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RESEARCH PAPER

Regulatory mechanism of feed conversion efficiency in largemouth bass (*Micropterus salmoides*): Insights from an integrated analysis of whole-genome resequencing, transcriptomics, and metabolomics

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Abstract

Improving feed efficiency is crucial for the sustainable development of aquaculture. In this study, the feed conversion efficiency (FCE) of 240 largemouth bass (*Micropterus salmoides*) was individually assessed after 60-day independent culture. 14 individuals with the highest and the lowest FCE were defined as the High- and Low-FCE groups, respectively. The differences between the two groups in growth, feed utilization, whole genome, transcriptome, and metabolome were analyzed. The High-FCE group exhibited significantly better weight gain, specific growth rate, and protein efficiency ratio, while no significant difference was found in feeding rate, indicating that the growth advantage may originate from superior FCE. Genome-wide association analysis identified four significant SNPs and five candidate genes associated with FCE. Transcriptome profiling revealed that genes related to digestion, absorption, and immunity were significantly up-regulated, while genes related to energy-consuming biosynthesis and processing were significantly suppressed in the High-FCE group. Metabolome analysis indicated that the purine metabolites were accumulated and purine metabolic pathway was activated in the High-FCE group, suggesting optimized energy metabolism. The increase in adenine and glucuronic acid content in liver may be beneficial to the improvement of FCE. Furthermore, common differential genes and metabolites identified in the liver and brain were involved in regulating energy metabolism, fat metabolism, immunity, and signal transduction, indicating the existence of a liver–brain synergistic regulatory network for FCE. In conclusion, high feed efficiency in largemouth bass resulted from the synergistic integration of enhanced nutrient digestion and absorption, optimized energy metabolism, improved immune capacity, and efficient signal transduction.

Keywords *Micropterus salmoides* · Feed conversion efficiency · Regulatory mechanism · Transcriptome · Metabolome

Introduction

Feed cost typically accounts for over 50% of the total production cost, representing the largest expense in aquaculture (Vassiliou et al. 2015). In recent years, with the continuous increase in the price of feed ingredients, especially fishmeal and fish oil, feed prices have been climbing year by year, leading to declining profitability for aquaculture enterprises (Nunes et al. 2022). Under such a background, feed efficiency (FE), as a core economic trait, has become increasingly important in aquaculture breeding and production (de Verdal et al. 2018a; Xu et al. 2025). Improving FE means significantly reducing feed input and emissions of nutrients, such as nitrogen and phosphorus, under the same output, thereby effectively lowering production costs, enhancing the utilization efficiency of nutrients, and alleviating

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environmental pressure (Liu et al. 2023; Silverstein 2006). Therefore, exploring the regulatory mechanism of FE in fish is a key scientific issue for promoting the efficient, healthy, and sustainable development of the aquaculture industry.

FE is a highly complex comprehensive trait regulated by multiple genes, often evaluated by feed conversion efficiency (FCE)—the ratio of weight gain to feed intake (FI) (Doupé and Lymbery 2004). The formation of FE involves multiple levels of animal physiological processes: from feeding, digestion, and absorption, to metabolism of nutrients, energy allocation and storage, as well as waste excretion (Cantalapiedra-Hijar et al. 2018; Hancz 2020). This series of processes is co-regulated by genetic factors and physiological metabolic states (de Verdal et al. 2018a; Mota et al. 2022). Genetic background determines the metabolic capacity and potential of the organism, while metabolic state reflects the actual conversion efficiency of nutrients under specific physiological conditions. Therefore, the variation in FE is essentially the result of the expression of genetic potential under specific physiological and metabolic conditions, and understanding its regulatory mechanism must integrate perspectives from genetics and metabolic physiology (Cantalapiedra-Hijar et al. 2018).

Generally, genetic factors are considered the main causes of differences in FE between individuals (de Verdal et al. 2018a; Liu et al. 2023). Previous studies have shown that FE exhibited moderate-to-high heritability, implying great potential for breeding new varieties of aquatic animals with high FE through genetic improvement (de Verdal et al. 2018b; Wang et al. 2025; Zhou et al. 2019a). With the advancement of molecular biology technologies, Genome-Wide Association Study (GWAS) has become a powerful tool for exploring the genetic basis of complex traits (Barría et al. 2021; Liu et al. 2023). GWAS scans a large number of single-nucleotide polymorphisms (SNPs) across the genome in a population to identify genetic loci significantly associated with target phenotypes, thereby locating quantitative trait loci (QTLs), which can be incorporated into aquaculture breeding programs and applied through marker-assisted selection (MAS) (Yanez et al. 2023). To date, researchers have conducted GWAS studies on FE in several aquatic species. For example, in turbot (*Scophthalmus maximus*), two significant SNPs and four candidate genes were identified to be correlated with feed conversion ratio (FCR) (Liu et al. 2023). In Pacific abalone (*Haliotis discus hannai*), *apol3* was identified as being associated with FE by combining the results of GWAS and comparative transcriptomic analyses (Yu et al. 2022).

Among the various organs involved in regulating FE in fish, both the intestine and the liver play a very important regulatory role (Fu et al. 2026; Moraes and de Almeida 2020). The intestine has the largest contact area between the fish and the external environment, which is an important place for digestion and absorption of nutrients (Moraes and de Almeida 2020). As the largest metabolic organ in fish, the liver governs crucial physiological processes, such as glycogenesis and glycogenolysis, fatty acid synthesis and β-oxidation, amino acid deamination and transamination, urea/uric acid cycle, and detoxification of exogenous substances (Bruslé and i Anadon 1996). In addition, the liver also senses and responds to energy status and hormone signals, maintaining energy and nutrient homeostasis by regulating the metabolic pathways (Tseng and Hwang 2008). Consequently, the differences in liver functional status directly impact the efficiency of nutrient utilization and energy allocation strategies, serving as a key physiological basis for individual variation in FE (Ling et al. 2023; Wang et al. 2025).

In recent years, multi-omics approaches have been widely applied to investigate the regulatory mechanisms underlying target traits in aquatic animals, including FE (Jin et al. 2025; Ling et al. 2023). Integrated analysis of transcriptomic and metabolomic data enables the construction of association networks linking gene expression, metabolites, and phenotypes, significantly deepening our understanding of the molecular mechanisms underlying complex traits (Liu et al. 2022). In the oriental river prawn (*Macrobrachium nipponense*), a combined transcriptomic and metabolomic analysis of the hepatopancreas and muscle revealed that the differences in lipid metabolism, amino acid metabolism, and immune response might lead to changes in FE (Ling et al. 2023). In the large yellow croaker (*Larimichthys crocea*), combined transcriptome and metabolome analysis showed that high-FE individuals exhibited enhanced digestion, efficient nutrient absorption, and stable liver function (Jin et al. 2025).

The largemouth bass (*Micropterus salmoides*) has developed into a large-scale aquaculture industry in China and many other countries due to its fast growth, delicious meat, short culture cycle, and high market value (Bai and Li 2018). According to China Fishery Statistical Yearbook, the aquaculture production of largemouth bass reached 938,509 tons in 2024. However, similar to most farmed fish species, the largemouth bass aquaculture industry is also significantly challenged by high feed costs (Liu et al. 2025). Therefore, this study aims to investigate the regulatory mechanism of FE in largemouth bass by performing whole-genome resequencing, and liver transcriptomic and metabolomic analyses on individuals with different FCE levels. This study will identify FCE-associated SNPs, differentially expressed genes (DEGs), differential metabolites (DMs), and related pathways through integrated multi-omics analyses. These findings will not only help comprehensively clarify the FCE regulatory network but also provide a scientific basis for the selective breeding of high-FCE trait and the development of precise nutritional regulation strategies in largemouth bass.

Materials and methods

Experimental animals

500 juvenile largemouth bass ("Youlu No. 3") were sourced from Guangdong Liangshi Aquatic Seed Industry Co., Ltd. For the cultural experiment, 240 healthy and uniformly sized individuals were chosen and subjected to a 14-day acclimation in an aquaculture system which can culture fish individually, following the design of Shi et al. (2024). During acclimation, they were fed a commercial diet (Huifu Largemouth Bass Extruded Compound Feed). The nutritional components of the feed were measured according to the method described in Jia et al. (2022). The actual nutritional levels and amino acid contents of the experiment feed are shown in Supplementary Tables S1 and S2.

Experimental procedure

Following acclimation, 240 individuals were subjected to a 60-day feeding trial. Before the experiments, all experimental fishes were weighed individually after anesthetization, yielding a mean initial body weight (IBW) of 23.91 ± 0.42 g. Then, each fish was transferred to its corresponding aquaculture tank (25 cm high, 12 L) for individual cultivation. During the experiment, the FI of each fish was recorded. At the end of the experiment, the final body weight (FBW) of the fish was measured, and weight gain rate (WGR), specific growth rate (SGR), feeding rate (FR), and protein efficiency rate (PER) were calculated. All experimental procedures for the culture experiment were conducted as described in Shi et al. (2024). During the experiment, the water parameters were as follows: temperature 24–28 ℃; dissolved oxygen ≥ 5.0 mg/L; pH 7.6–8.1; total ammonia-nitrogen < 0.1 mg/L.

The calculation formulas for the relevant indicators were as follows:

$$\mathrm{WGR}\ (\%) = 100 \times (\mathrm{FBW} - \mathrm{IBW})/\mathrm{IBW};$$

$$\mathrm{SGR}\ (\%\ \mathrm{d}^{-1}) = 100 \times (\ln(\mathrm{FBW}) - \ln(\mathrm{IBW}))/\mathrm{days};$$

$$\mathrm{FR}\ (\%\ \mathrm{BW}\ \mathrm{d}^{-1}) = 100 \times \mathrm{FI} / (\mathrm{days} \times (\mathrm{IBW} + \mathrm{FBW})/2);$$

$$\mathrm{FCE} = (\mathrm{FBW} - \mathrm{IBW})/\mathrm{FI};$$

$$\mathrm{PER} = (\mathrm{FBW} - \mathrm{IBW})/(\mathrm{FI} \times \text{crude protein content of feed}).$$

Sampling

All experimental individuals were ranked in descending order based on their FCE values. The top 14 and the bottom 14 individuals were ranked as the High and Low-FCE groups, respectively. Fishes from each group were anesthetized using 100 mg/L MS-222 and subsequently dissected on the ice. Dorsal muscle tissues of all 14 individuals in each group were collected and preserved in 95% ethanol for whole-genome resequencing. Six fishes with the highest FCE in the High-FCE group and six fish with the lowest FCE in Low-FCE group were sampled to obtain liver tissues, with each liver sample divided into two portions: one for transcriptome analysis and the other for metabolome analysis. The liver samples of two fishes in each group were mixed and set as a biological replicate, and each group has three replicates. The portion intended for transcriptome analysis was immediately stabilized in RNA Keeper and then frozen at − 80 °C for later RNA extraction and sequencing. The portion designated for metabolome analysis was immediately flash-frozen in liquid nitrogen and then stored at − 80 °C for the determination of metabolites.

Whole-genome resequencing and SNP calling

DNA was extracted from the muscle tissue and assessed for integrity and concentration using 1% agarose gel electrophoresis and NanoDrop® 2000 spectrophotometer, respectively. Then, the quality-controlled DNA was used to construct the DNA library after some steps: DNA fragmentation, end-repair, dA-tailing, ligation of indexed sequencing adapters, and PCR amplification. Library construction and sequencing were performed by Beijing Compass Agritechnology Co., Ltd. Clean data were obtained by filtering the raw data. The clean reads were aligned to the genome of largemouth bass (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_014851395.1/) using BWA software, and sequencing depth and genome coverage were calculated for each sample. SNP variants were called using GATK software and subjected to strict filtering. SNPs obtained from different samples were summarized, and the final high-quality SNPs were used for subsequent GWAS analysis.

Genome-wide association analysis and candidate gene identification

GWAS was performed using PLINK (v1.09) software (https://www.cog-genomics.org/plink2/) with a Mixed Linear Model (MLM) (Omeka et al. 2022). The Manhattan plot of the association data was created by self-developed R program. The genome-wide significance threshold in this study was defined according to Bonferroni correction method (1/N, N represented the number of SNPs used for GWAS analysis), where the SNPs with a *P*-value less than 2.74×10^{-5} (1/36,480) were considered to be significantly associated with the target trait (Bland and Altman 1995; Zhou et al. 2019b). The threshold in the Manhattan plot was set as 4.56, which was obtained by $-\log_{10}(1/N)$. The SNPs were mapped to the reference genome, and the genes near the SNPs were annotated using the Annovar software (http://annovar.openbioinformatics.org/en/latest/user-guide/downl

oad/). The genes annotated with significant SNPs were identified as candidate genes.

Transcriptomic sequencing and analysis

Total RNA was extracted using Trizol method. RNA concentration was determined using a NanoDrop® 2000 spectrophotometer. RNA integrity was evaluated by agarose gel electrophoresis. The RNA of two fishes was mixed in equal amounts to form a single sample, with three biological replicates used for transcriptomic sequencing and analysis. The subsequent cDNA library preparation and transcriptome sequencing were conducted by Nanjing Genepioneer Biotechnologies Co., Ltd. Sequencing was performed using the PE150 method on the DNBSEQ-T7 RS platform (MGI, Shenzhen, China).

Bioinformatic analysis of the transcriptome data was performed according to the method described in Shi et al. (2025). Briefly, Fastp software (V0.20.0) was used for quality control of the raw data. The clean reads were aligned to the reference genome using HISAT2 software (V2.1.0). Transcript assembly, structural annotation, and gene expression quantification were performed using StringTie software. DEGs were identified using the DESeq2 software with the threshold of $|\text{Log}_2 \text{ Fold Change}| \geq 1$ and a false discovery rate (FDR) ≤ 0.05. Functional annotation of all genes was performed by aligning them against public databases (GO, KEGG, COG, KOG, NR, Swiss-Prot, Pfam) with BLAST-ALL (v2.2.26). Subsequently, significant enrichment of GO terms and KEGG pathways among the DEGs was analyzed using the clusterProfiler R package, with an adjusted P-value < 0.05 defining statistical significance.

RNA-seq data validation

To validate the RNA-seq results, ten DEGs were analyzed by RT-qPCR following the reaction system and conditions described in our previous study (Shi et al. 2025). Primer sequences were provided in Supplementary Table S3. Relative expression levels were quantified via the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001), with *eef1a1* and *18S rRNA* serving as internal reference genes.

Metabolomic analysis

Untargeted metabolomic analysis of liver tissue was performed using a liquid chromatography-tandem mass spectrometry (LC–MS/MS), following the procedure described in our previous study (Shi et al. 2025). Briefly, the liver samples of six samples were extracted and homogenized to obtain supernatants for LC–MS/MS analysis. Metabolites were separated using an ultra-high-performance liquid chromatography equipped with an Acquity UPLC BEH Amide column. Spectra data were acquired using an Orbitrap Exploris 120 mass spectrometer controlled by the acquisition software (Xcalibur, V4.4, Thermo Fisher Scientific). Raw data were filtered using a perl program. Data normalization, visualization, and multivariate statistical analysis were performed using SIMCA software (V14.1). Metabolite annotation was conducted against the HMDB and KEGG COMPOUND databases. Variable Importance for the Projection (VIP) values were obtained through the orthogonal projections to latent structures-discriminant analysis (OPLS-DA). A perl program was used to perform Student's t test and fold change (FC) analysis. Those metabolites with VIP > 1 and $P < 0.05$ were deemed statistically significant. Pathway enrichment analysis was carried out based on the KEGG PATHWAY database.

Integrated transcriptomic and metabolomic analysis

Integrated transcriptomic–metabolomic analysis followed Shi et al. (2025). The common KEGG pathways were identified using the top 50 DEGs (lowest FDR) and top 50 DMs (highest VIP). The regulatory networks were built and visualized using Cytoscape (v3.7.2), based on a Spearman's correlation coefficient > 0.9 and P-value < 0.001.

Statistical analysis

Statistical analysis was performed using SPSS 21.0 for Windows. Significant differences between the two groups were assessed using Student's t test. Differences were considered statistically significant at $P < 0.05$ and highly significant at $P < 0.01$.

Results

Growth and feed utilization

As shown in Table 1, compared with the Low-FCE group, the High-FCE group showed better growth performance with significantly higher WGR and SGR ($P < 0.01$). Specifically, the WGR of the High-FCE group was 2.23 times that of the Low-FCE group. For feed utilization, the High-FCE group also exhibited significantly improved FCE and PER ($P < 0.01$). The FCE of the High-FCE group was 1.49 times that of the Low-FCE group. However, no significant difference in FR was observed between the two groups ($P > 0.05$).

Whole-genome resequencing analysis

Sequencing data

Whole-genome resequencing of the 28 individuals generated 368.08 Gb raw bases and 2453.97 Mb raw reads, averaging

Table 1 Growth and feed utilization of the Low- and High-FCE groups

Group	WGR (%)	SGR (% d^{-1})	FR (% BW d^{-1})	FCE	PER
Low-FCE	110.45 ± 16.37	1.24 ± 0.13	1.35 ± 0.12	0.87 ± 0.05	1.75 ± 0.09
High-FCE	246.21 ± 34.08**	2.06 ± 0.17**	1.41 ± 0.08	1.30 ± 0.02**	2.59 ± 0.05**

Values are mean of three replicate groups and presented as means ± SD. Values with asterisks mean significant differences between two groups (**$P < 0.01$)

WGR weight gain rate, *SGR* specific growth rate, *FR* feeding rate, *FCE* feed conversion efficiency, *PER* protein efficiency ratio

13.15 Gb raw bases and 87.64 Mb raw reads per individual (Supplementary Table S4). Following quality control, 362.88 Gb clean bases and 2,432.04 Mb clean reads were obtained, averaging 12.96 Gb of clean bases and 86.86 Mb clean reads per individual. The average GC content, Q20, Q30, and mapped ratio were 41.56%, 98.83%, 96.13%, and 99.90%, respectively (Supplementary Table S4).

GWAS analysis

Following sequence alignment, a total of 30,129,778 SNPs were obtained, averaging 1,076,064 SNPs per individual. Among all SNPs, those with heterozygosity were the most abundant (Supplementary Table S5). Regarding the genomic locations of the SNPs, the majority were located within introns (Supplementary Table S6). After filtering, a final set of 1,785,124 SNPs was used for the GWAS analysis (Supplementary Table S7). The number of SNPs on each chromosome, the average distance between SNPs, and the SNP density per chromosome are shown in Fig. 1. Chromosome 1 had the highest number of SNPs, while chromosome 10 had the smallest average distance between SNPs (Fig. 1). A Manhattan plot of SNPs associated with FCE was constructed (Fig. 2A). The results revealed four SNPs above the significance threshold (4.56). Three SNPs (Chr21: 7,735,504; Chr21: 7,738,837; Chr21: 26,609,486) were located on chromosome 21, and one SNP (Chr23: 1,305,555) was located on chromosome 23 (Fig. 2A).

Candidate gene

Based on the genomic positions of the significant SNPs associated with FCE, the nearest candidate genes upstream and downstream were identified. Three SNPs on chromosome 21 were all located in intergenic regions, while the SNP on chromosome 23 was located within an intron region. Through gene annotation, a total of five candidate genes were identified: solute carrier family 2, facilitated glucose transporter member 11-like (*slc2a11-like*); transcription factor Sox-9-A-like (*sox9a-like*); syntaxin-binding protein 4-like (*stxbp4-like*); ankyrin-repeat and fibronectin type III domain containing 1 (*ankfn1*); DENN domain-containing protein 5B (*dennd5b*) (Table 2).

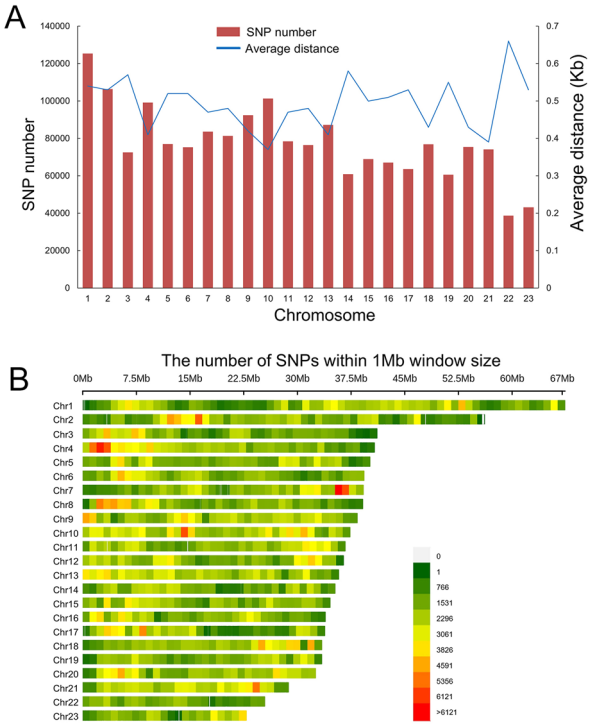

Fig. 1 Number, average distance, density and distribution of SNPs on each chromosome of largemouth bass. **A** SNP number (left *Y*-axis) and average distance (right *Y*-axis) of SNPs on each chromosome. **B** SNP density and distribution of each chromosome

Transcriptomic analysis

Sequencing data

Transcriptome sequencing generated 40.88 Gb of clean data in total, averaging 6.81 Gb per sample. The average GC content was 48.35%, with Q20, Q30, and mapping ratios of 96.73%, 91.49%, and 96.30%, respectively (Supplementary Table S8).

DEGs analysis

Comparative transcriptome analysis identified 861 DEGs between the two groups. Of these, 681 were significantly

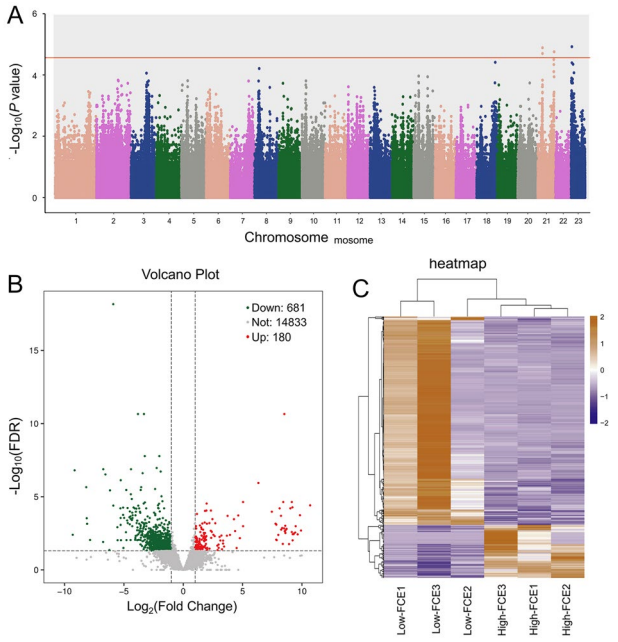

Fig. 2 Manhattan plots of genome-wide association study for FCE trait and DEGs identified in the liver of the Low- and High-FCE groups in largemouth bass. **A** Manhattan plots of GWAS. Different colored dots represent the loci on different chromosomes. The horizontal red line represents the significance threshold (4.56). **B** DEGs volcano plot. **C** DEGs cluster heatmaps. *DEGs* differentially expressed genes

down-regulated and 180 were up-regulated in the High-FCE group compared to the Low-FCE group (Fig. 2B). The hierarchical clustering clearly distinguished the two groups based on global expression profiles, affirming the reliability of the dataset (Fig. 2C).

GO enrichment analysis showed that the cellular component category was dominated by the general terms "cell" and "cell part" (Supplementary Fig. S1). The terms of transport vesicle, extracellular space, cell body fiber, anchored component of plasma membrane, MHC class I protein complex, and growth cone membrane were significantly up-regulated (Fig. 3A; Supplementary Table S9), and the terms of mitochondrion, mitochondrial inner membrane, Golgi membrane, Golgi apparatus, endoplasmic reticulum membrane, and endoplasmic reticulum lumen were significantly down-regulated (Fig. 3D; Supplementary Table S9) in the High-FCE group. For molecular function, the DEGs were predominantly involved in processes related to "banding" and "catalytic activity" (Supplementary Fig. S1). The terms of metallocarboxypeptidase activity, glycerol transmembrane transporter activity, and serine type endopeptidase activity were significantly up-regulated (Fig. 3B; Supplementary Table S9), and the terms of phospholipid transporter activity, nucleotide binding, ER retention sequence binding, and ATP binding were significantly down-regulated (Fig. 3E; Supplementary Table S10) in the High-FCE group. For biological process, the DEGs were mainly distributed in "cellular process" and "metabolic process" of the second category (Supplementary Fig. S1). The term of digestion was significantly up-regulated (Fig. 3C; Supplementary Table S9), and the terms of protein transport, and cholesterol biosynthetic process were significantly down-regulated (Fig. 3F; Supplementary Table S10) in the High-FCE group. The related DEGs in each key up-regulated and down-regulated GO term were presented in Supplementary Tables S9 and S10.

KEGG enrichment analysis mapped a total of 310 DEGs to various biological pathways, such as signal transduction, transport and catabolism, lipid metabolism, and translation (Supplementary Fig. S2). Among these, the TGF-beta and Notch signaling pathways were notably activated in the High-FCE group (Fig. 4A; Supplementary Table S11). In contrast, pathways including steroid biosynthesis, terpenoid backbone biosynthesis, glutathione metabolism, cysteine and methionine metabolism, Aminoacyl-tRNA biosynthesis, ribosome biogenesis in eukaryotes, protein processing in endoplasmic reticulum, and protein export were significantly suppressed in the High-FCE group (Fig. 4B; Supplementary Table S12). The related DEGs in each key up-regulated and down-regulated KEGG pathway were presented in Supplementary Tables S11 and S12.

Table 2 Candidate SNPs and genes associated with FCE trait of largemouth bass identified by the genome-wide association study

SNP name	SNP type	*P*-value	Region	Gene name	Gene annotation
Chr21: 7,735,504	A/G	0.0000130	intergenic	*slc2a11-like*; *sox9a-like*	Solute carrier family 2, facilitated glucose transporter member 11-like; transcription factor Sox-9-A-like
Chr21: 7,738,837	A/G	0.0000200	intergenic	*slc2a11-like*; *sox9a-like*	Solute carrier family 2, facilitated glucose transporter member 11-like; transcription factor Sox-9-A-like
Chr21: 26,609,486	G/T	0.0000175	intergenic	*stxbp4-like*; *ankfn1*	syntaxin-binding protein 4-like; Ankyrin-repeat and fibronectin type III domain containing 1
Chr23: 1,305,555	A/G	0.0000120	intronic	*dennd5b*	DENN domain-containing protein 5B

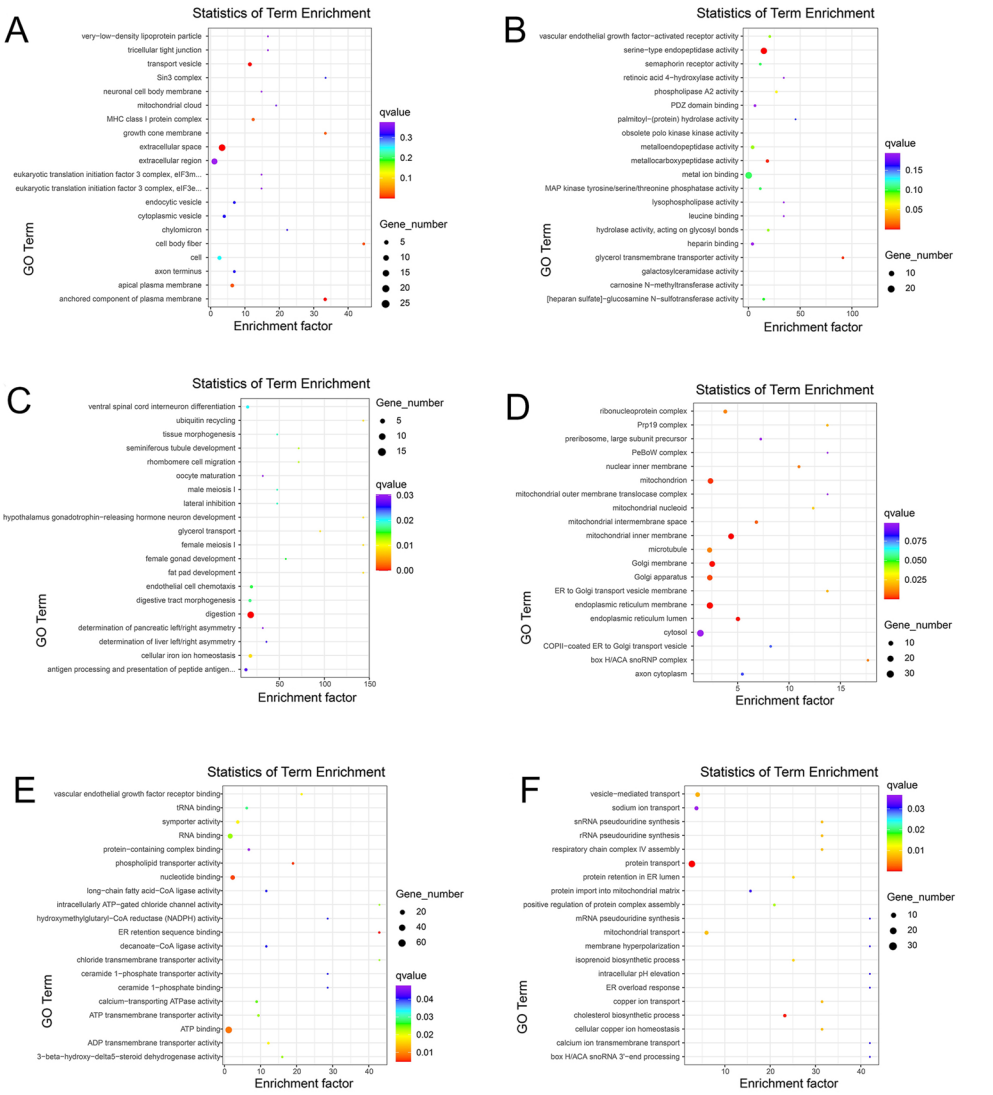

Fig. 3 GO enrichment of DEGs identified in the liver of the Low- and High-FCE groups of largemouth bass. **A–C** Up-regulated GO terms in the High-FCE group in cellular component, molecular function, and biological process, respectively. **D–E** Down-regulated GO terms in the High-FCE group in cellular component, molecular function, and biological process, respectively

RNA-seq data validation

Ten DEGs were chosen for RT-qPCR verification. These genes were coxsackievirus and adenovirus receptor-like (*carl*), chymotrypsin like elastase 2A (*cela2a*), phosphodiesterase 10A (*pde10a*), cytochrome P450 family 2 subfamily R member 1 (*cyp2r1*), elastase (*ela2*), protein disulfide isomerase family A member 2 (*pdia2*), cytokine receptor-like factor 3 (*crlf3*), phytochrome kinase substrate 1 (*pks1*), phospholipase A and acyltransferase 1-like (*plaat1l*), and thioesterase superfamily member 6 (*them6*). Their expression trends were in good agreement with the RNA-seq results, confirming the reliability of the transcriptome results (Fig. 5).

Metabolomic analysis

Due to sample contamination, only five metabolomic samples from each group were available for final analysis. The significant metabolic separation between the two groups was observed in both positive and negative ionization modes, as evidenced by the OPLS-DA scores plot (Fig. 6A) and validated through permutation tests (Fig. 6B). 93 DMs were identified by LC–MS analysis, with 46 down-regulated and 47 up-regulated in the High-FCE group (Fig. 6C).

A total of 66 pathways were enriched after KEGG enrichment analysis. These pathways were primarily associated with metabolic processes, such as nucleotide metabolism, biosynthesis of other secondary metabolites, lipid metabolism, and carbohydrate metabolism (Fig. 6D). Notably, in the High-FCE group, levels of adenine and xanthine were significantly elevated and some pathways including regulation

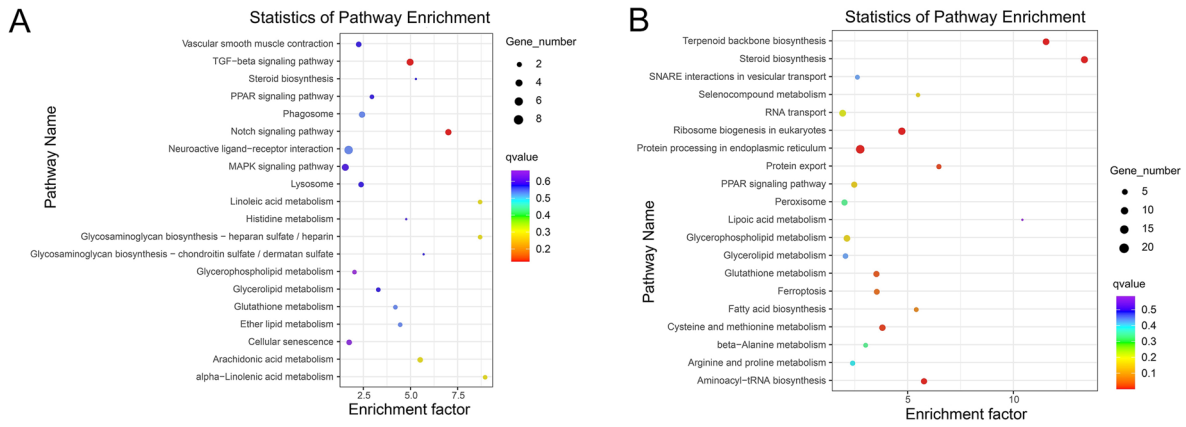

Fig. 4 KEGG pathway analysis of DEGs identified in the liver of the Low- and High-FCE groups of largemouth bass. **A** Up-regulated KEGG pathways in the High-FCE group. **B** Down-regulated KEGG pathways in the High-FCE group. *DEGs* differentially expressed genes

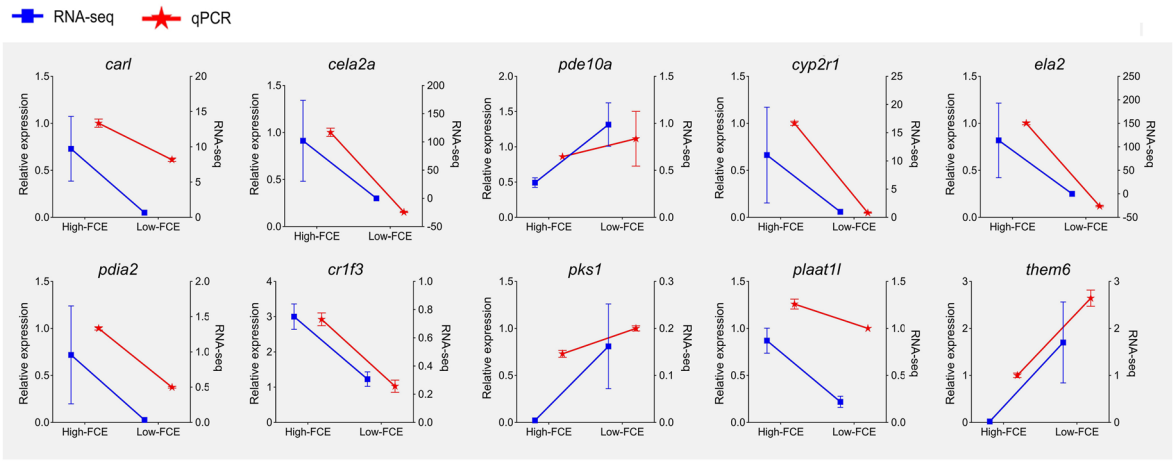

Fig. 5 Validation of RNA-seq results by RT-qPCR analysis of ten genes of largemouth bass. Values are mean of six replicate individuals in each group and presented as means ± SD

of lipolysis in adipocytes, purine metabolism, and nucleotide metabolism were significantly up-regulated (Fig. 6E). Detailed information on key DMs and their associated pathways was provided in Supplementary Table S13.

Integration of transcriptomics and metabolomics

The integrative KEGG analysis demonstrated that both DMs and DEGs were co-enriched in 20 pathways (Fig. 7A). Among these, pathways including glycerophospholipid metabolism, purine metabolism, neuroactive ligand-receptor interaction, propanoate metabolism, arachidonic acid metabolism, linoleic acid metabolism, and the pentose phosphate pathway contained a higher number of associated DEGs and DMs (Fig. 7A; Table 3). DEGs were prominent in pathways, such as glycerophospholipid metabolism, cysteine and methionine metabolism, and aminoacyl-tRNA biosynthesis, while DMs were distinctly associated with purine metabolism (Fig. 7A). Correlation analysis between 5 key DMs and 10 key DEGs indicated strong correlations between them, with each DM correlating with multiple DEGs (Fig. 7B). A regulatory network was constructed based on the correlations between DMs and DEGs, which showed that Methyl 9H-purine-6-carboxylate, adenosine, caylin-2, lysine, and adenosine dialdehyde were significantly correlated with 10, 3, 3, 2, and 2 DEGs, respectively (Fig. 7C).

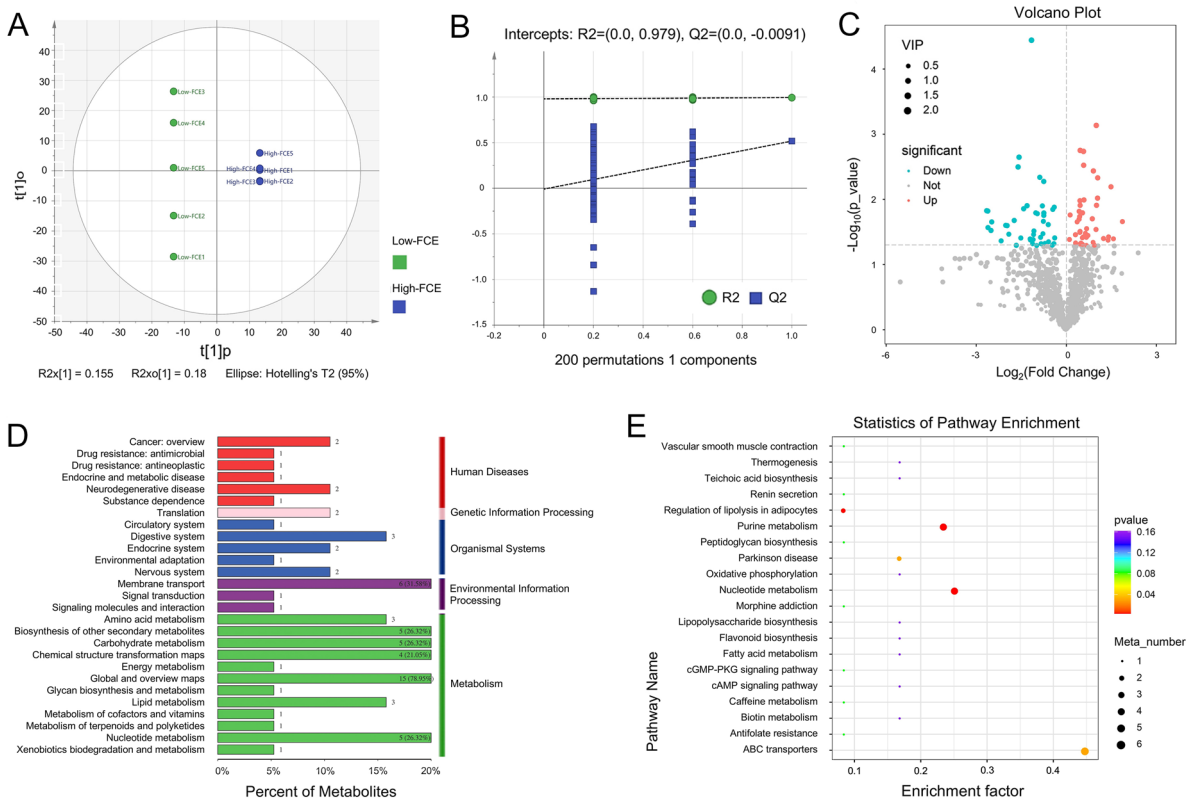

Fig. 6 DMs identified in the liver of the Low- and High-FCE groups of largemouth bass. **A** OPLS-DA scores plot. **B** 200 permutation tests of OPLS-DA model. **C** DMs volcano plot. **D** KEGG classifications. **E** Top 20 KEGG pathways. *DMs* differential metabolites

Analysis of common DEGs and DMs in liver and brain

DEGs and DMs identified in the liver in this study were compared with those obtained from brain tissue in a previous study (Shi et al. 2025) to identify common FCE-related DEGs and DMs shared between the two tissues. The results showed that a total of ten common DEGs were identified in both tissues (Table 4). Among these, nine genes exhibited consistent expression trends in both tissues: six genes were up-regulated (*plaat1l*, *crlf3*, *galectin-3-like*, *h2q9-like*, *tspan8-like*, *cxadr-like*) and three genes were down-regulated (*srp9*, *pde10a*, *noct-like*). However, one gene, *sh3pxd2b-like*, showed opposite expression trends between the two tissues (Table 4).

Regarding metabolites, six common DMs were identified in both tissues (Table 5). All shared DMs exhibited consistent expression trends: four DMs were down-regulated (Acetylcarnitine (Car (2:0)), 5-(*o*-Nitrobenzylidene) barbituric acid, 2'-deoxyguanosine 5'-diphosphate (dGDP), 6,6'-dihydroxy-5,5'-dimethoxybiphenyl-3,3'-dicarboxylic acid) and two DMs were up-regulated (*N*-Acetylsulfamethoxazole, *N*-(4-Chlorobenzyl)-4-quinazolinamine) (Table 5).

Discussion

Improvement of FCE promoted growth

Generally, both increased FI and improved FCE can significantly enhance the growth performance of fish (Besson et al. 2019; Silverstein 2006). This study found that the High-FCE group exhibited significantly better WGR, SGR, and PER compared to the Low-FCE group, while no significant difference was observed in the FR between the two groups. These results indicated that the growth advantage of the High-FCE group may stem not from the increased feed consumption, but from a higher metabolic conversion efficiency of nutrients. This was consistent with many studies on aquatic animals, which suggested that differences in FE were more closely related to metabolic regulation and energy allocation rather than simply feeding behavior (Elvy et al. 2022). Furthermore, the significantly higher PER in the High-FCE group suggested that it may enhance growth performance by optimizing protein synthesis or reducing catabolism (Kaushik and Seiliez 2010). Additionally, the transcriptomic results indicated that the expression levels of protease-encoding genes, such as *ela2* and *cela2a*, were

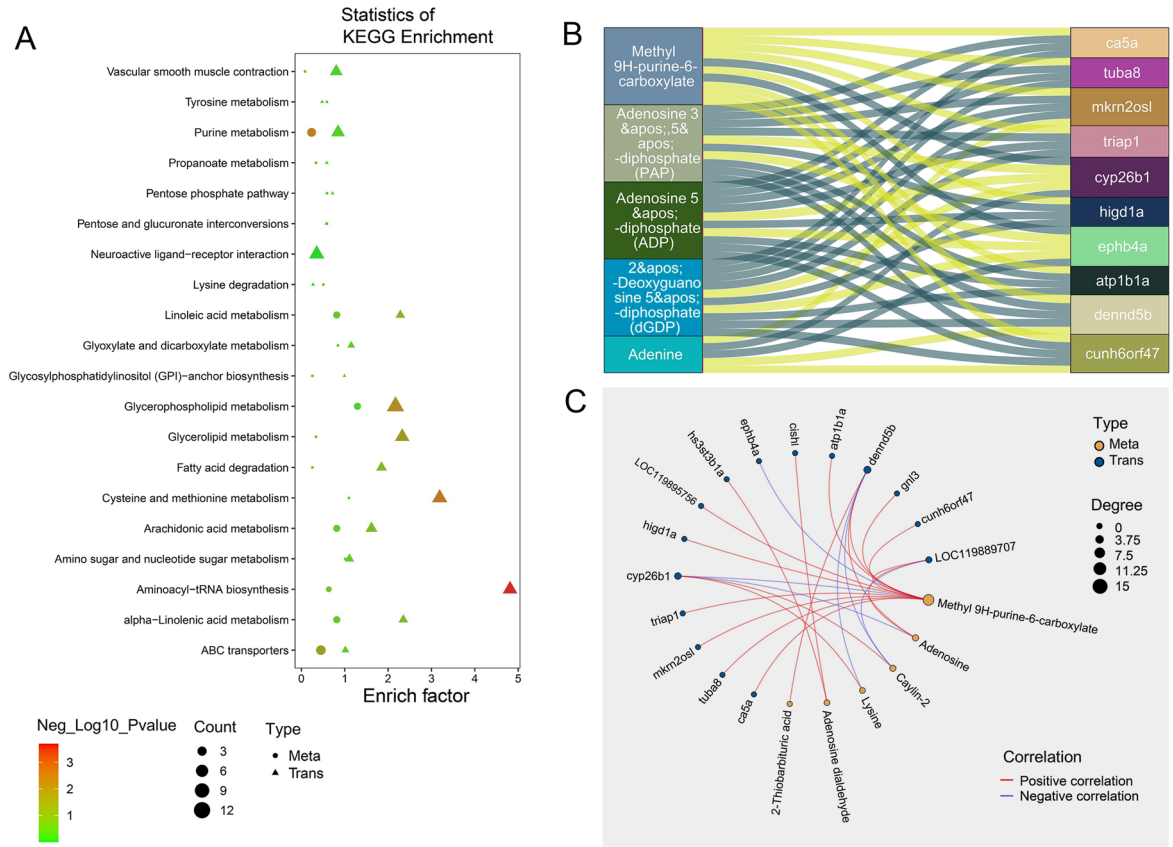

Fig. 7 Analysis of the integrative transcriptomics and metabolomics of the liver in the Low- and High-FCE groups of largemouth bass. **A** DEGs and DMs KEGG enrichment analysis. ▲ represents transcriptome; ● represents metabolome. **B** Sankey diagram of DMs and DEGs in the shared significant pathways. **C** Correlation of DEGs and DMs. Omics1 means DMs, and omics2 means DEGs. *DEGs* differentially expressed genes, *DMs* differential metabolites

Table 3 DEGs and DMs in the shared KEGG pathways in largemouth bass

Pathways	DMs	DEGs
Glycerophospholipid metabolism	PC (16:0/16:0) (−)	*plpp1-like* (−); *lpgat1-like* (−); *lpin1-like* (−); *pla2-like* (+); *agpat2* (−); *chkb* (−); *etnk2* (−); *lpgat1* (−); *phospho1* (−); *pla2g3* (−); *plaat1l* (+); *plpp5* (−)
Purine metabolism	Adenine (+); Adenosine (+); Deoxyinosine (+); Inosine (+); Xanthine (+)	*atase-like* (−); *adcy8-like* (+); *gda* (−); *nme6* (−); *papss1* (−); *pde10a* (−); *pde4ba* (+)
Neuroactive ligand-receptor interaction	Adenosine (+)	*trypsin-like* (+); *trypsin-2-like* (+); *trypsin-2* (+); *trypsin-1-like* (+); *klk11-like* (+); *adra2db* (+); *crhr1* (−); *edn2* (+)
Propanoate metabolism	2-Hydroxybutyric acid (−)	*acss2l* (−)
Arachidonic acid metabolism	PC (16:0/16:0) (−)	*pla2-like* (+); *gpx1b* (+); *pla2g3* (−); *plaat1l* (+); *ptges3a* (−)
Linoleic acid metabolism	PC (16:0/16:0) (−)	*pla2-like* (+); *pla2g3* (−); *plaat1l* (+)
Pentose phosphate pathway	Deoxyribose 5-phosphate (+)	*tkt-like* (+)

"+": up-regulation of gene or metabolite in the High-FCE group; "−": down-regulation of gene or metabolite in the High-FCE group

DEGs differentially expressed genes, *DMs* differential metabolites

Table 4 Common DEGs identified in the liver and brain of the High- and Low-FCE groups of largemouth bass

Common DEGs	Liver			Brain		
	Log_2 FC	*P*-value	Significant	Log_2 FC	*P*-value	Significant
plaat1l	1.8279	2.44E-04	up	1.7991	4.70E-05	up
sh3pxd2b-like	1.3393	3.16E-04	up	−1.8341	6.07E-14	down
crlf3	1.1554	1.63E-03	up	1.8445	9.08E-06	up
galectin-3-like	2.2097	1.73E-03	up	2.3150	3.80E-05	up
h2q9-like	2.6464	8.89E-05	up	2.0582	2.14E-04	up
tspan8-like	1.1561	6.09E-07	up	1.1516	6.90E-08	up
cxadr-like	3.6786	7.87E-05	up	4.4320	6.16E-04	up
srp9	−4.4015	7.32E-11	down	−1.6956	1.81E-05	down
pde10a	−1.4162	7.52E-04	down	−1.2089	1.13E-03	down
noct-like	−2.3600	7.66E-07	down	−1.4546	9.64E-04	down

Table 5 Common DMs identified in the liver and brain of the High- and Low-FCE groups of largemouth bass

Common DMs	Liver			Brain		
	VIP	Log_2 FC	Significant	VIP	Log_2 FC	Significant
Acetylcarnitine (Car (2:0))	1.7832	−0.7140	down	1.5154	−0.8185	down
5-(*o*-Nitrobenzylidene) barbituric acid	1.5868	−0.8251	down	1.4336	−0.3605	down
2'-Deoxyguanosine 5'-diphosphate (dGDP)	2.0819	−1.1222	down	1.5035	−0.5382	down
6,6'-Dihydroxy-5,5'-dimethoxybiphenyl-3,3'-dicarboxylic acid	1.8031	−0.7164	down	1.5715	−0.5073	down
N-Acetylsulfamethoxazole	1.5549	0.7098	up	1.6768	1.0169	up
N-(4-Chlorobenzyl)-4-quinazolinamine	1.8664	0.6124	up	1.5391	1.0443	up

significantly up-regulated in the High-FCE group, which may enhance the ability to digest dietary protein, thereby contributing to a significant improvement in protein efficiency. Traditionally, improving the growth performance of aquaculture species was usually achieved by increasing FI or dietary protein levels (El-Saidy and Gaber 2005). However, the results of this study suggested that improving the FCE of farmed aquatic animals may represent a more sustainable development pathway.

Genomic variation and genetic basis of FCE

In this study, four SNPs significantly associated with FCE were identified on two chromosomes. After gene annotation, five candidate genes potentially related to FCE were identified, which may influence FCE through multiple levels. For instance, *slc2a11-like* encodes a glucose transporter protein, which mainly mediates transmembrane transport of glucose. This gene may enhance glucose uptake, thereby improving glycolysis and ATP generation efficiency to provide more energy for growth (Doege et al. 2001). *Sox9a-like*, as a transcription factor, may interact with other transcription factors and DNA sequences to regulate processes, such as cell growth, cartilage development, and gonadal differentiation (Li et al. 2023; Yan et al. 2005). *Stxbp4-like* encoded syntaxin-binding protein 4, which can participate in vesicle transport and cell signal transduction (Otaka et al. 2017). *Ankfn1* was reported to be associated with human height and developmental abnormalities, and played a role in cell proliferation (Wang et al. 2022). The protein encoded by *dennd5b* was a member of the DENN (Differentially Expressed in Normal and Neoplastic cells) protein family, which was involved in various biological processes including neuronal development, synaptic formation, synaptic vesicle transport, and lipid metabolism (Huang et al. 2021). Therefore, these genes identified by GWAS may collectively influence the FCE of largemouth bass by synergistically regulating key biological processes, such as nutrient absorption, energy metabolism, cell growth and differentiation, and intercellular signal transduction. The identification of these genes provided potential targets for the genetic improvement of FCE in largemouth bass. It was noteworthy that most of the significant SNPs were located in intergenic or intronic regions, suggesting they might exert their effects by regulating the expression of adjacent genes or influencing the function of non-coding RNAs. Future studies should focus on functional verification to further elucidate the biological effects of these SNPs.

Transcriptional regulation of FCE

Serine-type endopeptidases is a proteolytic enzyme that can break down polypeptides into shorter chains (Page and di Cera 2008); while metallocarboxypeptidases can remove C-terminal amino acids from proteins and/or peptides, playing a central role in the breakdown of peptides in food (Vendrell et al. 2011). In this study, the High-FCE group exhibited the up-regulated activities of these two enzymes and the "digestion" process, suggesting that fish with high FCE may possess enhanced protein digestive capacity. This was further supported by the significant up-regulation of protease-encoding genes, such as *cela2a* and *ela2*, in the High-FCE group. Additionally, in the High-FCE group, some GO terms including "transport vesicle", "glycerol transmembrane transporter activity", and "anchored component of plasma membrane" were significantly up-regulated. The expression of the *dennd5b* gene identified by GWAS analysis was also significantly up-regulated in the High-FCE group. The up-regulation of these genes related to vesicle transport, substance transport, and membrane structure indicated that the High-FCE fish may have advantages in digestive enzyme secretion, nutrient transport, and intercellular signaling, thereby improving absorption efficiency (Xu et al. 2025). MHC class I molecules present protein fragments of cytosolic and nuclear origin at the cell surface, activating $CD8^+$ T cells to initiate cellular immune responses (Neefjes et al. 2011). The significant up-regulation of the MHC class I protein complex in the High-FCE group suggested that these fish may have a stronger cellular immune capacity. Furthermore, the *carl* gene was reported to play crucial roles in multiple biological processes, such as cell adhesion, immune cell activation, synaptic transmission, and signaling (Excoffon 2020). The significant up-regulation of the *carl* gene in the High-FCE group implied putative improvements in nutrient digestion and absorption, secretion/transport systems, and immune and intercellular signal transduction capabilities, ultimately fostering greater nutrient use efficiency.

Conversely, some genes and pathways were down-regulated in the High-FCE group. First, pathways related to the biosynthesis of various substances, including cholesterol biosynthetic process, steroid biosynthesis, terpenoid backbone biosynthesis, aminoacyl-tRNA biosynthesis, as well as genes, such as *pks1* and *them6*, were down-regulated. The *pks1* gene encodes polyketide synthase, which is involved in biofilm formation (Pang et al. 2012) and melanin biosynthesis (Takano et al. 1995). The *them6* gene belongs to the THEM family, which regulates intracellular lipids and affects fatty acid synthesis and β-oxidation processes (Lu et al. 2023). The biosynthesis of these substances is a highly energy-consuming process, and their down-regulation may imply a reduction in energy consumption, allowing High-FCE fish to allocate more energy to growth. Second, genes and pathways related to ribosome, mitochondrion, and endoplasmic reticulum function were suppressed in the High-FCE group, such as ribosome biogenesis in eukaryotes, mitochondrion, mitochondrial inner membrane, endoplasmic reticulum membrane, and protein processing in endoplasmic reticulum. The endoplasmic reticulum and ribosomes are factories for protein processing and are among the most energy-consuming processes in cells (Yonath et al. 2023). The down-regulation of their functional genes suggested that the High-FCE fish may have higher protein synthesis and assembly efficiency. The down-regulation of mitochondrial-related genes may decrease the basal metabolic rate, reducing energy consumption (Casanova et al. 2023). Additionally, some metabolic processes were inhibited in High-FCE fish, such as glutathione (GSH) metabolism, and cysteine and methionine metabolism. GSH can participate in cellular redox reactions, providing antioxidant defense (Dröge et al. 2000), and its reduction indicated that the High-FCE fish may have a lower basal oxidative stress level, and thus the demand for GSH-dependent antioxidant defense may be reduced. Therefore, fish with high FCE may have systematically reduced energy-intensive processes including biosynthesis and processing as well as oxidative defense, optimizing energy allocation and reducing maintenance costs, thereby improving FCE.

Metabolic regulation of FCE

At the metabolic level, the most prominent feature of the High-FCE group in this study was the accumulation of purine metabolites (adenine and xanthine) and the activation of nucleotide metabolism and purine metabolic pathways. Similarly, in the brain of largemouth bass, the purine metabolism of high FCE individuals was also significantly enhanced (Shi et al. 2025). Adenine, as the core component of adenosine nucleotides, plays an important role in energy metabolism (Atkinson 1971). The increase in adenine content in High-FCE fish can provide more energy for protein synthesis in the organism. Additionally, the activation of the purine salvage synthesis pathway reduced the energy consumption of nucleotide de novo synthesis (Aggrey et al. 2014). It is noteworthy that this metabolic signature is highly consistent with reports in bird species with high feed utilization, suggesting that energy metabolism optimization may be a conserved factor affecting FCE across species (Aggrey et al. 2014).

L-Palmitoylcarnitine is an ester derivative of carnitine, which is involved in fatty acid metabolism (Lu et al. 2025). The reduction of L-palmitoylcarnitine in the liver of the High-FCE group implied that these individuals may have reduced non-essential fatty acid oxidation and instead prioritized the utilization of carbohydrates as energy sources, which corresponded with the enhanced glucose uptake

mediated by the *slc2a11-like* gene. In the present study, the content of glucuronic acid was significantly increased in High-FCE fish. Studies have shown that glucuronic acid possesses various physiological functions, particularly in antioxidation, anti-inflammation, anti-bacteria, and detoxification (Hu et al. 2024). The increase of glucuronic acid in the High-FCE group reflected the enhancement of liver detoxification and antioxidant capacity. This adaptive change may reduce the inhibition of metabolic wastes such as ammonia nitrogen on growth and lower the damage to biological macromolecules caused by oxidative stress, thereby contributing to the improved FCE.

Multi-omics revealed FCE regulatory network

This study initially revealed the gene-transcription-metabolism regulatory network for the FCE trait of largemouth bass through multi-omics analysis. For example, the *dennd5b* gene was concurrently identified by GWAS and RNA-seq, showing significant upregulation in the High-FCE group and strong correlations with critical DMs. The identification of the *dennd5b* gene convincingly demonstrated how genetic variants (GWAS) influence gene expression (transcriptome), which in turn modulates metabolic pathways (metabolome) to enhance FCE. In summary, the regulatory network of FCE was mainly manifested in three aspects: the reprogramming of energy metabolism networks, the remodeling of membrane transport systems, and the systematic optimization of signal transduction networks. Firstly, the accumulation of adenine, the up-regulation of key genes in the purine salvage synthesis pathway, and the significant enrichment of the purine metabolism pathway, together with the *slc2a11-like* gene identified by GWAS, formed a "glucose uptake-purine synthesis-ATP supply" metabolic axis. This axis collectively maintained a high energy charge state, providing an energy support for energy-consuming processes such as protein synthesis (Shi et al. 2025; Zhang et al. 2019). Second, the enhanced transmembrane transport function mediated by *slc2a11-like* and *dennd5b*, the significant enrichment of GO terms, such as transport vesicle, glycerol transmembrane transporter activity, the co-enrichment of glycerophospholipid metabolism in both DEGs and DMs, along with the increased content of PC(33:1), collectively indicated that the High-FCE fish may promote the absorption and transport of nutritive materials by enhancing membrane fluidity and transport capacity (Ugolev et al. 1973). Finally, the significantly improved expression of *dennd5b* gene identified by GWAS, the significant up-regulation of *carl* gene, and the co-enrichment of neuroactive ligand-receptor interaction in both DEGs and DMs, jointly suggested that the High-FCE group had constructed an efficient signal transduction system, achieving precise regulation of nutrient absorption, distribution, and utilization (Hou et al. 2018; Shi et al. 2025).

Integrated regulation of FCE by liver and brain

By comparing multi-omics data from liver and brain tissues, this study for the first time revealed the liver–brain coordinated regulatory network of the FCE trait in largemouth bass. This finding provided a new perspective for understanding the neuroendocrine regulatory mechanism of nutrient utilization in fish. At the gene expression level, 90% of the common DEGs showed consistent expression trends; at the metabolite level, all six common DMs presented consistent expression trends, indicating that the liver–brain coordinated regulation of FCE was highly conserved. The *plaat1l* gene was reported to participate in lipid metabolism and fatty acid β-oxidation (Uyama et al. 2012), subsequently generating ATP for the use of the organism through the tricarboxylic acid cycle (Grabner et al. 2021). The expression level of *plaat1l* in the High-FCE group significantly increased, indicating that the lipid metabolism and energy conversion ability of these fish were significantly enhanced. Studies have shown that *galectin-3* can participate in processes, such as cell proliferation, differentiation, immune regulation, and inflammatory response (Díaz-Alvarez and Ortega 2017); while the protein encoded by the *cxadr* gene mainly participates in cell adhesion, immune cell activation, synaptic transmission, and signaling (Excoffon 2020). The significant up-regulation of *galectin-3* and *cxadr* in High-FCE fish suggested a marked strengthening of their immune regulation capacity. Furthermore, several up-regulated genes including *cxadr*, *tspan8*, and *crlf3* in High-FCE fish might be mainly involved in intracellular signal transduction processes (Liongue and Ward 2025; Zhang et al. 2020). Therefore, at the transcriptional level, the common DEGs in the liver and brain collectively shaped the molecular basis for the regulation of FCE through synergistic effects of energy metabolism, immune regulation, and signal transduction.

Acetylcarnitine participates in fatty acid metabolism and energy balance, primarily functioning as a carrier for fatty acids entering the mitochondria, facilitating the transport of long-chain fatty acids into the mitochondrial matrix for β-oxidation (Adeva-Andany et al. 2017). Generally, an increase of acetylcarnitine in animals or exogenous supplementation can lead to weight loss (Center et al. 2000). In this study, the increased levels of acetylcarnitine in the brain and liver of the Low-FCE fish indicated enhanced fat decomposition. Excessive fatty acid β-oxidation leaded to inefficient consumption of energy substrates, thereby reducing FCE. Sulfamethoxazole is a sulfonamide antibacterial agent widely used in aquaculture, which can inhibit the bacterial enzyme dihydropteroate synthetase (Liu et al. 2021). *N*-acetyl-sulfamethoxazole (N-ac-SMZ) is the predominant metabolite of SMZ (Samuelsen et al. 1997). The increase in N-ac-SMZ in High-FCE fish suggested enhanced antibacterial efficacy and improved drug detoxification capacity.

Thus, at the metabolic level, the common liver–brain DMs collectively form the metabolic basis for FCE regulation by influencing lipid metabolism and antibacterial activity.

Conclusions

This study comprehensively revealed the FCE regulatory mechanism in largemouth bass. The core lied in the efficient metabolic conversion of nutrients rather than the increase in FI. At the genetic level, genes, such as *slc2a11-like*, *sox9a-like*, *stxbp4-like*, *dennd5b*, and *ankfn1*, collectively influenced FCE by regulating processes including glucose uptake, cell growth, vesicle transport, and signal transduction. At the transcriptomic level, High-FCE individuals exhibited up-regulation of protease genes (e.g., *ela2*, *cela2a*) and genes related to nutrient transport, enhancing protein digestion and absorption capacity, while the high energy-consuming processes, such as cholesterol synthesis, ribosome biogenesis, mitochondrial function, and glutathione metabolism, were down-regulated, reducing maintenance consumption. At the metabolic level, the accumulation of purine metabolites and the activation of the salvage synthesis pathways optimized energy supply. The reduction in L-palmitoylcarnitine indicated a decrease in fatty acid oxidation, while the increase in glucuronic acid and N-ac-SMZ enhanced detoxification and antioxidant capacity. Furthermore, liver–brain coordinated regulatory network further strengthened the systematic regulation of FCE through regulating energy metabolism, immune regulation, and signal transduction. In summary, the realization of high FCE in largemouth bass was attributed to the coordinated optimization of multiple aspects, including nutrient digestion and absorption, energy metabolism, immune capacity, membrane transport, and signal transduction.

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Author contributions XS conceived the project and designed experiments. BZ and SY performed experiments, analyzed data and wrote the original draft. DY helped to perform experiments and collect samples. WM, XM, LW, and XT provided the experimental methods and participated in the figure creation. KW, HF, RX, and XL reviewed, checked, and edited the manuscript.

Data availability The raw sequencing data of the whole-genome resequencing and transcriptome sequencing have been uploaded to the NCBI database under accession numbers of PRJNA1303634 and PRJNA1304617.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Animal and human rights statement Animal experiments in this study were performed following the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China), and all experiments procedures involving animals were approved by the Animal Care and Use Ethics Committee of Henan Normal University (approval No. HNSD-2023–08-11).

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