RESEARCH ARTICLE

Metabolic and transcriptional disruption of American shad (*Alosa sapidissima*) by enrofloxacin in commercial aquaculture

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Abstract

Antibiotics, including enrofloxacin (ENR), are widely used for bacterial disease treatment in aquaculture in China, resulting in their discharge into various aquatic environments. Therefore, researchers have focused their attention on the harmful effects of antibiotics on aquatic animal metabolism. To understand the impacts of ENR on the metabolism of cultured American shad, the guts of these fish were sampled to test the toxicity of ENR through the combined results of metabolomics and transcriptomics analyses. In this study, adult American shad were exposed to ENR (200 mg/kg) for 30 days. The results showed that ENR could significantly ($p < 0.05$) increase the body weight of American shad. ENR exposure also contributed to the dysregulation of intestinal metabolism (approximately 49 metabolites and 963 genes). Multiomics analyses of the responses indicated that the specific metabolic dysregulation caused by ENR affects many signaling pathways, such as the glycolysis/gluconeogenesis and pentose phosphate pathways, which are known to be linked with body weight gain through protein and lipid accumulation. In conclusion, ENR treatment affected the metabolic system in the gut of American shad. The transcriptomics and metabolomics analysis results provide essential data for evaluating antibiotic hazards in the guts of aquatic organisms.

Keywords Enrofloxacin · American shad · Intestinal metabolism · Metabolic pathways · Body weight

Introduction

Enrofloxacin (ENR) has been detected in the environment, especially in water, sediment, and fish consumed as food, and the concentrations range from 3.49 to 660.13 ng/L, 1.03 to 722.18 μg/kg, and 6.73 to 968.66 μg/kg, respectively

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(Huang et al. 2020). With societal development, ENR and other antibiotics flow into aquatic environments through sewerage system from human activity, poultry production, animal husbandry, hospitals, pharmaceutical industries, and aquaculture, posing enormous threats to the environment and to life (Fiaz et al. 2021; Mahamallik et al. 2015; Qin et al. 2012; Huang et al. 2020). ENR may spread through the entire length of the food chain and ultimately impacts on biological health and human health (Qin et al. 2012; Hu et al. 2014; Huang et al. 2020). In addition, ENR has been associated in some cases of diseases like spinal abnormality, growth inhibition, metabolic disorders, and cell apoptosis in the organism (Wang et al. 2016; Hu et al. 2014; Gomes et al. 2020; Chen et al. 2020; Strzepa et al. 2017). ENR is widely used in commercial aquaculture in China due to its intense antibacterial activity, few side effects, rapid action, and low cross-resistance (Wang et al. 2016). ENR is considered a potential threat to ecosystems and its antibacterial activity does arrange from 0.125 to 512 μg/mL (Park et al. 2018; Hu et al. 2014). In addition to its assumed induction of oxidative stress and developmental toxicity in algae and other organisms (Gomes et al. 2020; Chen et al. 2020), ENR exposure has been found to disrupt early development and metabolic systems in fish (Qiu et al. 2020a),

affect the regulation of the NF-kappa B signaling pathway in fish (Qiu et al. 2020b), and alter reproductive behavior in earthworms (Parente et al. 2021). However, the mechanism of ENR toxicity to organisms is not fully understood.

Diverse organisms and biological systems, including fish (Wang et al. 2009; Rostang et al. 2021), crops (Marques et al. 2021), goats (Quintanilla et al. 2021), *Escherichia coli*, and other organisms (Aprilia et al. 2021; Gomes et al. 2020), have been used to study the toxic effects of ENR. Many recent studies have attracted attention to the biological impacts of ENR exposure on the gut. Elokil et al. (2020) reported persistent significant changes in the abundance of functional gut metabolites in chickens due to exposure to ENR and diclazuril. Significant metabolic shifts were found in the guts of mice in the ENR- and vancomycin-treated groups (Sun et al. 2019). Intestinal metabolism homeostasis plays essential roles in maintaining host health (Fan and Li 2019; Nicholson et al. 2012). In addition, ENR threatens biological health through the food chain via several species of shrimp, bivalves, fish, algae, and crustaceans (Ben et al. 2019). Aquatic organisms are the best choice for toxicity testing in the aquaculture industry (Lam et al. 2011; Nagato et al. 2016; Couch and Hargis 1984).

American shad (*Alosa sapidissima*) is an economically important fish that is widely distributed in the Pacific and Atlantic basins (Nack et al. 2019) and has become a popular and valuable aquaculture species in China since 2003 (Jia et al. 2009). Means for the broodstock culture and reproduction of American shad have already been developed in China, and shad have been sold to restaurants for human consumption. Although many studies have reported the importance of the culture system, temperature, light, and dissolved oxygen content on the growth and gonadal development of American shad (Liu et al. 2021), no investigation has combined both metabolomics and transcriptomics analyses to investigate the correlations between metabolic alterations and gut gene expression in American shad after exposure to antibiotics. Transcriptomics and metabolomics play significant roles in identifying the key signaling pathways and gene regulation networks affecting fish development and phenotypic adverse effects and can help provide a better understanding of the complex process of fish reproduction (Leng et al. 2019). Therefore, it is important to understand the metabolite profiles in the gut of American shad; these profiles can provide useful information to evaluate the health and productivity of this commercially valuable freshwater species.

The goal of this study was to determine the toxic effects of ENR exposure on adult American shad for more than 30 days, particularly the effects on metabolic alterations, using data from transcriptomics and metabolomics analyses. First, the metabolomics approach was used to evaluate the changes at the metabolome level. Second, high-performance RNA sequencing (RNA-Seq) was used to evaluate the mRNA

abundances. Finally, integration of the results at both omics levels was used to characterize relevant molecular biomarkers with high reliability and to identify the regulatory pathways underlying the observed ENR toxicity-induced changes in adult American shad. This research provides a unique perspective on the threat of ENR exposure toxicity on metabolism and gut gene expression in American shad. In addition, this study is the first to explore the metabolic disruption and developmental toxicity of ENR in this species.

Materials and methods

Reagents and chemicals

Formic acid, methanol, water, and acetonitrile were all purchased from CNW Technologies GmbH (Düsseldorf, Germany). ENR was Zhongyi Tech, Ltd. (Wuxi, China). L-2-chlorophenylalanine was purchased from Shanghai Hengchuang Bio-technology Co., Ltd. (Shanghai, China). The chemicals and solvents were analytical or HPLC grade.

Fish culture conditions, treatments, and sample collection

Fish were raised in a commercial recirculating aquaculture system (RAS) and underwent sixth-generation selection in indoor concrete tanks at Suzhou Fishseeds Biotech. Inc., Jiangsu, China (Liu et al. 2021). The system was a specially designed dual-drain RAS equipped with an online infrared dome camera to monitor the growth, maturation, and natural spawning behavior of shad broodstocks. Temperature and dissolved oxygen were measured by a YSI 550A (Y.S.I. Environmental Inc.) every morning. Water quality was monitored by testing ammonia and nitrite levels every 4 days. The recirculating aquaculture system was maintained under a natural photoperiod in the greenhouse, with the sunlight intensity attenuated by a roof cover with 40% light penetration. The daily average light intensity ranged from 800 to 1600 lux. Fish were fed commercial pellets three times a day for 20 min each time during the entire culture period.

Sixty fish (11 months postfertilization) were randomly selected and divided into 6 tanks (10 fish per tank). In the preliminary experiment, we found that a concentration of 200 mg/kg ENR could achieve the most effective cure rate of dermatitis and the lowest mortality rate of American shad (Supplementary material 3). Therefore, three tanks were dosed with ENR at 200 mg/kg, and the remaining 3 tanks were maintained as controls without ENR exposure. ENR was mixed with pellets (200 mg/kg) containing 42% protein in the ENR exposure groups, and fish were fed three times a day for 30 days. The ratio of feed to ENR in the mixtures was approximately 100:1. In addition, fish in the control

groups were fed only commercial pellets three times a day for 30 days.

After 30 days of exposure, thirty fish from the ENR treatment and control groups were sampled to measure their body weight. After weighing, all the fish were washed three times with purified water and euthanized by immersion in ice water. The intestines were dissected and treated with different methods according to different analysis approaches. Four replicates per group condition were used for metabolomics analyses, and three replicates per group condition were used for RNA-Seq analyses.

Sample preparation and analysis

Metabolite extraction

The process of metabolite extraction was performed as described in Lu et al. (2016). An ACQUITY UHPLC system (Waters Corporation, Milford, USA) coupled to an AB SCIEX Triple TOF 5600 System (AB SCIEX, Framingham, MA) was used to analyze the metabolic profiles with electrospray ionization (ESI) in both positive and negative ion modes. An ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm) was employed in both positive and negative ion modes. The binary gradient elution system consisted of (A) water (containing 0.1% formic acid, v/v) and (B) acetonitrile (containing 0.1% formic acid, v/v), and separation was achieved using the following gradient: 0 min, 5% B; 2 min, 20% B; 4 min, 60% B; 11 min, 100% B; 13 min, 100% B; 13.5 min, 5% B; and 14.5 min, 5% B. The flow rate was 0.4 mL/min, and the column temperature was 45 °C.

RNA extraction and RNA-Seq analyses

RNA was extracted from 3 mg of intestinal tissue using a mirVana miRNA Isolation Kit (Ambion). The RNA-Seq process was performed as described in Zhang et al. (2021). RNA integrity was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Samples with an RNA integrity number (RIN) ≥ 7 were used for subsequent analysis. Libraries were constructed using a TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Then, these libraries were sequenced on an Illumina sequencing platform (Illumina HiSeq X Ten), and 125 bp/150 bp paired-end reads were generated.

Data preprocessing and statistical analysis

LC-MS metabolomics data analysis

LC-MS metabolomics analysis was conducted by OE Biotech Co., Ltd. (Shanghai, China). The acquired LC-MS raw data

were analyzed using Progenesis QI software (Waters Corporation, Milford, USA). Metabolites were identified by Progenesis QI (Waters Corporation, Milford, USA) Data processing software was based on public databases such as http://www.hmdb.ca/ and http://www.lipidmaps.org/ and in house-developed databases. The changes in metabolites were analyzed by principal component analysis (PCA) and orthogonal partial least squares discriminate analysis (OPLS-DA). Hotelling's T2 region shows the 95% confidence interval in the model. Variable importance in projection (VIP) shows the contribution rate of each variable in the OPLS-DA model. The differential metabolites were obtained based on the combination of a statistically significant threshold of VIP values selected from the OPLS-DA model and p values from a two-tailed Student's t test in the normalized peak areas. The differential metabolites had VIP > 1 and p values < 0.05.

RNA sequencing data analysis

Transcriptome sequencing and analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China). Raw data (raw reads) were processed using Trimmomatic (Bolger et al. 2014). Reads containing poly-N sequences and low-quality reads were removed to obtain clean reads. Then, the clean reads were mapped to the reference genome using hisat2 (Kim et al. 2015). Transcript-level quantification, analysis of differentially expressed genes (DEGs), cluster analysis, gene ontology (GO), and Kyoto encyclopedia of genes and genomes (KEGG) enrichment were performed with the following considerations. For transcript-level quantification, fragment per kilobase of transcript per million reads mapped (FPKM) and read count values of each transcript (protein-coding) were calculated using bowtie2 and expressed (Kim et al. 2015). DEGs were identified using the DESeq (2012) functions estimate SizeFactors and nbinom Test. A p value < 0.05 and fold change >2 or fold change < 0.5 were set as the thresholds for significantly differential expression. Hierarchical cluster analysis of DEGs was performed to explore transcript expression patterns. GO term enrichment and KEGG pathway enrichment analyses of DEGs were performed using R based on the hypergeometric distribution (Kanehisa et al. 2008).

Integration of LC-MS metabolomics analyses and RNA-Seq analyses

KEGG database (https://www.kegg.jp/) was used to define metabolic pathway and integrated of metabolomics and transcriptomics datasets. The markers list was introduced into the KEGG database to find out metabolic pathways induced by ENR treatment. The specific metabolite is related to a specific gene if they shared one common KEGG pathway.

Results

Body weight

As shown in Fig. 1, the body weight (590.11 ± 33.02 mg) ($p < 0.05$) was significantly increased in the ENR treatment group compared to the control group (573.28 ± 28.01 mg).

Metabolomics alterations

LC-MS analysis was performed to analyze the alterations in the gut metabolic profiles of American shad. Forty-nine metabolites were significantly ($p < 0.05$) changed after ENR exposure (5 metabolites increased and 44 metabolites decreased) (Supplementary figure 1). Most of the metabolites were closely related between the control and ENR exposure groups (Supplementary figure 2). Hierarchical clustering analysis of the peak areas of these metabolites (biomarkers) grouped the samples that presented similar behavior according to the observed metabolite changes (Fig. 2A). The relative changes in the metabolites are represented by the colors in the heatmap (Fig. 2A). Down- and upregulation of metabolite concentrations are shown in blue and red, respectively. Similar patterns of metabolite changes are shown in the hierarchical clustering heat map, and these data are provided by the OPLS-DA score plot, which shows significant separation of metabolic profiles between the ENR treatment group and the control group (Fig. 2B). In the OPLS-DA model, $Q2= 0.683$, $R_2Y = 0.573$, $R_2X = 0.73$, and $R2= 0.998$, suggesting the acceptability of the model. The VIP values and coefficients of the OPLS-DA model were analyzed to determine the effects of metabolites on metabolic alterations (Supplementary material 1). Functional analysis of these results indicated that most metabolites changed in commercial aquaculture were related to primary bile acid biosynthesis. Figure 2C showed that 12 metabolic pathways were identified based on their enrichment in the differential metabolites. Two metabolic pathways were significantly different at $p < 0.05$, while one metabolic pathway was significantly different at $p < 0.01$. The coefficients and p values of the metabolic pathways were analyzed to determine the effects of the metabolites on the metabolic alterations after ENR treatment (Supplementary material 2). The significantly ($p < 0.05$) enriched pathways were selected to construct a bubble diagram. Figure 2D showed that among the altered metabolic pathways, primary bile acid biosynthesis and amino sugar and nucleotide sugar metabolism pathways showed the largest enrichment factors.

Transcriptomics alterations

The changes in mRNA levels between the ENR exposure groups and the control showed 963 significant DEGs ($p < 0.05$), specifically 319 upregulated and 644 downregulated genes. Hierarchical clustering according to differential mRNA expression levels showed two main clusters due to ENR exposure (Fig. 3A). The red cluster contains upregulated genes, whereas the blue cluster contains downregulated genes. As shown in Table 1, the top twenty DEGs were related to different molecular functions, including zinc ion binding, lipid transport, protein kinase activity, GTP binding, and DNA binding. A volcano plot was constructed to reveal the overall distribution of the DEGs (Supplementary figure 3). The distribution comparison diagram of DEGs and all genes by GO enrichment analysis at level 2 is shown in Fig. 3B. The terms "cellular process" and "metabolic process," "cell" and "cell part," and "binding" and "catalytic activity" were dominant in the three major categories. The distribution comparison diagram of up- and downregulated DEGs is shown for GO level 2 analysis (Supplementary figure 4). Among the identified pathways, we focused on the thirty most important pathways involved in lipid metabolism and energy metabolism (Fig. 3C) and some DEGs involved in growth and development. To further functionally characterize the DEGs, pathway analyses were conducted using the KEGG database. The results revealed a diverse range of pathways, with the 963 DEGs between the control and exposure groups assigned to 236 pathways. Among these pathways, the top twenty pathways identified by KEGG enrichment analysis (among pathways with more than two DEGs) were chosen for focused analyses. A bubble diagram was constructed using the -log10 (p value) to visualize the expression patterns of DEGs mainly involved in glycolysis/gluconeogenesis, the pentose phosphate pathway, the calcium signaling pathway, and cardiac muscle contraction (Fig. 3D).

Association between the transcriptomics pathway and metabolomics pathways

In the study, datasets of significantly ($p < 0.05$) changed metabolites and genes were calculated using the metabolic
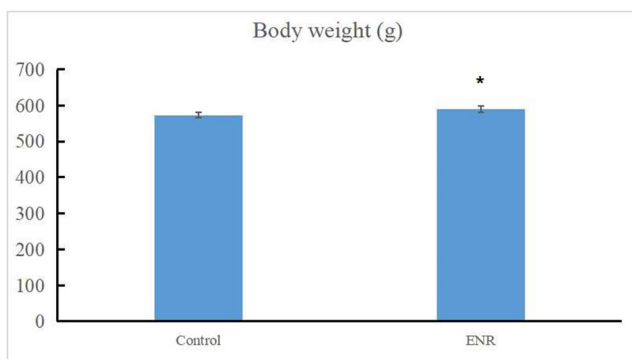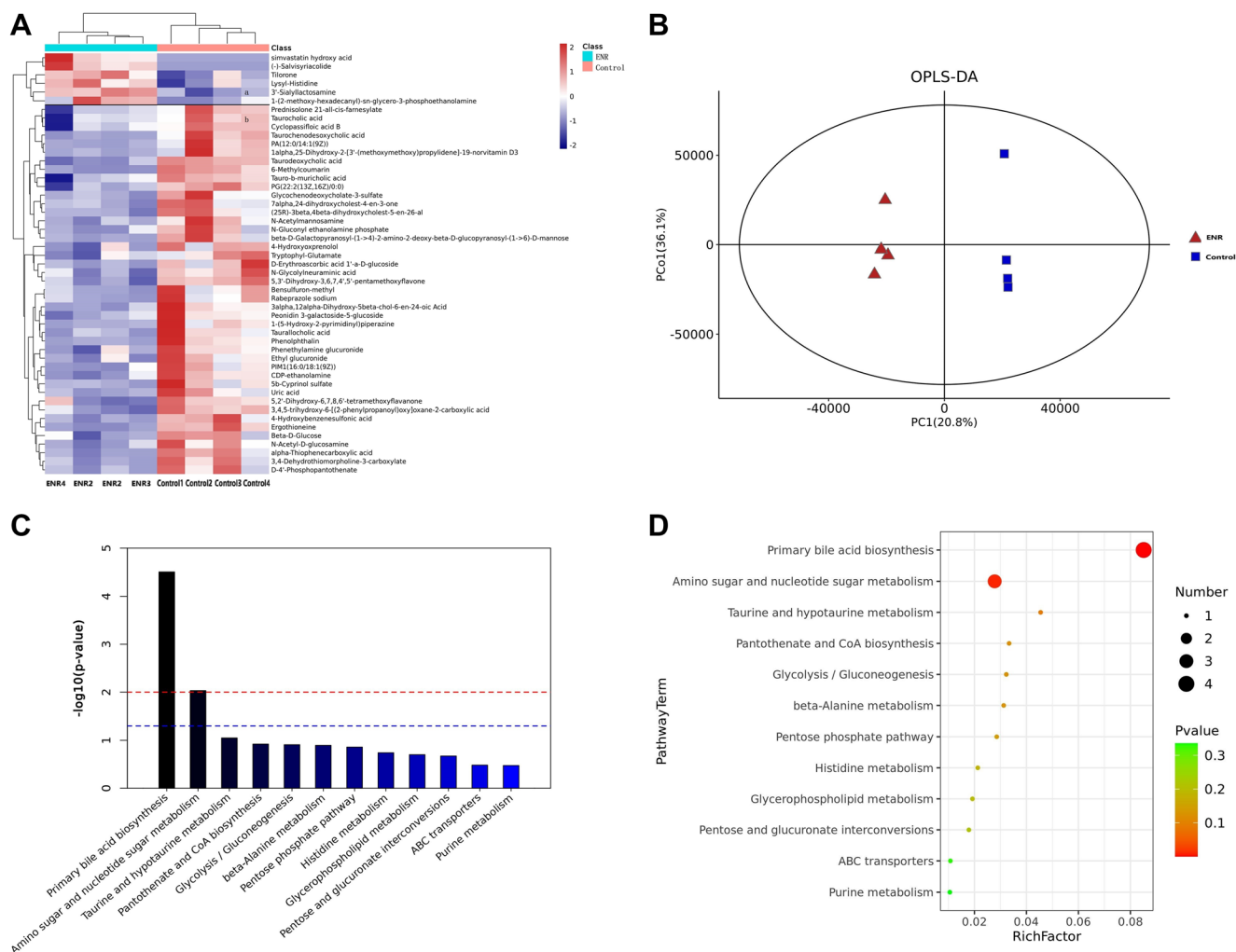

Fig. 1 Body weight in ENR-treated group and the control group. Asterisk denotes a significant difference between ENR-treated group and the control group ($p < 0.05$)

Fig. 2 (A) Heatmap of the statistically significant tentatively identified metabolites whose concentrations changed between the control and ENR-treated groups, (a) downregulated metabolites and (b) upregulated metabolites. (B) OPLS-DA score plot shows a clear separation of metabolic profiles between the control and ENR-treated groups. (C) Top-20 metabolic pathways enrichment map (the red line indicates p =0.01, and the blue line indicates p=0.05). (D) Bubble diagram of metabolic pathways, the ordinate indicates the name of metabolic pathways and the abscissa indicates the enrichment factors (rich factor, rich factor=significantly the different number of metabolites/total number of metabolites in the pathway)

pathways contained in the KEGG database. ENR exposure induced significant alterations in metabolism and transcription in the gut of American shad. To investigate potential associations between metabolites and transcriptomics pathways, Venn diagrams (Fig. 4A) were generated by identifying the common KEGG pathways. Eight of the common pathways shared three genes (As06G007597, As05G006999, and As08G010701), two metabolites (C00221, beta-D-glucose; C00140, N-acetyl-D-glucosamine), and two metabolite pathways (pentose phosphate pathway, ko00030; glycolysis/gluconeogenesis, ko00010). The total metabolite levels (beta-D-Glucose and N-acetyl-D-glucosamine) decreased upon ENR exposure, and the three genes (detailed in Table 2) related to these metabolic pathways were downregulated. Based on the results of the association analysis of differential genes

and differential metabolites, an association network map was constructed, as shown in Fig. 4B. The physiological meaning of the observed changes in the levels of the different biomarkers can be inferred by detecting which genes or metabolites increased (red symbols) or decreased (blue symbols) upon treatment with ENR (Fig. 4B). The total beta-D-glucose level decreased after ENR exposure, while 7 genes (As13G016970, As06G007597, As11G013909, As21G024098, As05G006999, As08G010701, and As15G018212) related to the glycolysis/gluconeogenesis pathway appeared to be downregulated (detailed in Table 2 and Fig. 4C). In addition, the total beta-D-glucose level decreased after ENR exposure, while 4 genes (As06G007597, As09G011968, As05G006999, and As08G010701) related to the pentose phosphate pathway appeared to be downregulated
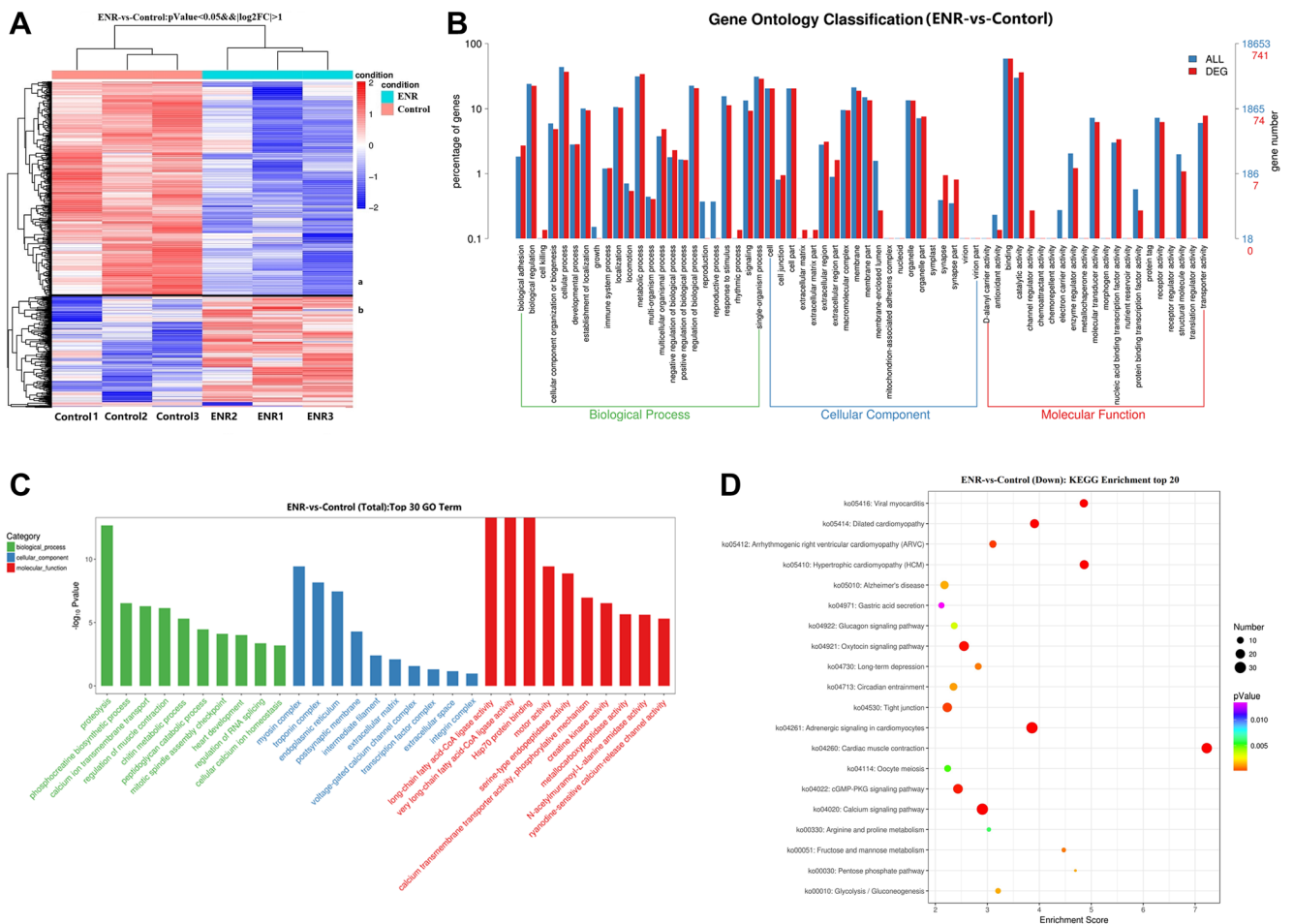
Fig. 3 (A) Heatmap of the DEGs after ENR exposure; a represents downregulation and b represents upregulation. (B) The distribution comparison diagram of DEGs and all genes by GO enrichment analysis at level 2. (C) GO enrichment analysis: the horizontal axis represents Go entry name and the vertical axis represents -log10(p value). (D) Bubble diagram of KEGG enrichment analysis TOP-20 of DEGs

(detailed in Table 2 and Fig. 4D). It is widely known that beta-D-glucose levels affect the growth and development of organisms, but the beta-D-glucose-disrupting effect of ENR remains to be studied in the future.

Discussion

This study investigated the toxicity of ENR in the gut of American shad. Based on our analysis, numerous genes and metabolites were significantly ($p < 0.05$) differently expressed between the ENR treatment groups and the control, in agreement with other related studies. For example, ENR can completely alter the intestinal microbiota and metabolism of chickens (Ma et al. 2020). Eight metabolites changed in fecal extracts of mice treated with ENR; these changes included depletion of amino acids produced by microbial proteases, a reduction in metabolites generated by lactate-utilizing bacteria, and an increase in urea caused by

loss of microbial ureases (Romick-Rosendale et al. 2009). Statistical assessment of the changes in the mRNA expression levels between the control and ENR groups revealed 963 DEGs ($p < 0.05$). Previous studies have shown that general changes in gene expression can be observed upon organism exposure to ENR. For instance, hub gene expression changes revealed that the metabolic system of zebrafish was seriously damaged by ENR exposure (Qiu et al. 2020c). Functional analysis showed that most metabolites affected by ENR were related to amino acid or glucose metabolic pathways. Relevant changes have already been reported in grass carp (*Ctenopharyngodon idellus*). For example, most of these metabolic alterations were related to amino acid synthesis and glucose, which are major components of cells and might be related to the cytotoxicity of ENR exposure (Liu et al. 2015). These changes are likely related to the changes in the expression levels of genes in the same metabolic pathways, but the direct correlations need to be proven by further research.

Table 1 Molecular functions of the top twenty DEGs after ENR exposure

Gene_id	Gene name	Gene symbol	Base Mean	p value	Fold change	Molecular function
Upregulated						
As21G024511	FCGBP	IgGFc-binding protein	7880.320028	0.021985184	1251.849108	Protein binding
As21G024508	Tecta	Alpha-tectorin	34024.59571	0.000317509	967.3511664	Protein binding
As08G009942	Nkx6-3	Homeobox protein	58.28885297	0.018327465	219.5726367	DNA binding
As21G024494	FCGBP	IgGFc-binding protein	5012.822607	0.004062294	186.9906406	Unknown
As01G001271	Unknown	Unknown	78.61605319	0.038339605	114.6224449	Unknown
As04G004894	Rnh1	Ribonuclease inhibitor	14.31367082	1.77E-08	107.3296018	Protein binding
As05G006239	Unknown	Unknown	94.62846591	0.009371775	75.37813908	Unknown
As21G024507	Ecm1	Extracellular matrix protein 1	155.3674234	0.001897908	70.67113383	Extracellular region | Extracellular space | signal transduction
As21G024269	Stmnd1	Stathmin domain-containing protein 1	8.297522136	3.73E-05	61.79781614	Regulation of microtubule polymerization or depolymerization
As11G014010	Unknown	Unknown	20.55080255	0.028517763	49.54872072	Transcription corepressor activity
Downregulated						
As10G012998	MATK	Megakaryocyte-associated tyrosine-protein kinase	73.05528577	0.028763713	0.499944219	Protein kinase activity
As21G024008	hdac9b	Histone deacetylase 9-B	44.1922097	0.049588204	0.499681407	Unknown
As19G022339	PRICKLE1	Prickle-like protein 1	91.14458867	0.019761656	0.499404125	Zinc ion binding
As14G017288	e2f8	Transcription factor E2F8	58.68985066	0.037442033	0.496821604	DNA-binding transcription factor activity
As03G004012	APOB	Apolipoprotein B-100	44.71129972	0.044405718	0.496596181	Lipid transporter activity | lipid transport
As21G023960	SYNC	Syncoilin	896.3416007	0.008240696	0.495206594	Intermediate filament
As10G013027	Unknown	Ras-related protein Rab-11B	64.09653444	0.027646747	0.494737588	GTPase activity | GTP binding
As17G020322	SLITRK3	SLIT and NTRK-like protein 3	158.210437	0.009284182	0.494524263	Protein binding
As09G011290	Unknown	Unknown	126.3699996	0.010631267	0.493682127	Unknown
As15G018433	NT5C2	Cytosolic purine 5'-nucleotidase	201.6811092	0.044924264	0.492071888	Unknown

The data from the metabolomics and transcriptomics analyses revealed a defined metabolic disruption in the gut of American shad after exposure to ENR. Integration of the data at the pathway level showed the changed metabolic routes in the gut of American shad after ENR treatment. This work revealed significant changes in some pathways, with possible markers of intestinal metabolic disorders, induced by ENR. These results are in agreement with some studies suggesting the pleiotropic toxic effect of ENR and ENR-related antibiotics, affecting beta-alanine metabolism and the alanine, aspartate, and glutamate metabolism pathways; apoptosis and oxidative stress pathways; steroid biosynthesis pathways; the NF-kB pathway; and apoptotic signaling pathways (Qiu et al. 2020a; Sun et al. 2019; Guo et al. 2020; Qiu et al. 2020b). Several ENR-affected signaling pathways (amino acid, glucose, and lipid pathways) were also detected by either single analysis or integrated analyses. These metabolic pathways have significant enrichment responses that are necessary for antibiotic biodegradation in organisms. These results also suggest that perturbation of beta-D-glucose and N-acetyl-D-glucosamine synthesis can affect several downstream glycolysis- and gluconeogenesis-, metabolic-, amino sugar-, and nucleotide sugar metabolism-dependent pathways. These results are similar to those of Su et al. (2021), who found that gluconeogenesis and glycolysis were important metabolic pathways related to glucose. The potential effect on the glycolysis/gluconeogenesis pathways is likely related to the step of blocking the effects of antibiotics, which could be related to dietary carbohydrate intake (Li et al. 2016). We speculated that the observed alterations in glycolysis/gluconeogenesis pathways might be related to the observed body weight gain. Mechanisms explaining the link between disorders of glucose metabolism and body weight gain have been suggested previously. For example, Su et al. (2021) found that reduced glycogenolysis with downregulation of the transcription of phosphorylase kinase regulatory subunit alpha and glycogen phosphorylase caused accumulation of glycogen and induced lipogenesis in Chinese longsnout catfish. The processes of digestion, glycolysis, gluconeogenesis, glycogen metabolism, and glucose-induced lipogenesis occurred in sequence in organisms upon the utilization of dietary carbohydrates. Kamalam et al. (2017) investigated the
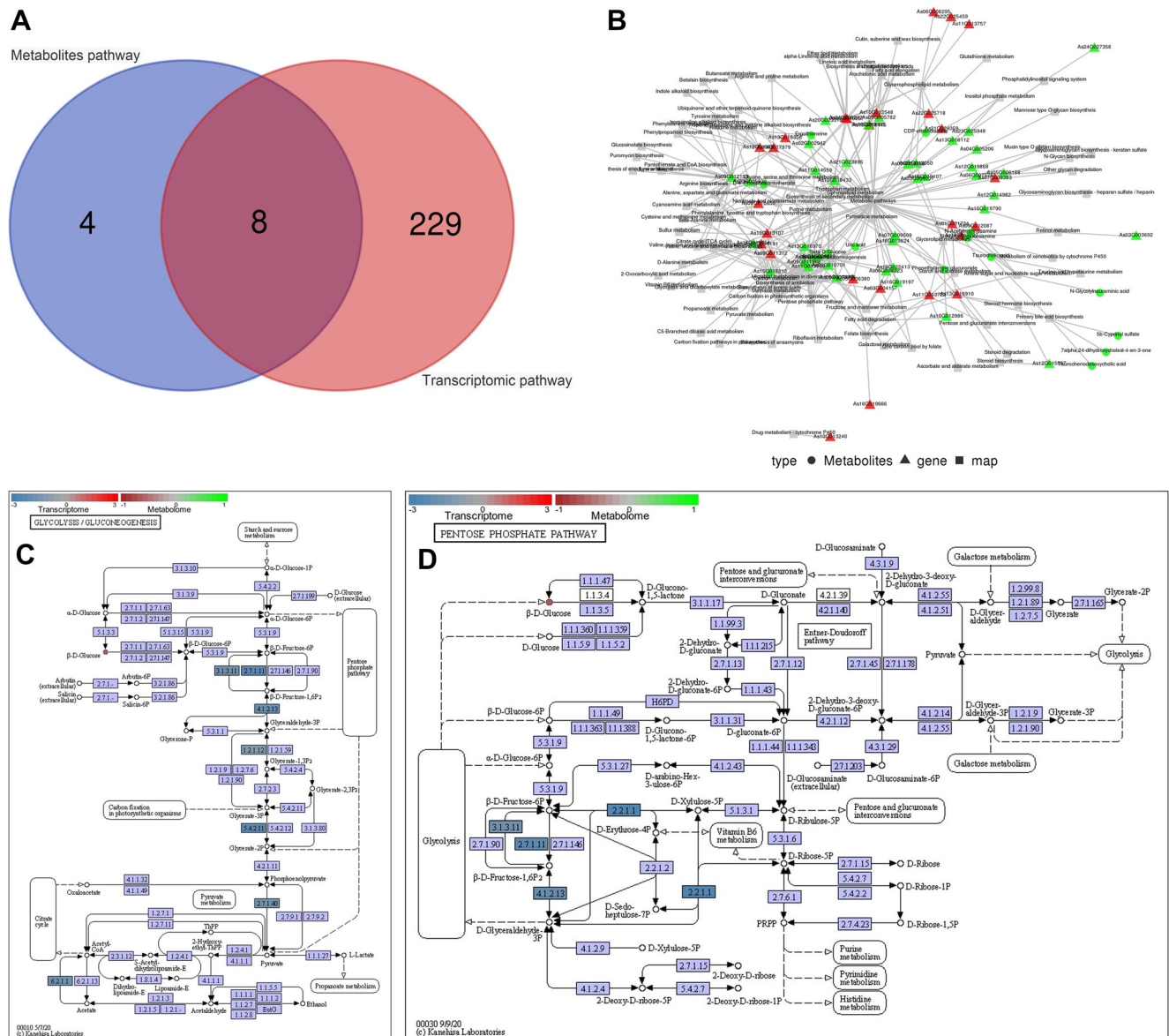
Fig. 4 (A) Venn diagrams of the common KEGG pathways (blue represents metabolites pathways and red represents transcriptomics pathways). (B) The association network map of metabolites (represented by their KEGG C-codes) and genes (represented by their gene ID) in ENR-treated group. (C) glycolysis/gluconeogenesis pathway. (D) pentose phosphate pathway. Increased (red) or decreased (blue) metabolite concentrations are represented by dots, and enzymes/genes are represented by boxes

utilization of dietary carbohydrates in farmed fishes and found that glucose-induced lipogenesis and glycogen metabolism are two important pathways related to glucose metabolism. Carbohydrates are an important source of energy in the growth and development of fish. The ability to utilize dietary carbohydrates determines the nutritional profile and quality of fish meat. Therefore, it is necessary to understand the regulation of glycogen metabolism by dietary carbohydrates in fish. In the end, we also found some unexpected pathways, such as ATP-binding cassette (ABC) transporter metabolism, glycerophospholipid metabolism, and purine metabolism; these data highlight the complexity of ENR ecotoxicity. We

speculated that ENR exposure would affect the metabolic system and the development of American shad through the interaction of hub genes with other genes, which might then play an essential role in linking the gluconeogenesis and glycolysis pathways to other signaling pathways.

The results of this study also showed how LC-MS metabolomics analysis and RNA sequencing analysis results can be coupled to analyze the hazards of compounds and to identify the related pathways. The metabolic processes in the gut of American shad were affected by ENR treatment through interactions of glycolysis/gluconeogenesis and pentose phosphate pathways with other pathways. For instance, changes

Table 2 Molecular functions of the common DEGs

Gene_id	Gene name	Gene symbol	Regulation	Molecular function
As06G007597	PFKM	ATP-dependent 6-phosphofructokinase	Downregulated	6-Phosphofructokinase activity
As05G006999	Unknown	Fructose-bisphosphate aldolase A	Downregulated	Catalytic activity
As08G010701	FBP2	Fructose-1,6-bisphosphatase isozyme 2 O	Downregulated	Carbohydrate metabolic process
As13G016970	PGAM2	Phosphoglycerate mutase 2	Downregulated	Catalytic activity
As06G007597	PFKM	ATP-dependent 6-phosphofructokinase	Downregulated	6-Phosphofructokinase activity
As11G013909	pkm	Pyruvate kinase	Downregulated	Magnesium ion binding
As21G024098	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Downregulated	Glucose metabolic process
As05G006999	Unknown	Fructose-bisphosphate aldolase	Downregulated	Catalytic activity
As15G018212	Acss1	Acetyl-coenzyme A synthetase 2-like	Downregulated	Catalytic activity
As08G010701	FBP2	Fructose-1,6-bisphosphatase isozyme 2	Downregulated	Carbohydrate metabolic process
As09G011968	TKT	Transketolase	Downregulated	Catalytic activity

in the glycolysis/gluconeogenesis pathway could predict changes in the metabolite beta-D-glucose. Pathway-based integration of LC-MS metabolomics analysis and RNA sequencing analysis data revealed the correlations between pathway behaviors. Previous studies demonstrated that changes in gene transcription in the gut played key roles in regulating metabolism (Peng et al. 2021). Although linear relationships between gene transcription and metabolic disorders have been found in fish (Peng et al. 2021), data on the effects of altered gene expression on glycogen metabolism are still relatively scarce. Further studies should be performed to correlate the gene expression and metabolite beta-D-glucose changes related to ENR exposure. In addition, some researchers found that ENR could induce microbial dysbiosis in the guts of chickens, pigs, and mice (Li et al. 2017; Strzepa et al. 2017; De Smet et al. 2020). The composition of the gut microbiota is closely related to changes in metabolites in fish (Karisson et al. 2013). The toxicity against the gut microbiota and the development of metabolic disorders after exposure of American shad to ENR also need to be determined in the future.

Conclusions

This study highlighted the impact of the commonly consumed antibiotic ENR on metabolism in the gut of American shad. These results are the first to prove that ENR affects glycolysis/gluconeogenesis and the pentose phosphate pathway, which indirectly affect nutrient absorption and meat quality, in the gut of American shad. In addition, we speculated that glycolysis/gluconeogenesis and the pentose phosphate pathway could be linked with body weight in American shad.

However, the ecotoxicity of antibiotics in the aquaculture industry is not fully understood and deserves further study. Furthermore, the effects of toxicity and weight gain after antibiotic exposure on the behavior of aquatic animals, such as predation, evasive behavior, and swimming, should be investigated in the future.

Declarations

Ethics approval   Not applicable.

Consent to participate   Not applicable.

Consent of publication   Not applicable.

Competing interests   The authors declare no competing interests.

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