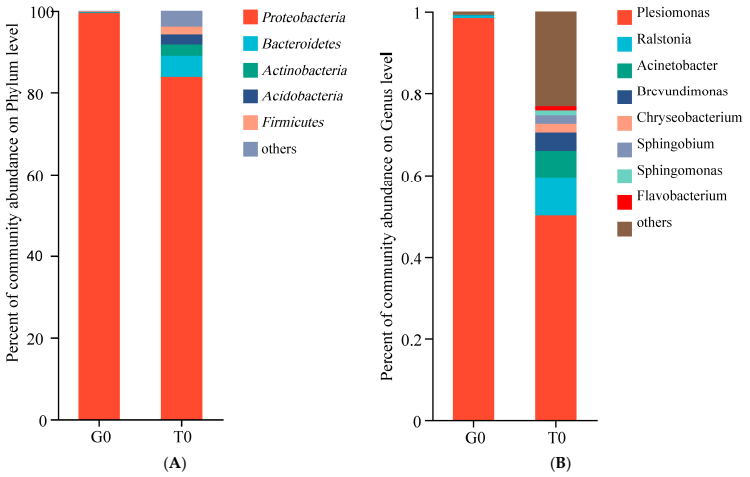


Figure 7. Column chart of relative species abundance at phylum (**A**) and genus (**B**) levels of *T. fulvidraco*.

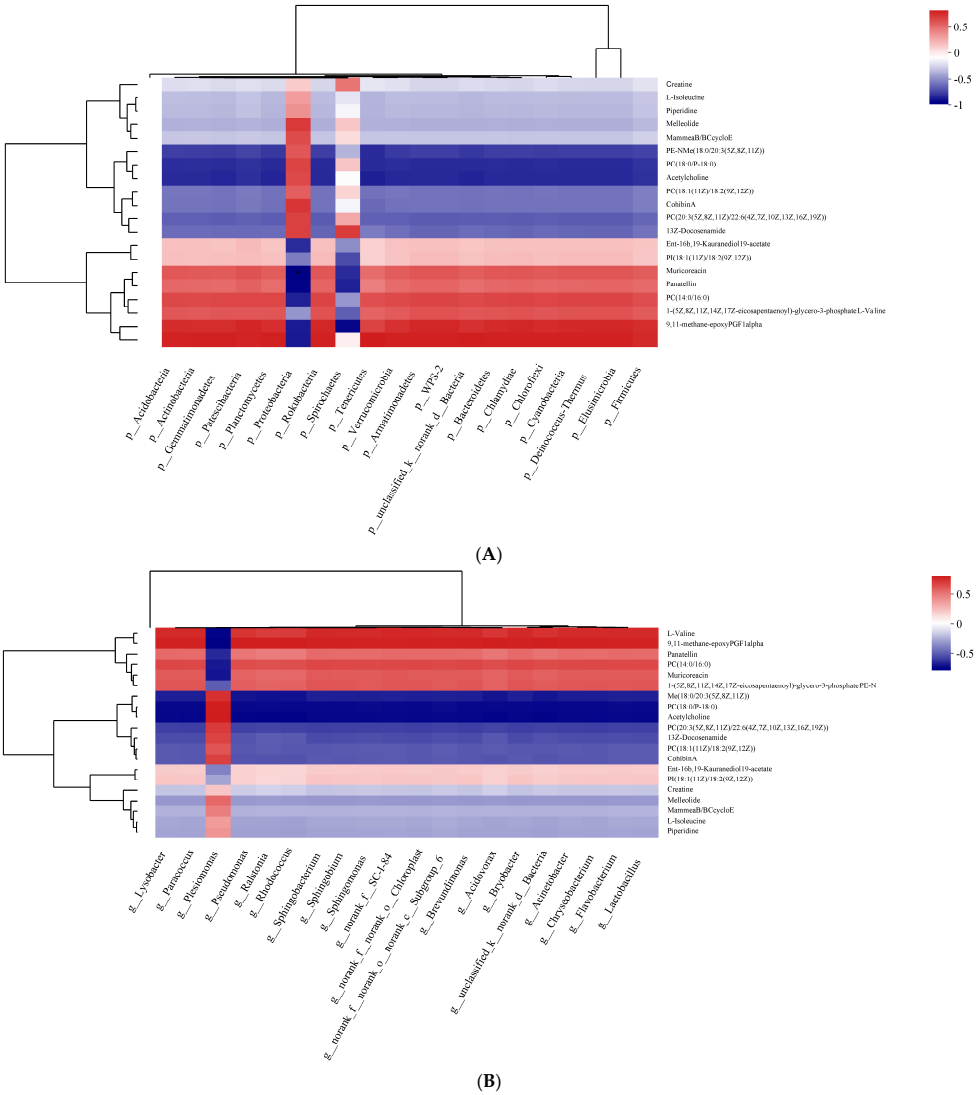


Figure 8. Correlation between intestinal metabolites and microflora at phylum (**A**) and genus (**B**) levels of *T. fulvidraco*.

4. Discussion

4.1. Growth Performance

Selenium is an essential trace element in *T. fulvidraco*. Previously, an 8-week feeding trial showed that the addition of 0.23 mg/kg of selenium to the diet significantly improved the growth performance of *T. fulvidraco* [16]. In this study, PWG and SGR were higher in the T0 group than in the G0 group. However, the difference was not significant, which may be attributable to the short feeding period (only 6 weeks). Nevertheless, these results suggest that selenium can improve the growth performance of *T. fulvidraco*.

4.2. Lipid Metabolism

Lipid metabolism is crucial for the conversion of macronutrients into metabolic intermediates for membrane biosynthesis, energy storage, signal transduction, and resistance to low-temperature stress [17,18]. Maintaining the relative fluidity and integrity of cell membranes for aquatic animals under low-temperature stress is vital for maintaining normal cellular physiological functions and improving stress resistance [19–21]. To adapt to low temperatures, poikilotherms use different mechanisms, including enhanced sphingolipid metabolism, steroid biosynthesis, cholesterol transport, and increased accumulation of unsaturated fatty acids, to maintain the fluidity and integrity of cell membranes [22–24]. Glycerophospholipids and sphingolipids are the first and second major classes of membrane lipids, respectively, and together form the principal components of the biological membrane matrix to maintain the stability of cell membranes [25,26]. Lipids and lipid-like molecules accounted for the highest percentage of selenium-induced DEMs, while some glycerophospholipids and sphingolipids were also significantly upregulated and may be attributed as a mechanism to maintain the stability of *T. fulvidraco* cell membranes under low-temperature stress. Moreover, the target gene of selenium-regulated miR-143 is involved in the metabolism of lipids, fatty acids, sphingolipids, and glycerophospholipids in the mesenteric tissue of *T. fulvidraco* [27]. Furthermore, in mammals, selenium supplementation increased the intestinal lactic acid bacteria content in mice, causing a significant enrichment of the metabolite phosphatidylglycerol [28].

The fatty acid composition of the cell membrane affects its fluidity, and the proportion and content of unsaturated fatty acids are closely related to the resistance of fish to low temperatures. Increasing the content of unsaturated fatty acids and controlling fatty acid desaturation promotes the resistance of fish to low-temperature stress [17]. In this study, the portion of glycerophospholipids was significantly reduced in the cell membranes of fish in the T0 group. Glycerophospholipids are hydrolyzed by phospholipases A1, A2, C, and D to produce glycerol, fatty acids, and phosphoric acid; phospholipase A2 helps release highly unsaturated fatty acids with crucial physiological functions bound at the sn-2 site of the phospholipid backbone [29]. We found that the contents of the polyunsaturated fatty acids EPA and DPA in the T0 group were increased. We hypothesize that the consumed glycerophospholipids in our study may have been catabolized to generate unsaturated fatty acids for maintaining cell membrane fluidity in *T. fulvidraco* under low-temperature stress. The involvement of selenium in regulating unsaturated lipid production has been long reported. In broilers, selenium resulted in significantly high levels of long-chain polyunsaturated fatty acids such as EPA (20:5), DPA (22:5), and DHA (22:6) in muscles [30]. However, in this study, we found that the levels of serum unsaturated fatty acid DHA were significantly reduced in *T. fulvidraco* in the T0 group. After analyzing this result, we speculated that unsaturated fatty acids may have differential catabolism or anabolism in response to low-temperature stress generated by selenium. Similarly, this deduction was consistent with the report that selenium reduced the adverse effects of heat stress on the lipid metabolism of *Oncorhynchus mykiss* [13].

4.3. Carbohydrate Metabolism

The decarboxylation reaction is exergonic and may occur in two forms—simple decarboxylation and oxidative decarboxylation [31]. We observed that the number of downregu-

lated carboxylic acid and its derivatives (13 DEMs) was higher than that of the upregulated counterparts (6 DEMs) in the T0 group compared to the G0 group, indicating that selenium promoted decarboxylation and energy release under low-temperature stress in *T. fulvidraco*. In addition, we also observed the significant enrichment of central carbon metabolic pathways, which primarily include the glycolytic pathway pentose phosphate pathway, and the tricarboxylic acid cycle that supplies the energy and precursors for other metabolic pathways [32,33]. These findings suggest that selenium regulates the central carbon metabolic pathways to provide energy for improving *T. fulvidraco* resistance to low-temperature stress.

4.4. Amino Acid Metabolism

Free amino acids play several functions, including protein synthesis, degeneration, growth, osmotic pressure homeostasis, and energy metabolism, in poikilotherms [34]. The increase in certain amino acids is positively correlated with the decrease in water temperature, which enhances resistance to low-temperature stress [35]. We observed that valine, leucine, and isoleucine degradation/biosynthesis; D-arginine and D-ornithine metabolism; protein digestion; and absorption pathways were significantly enriched in the T0 group under low-temperature stress. In addition, the serum levels of ornithine and L-valine were significantly upregulated, and the serum levels of L-isoleucine were significantly downregulated in the T0 group. The aforementioned results showed that the levels of different amino acids exhibited different trends under the influence of selenium. Selenium metabolism is closely linked to the 1C metabolic pathway [36]. The folate cycle and methionine cycle are the two principal components of 1C metabolism; selenium supplementation increased the levels of cysteine and cysteinyl-glycine and decreased the levels of some other essential free amino acids in the muscle tissues of *Oncorhynchus mykiss* [37]. The effect of selenium on amino acid metabolism may be evident during the synthesis of selenoproteins. The insufficient intake of exogenous selenium or a stressful state leads to the increased participation of amino acids in the synthesis of antioxidant enzymes, thereby promoting the metabolism of antioxidant-like amino acid derivatives, resulting in a decrease in certain amino acids [38].

4.5. Nucleotide Metabolism

Nucleotides are small molecules playing diverse biological functions and are involved in genetic material synthesis, biochemical reaction catalysis, and energy provision and transformation. Low-temperature stress causes significant alterations in nucleotide metabolism in aquatic animals such as *Sparus aurata* [39], *Nibea albiflora* [40], and *Litopenaeus vannamei* [41]. Research findings showed that selenium affected the metabolism of urinary purine derivatives in sheep and cattle [42,43]. Purine nucleoside (Deoxyhypoxanthine) was significantly downregulated, whereas pyrimidine nucleoside (cytosine) was significantly upregulated. Furthermore, the pyrimidine metabolic pathway was significantly enriched in the T0 group under low-temperature stress. Inosine and hypoxanthine are oxidized to xanthine and uric acid via the degradation pathway of purine metabolism, and higher levels of uric acid may cause adverse effects on liver and kidney function [44]. We found reduced contents of purine nucleosides (Deoxyhypoxanthine) in the T0 group, suggesting that selenium exerted a protective effect on the liver and kidney function of *T. fulvidraco* under low-temperature stress.

4.6. Oxidation Products and Other Metabolites

Low-temperature stress disrupts animal homeostasis and produces large amounts of free radicals. Failure to scavenge these excessive oxygen radicals leads to oxidative damage to proteins, lipids, and other biomolecules, and the production of oxidation products. The antioxidant system and non-specific immune system are vital defense mechanisms and exert a mitigating effect on environmental stress-induced oxidative damage. Selenium is a central element for GSH-Px activity; GSH-Px uses reduced GSH as a substrate to

reduce the generation of harmful hydroperoxides and free radicals, reducing peroxide damage to biomacromolecules and cell membranes [45]. Previous studies showed that the addition of selenium to the diet significantly increased the activity of liver GSH-Px and SOD enzymes in *T. fulvidraco* [14] and reduced lipid peroxide malondialdehyde and reactive oxygen species production in *Oncorhynchus mykiss* [46]. We observed that organic oxygen compounds, nitrogen-containing organic compounds, lotaustralin, and coumarin and their derivatives were significantly downregulated in the T0 group, suggesting that selenium is beneficial in scavenging free radicals produced by low-temperature stress and reducing organism damage.

4.7. Intestinal Microbes Diversity

The intestinal microbes, a crucial microbial barrier in the fish intestine, have diverse physiological functions, including regulating fish nutrient metabolism and immune response and inhibiting the proliferation of harmful bacteria. Increased intestinal microbe diversity is beneficial in maintaining intestinal health, while the lack of diversity can cause intestinal dysbiosis [47]. The nutritional composition and feeding environment can affect the abundance of intestinal microbes and metabolite levels. Low-temperature stress significantly reduced the intestinal microbial diversity in *Oncorhynchus mykiss* [48], *Seriola lalandi* [49], and *Apostichopus japonicus* [50]. In our previous study, the intestinal microbe diversity of *T. fulvidraco* was also significantly reduced, and the pathogenic bacteria of *plesiomonas* was rapidly in abundance [51]. In this study, we found that selenium supplementation was helpful in increasing the diversity of *T. fulvidraco* intestinal microbes and reducing the relative abundance of *Plesiomonas*, while other microbes, including *Brevundimonas*, *Chryseobacterium*, *Sphingobium*, *Sphingomonas*, and *Flavobacterium*, demonstrated significant proliferation. Similarly, Baia et al. (2018) [52] observed that selenomethionine supplementation at a dose of 5.03 mg/kg significantly affected the intestinal microbe diversity in *Carassius auratus*, increasing the abundance of *Cetobacterium* and decreasing that of *Ralstonia Cetobacterium*, a species of anaerobic bacteria present in the intestine of many fish, is involved in synthesizing vitamin B_{12} [53,54]. *Plesiomonas* and *Ralstonia* are present in the intestinal tract of fish and are opportunistic pathogens of fish [55,56]. However, a study on rainbow trout observed that selenium had a lower effect on intestinal microbe diversity under normal feeding conditions [52]. These studies suggested that selenium supplementation for different fish has differential effects on intestinal microbes and the health of fish. This may be attributed to the intestinal environment in which microbial colonization species in the fish gut act with selenium. Currently, few studies have been conducted on *Brevundimonas*, *Chryseobacterium*, *Sphingobium,* and *Flavobacterium*, and the function of these microbes is not exactly clear. However, some research showed that endogenous sphingolipid metabolites and indoleacetic acid, l-tryptophan, N-acetylserotonin, l-histidine, l-aspartic acid, phosphatidylserine, 5-methoxyindoleacetate, and L-serine were related to *Sphingomonas* [57,58]. However, correlation analysis showed no association between the intestinal microbes and the metabolites in this experiment. Thus, the selenium-induced changes in the metabolome of *T. fulvidraco* under low-temperature stress may be primarily related to the antioxidant capacity and the function of regulating lipid and protein metabolism of selenium but not so much related to the changes in the intestinal microbes.

5. Conclusions

Selenium primarily regulated lipid, organic heterocyclic compounds, and amino acid metabolism through linoleic acid metabolism, glycerophospholipid metabolism, amino acid metabolism, and pyrimidine metabolism pathways to maintain the fluidity and integrity of cell membrane and energy substance provision and concurrently decreased the production of organic oxides, nitrogenous compounds, purine nucleosides, and other harmful metabolites to improve the low-temperature stress resistance of *T. fulvidraco*. Moreover, selenium also increased the intestinal microbe diversity and reduced the relative abundance of *T. fulvidraco*. Overall, this study revealed that selenium affects the occurrence regularity

of all small molecular metabolites and the changes in the intestinal microbe community of *T. fulvidraco*, which provided a scientific basis for further revealing the mechanism of selenium in improving the resistance of *T. fulvidraco* to low temperature.

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