Dynamic Transcriptomic Profiling During Liver Development in Schizothorax Prenanti

Jiahui Ni1, Peng Zhu1, Qilang Mo1, Wei Luo1, Zongjun Du1, Jun Jiang1, Song Yang1, Liulan Zhao1, Quan Gong2 and Yan Wang1*

1College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, China, 2Fisheries Institute, Sichuan Academy of Agricultural Sciences, Chengdu, China

Liver is an important organ for glucose and lipid metabolism, immunity, and detoxification in fish. However, the gene regulatory network of postnatal liver development still remains unknown in teleost fish. In this study, we performed transcriptome analysis on the liver of S. prenanti at three stages. A total of 1692 differentially expressed genes (DGEs) were identified across three liver developmental stages. The oil red O staining and PAS staining revealed that the lipid content of liver was increased and the glycogen content of liver was decreased during liver development. The fatty acids biosynthesis related genes were upregulated in adult and young stages compared with juvenile stage, while lipid degradation related genes were downregulated. The genes related to glycolysis, gluconeogenesis and glycogenolysis were upregulated in juvenile or young stages compared with adult stage. Further pathway analysis indicated that the CYP450 pathway, cell cycle and amino acid metabolic pathway were induced in the process of liver maturation. Our study presents the gene expression pattern in different liver development stages of S. prenanti and may guide future studies on metabolism of S. prenanti liver.

Keywords: liver, development, RNA-seq, S. prenanti, metabolism

INTRODUCTION

The liver is an important digestive and metabolic organ of fish. Previous studies have been conducted on the effects of dietary and metabolic process on lipid deposition in liver of fish (Dai et al., 2015; Jia et al., 2020; Sun et al., 2021). It is not only an important site of glucose and lipid metabolism in fish, but also has the function of immunity and detoxification (Enes et al., 2009; Freitas-Lopes et al., 2017). Liver is mainly a hematopoietic organ during the embryonic stage, and is transformed into a major metabolic organ during the mature stage (Chapple et al., 2013; Lv et al., 2014). Wu et al. revealed that

Abbreviations: Aldehyde dehydrogenase family 9 member A1, ALDH9A1; alpha-methylacyl-CoA racemase, AMACR; Aldehyde oxidase, AOX; 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase, PGAM; Carbonyl reductase 1, CBR1; Dihydrodial dehydrogenase, DHDH; Elongation of very long chain fatty acids protein 1, ELOVL1; Enoyl-CoA hydratase, ECHS1; Enoyl-CoA hydratase, HADHA; Fatty acid transporter, FATP; Fatty Acid Binding Protein 1, FABP; Fructose-1,6-bisphosphatase, FBPase; Glycogen debranching enzyme, AGL; Glyceraldehyde 3-phosphate dehydrogenase, GAPDH; Glucuronosyltransferase, UGT; Hexokinase, HK; Lysosomal alpha-glucosidase, GAA; Long-chain acyl-CoA synthases, ACSLs; Monoamine oxidase, MAO; 6-phosphofructokinase 1, PFK; Phoshoenolpyruvate carboxykinase, PEPCk; Sterol regulatory element binding protein, SREBP1; UTP-glucose-1-phosphate uridylyltransferase, UGP2.
the hepatic immune functional, cell proliferation, and apoptotic related pathways are involved in postnatal liver maturation of breeder roosters (Wu et al., 2018). In addition, liver antioxidant components are decreased during liver postnatal development to protect juvenile animals from the oxygen environment and toxic stimuli (Wu et al., 2019). Previous studies have proved that there are significant changes in the expression levels of CYP450 isoforms, suggesting that different transcripts are regulated during postnatal liver maturation (Cui et al., 2012a; Peng et al., 2012). In addition, some other important factors, such as IGF2BP1 (Hammerle et al., 2013), hepatocyte growth factor (HGF) (Ishikawa et al., 2001), farnate X receptor (FXR) (Peng et al., 2017), also can regulate the process of liver development. Compared with tremendous researches on liver development of mammals, the studies about gene regulatory network of postnatal liver development still remains unknown in teleost fish.

RNA-seq is a kind of high-throughput sequencing, which is an important tool for gene expression analysis in biology (Blencowe et al., 2009). With the help of RNA-seq, there has been more insight in the field of liver development in recent years. Xin et al. (Wang et al., 2020) revealed that there are the similarities and differences between human and mouse liver development using RNA-seq. Besides, the mRNA abundance of transporters in liver was revealed, and the expression of liver transporters was demonstrated to both age and isoform specific (Cui et al., 2012b). The RNA-seq results of chicken liver from the prenatal to the postnatal stages indicated that antioxidant defenses pathway is activated during chicken postnatal liver mature (Xu et al., 2019).

**Schizothorax prenanti** (S. prenanti) is commonly known as “Ya-fish”, belonging to the Schizothoracinae subfamily of the Cyprinidae family. It is cultural symbol of Ya’an and popular with the breeding industry (Li et al., 2018). In our previous study, single molecule real-time sequencing is performed to generate full-length transcriptome of **S. prenanti** (Wang et al., 2022). In the present study, we obtained the overall gene expression patterns of the three stages of liver by RNA-seq technique, then identified and annotated the differentially expressed genes. These results will further deepen our understanding of the liver metabolism of **S. prenanti** in different stages, and provide a reference for the liver health of **S. prenanti** in the artificial cultivation in the future.

**METHODS**

**Experimental Animals and Feeding Management**

The study was performed with **S. prenanti** of three different ages: 6 months (juvenile, 9.41 ± 0.60 g, n = 3), 1.5 years (young, 110.11 ± 10.82 g, n = 3), and 3 years (adult, 673.33 ± 25.17 g n = 3) of age, denoted as S, M, and L, respectively. All the fish were maintained for cultivation at the Fish Breeding Center of Sichuan Agriculture University (Ya’an, China) and were kept at 17 ± 1°C. Commercial fish food pellet was resupplied twice daily (9:00 a.m. and 6:00 p.m.). After acclimation for 2 weeks, healthy **S. prenanti** for normal feeding are used for experiments. Prior to sampling, fish were fasted for 24 h. Each fish was anesthetized with MS-222 (80 mg/L) and weighed. After dissection, liver was washed with PBS buffer solution, then placed in a 2 ml centrifuge tube and quickly preserved in liquid nitrogen.

**Histological Structure and Oil Red O Staining of Liver**

Liver samples from three stages were fixed in 4% paraformaldehyde and sections (4 μm) were cut and stained with PAS staining. Then cryo sectioned and frozen sections were subjected to standard Oil Red O staining (Servicebio, Wuhan, China). The slides were seal-capped with glycerogelatin and were photographed by a Nikon Eclipse Ti-SR inverted microscope.

**Triglyceride and Glycogen Concentration Measurement**

The contents of triglycerides were measured by Triglyceride Assay Kit (Jiancheng Biotech Co, Nanjing, China). Briefly, the liver samples treated with anhydrous ethanol were co-incubated with GPO-PAP at 37°C for 10 min, and the absorbance value was measured at 510 nm. The content of glycogen was measured by Glycogen Assay Kit (Jiancheng Biotech Co, Nanjing, China). Briefly, the glycogen detection solution was prepared, and mixed with the chromogenic solution, then incubated at 100°C for 5 min. The absorbance of samples was measured at 620 nm by Varioskan LUX Microplate Reader.

**RNA Preparation, Illumina Library Construction and Sequencing**

Total RNA was extracted by the RNAiso Pure RNA Isolation Kit (TaKaRa, Tokyo, Japan). The quality of total RNA was measured by Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, United States). The eukaryotic mRNA was enriched with Oligo (dT) beads by A-T complementary pairing principle. The first-strand cDNA was synthesized by random hexamers based on the mRNA template, and then the second-strand cDNA was synthesized by adding buffer, DNTPs, RNase H and DNA Polymerase I. Then the double-stranded cDNA was purified and a-tailed. After that, sequencing adaptors were attached and the fragment size was selected by AMPure XP beads. Finally, the cDNA library was obtained by PCR and sequenced on the Illumina NovaSeq 6000 platform.

**Identification and Expression Analysis of Differentially Expressed Genes**

Clean reads were obtained after removing ply-N and low-quality reads from raw data. Then, the base quality of the clean reads was evaluated by GC content and Q30. The clean reads were mapped onto transcripts of full-length transcriptome using the RNA-seq comparison software STAR (Dobin et al., 2013). We aligned the transcript sequence to NR (Deng et al., 2006), Swissprot (Rolf et al., 2004), GO (Ashburner et al., 2000), COG (Tatusov et al., 2000), KOG (Koonin et al., 2004), Pfam (Finn et al., 2014), KEGG
(Minoru et al., 2004) databases by BLAST software (version 2.2.26) to obtain the annotation information of the transcript. The FPKM distribution density was quantified using RSEM (Li and Dewey, 2011). Subsequently, DESeq software package was used to analyze differentially expressed genes, which could remove genes that were not expressed (counts = 0) at least in two samples (Anders and Huber, 2010), and then the p-values were adjusted for controlling the false discovery rate (FDR) by the Benjamini and Hochberg method (Storey and Tibshirani, 2003). The screening criteria were fold change ≥2 and FDR <0.05.

KOBAS 2.0 software was used to test the statistical enrichment of differentially expressed genes in KEGG pathways (Xie et al., 2011).

**Quantitative Real-Time PCR**
The cDNA synthesis was used by HiScript® III RT SuperMix for qPCR (Vazyme, Nanjing, China). CFX96TM Real-Time PCR Detection System (Bio-Rad, United States) was used to perform qPCR in a final volume of 10 μl: 3.8 μl of sterilized double-distilled water, 5 μl of SYBR qPCR Master, 0.8 μl of cDNA and 0.4 μl of each primer. The primers as shown in Supplementary Table S1. Transcript levels were normalized to the expression of the reference gene β-actin by the 2−ΔΔCt method.

**Enzyme Assay**
The activity of PEPCK and PFK was determined with Phosphoenolpyruvate Carboxykinase Activity Assay Kit (Jiancheng Biotech Co, Nanjing, China) and Phosphofructokinase Activity Assay Kit (Jiancheng Biotech Co, Nanjing, China), respectively. The mixture of 0.1 g tissue and 1 ml extraction solution was used for ice bath homogenization. Take the upper part and put it on ice for test. Then we added the reagent and sample into 1 ml quartz cuvette according to the instructions, and read the absorbance at 340 nm by a UV-2800 spectrophotometer (BMS Biotechnology Medical Services, Madrid, Spain).

**Statistical Analysis**
All data are expressed as the mean ± SEM. The statistical analyses of one-way ANOVA were performed using SPSS 19.0 (IBM, NY, United States). All data were represented as mean ± SEM. And groups denoted by different letters represent a significant difference at p < 0.05.

**RESULTS**

**Illumina RNA-Seq Quality Validation**
To identify the difference of gene expression in different stages of *S. prenanti*, a total of 9 liver cDNA libraries in three periods were constructed. After quality control of sequencing data, a total of 57.22G clean data were obtained, with Q30 reaching more than 85% (Table 1). Alignment results of clean reads and transcripts are presented in Table 2. Normalize the number of reads mapped to all genes and calculate it as FPKM for evaluating gene expression. The FPKM distribution density displayed the overall distribution of different transcripts expression levels in 9 liver samples (Figure 1A). The dispersion degree of the different samples was also generated with box plots (Figure 1B), which measured the expression level of each sample from the overall discrete expression level. The PCA score plot showed that the three stages were clearly separated with the main principal component (PC) scores as follows: PC1 = 36.90%, PC2 = 20.90% (Figure 1C).

**Analysis of Differentially Expressed Genes**
A total of 1692 DEGs were detected across three liver developmental stages (Supplementary Table S2). The expression levels of genes among the three groups in the pairwise comparisons were viewed through the Volcano Plot. As shown in Figure 1D, the highest number of DEGs was found between juvenile and young fish, including 511 upregulated and 549 downregulated genes. Between the juvenile and adult fish, 417 genes were upregulated and 669 downregulated genes. There were only 95 DEGs between the young and adult fish, including 36 upregulated and 59 downregulated genes. A Venn diagram was constructed to identify the joint DEGs among three pair-wise stage comparisons. Through Venn diagram analysis, we identified 470 DEGs that were co-altered in both juvenile vs. young and juvenile vs. adult. A total of 25 and 58 DEGs presented the same expressed trends between juvenile vs. young and young vs. adult, young vs. adult and juvenile vs. adult, respectively, (Figure 1E).

**GO and KEGG Pathway Enrichment Based on DEGs**
To explore the pathway of differential gene enrichment in different periods, the sequences were annotated by GO and KEGG databases. GO enrichment analysis was conducted on DEGs identified in three stages, and the results were divided into three categories: biological process (BP), cellular component (CC) and molecular function (MF) (Figures 2A–C). GO enrichment analysis showed that the major category represented was cellular process (GO:0009987) among biological processes. And among molecular functions, the major categories were catalytic activity (GO:0003824).

Finally, KEGG pathways databases were performed to determine the enrichment of differentially expressed genes. The top 20 pathways of DEGs enrichment can be seen in Supplementary Table S3 between different stages. In the comparison of young vs. adult, only the cell cycle pathway was significantly enriched for the differentially expressed genes (Figure 3A). Comparing juvenile vs. young, the differentially expressed genes were significantly enriched in three pathways, including drug metabolism-cytochrome P450, protein processing in endoplasmic reticulum, metabolism of xenobiotics by cytochrome P450 (Figure 3B). In contrast, a number of pathways were significantly enriched for juvenile vs. adult. The top 20 KEGG pathways were shown in Figure 3C, including drug metabolism-cytochrome P450, metabolism of xenobiotics by cytochrome P450, carbon metabolism, tyrosine metabolism, arginine and proline metabolism, tryptophan
TABLE 1 | Statistical table for sample sequencing data evaluation.

| Samples      | BMK-ID | Read Number | Base Number | GC Content % | ≥Q30 |
|--------------|--------|-------------|-------------|--------------|------|
| Adult1 Liver | L1 Liver | 21,498,046  | 6,408,391,356 | 47.28 | 93.66 |
| Adult2 Liver | L2 Liver | 21,224,260  | 6,345,019,826 | 47.85 | 93.84 |
| Adult3 Liver | L3 Liver | 21,873,325  | 6,534,813,036 | 47.85 | 93.83 |
| Young1 Liver | M1 Liver | 20,927,905  | 6,281,676,092 | 47.57 | 93.93 |
| Young2 Liver | M2 Liver | 21,009,982  | 6,291,554,036 | 48.49 | 93.87 |
| Young3 Liver | M3 Liver | 21,748,283  | 6,483,382,690 | 47.96 | 93.07 |
| Juvenile1 Liver | S1 Liver | 21,527,874  | 6,433,382,690 | 47.95 | 92.97 |
| Juvenile2 Liver | S2 Liver | 21,070,071  | 6,291,554,182 | 48.27 | 92.97 |
| Juvenile3 Liver | S3 Liver | 20,741,711  | 6,195,120,812 | 48.27 | 93.1  |

TABLE 2 | Statistical table of comparison results between the second-generation sequencing data and the third-generation non-redundant transcripts.

| Sample         | Total Reads | Uniquely Mapped reads (%) | % of reads Mapped to Multiple Loci | % of reads Mapped to too Many Loci |
|----------------|-------------|--------------------------|-----------------------------------|----------------------------------|
| Adult1 Liver   | 21,498,046  | 44.61                    | 27.32                             | 0.03                             |
| Adult2 Liver   | 21,224,260  | 49.83                    | 24.67                             | 0.04                             |
| Adult3 Liver   | 21,873,325  | 49.92                    | 24.47                             | 0.04                             |
| Young1 Liver   | 20,927,905  | 49.55                    | 21.65                             | 0.08                             |
| Young2 Liver   | 21,748,283  | 51.26                    | 22.84                             | 0.09                             |
| Young3 Liver   | 21,527,874  | 46.27                    | 21.77                             | 0.05                             |
| Juvenile1 Liver | 21,009,982 | 53.07                    | 20.02                             | 0.05                             |
| Juvenile2 Liver | 21,070,071 | 52.05                    | 21.27                             | 0.04                             |
| Juvenile3 Liver | 20,741,711 | 52.06                    | 20.41                             | 0.04                             |

FIGURE 1 | Quality assessment and analysis of global gene expression among liver samples at three stages. (A) Intuitive display genes expressed at different FPKM levels. (B) Boxplot of FPKM distribution among liver samples. (C) Principal component analysis (PCA) plots of all transcripts at different stages in S. prenanti. (D) Volcano plot of differential expression genes among the three stages in the pairwise comparisons. (E) Venn diagram showing the number of DEGs in transcript abundance in liver of S. prenanti. Yellow circles indicate the samples of juvenile vs young, green circles indicate the samples of young vs adult, and blue circles indicate samples of juvenile vs adult.
metabolism. We used heatmaps to characterize the DEGs in the enriched pathway drug metabolism—cytochrome P450. The mRNA expression levels of the CYP450 pathway related genes such as GST, UGT, DHDH, CBRI, AOX, MAO and CYP450 subtypes CYP1a1 were lower level in adult stage compared with juvenile and young stages (Figure 3D).
Genes Involved in Lipid Metabolism
To investigate the contents of triacylglycerol among three stages, we performed oil red O staining to the liver section. As shown in Figures 4A–C, lipid accumulation was remarkably increased in the liver of young and adult stages compared with juvenile stage. Meanwhile, the content of liver triglyceride was remarkably increased at young and adult stages compared with juvenile stage (Figure 4D) (p < 0.05). Expression levels of genes involved in the pathways associated to fatty acid metabolism were showed in Figure 4E. Compared with juvenile stage, the mRNA expression levels of fatty acid synthesis related genes such as FATP, ACSL, SREBP1, ELOVL1 were upregulated in adult and young stages, while expression of fatty acid β oxidation related genes such as ALDH9A1, AMACR, ECHS1, and HADHA were downregulated in young and adult stage compared with juvenile (Figure 4E).

Genes Involved in Carbohydrate Metabolism
To investigate the contents of glycogen during three stages, we performed PAS staining to the liver section. As shown in Figures 5A–D, glycogen content was higher in the livers of juvenile and young stages, compared with adult stage (p < 0.05). Expression levels of genes involved in the main pathways associated to carbohydrate are given in Figure 5E. The pathways include glycolysis (HK, PFK, GAPDH, and PGAM), gluconeogenesis (PEPCK and FBPase) and glycogenolysis (AGL, UGP2, GAA, and TREH) were upregulated in juvenile or young stages compared with adult stage. Moreover, the activity of PFK and PEPCK was at the lowest level in juvenile and then increased to a peak in young and was downregulated from young to adult (Figures 5F,G).

Validation of Differential Gene Expression By qPCR
To validate the accuracy of the transcriptome analysis, five differentially expression genes were selected for qPCR. As shown in Figures 6A,B, the mRNA expression levels of FABP7 and FBPase were downregulated from juvenile to adult. PFK, PEPCK and SREBP genes were expressed at the lowest level in juvenile and then increased to a peak in young, and was downregulated from young to adult (Figures 6C–E). As the qPCR results shown, the expression of all the tested genes presented the same expressed trends with the results of transcriptome analysis, suggesting that the results of RNA-seq were accurate and credible.

DISCUSSION
In vertebrates, the liver is the main place for metabolic clearance of foreign compounds, and is the richest source of drug
metabolizing enzymes (Parkinson et al., 2001). In this study, the differentially expressed genes were significantly enriched in drug metabolism-cytochrome P450 and metabolism of xenobiotics by cytochrome P450 pathways. Drug metabolic enzyme can be divided into cytochrome P450 enzymes and non-cytochrome P450 enzymes. CYP450 is generated by liver microsomes, which is an important enzyme in the metabolism of environmental pollutants and endogenous substances (Waxman, 1999). The expression and activity of CYP isoforms in liver alter significantly with age during development, and CYP1A1 is detected only in 3-week-old rats (Yun et al., 2010). In rainbow trout, the concentration of CYP450 is negatively correlated with body weight in the process of growing (Fitzsimmons et al., 2018). The mRNA expression of CYP450 subtypes CYP1a1 were lower level in adult stage compared with juvenile and young stage.

Non-cytochrome P450 enzymes includes DHDH, CBR1, GST, MAO, AOX, UGT, and so on (Pang et al., 2022). Dihydrodiol dehydrogenase (DHDH) is involved in the metabolism of polycyclic aromatic hydrocarbons in the liver (Carbone et al., 2008), but there is no report on the development change of DHDH in teleost fishes. In the present study, the mRNA expression of DHDH were lower level in adult stage compared with juvenile and young stage.

Liver is one of the important sites of amino acid metabolism. Amino acids play crucial roles on the growth, reproduction, and immune responses in fish (Li et al., 2009). Arginine and proline are amino acids with crucial roles in protein deposition and the immune response (Wu et al., 2011). In addition, dietary tryptophan attenuated stress induced anorexia and reduce aggressive behavior in brown trout (Andersen et al., 2016). Arginine and proline metabolism pathway and tryptophan metabolism pathway were significantly enriched during chicken liver development (Wu et al., 2018). Consistent with this, arginine and proline metabolism pathway and tryptophan metabolism pathway were significantly enriched based on the DEGs, which suggested that amino acids pathway was involved in development of S. prenanti.
the synthesis of acyl CoA from fatty acids, which is the key enzyme of triglyceride synthesis and fatty acid β oxidation (Grevengoed et al., 2014). Our results revealed that the lipid content of liver increased in adult and young stages compared with juvenile. Consistent with the oil-red O staining results, the mRNA expression levels of SREBP1 and ACSLs increased in young and adult stage compared with juvenile, indicating the fatty acid and triglyceride synthesis maybe active in young and adult stage. Previous studies proved that ELOVL1 can elongate very long chain monounsaturated fatty acid and very long chain saturated fatty acids (Moon et al., 2009; Ohno et al., 2010). In the present study, the mRNA expression of ELOVL1 was increased during liver development, which suggested that liver fatty acid elongation may be induced during the liver development. FATP is a key factor involved in fatty acid transport and fat deposition, which function is to transport of long-chain and very long chain fatty acids into cells (Dutta-Roy, 2000). In the conditions of low-fat feeding, the expression level of FATP is upregulated in the liver of 18 months mice comparing to 3 months mice (Martin et al., 2008). In our RNA-seq results, we found that the mRNA expression of FATP was also increased in young and adult stage comparing with juvenile. FABP can bind fatty acids and transport them to peroxisome, mitochondria and endoplasmic reticulum for fatty acid β oxidation (Veerkamp et al., 1993). In mice, L-FABP gene ablation increases the concentration of liver lipid concentrations (Martin et al., 2008). In the resent study, mRNA expression of FABP was the lowest in adult of the three periods. Meanwhile, the mRNA expression of fatty acid β oxidation related genes including ALDH9A1, AMACR, ECHS1, HADHA were consistent with the results of FABP, which suggested that fatty acid β oxidation pathway was downregulated in adult stage. Collectively, the results revealed that the pathways of lipid synthesis and fatty acid elongation was elicited, and fatty acid β oxidation pathway was downregulated in the liver development.

Liver is the main site of carbohydrate metabolism in fish. Our PAS staining revealed that the glycogen content of liver decreased during liver development. Hexokinase (HK) and phosphofructokinase (PFK) are the rate-limiting enzymes of glycolysis. In rats, hexokinase activity has no significant change during liver postnatal development (Dileepan et al., 1979). In our study, mRNA expression levels encoding these two enzymes and the activity of PFK were the highest in young stage, suggesting that glycolysis ability may be enhanced in young stage. PEPCK and FBPase are the rate-limiting enzymes of gluconeogenesis, and catalyzes oxaloacetic acid to form phosphoenolpyruvate and the conversion of fructose-1, 6-diphosphate to fructose-6-phosphate (El-Maghrabi et al., 1995; Hanson, 2009). We found that the mRNA expression of PEPCK and FBPase as well as the activity of PEPCK were decreased at the adult stage, which indicated that the gluconeogenesis pathway was inhibited in adult stage. Glycogen degradation is catalyzed by many enzymes, which including AGL, GAA and TREH (Yong et al., 1996; Lombard et al., 2014). In this study, the mRNA of these genes showed that glycogenolysis decreased in adulthood. These results indicated that glycogenolysis, gluconeogenesis and glycogenolysis pathway were inhibited during liver development of S. prenanti. With the growth of individuals, the demand for feed lipid and carbohydrate varies at different stages. In fish, many studies have confirmed that high carbohydrate and high fat diets can lead to the increase of lipid accumulation and glycogen content in fish liver (Prisingkorn et al., 2017; Jia et al., 2020; Sun et al., 2021). Therefore, suitable nutrient levels are able to affect fish health and promote fish growth. Previous study showed that fatty acid deficiencies manifest themselves faster in juvenile fish (Dabrowski, 1986). For example, essential fatty acid (EFA) deficiency occurs only within 2 weeks when young juvenile barramundis (Lates calcarifer) are fed with fish oil free diet, or with a low inclusion of fish oil (Salini et al., 2015). In seabass (Dicentrarchus labrax) juveniles, reducing dietary fish oil levels from 6% to 3% increases gut bacterial translocation rates (Torrecillas et al., 2017). In this study, lipid accumulation was remarkably increased in the liver of young and adult stages compared with juvenile stage, and we speculated that the crude lipid requirement of juvenile is higher than that of young and adult. In gilthead sea bream (Sparus aurata) juveniles, the growth performance, feed efficiency, and protein efficiency ratio of 30% gelatinized maize starch were lower than those of 20 and 10% gelatinized maize starch fish fed diet (Couto et al., 2008). In this study, glycogen content was higher in the livers of juvenile and young stages, compared with adult stage, and we speculated that the carbohydrate requirement of juvenile and young is lower than that of adult.

In this study, we obtained mRNA expression patterns of three stages in S. prenanti liver by RNA-seq technology and have identified a total of 1692 differentially expressed mRNAs across three stages of S. prenanti liver. In addition, we revealed that lipid accumulation was increased and glycogen content was decreased during liver development. Moreover, based on KEGG analysis we found that the differentially expressed mRNAs were involved in the CYP450 pathway, lipid metabolism and carbohydrate metabolism in liver maturation of S. prenanti. Overall, our data enriched sequences information of S. prenanti and provided a broad and novel vision for future research at the transcription level in fish.

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 in the article/Supplementary Material.

AUTHOR CONTRIBUTIONS

JN and PZ designed the study and wrote the manuscript. QM contributed to concept and experimental design. WL and ZD collected the samples. JJ, SY, and LZ conducted bioinformatics analysis. QG contributed materials/analysis tools. YW contributed supervision, funding acquisition, and project administration.
