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RESEARCH PAPER

Liver Transcriptome and Molecular Responses of Giant Gourami (*Osphronemus goramy*) Fed with Different Dietary Carbohydrate Levels

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Abstract

Giant gourami (*Osphronemus goramy*) has been known to have a high ability to utilize high dietary carbohydrates. However, its molecular information related to carbohydrate metabolism is still limited. The present study elucidated Giant gourami's liver transcriptome and molecular responses to high dietary carbohydrates. Two semi-purified diets with different levels of dietary carbohydrate, normal carbohydrate (34%, N34) and high carbohydrate (53%, H53), were offered to the fish for 60 days. A total of 87,430,553 clean reads were collected and assembled into 49,034 cDNA contigs. Annotation was performed, and a total of 33,104 unigenes were obtained. High dietary carbohydrates generally down-regulated the differentially expressed genes (DEGs). The genes expression related to glycolysis and the antioxidant system were significantly upregulated ($P<0.05$). Meanwhile, the genes expression related to insulin signaling and gluconeogenesis were significantly downregulated ($P>0.05$). The results showed that Giant gourami has several adaptabilities for high carbohydrates. The upregulation of the glycolytic, *gpx*, and *noa1* genes, downregulation of insulin signaling, and gluconeogenic genes indicated a good glucose homeostasis maintenance in Giant gourami.

Introduction

Carbohydrate is the cheapest energy source and can spare the use of protein, leading to a reduction in feed cost and negative impacts on the environment caused by metabolic waste from protein deamination (Babaei et al., 2016; Boonanuntanasarn et al., 2018). However, most studies demonstrated that carbohydrate utilization in most aquatic organisms, including aquaculture species relatively low (Li et al., 2016). Kamalam *et al.* (2017) suggested that the low utilization of carbohydrates in aquaculture species is related to several biological aspects, dietary conditions, and environmental factors.

Previous studies reported that the metabolic mechanism of nutrients in aquatic animals could be revealed by studying the regulation mechanism of key metabolic enzymes (Liu et al., 2019; Yan, 2015). Several genes related to nutrient metabolism in fish have been studied in recent years. For example, the glucose metabolism genes have been cloned in fish, including hexokinase (*hk*) (Blin et al., 2000), pyruvate kinase (*pk*) (Yuan et al., 2013), glucokinase (*gck*) (Li et al., 2016; Sari et al., 2021), and glucose transporter 1 (*glut1*) (Liu et al., 2016). However, the traditional molecular cloning method is inefficient and time-consuming, and the regulation mechanism of the whole metabolic pathway cannot be well understood from the macro perspective

(Liu et al., 2019).

RNA-sequencing (RNA-seq) is a next-generation sequencing-based technology that can detect the whole transcriptional activity of the nutrient metabolism provide high accuracy digital information and the most comprehensive information about gene expression (Liu et al., 2019). This method has been used in various research in some aquaculture species, such as rainbow trout (*Oncorhynchus mykiss*) (Hu et al., 2016), Atlantic salmon (*Salmo salar*) (Betancor et al., 2017), grass carp (*Ctenopharyngodon idella*) (Zhao et al., 2019), and common carp (*Cyprinus carpio*) (Zhao et al., 2020). However, study about transcriptome sequencing in omnivorous fish is still scarce.

Giant gourami *Osphronemus goramy* is an omnivorous fish that is one of the primary cultured freshwater fish in Southeast Asia, including Indonesia (Arifin et al., 2019). Due to its high capability to utilize carbohydrates and can directly be fed by fresh plant-based fed, such as giant taro *Alocasia macrorrhiza* (Arifin et al., 2019; Kristanto et al., 2019), the formulated diet for this fish in Indonesia usually contain high carbohydrate to reduce the feed cost. However, excessive carbohydrates have been reported to disturb the metabolic process and suppress antioxidant activity, health status, and growth rate, as reported in several fish (Anand et al., 2018; Li et al., 2012). Therefore, it is urgent to investigate this species's carbohydrate metabolism, which, unfortunately, is still unavailable. In order to provide a theoretical reference for the study of carbohydrate metabolism in omnivorous fish and obtain more abundant molecular information, we conducted a transcriptomic analysis using the RNA-sequencing method. We then focused on investigating the tolerance mechanism of Giant gourami to high carbohydrate by transcriptome and genes expression data.

Material and Methods

Experimental Diets

Two semi-purified diets were formulated to contain two dietary carbohydrate levels, normal carbohydrate (34%, N34) and high carbohydrate (53%, H53), to evaluate the effect of different carbohydrate levels (Table 1). Dextrin and casein were used as carbohydrate and protein sources, respectively. The experimental diets nutritional contents were subsequently determined according to AOAC (1990). All ingredients were carefully weighed, mixed, and added about 30–40% of water to form a stiff dough. The dough was then pelleted into a 2 mm diameter of pellet. Then, the experimental diets were dried using an oven at 45-50°C for 8 hours. After drying, the experimental diets were stored in plastic bags at –20°C until used.

Feeding Trial and Sample Collection

Giant gourami with an initial average body weight of 25.48±0.84 g obtained from a local hatchery were randomly distributed into ten units of aquarium (135 L working capacity) at a stocking density of eight fish per aquarium. Each aquarium was equipped with an individual filtration system and a thermostat set at 28–30°C. During the experimental period, the dissolved oxygen was kept to be not less than 5.0 mg L^{-1}, and the pH value ranged from 6.9–7.3. An additional 50% water exchange was done once a week to ensure good water quality throughout the experiment. Prior to experimentation, the fish were acclimatized to the laboratory condition for a week, during which feeding was done using a commercial feed (47% nitrogen-free extract, NFE; 32% protein) two times a day with an

Table 1 Experimental diets composition (%)

Ingredients	N34	H53
Casein	2.00	24.50
Gelatin	1.00	6.05
Pollard meal	39.25	6.75
Dextrin	0.00	42.20
Soybean meal	30.00	6.50
Meat bone meal	19.75	5.50
Corn oil	1.50	2.00
Fish oil	3.00	3.00
Beef tallow	1.00	1.00
Premix	2.00	2.00
Choline Chloride	0.50	0.50
Proximate analyses (%, dry weight-based)		
Protein	33.70	32.67
Lipid	11.77	6.14
Fiber	7.64	1.89
Ash	12.64	6.37
NFE[a]	34.25	52.92
GE (kcal.kg^{-1})[b]	4398	4577

[a] NFE (Nitrogen-free extract) = 100 – protein – lipid – fiber – ash,

[b] Gross energy; 1 g protein= 5.6 kcal, 1 g carbohydrate= 4.1 kcal, 1 g lipid= 9.4 kcal (Halver & Hardy, 2002).

addition of about 5-7 g of giant taro leaves (*A. macrorrhiza*) (35-40% NFE, 18% protein) as a supplement to improve the fish fitness and health (Kristanto et al., 2019), once a day until apparent satiety. During the experiment, the fish were subsequently fed with the experimental diets until apparent satiety three times a day for a period of 60 days. Fish survival was monitored daily.

On day 60, the fish were weighed individually to assess the weight gain, feed conversion rate, and growth rate; the number of remaining fish is counted to determine the survival rate; and some samples of liver were collected from 6 hours fasted fish, which was set according to the results of a preliminary experiment to determine the peak point of postprandial glycemia. Prior to sample collection, the fish were euthanized with 200 mg L^{-1} of MS-222 (Polar Calm, Indonesia). All experimental animal and rearing procedures were carried out following the ethical guidelines from the Animal Care and Use Committee of IPB University, Indonesia (Grant No. 192-2021 IPB).

RNA Extraction

Liver tissues for RNA-sequencing were bulk collected from five fish in each group at the end of the experiment. The samples were stored in a DNA/RNA shield solution (Zymo Research, USA) −80 °C until further analysis. Total RNA was extracted using TRizol Reagent (Invitrogen, USA) according to the manufacturer's protocol. The RNA quality and purity were analyzed using 1% agarose gel electrophoresis and NanoPhotometer® (IMPLEN, USA), respectively. Integrity and quantity of RNA were analyzed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, USA).

Meanwhile, liver tissues for quantitative real-time polymerase chain reaction (qPCR) analysis were collected from five fish for each group and stored separately in a microtube containing 200 µL of RNA kit extraction. Total RNA was extracted, and its integrity was verified on 1% agarose gel. An aliquot of 50 ng μL^{-1} of total RNA was used for the first-strand cDNA synthesis using ReverTraAce®qPCR RT Master Mix with gDNA Remover kit (Toyobo, Japan).

RNA-seq Library Construction and Sequencing

An aliquot of 1 µg of RNA sample was taken from each group and then analyzed separately using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations for cDNA library preparation. The cDNA was purified with the AMPure XP (Beckman Coulter, USA) to obtain cDNA fragments in length 150-200 base pair (bp). This length was selected for sequencing to better understand the gene expression profile and for suitable assembly of the entire transcriptome de novo. The cDNA library was sequenced using Illumina HiSeq 4000 platform (Illumina, USA) at the Novogene Laboratorium (Beijing, China).

De novo Assembly and Functional Annotation

Raw reads from each library were cleaned by trimming and removing reads containing adapter, poly-N sequences, and low quality (cut-off phred=30). High-quality sequences were selected for subsequent assembly using Trinity software, and the redundancy results were removed using CORSET software. Then, the obtained unigenes were used for annotation. Seven databases were used for that purpose, including NT and NR (Altschul et al., 1997); SWISS-PROT (Buchfink et al., 2015), euKaryotic Orthologous Groups (KOG) (Moriya et al., 2007); PFAM (Punta et al., 2012); Gene Ontology (GO) (Götz et al., 2008); and Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2008). NT annotation used NCBI BLAST with E-value cut-off of $1e^{-5}$ (each unigene shows top10 alignment results); NR, KOG, and SWISS-PROT used Diamond with E-value cut-off of $1e^{-5}$ (each unigene shows top10 alignment results). PFAM used HMMER 3.1 package on hmmscan with E-value cut-off of 0.01; GO used Blast2GO with E-value cut-off $1e^{-6}$; and KEGG used KASS (KEGG Automatic Annotation Server) with E-value cut-off $1e^{-5}$. The biological processes, molecular functions, and cellular components were described using GO annotations of unique assembled unigenes.

Validation of Quantitative Real-time PCR

Fifteen genes related to the carbohydrate metabolism (*irs1, glut2, igf-1, hk, gck, pfk, pk, pepck, fbp,* and *g6pc*) and antioxidant systems (*sod1, cat, gpx,* and *noa1*) were selected to verify the RNA-seq results. The verification was performed by the quantitative real-time polymerase chain reaction method (qPCR). The specific qPCR primers were designed using PrimerQuest Tool (https://sg.idtdna.com//primerquest) based on the RNA-seq sequences (BioSample accession number SAMN19588551). Primers quality was confirmed using IDT (https://www.sg.idtdna.com/calc/analyzer) and primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast) tools. The primers specificity was verified on 1.0% agarose gel and qPCR-melting curve analysis. All primers sequences are listed in Table 2.

The qPCR reaction was performed in the Rotor-Gene 6000 machine (Qiagen, USA) with a reaction mixture of 10 µL of SensiFAST SYBR®NO-ROX (Bioline, UK), 0.8 µL of each primer (10 µM), 4.4 µL nucleases-free water, and 4 µL of cDNA template (5 ng μL^{-1}). All reactions were run with no-template control and were prepared using filtered microtip (Genmax Biotech, China) to prevent contamination. The qPCR protocol was initiated at 95°C for 5 min for initial DNA denaturation; followed by 40 cycles of three-step amplification program (5 s at 95°C; 15 s at 60°C; 15 s at

Table 2. The qPCR primer used in this study

Gene abbrev	Gene name	Sequence (5'-3')	Amplicon size (bp)
β-actin	β-actin	F: ACC GGA GTC CAT CAC AAT ACC AGT R: GAG CTG CGT GTT GCC CCT GAG	192
irs1	Insulin receptor substrate 1-β-like	F: GGC AGG CAG TGA CTA TAT GAA R: GGG CTT GCT GTT GAG AAC TA	113
glut2	Solute carrier family 2, facilitated glucose transporter member 2	F: GCA ACT GGA CAA GCA ACT TC R: CAC AGC AAA CAG GAT GAA CAC	89
igf-1	Insulin-like growth factor I	F: GAG AGC ACC TAA GAG ACC TTT G R: TGT GTT GCC TCG ACT TGA GTT T	85
hk	Putative hexokinase HKDC1	F: CTC AAC CCT GGG AGA CAA ATC R: CCG TCC TTG TTT AGC CAT CTT	99
gck	Glucokinase	F: GGA GTA TGA CAG AGT GGT GGA CGA G R: CAG AAC AAG CCT GAC CAG CTC ACC	106
pfk	ATP-dependent 6-phosphofructokinase, liver type-like	F: GAA GGG AAG GGC ATC TTT GA R: AAT AGC TCT CAC ACC CAA CTT AG	114
pk	Pyruvate kinase	F: GTG CCA CTC AGA TGC TGG AGA R: ATT GCT GCG TCT GCT TCA CTG C	190
pepck	Phosphoenolpyruvate carboxykinase, cytosolic	F: CCT GAC TCC CTC CAC ATC TG R: GCC AAC CAG CAG TTT TCA T	116
fbp	Fructose-1,6-bisphosphatase 1-like	F: TGC TGG TGT CTG AAG AGA ATG R: GAG CCA TCC AAC GGA TCA A	91
g6pc	Glucose-6-phosphatase	F: CCT CTT TAC ACT GAG ACC AAG AA R: GGA GGC TTG AAG GAG TCA AA	104
sod1	Superoxside dismutase-1	F: GAT CCA TGA GAA GGC TGA TGA C R: TAC TCG GCG ATG CCA ATA AC	108
cat	Catalase	F: GCA GAT GTC GCT CGT TAC A R: CAA AGT CTC TGA CGT TCC TCT T	101
gpx	Glutathione peroxidase	F: TTT GGG TGT ACC CTG CAA TC R: ATT CCC TGG ACG GAC ATA CT	101
noa1	Nitric oxide-associated protein	F: CTG GAG GAA CAG TTC GTG TTT R: AGC TGA TGG AGG AGG TCT TT	83

Table 3 KEGG classification of significantly different of unigenes in high dietary carbohydrate group against normal dietary carbohydrate group

Term	Gene count	ID	Statistic
Carbon metabolism	12	ko01200	upregulated
Biosynthesis of amino acids	11	ko01230	upregulated
Arginine and proline metabolism	8	ko00330	upregulated
Glycolysis/Gluconeogenesis	7	ko00010	upregulated
Arginine biosynthesis	5	ko00220	upregulated
Pathways in cancer	41	ko05200	downregulated
PI3K-Akt signaling pathway	33	ko04151	downregulated
Complement and coagulation cascades	30	ko04610	downregulated
Viral carcinogenesis	29	ko05203	downregulated
Focal adhesion	29	ko04510	downregulated
Herpes simplex infection	28	ko05168	downregulated
MicroRNAs in cancer	24	ko05206	downregulated
Cell cycle	23	ko04110	downregulated
Phagosome	19	ko04145	downregulated
Staphylococcus aureus infection	18	ko05150	downregulated
DNA replication	17	ko03030	downregulated
Prostate cancer	17	ko05215	downregulated
Chagas disease (American trypanosomiasis)	15	ko05142	downregulated
ECM-receptor interaction	14	ko04512	downregulated
Leishmaniasis	14	ko05140	downregulated
Pertussis	14	ko05133	downregulated
Fanconi anemia pathway	13	ko03460	downregulated
Systemic lupus erythematosus	13	ko05322	downregulated
Protein digestion and absorption	13	ko04974	downregulated
Legionellosis	11	ko05134	downregulated
Meiosis – yeast	10	ko04113	downregulated
Nucleotide excision repair	9	ko03420	downregulated
Mismatch repair	6	ko03430	downregulated

72°C); and then followed by melting curve analysis. Analysis of gene expression was determined from the three best data of mRNA levels using the optimized comparative Ct ($2^{-\Delta\Delta ct}$) value method, according to Livak and Schmittgen (2001). The relative gene expression of the *β-actin* gene was used to normalize the measured mRNA.

Differential Expressed Genes and Statistical Analysis

All clean reads from the N34 and H53 libraries were determined by mapping the transcriptome assembly. Prior to differential expression analysis, the read counts were adjusted by the Trimmed Mean of M-values (TMM) through one scaling normalized factor. Differential expression analysis between the groups was performed using the edgeR package (Robinson et al., 2010). The P values were adjusted using the Benjamini and Hochberg methods. Corrected p-value of 0.005 and |log2 (Fold Change) | of 1 were set as the threshold for significantly differential expression. All the remaining data were analyzed by the *independent sample T-test* (P<0.05) in SPSS *Statistic* version 21 (SPSS Inc, USA). Abnormal and inhomogeneous data were analyzed by the Mann-Whitney test (P<0.05).

Results

Growth Performance

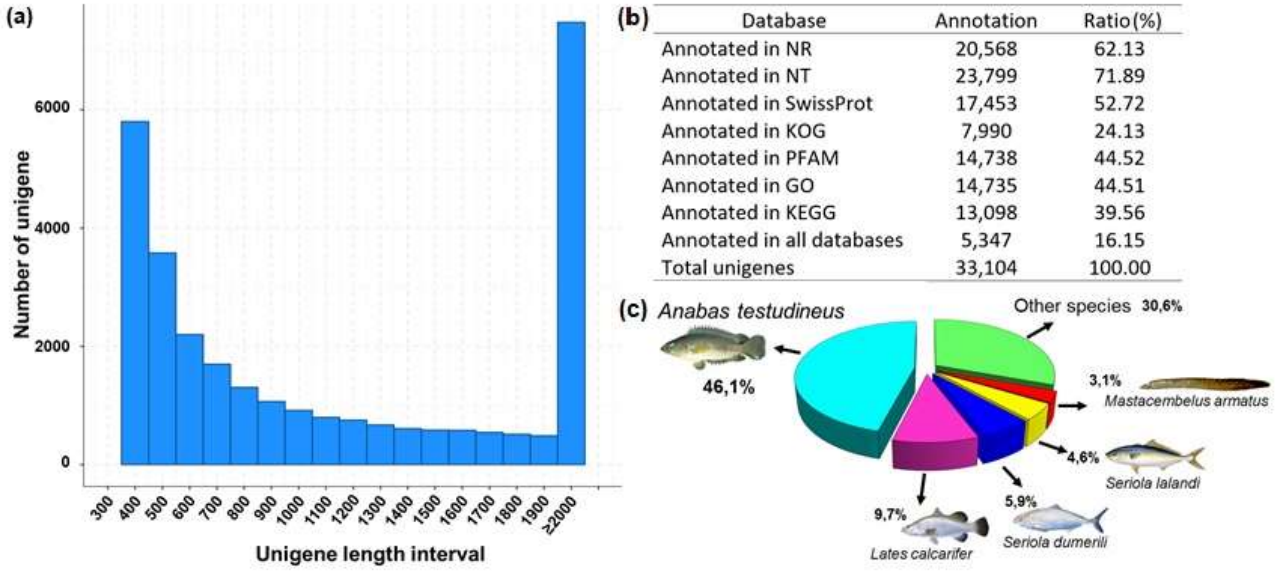
The high dietary carbohydrate (H53) significantly reduce the growth rate (P<0.05), however significantly increase the feed efficiency (Table 3). Compared to the normal carbohydrate group, the final body weight and specific growth rate in the high carbohydrate group decreased by 19.18% and 10.36%, respectively. However, the Giant gourami's feed efficiency was significantly increased by 33.47% after high carbohydrate feeding. No mortality was observed during the trial.

De novo Assembly, Functional Annotation, and Differential Gene Expression

The present study yielded a total of 89,373,579 raw reads from Giant gourami's liver. After filtering the raw reads, 87,430,553 clean reads were obtained with a Q20 of 97.80% and an average GC content of 46.66%. All the clean reads were assembled into 49,034 transcripts and 33,104 unigenes, with average unigenes length 1319 bp (Figure 1a).

All the unigenes were used as queries for BLAST against seven databases, including NT, NR, SWISS-PROT, KOG, PFAM, GO, and KEGG annotation (Figure 1b). Out of 33,103 unigenes, 26,138 unigenes (78.95%) successfully annotated in at least one database, and 5,347 unigenes (16.15%) were annotated in all databases. According to the species distribution, Giant gourami's unigenes had a high homology to genes in five fishes with the highest similarity with *Anabas testudineus* (46.1%) (Figure 1c).

After Gene Ontology (GO) annotation, 14,735 unigenes were successfully annotated and grouped into three main GO domains: biological process (33,542 unigenes; 50.87%), molecular function (17,820 unigenes; 27.02%), and cellular component (14,577 unigenes; 22.11%) (Figure 2a). The most abundant transcripts in the biological process category are associated with the cellular process, followed by the metabolic process and biological regulation. The transcripts related to the cellular anatomical entity and binding were the most abundant in the cellular component and molecular function category, respectively.



Database	Annotation	Ratio(%)
Annotated in NR	20,568	62.13
Annotated in NT	23,799	71.89
Annotated in SwissProt	17,453	52.72
Annotated in KOG	7,990	24.13
Annotated in PFAM	14,738	44.52
Annotated in GO	14,735	44.51
Annotated in KEGG	13,098	39.56
Annotated in all databases	5,347	16.15
Total unigenes	33,104	100.00

Figure 1. Length distribution of unigenes (a), ratio of successfully annotated unigenes (b), and species distribution of a similarity search of Giant gourami's unigenes against NR database (c).

A total of 2,723 unigenes were significantly different in both groups. In H53 group, 1,438 genes were down-regulated, and 1,285 genes were up-regulated compared to N34 (Figure 2b). Further analysis showed that the two groups have a similar transcript (Figure 2c). About 7,538 DEGs were only found in N34, 6,097 in H53, and 15,946 DEGs were similar in both groups (Figure 2b and 2c).

Enrichment Analysis

According to *euKaryotic Orthologous Groups* (KOG) annotation, a total of 7,990 unigenes were successfully annotated into 24 categories (Figure. 3a). Among these, the largest group is the "signal transduction mechanisms" (1,249 unigenes; 15.63%), followed by "general function prediction only" (1,232 unigenes;

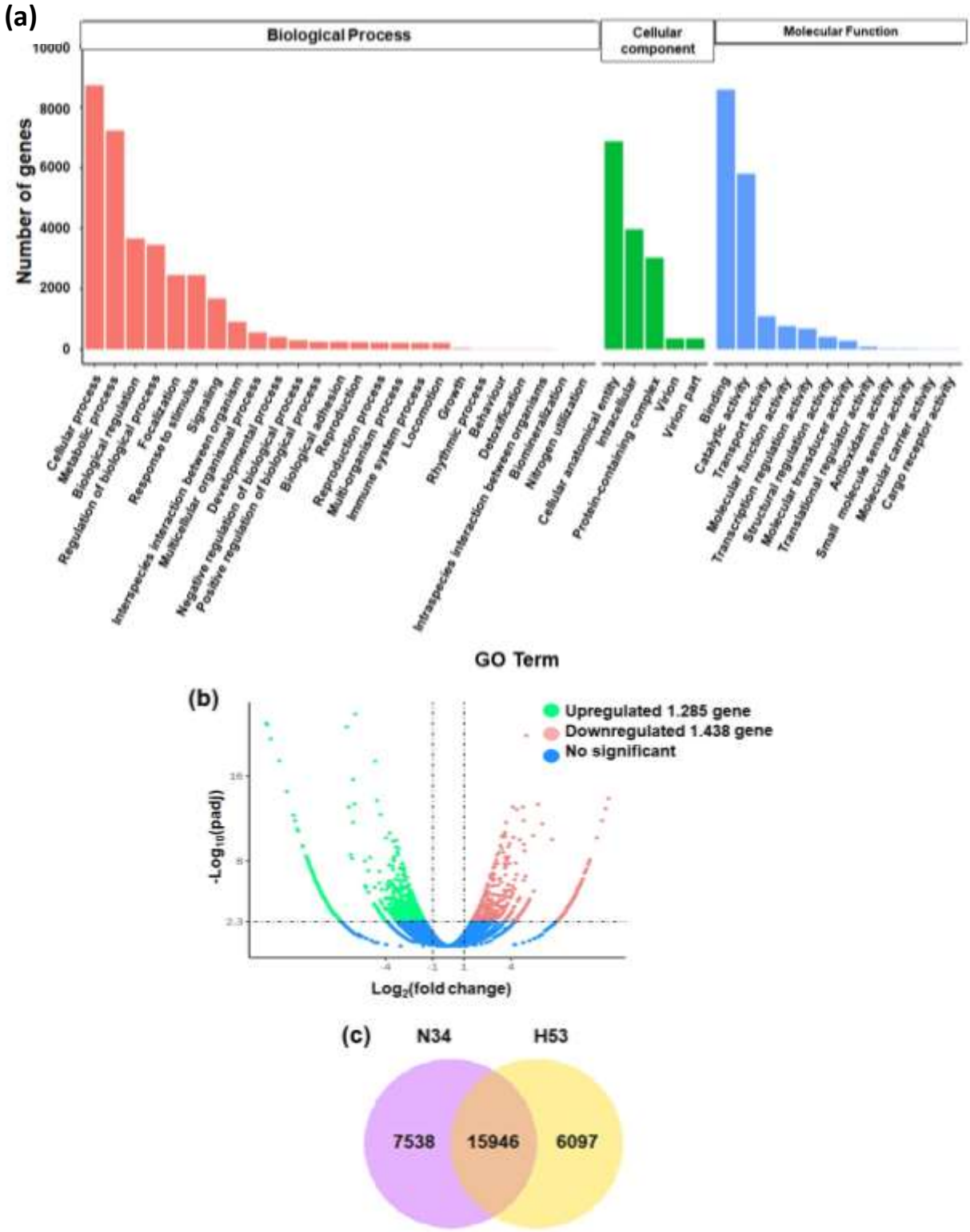


Figure 2. Gene ontology classification of assembled unigenes (a), volcano plot of differential expression analysis of gene between N34 and H53 (b), and Venn diagram of expressed genes in N34 and H53 (c) of Giant gourami fed with different dietary carbohydrate levels.

15.42%). The "carbohydrate transport and metabolism" category were assigned from a total of 276 genes (3.45%). Classification of the biological system was conducted against the *Kyoto Encyclopedia of Genes and Genome* (KEGG) database. A total of 13,098 unigenes were annotated into 291 pathways and divided into five branches: cellular process, environmental information processing, genetics information processing, metabolism, and organismal systems (Figure 3b).

According to the KEGG database, high dietary carbohydrates significantly increased five pathways, including pathways related to the carbon, carbohydrate, and amino acids metabolism. In addition, a total of 22 pathways related to the immune systems, DNA replication, cell cycles, protein digestion and absorption were significantly decreased in the high dietary carbohydrate group (Table 4).

Validation of Quantitative Real-time PCR

A total of 15 key genes related to insulin-signaling (*irs1* and *igf-1*), glucose transportation (*glut2*),

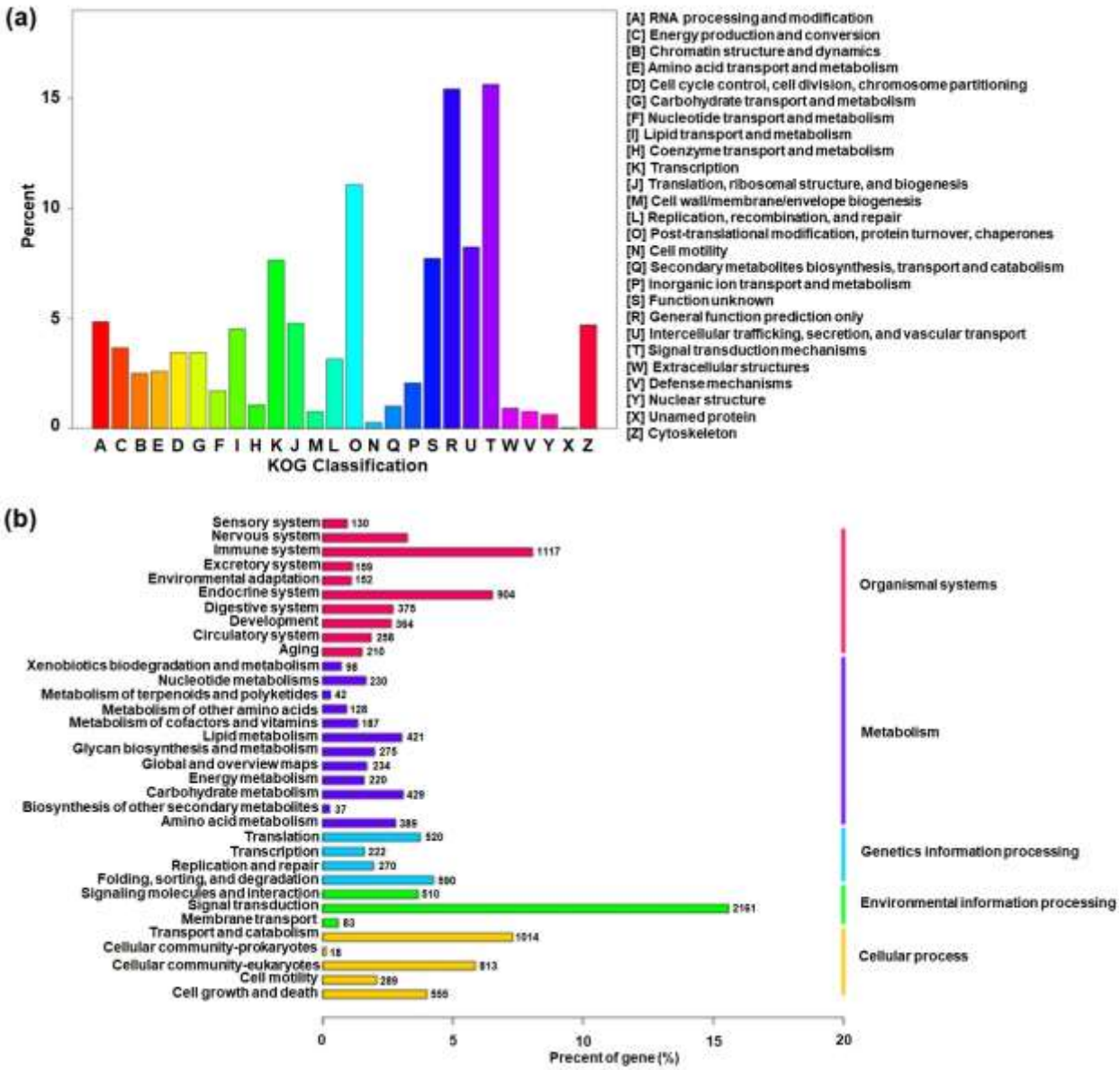


Figure 3. Classification of Giant gourami's unigenes according to euKaryotic Orthologous Groups (KOG) (a) and the Kyoto Encyclopedia of Genes and Genome (KEGG) (b).

Table 4 Effects of different dietary carbohydrate levels on growth performance of Giant gourami

Diet	IBW (g)	FBW (g)	SGR (%)	FE (%)	SR (%)
N34	25.80±0.76	114.31±3.00*	2.51±0.06*	59.33±0.78	100±0.00
H53	25.17±0.95	95.94±7.81	2.25±0.09	79.19±1.41*	100±0.00

Data were expressed as mean ± SD (standard deviation) (n=3). IBW: initial body weight, FBW: final body weight, SGR: specific growth rate, FE: Feed Efficiency, SR: survival rate. Means with an asterisk (*) at same column indicate significant differences between groups (P<0.05).

glycolysis/gluconeogenesis (*gck, hk, pfk, pk, pepck, fbp, and g6pc*), and antioxidant systems (*sod1, cat, gpx,* and *noa1*) were selected for qPCR confirmation. In general, the qPCR results were consistent with the RNA-Seq results. These results show that the carry-out *de novo* transcriptome assembly provides accurate results and further confirmed the RNA-seq reliability and the Trinity assembly accuracy (Figure 4).

Discussion

Giant gourami has been known to have the capability to utilize high carbohydrates (Mokoginta et al., 2004). In this study, Giant gourami fed with high carbohydrates have significantly higher feed efficiency but significantly lower growth rate. This result might indicate that the protein-sparing effect mechanism from carbohydrates has been well established, and the gelatinization degrees did not significantly affects this fish's carbohydrate metabolism. However, the semi-purified diet might not meet the optimal nutrient requirement for Giant gourami growth, especially amino acid composition, due to the differences in protein sources other than casein. The previous study also found a lower growth rate after high semi-purified carbohydrate feeding in Giant gourami (Mokoginta et al., 2004).

Transcriptome profiling is an essential method for obtaining information about the functional component of the genome and revealing the molecular constituents of cells and tissues from non-model organisms with no available reference genome (Liu et al., 2019). Among the 33,104 unigenes, a total of 26,138 unigenes (78.96%) were successfully annotated at least in one database. The annotation success rate in this study was higher than that obtained in largemouth bass (*Micropterus salmoides*) (52.98%) (Zhang et al., 2019) and golden pompano (*Trachinotus ovatus*) (44.3%) (Liu et al., 2019).

GO annotation showed that the genes that frequently appeared in the cellular process are those related to cellular process, metabolic process, and biological regulation. These results are consistent with the liver's role in glucose and lipid metabolism (Zhang et al., 2019). The higher frequencies of cellular component and molecular function were cellular anatomical entity and binding, respectively. This result was different from other studies reported in fish, such as grouper (*Epinephelus akaara*) (Yang et al., 2018), largemouth bass (Zhang et al., 2019), and rohu (*Labeo rohita*) (Rasal et al., 2020), suggesting that the diet preference, treatment and the source of tissue significantly affect the transcriptome. This result also confirms the suitability of RNA-seq technology in producing an accurate transcriptional database (Zhang et al., 2019). About 39.56% of unigenes were annotated in the KEGG database, which further enriched our knowledge of the biological characteristics of omnivorous fish. The obtained data in the present study was higher than that in largemouth bass (26.96%) (Zhang et al., 2019) and golden pompano (22.02%) (Liu et al., 2019). According to the author's knowledge, Giant gourami has not been reported in any genomic library. So, the results in this study provide a great number of novel transcription data to study carbohydrate metabolism in Giant gourami.

High dietary carbohydrate feeding in Giant gourami generally downregulated DEGs expression level (52.81%). This result corresponds to the high dietary carbohydrate content (53%) that may negatively interfere with liver function, causing metabolic stress, and inhibiting the expression of some genes that are related to metabolism and immune systems (Ma et al., 2019; Zhang et al., 2019). The current study agrees with

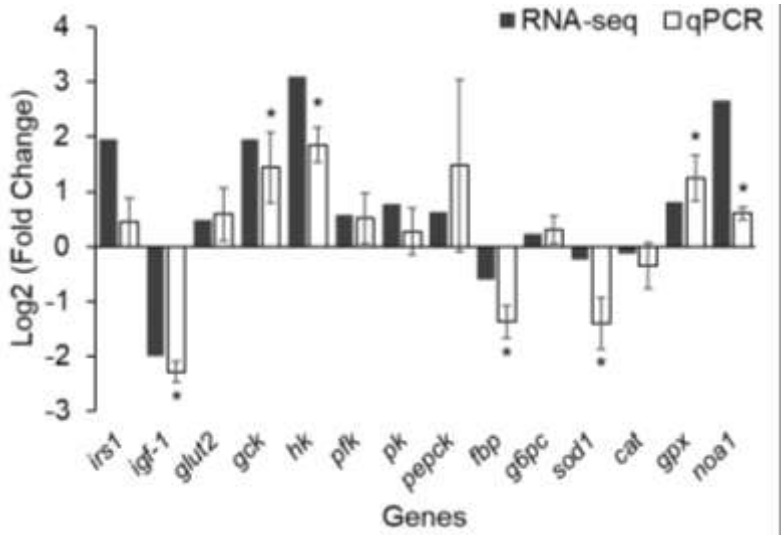


Figure 4. Validation of RNA-seq data by qPCR. The relative mRNA levels of key genes related to carbohydrate metabolism and antioxidant systems in the liver of Giant gourami were examined by qPCR. The mRNA levels by qPCR are presented as fold change of H53 compared to N34 after normalized with β-actin. The relative expression levels from the RNA-seq analysis were calculated as fold change of read count of H53 compared to N34. The asterisk (*) denotes the presence of a statistically significant difference ($P<0.05$) in mRNA levels between H53 and N34.

the transcriptome response in largemouth bass fed with high carbohydrates (Zhang et al., 2019). Different results were found in golden pompano, which shows that high carbohydrate feeding mainly upregulated the unigenes (Liu et al., 2019). Further analysis using the KEGG database showed that high dietary carbohydrates significantly increased five pathways and significantly decreased 22 pathways, mainly those related to immune systems. These results further reinforce the adverse effects of high carbohydrates on immune-related gene expression.

For qPCR, 15 key genes related to insulin signaling, glucose transportation, glycolysis/gluconeogenesis, and antioxidant systems were selected. *Irs1, igf-1,* and *glut2* play a role in the glucosensing mechanism that promotes glucose uptake (Aguirre et al., 2016; Kamalam et al., 2017; Polakof et al., 2010). The significant downregulation of *igf-1* expression after high carbohydrate feeding in this study might be correlated to sampling time at 6 hours after feeding. Liu *et al.* (2018) reported that the significant effect of high carbohydrates in omnivorous fish was reached one hour after feeding. The *gck, hk, pfk,* and *pk* have been known as the rate-limiting enzyme genes in glycolysis (Kamalam et al., 2017). Glucokinase is one of the hexokinase family, which catalyzes the first step in glycolysis (Enes et al., 2009). This enzyme has a low Km value to catalyze glucose phosphorylation at high glucose concentrations when other hexokinase variants cannot do that (Liu et al., 2019). In this study, the *gck* expression levels were significantly upregulated after high carbohydrate feeding and positively correlated with plasma glucose as reported in blunt snout bream (*Megalobrama amblycephala*) (Li et al., 2016). The non-carbohydrate carbon sources were converted into glucose by gluconeogenesis to maintain the blood glucose level (Kamalam et al., 2017; Liu et al., 2019). The *pepck, fbp,* and *g6pc* are the rate-limiting enzyme genes in gluconeogenesis (Kamalam et al., 2017). A study in largemouth bass showed that a high glucose level would downregulate the *fbp* enzyme (Lin et al., 2018) and is consistent with the result in this study.

Hyperglycemia and overproduction of reactive oxygen species (ROS) during carbohydrate metabolism depress the endogenous antioxidant system and expose cells to damage due to oxidative stress (Giacco & Brownlee, 2010; Zhang et al., 2019). Fish has several antioxidant defence systems against ROS, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Yang et al., 2019). In our study, DEGs related to the immune system were dominantly downregulated. Further analysis using qPCR showed that the level expression of *sod1* was also significantly downregulated after high carbohydrate feeding. Contrarily with the *sod1* gene, the *gpx* and *noa1* were significantly upregulated after high carbohydrate feeding. A high expression of *noa1* indicated that the respiratory chain in mitochondria was in control due to the role of this gene in stabilizing the respiratory chain in mitochondria, oxidative stress, and cell death (Heidler et al., 2011; Kolanczyk et al., 2011). The present study results confirmed that giant gourami could utilize high carbohydrates well. Other studies in blunt snout bream and Indian major carp (*L. rohita*) also showed that high carbohydrates do not generate oxidative stress (Anand et al., 2018; Zhou et al., 2013).

Conclusion

In conclusion, this study obtained liver transcriptomes and gene expression patterns of carbohydrate metabolism for the first time in Giant gourami after high carbohydrate feeding using Illumina HiSeq 4000 platform. The results showed that Giant gourami has several adaptabilities for high carbohydrates. The upregulation of the glycolytic, *gpx*, and *noa1* genes, downregulation of insulin signaling, and gluconeogenic genes indicated a good glucose homeostasis maintenance in Giant gourami.

Ethical Statement

The protocol was approved by the Animal Care and Use Committee of IPB University, Indonesia (Grant No. 192-2021 IPB). All surgery was performed under MS-222 anesthesia, and all efforts were made to minimize suffering.

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Author Contribution

Conceptualization, D.N.S, J.E., A.A, M.A.S.; methodology, software, and formal analysis: D.N.S, H.N.; investigation, writing-original draft preparation, D.N.S; writing-review, editing, and validation: H.N., J.E., M.A.S, A.A. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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