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Research Article

Effects of Salinity on Muscle Nutrition, Fatty Acid Composition, and Substance Anabolic Metabolism of Blue Tilapia *Oreochromis aureus*

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A 60-day culture in brackish water (0, 3, 5, 7, 9, and 11 ppt) was conducted to study the effects of salinity on muscle nutrition, fatty acid composition, and anabolic metabolism of blue tilapia *Oreochromis aureus*. Current research showed that WGR, SGR, and FCR indicators of tilapia were better improved in brackish water. In addition, the physical properties of *O. aureus* muscle demonstrated a positive correlation with salinity levels. The content of amino acids and fatty acids in *O. aureus* muscles grown under salinity was higher than that in freshwater ($P > 0.05$). Venn analysis showed that 2343 common differentially expressed genes (DEGs) were identified in the four groups (0, 3, 7, and 11 ppt), of which 767 were up-regulated and 1576 were down-regulated. GO and KEGG analysis revealed 39 significant pathways, mainly including unsaturated fatty acid biosynthesis, fatty acid elongation, and α-linolenic acid metabolism. The heat map and trend analysis showed that the expression levels of key genes involved in the physiological processes of ELOVL, SPLA2, and FADS2 in the test group were significantly higher than those in the control group ($P > 0.05$). The above results suggested that *O. aureus* can not only adapt to the saline habitat but also improve muscle quality by regulating the body's metabolic pathways.

1. Introduction

Taste and nutrition are important indexes for evaluating fish flesh quality in fish processing and farming [1, 2]. While the genetic characteristics of species are primarily responsible for these qualities, they can also be influenced by factors such as feed composition and rearing environment [3, 4]. Among them, ambient salinity is a crucial factor affecting fish flesh quality. Previous studies have found that the amino acid and fatty acid content in the muscles of channel catfish *Ictalurus punctatus* and turbot *Scophthalmus maximus* increased with increasing salinity [5, 6]. Similarly, studies on Nile tilapia *Oreochromis niloticus* and rainbow trout *Oncorhynchus mykiss* found significant differences in amino acid and fatty acid content between seawater and freshwater aquaculture environments [7, 8]. Simultaneously, under salinity conditions, the total polyunsaturated fatty acids (PUFA) in grass carp *Ctenopharyngodon idella*, Japanese seabass *Lateolabrax japonicus* and Nile tilapia, especially docosahexaenoic acid (DHA) and eicosapentaene acid (EPA) increased compared to fresh water [9–11].

Saline land refers to areas with salt accumulation in the soil [12]. In China, there are over 99 million hectares of saline land, which poses a serious threat to the survival and reproduction of animals and crops, leading to extremely low resource utilization [13]. Fortunately, researchers have discovered that the blue tilapia *Oreochromis aureus*, a freshwater fish, exhibits high tolerance to harsh environments and has great potential for fisheries development in saline lands [14]. *O. aurea* is an euryhaline fish known for its ease of reproduction, rapid growth, and good quality, making it highly popular among farmers and consumers in China [15, 16]. Furthermore, tilapia has been successfully farmed in saline and alkali waters with good

economic benefits [17, 18]. In addition, the muscle nutrition (amino acids, moisture, and collagen) and physical properties (mouthfeel, elasticity, and chewiness) of tilapia cultured in saline-alkali waters are significantly improved compared to freshwater [7, 19].

Euryhaline fish adapt to salinity mainly through physiological functions such as osmotic adjustment, ion transport, and energy metabolism [20, 21]. However, the specific mechanism by which salinity factors influence changes in fish muscle nutrition remains unclear, particularly regarding the variations in amino acid and fatty acid composition. Herein, a 60-day feeding experiment was conducted in brackish water (0, 3, 5, 7, 9, and 11 ppt, parts per thousand) to explore the effects of salinity on muscle nutrition and the amino acid and fatty acid composition of *O. aurea*. Meanwhile, this study also collected livers from different treatment groups for second-generation transcriptome analysis and explored some pathways related to amino acid and fatty acid metabolism and key gene expression. The objective of this research is to examine how salinity influences the muscle quality of tilapia and to provide a foundation for understanding the regulation mechanism of meat quality as well as the development of fisheries in brackish waters.

2. Materials and Methods

2.1. Ethics Statement. All operations on animals in this study followed the standard practices of animal care and use. The practices related to the animals in this study were approved by the Guangxi Institutional Animal Care and Use Committee (GACUC number 201703021).

2.2. Experimental Design. A total of 900 healthy and vigorous juvenile tilapia (average weight 15.52 ± 3.90 g, length 12.01 ± 0.43 cm) were sourced from the Hainan Nanfan breeding base in Hainan, China. Fish were kept in the pond ($4.5 \times 1.8 \times 1$ m) for a week before the experiment started and then randomly assigned to 6 treatment groups, including a control group (0 ppt) and 5 salinity test groups (3, 5, 7, 9, and 11 ppt). Each group has three replicates containing 150 fish. The salinity group was increased at a rate of 1 ppt per day so that fish could better adapt to the new salinity and then maintained for 2 days before the formal 60-day feeding begins. Calibrate with a salinity meter (Hengxing, Taiwan, China) every week to ensure the salinity level of each treatment. Fish were fed 3 times a day (8: 00 am, 12:00 pm, and 17:00 pm) with 2.5% biomass, and the remaining bait was collected one hour after feeding. The body weight was measured every two weeks to adjust the feeding amount. Each group was fed a uniform commercial feed (protein 35%, lipid 3%), which was purchased from Hainan Hengxing Feed Co., Ltd. During the experiment, the water temperature was maintained at 26–28°C, and the dissolved oxygen was greater than 5.0 mg/L.

2.3. Sample Collection and Analysis

2.3.1. Sample Collection. After the 60-day feeding period, 30 fish were randomly selected from each group and anesthetized with 0.01% methanesulfonate (MS-222) which destroyed the brain to death. Then, back muscles and liver samples were taken and stored at −20 and −80°C, respectively, for further analyses.

2.3.2. Statistical Analysis of Growth. The average initial and final weight (wet weight) of fish, weight gain rate (WGR, %), specific growth rate (SGR, %), feed conversion ratio (FCR, %), net weight (NW, %), and condition factor (CF, g/cm) were calculated as follows:

$$\begin{aligned}
\mathrm{WGR}(\%) &= 100\% \times \frac{\left(W_f - W_0\right)}{W_0}, \\
\mathrm{CF}\left(\frac{g}{\mathrm{cm}}\right) &= 100\% \times \frac{W_f}{L_f^3}, \\
\mathrm{NW}(\%) &= 100\% \times \frac{W_{f_n}}{W_f}, \\
\mathrm{SGR}(\%) &= 100\% \times \frac{\left(\ln W_f - \ln W_0\right)}{t}, \\
\mathrm{FCR}(\%) &= \frac{W_{\mathrm{ft}}}{\left(W_f - W_0\right)}.
\end{aligned} \tag{1}$$

In formulae, W_0 is the average initial weight. W_f is the average final weight. L_f^3 is the body length (cm) after 60 days. W_{f_n} is the visceral weight (g). W_{ft} is the total amount of feed intake after 60 days of rearing (g), and t is the rearing time.

2.3.3. Analysis of Physical Characteristics of Muscle. In order to understand the physical characteristics of different groups of muscles, a texture analyzer (CNS Farnell QTS-25, UK) was used for quality composition analysis. The back muscles of tilapia were taken and cut into $10 \times 10 \times 10$ mm squares. The texture profile analysis (TPA) mode was used to compress the sample twice. The experimental parameters were set according to Zhang et al. [6] to record the constituent flesh quality parameters such as hardness, adhesiveness, springiness, chewiness, and cohesiveness.

2.3.4. Analysis of the Water Loss Rate of Muscle. The water loss rate of muscles from different groups was investigated in this study. The specific operational method refers to Li et al. [10]. Briefly, take the back muscles and weigh them (W_0) before boiling. Then boil them for 5 min, remove them to cool, and weigh them (W_t). Water-loss rate (%) = $(W_0 - W_t)/W_0 \times 100$.

2.3.5. Analysis of the Proximate Composition of Muscle. The AOAC method was used to detect the proximate components in the muscle of fish. Briefly, an automatic Kjeldahl analyzer (Shimadzu UDK132, Japan) was used crude protein content. Determination of moisture content by constant temperature drying at atmospheric pressure 105°C. Crude ash content was determined by high temperature burning method of 550°C muffle furnace, and lipid was analyzed by ethyl ether extraction method.

2.3.6. Fatty Acid Analysis. The fatty acid composition of muscle samples was determined referring to the description of Wu et al. [22]. Briefly, chloroform-methanol (2 : 1, V/V) was used to extract total lipids (TL) from the pretreated samples. After grinding and standing for 2 h, the solution was separated on qualitative filter paper and centrifuged at 3000 r/min for 5 min with 4 mL of distilled water to obtain the lower liquid layer. Then, remove water and residual methanol and chloroform from the solution in a water bath at 40°C. Next, 1 mL of chromatographically pure n-hexane was added to the tube, and after the oil at the bottom was dissolved, 1 mL of 0.4 mol/L KOH-methanol solution was added, and methyl esterification was carried out by shaking for 30 min. After methyl esterification, 2 mL of ddH_2O_2 was added and mixed well, and then centrifuged at 3000 r/min for 5 min, and the supernatant was taken for testing. The prepared fatty acid methyl ester (FAME) was transesterified with boron trifluoride ether, and then gas chromatograph (Shimadzu, Kyoto, Japan) with methyl decarbonate as the internal standard. By comparing with commercial fatty acid standard solution (NU-CHEK, USA), the content of individual fatty acids was identified and quantified using CLASSGC10 GC-17A workstation (Shimadzu, Kyoto, Japan).

2.3.7. Amino Acid Analysis. The amino acid composition of the muscle sample was detected using the amino acid analyzer (Hitachi L-8900, Japan). In brief, add 10 ml of 6 mol/L hydrochloric acid and 3 drops of phenol were added to 2 g of homogeneous sample, frozen for 5 min before adding nitrogen, and hydrolyze at 110°C for 22 h. After filtration, take 1 ml of the filtrate was taken and evaporate to dryness in a parallel evaporator at 45°C, then add 2 ml of sodium citrate buffer was added shake evenly, and used for determination after 0.22 membrane filtration. The amount of amino acids was expressed as milligrams of amino acids per Gram of dry tissue (mg/g dry weight).

2.3.8. Evaluation Method of Muscle Nutrition Value. The chemical score (CS), amino acid score (AAS), and essential amino acid index (EAAI) of muscle refer to the FAO/WHO amino acid score standard model and the amino acid model of egg protein [23], the calculation formula was as follows:

$$\mathrm{EAAI} = \left[\frac{\mathrm{Lys}(t)}{\mathrm{Lys}(s)} \times 100 \times \frac{\mathrm{Met}(t)}{\mathrm{Met}(s)} \times 100 \times \frac{\mathrm{Val}(t)}{\mathrm{Val}(s)} \times 100\right]^{1/n}. \quad (2)$$

CS = Protein amino acid content of samples to be evaluated (mg/g Protein)/Egg protein amino acid content (mg/g Protein).

AAS = Protein amino acid content of samples (mg/g Protein)/(WHO/FAO Scoring pattern amino acid content) (mg/g Protein).

Abbreviations in the above formula: n = the number of amino acids, s = standard protein, WHO = World Health Organization, and FAO = World Food and Agriculture Organization.

2.4. Transcriptome Data Analysis. The transcriptome data from various salinity test groups (0, 3, 7, and 11 ppt) were obtained from our laboratory's experiments and were analyzed as outlined by Zhou et al. [24]. Initially, the edgeR package (https://www.r-project.org/) was employed to identify differentially expressed genes (DEGs) across the groups. Genes exhibiting a fold change >2 and a false discovery rate (FDR) < 0.05 in comparisons were deemed significant DEGs. Subsequently, all DEGs were subjected to gene ontology (GO) enrichment analysis using the gene ontology database (https://www.geneontology.org/). In addition, pathway enrichment analysis was conducted using the Kyoto Encyclopedia of Genes and Genomes pathway database (KEGG) [25]. This analysis aimed to identify significantly enriched metabolic and signal transduction pathways in DEGs compared with the whole genome background (COG/KOG, https://www.ncbi.nlm.nih.gov/COG/). The analysis was complemented by heatmap and trend analysis (series test of cluster) using OmicShare tools, an online platform for data analysis (https://www.omicshare.com/tools).

2.5. Quantitative Real-Time PCR. The sequences of the six primers (including Unigene0030910 reference genes) are presented in Table S1 [24]. Total RNA was prepared using the Trizol reagent (Invitrogen, Shanghai, China). RNA was used for reverse transcription after identification of RNA quality by electrophoresis and spectrophotometry. The first-strand cDNA synthesis was performed using the HiScript II Q RT SuperMix for qPCR (+gDNA wiper) kit (Vazyme Biotech, Nanning, China). The reaction system was 20 μl, which included 0.4 μl each of the forward and reverse primers, 10 μl of 2× ChamQ SYBR qPCR Master Mix, 4 μl of cDNA samples, and 5.2 μl of ddH_2O. The qPCR reacted at 95°C for 90 s and followed by 40 cycles at 95°C for 5 s, 60°C for 15 s, and 72°C for 20 s. The $2^{-\Delta\Delta ct}$ method was used to calculate the relative expression level of each sample, and each reaction has three independent biological replicates [24].

2.6. Statistical Analysis. All data were displayed as the mean ± standard deviation (SD). Salinity as a factor, one-way ANOVA was performed using SPSS 20 statistics, and then polynomial linear contrast and Duncan's multiple comparison test were used to determine significant differences among groups [26]. When $P < 0.05$, the difference was considered significant. The pictures were made in GraphPad Prism 8.0.2 software.

3. Results

3.1. Fish Performance. Growth data were collected after the fish had been fed for 60 days (Table 1). The final weight (61.98 g), specific growth rate (SGR, 2.31%), and weight gain rate (WGR, 299.03%) of 9 ppt were the highest, which were statistically significantly higher than 3 ppt ($P < 0.05$), but there were no significant differences among other groups ($P > 0.05$). Then, we observed that with the increase in

Table 1: The effect of different salinity on the growth performance of *Oreochromis aurea*.

Parameters	Groups					
	0	3	5	7	9	11
Initial weight (g)	15.65 ± 4.21	15.57 ± 3.4	15.43 ± 3.29	15.44 ± 4.98	15.53 ± 4.3	15.52 ± 3.23
Final weight (g)	56.78 ± 5.68^{ab}	51.26 ± 2.39^{a}	55.33 ± 4.91^{ab}	56.11 ± 4.46^{ab}	61.98 ± 2.89^{b}	58.67 ± 3.56^{ab}
WGR (g)	262.81 ± 36.31^{ab}	229.15 ± 15.36^{a}	258.43 ± 31.8^{ab}	263.42 ± 28.91^{ab}	299.03 ± 18.65^{b}	277.89 ± 22.95^{ab}
SGR (%/day)	2.13 ± 0.16^{ab}	1.98 ± 0.07^{a}	2.12 ± 0.15^{ab}	2.14 ± 0.13^{ab}	2.3 ± 0.07^{b}	2.21 ± 0.1^{ab}
FCR (%)	1.02 ± 0.13^{ab}	1.13 ± 0.07^{a}	1.03 ± 0.13^{ab}	0.99 ± 0.11^{ab}	0.86 ± 0.06^{b}	0.93 ± 0.09^{ab}
CF (g/cm^3)	3.22 ± 0.18^{ab}	3.27 ± 0.18^{ab}	3.03 ± 0.16^{a}	3.15 ± 0.07^{a}	3.48 ± 0.10^{b}	3.49 ± 0.14^{b}
NW (%)	88.52 ± 0.88^{ab}	88.95 ± 0.71^{b}	86.39 ± 0.49^{c}	87.02 ± 1.15^{ac}	86.84 ± 0.34^{ac}	87.79 ± 0.89^{abc}

Note. Values are mean ± SD; WGR: weight gain rate; SGR: specific growth rate; FCR: feed conversion ratio. CF: condition factor; EW: net weight; 0 : 0 ppt salinity; 3 : 3 ppt salinity; 5 : 5 ppt salinity; 7 : 7 ppt salinity; 9 : 9 ppt salinity; 11 : 11 ppt salinity; different superscript letters in the same row indicate significant differences ($P < 0.05$).

salinity, the feed coefficient showed a slight downward trend. Among them, the feed coefficient of 9 ppt was the lowest (0.86), and it was significantly less than that of 3 ppt (1.13) ($P < 0.05$). The condition factor of 9 and 11 ppt (3.48 and 3.49 g/cm^3) was significantly different from 5 to 7 ppt (3.03 and 3.15 g/cm^3) ($P < 0.05$). In addition, the eviscerated weight after removal of the viscera was significantly higher at 3 ppt than at 5 and 9 ppt ($P < 0.05$). In short, these data indicate that *O. aurea* grows best at 9 ppt.

3.2. Physical Properties and Proximate Composition of Muscle. The results for the physical properties of the muscle were detailed in the figure (Figure 1(a)). The water-loss rate of each group was 24.7%–30.15%, and the water-loss rate of the treatment group was lower than that of the control (30.15%), and the 5 ppt group was significantly lower than the other groups ($P < 0.05$). This indicates that the saline environment can improve the water-holding capacity of *O. aureus* muscles, and the salinity of 5 ppt (24.7%) has the best effect in this study. In addition, the same muscle on the back of the fish was collected for physical property testing after 60 days of culture. We found that the hardness, adhesiveness, and chewiness index showed an overall upward trend with increasing salinity. Compared with the control, the hardness and chewiness values of all treatments were significantly increased, among which the 3 ppt group (1,492 g, 515 gf) had the highest ($P < 0.05$). Apart from the 3 ppt group, the other treatment groups had significantly higher chewing force than the control, the highest being the 5 ppt group (15.24 gf/mm) ($P < 0.05$). The group with the highest springiness was the 3 ppt group (0.57), which was significantly higher than the control and 9 ppt groups ($P < 0.05$). There was no significant difference in cohesiveness between the groups, and the 7 ppt group (0.55) had the lowest value ($P > 0.05$). The above results reveal that salinity can greatly improve the evaluation index of the stickiness, chewiness, and hardness of fish muscles, thereby improving the flesh quality.

After 60 days of cultivation, the contents of moisture, ash, crude protein, and crude lipid in the muscles of each group were observed (Figure 1(b)). The moisture content showed a decreasing trend with increasing salinity (maintained between 76.33% and 77.07%), and the difference between the groups was not significant ($P > 0.05$). The ash value of each test group was not significantly different from that of the control, but the 5 ppt group (1.43%) was significantly higher than 3 ppt, 9 ppt, and 11 ppt ($P < 0.05$). The amount of crude protein in each group was 19.37%–20.6%, and the 3 ppt group (20.6%) was significantly higher than the other four salinity levels, but the control group was not included ($P < 0.05$). Curiously, no significant difference was observed in crude fat between all groups ($P > 0.05$), with the lowest in the control (1.57%) and the highest in the 5 ppt (1.87%).

3.3. Amino Acid Composition in Muscle. The amino acid composition of *O. aurea* muscle is detailed in Table 2. Apart from the destruction of tryptophan and the low cysteine content during acid hydrolysis, a total of 16 amino acids were detected, with glutamic acid (2.15–2.51 g) and aspartic acid (1.69–1.71 g) being the highest in all samples, and methionine and histidine being the lowest. However, only isoleucine showed significant differences between the groups ($P < 0.05$), with 7 ppt being the highest (0.76 g). Surprisingly, maximum values of all four indicators were recorded at 5 ppt salinity, including total amino acids (TAA, 15.97 g), essential amino acids (EAA, 6.43 g), nonessential amino acids (NEAA, 9.51 g), and palatable amino acids (DAA, 6.23 g). Simultaneously, the maximum DAA/TAA ratio was also observed at 5 ppt, indicating that the quality of tilapia cultured under this condition was the best. In addition, we observed that the ratio of EAA/NEAA was 67.1% on average, of which 68.7% at 7 ppt was significantly higher than the other groups ($P < 0.05$), and the ratio of EAA/TAA was about 40.1%, and the maximum value also appeared at 7 ppt (40.6%), which was significantly higher than that of the control, 9 ppt and 11 ppt ($P < 0.05$). It is generally accepted that EAA/NEAA (>60%) and EAA/TAA (about 40%) are important evaluation indices of high-quality protein [27]. These results indicate that *O. aurea* is a high-quality protein source and that culture at moderate salinity can further improve protein quality.

The nutritional evaluation of the essential amino acids in the muscles of *O. aurea* is shown in Table 3. We observed that the EAAI value remained between 89.56 and 93.01, with the highest and lowest in the 5 ppt and 11 ppt groups, respectively. This indicates that the closer the cultured salinity was to 5 ppt, the more amino acids in *O. aurea* muscle met the FAO/WHO Essential Amino Acid Index scoring

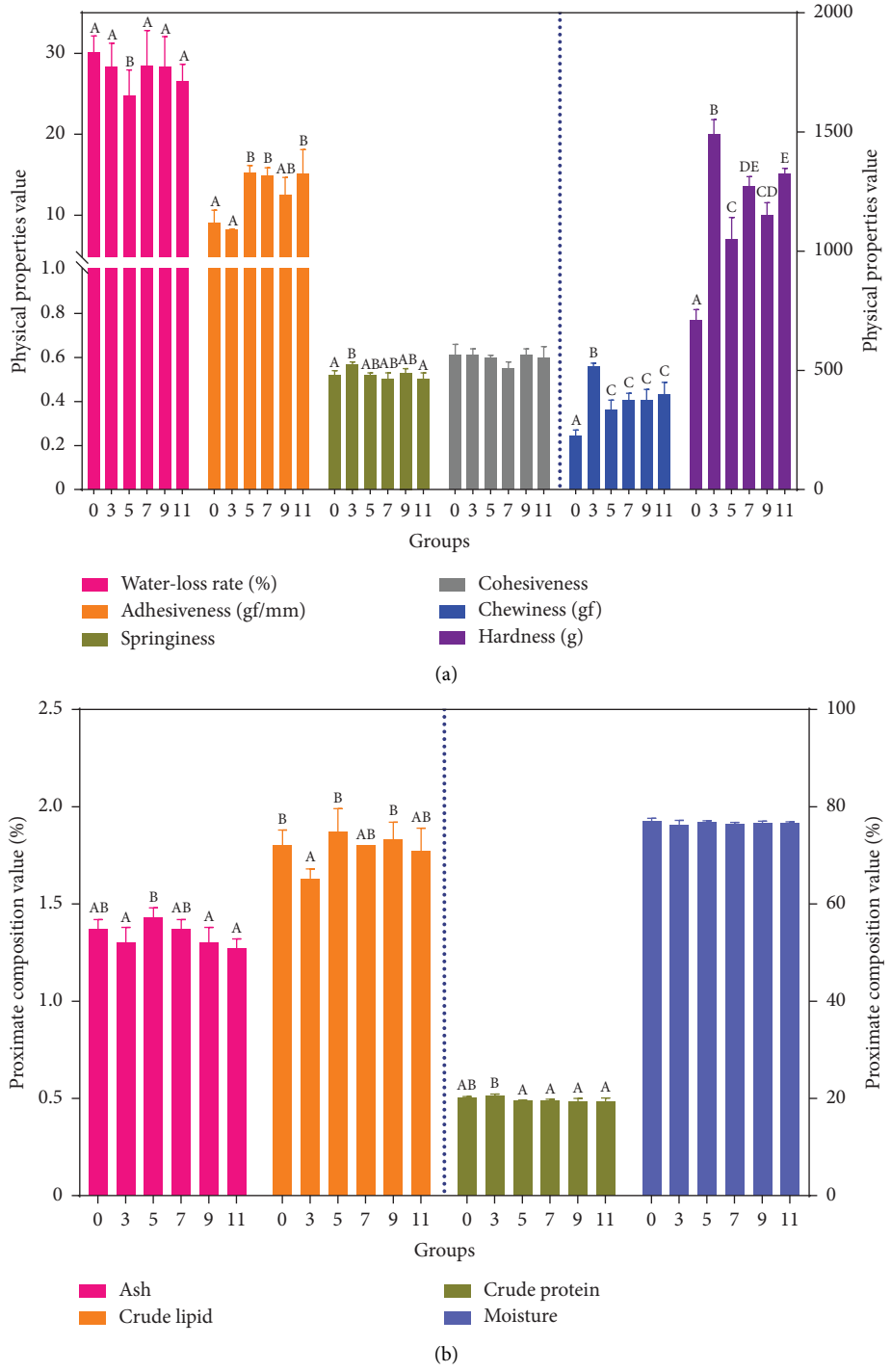

FIGURE 1: The physical properties (a) and proximate composition (b) of the muscles of *Oreochromis aurea* after 60 days culture at different salinities. *Note*. 0 : 0 ppt salinity; 3 : 3 ppt salinity; 5 : 5 ppt salinity; 7 : 7 ppt salinity; 9 : 9 ppt salinity; 11 : 11 ppt salinity; different letters in the superscript indicate significant differences across groups ($P < 0.05$).

standard. In addition, according to the results of CS and AAS, it was found that (methionine + cysteine) scored the lowest in each group, followed by valine. Therefore, we speculate that the first restricted amino acid in *O. aurea* muscle was (methionine + cysteine) and the second restricted amino acid was valine.

3.4. Fatty Acid Composition in Muscle. The fatty acid composition of *O. aurea* muscle is detailed in Table 4. A total of 23 fatty acids were detected, including 7 saturated fatty acids (SFA), 6 monounsaturated fatty acids (MUFA), and 10 polyunsaturated fatty acids (PUFA). The total amount of fatty acids (TFA) was between 95.95% and 97.99%, and there

Table 2: Amino acid composition in muscle of *Oreochromis aurea* after 60 days culture at different salinities (g/100 g, $n > 3$).

Category	Amino acid	0	3	5	7	9	11
EAA	Threonine	0.74 ± 0.04	0.74 ± 0.01	0.75 ± 0	0.74 ± 0.01	0.75 ± 0.03	0.7 ± 0.005
	Valine	0.80 ± 0.04	0.82 ± 0.03	0.83 ± 0.03	0.84 ± 0.02	0.82 ± 0.03	0.80 ± 0.01
	Methionine	0.48 ± 0.03	0.48 ± 0.02	0.5 ± 0.01	0.50 ± 0.02	0.48 ± 0.03	0.47 ± 0.01
	Isoleucine	0.70 ± 0.04^{a}	0.73 ± 0.03^{ab}	0.74 ± 0.03^{ab}	0.76 ± 0.01^{b}	0.73 ± 0.03^{ab}	0.70 ± 0.01^{a}
	Leucine	1.27 ± 0.06	1.28 ± 0.03	1.31 ± 0.01	1.29 ± 0.02	1.29 ± 0.05	1.2 ± 0.01
	Phenylalanine	0.69 ± 0.04	0.69 ± 0.01	0.72 ± 0.005	0.69 ± 0.01	0.68 ± 0.03	0.67 ± 0.01
	Lysine	1.54 ± 0.06	1.53 ± 0.02	1.58 ± 0.01	1.55 ± 0.03	1.54 ± 0.07	1.55 ± 0.02
NEAA	Asparagic acid	1.71 ± 0.08	1.69 ± 0.03	1.76 ± 0.01	1.71 ± 0.02	1.71 ± 0.07	1.71 ± 0.02
	Serine	0.66 ± 0.03	0.65 ± 0.01	0.66 ± 0.01	0.6 ± 0.01	0.66 ± 0.02	0.6 ± 0.01
	Glutamic acid	2.45 ± 0.08	2.45 ± 0.03	2.51 ± 0.01	2.45 ± 0.05	2.47 ± 0.09	2.48 ± 0.02
	Glycine	0.89 ± 0.00	0.92 ± 0.02	0.91 ± 0.01	0.88 ± 0.01	0.92 ± 0.02	0.89 ± 0.03
	Alanine	1.03 ± 0.04	1.04 ± 0.03	1.06 ± 0.005	1.05 ± 0.02	1.03 ± 0.04	1.04 ± 0.02
	Tyrosine	0.55 ± 0.02	0.56 ± 0.02	0.58 ± 0.01	0.55 ± 0.01	0.55 ± 0.02	0.55 ± 0.01
	Histidine	0.43 ± 0.02	0.41 ± 0.01	0.42 ± 0.001	0.42 ± 0.01	0.43 ± 0.01	0.41 ± 0.01
	Arginine	0.98 ± 0.03	1.00 ± 0.03	1.00 ± 0.005	0.97 ± 0.03	1.01 ± 0.04	1.00 ± 0.02
	Proline	0.63 ± 0.05	0.61 ± 0.03	0.63 ± 0.02	0.5 ± 0.005	0.63 ± 0.02	0.63 ± 0.02
Total TAA		15.57 ± 0.61	15.57 ± 0.33	15.97 ± 0.09	15.6 ± 0.17	15.7 ± 0.57	15.6 ± 0.08
Total EAA		6.22 ± 0.29	6.26 ± 0.14	6.43 ± 0.07	6.37 ± 0.10	6.28 ± 0.28	6.21 ± 0.02
Total NEAA		9.33 ± 0.36	9.33 ± 0.19	9.51 ± 0.01	9.26 ± 0.11	9.42 ± 0.32	9.37 ± 0.07
DAA		6.08 ± 0.20	6.1 ± 0.09	6.23 ± 0.04	6.09 ± 0.09	6.13 ± 0.22	6.11 ± 0.04
BCAA		2.77 ± 0.13	2.83 ± 0.08	2.88 ± 0.07	2.89 ± 0.06	2.83 ± 0.12	2.78 ± 0.02
EAA/TAA		39.9 ± 0.30^{a}	40.2 ± 0.13^{ab}	40.2 ± 0.23^{ab}	40.6 ± 0.18^{b}	39.9 ± 0.32^{a}	39.8 ± 0.18^{a}
EAA/NEAA		66.6 ± 0.54^{ab}	67.0 ± 0.32^{ab}	67.5 ± 0.79^{b}	68.7 ± 0.23^{c}	66.6 ± 0.67^{ab}	66.2 ± 0.44^{a}
DAA/TAA		39.09 ± 0.23	39.17 ± 0.26	39.04 ± 0.23	38.8 ± 0.15	39.07 ± 0.01	39.1 ± 0.09

Note. 0 : 0 ppt salinity; 3 : 3 ppt salinity; 5 : 5 ppt salinity; 7 : 7 ppt salinity; 9 : 9 ppt salinity; 11 : 11 ppt salinity; EAA stands for essential amino acids, including threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine; NEAA stands for nonessential amino acids; DAA stands for delicious amino acids, including asparagus Amino acid, glutamic acid, glycine, and alanine; TAA means total amino acids; BCAA means branched chain amino acids, including valine, isoleucine, and leucine; different English letters in the upper right corner of the same row of values indicate significant difference ($P < 0.05$).

Table 3: Nutritional evaluation of essential amino acids in muscle of *Oreochromis aurea* after 60 days of culture under different salinities (mg/g).

Groups	Item	Thr	Val[#]	Met + Cys[*]	Ile	Leu	Phe + Tyr	Lys	EAAI
0	CS	1.23 ± 0.01	0.89 ± 0.02	0.55 ± 0.01	0.94 ± 0.02	1.01 ± 0.00	0.86 ± 0.01	1.59 ± 0.02	89.78 ± 4.34
	AAS	0.96 ± 0.01	0.84 ± 0.02	0.71 ± 0.01	0.91 ± 0.02	0.94 ± 0.00	1.05 ± 0.02	1.47 ± 0.02	
3	CS	1.22 ± 0.01	0.91 ± 0.01	0.54 ± 0.01	0.97 ± 0.01	1.01 ± 0.00	0.87 ± 0.01	1.57 ± 0.01	90.58 ± 2.13
	AAS	0.95 ± 0.01	0.85 ± 0.01	0.7 ± 0.01	0.94 ± 0.01	0.93 ± 0.00	1.05 ± 0.01	1.45 ± 0.00	
5	CS	1.21 ± 0.01	0.89 ± 0.02	0.55 ± 0.01	0.96 ± 0.03	1 ± 0.00	0.88 ± 0.01	1.58 ± 0.00	93.01 ± 0.86
	AAS	0.94 ± 0.01	0.83 ± 0.02	0.71 ± 0.02	0.93 ± 0.03	0.93 ± 0.00	1.06 ± 0.01	1.46 ± 0.00	
7	CS	1.2 ± 0.01	0.91 ± 0.02	0.56 ± 0.02	1 ± 0.01	1 ± 0.01	0.85 ± 0.00	1.56 ± 0.01	92.24 ± 1.23
	AAS	0.93 ± 0.01	0.85 ± 0.02	0.71 ± 0.03	0.96 ± 0.01	0.93 ± 0.01	1.04 ± 0.00	1.44 ± 0.01	
9	CS	1.24 ± 0.01	0.9 ± 0.01	0.54 ± 0.01	0.97 ± 0.01	1.01 ± 0.00	0.85 ± 0.00	1.58 ± 0.00	90.84 ± 4.09
	AAS	0.96 ± 0.01	0.84 ± 0.01	0.7 ± 0.01	0.93 ± 0.01	0.94 ± 0.00	1.04 ± 0.00	1.45 ± 0.00	
11	CS	1.24 ± 0.01	0.89 ± 0.01	0.54 ± 0.01	0.95 ± 0.01	1.01 ± 0.00	0.86 ± 0.00	1.6 ± 0.02	89.56 ± 0.17
	AAS	0.96 ± 0.01	0.83 ± 0.01	0.69 ± 0.01	0.91 ± 0.01	0.94 ± 0.00	1.04 ± 0.01	1.48 ± 0.02	
Egg protein		404	603	587	501	848	960	653	—
FAO/WHO		250	310	220	250	440	380	340	

Note. 0 : 0 ppt salinity; 3 : 3 ppt salinity; 5 : 5 ppt salinity; 7 : 7 ppt salinity; 9 : 9 ppt salinity; 11 : 11 ppt salinity; CS: the chemical content of muscle protein; AAS: the amino acid content of muscle protein; EAAI: the essential amino acid index of muscle protein; Thr: threonine; Val: valine; Met: methionine; Cys: cysteine; Ile: isoleucine; Leu: leucine; Phe: phenylalanine; Tyr: tyrosine; Lys: lysine; [*]: the first restricted amino acid; [#]: the second restricted amino acid.

was no significant difference between the groups ($P > 0.05$), the highest being 5 ppt and the lowest being 11 ppt. The total amount of essential fatty acids (EFA) showed an overall upward trend with increasing salinity, with 11 ppt being the highest (20.67%) and the control being the lowest (19.33%). On the contrary, the total amount of nonessential fatty acids (NEFA) showed a downward trend with increasing salinity, with the highest value in the control (78.24%) and the lowest value in the 11 ppt (75.29%). We also observed that the SFA content was highest at 3 ppt and lowest at 11 ppt. Then the

Table 4: Fatty acids composition in muscle of *Oreochromis aureus* after 60 days of culture under different salinities (%).

Fatty acid	0	3	5	7	9	11
C14 : 0	1.87 ± 0.05^{b}	2.03 ± 0.12^{a}	1.93 ± 0.05^{ab}	$2.\pm0.08^{ab}$	2.07 ± 0.05^{a}	2.07 ± 0.05^{a}
C15 : 0	0.27 ± 0.01	0.28 ± 0.04	0.28 ± 0.03	0.27 ± 0.02	0.25 ± 0.02	0.26 ± 0.01
C16 : 0	21.07 ± 0.40	21.13 ± 0.5	20.53 ± 0.40	20.87 ± 0.26	21.3 ± 0.29	20.67 ± 0.17
C17 : 0	0.46 ± 0.04^{b}	0.39 ± 0.05^{ab}	0.42 ± 0.07^{ab}	0.39 ± 0.02^{ab}	0.357 ± 0.03^{a}	0.36 ± 0^{a}
C18 : 0	5.4 ± 0.29	5.47 ± 0.12	5.3 ± 0.14	5.27 ± 0.21	5.3 ± 0.08	5.23 ± 0.05
C20 : 0	0.16 ± 0.01^{b}	0.2 ± 0.01^{a}	0.23 ± 0.01^{c}	0.21 ± 0.01^{a}	0.20 ± 0.01^{a}	0.21 ± 0.01^{a}
C24 : 0	0.11 ± 0.01	0.1 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.11 ± 0.01
C14 : 1	0.1 ± 0.01	0.12 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
C16 : 1	4.67 ± 0.4	4.67 ± 0.54	4.23 ± 0.25	4.33 ± 0.12	4.57 ± 0.25	4.43 ± 0.12
C17 : 1	0.33 ± 0.01^{ab}	0.39 ± 0.06^{b}	0.37 ± 0.02^{b}	0.31 ± 0.01^{a}	0.3 ± 0.02^{a}	0.37 ± 0.02^{b}
C18 : 1	34.63 ± 0.58	34.4 ± 0.37	34.23 ± 1.05	33.93 ± 0.34	34.1 ± 0.83	32.43 ± 2.47
C20 : 1	1.53 ± 0.12	1.63 ± 0.09	1.5 ± 0.08	1.6 ± 0.00	1.53 ± 0.05	1.57 ± 0.12
C22 : 1	0.09 ± 0.02^{d}	0.1 ± 0.01^{abcd}	0.11 ± 0.01^{abc}	0.12 ± 0.01^{ac}	0.12 ± 0.01^{c}	0.09 ± 0.01^{bd}
C18 : 2	16.33 ± 1.68	16.73 ± 0.77	17.73 ± 0.45	17.87 ± 0.59	17.1 ± 0.85	17.93 ± 0.62
C18 : 3	3 ± 0.16^{b}	2.73 ± 0.12^{a}	2.77 ± 0.12^{ab}	2.8 ± 0.08^{ab}	2.77 ± 0.05^{ab}	2.73 ± 0.12^{a}
C18 : 4	0.31 ± 0.01	0.3 ± 0.01	0.40 ± 0.11	0.3 ± 0.01	0.32 ± 0.02	0.34 ± 0.05
C22 : 2	0.69 ± 0.07	0.67 ± 0.05	0.74 ± 0.06	0.72 ± 0.03	0.69 ± 0.07	0.73 ± 0.01
C22 : 3	1.13 ± 0.05	1.1 ± 0.00	1.2 ± 0.08	1.17 ± 0.05	1.17 ± 0.09	1.2 ± 0.00
C20 : 4n-6 ARA	1.3 ± 0.08	1.17 ± 0.05	1.33 ± 0.09	1.27 ± 0.05	1.27 ± 0.12	1.23 ± 0.05
C20 : 5n-3 EPA	0.11 ± 0.04	0.15 ± 0.05	0.24 ± 0.06	0.14 ± 0.01	0.14 ± 0.04	0.19 ± 0.03
C22 : 4	0.91 ± 0.02	0.82 ± 0.06	0.87 ± 0.04	0.89 ± 0.03	0.88 ± 0.06	0.85 ± 0.02
C22 : 5n-3 DPA	1.7 ± 0.08	1.57 ± 0.05	1.77 ± 0.19	1.63 ± 0.05	1.63 ± 0.17	1.57 ± 0.09
C22 : 6n-3 DHA	1.4 ± 0.29	1.17 ± 0.05	1.57 ± 0.26	1.3 ± 0.08	1.3 ± 0.14	1.27 ± 0.05
EFA	19.33 ± 1.82	19.47 ± 0.9	20.5 ± 0.57	20.67 ± 0.66	19.87 ± 0.87	20.67 ± 0.69
NEFA	78.24 ± 1.74	77.87 ± 0.71	77.49 ± 1.67	76.94 ± 0.77	77.69 ± 0.69	75.29 ± 2.90
TFA	97.57 ± 0.14	97.34 ± 0.36	97.99 ± 1.10	97.61 ± 0.16	97.56 ± 0.19	95.95 ± 2.26
SFA	29.33 ± 0.69	29.61 ± 0.58	28.81 ± 0.52	29.12 ± 0.49	29.57 ± 0.34	28.91 ± 0.23
MUFA	41.35 ± 0.78	41.31 ± 0.15	40.56 ± 1.34	40.40 ± 0.43	40.73 ± 0.93	39.01 ± 2.5
PUFA	26.89 ± 1.5	26.41 ± 0.89	28.62 ± 0.97	28.09 ± 0.62	27.2 ± 1.41	28.04 ± 0.62
EPA + DHA	1.51 ± 0.33^{ab}	1.32 ± 0.09^{a}	1.81 ± 0.32^{b}	1.44 ± 0.09^{ab}	1.44 ± 0.18^{ab}	1.45 ± 0.08^{ab}

Note. 0 : 0 ppt salinity; 3 : 3 ppt salinity; 5 : 5 ppt salinity; 7 : 7 ppt salinity; 9 : 9 ppt salinity; 11 : 11 ppt salinity; ARA: arachidonic acid;EPA: eicosapentaenoic acid; DPA: docosapentenoic acid; DHA: docosahexenoic acid; EFA: essential fatty acids; NEFA: nonessential fatty acids; TFA: total fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; different letters on the same line indicate significant differences ($P < 0.05$).

highest PUFAS content was in the 5 ppt group, which was 2.21% higher than the lowest group (3 ppt). Curiously, we found that the content of EPA + DHA increased by 0.3% compared to the control group at 5 ppt salinity and was significantly higher than that at 3 ppt ($P < 0.05$). In general, the fatty acid composition of *O. aureus* muscle was affected by salinity, and the fatty acid content in the saline environment was higher than that in freshwater.

3.5. Transcriptome Analysis of the O. aureus Liver. This study was based on *O. aureus* liver transcript data obtained from previous studies to further explore the relevant pathways or genes of fatty acid and amino acid metabolism. It has been reported that a total of 168,308,850 clean reads (average 42,077,212) were obtained from the four treatment groups (0, 3, 7, and 11 ppt), with a mapping ratio of 87.36% [24]. We then extracted the differentially expressed genes (DEGs) between the control and the three treatment groups to construct a Venn diagram, obtained 2343 common DEGs (Figure 2(a)), and then performed in-depth GO and pathway analysis.

Subsequently, in the GO analysis results, we found that the shared genes were divided into three categories, namely biological processes (19 types), cellular component (13 types), and molecular function (10 types) (Figure 2(b)). In addition, 2,343 shared genes were mapped to 293 pathways in the pathway analysis results (Table S2), among which 39 significantly different pathways were observed ($P < 0.05$) (Figure 2(c)). Furthermore, we combined the GO and KEGG results with previous studies to explore candidate genes involved in the anabolic metabolism of related substances under salinity conditions. Here, we constructed a heat map and performed trend analysis to visually show the changes in the expression of candidate genes under different salinities (Figures 2(d) and 2(e)). We were surprised to find that the expression levels of 3 genes, including transketolase (TKT), phosphoserine phosphatase (PSPH), and carbamoyl-phosphate synthase (CPS1), which are involved in the amino acid synthesis pathway, all increased compared to the control ($P < 0.05$). This shows that the activity of related enzyme genes in the amino acid synthesis pathway continuously increased under the effect of salinity, and this result was consistent with the change in amino acid composition. We then observed several amino acid metabolism and catabolism pathways, including glycine, serine, and threonine metabolism; alanine, aspartate, and glutamate metabolism; and valine, leucine, and isoleucine catabolism. And the expression of the genes alanine glyoxylate transaminase (AGXT), hydroxymethylglutaryl-CoA synthase

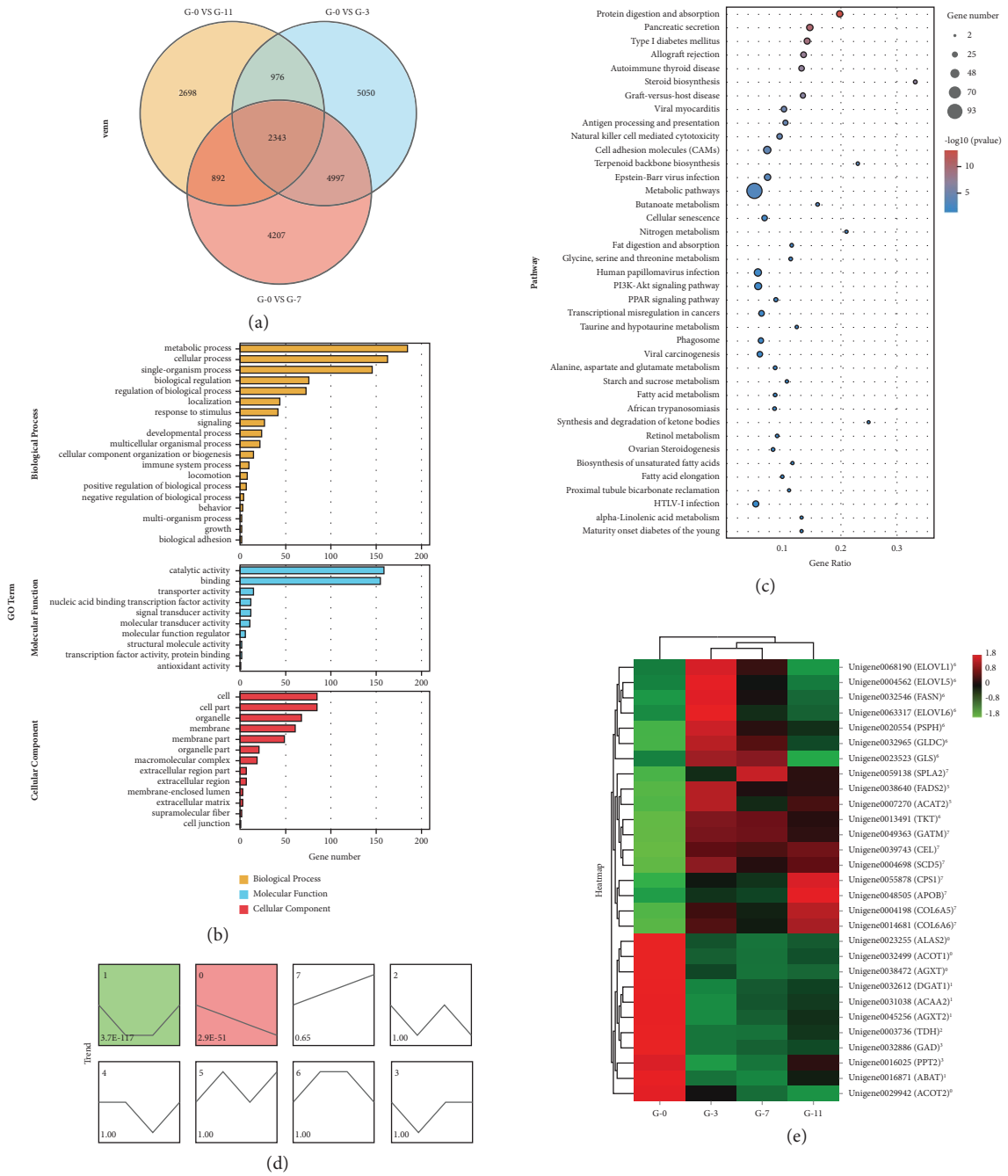

FIGURE 2: The venn diagram (a), GO (b), KEGG (c), trend (d) and heatmap (e) analysis of the liver transcripts of *Oreochromis aurea* after 60 days of culture at different salinities. *Note*. The total DEG number identified by FDR-adjusted P-value <0.05 was shown. Scatter plot (only $P < 0.05$ displayed) indicating the pathway categories on the vertical axis and the enrichment factor on the horizontal axis; larger symbols indicate a greater number of DEGs in the pathway; the symbol colours indicate different Q values as shown on the right. Metabolic pathways contained the most DEGs. The trend graph shows eight trends of genetic changes; the numbers in the upper left corner each represents a trend; the numbers in the lower left corner represent P-values for each trend type; colour represents a significant trend ($P < 0.05$). The superscript numbers of the gene names in the heatmap indicate to which type of trend change they correspond.

(HMGCS1), acetoacetyl-CoA synthetase (AACS), and 4-aminobutyrate aminotransferase (ABAT) involved in these pathways was significantly reduced in salinity ($P < 0.05$), indicating that salinity has weakened the ability of fish to metabolise amino acids. In addition, we found that elongation of the very long-chain fatty acid protein (ELOVL), stearoyl-CoA desaturase (SCD), and acyl-CoA 6-desaturase (FADS2) were involved in several fat anabolic pathways (e.g., unsaturated fatty acid biosynthesis, fatty acid elongation, and fatty acid metabolism). Compared with freshwater, the expression levels of ELOVL, SCD, and FADS2 were significantly increased in brackish water

($P < 0.05$), and we also found that the expression level of both was higher under low salinity than that under high salinity ($P < 0.05$). Conversely, we found that the expression levels of acyl-coenzyme A thioesterase (ACOT1 and ACOT2), palmitoyl-protein thioesterase (PPT), and acetyl-CoA acyltransferase 2 (ACAA2), which are involved in unsaturated fatty acid synthesis and fatty acid elongation pathways, all of which showed a downward trend with salinity ($P < 0.05$). In addition to the discovery of FASD2, we also observed that secretory phospholipase A2 (SPLA2) is also involved in the alpha-linolenic acid pathway, and its expression level continues to increase with increasing salinity ($P < 0.05$). In conclusion, the fluctuation of salinity in aquaculture affects the synthesis and metabolism pathways of amino and fatty acids in *O. aureus*, thus affecting the amino acid and fatty acid composition of muscle.

3.6. Validation of RNA-Seq Data through qPCR. In this study, 5 DEGS were selected from the liver transcripts for qPCR analysis to verify the accuracy of the RNA-Seq data. In Figure 3, the gene expression level obtained by RNA-Seq was highly consistent with the change trend of the relative expression of the selected verification gene, showing that the RNA-Seq data was accurate and reliable.

4. Discussion

4.1. Effect of Salinity on the Growth Performance of O. aureus. The growth process of fish involves several material cycles and energy conversions, leading to an increase in both the size and mass of the fish. To enhance fish growth and improve bait utilization, it has been suggested that manipulating the external environment can minimize the energy consumption associated with ion and osmotic pressure regulation [28]. Previous studies have shown that the growth rate of *Lateobrax japonicus* is highest at a salinity of 7.5‰, while Nile tilapia has been observed to grow as the results were observed, with the specific growth rate (SGR), weight gain rate (WGR), and net weight (NW) values being the highest at a salinity of 9 ppt. In addition, the feed conversion ratio (FCR) was found to be the lowest at this salinity. These findings suggest that *O. aureus* may require the least metabolic energy for osmotic pressure adjustment at 9 ppt, thus allowing more energy to be allocated towards promoting its growth.

4.2. Effects of Salinity on the Physical Properties and Proximate Composition of O. aureus. Salinity has a significant impact on the physiological and metabolic processes of fish, leading to changes in the nutrient composition of their muscles [9]. In response to high osmotic stress, *O. aureus* excretes excess water into the environment, resulting in a lower moisture content in the muscles compared to freshwater species. This is in contrast to marine organisms that adapt to low osmotic pressure, such as *Uca tangeri* crabs, *Scylla paramamosain* mud crabs, and *Sinonovacula constricta* [29–31]. When fish experience salinity-induced stress, ash content plays a crucial role in regulating osmotic ions. In this study, it was observed that the ash content was highest at a salinity of 5 ppt, indicating that *O. aureus* exhibited the most active osmotic adjustment at this salinity. Furthermore, studies have confirmed that an increase in ash content in fish muscle is beneficial for human health [32]. It has been reported that eutrophic fish will consume protein and lipids to meet the energy demands during salinity stress [33]. Consequently, the crude protein and crude lipid content in the muscle of *O. aureus* are reduced under varying salinity conditions.

Water-holding capacity is a crucial indicator for evaluating muscle quality. Zhang et al. [6] found that as aquaculture salinity increases, the water-holding capacity of *Letalurus punetaus* decreases, and similar results have been observed in *Ctenopharyngodon idella* [10]. However, in our experiment, we observed that the muscle water-holding capacity of *O. aureus* cultured under salinity was better than that of freshwater. Previous studies have shown that muscles with strong water-holding capacity not only reduce the rate of protein degradation but also extend the storage period. Furthermore, some scholars have reported a positive correlation between collagen content in muscle and water-holding capacity [34, 35]. Based on these findings, we speculate that a moderate increase in culture salinity may enhance the collagen content and improve the water-holding capacity of *O. aureus* muscles.

The texture analysis method is used to simulate the chewing movement of the human mouth and obtain a set of physical parameters for muscles [36]. In our study, we found that the hardness, stickiness, and chewiness values of *O. aureus* muscles increased with increasing salinity. This indicates that proper cultivation under specific salinity conditions can enhance the muscle's ability to resist damage and maintain integrity, ultimately improving its taste quality [37]. Overall, both the nutritional composition and physical properties of *O. aureus* muscles can be improved through salinity farming. The promotion effect is particularly prominent at low salinity levels, specifically 5 ppt. This not only leads to better-tasting muscles but also offers cost advantages for farmers in terms of lower cultivation expenses.

4.3. Effect of Salinity on the Content of Amino Acid of O. aureus. The composition of amino acids, particularly essential amino acids (EAA) and nonessential amino acids (NEAA), is an important indicator for evaluating the protein nutritional value of aquatic products [38]. The EAA/TAA ratio, which should be around 40%, and the EAA/NEAA ratio, which should be over 60%, are considered indicative of high-quality protein according to WHO/FAO standards. In our study, all groups of *O. aureus* reached these standards, indicating that *O. aureus* muscle is a high-quality protein source. This finding aligns with the nutritional evaluation of other tilapia species conducted by other researchers [7, 39]. Interestingly, the EAA/TAA ratio (40.6%) and EAA/NEAA ratio (68.7%) at a salinity of 7 ppt were significantly higher than those in the control group (39.9%, 66.6%). This suggests that the quality of *O. aureus* raised at this salinity level is better. In addition, it is generally believed that nonessential amino acids (DAA) play a crucial role in the development of

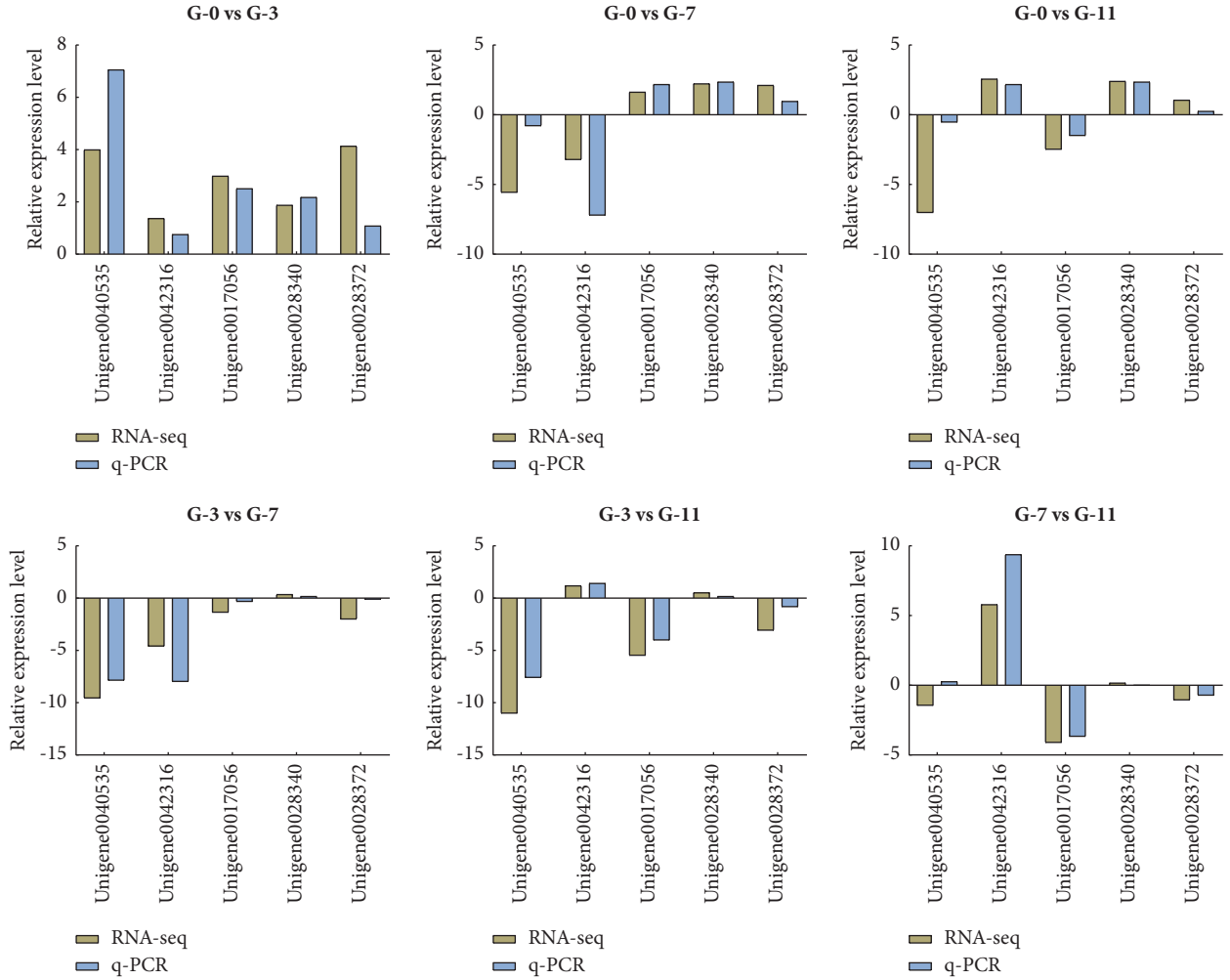

Figure 3: The relative expression of the qPCR validation genes and RNA-Seq data. *Note*. Comparison of the relative log2 (fold changes) between RNA-Seq and qPCR after salinity stress compared with the control, normalized to expression of the unigene0030910 gene. 0 : 0 ppt salinity; 3 : 3 ppt salinity; 5 : 5 ppt salinity; 7 : 7 ppt salinity; 9 : 9 ppt salinity; 11 : 11 ppt salinity.

muscle flavor. Zeng et al. [40] observed an increase in DAA content with increasing salinity in *Scophthalmus maximus* over a period of 60 days. In our research, the DAA content of *O. aureus* raised under salinity conditions was higher than that of freshwater, further confirming that salinity can enhance the flavor of *O. aureus* flesh.

Among all amino acids, only the Ile (isoleucine) content at a salinity of 7 ppt was significantly higher than in the control group ($P < 0.05$). Branched-chain amino acids (BCAA), including Val (valine), Leu (leucine), and Ile (isoleucine), can be oxidized to produce glucose, thereby providing energy to the body. Ile is considered the most effective member of BCAA [41]. In our study, we conducted further analysis using the KEGG database and found that six differentially expressed genes (DEGs) are involved in the pathway of valine, leucine, and isoleucine degradation (Table S2). Among them, the expression of five DEGs (AGXT, ACAA2, ABAT, AACS, and HMGCS1) significantly decreased in the salinity group (Figure 2(e), Table 2), suggesting that salinity may reduce the activity of these enzymes in the pathway. This could potentially weaken *O. aureus*' ability to degrade BCAA and lead to its accumulation in the body. On the other hand, the expression of DEGs (TKT, PSPH, and CPS1) involved in the amino acid synthesis pathway was significantly increased under salinity conditions. This could contribute to the accumulation of amino acids in *O. aureus*, as observed by the higher amino acid content in the salinity group compared to the control group (Table 2).

4.4. Effect of Salinity on the Content of Fatty Acid of O. aureus. Salinity has an impact on the fatty acid composition by altering the physiological metabolic pathways in aquatic animals, potentially related to providing energy for osmotic regulation [9, 42]. The content of polyunsaturated fatty acids (PUFA) is reflective of the nutritional value of aquatic products, particularly long-chain polyunsaturated fatty acids (LC-PUFA), which are considered essential fatty acids (EFA) for organism development and growth [43]. Cheng et al. [7] reported that the muscle PUFA content of Nile tilapia fed under 12 ppt salinity for 56 days was significantly higher than that of freshwater. Similarly, a significant increase in

PUFA content was observed in rainbow trout and Atlantic salmon Salmo salar living in brackish water [8, 44]. Current research suggests that euryhaline fish can utilize linoleic and linolenic acids to synthesize PUFA. For example, Li et al. [45] found that the growth performance of *Siganus canaliculatus* fed with D2-D4 diets (containing no highly unsaturated fatty acids but linoleic and linolenic acids) was comparable to that of the D1 diet (containing highly unsaturated fatty acids), confirming that euryhaline fish can effectively utilize linoleic and linolenic acids to meet their EFA requirements. In our study, except for a salinity of 3 ppt, the PUFA content of *O. aureus* at other salinities was higher than that of the control group, indicating that appropriate salinity does have a positive effect on the synthesis of PUFA in fish. Additionally, we were surprised to find that the linolenic acid (C18 : 3) content in the control group (3%) was the highest, significantly surpassing the salinities of 3 ppt (2.7%) and 11 ppt (2.7%) ($P < 0.05$). Furthermore, the content of linoleic acid (C18 : 2) in all treatment groups was higher than that of the control group. These findings suggest that *O. aureus* primarily utilizes linolenic acid for PUFA synthesis rather than linoleic acid, and the utilization efficiency of linolenic acid by fish in freshwater is not as efficient as at salinity. We further confirmed this notion at the transcriptional level. The KEGG results revealed a related α-linolenic acid metabolism pathway where the expression levels of secretory phospholipase A2 (SPLA2) and Acyl-CoA 6-desaturase (FADS2 or Δ6 FAD) genes were significantly upregulated ($P < 0.05$). Specifically, SPLA2 can hydrolyze extracellular phosphatidylcholine into α-linolenic acid, which then undergoes the first dehydrogenation by FADS2 to form stearidonic acid, providing raw materials for subsequent PUFA synthesis [46–48]. Therefore, we speculate that *O. aureus* predominantly utilizes linolenic acid for PUFA synthesis instead of linoleic acid, with a stronger ability to utilize linolenic acid under salinity compared to freshwater conditions.

4.5. Effect of Salinity on the Content of LC-PUFA of O. aureus. In fish, long-chain polyunsaturated fatty acids (LC-PUFA) with a carbon length of 20 or more and at least 3 double bonds are typically synthesized from their 18-carbon polyunsaturated fatty acid precursors through the action of Δ5/Δ6 FAD and elongation of very long-chain fatty acids enzymes (ELOVL) [49, 50]. LC-PUFA, especially n-3 PUFA (e.g., DHA, EPA), plays a crucial role in the development and membrane enhancement of aquatic animals living in a saline environment. They are particularly important for animal health during lactation [33, 51]. In our results, the EPA content in all experimental groups (mean 0.17%) was higher than that of the control group (0.11%), but only the DHA content at 5 ppt salinity (1.57%) was higher than that in freshwater (1.4%). Additionally, only at 5 ppt salinity (1.81%) was the EPA + DHA content higher than the control (1.51%) and significantly higher than at 3 ppt (1.32%). These findings indicate that evaluating the cultivation of *O. aureus* at 5 ppt salinity based solely on the content of n-3 PUFA would be beneficial.

Furthermore, through transcriptomic analysis, we unexpectedly discovered two pathways related to fatty acid elongation and the biosynthesis of unsaturated fatty acids ($P < 0.05$). Based on heatmap and trend analysis, we found that key enzymes such as FADS2, ELOVL1, ELOVL5, and ELOVL6 involved in the synthesis of LC-PUFA exhibited better activity at low salinity (3 ppt) compared to high salinity (7 and 11 ppt) and freshwater (Figures 2(d) and 2(e)). Similar findings have been reported for steelhead trout *Salmogairdnerii richardson*, red sea bream *Pagrus major*, and mullet sea fry *Mugil cephalus*, where under low salinity conditions, the activity of elongase and Δ5/Δ6 desaturase in fish can be enhanced, leading to improved PUFA synthesis ability rather than under high salinity conditions [52–54]. Therefore, we speculate that low salinity may enhance the enzyme activity of the ELOVL family and FADS2, thereby improving *O. aureus*' ability to synthesize LC-PUFA and increasing its content in muscle.

Moreover, some scholars suggest the existence of a synthetic pathway from EPA to DHA, where EPA is converted to C24 : 6n-3 in the presence of ELOVL2 (or ELOVL4) and Δ6 FAD, which is then converted to DHA through β-oxidation [55]. However, in our analysis, we did not find ELOVL2 and ELOVL4 among all differentially expressed genes (DEGs), suggesting that this pathway may not exist or may be incomplete in *O. aureus*. Fortunately, we found another pathway involving acyl-coenzyme A thioesterase (ACOT) in the synthesis of DHA. ACOT can hydrolyze docosahexaenoyl-CoA into DHA and coenzyme A (CoASH) [56]. We observed that the expression levels of ACOT1 and ACOT2 were significantly lower in salinity groups compared to freshwater, suggesting that it affects the synthesis of DHA in *O. aureus* and leads to reduced content. However, the reason for the additional increase in DHA content at 5 ppt salinity has not been determined, and further investigation is needed.

5. Conclusions

This study demonstrates that *O. aureus* is a high-quality protein source for humans, and the flesh quality of *O. aureus* reared in salinity conditions is significantly better than that of freshwater, particularly in terms of taste and nutritional value. Furthermore, the results indicate that salinity fluctuations indeed have a positive impact on the amino acid and fatty acid composition of *O. aureus* muscles. The salinity factor activates the synthesis pathway of amino acids and PUFA, leading to an enhancement in the expression levels of related genes such as FADS2, ELOVL, SCD, and SPLA2, among others. This improvement in gene expression contributes to the enhancement of the nutritional value of muscles and the improvement of flesh flavor. Based on a comprehensive comparison of data under various salinities, it is recommended that farmers consider raising *O. aureus* under low salinity conditions, approximately around 5 ppt. This approach not only reduces input costs but also improves economic benefits.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. The transcriptome data are available in the GenBank database with accession numbers SRR11783565, SRR11783564, SRR11783563, and SRR11783562.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Kang-qi Zhou and Xian-hui Pan jointly carried out this research, data analysis, and article writing work. Yong Lin and Zhong Chen carried important background information and provided technical support. Xue-song Du, Yin Huang, Jun-qi Qin, and Cai-qun Zhang assist in raising tilapia and data collection. All authors read and approved the final manuscript. Kangqi Zhou and Zhong Chen contributed equally to this work and are the co-first authors.

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Supplementary Materials

Supplementary data-Table S1: Primer information used for the quantitative real-time PCR. Supplementary data-Table S2: Analysis of 2343 pathways sharing unigene in *Oreochromis aureusa* liver transcriptome. (*Supplementary Materials*)

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