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# Brain and intestine transcriptome analyses and identification of genes involved in feed conversion efficiency of Yellow River carp (*Cyprinus carpio haematopterus*)☆,☆☆

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ABSTRACT

Feed cost is one of the largest variable input costs in aquaculture. In general, dietary energy is directed toward protein deposition and muscle growth. However, most of the dietary energy will be used to support body maintenance if feed conversion efficiency (FCE) is relatively low. Thus, improving feed efficiency will make great contributions to the productivity, profitability, and sustainability of fish farming industry. In the present study, we performed comparative transcriptome analyses of brain and intestine tissues from extreme FCE groups and identified differentially expressed genes (DEGs) and regulatory pathways that may be involved in FCE and related traits in one of the important common carp strains of China, the Yellow River carp (*Cyprinus carpio haematopterus*). Totally, 557 and 341 DEGs between high and low FCE groups were found in brain and intestine tissues, respectively, including 66 up- and 491 down-regulated in brain of high FCE group and 282 up- and 59 down-regulated in intestine of high FCE group ($p < 0.01$, FDR $< 0.05$). These DEGs are mainly involved in metabolic pathway, organismal system and genetic information processing pathway. Finally, 20 key DEGs potentially involved in FCE of Yellow River carp were identified from these two tissues. Expression patterns (up or down regulation in the high or low FCE group) of these DEGs have been successfully validated by quantitative real-time PCR of 10 unigenes. This study provides insights into the genetic mechanisms underlying feed efficiency in Yellow River carp and supplies valuable FCE-related candidate gene resources for potential molecular breeding studies.

## 1. Introduction

Feed conversion efficiency (FCE) is an significant measurement for livestock in converting feed into live weight, and also plays vital roles in feed efficiency for many cultured species (Wilkinson, 2011). High feed efficiency contributes a lot to the reduction of input costs and the increase of financial returns for producers. Feed cost is one of the major input costs, often accounting for 30% - 70% of the variable costs in animal production systems (Doupé and Lymbery, 2010). As we all know, even animals from the same family rear under the same environmental conditions, their body weight gains vary from each other because of different FCE performance (Singh et al., 2014). Improvement in FCE performance means reducing the amount of feed resources needed to produce meat and contributing to environmental sustainability.

Aquaculture has risen from relative obscurity to become a major component of the global food system, providing more than half of all fish destined for direct consumption by humans worldwide (FAO, 2016). However, increasing aquatic production is linked to escalating environmental degradation (Henriksson et al., 2018). Thus, as demand for aquatic production grows, there is an urgent need to develop the sustainable intensification of aquaculture (Engle et al., 2017). Improvement in FCE performance of aquatic animals will potentially help human to find a golden balance between the friendly environment and financial returns. FCE performance is a heritable trait for fish, and very high genetic correlations (often from 0.80 to 0.95) are found between growth rate and feed conversion (Andersen et al., 1977; Archer et al., 1999; Crawford et al., 2008; Zhang et al., 2017). Thus, it is a feasible

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method to develop the sustainable intensification culture by selective breeding fish with high FCE performance.

The brain and intestine tissues of vertebrates play important roles in growth and metabolism. As part of the central nervous system (CNS), the brain contributes a lot to the alteration of food intake (Tannenbaum and Goltzman, 1985). Feed efficiency could be influenced by feed intake to a large extent (Ogata et al., 2002), so fish with significantly different FCE performance may be reflected by RNA expression profiles in brain. In addition, as a major metabolic organ, the intestine of zebrafish harbours a rich collection of genes involved in metabolism, molecular transport and localization and catalytic activities (Rainieri et al., 2018). The humoral factors produced by brain and intestine tissues operate in parallel to direct digestion process (secretion of digestive enzymes, and motility), to regulate nutrient and ion/water absorption, to influence the secretion of other hormones, to modulate drinking and feeding, and to regulate metabolism, growth and other essential vital physiological processes of other tissues and organs (Takei and Loretz, 2010). As a result, there are numerous factors that can impact FCE performance of the brain and intestine tissues, which can be evaluated using the transcriptomic approach (Martin et al., 2016). Compared with the previous study, both brain (a major neural organ) and intestine (a critical target organ) tissues associated with FCE performance are necessary to be detected to identify the key pathways and genes comprehensively.

The Yellow River carp (*Cyprinus carpio haematoperus*) is an important aquaculture fish in north-central China. It has great economic value because of its nutrient content and delicious taste (Wang et al., 2018). However, the natural populations are declining because of excessive exploitation and habitat fragmentation. To date, few genetic and genomic studies were performed for Yellow River carp (Wang et al., 2018). Moreover, existing literatures about improving feed efficiency of fish focused on external condition aspects, such as changing feed ingredients (Dumas et al., 2018) and different feeding ways (Pereira et al., 2018). Just few literatures focused on internal molecular genetic mechanism of FCE performance in fish (Lu et al., 2016; Pang et al., 2017; Pang et al., 2018). So far, no analysis has been reported on internal molecular genetic mechanism of FCE performance in Yellow River carp.

As one of the next-generation sequencing (NGS) platforms, RNA-sequencing (RNA-Seq) technique has increased the sequencing throughput enormously and allowed collection of broad genome coverage and large amount of data cost-effectively (Song et al., 2015). In previous studies, candidate genes associating with FCE and its relevant traits were identified from livestock and poultry by RNA-Seq, such as carboxylesterase 1 (*ces1*), phosphoenolpyruvate carboxykinase 1 (*pck1*) and cardiac muscle myosin heavy chain 6 alpha (*myh6*), involved in several biological functions (Louveau et al., 2016; Zhu et al., 2016). When it comes to fish, few literatures on food intake by transcriptome analyses were reported (Tacchi et al., 2011; Morais et al., 2012; Pang et al., 2018). To date, the only one literature on FCE of crucian carp using transcriptome analysis by our laboratory focused on internal molecular genetic mechanism of FCE performance, which did not focused on feed ingredients and feeding ways (Pang et al., 2018). No transcriptome analysis has been conducted for FCE performance in Yellow River carp. In this study, we performed comparative transcriptome analyses of brain and intestinal tissues in Yellow River carp with extreme FCE performance. Our aim was to identify key genes and biological pathways associating with FCE performance of Yellow River carp, which would shine a light on the genetic mechanisms underlying FCE performance of fish.

## 2. Materials and methods

### 2.1. Sample selection and tissue collection

A total of 120 individuals at the age of 4 months from 4 half-sib families were used in this study, which were generated by parents from Yellow River (Zhengzhou, China) in April 2017. In order to achieve accuracy of feed consumption, these fishes were stocked individually in a series of 0.2 $m^3$ re-circulating aquarium tanks under the same environment. Detailed conditions of the aquarium tanks, such as water temperature (27–28 °C) and dissolved oxygen (7–8 mgL), were maintained throughout the feeding test for four weeks. The experimental fishes were fed with pellet feed thrice a day (9:00 am, 15:00 pm and 21:00 pm) by the same fish-feeder, which contains 34.25% crude protein, 9.93% crude lipid and 7.44% ash, meeting aquaculture industry standard of China. The feeder observed carefully during feeding time and stopped feeding when the fish no longer show apparent behavior of snatching food, which was the criterion of “satiation”. According to this practice, no feed left in the tanks and therefore no waste of feeds would happen in the experiment. Therefore, feed intake of each fish is measured simply by summing up all feed weights it consume during the experiment period. Faeces in each tank were siphoned out daily and a complete water change was made once a week. The individual body weights (BW) were measured at the start and the end of the experiment as initial body weight (IBW) and final body weight (FBW), respectively. The feed conversion efficiency (FCE) index was estimated with the model as follows:

$$FCE = (FBW - IBW)/FI$$

Here FCE = feed conversion efficiency, FBW = final body weight, IBW = initial body weight, FI = total feed intake of each individual.

At the end of the whole experimental period, six fishes with two divergent FCE performance (high FCE group: H1–H3 and low FCE group: L1–L3), were selected for the following study. Detailed information of FCE performance and relevant traits of the two groups was showed in Table 1. Brain and intestine tissues were collected immediately from the six sacrificed fishes (H1–H3 and L1–L3), snap-frozen in liquid nitrogen and then stored at 80 °C, which were used for RNA-Seq analysis. This experiment involved in fishes in this study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Institute of Hydrobiology, the Chinese Academy of Sciences, China (20170303-1). All efforts were made to minimize suffering of the fishes.

### 2.2. Total RNA extraction and transcriptome sequencing

Total RNA of each sample (brain or intestine) was extracted using TRIzol Reagent (Invitrogen, CA, USA) according to the manufacturer's instruction. The RNA degradation and concentration were determined by 1% agrose gel and NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA), respectively. RNA quality was monitored using the Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA) and only samples with an RNA Integrity Number (RIN) value higher than 8.0 were used for RNA library construction. Twelve cDNA libraries were prepared from the brains and intestines of two groups according to the manufacturer's recommendation (NEB, Ipswich, MA, USA). The libraries were then sequenced by GENEWIZ company (Jiangsu, China) using the Illumina sequencing platform 150 bp paired-end (PE) configuration. Raw reads

**Table 1**
Descriptive statistics of feed conversion efficiency and relevant traits in Yellow River carp.

| Groups | FBW (g) | IBW (g) | FI (g) | FCE |
|---|---|---|---|---|
| Low group | 1.76 ± 0.17 | 8.11 ± 1.02 | 14.16 ± 0.87 | 0.448 ± 0.068[a] |
| High group | 3.33 ± 0.79 | 16.73 ± 0.88 | 14.63 ± 0.34 | 0.916 ± 0.013[b] |

Here FCE = feed conversion efficiency, FBW = final body weight, IBW = initial body weight, FI = total feed intake of each individual. a, b: the different superscript lowercase letters within a column mean significant difference, $p < 0.01$.

of fastq format were firstly processed through in-house Perl scripts, which removed all those reads with sequencing adapter and nucleotides in reads with quality value < 20 in both end. In this step, high quality sequences of clean reads were obtained by eliminating reads containing adapter, ploy-N and low-quality reads from raw data. At the same time, Q20, Q30, GC-content and sequence duplication level of the clean data were calculated. All downstream analyses were based on high quality clean reads.

### 2.3. Transcriptome alignment and functional annotation

The clean RNA-seq data were aligned to the reference genome of common carp (https://www.ncbi.nlm.nih.gov/genome/genomes/10839) using Hisat2 (version 2.0.1) with an allowance of two mismatches. Other parameters of Hisat2 (version 2.0.1) were set as default. Sequence reads were discarded when they were mapped to multiple genes or positions. HTSeq (v.0.6.1) was used to estimate gene and isoform expression levels from the pair-end clean data according to the reference file converted from known gff annotation file (Anders et al., 2015). Differentially expressed genes (DEGs) between the two groups (high and low FCE groups) were analyzed by DESeq Bioconductor package. DEGs were screened with the criteria of $|\log2Ratio| \geq 1$ and FDR ≤ 0.05. Annotation of the DEGs was obtained through BLASTN similarity searches against the whole-genome sequence of the common carp. To map the DEGs with Gene Ontology (GO) terms, GO enrichment analysis was conducted by the GOseq R package (Matthew et al., 2010). Significantly enriched GO terms were selected using corrected p-values < 0.05. The Kyoto Encyclopedia of Genes and Genomics (KEGG) orthology database was adopted for pathway analyzes, and the enrichment p-values were adjusted using the Benjamini and Hochberg method.

### 2.4. Validation of RNA-seq results by quantitative real-time PCR (qPCR)

In order to examine the reliability of the RNA-Seq results, 10 DGEs were randomly selected for validation by qPCR. Total RNA from each sample (brain or intestine) was extracted individually from the high- and low-FCE groups. The cDNA was synthesized from 1 μg of total RNA for each sample using Prime-ScriptTM RT reagent Kit (TaKaRa, Dalian, China). 10 pairs of specific primers were designed using Premier 5.0 software (Table S1). QPCR was performed on a StepOneTM Real-Time PCR System (Applied Biosystems, USA). The qPCR reaction solution consisted of 6.5 μL Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.2 μM of each forward and reverse primer, 1.2 μL diluted cDNA and 4.5 μL sterile distilled water. The PCR reaction condition was performed at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, 72 °C for 45 s. RNA samples from each group were run in three times biological replicates and three technical replicates for qPCR. The relative expression levels were normalized toward the internal control gene of *β-actin*, and expression ratios were calculated by using the $2^{-\Delta\Delta CT}$ value method.

## 3. Results

### 3.1. Illumina sequencing and alignment quality assessment

The Illumina sequencing yielded 499.40 million and 408.33 million raw reads from the brain and intestine cDNA libraries in high FCE and low FCE groups, respectively. After filtering the low-quality reads, a total of 498.14 million and 407.29 million clean reads were obtained from the brain and intestine cDNA libraries, respectively. An average of 84.46 million, 81.58 million, 61.55 million and 74.21 million clean reads were obtained from the brain cDNA libraries of high FCE group (HB group), brain cDNA libraries of the low FCE group (LB group), intestine cDNA libraries of high FCE group (HI group) and intestine cDNA libraries of the low FCE group (LI group), respectively. Totally, 37.74 GB, 36.44 GB, 27.51 GB and 33.18 GB of clean bases were generated in HB group, LB group, HI group and LI group, respectively. More than 97% of clean bases had a base accuracy of 99% and > 95% of clean bases had a base accuracy of 99.9%. These clean reads were aligned to the annotated genome of the common carp. A total of 377.77 million and 296.52 million clean reads from the brain and intestine cDNA libraries in high FCE and low FCE groups were successfully mapped, respectively. A total of 313.06 million and 237.52 million clean reads from the brain and intestine cDNA libraries were uniquely mapped (Table 2).

**Table 2**
Summary statistics for sequence quality and alignment information of samples.

| Groups | HB | LB | HI | LI |
|---|---|---|---|---|
| Raw reads | 254,067,000 | 245,335,474 | 185,133,228 | 223,190,094 |
| Total clean reads | 253,392,390 | 244,755,912 | 184,642,746 | 222,643,852 |
| Total bases | 3,774,564,656 | 3,643,775,872 | 2,750,603,255 | 3,317,690,928 |
| Q30% | 95.88% | 95.81% | 96.15% | 95.91% |
| GC% | 49.52% | 46.62% | 49.32% | 49.65% |
| Map read | 192,899,497 (76.13%) | 184,869,949 (75.53%) | 133,798,909 (72.49) | 162,724,839 (73.06%) |
| Unique mapped reads | 158,868,450 (62.69%) | 154,202,128 (62.98%) | 105,971,572 (57.48%) | 131,556,848 (59.05%) |

Here HB = brain cDNA libraries of the high FCE group, LB = brain cDNA libraries of the low FCE group, HI = intestine cDNA libraries of the high FCE group, LI = intestine cDNA libraries of the low FCE group. Q30: the percentage of bases with quality value larger than 30. GC%: the percentage of proportion of guanidine and cytosine nucleotides among total nucleotides.

### 3.2. Differential gene expression analysis

In total, the numbers of DEGs from brain and intestine tissues between high FCE and low FCE groups were as follows: 557 from brain tissues (66 up- and 491 down-regulated in the high FCE group) and 341 from intestine tissues (282 up- and 59 down-regulated in the high FCE group). Compared with DEGs from brain tissues, more up-regulated genes and less down-regulated genes were identified from intestine tissues between high FCE and low FCE groups. When we compared DEGs from brain and intestine tissues, the majority of them were tissue specific, with 27 DEGs being shared in brain and intestine tissues between high FCE and low FCE groups (Fig. 1, Supplementary Table S2). These shared DEGs were associated mainly with electron transport and glycometabolism, such as calcium-binding mitochondrial carrier protein Aralar1 (*aralar1*), succinate dehydrogenase assembly factor 2 (*sdhaf2*), transient receptor potential cation channel subfamily V (*trpv5*), and aconitase (*aco1*).

### 3.3. Functional annotation of DEGs

To elucidate the biological events of the DEGs from brain and intestine tissues, which would be mainly involved in FCE performance of Yellow River carp, GO term enrichment analyzes were conducted (Fig. 2). Under the biological process category in both brain and intestine tissues, cellular process (GO: 0009987), biological regulation (GO: 0065007) and metabolic process (GO: 0008152) have the most abundant GO function items. Within the cellular component category in both brain and intestine tissues, higher percentages of genes were commonly clustered into cell part (GO: 0044464) and membrane part (GO: 0044425). In the molecular function category in both brain and intestine tissues, most genes were assigned to binding (GO: 0005488) and catalytic activity (GO: 0003824). These common GO terms of DEGs from these two tissues might suggest brain and intestine tissues play roles in FCE performance of Yellow River carp cooperatively.

KEGG pathway-based analyses help to identify the biological

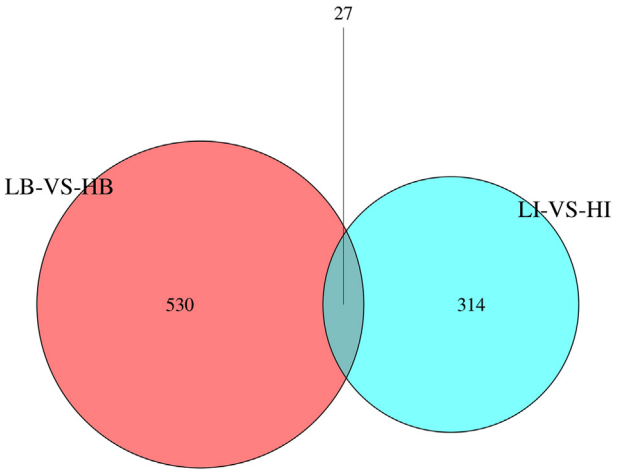


**Fig. 1.** Venn diagrams describing overlap of DEGs between brain and intestine tissues of Yellow River carp.

pathways that are related to DEGs. Totally, 124 and 121 DEGs of brain and intestine tissues between high FCE and low FCE groups were assigned to 86 and 35 KEGG pathways, respectively. Pathway enrichment analysis identified the first twenty-five enriched pathways from brain and intestine tissues, respectively (Fig. 3). Among these pathways, metabolic pathways were the most frequently represented pathways in response to high FCE and low FCE performances of Yellow River carp, followed by organismal systems, diseases, and genetic information processing pathways. These observations disclosed the vital implications of amino acid metabolism, vitamins metabolism, starch and sucrose metabolism, genetic information processing and organismal system in brain and intestine tissues between high FCE and low FCE performances of Yellow River carp.

### 3.4. Identification of DEGs related to FCE performance

19 key pathways associated with the FCE performance of Yellow River carp were identified from the brain and intestine tissues, respectively (Supplementary Table S3). These key pathways were mainly involved in the metabolic-related pathways, such as amino acid, lipid and carbohydrate metabolism. Totally, these important DEGs were selected by following strategies simultaneously, which were considered to be related to the FCE performance of Yellow River carp: (i) 6 important DEGs in 19 key pathways of brain tissues, including synthesis and degradation of ketone bodies (hydroxymethylglutaryl - CoA lyase, *hmgcl*), fructose and mannose metabolism (phosphofructokinase muscle, *pfkm*), glyoxylate and dicarboxylate metabolism (*aco1*); Genetic information processing, for example, RNA transport (eukaryotic translation initiation factor 4B, *eif4b*); and organismal systems, such as salivary secretion (sodium/potassium transporting ATPase subunit beta, *atp1b1*) and insulin signaling pathway (phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit delta, *pik3cd*). (ii) another 15 important DEGs in 19 key pathways of intestine tissues, including succinyl-CoA: 3-ketoacid coenzyme A transferase 1 (*scot1*), fructose-1,6-bisphosphatase (*fbp*), glycogen phosphorylase (*glyp*), alpha-amylase (*amy2*), *aco1*, serine–pyruvate aminotransferase (*spt*), biotinidase (*btd*), phenylalanine-4-hydroxylase (*pah*), medium-chain specific acyl-CoA dehydrogenase (*acadm*), glucose-6-phosphatase catalytic (*g6pc*), alanine-glyoxylate aminotransferase 2 (*agxt2*), trans-activator (*tat*), glycogen synthase 2 (*gys2*), serine hydroxymethyltransferase (*shmt1*), regucalcin (*rgn*); (iii) 1 great potentially DEG (*aco1*) which was commonly identified in these key pathways from both brain and intestine tissues. Then, the 20 important DEGs in total were obtained from brain and intestine tissues, which were considered as key candidate genes involved in FCE performance of Yellow River carp.

Compared with DEGs from low FCE group, 16 and 4 of 20 candidate genes were up-regulated and down-regulated in high FCE group, respectively. The detailed information of these 20 key candidate genes was showed in Table 3. These key candidate genes need further studies to clarify the associations between genes and the FCE performance of

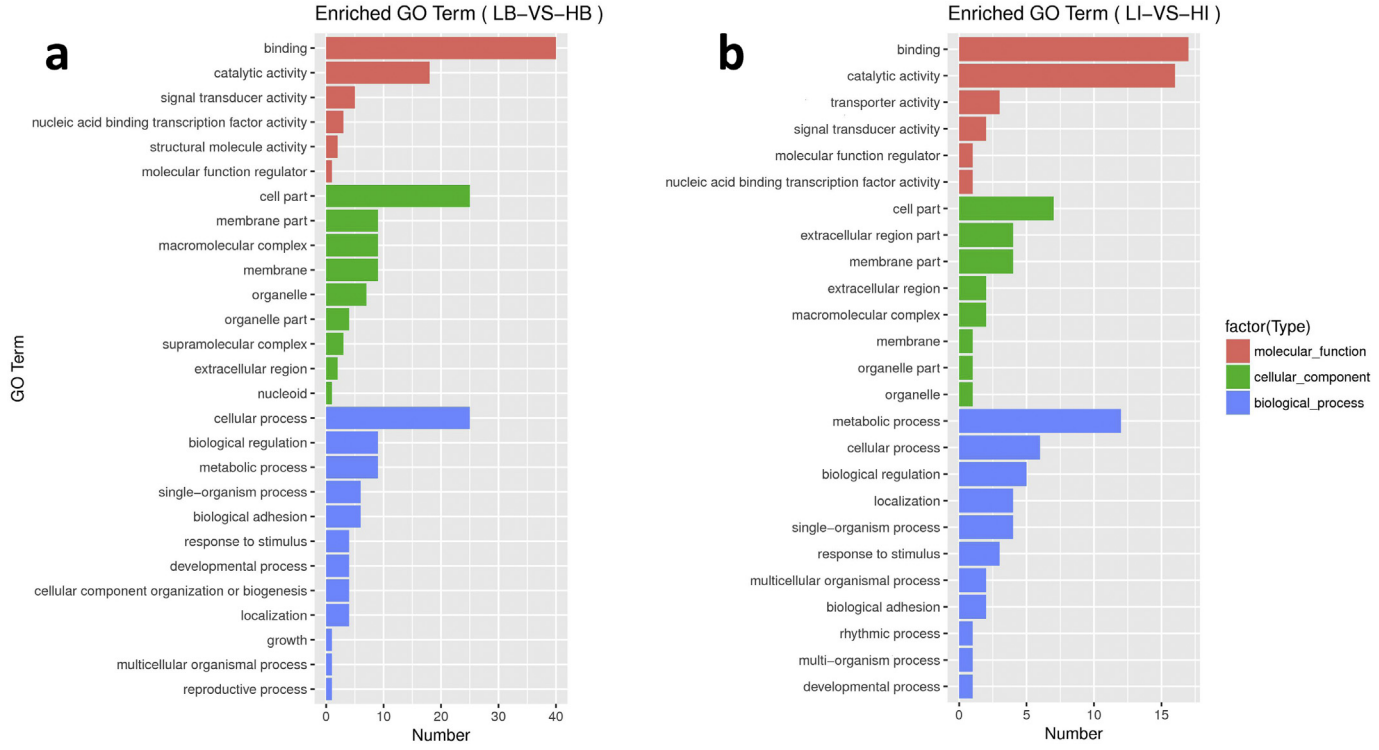


**Fig. 2.** GO classifications of DEGs associating with feed conversion efficiency from brain and intestine samples of Yellow River carp.

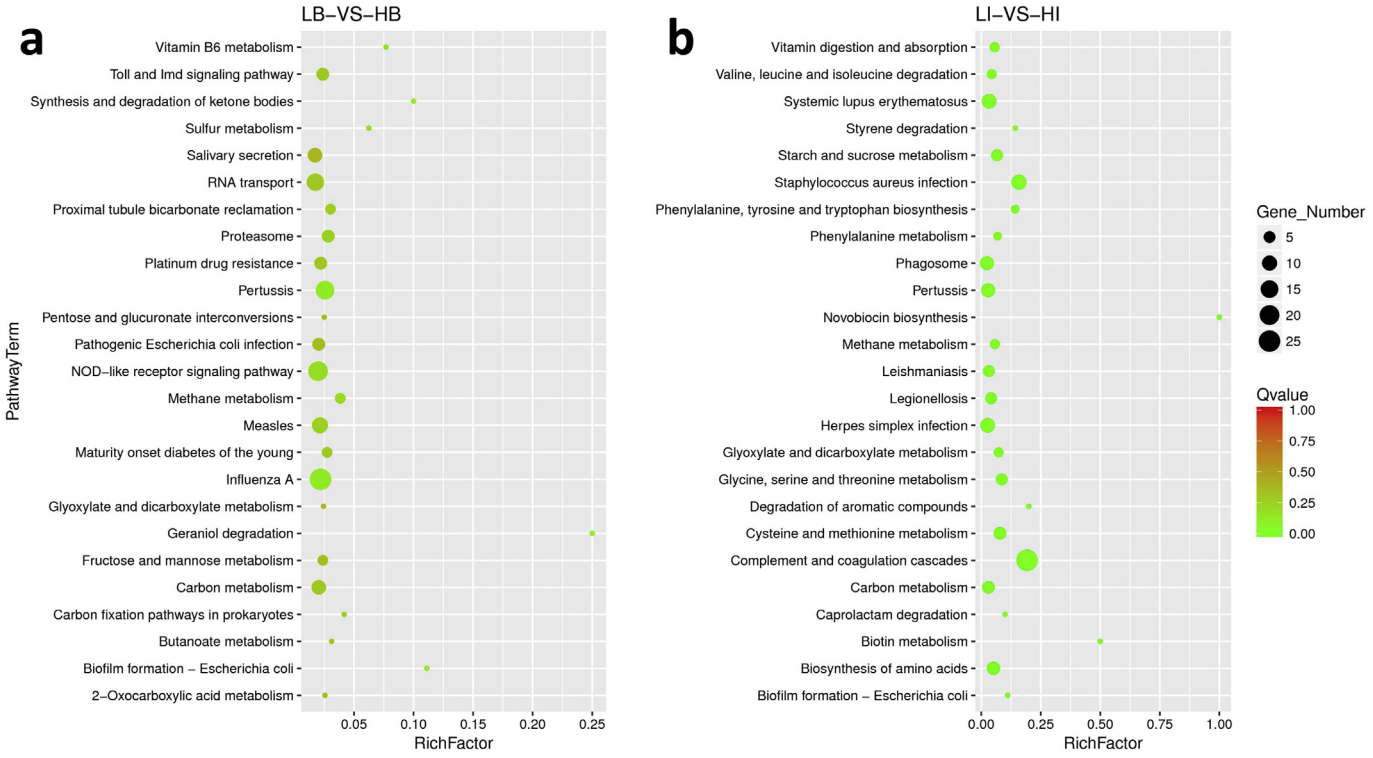


**Fig. 3.** KEGG enrichment analysis of DEGs associated with feed conversion efficiency from brain and intestine samples of Yellow River carp.

**Table 3**
The key candidate genes associated with feed conversion efficiency of Yellow River carp.

| Organization | Gene ID | Gene name | log2 FC | p value |
|---|---|---|---|---|
| Brain | gene45160 | *pfkm* | 1.713 | 6.00E−07 |
| | gene47930 | *aco1* | 1.232 | 2.46E−04 |
| | gene13577 | *atp1b1* | −1.015 | 2.63E−06 |
| | gene36032 | *eif4b* | 1.448 | 1.17E−09 |
| | gene47118 | *pik3cd* | −1.153 | 4.34E−05 |
| | gene23128 | *hmgcl* | −1.273 | 1.24E−04 |
| Intestine | gene34461 | *scot1* | 3.144 | 0 |
| | gene38879 | *fbp* | 2.771 | 1.00E−12 |
| | gene41212 | *glyp* | 2.649 | 2.32E−10 |
| | gene22668 | *amy2* | 2.344 | 1.09E−09 |
| | gene47930 | *aco1* | 2.178 | 7.81E−09 |
| | gene46417 | *spt* | 2.296 | 9.97E−08 |
| | gene60435 | *btd* | 1.228 | 0.0000211 |
| | gene5738 | *pah* | 1.794 | 0.0000296 |
| | gene2168 | *acadm* | 1.451 | 0.0000297 |
| | gene47013 | *g6pc* | 1.654 | 0.0000472 |
| | gene50886 | *agxt2* | 1.588 | 0.0000649 |
| | gene25259 | *tat* | 1.669 | 0.0000844 |
| | gene5398 | *gys2* | 1.501 | 0.000308877 |
| | gene2874 | *shmt1* | 1.357 | 0.000400419 |
| | gene45688 | *rgn* | −1.148 | 0.000012 |

FC is fold change, positive values denote up-regulation in high FCE group compared to low FCE group.

Yellow River carp.

### 3.5. Validation of RNA-seq results by qPCR

QPCR experiment of 10 FCE-related DEGs was performed to validate the RNA-Seq data obtained in this study, including RNA binding protein 4.1 (*rbp4.1*), apolipoprotein M (*apom*), acid phosphatase 5 (*acp5*), protoporphyrinogen oxidase (*ppox*), glutathione *S*-transferase mu 3 (*gstm3*), alpha-amylase-like 2 (*amy2*), tartrate-resistant acid phosphatase type 5 (*trap*), regucalcin (*rgn*), UTP-glucose-1-phosphate uridylyltransferase (*upg1*) and apolipoprotein A1 (*apoa1*). Results obtained by qPCR were compared to data obtained by RNA-seq (Fig. 4). The expression patterns for the 10 DEGs obtained using the two methods were similar, confirming the reliability of the data obtained by RNA-seq.

## 4. Discussion

Efficient production of aquatic animals is one of the most important issues for aquaculture. Efficient production implies the reduction of the amount of feed resources needed to produce meat and contributes to increase production efficiency (Jégou et al., 2016). Although several QTLs and mRNA-seq associated with FCE have been identified in fish (Wang et al., 2012; Pang et al., 2017; Pang et al., 2018), further refined exploration of genes and pathways involved in it is still required. According to the results of previous work on transcriptome analysis of brain tissues for FCE performance in crucian carp (Pang et al., 2018), KEGG analysis mainly classified DEGs into metabolism and genetic information processing, which were validated in our study. Key DEGs identified from the brain tissues of Yellow River carp play important roles in food intake, digestion and absorption, which would benefit to the growth and weight gain of Yellow River carp. Amino acids, such as Pro, Gly, Ala and Trp, are vital substances for energy metabolism in fish or invertebrates (Fair and Sick, 1982; Walton and Cowey, 1982). Amino acid absorption is related to the activation of ATP1b1 by glucocorticoids, which are liberated during stress (Field, 1978). Amino acid absorption is also sodium dependent (Mepham and Smith, 1966) and the atp1b1 controls this sodium concentration (Charney et al., 1975). Moreover, *pik3cd* associated with phosphatidylinositol phosphate shows an important role in signal transmission, and the homologous gene (*pip5kl1*) has also been identified in previous work on FCE performance in common carp using QTL mapping method (Lu et al., 2016). The coordination of these enriched KEGG pathways and DEGs would contribute a lot to the FCE performance of Yellow River carp.

The intestine is the major place where fish digests and absorbs nutrition, which may directly affect feed conversion. Lipoproteins, such as

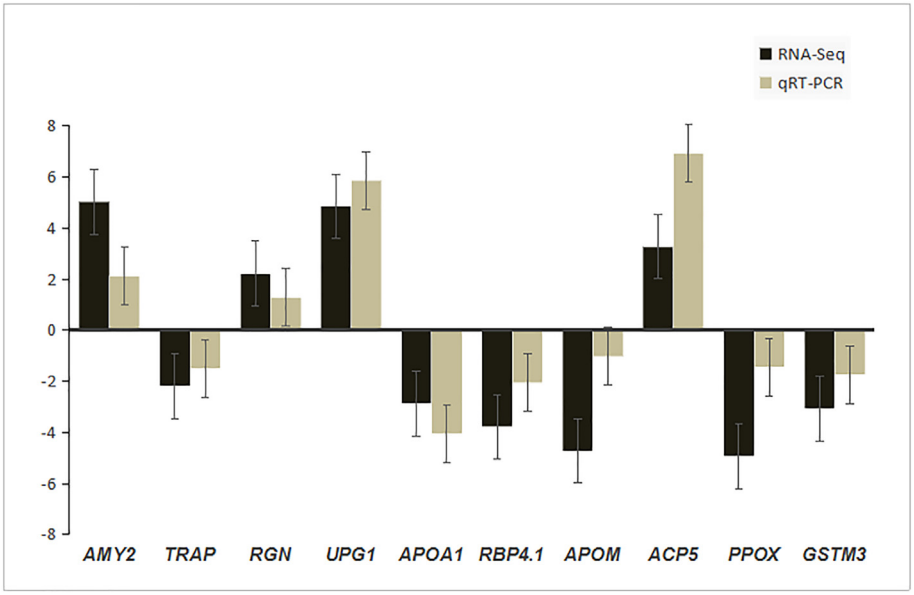


**Fig. 4.** Illustration of qRT-PCR confirmation results for 10 randomly selected DEGs. Gene expression levels were expressed as mean normalized ratios (n = 3, ± SE). β-Actin was used as reference gene. Positive values denote up-regulation high FCE group compared to low FCE group.

the chylomicrons, involved in packaging and transport of dietary lipid from the intestine to other tissues through the circulatory system (Tocher et al., 2008). In this study, some biological pathways associated with FCE performance in intestine tissues were also identified. Novobiocin biosynthesis (*tat*), Cysteine and methionine metabolism (cystathionine beta, *cbs*), Glycine, serine and threonine metabolism (*spt* and *pah*), Biosynthesis of amino acids (*agxt2*, *aco1*, *cbs* and *tat*), and Starch and sucrose metabolism (*glyp* and, *gys2*), were the most significant pathways identified in intestine tissues. Previous studies have shown that the metabolic pathway was significantly associated with both residual feed intake traits (RFI) in pigs and other animals (e.g., energy, carbohydrate and lipid, nucleotide and amino acid) (Do et al., 2014). It has also been reported that energy and lipid metabolism pathways were critically important for feed efficiency in crucian carp (Pang et al., 2017). These pathways regulate the transport and oxidation, digestion and absorption of fatty acid, protein and carbohydrates, which influence energy transformation in the intestine tissues of Yellow River carp.

Carbohydrates are used in fish diets primarily as energy sources for their binding properties, such as starches and pectins (Krogdahl et al., 2015). Considering that it is too redundant to discuss all the key DEGs associated with FCE performance in the intestine tissues, we only select four representative genes, which were mainly metabolic enzymes in the pathways of carbohydrate metabolism. Succinyl-CoA: 3-ketoacid CoA transferase-1 (*scot1*), glucose-6-phosphatase (*g6pc*, *gys2* and *fbp*) play important roles in gluconeogenesis, tricarboxylic acid cycle, pentose phosphate pathway, glycogenesis and glycogenolysis. *Scot* is a mitochondrial enzyme which can catalyze the reversible transfer of coenzyme-A from acetoacetyl-CoA to succinate to form acetoacetate and succinyl-CoA. Hasan et al. (2010) identified that knockdown of *scot* gene inhibited insulin release in rat insulinoma cells. In the control of glycogen metabolism, GYS is the key enzyme showing a high positive correlation with the intracellular concentration of G6PC, which plays the important role of providing glucose during starvation (Van and Gerin, 2002). FBP, a key enzyme of glycolysis-gluconeogenesis, plays important roles in carbohydrate metabolism. Baanante et al. (1991) identified that starvation, re-feeding, and diet composition studies provided a general view of the *fbp* in fish system from which the differences between fish and mammal glycolysis-gluconeogenesis may be ascertained. In this study, these genes (*scot1, g6pc, gys2* and *fbp*) were up-expressed in high FCE group compared with low FCE group, suggesting these genes may be considered as potentially critical candidate genes affecting FCE performance in Yellow River carp.

Aconitase (ACO) is a vital enzyme that catalyzes the isomerization of citrate to isocitrate in the tricarboxylic acid (TCA) and glyoxylate cycles (Wang et al., 2016). Animal cytosolic ACO is able to switch to RNA-binding proteins [IRPs (iron-regulatory proteins)], thereby playing a key role in the regulation of iron homoeostasis (Arnaud et al., 2007). Iron as the major cofactor of proteins involved in essential processes like respiration, DNA replication or nitrogen fixation, is regulated by ACO. Research showed that knock-down of *aco2* gene expression not only created oxidative stress, it also led to a reduction in ATP and NAD production (Dhami et al., 2018). In this study, *aco1* showed a significantly higher expression level in high FCE group than low FCE group in both tissues, indicating that *aco1* plays a significant role in FCE of Yellow River carp.

To confirm the putative results from RNA-Seq, we selected 10 FCE-related DEGs for qPCR. Overall, there was a high agreement and concordance between the expression profile results of RNA-Seq and qPCR, which was similar to some previous results in fish (Żarski et al., 2017; Pang et al., 2018) and revealed the data reliability of RNA-Seq.

In summary, we conducted comprehensive brain and intestine tissues transcriptome analyses in Yellow River carp with extremely low and high FCE performance. Totally, 557 and 341 DEGs between high and low FCE groups were found in brain and intestine tissues, respectively. These DEGs are mainly assigned to metabolic pathway, organismal system and genetic information processing pathway. Finally, 20 key DEGs mainly involved in amino acid, lipid and carbohydrate metabolisms were considered as potential candidate genes affecting FCE performance of Yellow River carp. Our findings provide a basis for comprehensive understanding the genetic mechanisms of FCE performance in fish, which would be beneficial to assist in optimizing current gene models and guide future molecular breeding programs for feed efficiency.

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## Conflict of interest

Authors have no conflict of interest to declare.

## References


Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq-a Python framework to work with high-throughput sequencing data. Bioinformatics 31 (2), 166–169.

Andersen, B.B., Liboriussen, T., Kousgaard, K., Buchter, L., Møller, A.J., 1977. Crossbreeding experiment with beef and dual-purpose sire breeds on Danish dairy cows III. Daily gain, feed conversion and carcass quality of intensively fed young bulls. Livest. Prod. Sci. 4 (1), 19–29.

Archer, J.A., Richardson, E.C., Herd, R.M., Arthur, P.F., 1999. Potential for selection to improve efficiency of feed use in beef cattle: a review. Aust. J. Agric. Res. 50, 147–162.

Arnaud, N., Ravet, K., Borlotti, A., Touraine, B., Boucherez, J., Fizames, C., Briat, J., Cellier, F., Gaymard, F., 2007. The iron-responsive element (IRE)/iron-regulatory protein 1 (IRP1)-cytosolic aconitase iron-regulatory switch does not operate in plants. Biochem. J. 405 (3), 523.

Baanante, I.V., Frutos, P.G.D., Bonamusa, L., Fernandez, F., 1991. Regulation of fish glycolysis-gluconeogenesis: role of fructose 2,6 P2 and PFK-2. Comp. Biochem. Phys. B Biochem. Mol. Biol. 100 (1), 11–17.

Charney, A.N., Kinsey, M.D., Myers, L., Giannella, R.A., 1975. $Na^+$-$K^+$-activated adenosine triphosphatase and intestinal electrolyte transport. J. Clin. Invest. 56, 653–660.

Crawford, G.I., Keeler, C.D., Wagner, J.J., Krehbiel, C.R., Erickson, G.E., Crombie, M.B., Nunnery, G.A., 2008. Effects of calcium magnesium carbonate and roughage level on feedlot performance, ruminal metabolism, and site and extent of digestion in steers fed high-grain diets. J. Anim. Sci. 86 (11), 2998–3013.

Dhami, N., Trivedi, D.K., Goodacre, R., Mainwaring, D., Humphreys, D.P., 2018. Mitochondrial aconitase is a key regulator of energy production for growth and protein expression in Chinese hamster ovary cells. Metabolomics 14 (10), 136.

Do, D.N., Strathe, A.B., Ostersen, T., Pant, S.D., Kadarmideen, H.N., 2014. Genome-wide association and pathway analysis of feed efficiency in pigs reveal candidate genes and pathways for residual feed intake. Front. Genet. 5, 307.

Doupé, R.G., Lymbery, A.J., 2010. Toward the genetic improvement of feed conversion efficiency in fish. J. World Aquacult. Soc. 34 (3), 245–254.

Dumas, A., Raggi, T., Barkhouse, J., Lewis, E., Weltzien, E., 2018. The oil fraction and partially defatted meal of black soldier fly larvae (*Hermetia illucens*) affect differently growth performance, feed efficiency, nutrient deposition, blood glucose and lipid digestibility of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 492, 24–34.

Engle, C.R., Mcnevin, A., Racine, P., Boyd, C.E., Paungkaew, D., Viriyatum, R., Tinh, H.Q., Minh, H.N., 2017. Economics of sustainable intensification of aquaculture: evidence from shrimp farms in Vietnam and Thailand. J. World Aquacult. Soc. 48 (2), 227–239.

Fair, P.H., Sick, L.V., 1982. Serum amino acid concentration during starvation in the prawn, *Macrobrachium rosenbergii*, as an indicator of metabolic requirements. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 73 (2), 195–200.

FAO, 2016. FishStatJ–software for fishery statistical time series. http://www.fao.org/fishery/statistics/en, Accessed date: 22 January 2017.

Field, M., 1978. Corticosteroids, Na-K-ATPase and intestinal water and electrolyte transport. Gastroenterology 75, 317–325.

Hasan, N.M., Longacre, M.J., Seed, A.M., Kendrick, M.A., Gu, H., Ostenson, C.G., Fukao, T., MacDonald, M.J., 2010. Lower succinyl-CoA: 3-ketoacid-coa transferase (SCOT) and ATP citrate lyase in pancreatic islets of a rat model of type 2 diabetes: knockdown of SCOT inhibits insulin release in rat insulinoma cells. Arch. Biochem. Biophys. 499 (1), 62–68.

Henriksson, P.J.G., Belton, B., Jahan, K.M., 2018. Measuring the potential for sustainable intensification of aquaculture in Bangladesh using life cycle assessment. Proc. Natl. Sci. USA 115 (12), 2958–2963.

Jégou, M., Gondret, F., Vincent, A., Tréfeu, C., Gilbert, H., Louveau, I., 2016. Whole blood transcriptomics is relevant to identify molecular changes in response to genetic selection for feed efficiency and nutritional status in the pig. PLoS One 11 (1), e0146550.

Krogdahl, A., Hemre, G., Mommsen, T.P., 2015. Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. Aquac. Nutr. 11 (2), 103–122.

Louveau, I., Vincent, A., Tacher, S., Gilbert, H., Gondret, F., 2016. Increased expressions of genes and proteins involved in mitochondrial oxidation and antioxidant pathway in adipose tissue of pigs selected for a low residual feed intake. J. Anim. Sci. 94 (12), 5042.

Lu, C., Laghari, M.Y., Zheng, X., Cao, D.C., Zhang, X.F., Kuang, Y.Y., Li, C., Cheng, L., Mahboob, S., Al-Ghanim, K.A., Wang, S., Wang, G.L., Sun, J., Zhang, Y., Sun, X.W., 2016. Mapping quantitative trait loci and identifying candidate genes affecting feed conversion ratio based onto two linkage maps in common carp (*Cyprinus carpio* L.). Aquaculture 468, 585–596.

Martin, S.A.M., Dehler, C.E., Król, E., 2016. Transcriptomic responses in the fish intestine. Dev. Comp. Immunol. 64, 103–117.

Matthew, I.R., Pollard, D.J., Frame, J.W., 2010. Development and evaluation of a computer-aided learning package for minor oral surgery teaching. Med. Educ. 32 (1), 89–94.

Mepham, T.B., Smith, M.W., 1966. Regulation of amino acid transport across intestine of goldfish acclimatized to different environmental temperatures. J. Physiol. 184, 619–631.

Morais, S., Edvardsen, R.B., Tocher, D.R., Bell, J.G., 2012. Transcriptomic analyses of intestinal gene expression of juvenile Atlantic cod (*Gadus morhua*) fed diets with camelina oil as replacement for fish oil. Comp. Biochem. Phys. B Biochem. Mol. Biol. 161 (3), 283–293.

Ogata, H.Y., Oku, H., Murai, T., 2002. Growth, feed efficiency and feed intake of offspring from selected and wild Japanese flounder (*Paralichthys olivaceus*). Aquaculture 211, 183–193.

Pang, M.X., Fu, B.D., Yu, X.M., Liu, H.Y., Wang, X.H., Yin, Z., Xie, S.Q., Tong, J.G., 2017. Quantitative trait loci mapping for feed conversion efficiency in crucian carp (*Carassius auratus*). Sci. Rep. 7, 16971.

Pang, M., Luo, W., Fu, B., Yu, X., Zhou, Y., Tong, J., 2018. Transcriptomic profiles of brain provide insights into molecular mechanism of feed conversion efficiency in crucian carp (*Carassius auratus*). Int. J. Mol. Sci. 19 (3), 858.

Pereira, F.A., Ha, N., Antunes, H., Valenti, W.C., Fabregat, T.E.H.P., 2018. Can the polyculture with south American catfish improve the feeding efficiency of rainbow trout culture? Aquac. Int. 26 (2), 487–493.

Rainieri, S., Conlledo, N., Larsen, B.K., Granby, K., Barranco, A., 2018. Combined effects of microplastics and chemical contaminants on the organ toxicity of zebrafish (*Danio rerio*). Environ. Res. 162, 135.

Singh, K.P., Tripathi, N., Singh, M.K., 2014. Effect of gestational exposure to novel antipsychotics on body weight gain in rats. Natl. Acad. Sci. Lett. 37 (3), 227–232.

Song, W., Jiang, K., Zhang, F., Lin, Y., Ma, L., 2015. Transcriptome sequencing, de novo assembly and differential gene expression analysis of the early development of *Acipenser baeri*. PLoS One 10, e0137450.

Tacchi, L., Bickerdike, R., Douglas, A., Secombes, C.J., Martin, S.A., 2011. Transcriptomic responses to functional feeds in Atlantic salmon (*Salmo salar*). Fish Shellfish Immunol. 31 (5), 704–715.

Takei, Y., Loretz, C.A., 2010. The gastrointestinal tract as an endocrine/neuroendocrine/paracrine organ: organization, chemical messengers and physiological targets. Fish Physiol. 30, 261–317.

Tannenbaum, G.S., Goltzman, D., 1985. Calcitonin gene-related peptide mimics calcitonin actions in brain on growth hormone release and feeding. Endocrinology 116 (6), 2685–2687.

Tocher, D.R., Bendiksen, E.Å., Campbell, P.J., Bell, J.G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture 280, 21–34.

Van, S.E., Gerin, I., 2002. The glucose-6-phosphatase system. Biochem. J. 362 (3), 513–532.

Walton, M.J., Cowey, C.B., 1982. Aspects of intermediary metabolism in salmonid fish. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 73 (1), 59–79.

Wang, X.P., Zhang, X.F., Li, W.S., Zhang, T.Q., Li, C., Sun, X.W., 2012. Mapping and genetic effect analysis on quantitative trait loci related to feed conversion ratio of common carp (*Cyprinus carpio*). China Acta Hydrobiol. Sin. 36 (2), 177–196.

Wang, Y.M., Yang, Q., Liu, Y.J., Yang, H.L., 2016. Molecular evolution and expression divergence of the aconitase (ACO) gene family in land plants. Front. Plant Sci. 7, 1879.

Wang, X., Fu, B., Yu, X., Qu, C., Zhang, Q., Tong, J., 2018. Fine mapping of growth-related quantitative trait loci in yellow river carp (*Cyprinus carpio haematoperus*). Aquaculture 484, 277–285.

Wilkinson, J.M., 2011. Re-defining efficiency of feed use by livestock. Bioacoustics 5 (7), 1014.

Żarski, D., Nguyenc, T., Cam, A.L., Montfort, J., Dutto, G., Vidal, M.O., Fauvel, C., Bobe, J., 2017. Transcriptomic profiling of egg quality in sea bass (*Dicentrarchus labrax*) sheds light on genes involved in ubiquitination and translation. Mar. Biotechnol. 19 (1), 102–115.

Zhang, Y., Guo, Z.B., Xie, M., Zhang, Z., Hou, S., 2017. Genetic parameters for residual feed intake in a random population of Pekin duck. Asian Austral. J. Anim. 30, 167–170.

Zhu, F., Yuan, J.M., Zhang, Z.H., Hao, J.P., Yang, Y.Z., Hu, S.Q., Yang, F.X., Qu, L.J., Hou, Z.C., 2016. *De novo* transcriptome assembly and identification of genes associated with feed conversion ratio and breast muscle yield in domestic ducks. Anim. Genet. 46 (6), 636–645.