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Transcriptome analysis reveals high concentration of resveratrol promotes lipid synthesis and induces apoptosis in Siberian sturgeon (*Acipenser baerii*)

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Abstract

Resveratrol has been reported to promote immunity and decrease oxidative stress, but which demonstrates biphasic effects relied on the use concentration. In this study, the effects of diet supplement with a relative high concentration of resveratrol (0.32 mg/kg) on metabolism, antioxidation and apoptosis of liver were investigated in Siberian sturgeon. The results showed that resveratrol significantly increased the lipid synthesis and the apoptosis, but did not either activate the antioxidant NRF2/KEAP1 pathway or enhance the antioxidant enzyme activity. Transcriptome analysis revealed significant changes in regulatory pathways related to glycolipid, including PPAR signaling pathway, Insulin signaling pathway, Fatty acid biosynthesis, and Glycolysis/Gluconeogenesis. In addition, resveratrol significantly increased the lipid synthesis genes (*acca* and *fas*), fatty acid transport gene (*fatp 6*) and gluconeogenesis gene (*gck*), but decreased the survival-promoting genes (*gadd45β* and *igf 1*). These findings highlight a significant effect of resveratrol on glycolipid metabolism in Siberian sturgeon. Moreover, this study also demonstrated that 0.32 mg/kg resveratrol has physiological toxicity to the liver of Siberian sturgeon, indicating that this dose is too high for Siberian sturgeon. Thus, our study provides a valuable insight for future research and application of resveratrol in fish.

Keywords Sturgeon, Resveratrol, Lipid metabolism, Apoptosis, Anti-oxidation

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Introduction

China has become the foremost producer and exporter of sturgeon caviar globally [1]. Among sturgeon species, the Siberian sturgeon (*Acipenser baerii*) is particularly valuable in aquaculture for its ability to thrive in various temperature ranges and its fast growth rate [2–4]. Environmental stressors like temperature fluctuations, ammonia nitrogen, and toxicants can disrupt the fish's metabolic balance, increase reactive oxygen species and inflammation levels, and ultimately impact yield, quality, and even survival [5–10]. To address these challenges, numerous studies have proposed using dietary additives such as plant and animal extracts, beneficial probiotics, microalgae, and other substances [11–17].

Resveratrol (3,4,5-trihydroxy-trans-stilbene) is a dietary polyphenol commonly found in peanuts, mulberry fruits, tiger nuts, grapes, and red wine [18]. Research has indicated that resveratrol has potential benefits in improving fish immunity, preventing stress-related issues, and reducing economic losses due to diseases or stresses [14, 19–23]. As a result, it has been used as an additive in aquafeed to enhance the quality and economic value of aquatic products. Studies have shown that resveratrol can improve gut health in Siberian sturgeon by enhancing the structure of intestinal villi and the abundance and composition of microbial communities [14]. Furthermore, resveratrol has been found to mitigate the negative effects of a high-fat diet on hepatic antioxidant levels, inflammation, and lipid metabolism in carp (*Cyprinus carpio*) [22].

The effects of resveratrol are concentration-dependent, with varying outcomes based on exposure time and cell type [24]. Some studies have suggested that resveratrol demonstrates biphasic effects, with beneficial effects at low concentrations and toxic effects at high concentrations [25–32]. The toxic effects may include a decrease in antioxidant enzyme activity, an increase in reactive oxygen species production, lipid peroxidation, and potential cancer development in mammals [30, 33–37]. The optimal concentration of resveratrol for aquatic products remains unclear [38]. Therefore, adding an appropriate low concentration of resveratrol into the diet of fish can potentially yield beneficial effects and support the growth and health of fish breeding.

To investigate the effect of high concentration resveratrol on Siberian sturgeon, a daily diet containing a relative high concentration of resveratrol (0.32 mg/kg), was administered based on a previous study [14]. This study aimed to elucidate the potential effects of high concentration of resveratrol on histological changes, apoptosis, lipid metabolism, and antioxidant enzyme activity in liver of Siberian sturgeon. Furthermore, the study delved into the underlying molecular mechanisms through RNA-seq, Real-time PCR, and Western blot analysis. The findings would offer valuable insights that guide the utilization of resveratrol in the cultivation of Siberian sturgeon (Fig. 1).

Results

Resveratrol increases lipid deposition and apoptosis in liver

H&E staining revealed that resveratrol notably enhanced vacuolar degeneration of hepatocytes, meaning that resveratrol administration facilitated the triglyceride deposition in liver (Fig. 2A). This observation was further supported by Oil red O staining, providing direct visual evidence (Fig. 2B, D). Additionally, Immunofluorescence TUNEL assay demonstrated that resveratrol also triggered apoptosis of hepatocytes (Fig. 2C, E).

Resveratrol does not impact antioxidative level in liver

To investigate the effect of resveratrol on antioxidative level, antioxidative enzyme activity assay and immunohistochemical analysis of KEAP1 and NRF2 were conducted. The results revealed that no significant difference was found in the activity of relevant antioxidant enzymes, Catalase, Glutathione peroxidase (GSH-PX), Superoxide Dismutase (SOD), Total antioxidant capacity (T-AOC) or oxidative stress product Malonaldehyde (MDA) (Fig. 3D). The protein levels of KEAP1 and NRF2 was not significantly changed in liver tissue after resveratrol administration (Fig. 3A-C).

RNA-seq reveals resveratrol promotes hepatic lipid synthesis

In RNA-seq, clean reads were obtained by filtering reads containing adapters, reads with an N ratio greater than 10%, and reads containing a large number of low-quality reads. Differential expression analysis revealed 1,314 DEGs in liver, with 609 up-regulated and 705 down-regulated DEGs (Fig. 4A). GO Enrichment analysis showed that all the DEGs were enriched into 36 items, containing 16 biological processes, 12 cellular components, and 8 molecular functions. Highly expressed functions in the liver included "biological regulation", "cellular process", "metabolic process", "cell part", "membrane part", "organelle part", and "catalytic activity" (Fig. 4B).

The top 30 pathways enriched by KEGG database were presented. Among these pathways, metabolism-related pathways, including "PPAR signaling pathway", "Starch and sucrose metabolism", "Insulin signaling pathway", "Fatty acid biosynthesis", "Glucagon signaling pathway" and "Glycolysis/Gluconeogenesis", were highlighted, meaning that resveratrol might change the metabolism balance of liver. Additionally, the pathways associated with apoptosis were also found, including "p53 signaling pathway" and "Apoptosis" (Fig. 4C).

The expression of DEGs enriched in the glycolipid metabolism- and apoptosis-related pathways was

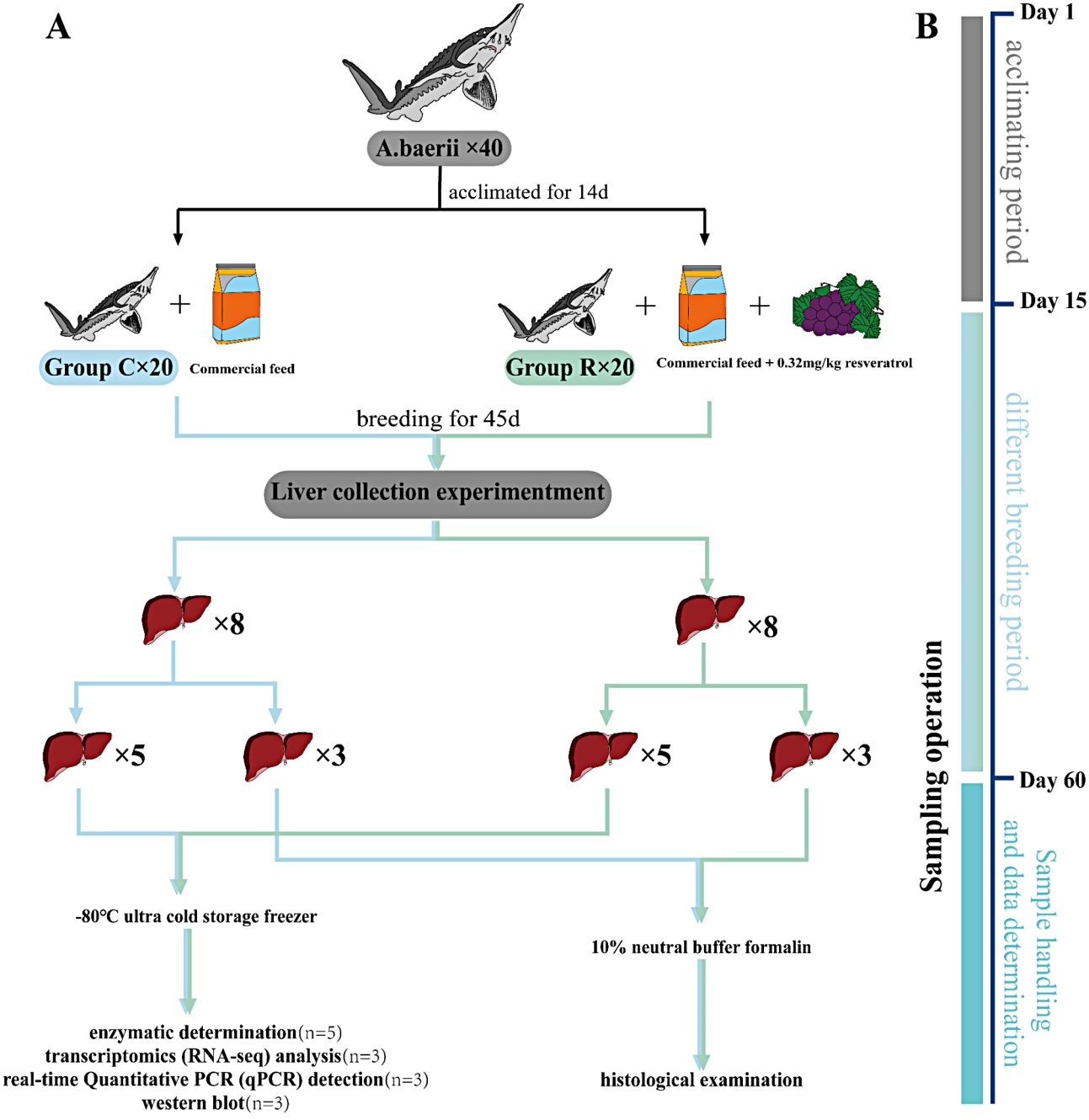

Fig. 1 Breeding and sample analysis processes of control (C) and resveratrol (R) groups. A total of 40 Siberian sturgeon underwent a 14-day acclimation period, followed by feeding 20 fish with a commercial diet and another 20 fish with a resveratrol-added diet for 45 days. After feeding, 8 fish were randomly selected from each group, of which 5 fish were used for enzymatic, RNA-seq, qPCR and western blot analysis, and 3 fish for histological examination

presented in heatmap (Fig. 4D). Based on their function, we classified into 4 categories, containing glycometabolism, lipid metabolism, apoptosis and anti-apoptosis. In the glycometabolism pathway, the expression levels of glycolysis genes (*hexok 4* and *gck*) and gluconeogenesis gene (*pck 1*) were significantly increased and decreased, respectively. In the lipid metabolism pathway, the expression levels of lipid synthesis genes (*accα* and *fas*), fatty acid transport gene (*fatp 6*) and unsaturated fatty acid synthesis genes (*fads 2* and *scdb 2*) were significantly increased after resveratrol administration. Especially for *accα*, *fas*, *fatp 6* and *gck*, which showed very high expression foldchange (2-3-foldchange) than the control group, revealing that lipid synthesis and carbohydrate decomposition were significantly increased. In the apoptotic and anti-apoptotic pathways, the apoptotic gene (*caspase 9*) and the anti-apoptotic genes (*gadd45β*, *gadd45γ*, *igf 1*, *igf*

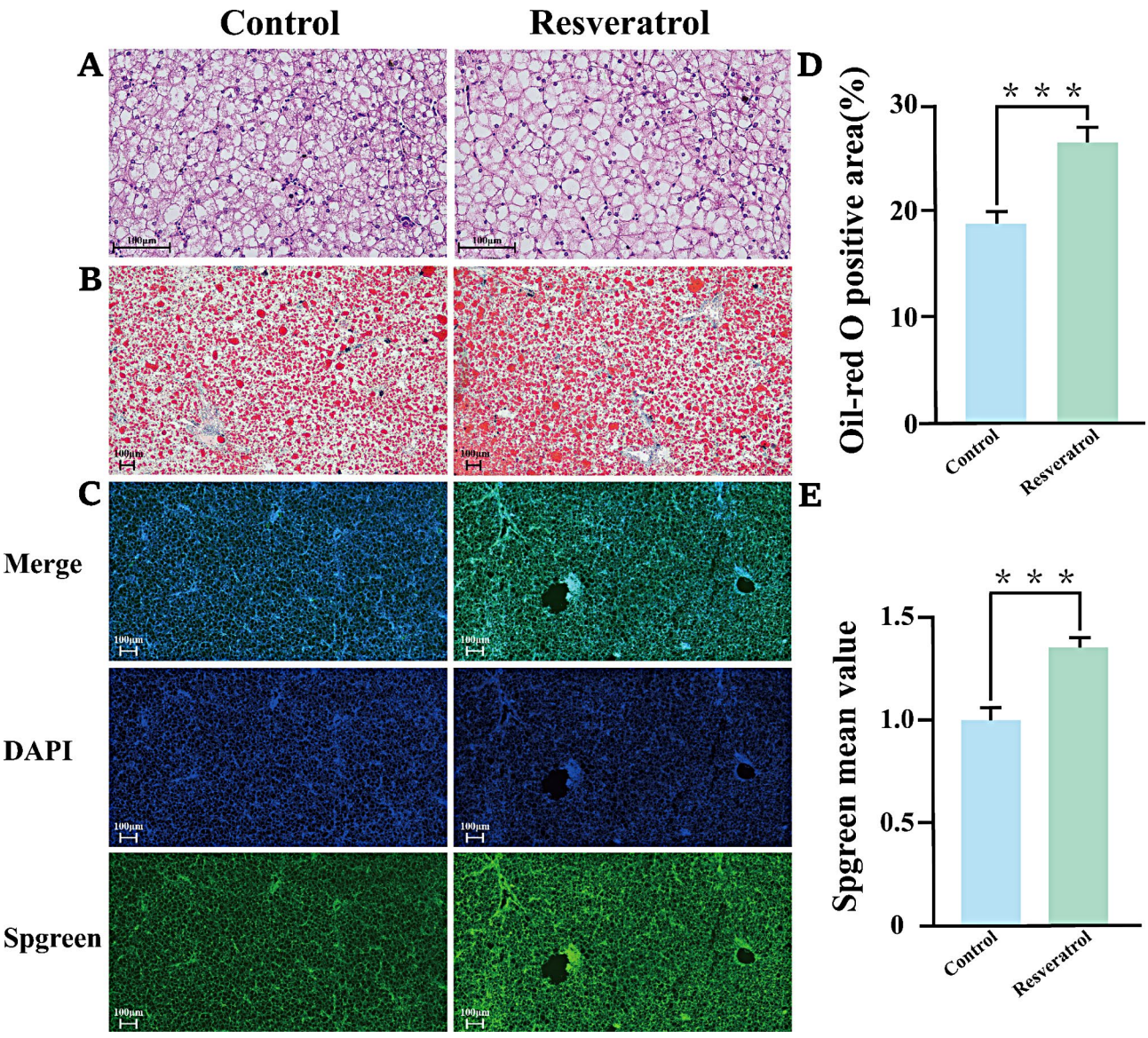

Fig. 2 Histological observation and TUNEL assay of liver in Siberian sturgeon. **A**-**B**: H&E and Oil red O staining of liver tissue section. **C**: Immunofluorescence TUNEL assay in liver (Spgreen: spectrum green; TUNEL can be detected by spectrum green). **D**: Relative Oil red O staining level assay of C and R groups. **E**: Relative Spgreen mean value of C and R groups. Data are presented as mean ± SD ($n = 3$), the significance analysis were performed by T-test, ***$p < 0.001$

2 and *igf binding protein 1/3*) were significantly increased and decreased, respectively.

Expression validation of DEGs associated to glycolipid metabolism and apoptosis in liver

To validate the expression of DEGs associated to glycolipid metabolism and apoptosis in liver, we performed qRT-PCR to detect the expression of 8 DEGs. As shown in Figs. 5 and 6, the expression of genes that promote lipid synthesis and fatty acid transport, *acca*, *fas* and *fatp 6*, was significantly elevated in resveratrol group. the expression of key genes related to glycolysis (*gck*) and gluconeogenesis (*pck 1*) was significantly increased and decreased, respectively (Fig. 5). Additionally, the expression of survival-promoting genes *gadd45β* and *igf 1*was notably decreased, but the expression of apoptosis-inducing gene *caspase 9* was not obviously changed (Fig. 6). Together, the expression of these genes was consistent with the RNA-Seq results, indicating that the transcriptome data is reliable (Figs. 5 and 6).

Protein expression of CASPASE 9, IGF 1 and PCK 1

The Western blot experiment revealed a notable decrease in the protein level of PCK 1 in the resveratrol group, a known rate-limiting enzyme of gluconeogenesis. Furthermore, a marked reduction in the protein level of IGF

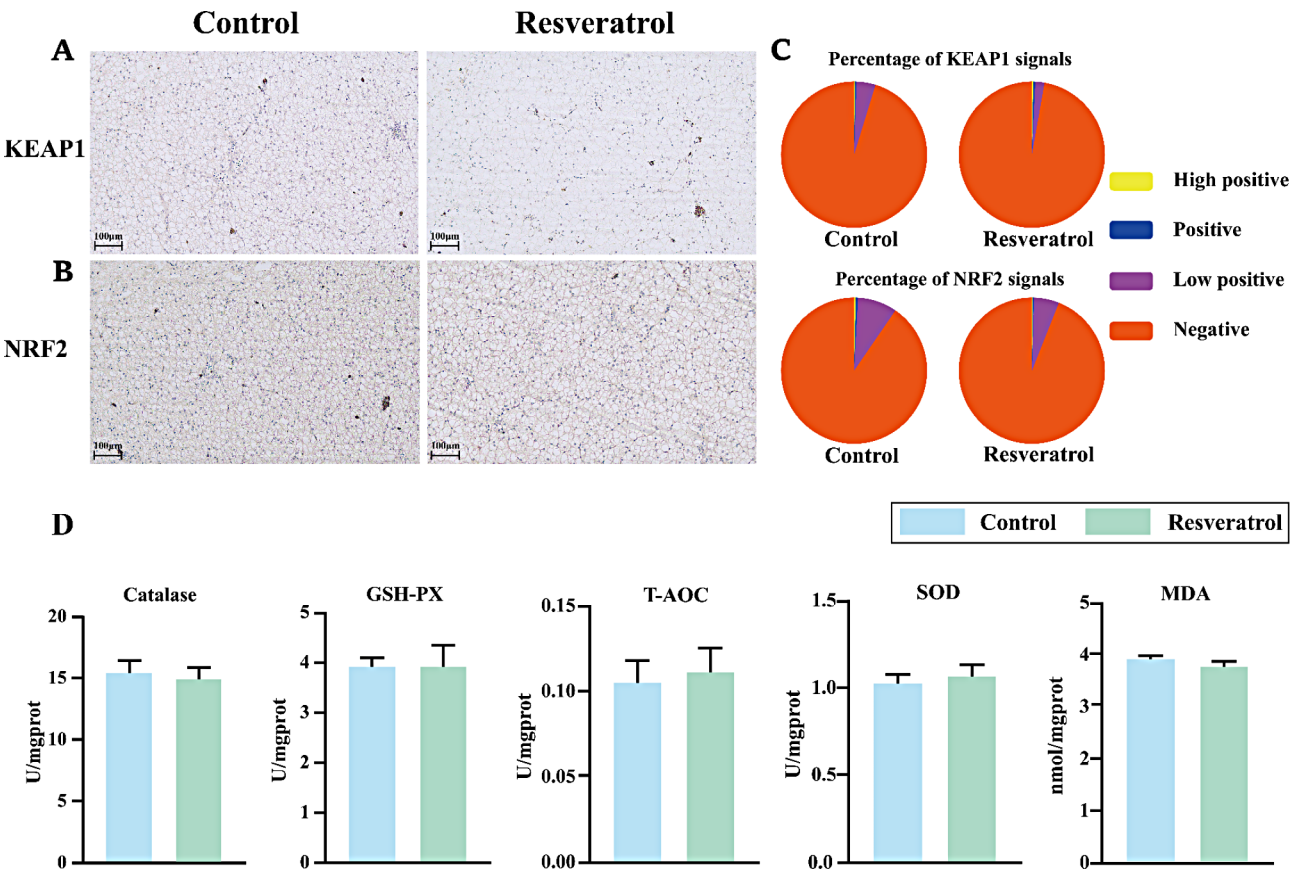

Fig. 3 Evaluation of antioxidative level in liver. **A**-**B**: Protein levels of KEAP1 and NRF2 in the liver of Siberian sturgeon from C and R groups. **C**: KEAP1/NRF2 signal statistics. **D**: Determination of antioxidant enzyme activity (Catalase, GSH-PX, T-AOC, SOD) and peroxidation markers (MDA). Data are presented as mean ± SD ($n = 5$), the significance analysis were performed by T-test

1, a protein associated with cell survival, was observed. However, there was no significant change in the protein level of CASPASE 9 between the control and resveratrol groups. These results are consistent with the previous findings in their expression levels conducted from qPCR and RNA-seq analysis (Fig. 7).

Discussion

Fatty liver disease has become a common issue in aquaculture [39–42]. Hepatic glycolipid metabolism plays a crucial role in maintaining the organism's metabolism balance [43, 44]. Resveratrol, when present in moderate concentrations, has a positive impact on the organism. It helps alleviate lipid accumulation in liver cells caused by poor diet, enhances glycogen storage, and promotes adipocyte lipolysis [45–47]. Additionally, resveratrol reduces hepatocyte vacuolization, and thus mitigates liver damage in Nile tilapia caused by a high-fat diet [48]. However, there are instances where high concentrations of resveratrol can lead to abnormalities in various aspects of the organism.

In fish, previous study has reported that long-term feeding with high concentration of resveratrol was found to cause congestion, hepatocellular fibrosis, and abnormalities in hepatic epidermal cells in cultured tilapia [49]. In our study, the stained sections using oil red O revealed that 0.32 mg/kg resveratrol administration led to an increase in lipid level in liver of Siberian sturgeon. To further investigate the underlying mechanisms, transcriptome analysis was conducted. Several significantly enriched pathways were acquired, which associated to glycolipid metabolism. Within these pathways, the expression of key rate-limiting genes related to lipid synthesis (*accα*, *fas*), lipid transportation (*fatp 6*) and unsaturated fatty acid synthesis (*fads 2* and *scdb 2*) was significantly upregulated. In juvenile golden pomfret, the decreased lipid synthesis was found in liver, because the expression of *fas* and *acc α* genes was significantly decreased [50]. Thus, our results reveal that high concentration of resveratrol promoted hepatic lipid synthesis in the liver of Siberian sturgeon.

Additionally, the expression of key rate-limiting genes for glycolysis (*gck* and *hexok*) was significantly increased, while the expression of key rate-limiting genes for gluconeogenic synthesis (*pck 1*) was significantly decreased. In zebrafish, knockdown of *pck 1* gene caused up-regulation

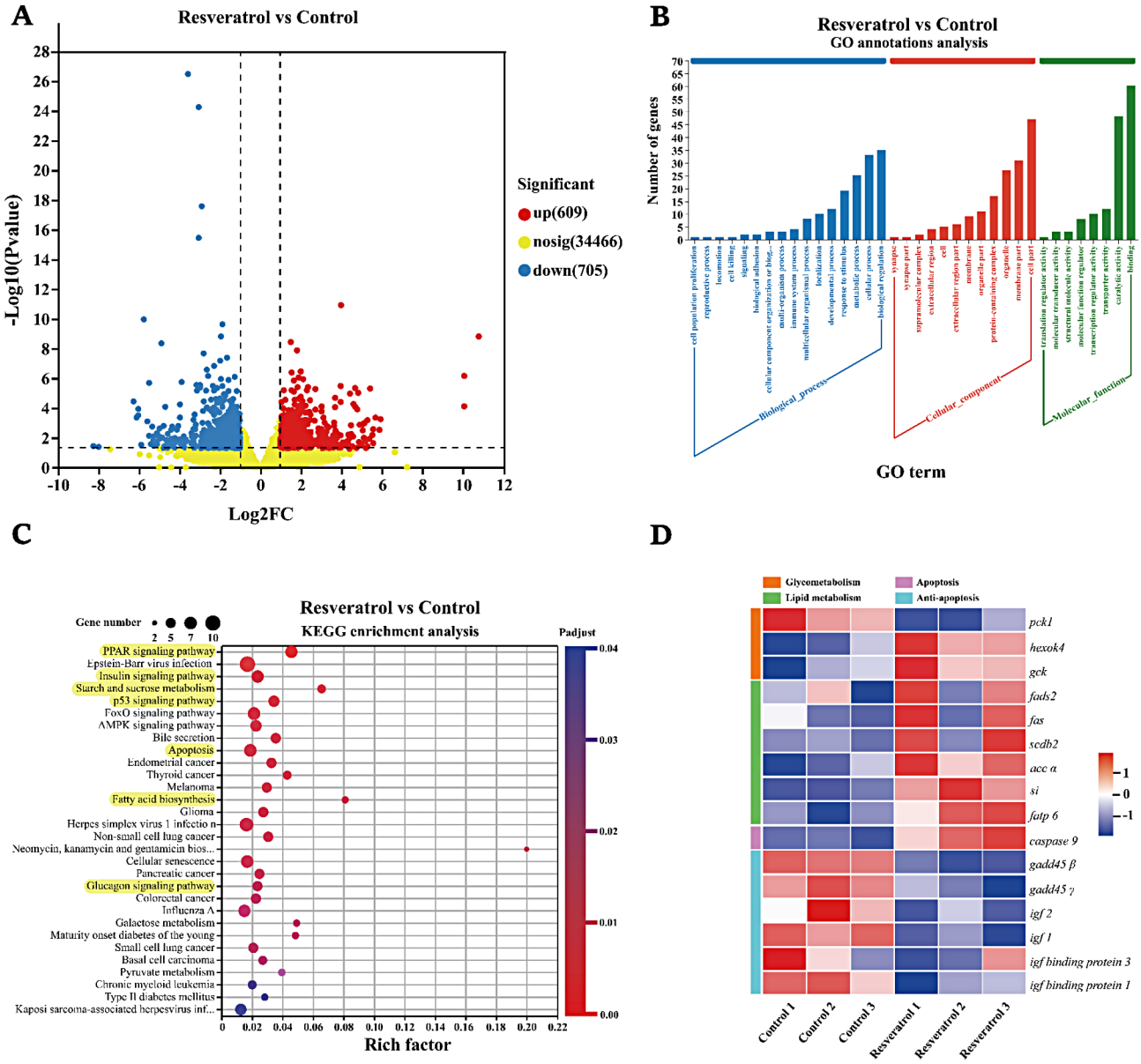

Fig. 4 Transcriptome profiles of liver. **A**: Differential gene volcano map. **B**: GO annotation analysis. **C**: KEGG enrichment analysis. The top 30 pathways enriched by KEGG database were presented based on the enriched significance. **D**: Heatmap of DEGs related to metabolism- and apoptosis-related pathways

of *gck* gene expression, resulted in increasing carbohydrate utilization of liver [51]. Thus, our findings suggest that high concentration of resveratrol promotes carbohydrate utilization in the liver of Siberian sturgeon.

Excessive lipid synthesis may lead to increase oxidative stress [52–54]. In an other hand, resveratrol was used as an antioxidant in aquatic animals [22, 55–57], in spite of some studies have shown that resveratrol may promote oxidative stress [24, 58, 59]. In this study, the effect of 0.32 mg/kg resveratrol on the anti-oxidative ability of liver was investigated in Siberian sturgeon. The results showed that the activity of antioxidative enzymes CAT, GSH, T-AOC and SOD, and the level of lipid peroxidation marker MDA did not change in the liver after resveratrol administration. In addition, the expression level of the key proteins (NRF2 and KEAP1) of antioxidative pathway also showed no significantly change in the liver. These results reveal that 0.32 mg/kg resveratrol promotes the lipid synthesis without inducing lipid peroxidation ultimately, speculating that resveratrol may relieve lipid synthesis-induced oxidative stress.

Excessive lipid synthesis may also cause cell apoptosis in liver [60–62]. In this study, TUNEL assay uncovers that resveratrol promotes the cell apoptosis in liver. In

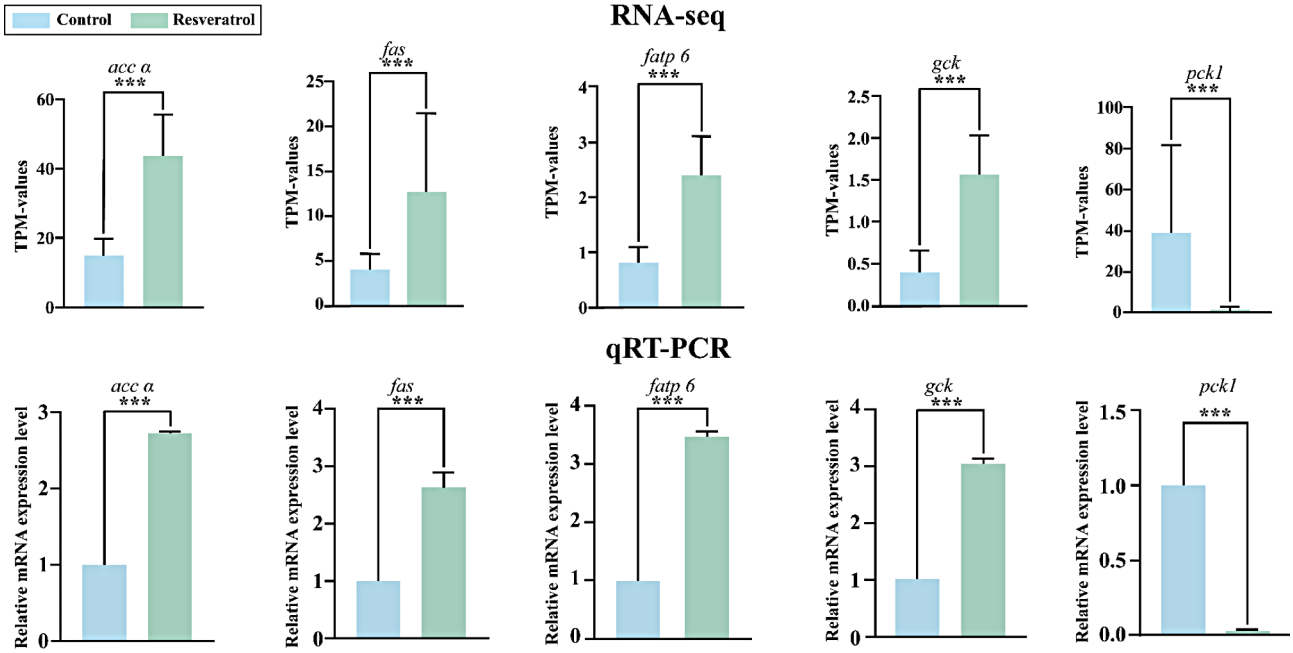

Fig. 5 Expression validation of DEGs associated to glycolipid metabolism in liver. Comparison of the expression of 5 selected DEGs by RNA-seq and qRT-PCR. The gene expression of RNA-seq was presented based on the TPM-values, while the qPCR results were calculated as foldchange compared to the control group after normalizing to the reference gene (*β-actin*), Data are presented as mean ± SD ($n = 5$), the significance analysis were performed by T-test, *** $p < 0.001$

transcriptome analysis, the significantly enriched pathways include apoptosis pathways, such as the p53 signaling pathway and Apoptosis. Gadd45 is a stress protein that responds to the environment and is able to repair damaged cells [63]. *Igf* 1 is a growth promoting factor, which has been shown to inhibit apoptosis through different control sites of apoptosis [64]. The expression of cell survival-promoting genes (*gadd45β* and *igf 1*) was significantly reduced in resveratrol group. However, the expression of *caspase 9* gene and protein were not significantly changed after resveratrol administration. These results suggest that resveratrol promotes cell apoptosis by suppressing survival-promoting signaling but not increasing the apoptosis signaling.

Taken together, diet supplement with a relative high concentration of resveratrol (0.32 mg/kg) promotes lipid synthesis by increasing the expression of lipid synthesis genes (*acc α*and *fas*), lipid transportation gene (*fatp 6*) and unsaturated fatty acid synthesis genes (*fads 2* and *scdb 2*), and enchances carbohydrate utilization by increasing the expression of glycolysis genes (*gck* and *hexok*) and decreasing the expression of gluconeogenic synthesis gene (*pck 1*) in liver of Siberian sturgeon. Yet, the promoted lipid synthesis did not induce lipid peroxidation in liver. In addition, resveratrol administration promotes cell apoptosis by suppressing the expression of survival-promoting genes (*gadd45β* and *igf 1*). Our study could provide a reference for the application of resveratrol on aquaculture by guiding the use concentration and showing the potential negative effects.

Materials and methods

Siberian sturgeon

Forty healthy juvenile sturgeons, with an average weight of 237.6±4.2 g, were obtained from Sichuan Tianquan Sturgeon Farm (Yaan, China). The experiment was conducted in a laboratory using round transparent glass tanks (1.5 m in diameter and 1 m in height) with continuous aeration for 24 h, and half of the water was renewed every day. The tank maintained the specific conditions, including a temperature of 16±1℃, dissolved oxygen level of 7.1±0.5 mg/L, pH level of 7.8±0.2, ammonia nitrogen concentration≤0.01 mg/L, and nitrite concentration≤0.05 mg/L. Prior to the experiment, the juvenile sturgeons were acclimated for 14 days under the conditions. The fish were fed three times per day at 8:00 am, 14:00 pm, and 20:00 pm, respectively, with a commercial feed provided by Haida Group Co., Ltd. The amount of feed given was 3% of the fish's body weight per day. The animal handling procedure for this experiment was approved by the Animal Protection and Utilization Committee of Sichuan Agricultural University.

Experimental administration

After 14 days of domestication, a total of 40 fish were randomly assigned to two groups: Control (C) and resveratrol (R) groups. Each group was further divided into

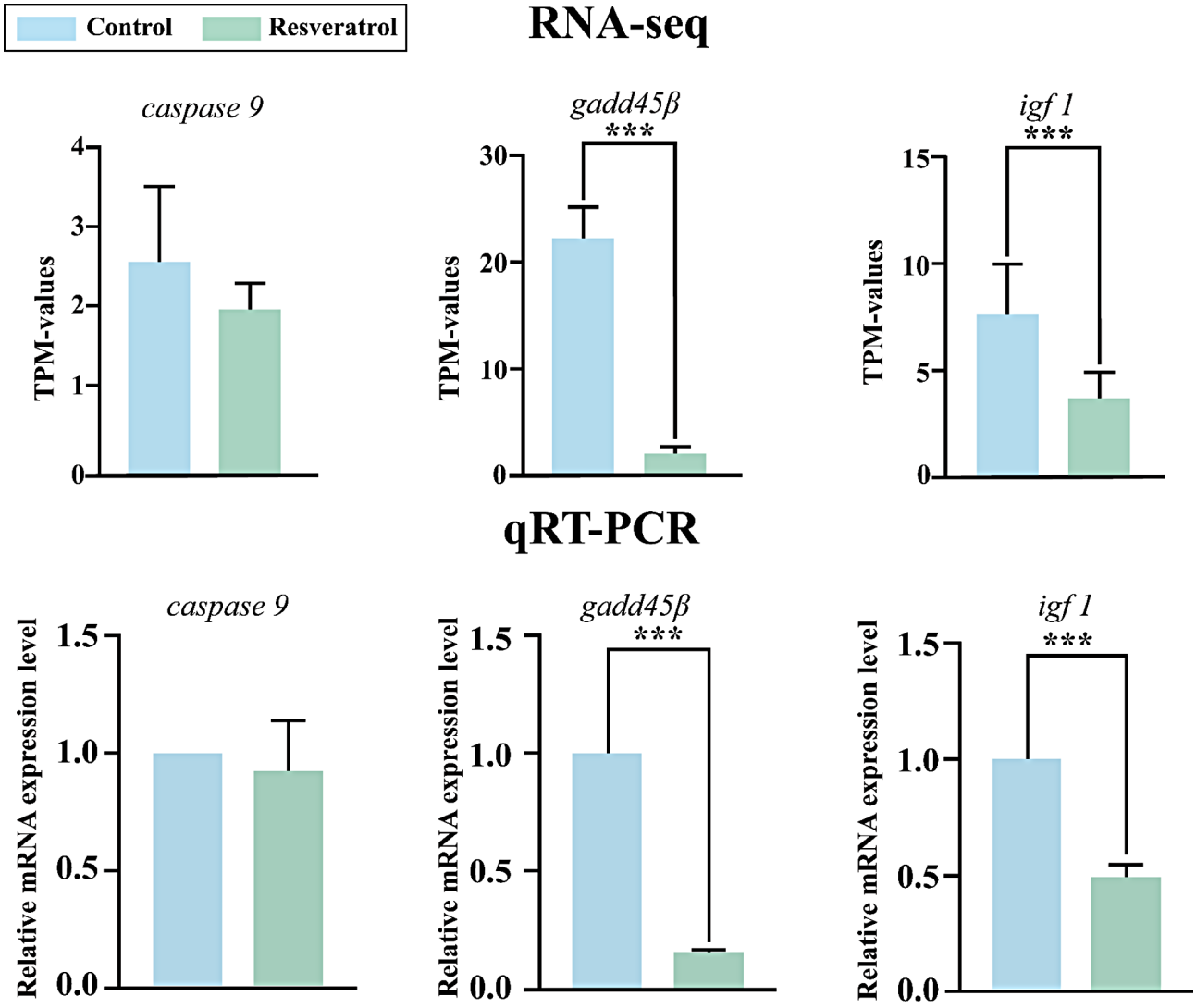

Fig. 6 Expression validation of DEGs associated to apoptosis in liver. Comparison of the expression of 3 selected DEGs by RNA-seq and qRT-PCR. The gene expression of RNA-seq was presented based on the TPM-values, while the qPCR results were calculated as foldchange compared to the control group after normalizing to the reference gene (*β-actin*). Data are presented as mean ± SD ($n = 5$), the significance analysis were performed by T-test, *** $p < 0.001$

two fish tanks (10 fish per tank). The fish of R group was fed with diets containing a concentration of 0.32 mg/kg resveratrol (purity ≥ 99%, McLean Biochemical Company), while C group fed with the commercial feed. After 45 days of diet administration, 8 fish were randomly selected from each group (4 fish per tank) and anesthetized with 70 mg/L MS-222 (Tricaine methanesulfonate; each fish belly up and unconscious), of which 5 fish were used for enzymatic analysis, RNA-seq, qPCR and western blot analysis, and 3 fish for histological examination (Fig. 1). Among the 5 fish, 5 fish were used for enzymatic analysis, but random 3 fish for RNA-seq, qPCR and western blot analysis.

HE staining

Liver samples were fixed in 10% neutral buffered formalin for at least 24 h. The fixed liver samples were then dehydrated, transparentized using xylene, and embedded in paraffin wax. The solidified wax blocks were subsequently cut into 5-mm slices and mounted on slides for H&E staining. Following staining, the slides were observed under an optical microscope (Nikon, Tokyo, Japan).

Oil red O staining

Frozen liver was sectioned as 10 μm slices. Then, the slices were kept at room temperature for 30 min and washed with distilled water three times to remove the embedding agent. Subsequently, they were soaked in 60% isopropyl alcohol for 2 min. The sections were stained

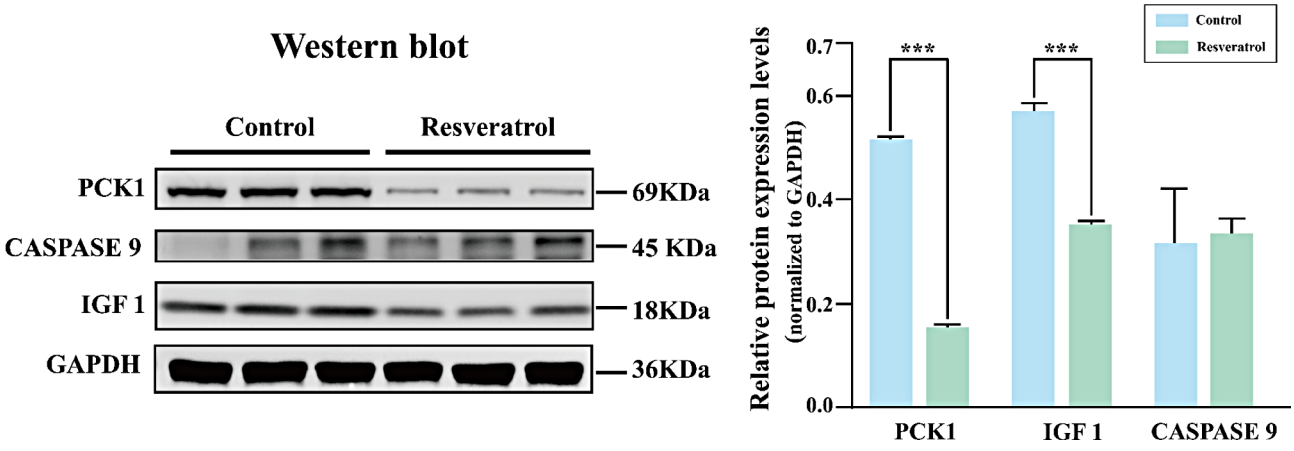

Fig. 7 Western blot analysis of CASPASE 9, IGF 1 and PCK 1 protein expression in liver. Gray values were analyzed by Image J, and the results were calculated as foldchange of gray values compared to the control group after normalizing to the reference protein (GAPDH). Data are presented as mean ± SD ($n = 3$), the significance analysis were performed by T-test, *** $p < 0.001$

with oil red working liquid, washed again with distilled water three times, restained with hematoxylin, sealed with glycerin gelatin, and observed under an optical microscope.

Immunohistochemistry

The paraffin sections were dewaxed in water and then placed in a repair box filled with citric acid antigen repair buffer (pH 6.0) for antigen repair in a microwave oven. Subsequently, the slices were incubated in a 3% hydrogen peroxide solution at room temperature, away from light, for 25 min, and then washed in PBS (pH 7.4) three times to block endogenous peroxidase. Next, a 3% BSA solution (Thermo Fisher, USA) was evenly applied to cover the tissue and left at room temperature for 30 min. The primary antibodies NRF2 Rabbit pAb (GB113808, Servicebio, China) and KEAP1 Rabbit pAb (GB113747, Servicebio, China) were added with PBS and incubated overnight in a wet box at 4 °C. Following washing, the tissue was covered with Goat Anti-Rabbit secondary antibody (G1213, Servicebio, China) and incubated at room temperature for 50 min. After DAB color rendering, the sections were re-stained the nucleus. Finally, the sections were observed under a Nikon Eclipse E200 (Japan) microscope and photographed for analysis. The mean optical density (IOD SUM/area) was evaluated using Image J.

TUNEL assay

Liver samples were stained using TUNEL method by TUNEL cell apoptosis assay kit (Xavier, China). The stained samples were observed under a fluorescence microscope (Eclipse Ci-L, Nikon, Japan). Following imaging, Image-Pro Plus 6.0 software was utilized for analysis, where apoptotic cells exhibited green fluorescence and normal cells displayed blue fluorescence. The positive rate was calculated as the number of positive cells divided by the total number of cells.

Detection of antioxidative biomarkers

The 1 g of liver tissue was ground with 9 mL of 0.85% NaCl solution in a homogenizer on ice. The antioxidative biomarkers were detected by commercial kits purchased from Nanjing Jiancheng Bioengineering Institute (China), which contained Malonaldehyde (A003-1), Catalase (A007-1-1), Glutathione peroxidase (A005-1), Superoxide Dismutase (A001-3), Total antioxidant capacity (A015-2-1), and Total protein(A045-2). All measurement procedures were strictly conducted following the manufacturer's instructions.

Western blot

The liver sample were lysated by RIPA buffer including phosphatase and protease inhibitors. The lysate was collected by centrifuge and determined the protein concentration using a BCA kit (PC0020, Solarbio). Electrophoresis was performed at 80 V/30 min and 120 V/60 min in a 12% SDS-PAGE gel. The proteins on the gel were transferred to a PVDF membrane, which was then seale in TBST containing 5% skim milk for 2 h. The PVDF membranes were incubated overnight with CASPASE9 (A21682, ABclonal), PCK1 (A2036, ABclonal), IGF1 (A11985, ABclonal), or GAPDH (GB15002, servicebio) antibody at 4 °C, following to incubate with a secondary antibody coupled with HRP at room temperature for 1 h. The protein bands were visualized using an ECL color development solution, and Image J software was used for quantitative analysis of the indicator gray value/the internal reference gray value.

Table 1 Primers for qPCR validation

Abbr.		Primer sequences (5′→3′)	Product size(bp)	Tm(℃)
pck 1	F	CCTGCACATCTGCGATGGAT	133	58.9
	R	TACATGGTGCGCCCTTTCAT		
fatp 6	F	ACCTCAGGAACCACAGGTCT	140	60.0
	R	GCAGCACTGTGATATAGAGGCA		
gck	F	ACAAGCTACACCCCAATTTCCA	136	61.2
	R	TTGCAAGCCACTGCTGAGAT		
hexok 4	F	GAGACCTCCACCAACAGTGG	200	60.4
	R	CGGTCCCCAGAATCACTTTCA		
fas	F	CTCAGTACTGTGCAAGCTGGA	205	60.8
	R	CCCATTGCTAGACAAAACCTCAA		
acc a	F	CGGAGGCTGCTCCTGGAATA	137	61.0
	R	TCCCAGAGGTACGCCTTGAC		
igf 1	F	CTCCTGTGTGTTCTGTGCCT	134	58.9
	R	CCATAGCCTGTTGGTTTGTTGAA		
caspase 9	F	CATGGCACAGAGGTGAACCA	274	59.5
	R	CTTTCCCAAGCTCCTCCCTG		
gadd45β	F	GCGAAGCTGATGAATGTCGAT	150	59.7
	R	GGACACTCGCAGGATGTTGA		
β-actin	F	GCCAGGGCTGTGATTTCTTT	275	57.6
	R	GCTGCTTCCTCTTCCTCCCT		

RNA extraction, cDNA synthesis and qPCR

Total RNA was isolated from the liver using an animal tissue total RNA extraction kit (Fuji, Chengdu, China). Then, the cDNA was synthesized from 1 μg of RNA using the RT Easy™II kit (Fuji, Chengdu, China). The qPCR analysis was performed in Thermo Cycler (BioRad, Hercules, CA, USA) using SYBR Green Real-Time PCR kit (Takara, Kyoto, Japan). The design of primers was based on a local database constructed from RNAseq (Table 1).

The reaction procedure involved an initial cycle at 95 °C for 2 min, followed by 40 cycles of the following steps: 10 s at 95 °C, and a melting temperature for 30 s based on the specific primer pair. After the 40 cycles, the reaction was held at 95 °C for 30 s and 72 °C for 5 s. The relative expression of the target gene was determined using *β-actin* as the internal reference gene. The relative changes in mRNA transcript expression in the qPCR results were calculated using the $2^{-\Delta\Delta CT}$ method.

RNAseq

Total RNA was extracted from the tissue using TRIzol® Reagent according the manufacturer's instructions. The quality of the extracted RNA was determined using the 5300 Bioanalyzer (Agilent) and quantified using the ND-2000 (NanoDrop Technologies). The libraries were size-selected for cDNA target fragments of 300 bp on a 2% Low Range Ultra Agarose gel, followed by PCR amplification using Phusion DNA polymerase (NEB) for 15 PCR cycles. After quantification using the Qubit 4.0, the paired-end RNA-seq sequencing library was sequenced with the NovaSeq 6000 sequencer.

To identify differentially expressed genes (DEGs) between two different samples, the expression level of each transcript were calculated by the transcripts per million reads (TPM) method. Gene abundances were quantified using RSEM [65]. Differential expression analysis was performed using DESeq2 [66]. Differential expressed genes (DEGs) with an absolute log2 fold change (|log2FC|) greater than or equal to 1 and a false discovery rate (FDR) less than 0.05 (DESeq2) or less than 0.001 (DEGseq) were considered as DEGs. Furthermore, we conducted functional enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

Statistic analysis

All data were expressed as mean±standard deviation (SD). Statistical analysis was performed using SPSS 27.0 software (IBM Corp., Armonk, NY, United States), with T-test, p-value of less than 0.05 was considered as statistical significance.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10698-0.

Supplementary Material 1

Author contributions

S.Y and C.Y designed the experiment and wrote the manu script. W.X and D.L designed the experiment. C.Y, X.P, W.S and Z.X carried out the experiment, organized the data, and carried out statistical analyses. C.Y, Z.Z and B.S carried out data visualization. Y.L, J.W, X.H and W.L provided the experimental samples. X.D, Y.L, S.Y and C.Y discussed the manuscript. All authors read and approved the fnal manuscript.

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Data availability

The datasets generated and analyzed during the current study are available in the Sequence Read Archive of National Center for Biotechnology Information database with accession number PRJNA1082803 (http://www.ncbi.nlm.nih.gov/bioproject/1082803).The datasets analyzed during this study are included in this published article and its supplementary information. Please contact Shiyong Yang (yangshiyong@sicau.edu.cn) if someone wants to request the data from this study.

Declarations

Ethics approval and consent to participate

All experimental protocols involved in fishes in this study were conducted in strict accordance with the recommendations in the Guide for the Care and

Use of Laboratory Animals of the Sichuan Agricultural University and ARRIVE guidelines (https://arriveguidelines.org). All procedures and investigations were reviewed and approved by the Animal Research and Ethics Committees of Sichuan Agricultural University and performed in accordance with the guidelines of the committee (Approval No.20190031).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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