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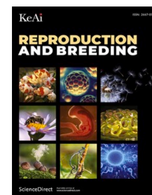

Identification of key genes affecting development and nutrient metabolism in the early seedling stage of Dongting catfish (*Silurus asotus*)

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A R T I C L E I N F O

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A B S T R A C T

As a carnivorous fish, the cannibalism are prone to happen in the early seedling stage in Dongting catfish (*Silurus asotus*), especailly when there is not enough food, which resulting in the low survival rate of Dongting catfish. To find clues to improve the survival rate of Dongting catfish offspring, the comparative transcriptomics analysis were conducted between normal and malformed larval fish, as well as normal larval fish before and after first food intake. In normal and malformed larval fish groups, a total of 881 differential genes were identified, including 634 up-regulated genes and 247 down-regulated genes. From these genes related to growth hormone synthesis and secretion were screened out as *SLA isoform X1*, *SOCS3*, *STAT1b*, *JUNB*, respectively. In the group of larval fish before and after the first food intake, 4901 up-regulated genes and 3660 down-regulated genes were found, from which the differential genes related to protein digestion and absorption pathway and pancreatic secretion pathway were screened out as *cathepsin D precursor*, *elastase 2 like precursor*, *elastase 2 precursor*, *LPL isoform X1*, *CTRB1 precursor*, *CTRL precursor*, *CPA precursor*, *FABP*, *SLC15A1*, *CEL tandem duplicate 2 precursor*. The differential genes screened for association with amino acid metabolism were *ASAOC precursor*, *PLD3*, *NDPKs*, *trehalase*, *UMP-CMP kinase*, *L-amino-acid oxidase*, *PLB1*, *FALDH isoform X2*, *PLA2 precursor*, *glucokinase*. Meanwhile, nine differential genes were selected for qPCR verification. The results confirmed that the relative expression trends of these genes were consistent with those found in the transcriptome. These findings provide an important reference for developing strategies to enhance the survival rate of Dongting catfish at early seedling stage.

1. Introduction

Catfishes are popular food-fish in various countries worldwide and good candidate for aquaculture. The catfish family Siluridae contains 107 described species distributed in Asia, but with some distributed in Europe [1]. The Dongting catfish is a local variety of *Silurus asotus* (*S. asotus*) in the Dongting Lake basin, which is an important fishery resource with highly valued economic benefits in Yangtze River in China. However, along with the ten-years ban on fishing in the Yangtze River, there is an increasing need for the artificial breeding of Dongting catfish.

At present, some progress has been reported in the artificial breeding of the local catfish in the Dongting Lake basin. For example, han et al., analyzed the growth and reproductive biological characteristics of *S. asotus* in the main stream of Yuanjiang River [2], which showed a power function relationship between body length and body mass for Yuanjiang *S. asotus* and the sexual maturity coefficients of females were higher than those of males. The artificial production and fry culture on catfish (*S. asotus*) in Dongting Lake has also been conducted [3].The results showed that after injected with mixture hormones of LRH-A_2 (luteinizing hormone releasing hormone analogue 2), HCG (human chorionic gonadotropin) and DOM (Diorone maleate), the spawning rate of *S. asotus* was 93.75 %, the fertilizing rate was 75.35 % and the hatching rate was 59.3 %. However, there are still some problems in the

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breeding process of Dongting catfish, such as the low survival rate of large-size seedlings, which greatly restrict the large-scale development of its breeding industry.

The development malformation during hatching and cannibalism in the early seedling stage are the main reasons for the low survival rate of Dongting catfish seedlings. So it is very important to find methods to reduce the ratio of deformity and the occurrence of cannibalism of Dongting catfish seedlings. During this critical early seedling stage, adequate feeds are needed to fulfill the nutritional requirements of fish larvae. In pikeperch, it has been shown that the structure and functional ability of the digestive tract are affected by the larval developmental stage as well as by the diet composition [4]. At present, the genes that affect the development and nutrient metabolism in the early seedling stage of Dongting catfish (*S. asotus*) is largely unknown. Transcriptome sequencing and analysis is a valuable tool for studying the structure, function, and expression of genes in individual organisms. It is also an important method for conducting phenotype-gene association studies [5]. For examle, shao et al., used RNA-Seq technology to analyze the transcriptome of *Pelteobagrus vachellii* (*P. vachellii*) under two different feeding time treatments [6]. The result showed that shift the feeding time from day-time (8:00) to night-time (20:00) may promote the expression of genes related to nutrient metabolism and thus contribute to the digestion, absorption, and transport of nutrients. The differentially expressed genes (DEGs) in the two groups of samples were screened to investigate the molecular mechanisms by which feeding time affects the digestion and absorption of proteins and lipids in *P. vachelli*.

In this study, we analyzed the transcriptome of Dongting catfish at different states and identified key genes affecting their development and nutrient metabolism in the early stage of opening. The findings provide an important reference for developing strategies to enhance the survival rate of Dongting catfish seedlings at the early stage.

2. Materials and methods

2.1. Experimental animal

Adult Dongting catfish were collected from the water area of Dongting Lake under the permission of fishing licences. The Dongting catfish seedlings were gained by artiticial reproduction. The first food (fairy shrimp, *Artemia salina*) supply were given at 48h after hatch. The fry that have in-taken the food which are characteristic by a distinct red color in the abdomen, are collected 24 h after the food supply. Normal Dongting catfish (control group) larvae and malformed Dongting catfish (treated group 1) larvae were collected 24 h after hatch and Dongting catfish larvae after first feeding (treated group 2) were collected 72 h after hatch. Three samples were taken for each group.

2.2. RNA extraction

The samples were used to extract total RNA following the manufacturer's instructions for TRIzol® Reagent. RNA quality was assessed using the 5300 Bioanalyser (Agilent) and quantified with the ND-2000 (NanoDrop Technologies). Only high-quality RNA samples (OD260/280 = 1.8–2.2, OD260/230 ≥ 2.0, RIN≥8.0, 28S:18S ≥ 1.0, RNA mass>1 μg) were used for sequencing library construction.

2.3. Library preparation and sequencing

RNA purification, reverse transcription, library construction and sequencing were performed at Shanghai Majorbio Bio-pharm Biotechnology Co., Ltd. (Shanghai, China) according to the manufacturer's instructions (Illumina, San Diego, CA). The Dongting catfish seedlings RNA-seq transcriptome library was prepared using Illumina® Stranded mRNA Prep Ligation kit from Illumina (San Diego, CA) with 1 μg of total RNA. Firstly, the mRNA was isolated using the poly A selection method with oligo (dT) beads and then fragmented using the fragmentation buffer. Double-stranded cDNA was synthesized using a SuperScript double-stranded cDNA synthesis kit (Invitrogen, CA) with random hexamer primers (Illumina) secondly. The cDNA was synthesized and then subjected to end-repair, phosphorylation, and 'A' base addition following Illumina's library construction protocol. The libraries were size-selected for cDNA target fragments of 300 bp on 2 % Low Range Ultra Agarose. Afterward, they were PCR amplified using Phusion DNA polymerase (NEB) for 15 PCR cycles. The paired-end RNA-seq sequencing library was sequenced using the NovaSeq 6000 sequencer (2 × 150 bp read length) after being quantified by Qubit 4.0.

2.4. Quality control and read mapping

The raw paired end reads were trimmed and quality controlled by fastp [7] with default parameters. Then clean reads were aligned separately to reference genome [8] of *Silurus asotus* downloaded from the NCBI website (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_024362625.1/) with orientation mode using HISAT2 [9] software. The mapped reads of each sample were assembled by StringTie [10] in a reference-based approach.

2.5. Differential expression analysis and functional enrichment

To identify DEGs (differentially expressed genes) between two different samples, the expression level of each transcript was calculated according to the transcripts per million reads (TPM) method. Gene abundances were quantified using RSEM [11]. Differential expression analysis was performed using DESeq2 [12] or DEGseq [13]. Genes with $|\log_2 FC| \geq 1.5$ and FDR < 0.05 (DESeq2) or FDR< 0.001 (DEGseq) were considered significantly differentially expressed. Additionally, functional enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), was conducted to identify differentially expressed genes (DEGs) significantly enriched in KEGG at P-adjust of less than 0.05 compared to the whole-transcriptome background. GO functional enrichment analysis and KEGG pathway analysis were performed using Goatools and Python scipy, respectively.

2.6. qRT-PCR

In this study, seven differential expressed genes were selected for experimental verification. The cDNA were synthesized using the RT-PCR kit (Takara, Japan). The qRT-PCR primers for the target genes were designed using the NCBI website (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The qRT-PCR was performed using the ChamQ Universal SYBR qPCR Master Mix (Vazyme, China). The amplification procedure of qRT-PCR was: 1 cycle of 95 °C/10 min; 40 cycles of 95 °C/15 s, 60 °C/1 min. The data were analyzed by the $2^{-\Delta\Delta CT}$ method. Data were normalized by β-actin expression as the internal control. Primers used for qRT-PCR assays are described in Table 1.

Table 1
Primer sequences used for real-time PCR.

Genes	Forward primer(5′-3′)	Reverse primer(5′-3′)
elastase 2 like precursor	TGGAGGAGATGGCATTGTGT	TGTGGGCTTCTTGAGGTAGT
CTRB1 precursor	TTCCCGCCATTTCTCCTGTC	TCTCCAAGGACCACAACGTG
FABP	CACTCAACGGCACATGGAAG	GGTTGTCATGTTCTGCCAGC
ASAOC precursor	GACCCATGGGAACCTGTTGT	CAGAGTTTCCGGGAGTTGCT
NDPKs	GATGGCCGCTGTAACACGAT	TCAGACCTACCAAGCGGAAC
trehalase	CACGGTGCTATTCTCAGCCT	TGCCTTCCATCGAGTCAAGC
PLB1	GCATCGTTCTATTGGTCTCT	CAAAGTTAATTCGCCTGACC
SOCS3	TGCGTTACAGCACACCTCTC	CACGCGGACATTCTTGATGC
STAT1b	TCCAATGAGAGAGGAGTCGC	CACCCACGTGATGGTGATCC
β-actin	CCACCATGTACCCAGGCATT	CGGACTCATCGTACTCCTGC

3. Result

3.1. Statistical analysis of transcriptome sequencing data

A total of 58.4 Gb of clean data was obtained from this transcriptome sequencing, and the clean data of each sample reached 6.12 Gb or more. The percentage of Q30 bases in each sample was above 92.95 % and the samples were of good quality for subsequent analysis. Sequence comparison of clean reads from each sample with the specified reference genome was performed separately, and the rate of comparison that could be localized to the genome ranged from 85.46 % to 89.85 %, with 2.87%–3.58 % of clean reads having multiple comparison positions on the reference sequence, and 81.88%–86.95 % of clean reads having a unique comparison position on the reference sequence (Table 2).

PCA analysis was performed among the samples, and principal component 1 (PC1) and principal component 2 (PC2), contributed 84.56 % and 5.31 % to differentiating the samples, respectively. The results showed high similarity between samples within groups and strong correlation between biological replicates, but exhibited very clear separation characteristics between groups, which indicated significant changes in the transcript levels of Dongting catfish seedlings at different states (Fig. 1).

3.2. Functional annotation analysis

All the genes obtained from the transcriptome assembly were compared with six major databases (GO, KEGG, EggNOG, NR, Swiss-Prot, and Pfam), of which 18172, 17686, 22268, 23113, 21296, and 21036 genes were obtained with functional annotations in the GO, KEGG, EggNOG, NR, Swiss-Prot, and Pfam databases, respectively (Fig. 2A).

Genes obtained by transcriptome assembly were categorized by functional annotation using the GO database, including biological processes (BP), cellular components (CC), and molecular functions (MF). The genes obtained from transcriptome assembly were mainly annotated to molecular functions of binding and contact reactions, cellular and membrane components in cellular components, and cellular processes, metabolic processes, and bioregulatory processes in biological processes (Fig. 2B).

The KEGG database was utilized to classify the functional annotations of genes obtained from transcriptome assembly, including Metabolism, Genetic Information Processing, Environmental Information Processing, Cellular Processes, Organismal Systems, Human Diseases, and Drug Development.

The genes obtained from transcriptome assembly are primarily annotated to signal transduction in Environmental Information Processing, immune and endocrine systems in Organismal Systems, and to a lesser extent, transport and catabolism in Cellular Processes, and cancer and viral infectious diseases in Human Diseases (Fig. 2C).

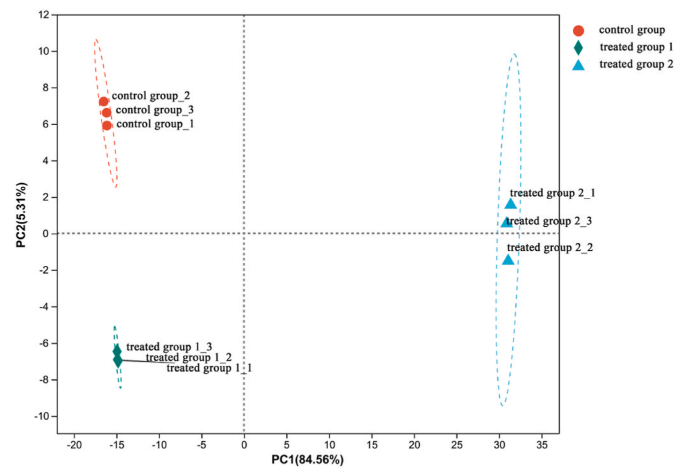

Fig. 1. PCA analysis of the tested samples (A). normal Dongting catfish larvae before first food intake. (B). normal Dongting catfish larvae after first food intake. (C). malformed Dongting catfish larvae.

3.3. Identification of differentially expressed genes

In this study, differentially expressed genes were screened by further comparing the transcriptome expression between samples from treated group 1 and control group, treated group 2 and control group ($p < 0.05$). There were a total of 881 genes that differed between treated group 1 and control group, including 634 up-regulated genes and 247 down-regulated genes (Fig. 3A). The total number of genes that differed between treated group 2 and control group was 8561, including 4901 up-regulated genes and 3660 down-regulated genes (Fig. 3B). The distribution of DEGs in Fig. 3 shows significant changes in the transcriptome of Dongting catfish at different states.

3.4. GO and KEGG enrichment analysis of DEGs

In this study, the differential genes were enriched and analyzed in KEGG and GO databases, differentially expressed genes between treated group 1 and control group were enriched and analyzed in the KEGG database ($p < 0.05$), and 75 of these pathways were found to be up-regulated and 7 pathways were down-regulated. The up-regulated pathways were mainly involved in osteoclast differentiation, TNF signaling pathway, IL-17 signaling pathway and Toll-like receptor signaling pathway (Fig. 4A). Down-regulated pathways mainly include Vitamin digestion and absorption and Fat digestion and absorption, Cholesterol metabolism, and Protein digestion and absorption (Fig. 4B).

In GO enrichment analysis, the 4901 up-regulated differential genes between treated group 2 and control group were mainly involved in immune system process, oxidoreductase activity, immune response, extracellular space and small molecule metabolic processes (Fig. 5A).

Table 2
A summary of transcriptome sequencing information of *S.asotus* larvae at three different states.

Group	Control group			Treated group 1			Treated group 2		
Sample	Control group-1	Control group-2	Control group-3	Treated group 1-1	Treated group 1-2	Treated group 1-3	Treated group 2-1	Treated group 2-2	Treated group 2-3
Raw reads	48134808	44685716	43679634	43014796	45432752	45201696	47494430	48926308	45933370
clean reads	47369646	43995246	42947172	42289568	44700740	44350714	46783194	48119540	45209244
Q30(%)	93.22	93.1	93	92.95	93.26	93.03	93.15	93.26	93.28
GC content(%)	49.03	48.98	49.06	48.94	48.82	48.93	49.2	49.18	49.19
Total mapped (%)	89.77 %	89.68 %	89.85 %	89.26 %	89.31 %	89.38 %	85.46 %	85.62 %	85.53 %
Multiple mapped(%)	2.98 %	2.91 %	2.9 %	2.92 %	2.87 %	2.93 %	3.58 %	3.48 %	3.52 %
Uniquely mapped(%)	86.79 %	86.77 %	86.95 %	86.34 %	86.44 %	86.44 %	81.88 %	82.15 %	82.01 %

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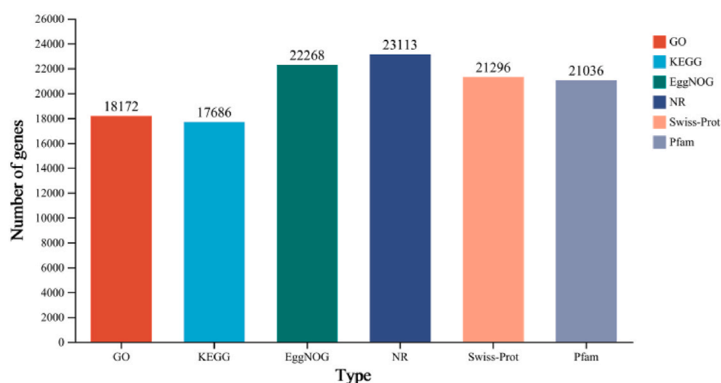

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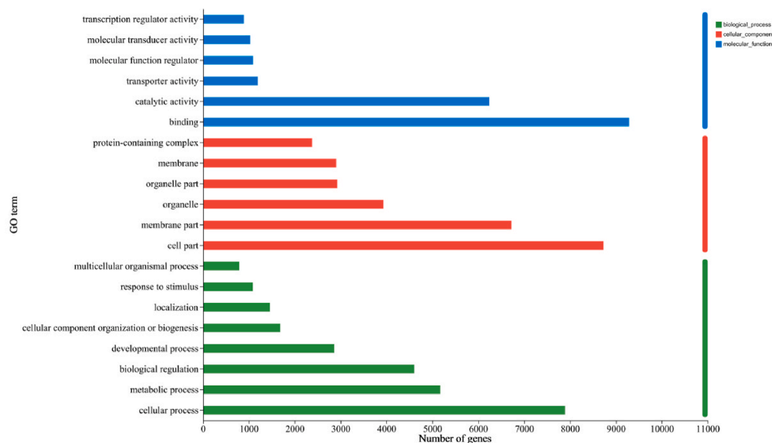

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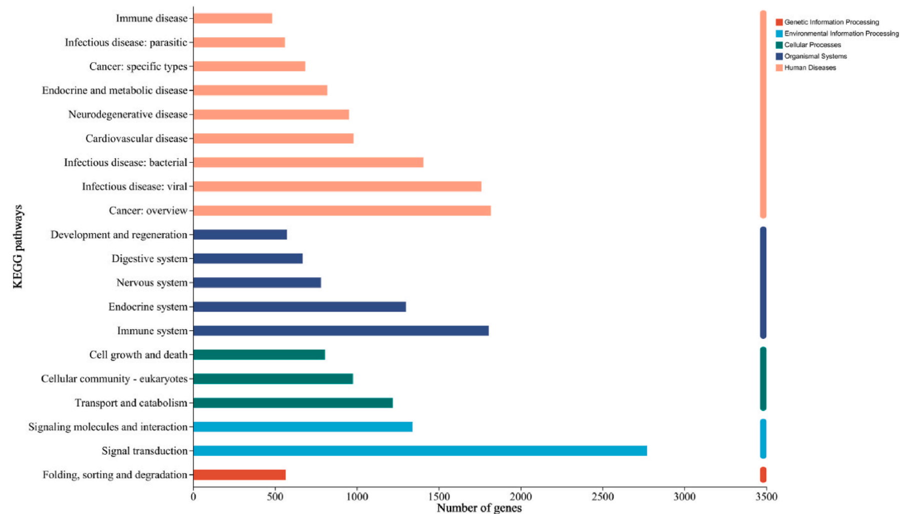


Fig. 2. Functional annotation results of the different Dongting catfish larvae samples (A). Unigenes annotated result of each functional database. (B). GO annotations analysis (Level 2). (C). KEGG annotations analysis.

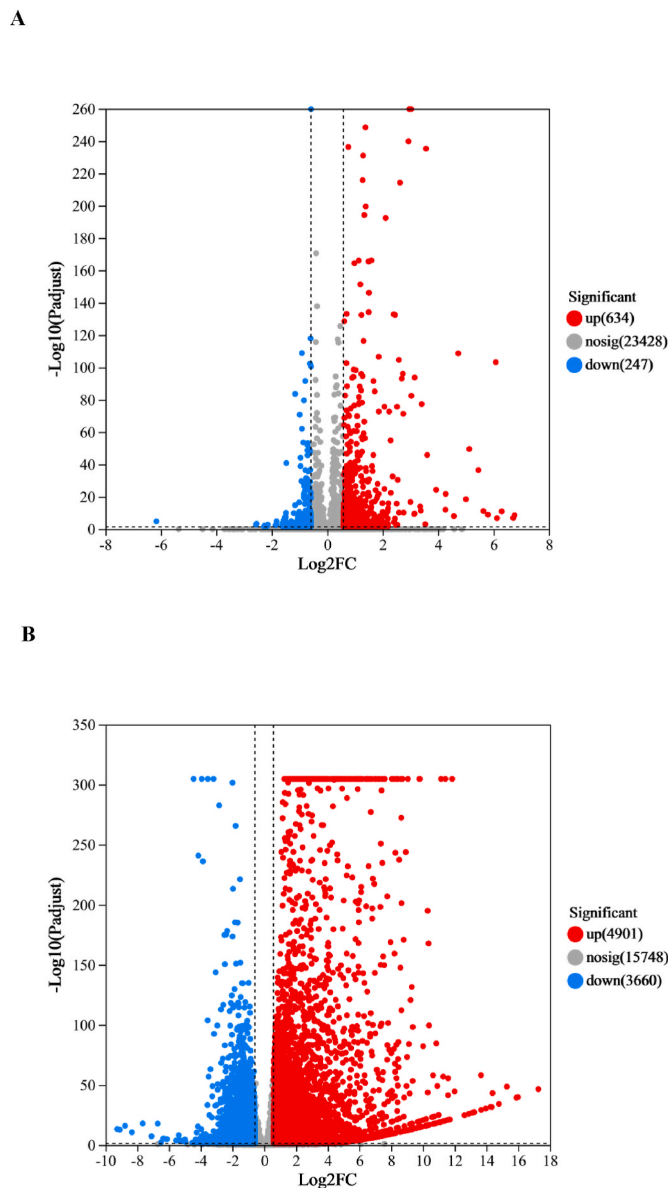


Fig. 3. The changes of DEGs in the different Dongting catfish larvae samples (A). Volcano plot of DEGs in malformed Dongting catfish larvae compared to normal Dongting catfish larvae. (B). Volcano plot of DEGs in normal Dongting catfish larvae after feeding compared to normal Dongting catfish larvae before first food intake.

The 3660 down-regulated differential genes between treated group 2 and control group were mainly involved in nucleus, DNA binding, and intracellular membrane-bounded organelle (Fig. 5B).

3.5. Screening for key DEGs

In order to study the key genes affecting the development of Dongting catfish, the four genes related to growth hormone synthesis, secretion and action were screened between normal and malformed Dongting catfish larvae. The fold change was analyzed and four key DEGs with the highest fold change were screened as *SLA isoform X1* (*somatolactin alpha isoform X1*), *SOCS3* (*suppressor of cytokine signaling 3*), *STAT1b* (*signal transducer and activator of transcription 1b*), *JUNB* (*JunB proto-oncogene*) (Fig. 6A).

In order to study the key genes affecting the nutrient metabolism of Dongting catfish seedlings, the pathways and genes related to digestion and metabolism were screened between seedlings before and after first food intake. Differential genes were obtained for the protein about digestion and absorption pathways and pancreatic secretory pathway, which were screened for *cathepsin D precursor*, *elastase 2 like precursor*, *elastase 2 precursor*, *LPL isoform X1* (*lipoprotein lipase-like isoform X1*), *CTRB1 precursor* (*chymotrypsin B1 precursor*), *CTRL precursor* (*chymotrypsin-like precursor*), *CPA precursor* (*carboxypeptidase A precursor*), *FABP* (*fatty acid-binding protein*), *SLC15A1* (*solute carrier family 15 member 1*), and *CEL tandem duplicate 2 precursor* (*carboxyl ester lipase, tandem duplicate 2 precursor*) (Fig. 6B). Ten key DEGs related to amino acid metabolism were obtained as *ASAOC precursor* (*amiloride-sensitive amine oxidase [copper-containing] precursor*), *PLD3* (*phospholipase D3*), *NDPKs* (*nucleoside diphosphate kinase, mitochondrial*), *trehalase*, *UMP-CMP kinase*, *L-amino-acid oxidase*, *PLB1* (*phospholipase B1, membrane-associated*), *FALDH isoform X2* (*fatty aldehyde dehydrogenase isoform X2*), *PLA2 precursor* (*phospholipase A2 precursor*), and *glucokinase* (Fig. 6C). These DEGs are mainly involved in histidine metabolism, arginine, proline metabolism and tryptophan metabolism.

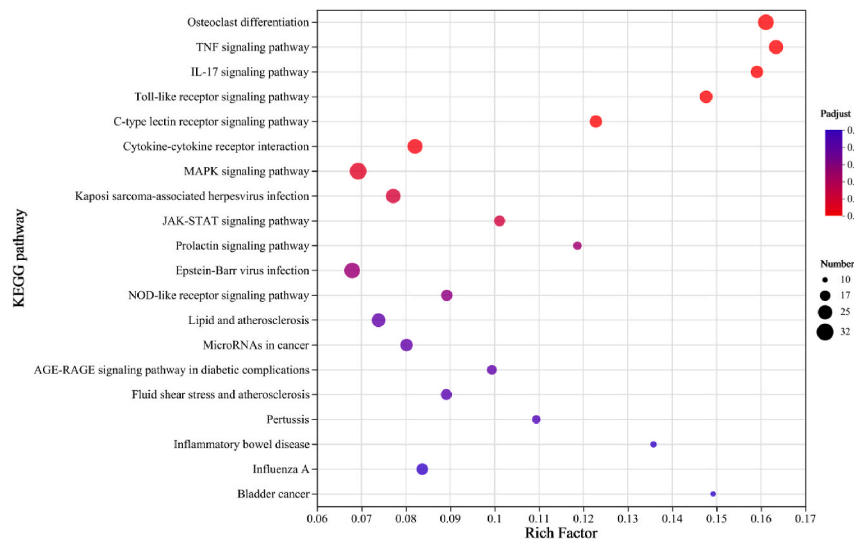
3.6. Validation of transcriptome data by RT-qPCR

Nine DEGs were screened from DEGs of Dongting catfish for RT-qPCR validation, which were *elastase 2 like precursor*, *CTRB1 precursor*, *FABP*, *ASAOC precursor*, *NDPKs*, *trehalase*, *PLB1*, *SOCS3*, *STAT1b*. The expression measured by qPCR was plotted by taking the logarithm with 2 as the base, as shown in Fig. 7, and the trend of the expression of the selected genes was consistent with the results of RNA-seq analysis.

4. Discussion

In this study, we sequenced the transcriptome of normal and malformed Dongting catfish seedlings before first food intake, and normal Dongting catfish seedlings after first food intake to investigate the key genes affecting development and nutrient metabolism in the early seedling stage of Dongting catfish. The key genes affecting the development of Dongting catfish seedlings were explored by analyzing differentially expressed genes between normal and malformed seedlings. The omics data showed the up-regulated pathways were mainly involved in osteoclast differentiation and immune response, such as TNF signaling pathway, IL-17 signaling pathway and Toll-like receptor signaling pathway. Osteoclasts play an important role in bone metabolism, and the differentiation and function of osteoclasts are regulated by a variety of cytokines and growth factors [14]. In addition, the down-regulated pathways were mainly involved in digestion and absorption and metabolism. In this study, four differential genes (*SLA isoform X1*, *SOCS3*, *STAT1b*, *JUNB*) related to growth hormone synthesis, secretion and action were screened from the DEGs between the two groups. SLA is a close relative of growth hormone (GH) secreted by the central pituitary gland in fish. In addition, Sasano Y found that *SLA* significantly affected the skin color phenotype of medaka [15]. The SOCS family is a key negative regulator of cytokine and growth factor signaling [16], and plays an important role in regulating a variety of signaling pathways in the body, including immunity and development [17].

For the pathways related to fish nutrient metabolism, ten key DEGs (*cathepsin D precursor*, *elastase 2 like precursor*, *elastase 2 precursor*, *LPL isoform X1*, *CTRB1 precursor*, *CTRL precursor*, *CPA precursor*, *FABP*, *SLC15A1*, and *CEL tandem duplicate 2 precursor*) related to protein digestion and absorption pathway and pancreatic secretion pathway were screened. Cathepsin D (CD) is a soluble lysosomal aspartic endopeptidase synthesized in the crude endoplasmic reticulum as a histone D precursor zymogen involved in nonspecific protein degradation. CD has an important role in the regulation of apoptosis, interacting with other important molecules and influencing cell signaling. It's not essential for seedling development, but is essential for seedlings (postnatal) tissue homeostasis [18]. It has been demonstrated that CD has protease



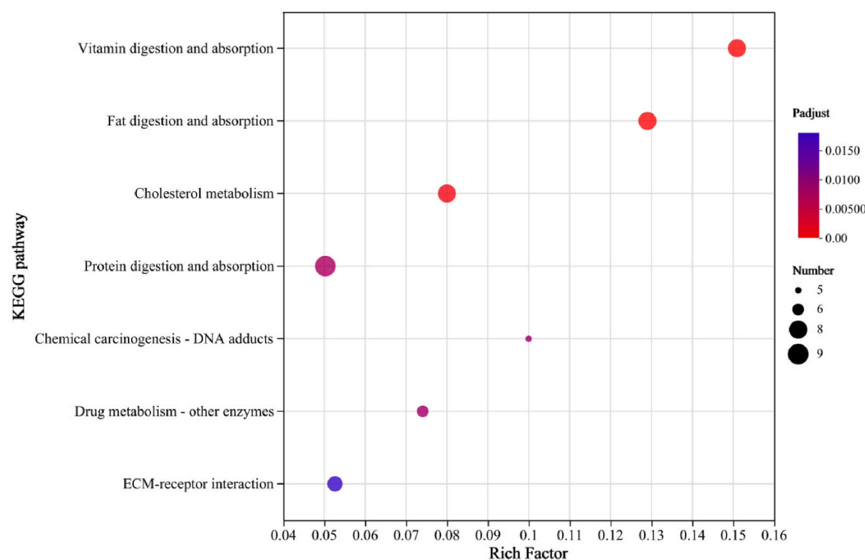


Fig. 4. KEGG pathway enrichment analysis of DEGs in malformed Dongting catfish larvae compared to normal Dongting catfish larvae. (A). Significantly enriched pathways of up-regulated DEGs in malformed Dongting catfish larvae compared to normal Dongting catfish larvae. (B). Significantly enriched pathways of down-regulated DEGs.

activity in fish [19–21]and plays an important role in the fish innate immune system [22]. Dong [23]found that the mRNA expression of CD gene showed a gradual increase during the development of grass carp fry. Elastase is stored as an zymogen in the pancreas of catfish [24], and it is closely related to the digestive and immunophysiology of grass carp. Xu [25] applied molecular biology techniques for gene cloning, sequence analysis, and tissue expression studies with *elastase*. LPL is an important digestive enzyme in fish and plays an important role in the absorption of nutrients as well as energy gain in fish [26]. FABPs are key genes in fat metabolism in *P. vachellii* [6]. It plays an important role in the uptake, transport, and oxidation of fatty acids and their derivatives in animals. Moreover, it is also considered a key fatty acid carrier protein in cells [27].

Fish require the participation and cooperation of a variety of functional enzymes in the metabolic process, such as amylase, protease, lipase, esterase, etc. In this study, ten key DEGs (*ASAOC precursor*, *PLD3*, *NDPKs*, *trehalase*, *UMP-CMP kinase*, *L-amino-acid oxidase*, *PLB1*, *FALDH isoform X2*, *PLA2 precursor*, and *glucokinase*) related to amino acid metabolism were identified between the Dongting catfish seedlings before and after first food intake. Partial of these genes had been reported to be involved in nutrient metabolism. For example, shao [6] screened key genes related to amino acid metabolism, including *ASAOC*

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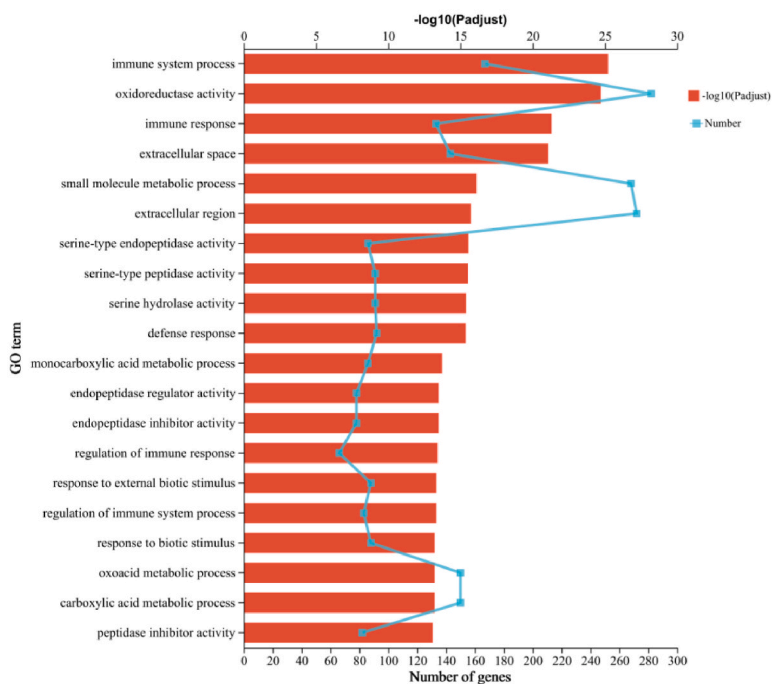

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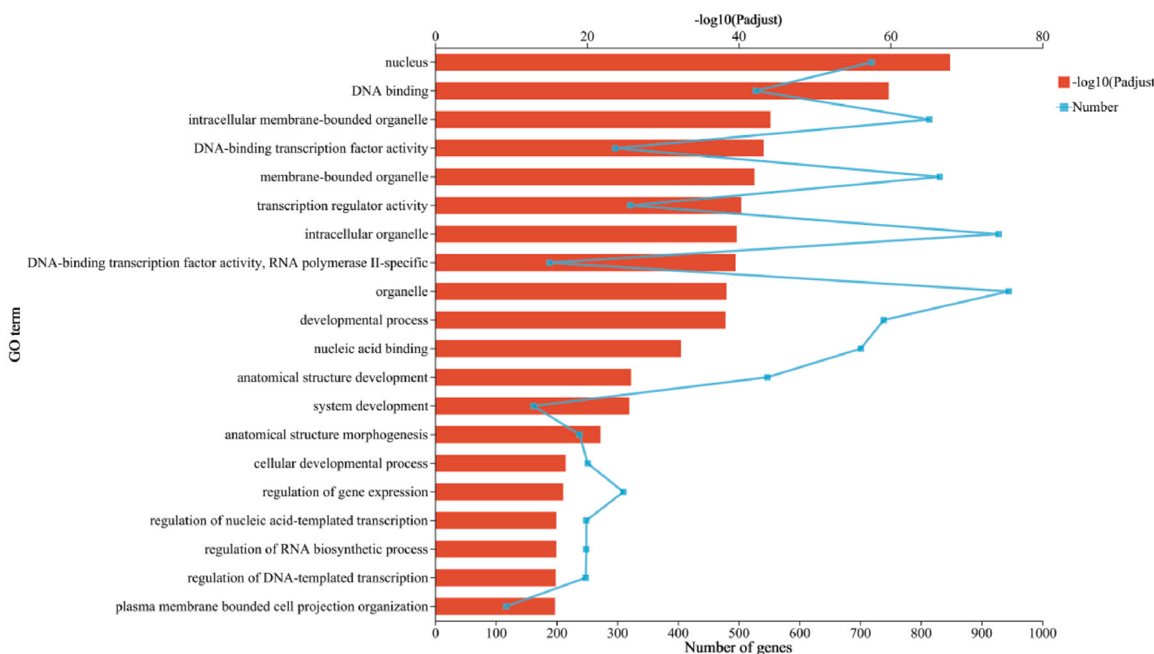

Fig. 5. GO enrichment analysis of DEGs between normal Dongting catfish larvae before and after first food intake. (A). Top20 significantly enriched GO terms of up-regulated DEGs in normal Dongting catfish larvae after first food intake compared to normal Dongting catfish larvae before first food intake. (B). Top20 significantly enriched GO terms of down-regulated DEGs.

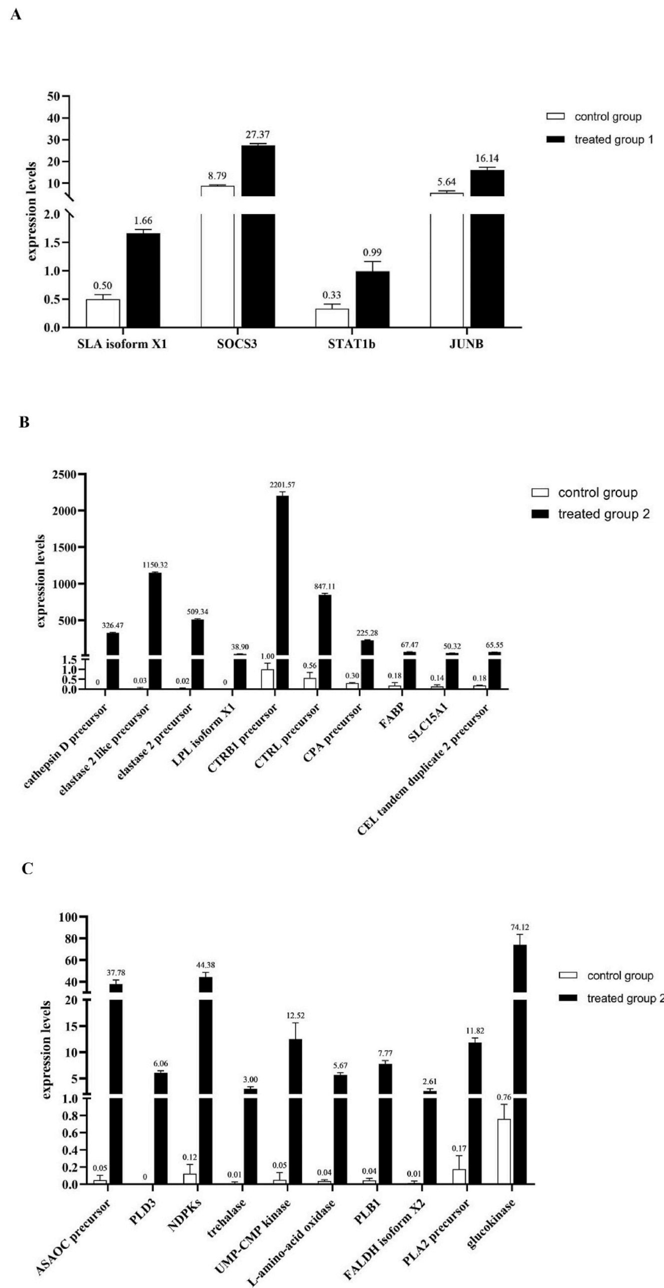

Fig. 6. DEGs in the different Dongting catfish larvae samples (A). DEGs involve in growth. (B). DEGs involve in digestion. (C). DEGs involve in metabolism.

precursor, *trehalase*, *PLB1*, *PLA2 precursor*, *glucokinase* from intestinal samples of *P.vachellii*, under the influence of different feeding times. NDPKs are highly evolutionarily conserved from yeast to humans, suggesting that members of this protein family have critical cellular and developmental functions. NDPKs are essential for the regulated uptake of objects for example micronutrients, and this evolutionarily conserved endocytosis contributes to nutrient supply, energy consumption and receptor internalization [28]. L-amino-acid oxidase, a flavonoid enzyme involved in amino acid metabolism, is widely distributed in a variety of organisms from bacteria to mammals [29]. In addition, I-amino acid oxidases extracted from several fish species has been found to be bioactive molecules with strong antimicrobial and antiparasitic activities [30–33]. The expression of *cPLA2* (*cytosolic PLA2*) gene, a key enzyme in phospholipid catabolism, showed regular changes during the early metamorphosis from larvae to juveniles of *Larimichthys crocea* (*L. croceal*), which may be important for the body to maintain the dynamic

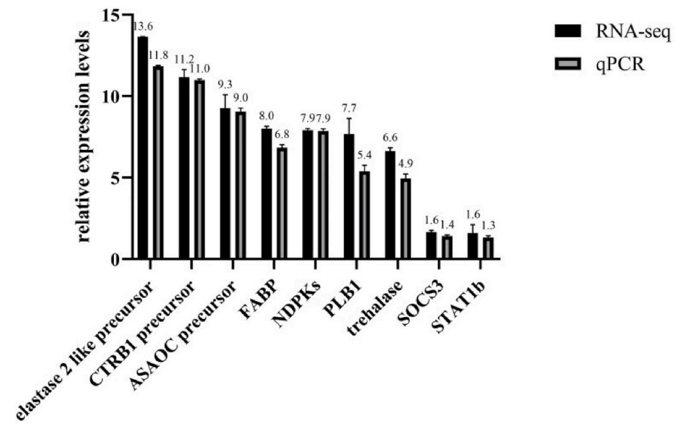

Fig. 7. Validation of DEGs by qRT-PCR
Fig. 7. Validation of DEGs by qRT-PCR. *SOCS3* and *STAT1b* are DEGs in treated group 1 vs control group. *Elastase 2 like precursor*, *CTRB1 precursor* and *FABP*, *ASAOC precursor*, *NDPKs*, *trehalase* and *PLB1* are DEGs in treated group 2 vs contrrol group.

balance of phospholipids in the body, and to maintain the fluidity of the cell membrane. To some extent, the trend of *cPLA2* gene expression in larvae and juveniles can be a measure of the development of *L.croceal* digestive system [34]. Glucokinase (GK) enzyme is essential for the utilization of dietary glucose as it is the first enzyme that phosphorylates excess glucose in different key tissues such as pancreas and liver [35].

Feeding is a direct way of obtaining nutrients and it is the basis of nutrition, so the condition of feeding directly affects the nutritional status of fish, which influences the growth, development, reproduction and survival of fish [36]. The investigation of key DEGs affecting the development and nutrient metabolism of Dongting catfish provides a theoretical basis for the development of artificial breeding feeds. Nutritional requirements of Dongting catfish are satisfied by artificial breeding feeds. It has been reported that nutritional deficiency and starvation were the key induce factor for cannibalism in the early seedling stage of carnivorous fishes. The results in this study provide information for the development of specific forage for Dongting catfish seedlings, which might fulfill the nutritional requirements and reduce starvation of Dongting catfish seedlings, avoiding the cannibalism and thus improve the survival rate of Dongting catfish seedlings in artificial breeding.

In this study, the transcriptomes of Dongting catfish seedlings at different states were analyzed by transcriptome sequencing technology. The key genes and signaling pathways that regulate the development and nutrient metabolism of Dongting catfish seedlings were explored, which provided important references for the molecular marker assisted selection on Dongting catfish and the development of composite catfish feeds to improve the survival rate of Dongting catfish seedlings.

Ethics statement

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals, and was approved by the Institutional Animal Care and Use Committee of Hunan Normal University. The fishes were anesthetized with MS-222 before been euthanized, and all efforts were made to minimize suffering.

CRediT authorship contribution statement

Yanfang Wu: Writing – original draft, Investigation, Formal analysis, Data curation. **Jiaxin Fu:** Methodology, Formal analysis. **Jixiang Chu:** Investigation, Data curation. **Jun Yan:** Visualization, Investigation. **Jun Xiao:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Can Yang:** Validation, Formal analysis.

Rui Song: Validation, Resources. **Hao Feng:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

Hao Feng is an editorial board member for Reproduction and Breeding and was not involved in the editorial review or the decision to publish this article. All authors declare that they have no conflict of interest.

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References

[1] S. Ditcharoen, et al., Genomic organization of repetitive DNA elements and extensive karyotype diversity of silurid catfishes (Teleostei: Siluriformes): a comparative cytogenetic approach, Int. J. Mol. Sci. 20 (14) (2019).
[2] Qing Han, et al., Analysis of reproductive biological characteristics of *Silurus asotus*, South China Fisheries Science (S5) (2023) 154–161 (in Chinese).
[3] Qing Han, et al., Test of artificial propagation and fry culture on catfish(*Silnrus asotus*) in Dongting Lake, Hubei Agric. Sci. (S11) (2009) 2801–2804 (in Chinese).
[4] N.E. Kertaoui, I. Lund, H. Assogba, et al., Key nutritional factors and interactions during larval development of pikeperch (Sander lucioperca), Sci. Rep. 9 (1) (2019).
[5] Xiaobai Li, et al., The strategy of RNA-seq, application and development of molecular maker derived from RNA-seq, Chinese Journal of Cell Biology 35 (5) (2013) 720–726, 740 (in Chinese).
[6] Ting Shao, Effect of Feeding Time on the Expression of Genes Related to Related to Protein and Lipids Metabolism in Pelteobagrus Vachellii, Sichuan Normal University, 2018 (in Chinese).
[7] J. Gu, et al., fastp: an ultra-fast all-in-one FASTQ preprocessor, Bioinformatics 34 (18) (2013) 884–890.
[8] S. Zheng, W. Tao, et al., Characterization of the male-specific region containing the candidate sex-determining gene in Amur catfish (Silurus asotus) using third-generation and pool-sequencing data, Int. J. Biol. Macromol. 248 (2023) 125908. Sep. 1.
[9] D. Kim, et al., HISAT: a fast spliced aligner with low memory requirements, Nat. Methods 12 (4) (2015) 357–360.
[10] M. Pertea, et al., StringTie enables improved reconstruction of a transcriptome from RNA-seq reads, Nat. Biotechnol. 33 (3) (2015) 290–295.
[11] B. Li, et al., RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome, BMC Bioinf. 12 (1) (2011) 323, 323.
[12] M.I. Love, et al., Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2, Genome Biol. 15 (12) (2014) 550.
[13] L. Wang, et al., DEGseq: an R package for identifying differentially expressed genes from RNA-seq data, Bioinformatics 26 (1) (2009) 136–138.
[14] X.C. Zhuang, J.C. Su, Transforming Growth factorβ1/Smad Signaling Pathway in Osteoclast Differentiation and Development:research Progress, vol. 5, Academic Journal of Naval Medical University, 2023, pp. 622–626 (in Chinese).
[15] Y. Sasano, et al., Reassessment of the function of somatolactin alpha in lipid metabolism using medaka mutant and transgenic strains, BMC Genet. 13 (1) (2012) 64.
[16] B. Wang, et al., Insights into the evolution of the suppressors of cytokine signaling (SOCS) gene family in vertebrates, Mol. Biol. Evol. 36 (2) (2019) 393–411.
[17] H.J. Jin, et al., Global identification and comparative analysis of SOCS genes in fish: insights into the molecular evolution of SOCS family, Mol. Immunol. 45 (5) (2008) 1258–1268.
[18] P. Benes, et al., Cathepsin D–many functions of one aspartic protease, Crit. Rev. Oncol.-Hematol. 68 (1) (2008) 12–28.
[19] S.T. Jiang, et al., Purification and characterization of a proteinase identified as cathepsin D from tilapia muscle (Tilapia nilotica X Tilapia aurea), J. Agric. Food Chem. 39 (9) (1991) 1597–1601.
[20] P.A. Wang, et al., Cathepsin D from Atlantic cod (Gadus morhua L.) liver. Isolation and comparative studies, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 147 (3) (2007) 504–511.
[21] Y. Jiao, et al., Characterization and expression of sweetfish (Plecoglossus altivelis) cathepsin D, Zool. Res. 35 (4) (2014) 294–299.
[22] W. Shina, et al., Advance on lysozyme and cathepsin of fish, Guangxi Sci. 25 (1) (2018) 32–35 (in Chinese).
[23] Qingdain Dong, Molecular Cloning and Functional Identification of MHC II B and Cathepsin D Gene of Grass Carp (Ctenopharyngodon idellus), Shandong Agricultural University, 2014 (in Chinese).
[24] R. Yoshinaka, et al., Purification and some properties of elastase from the pancreas of catfish, Nsugaf 48 (4) (1982) 573–579.
[25] Bo Xu, Cloning and Expression Analysis of Elastase cDNA from Grass Carp (Ctenopharyngodon Idella), Hunan Agricultural University, 2009 (in Chinese).
[26] Hongliang Yu, Molecular Studies of Two Kinds of Isozyme in Different Feeding Habits Fishes and the Study on LPL Gene of Grass Carp, Dalian Ocean University, 2016 (in Chinese.
[27] J. Luiken, et al., Cellular fatty acid transport in heart and skeletal muscle as facilitated by proteins, Lipids 34 (1999) 169–175.
[28] Takacs-Vellai, et al., Nucleoside diphosphate kinases (NDPKs) in animal development, Cellular & Molecular Life Sciences Cmls 72 (2015) 1447–1462.
[29] A.L. Hughes, Origin and diversification of the L-amino oxidase family in innate immune defenses of animals, Immunogenetics 62 (2010) 753–759.
[30] Y. Kitani, et al., Identification of an antibacterial protein as L-amino acid oxidase in the skin mucus of rockfish Sebastes schlegeli, FEBS J. 274 (2007) 125–136.
[31] F. Wang, et al., The serum of rabbitfish (Siganus oramin) has antimicrobial activity to some pathogenic organisms and a novel serum L-amino acid oxidase is isolated, Fish Shellfish Immunol. 30 (2011) 1095–1108.
[32] K. Kasai, et al., Novel Lamino acid oxidase with antibacterial activity against methicillin-resistant Staphylococcus aureus isolated from epidermal mucus of the flounder Platichthys stellatus, FEBS J. 277 (2010) 453–465.
[33] Y. Nagashima, et al., Isolation and cDNA cloning of an antibacterial L-amino acid oxidase from the skin mucus of the great sculpin Myoxocephalus polyacanthocephalus, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 154 (2009) 55–61.
[34] Shuoheng Feng, et al., Cytosolic phospholipase A_2 gene: cloning and expression level change with growth development of large yellow croaker(larmichthys crocea) larvae, Chinese Journal Of Animal Nutrition. 26 (9) (2014) 2883–2891 (in Chinese).
[35] S. Panserat, et al., Nutritional regulation of glucokinase: a cross-species story, Nutr. Res. Rev. 27 (1) (2014) 21–47.
[36] Mingde Li, Fish Morphology and Biology, Xiamen University Press, Xiamen, 2011 (in Chinese).