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Comparative Analysis of Hepatopancreas RNA-Seq of Juvenile Grass Carp (*Ctenopharyngodon idella*) Fed Different Starch Diets

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Abstract: This study aimed to explore the effects of different starch source diets on the growth performance and hepatopancreas RNA-seq of grass carp. Juvenile grass carp (initial body weight of 39.4 ± 1.6 g) were fed diets containing 25% corn (CO), potato (PO), and wheat (WH) starch for 8 weeks, respectively. The weight gain ratio (WGR) was significantly lower, whereas the visceral somatic index (VSI) and feed conversion ratio (FCR) were significantly higher in the CO group than those in the PO and WH groups. These indicators did not significantly differ between the PO and WH groups. Hepatopancreas RNA-seq analysis showed that 536, 514, and 647 differentially expressed genes (DEGs) were screened out in the comparisons of PO vs. WH, PO vs. CO, and CO vs. WH. The DEGs were mainly enriched in the several known pathways involved in steroid biosynthesis, cell cycle, fatty acid metabolism, and fat digestion and absorption according to Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis. The major DEGs related to lipid and carbohydrate metabolism were analyzed, in which lipogenesis-related DEGs (*fasn*, *acc1*, *scd1*, *elovl6*, and *me1*), fat digestion and absorption-related DEGs (*fabp7*, *apoa1*, *apoa4*, and *pla2*), and glycometabolism-related DEGs (*gk*, *g6pd*, and *pepck*) were down-regulated in the PO group compared with those in the CO and WH groups. Conversely, steroid synthesis-related DEGs (*hmgcs*, *fdft1*, *sqle*, *lss*, *cyp51*, *msmo1*, *nsdhl*, *ugt*, *cyp1b1*, and *cyp7a1*) were up-regulated in the PO group. These results indicate that the long-term PO ingestion could modulate hepatic lipid metabolism by reducing fatty acid biosynthesis and increasing bile acid biosynthesis. PO may be healthier in contrast to CO alone, which may not be suitable as a starch source in grass carp diet.

Keywords: grass carp; starch sources; growth; hepatopancreas; RNA-seq

Key Contribution: Wheat, potato, and corn starch are the major dietary starch sources used in aquafeed. Different starch types may alter nutritional value of aquafeed and thus affect growth performance in fish. In actual farming, corn starch source diets are more likely to cause fatty liver in fish, but the underlying mechanism is not clear. Therefore, this experiment was conducted to verify whether corn starch is more likely to cause fatty deposition in fish by evaluating the actual effects of three starch sources (corn, potato, and wheat) diets on growth parameters in grass carp (*Ctenopharyngodon idella*) and to investigate the transcriptome characteristics of different starch sources affecting the fatty deposition via RNA-seq. A precise understanding and assessment of the nutritional value of commonly used starch sources in aquafeeds can contribute to improving feed quality and utilization and reducing feed cost and the probability of fish disease.

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1. Introduction

Starch is a polymeric carbohydrate consisting of numerous glucose molecules linked by glycosidic bonds [1]. In poultry and livestock feeds, starch is the main form of dietary energy. In aquatic feed, starch is a less expensive energy source than lipid or protein [2,3]; it also improves the water environment by decreasing ammonia excretion due to protein catabolism [4,5]. Furthermore, starch is often added to feed as a stabilizer and a swelling agent during the pelleting process. However, most fish species, especially carnivorous fish, have a relatively low starch utilization capacity [6]. Thus, the dietary starch level is generally limited to 30–40% for omnivorous or herbivorous fish species and to <15–20% for carnivorous fish species [7,8]. Excess dietary starch has been reported to lead to poor growth, fatty liver symptoms, and visceral lipid accumulation in various fish species [9,10]. Previous study demonstrated that post-prandial hyperglycemia appeared in fish fed over 20% of dietary carbohydrate, especially carnivorous species, which is a common symptom associated with 'fatty liver' [11]. Compared to the fish fed diet containing normal levels of corn starch, Nile tilapia (*Oreochromis niloticus*) fed high starch diet (45% corn starch) showed higher hepatosomatic index and visceral somatic index, as well as higher levels of serum triglyceride, whole body and hepatic lipid, indicating that high-carbohydrate diets may be prone to induce liver damage characterized by abnormal hepatic fat accumulation [12]. Therefore, optimizing dietary starch levels is essential for developing cost-effective and nutritionally appropriate aquafeed in various fish species.

Wheat, potato, and corn starch are the major dietary starch sources used in aquafeed. Different starch types may alter nutritional value of aquafeed and thus affect growth performance and health in fish. The viscerosomatic index, hepatopancreatic fat content, and mesenteric fat percentage of grass carp fed 30% cornstarch diets were significantly higher than those in grass carp fed a glucose diet [13]. The viscerasomatic index of juvenile European sea bass (*Dicentrarchus labrax*) fed a corn starch diet was significantly higher than that in fish fed a pea starch diet [14]. Yellow perch (*Perca flavescens*) were fed diets with 20% corn, potato, or wheat starch for 8 weeks, in which the wheat starch diet led to noticeably bigger livers and increased the accumulation of fat in the body, viscera, and liver [15]. By contrast, corn and potato starch are regarded as suitable carbohydrate sources for yellow perch as they have less adverse effects on fish health, which are not easily attributable to higher blood glucose, fatty liver, and other diabetes-related symptoms in yellow perch [15]. Similar effects have also been observed in terrestrial animals [16,17]. In addition, starch is a mixture of amylose and amylopectin, in which amylopectin has a larger surface area and fewer intramolecular hydrogen bonds than those of the highly linear amylose. Therefore, it is more easily digested than amylose, causing a sharp rise in the levels of insulin and blood sugar [18]. High amylose/amylopectin ratio diets were found to dramatically increase starch consumption in obscure puffer (*Takifugu obscurus*) [18]. Similarly, a high-amylose diet reduced accumulation of fat in the liver and muscle of non-fish [16]. These results suggest that corn starch is more likely to cause fatty liver symptoms in fish, but the reason is unclear. Hence, it is very important to explore how different starch source feeds cause the deposition of body fat metabolism in grass carp, which in turn affects the growth performance of fish, which is very important for the selection of starch sources in the future.

Grass carp, a typical herbivorous freshwater fish, is an important aquatic animal in global aquaculture system, including China. Research has revealed that grass carp shows a good capacity to digest a relatively high level of wheat starch of up to 33–38% [19]. However, the ability to digest and absorb starch source in fish depends on the structural characteristics and physicochemical properties of starch used in the feed [20]. Given the differences in effects of various dietary starch previously documented in other fish species [20–22], the actual influences of specific dietary carbohydrate substances on grass carp and the underlying molecular mechanism still need to be further elucidated. Therefore, the hepatopancreatic transcriptome of juvenile grass carp fed three starch diets (containing 25% corn, potato, or wheat starch) were analyzed in combination with the growth parameters to explore the molecular mechanisms by which corn starch is more likely to lead to fat deposition in fish

than wheat and potato starch. Our findings will serve as a theoretical foundation for the appropriate use of carbohydrate sources in formula feed of juvenile grass carp and other fish species.

2. Materials and Methods

2.1. Ethics Statement

All experiments were conducted at the Jiangsu Ocean University (Lianyungang, China) in accordance with ethical standards for experimental animals of Jiangsu Ocean University and approved by the Animal Care and Use Committee of Jiangsu Ocean University (Approval Date: 1 September 2019; Approval Code: No. 2020-37).

2.2. Fish Diet

Experimental diets were prepared according to our previous studies with modifications [23,24]. The components of the experimental aquafeed are described in Table 1. Briefly, three kinds of equal nitrogen (35% crude protein) and equal lipid (6% crude lipid) diets were formulated with 25% corn (CO), potato (PO), or wheat (WH) starch as the main starch source, respectively. Three different sources of starch are 100% pure. Dry feed ingredients were pulverized to <40 mesh (0.425 mm), weighed according to feed formulas, and mixed using a mixer to form a homogeneous mixture. Then, the dry mixture was first blended with oils and then mixed with 250 mL/kg tap water to stir into dough. The dough was pelleted using a small meat grinder, and the pellets were dried in an oven at 45–55 °C until the moisture content decreased to 10–12%. The experimental feeds were bagged, numbered, and stored at 4 °C.

Table 1. Formulations of experimental diets (%, dry weight).

Dietary Component	CO	PO	WH
Ingredients			
Fish meal	15.00	15.00	15.00
Canola meal	24.70	24.70	24.70
Soybean meal	24.00	24.00	24.00
Blood meal	3.00	3.00	3.00
Corn starch	25.00		
Potato starch		25.00	
Wheat starch			25.00
Met	0.08	0.08	0.08
Lys	0.12	0.12	0.12
Choline (50%)	0.30	0.30	0.30
$Ca(H_2PO_4)_2$	1.80	1.80	1.80
Soybean oil	5.00	5.00	5.00
Premix [1]	1.00	1.00	1.00
Total	100	100	100
Nutrient levels (calculated value)			
Crude protein	35.00	35.00	35.00
Crude fat	6.10	6.10	6.10

[1] Premix (mg/kg diet): VA, 5000 IU; VD, 2000 IU; VE, 50 mg; VB_1, 8 mg; UK, 5 mg; VB_2, 10 mg; VB_{12}, 0.03 mg; VB_6, 8 mg; inositol, 100 mg; folic acid, 3 mg; pantothenic acid, 30 mg; nicotinic acid, 30 mg; biotin, 0.4 mg; VC, 180 mg; Cu, 4 mg; Fe, 170 mg; Zn, 150 mg; Mn, 22 mg; I, 1 mg; Co, 0.25 mg; Se, 0.4 mg; and Mg, 300 mg. Abbreviations: CO, corn starch; PO, potato starch; and WH, wheat starch.

2.3. Fish and Rearing Experiment

Juvenile grass carp were collected from the local Huandun fish farm (Lianyungang, China) and transferred to indoor tanks (240 L) at the Jiangsu Ocean University. The aquatic environmental and rearing conditions for acclimation and experiment were based on previous reports [23–25] with several changes. Grass carp were acclimatized for 2 weeks in tanks with recirculating aerated water and were fed commercial feed. After the acclimatization period, 270 fish (initial weight 39.4 ± 1.6 g) were selected and distributed into three groups

(30 fish/tank, 240 L/tank, three biological triplicates for each group). All fish were fed experimental feed four times a day (8:00, 11:00, 14:00, and 17:00) for 8 weeks. The daily feeding rate was approximately 3% of the fish's body weight, adjusted once a week according to the fish weight and intake. A third of the water in each tank was renewed every day. The conditions of water quality were maintained as follows: water temperature 20–25 °C, dissolved oxygen more than 5 mg/L, ammonia nitrogen <0.2 mg/L, and pH 7–8.

2.4. Sampling Collection

After the rearing experiment, the grass carp were fasted for 24 h before sampling. The fish from each tank were counted and weighed individually. Thereafter, six fish from each tank (18 fish per dietary treatment) were sampled randomly, anesthetized with MS-222, and dissected to obtain their visceral tissues. For RNA-seq analysis, hepatopancreas samples were frozen immediately in liquid nitrogen and then stored at −80 °C. The weight gain ratio (WGR), feed conversion ratio (FCR), and visceral somatic index (VSI) were calculated using the formulae described by Gao et al. [23]:

$$\text{WGR (\%)} = [\text{final body weight (g)} - \text{initial body weight (g)}]/\text{initial body weight} \times 100$$
$$\text{VSI (\%)} = \text{viscera weight (g)}/\text{final body weight (g)} \times 100$$
$$\text{FCR} = \text{feed consumption (g)}/\text{fish weight gain (g)}$$

2.5. RNA Extraction and Transcriptome Library Preparation

Total RNAs of hepatopancreas samples (3 treatments × 3 samples, mixture of 6 fish as one sample) were extracted using an RNAprep Pure Tissue Kit (TIANGEN, Beijing, China) according to the manufacturer's instructions. The RNA integrity was determined with 1.5% agarose gel electrophoresis, and the 28S/18S ratio, RNA concentration, and RNA Integrity Number (RIN) were determined using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) [26]. High purity samples with RIN values more than 8.0 were selected for further analysis as previously described [26]. The cDNA libraries were constructed for the CO, WH, and PO groups with three biological replicates, and digital gene expression profiling (DGE) were conducted using BGISEQ-500 platform (BGI, Wuhan, China). In addition, the partial RNAs of CO, WH, and PO groups were reverse transcribed to cDNA and used for RT-qPCR validation.

2.6. Sequencing Data Quality Control and Alignment

cDNA was sequenced on the BGISEQ-500 platform. The software SOAPnuke (https://github.com/BGI-flexlab/SOAPnuke (accessed on 31 August 2021)) [27] was used to filter the sequencing raw reads containing low-quality (more than 20% of the base qualities lower than 10), adaptor-polluted, and unknown bases (N bases more than 5%) in order to obtain clean reads for data analysis. The remaining clean readings were saved in the FASTQ format following read filtering [27] and then aligned to the reference genome using HISAT2 (parameters: -p 8 --phred64 --sensitive -I 1 -X 10000; website: http://www.ccb.jhu.edu/software/hisat (accessed on 20 October 2021)) [28]. All clean reads were submitted to the NIH Short Read Archive (SRA) and registered under the accession number PRJNA514443.

2.7. Differentially Expressed Genes Screening and Function Annotation

The *Ctenopharyngodon idellus* genome (assembly HZGC01, https://www.ncbi.nlm.nih.gov/genome/2658 (accessed on 30 October 2021)) was used as the reference genome for mapping reads. The expression levels of identified unigenes and transcripts were determined using the RSEM software (http://deweylab.biostat.wisc.edu/RSEM (accessed on 21 November 2021)) [29]. Pearson's correlation coefficient, hierarchical cluster analysis, and principal component analysis (PCA) were performed across all groups using the cor, hclust, and princomp functions of RSEM. The DEGseq algorithm was used to identify the differentially expressed genes (DEGs) [30] (parameters: $|\log2(\text{Fold Change})| \geq 1$ and adjusted p-value ≤ 0.05). The adjusted p-value was determined with the Benjamini–Hochberg proce-

dure, and false discovery rate (FDR) ≤ 0.01 was used to determine significant differences in gene expression.

The gene ontology (GO) enrichment analysis and Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis were accomplished using phyper R package for identifying and annotating the biological functions and the enriched pathways of the differentially expressed transcripts, which were defined as significant enrichment at the corrected p-value < 0.05.

2.8. RT-qPCR Validation

To validate the RNA-seq results, nine DEGs between groups based on the KEGG metabolic pathway and the fold change of DEGs between pairwise comparisons of three groups were screened and analyzed with RT-qPCR. The suitable reference gene was selected among elongation factor 1 alpha (*ef1a*), beta actin (*β-actin*), glyceraldehyde-3-phosphate dehydrogenase 1 (*gapdh1*), and ribosomal protein L13a (*rpl13a*). According to the amplification efficiency and dissociation curve of these genes in this study, *ef1a* and *rpl13a* were used as the internal control genes. The primers were designed using Primer 6.0 software (REMIERE Biosoft, San Francisco, CA, USA) according to the coding sequences described in the NCBI database; the primer sequences are listed in Table 2.

Table 2. Primers used for RT-qPCR analyses.

Gene Function	Genes	Primer Sequence (5′-3′)	Length (bp)	Accession No./Contig
Fat synthesis gene	*fasn*	(F) TATCGCATCGCTGGCTACT (R) TGGCTCTGTAACTCTGTGTATAAG	148	XM026276596.1
	acc1	(F) GTGGGCACAGAGTGTAATCGTAGG (R) CAGTCTTAAAAGCAGAGTCAGGGA	148	GU908475
	scd	(F) ACTGGAGCTCTGTATGGAC (R) CGTAGATGTCATTCTGGAAG	90	AJ243835
	me1	(F) GGTGTCTATGGGCGTCTACT (R) CTTCCAGGTCTTGTTCTTAATCT	97	EU569765.1
Glucose synthesis gene	*pepck*	(F) GAATCTCAGAGCCATCAACCCAG (R) TCCATGCCTTCCCAGTAAACG	159	XM039654372.1
	gk	(F) GAAGAGCGAGGCTGGAAGG (R) CAGAATGCCCTTATCCAAATCC	211	ADD52460
	g6pd	(F) GAAGGTAAAGGTGCTGAAGTG (R) GCAAATGTAGCCTGAGTGGA	153	KJ743994.1
Steroid synthesis	*cyp51*	(F) ACCTCAGAAAGTGGGCGAATA (R) GCGGCAGGGTTGTCATGTAG	141	XM026232302.1
	msmo1	(F) GGGAGAAACAGTGGAAGTGC (R) TCATAAGGGATGCTGAAGAAC	121	XM039692682.1
Reference genes	*β-actin*	(F) TTCGAGACCTTCAACACCCC (R) CCAAGTCCAGACGGAGGATG	172	XM051886219.1
	ef1a	(F) CGCCAGTGTTGCCTTCGT (R) CGCTCAATCTTCCATCCCTT	98	XM042745044
	gapdh1	(F) TGACCCGTGCTGCTTTCC (R) TTGCCGCCTTCTGCCTTA	145	XM051865633.1
	rpl13a	(F) CTTCTGGAGGACATAAGAGGTATGC (R) GGAGAGGGATGCCATCAAAGAC	93	XM042742284.1

Notes: primers were designed using the Primer 6.0 software. Abbreviations: *fasn*, fatty acid synthase; *pepck*, phosphoenolpyruvate carboxykinase; *acc1*, acetyl-CoA carboxylase 1; *scd*, stearoyl-CoA desaturase; *gk*, glucokinase; *g6pd*, glucose 6-phosphate dehydrogenase; *cyp51*, sterol 14 alpha-demethylase; *msmo1*, methylsterol monooxygenase 1; *me1*, cytosolic NADP malic enzyme; *β-actin*: beta-actin; *ef1a*, elongation factor 1 alpha; *gapdh1*: glyceraldehyde 3-phosphate dehydrogenase 1; and *rpl13a*, ribosomal protein L13a.

Total RNA (1 μg/sample) was reverse-transcribed to form cDNA using the FastKing gDNA Dispelling RT SuperMix kit (Tiangen, Beijing, China). The synthesized cDNAs were then used as the template for further RT-qPCR analysis. RT-qPCR was performed using QuantiNova SYBR Green Kits (QIAGEN) in StepOnePlus Real-Time PCR Systems (ABI, Foster City, CA, USA) according to previous experimental operation with modifications [31].

The 20-μL reaction consisted of 1 μL of cDNA, 10 μL of 2× SYBR Green PCR Master Mix, 2 μL of Rox, 0.6 μL of each forward and reverse primer, and 5.8 μL of RNase-free water. For each sample (mixture of 6 fish as one sample), the negative control using RNase-free water as template was set in this study. RT-qPCR was performed in triplicates. The RT-qPCR conditions were pre-denaturation for 2 min at 95 °C, followed by 40 cycles of denaturation for 5 s at 95 °C, and annealing for 10 s at 60 °C. Finally, the melting curve was generated by increasing temperature from 60 °C to 95 °C at a heating rate of 0.3 °C/s [24]. The relative expression levels of target genes were determined using the 2-ΔΔCt method.

2.9. Statistical Analysis

In this study, data were expressed as mean ± standard error (SE). Data were assessed with one-way ANOVA (Tukey test) using spss 23.0 software (SPSS Inc., Chicago, IL, USA) and plotted using the GraphPad Prism 8.0 (GraphPad, La Jolla, CA, USA). The statistical significance was set to $p < 0.05$.

3. Results

3.1. Growth Performance of Grass Carp

Juvenile grass carp were fed diets containing 25% CO, PO, or WH for 8 weeks. The final weight (FW) and WGR of the CO group were significantly lower, whereas the viscera somatic index (VSI) and feed conversion ratio (FCR) were significantly higher than those of the PO group ($p < 0.05$). Moreover, VSI in the PO group were significantly lower compared to the fish in the WH group ($p > 0.05$). These results are provided in Table 3.

Table 3. The growth performance of grass carp fed on the experimental diets of different starch sources.

Items	Experimental Group		
	CO	**PO**	**WH**
IW (g/fish)	39.41 ± 1.60	39.41 ± 1.60	39.41 ± 1.60
FW (g/fish)	84.97 ± 4.79 ^{b}	103.67 ± 4.25 ^{a}	98.23 ± 6.51 ^{ab}
SR (%)	96.67%	96.67%	100%
VSI (%)	8.18 ± 0.21 ^{a}	6.31 ± 0.21 ^{c}	7.13 ± 0.18 ^{b}
WGR (%)	115.63 ± 12.15 ^{b}	162.96 ± 10.79 ^{a}	149.32 ± 16.52 ^{ab}
FCR	1.72 ± 0.21 ^{a}	1.20 ± 0.07 ^{b}	1.33 ± 0.14 ^{ab}

Notes: Values in the same row with different lowercase letters indicate significant difference ($p < 0.05$). The used abbreviations are as follows: IW, initial weight; FW, final weight; SR, survival rate; VSI, viscera somatic index; WGR, weight gain ratio; FCR, feed conversion ratio; CO, corn starch; PO, potato starch; and WH, wheat starch.

3.2. RNA-Seq Assembly and Analysis

To provide an overview of the hepatopancreatic gene expression profile of grass carp, nine cDNA samples (CO, PO, and WH groups with three biological replicates) were prepared and sequenced with the BGISEQ-500 platform, generating an average of roughly 24.11 M reads for each sample. The average mapping ratios with the reference genome and the gene were 96.05% and 63.73%, respectively. A total of 27,611 genes were detected (Table 4).

Table 4. Summary statistics of transcriptome sequencing and assembly.

Items	CO1	CO2	CO3	PO1	PO2	PO3	WH1	WH2	WH3
Clean reads									
Total raw reads (Mb)	24.14	24.14	24.14	24.14	24.14	24.14	24.14	24.14	24.14
Total clean reads (Mb)	24.11	24.11	24.11	24.11	24.11	24.11	24.11	24.11	24.11
Total clean bases (Gb)	1.21	1.21	1.21	1.21	1.21	1.21	1.21	1.21	1.21
Clean reads ratio (%)	99.89	99.88	99.86	99.88	99.88	99.88	99.88	99.87	99.88
Genome mapping ratio (%)									
Total	95.90	95.94	96.29	95.86	95.80	96.00	96.02	96.20	96.19
Uniquely	85.77	85.44	87.09	85.85	85.81	86.50	85.80	87.18	86.85
Gene mapping ratio (%)									
Total	63.17	64.52	64.51	64.32	63.53	63.61	63.71	63.65	64.33
Uniquely	58.44	59.24	59.80	59.34	58.57	58.82	58.84	59.25	59.64
Gene expression amount	22,280	23,241	21,771	22,106	22,796	22,023	22,654	21,050	21,091

3.3. Analysis of DEGs

The DEGseq algorithm (parameters: $|\log2(\text{Fold Change})| \geq 1$ and adjusted p-value ≤ 0.05) was used to locate the DEGs. The DEGs identified among the three groups are shown in Figure 1. Compared with those in the WH group, 614 up- and 33 down-regulated genes were found in the CO group, and there were 536 DEGs (387 up-regulated and 149 down-regulated) in the PO group. When comparing with the CO group, there were 514 DEGs (108 up-regulated and 406 down-regulated) in the PO group.

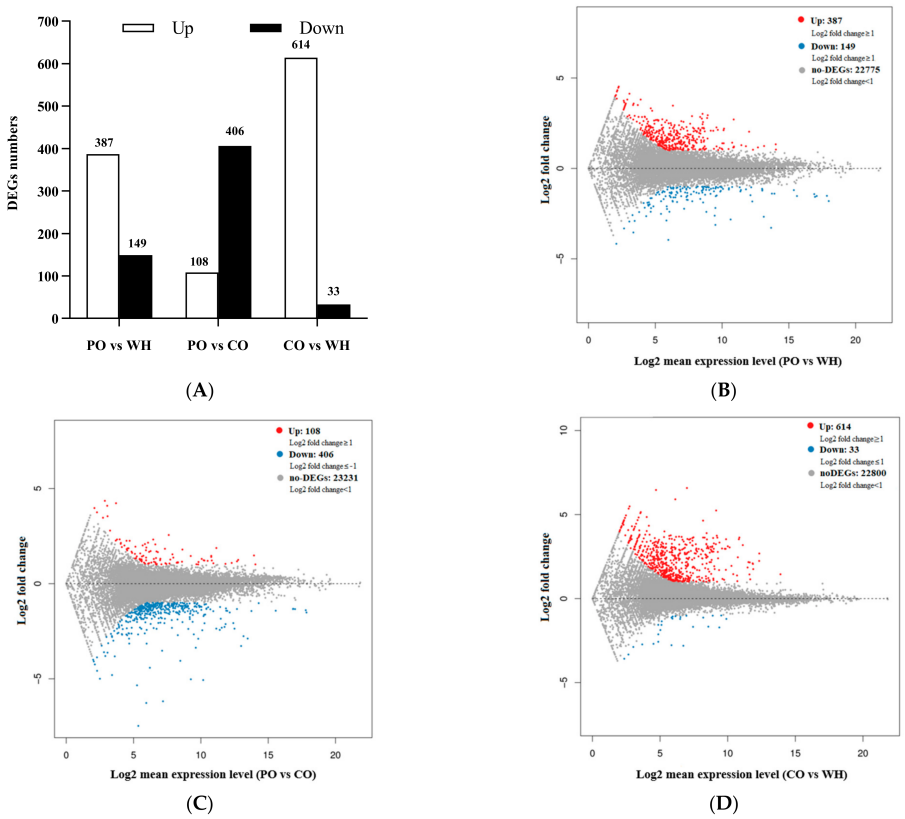
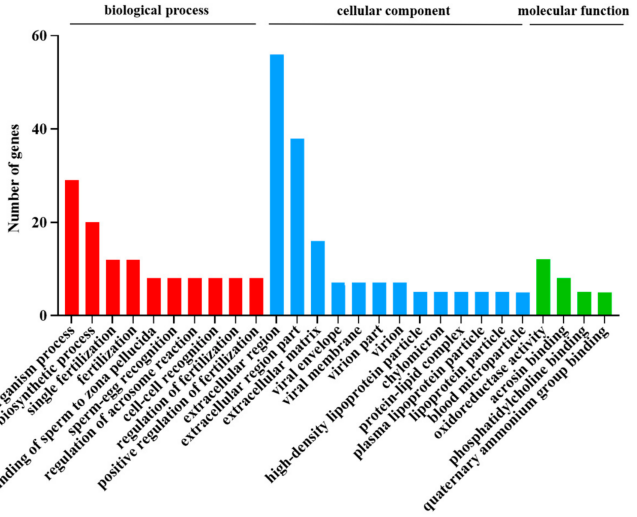


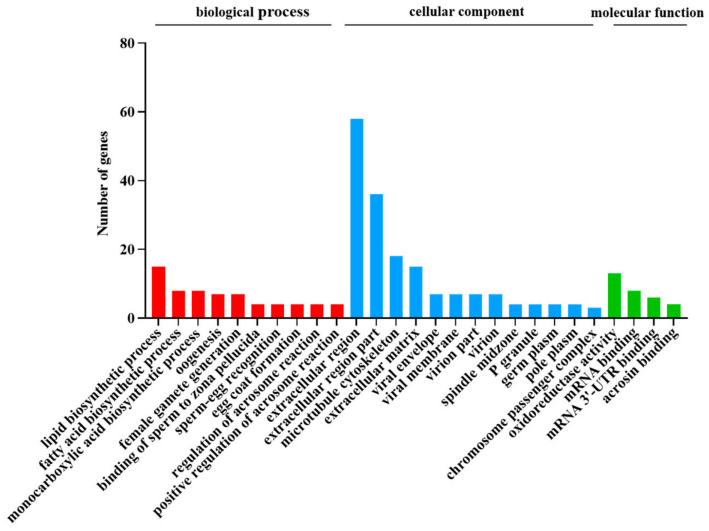
Figure 1. Summary of differentially expressed genes ($|\log2(\text{Fold Change})| \geq 1$ and adjusted p-value ≤ 0.05). (**A**) Bar graph of the corresponding number of DEGs (up-regulated and down-regulated DEGs) in the comparisons of PO vs WH, PO vs CO, and CO vs WH. (**B**) Scatter-plots distribution of DEGs in the comparison of PO vs WH. (**C**) Scatter-plots distribution of DEGs in the comparison of PO vs CO. (**D**) Scatter-plots distribution of DEGs in the comparison of CO vs WH. The gray points showed unigenes with no change in expression, while the red and blue points indicated up-regulated and down-regulated DEGs, respectively.

3.4. Gene Ontology (GO) Classification and Functional Enrichment of DEGs

Based on the GO database, the results of GO classification and functional enrichment analysis are shown in Figures 2, S1 and S2. In the biological process category, multi-organism process, lipid biosynthetic process, and single fertilization were top three GO terms in the PO vs. WH comparison, while lipid biosynthetic process, fatty acid biosynthetic process, and monocarboxylic acid biosynthetic process were top three GO terms in the PO vs. CO comparison. In the cellular components category, the GO terms with the greatest enrichment were extracellular region, extracellular region part, and extracellular matrix in the PO vs. WH comparisons. For the PO vs. CO comparison, extracellular region, extracellular region part, and microtubule cytoskeleton were the most highly enriched GO terms in the cellular components category. In the molecular function category, the top GO terms included oxidoreductase activity, acrosin binding, and mRNA binding between the different pairwise comparisons of three groups.



(**A**) PO vs. WH



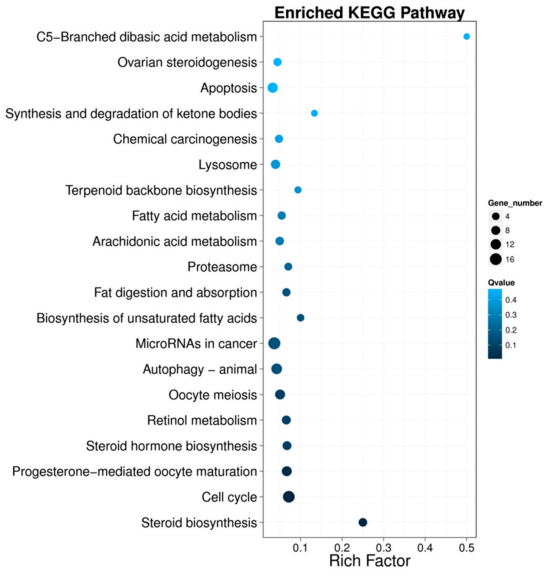
(**B**) PO vs. CO

Figure 2. Gene ontology (GO) classification and functional enrichment of DEGs between groups. (**A**) GO terms of DEGs in the comparison of PO vs WH. (**B**) GO terms of DEGs in the comparison of PO vs CO. The number of DEGs enriched GO term is shown on the Y axis. Three kinds of GO terms (biological process (red), cellular component (blue), and molecular function (green)) are visible in the figure.

3.5. Kyoto Encyclopedia of Genes and Genomes (KEGG) Analysis of DEGs

KEGG pathway enrichment was completed by mapping all DEGs to the terms of the KEGG databases. The analysis showed that the significant pathways ($p < 0.05$) in the PO vs. WH comparison mainly contained steroid biosynthesis, fat digestion and absorption (AMPK, and PPAR signaling pathways), cell cycle (p53 signaling pathway), retinol, and fatty acid metabolism (Figures 3A and S3). In the PO vs. CO comparison, the DEGs were significantly enriched in steroid biosynthesis, cell cycle, and progesterone-mediated oocyte maturation (Figures 3B and S4).

(**A**) PO vs. WH



(**B**) PO vs. CO

Figure 3. Kyoto encyclopedia of genes and genomes (KEGG) analysis of DEGs between groups. (**A**) Top enriched pathways of DEGs in the comparison of PO vs WH. (**B**) Top enriched pathways of DEGs in the comparison of PO vs CO. The percentage of DEGs annotated with KEGG pathway term is presented on the X axis. The annotated KEGG pathway term is presented on the Y axis. The number of DEGs annotated with a certain KEGG term and the enhanced significance are presented by the size and color of the bubbles, respectively.

Among the DEGs mapped to KEGG pathway with significant enrichment, the major genes related to lipid and carbohydrate metabolism are shown in Table 5. In the PO group, five lipogenesis-related DEGs (*fasn*, *acc1*, *scd1*, *elovl6*, and *me1*), four fat digestion and absorption-related DEGs (*fabp7*, *apoa1*, *apoa4*, and *pla2*), and three glycometabolism-related DEGs (*gk*, *g6pd*, and *pepck*) were down-regulated, compared with those in the WH and CO groups. Furthermore, *gk* was up-regulated and *pepck* was down-regulated in the CO group, compared to those in the WH group. Ten steroid synthesis-related DEGs (*cyp51*, *msmo1*, *hmgcs*, *fdft1*, *sqle*, *lss*, *nsdhl*, *ugt*, *cyp1b1*, and *cyp7a1*) were up-regulated in the PO group, compared with those in the WH and CO groups.

Table 5. The major DEGs related to lipid and carbohydrate metabolism.

Gene ID	Abbr.	Expression			Log2 Fold Change//q-Value			KEGG Orthology
		WH	PO	CO	PO vs. WH	PO vs. CO	CO vs. WH	
CI01000055_01852604_01874289	FASN	200,180	74,663	186,817	−1.4 ↓//0	−1.3 ↓//0	NS	K00665//fatty acid synthase, animal type [EC:2.3.1.85]
CI01000059_01463769_01531967	ACC1	84,872	29,407	79,055	−1.5 ↓//0	−1.4 ↓//0	NS	K11262//acetyl-CoA carboxylase/biotin carboxylase 1 [EC:6.4.1.2 6.3.4.14]
CI01154971_00000219_00002467	SCD1	236,272	81,974	207,622	−1.5 ↓//0	−1.3 ↓//0	NS	K00507//stearoyl-CoA desaturase (Delta-9 desaturase) [EC:1.14.19.1]
CI01000000_13579971_13586927	ELOVL6	22,531	3527	23,975	−2.7 ↓//0	−2.8 ↓//0	NS	K10203//elongation of very long chain fatty acids member 6 [EC:2.3.1.199]
CI01000027_09860217_09913586	ME1	40,803	4180	30,966	−3.3 ↓//0	−2.9 ↓//0	NS	K00029//Cytosolic NADP malic enzyme [EC:1.1.1.40]
CI01000053_05704944_05710574	GK	631	327	2440	NS	−2.9 ↓//0	$1.9 \uparrow//3.5 \times 10^{-24}$	K12407//glucokinase [EC:2.7.1.2]
CI01000021_01307370_01312008	G6PD	5287	2372	5850	$-1.2 \downarrow//1.0 \times 10^{-24}$	−1.3 ↓//0	NS	K00036//glucose-6-phosphate 1-dehydrogenase [EC:1.1.1.49 1.1.1.363]
CI01000340_19267148_19271146	PEPCK	310	66	97	$-2.2 \downarrow//1.6 \times 10^{-37}$	NS	$-1.7 \downarrow//9.9 \times 10^{-26}$	K01596//phosphoenolpyruvate carboxykinase (GTP) [EC:4.1.1.32]
CI01000304_01560571_01565345	HMGCS	10,453	26,282	9442	1.3 ↑//0	1.5 ↑//0	NS	K01641//hydroxymethylglutaryl-CoA synthase [EC:2.3.3.10]
CI01000029_08237701_08245368	FDFT1	1248	2887	1052	$1.2 \uparrow//1.7 \times 10^{-145}$	$1.5 \uparrow//1.7 \times 10^{-194}$	NS	K00801//farnesyldiphosphate farnesyltransferase1 [EC:2.5.1.21]
CI01000027_02950348_02954214	SQLE	1057	2832	1073	$1.4 \uparrow//6.0 \times 10^{-18}$	$1.4 \uparrow//7.0 \times 10^{-18}$	NS	K00511//squalene monooxygenase [EC:1.14.14.17]
CI01000009_10376105_10382009	LSS	4403	9838	4385	1.2 ↑//0	1.2 ↑//0	NS	K01852//lanosterol synthase [EC:5.4.99.7]
CI01000319_00143560_00150809	CYP51	5903	13,921	7209	1.2 ↑//0	1.0 ↑//0	NS	K05917//sterol 14-demethylase [EC:1.14.13.70]
CI01000300_07721516_07724293	MSMO1	11,675	24,027	11,951	1.0 ↑//0	1.0 ↑//0	NS	K07750//methylsterol monooxygenase 1 [EC:1.14.13.72]
CI01000110_03769078_03777682	NSDHL	611	1340	611	$1.1 \uparrow//4.6 \times 10^{-61}$	$1.1 \uparrow//1.6 \times 10^{-61}$	NS	K07748//sterol-4alpha-carboxylate 3-dehydrogenase [EC:1.1.1.170]
CI01153302_00000020_00004982	UGT	1224	2733	1179	$1.2 \uparrow//1.6 \times 10^{-128}$	$1.2 \uparrow//3.1 \times 10^{-139}$	NS	K00699//glucuronosyltransferase [EC:2.4.1.17]
CI01000012_01601665_01604244	CYP1B1	47	97	47	1.0 ↑//0.0002	1.1 ↑//0.0002	NS	K07410//cytochrome P450 family 1 subfamily B1 [EC:1.14.14.1]
CI01000004_06651958_06654716	CYP7A1	6926	10,758	4543	NS	1.2 ↑//0	NS	K00489//cholesterol 7alpha-monooxygenase [EC:1.14.14.23]
CI01000311_02173908_02174778	FABP7	2140	245	2533	−3.1 ↓//0	−3.4 ↓//0	NS	K08756//fatty acid-binding protein 7, brain
CI01000039_08052093_08053571	ApoA1	399,511	139,331	367,496	−1.5 ↓//0	−1.4 ↓//0	NS	K08757//apolipoprotein A-I
CI01000195_00989184_00990148	ApoA4	25,163	9555	18,052	−1.4 ↓//0	NS	NS	K08760//apolipoprotein A-IV
CI01000046_02066859_02072868	PLA2	155	21	109	$-2.9 \downarrow//3.7 \times 10^{-25}$	$-2.4 \downarrow//1.9 \times 10^{-14}$	NS	K01047//secretory phospholipase A2 [EC:3.1.1.4]
CI01000051_01785489_01787612	IGFBP3	1257	2816	1345	$1.2 \uparrow//2.0 \times 10^{-13}$	$1.1 \uparrow//7.9 \times 10^{-27}$	NS	K10138//insulin-like growth factor-binding protein 3
CI01000054_01227110_01229796	EFF1A	331,052	372,917	307,816	NS	NS	NS	K03231//elongation factor 1-alpha (Reference genes for qRT-PCR)
CI01000313_01716967_01719318	RPL13A	36,377	44,148	35,194	NS	NS	NS	K02872//ribosomal protein L13a (Reference genes for qRT-PCR)

'NS': No significant difference between two groups.

3.6. Validation of RNA-Seq Results using RT-qPCR

To validate the RNA-seq results, nine genes (*fasn*, *acc1*, *scd*, *pepck*, *me1*, *gk*, *g6pd*, *cyp51*, and *msmo1*) were randomly selected for RT-qPCR analysis using *ef1a* as the housekeeping gene. The RT-qPCR data were in agreement with the RNA-seq results (Figure 4).

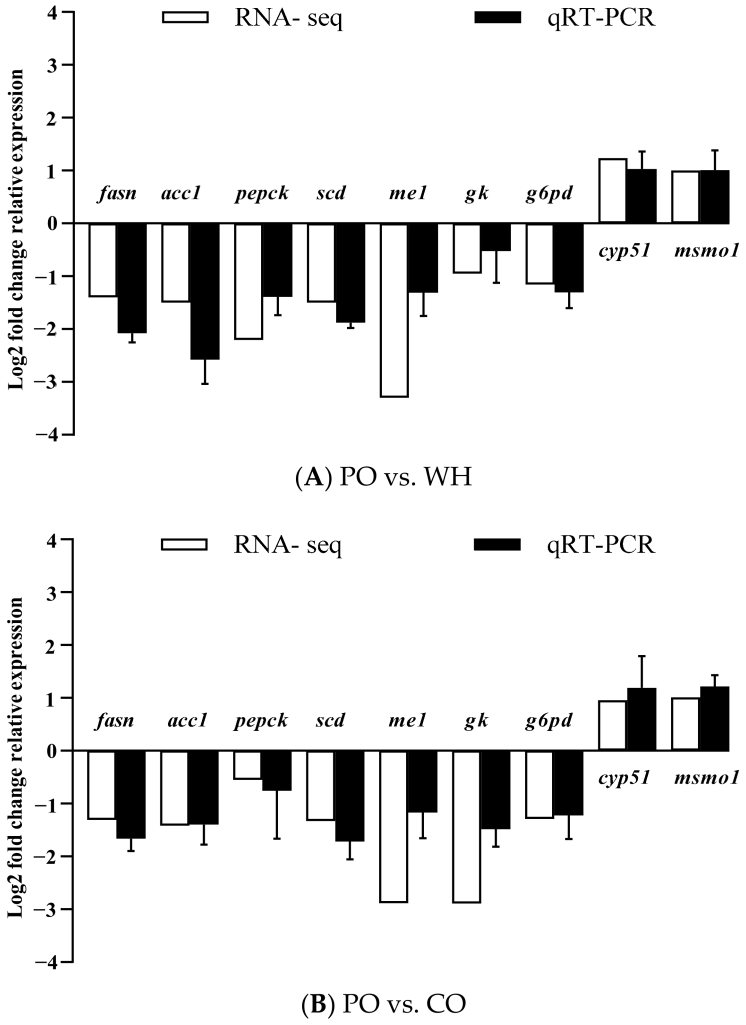


Figure 4. Validation of the expression of DEGs using RT-qPCR. (**A**). Relative expression levels of 9 DEGs validated using qRT-PCR in the comparison of PO vs. WH. (**B**). Relative expression levels of 9 DEGs validated using qRT-PCR in the comparison of PO vs. CO. Data are expressed as means ± S.E (n = 3).

4. Discussion

4.1. Effect of Different Starch Diets on Growth Performance of Grass Carp

Starch is a mixture of amylose and amylopectin, referred to as resistant and digestible starch, respectively. In livestock, the high-amylose starch is difficult to hydrolyze, whereas that consisting of only amylopectin is easily digested for further growth utilization. The starch digestibility and the related metabolic reactions in aquatic animals have also been reported to be influenced by the amylose/amylopectin ratio [32], swelling, viscosity, and other physicochemical characteristics of starch [32]. Thus, differences in composition and functional properties of dietary starch could cause variations in growth performance in aquaculture, as documented in several fish species [10,18,22,33]. In this study, the FW and WGR of juvenile fish fed the CO diet were significantly lower, whereas the values of VSI and FCR were significantly higher than those of fish fed the PO diet. Furthermore, VSI levels obtained from the PO group were significantly lower than those of the WH group. It indicated that grass carp has a better ability to utilize nutrients from potato than that from dietary wheat and corn. Similarly, the improved growth and feed utilization were observed in striped catfish (*Pangasianodon hypophthalmus*) receiving the potato-based diet [34]. In contrast, no significant alterations in weight gain and feed conversion were found in yellow perch [15], blunt snout bream (*Megalobrama amblycephala*) [35], or infoil

barb (*Barbonymus schwanenfeldii*) [36], when these fish were fed the potato-, corn-, or wheat-based diets, particularly the corn- and wheat-based diets. Additionally, gilthead seabream (*Sparus aurata*) fed 19.25% dietary corn starch exhibited lower feed efficiency and higher feed intake compared with those fed 19.25% dietary wheat starch [37]. In addition to differences in species and dietary starch level, these results may be because corn starch has low viscosity and is not conducive to the adhesion of aquatic pellet feeds, and may be easily lost from the aquafeeds. Another possible explanation is that raw corn starch and its cuticular starch are hard starch types, which are hard to be digested and absorbed by some aquatic animals [10,38]. Similar findings were also observed when juvenile grass carp (8.49 $\pm$ 0.04 g) were fed diets containing 30% corn, wheat, or rice starch for 80 days [13]. Our data may indicate that PO diet is more beneficial in promoting the growth of grass carp among the dietary starch examined in this study. This speculation could be partly supported by the expression of growth-related genes obtained from RNA-seq. For example, insulin-like growth factor-binding protein 3 (IGFBP3) plays an important role in the regulation of cell proliferation, growth, and development in animals [39,40]. In this study, the expression of *igfbp3* gene among DEGs was significantly higher in fish fed potato starch diet than in fish fed corn starch diet (Table 5), which was consistent with the growth results of this experiment.

Wheat and corn starch were more likely to cause fat deposition in the liver and mesentery compared to other starch types [13]. Striped catfish fed a corn-based diet exhibited significantly higher FCR than that of the fish fed potato- and wheat-based diets [34]. A 25% corn starch/yellow dextrin (2:1) diet induced more fat deposits in the livers of European sea bass (*Dicentrarchus labrax* L.) than those in the 12.6% and 0.2% groups [14], further demonstrating that lipid accumulation in fish body was affected by dietary carbohydrate sources. In another study regarding juvenile yellow perch fed three diets containing 200 g/kg of corn, potato, or wheat starch, the wheat starch diet resulted in significantly enlarged livers and increased accumulation of lipid in the liver, viscera, and whole body, while CO or PO starch had no effect on the fish's health [15]. This is inconsistent with the results of this experiment, and the reason may be because of the different feeding habits of the fish and fish size used in different studies. In the present and others studies [15,18], it was found that corn starch significantly affected the growth of fish and caused more fat deposition compared with other starch. These results indicate that among tested starch sources, PO is the best starch source, and that CO alone may not be suitable as a starch source for grass carp, in terms of growth and feed utilization.

4.2. Analysis of Lipid and Carbohydrate Metabolism-Related DEGs

4.2.1. Analysis of Carbohydrate Metabolism-Related DEGs

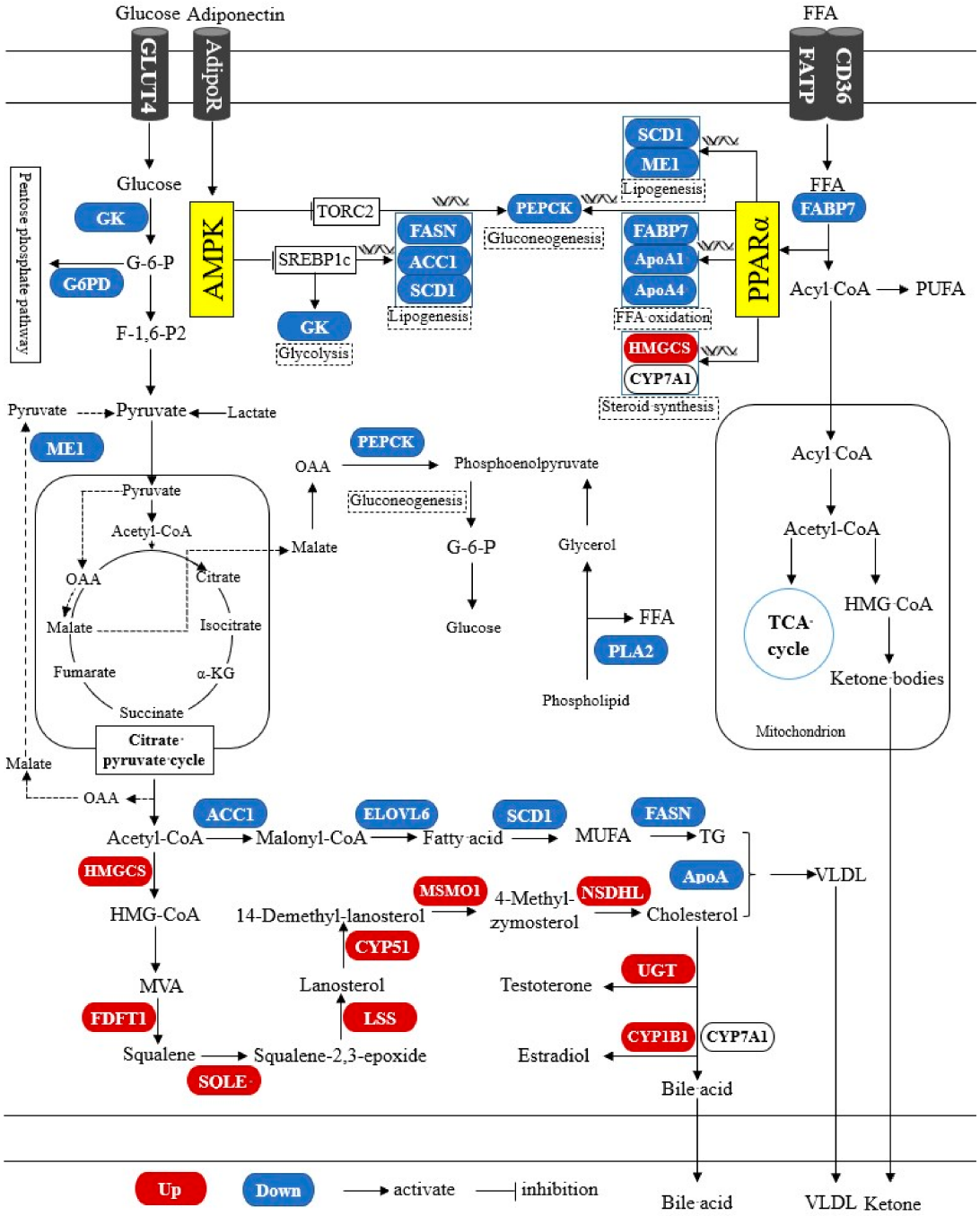
Carbohydrates, an indispensable source of nutrients in feed, can provide sufficient energy for the body. Glucose is one of the most important carbohydrates, and the dynamic balance of glucose metabolism depends on the activity and gene expression of many enzymes involved in glycogenolysis and gluconeogenesis [41]. In the liver, glucose is absorbed by hepatocytes with the assistance of glucose transporter 4 (GLUT4), and further converted to pyruvate via glycolysis, which can be oxidized in case of insufficient energy or used to synthesize fatty acids when energy is available [42]. Glucokinase (GK), glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase (PK), and phosphofructokinase (PFK) are the key regulatory enzymes that play crucial roles in glycolysis [43]. The enzyme activities and metabolic capacities of these glycolytic enzymes are closely associated with the dietary macronutrient levels in mammal and fish [42–47]. For example, high glucose level within a certain concentration elevated G6PD activity and stimulated glucose entry into pentose phosphate shunt in mammals [44,46]. However, excess glucose intake caused lower G6PD activity/expression via phosphorylation [44,47], which could result in glycometabolism disorder and oxidative stress in the metabolically critical organs of mammals [44]. In line with that, the GK activity and *gk* gene expression in fish are regulated by exogenous nutrients [48,49]. Diets with high sugar content have been demonstrated

to promote the expression of *gk* gene in the liver of grass carp [48,49]. High levels of dietary carbohydrate also inhibited gluconeogenesis in blunt snout bream by repressing the expression of *pepck* and *g6pd* [50]. The elevated expressions and enzyme activities of glucose-6-phosphatase catalytic subunit (G6PC) and phosphoenolpyruvate carboxykinase (PEPCK) in the liver were easily observed in some rodent and fish species [44,51–53] when the carbohydrate content of the diet was increased. Meanwhile, the mRNA expression of *g6pc* and *pepck* decreased significantly under dietary glucose excess condition [44,51,53,54]. In this study, fish fed the PO diet showed lower mRNA levels of *gk*, *pepck*, and *g6pd* than those fed the WH and CO diets (Table 5). These findings are in accordance with those of a study on rice field eel (*Monopterus albus*), in which the 40% corn starch diet significantly upregulated the expressions of *gk*, *pk*, *pfk*, and *g6pd* in the liver [55]. The results obtained in this study imply that the glycolysis response was relatively weak in the PO group, thereby retaining more sugar and other related carbohydrate substrates in the liver of grass carp.

4.2.2. Analysis of Lipogenesis-Related DEGs

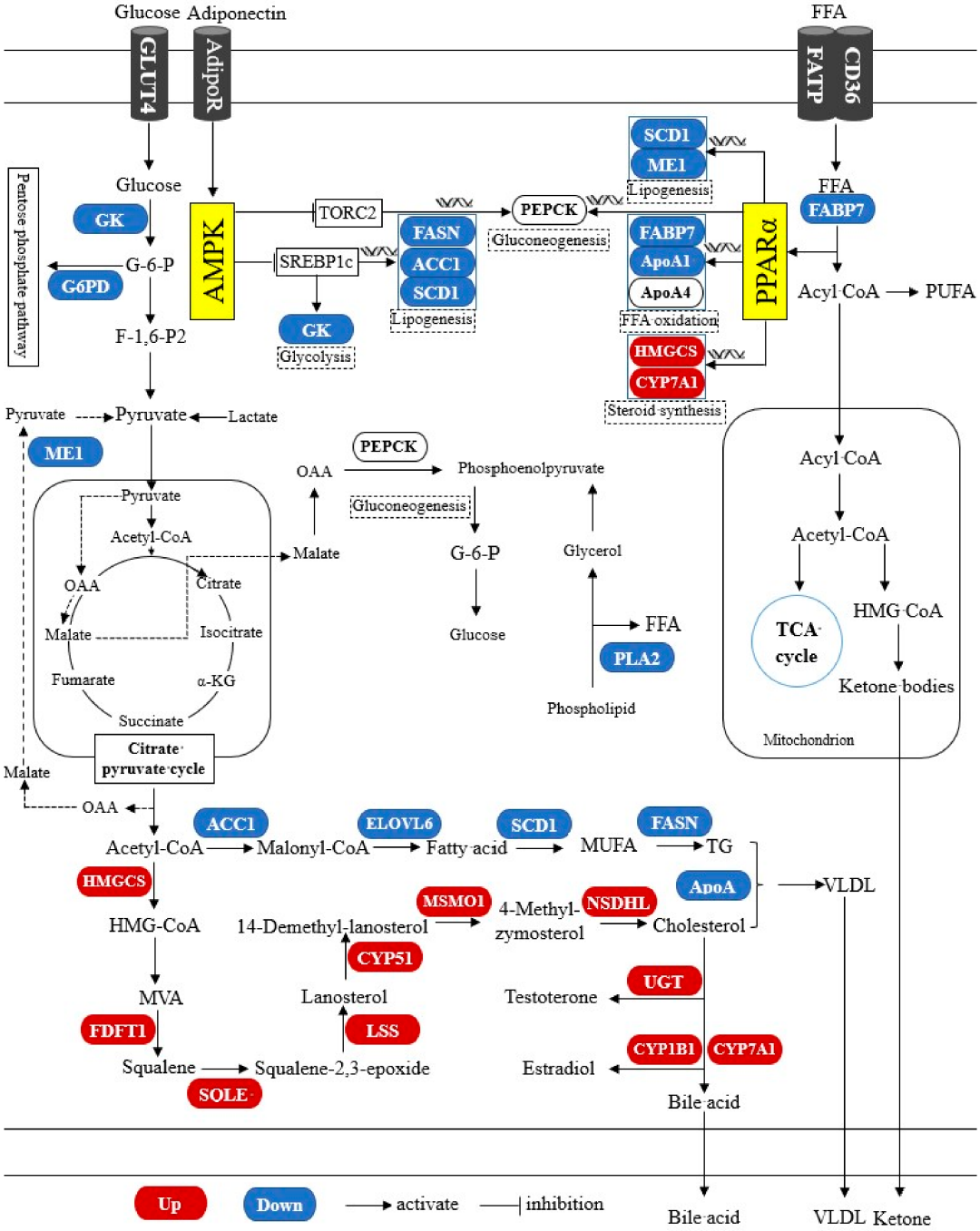
The PPAR and AMPK signaling pathways are two of the signaling pathways that control lipid homeostasis in organisms, including fish [56,57]. The de novo lipogenesis pathway in the liver is responsible for converting excess glucose into fatty acids [58,59] and involves the rate-limiting processes tightly catalyzed by acetyl CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl CoA desaturase (SCD).

AMPK, the master regulator of metabolism, is activated in cases of energy and glucose shortage [56,57,60,61], but suppressed in the case of over-nutrition [57]. Jiang [62] has confirmed that high glucose levels repress AMPK signaling pathway via MG53 E3 ubiquitin ligase-induced AMPK degradation and inactivation. When AMPK is inhibited, the expression of SREBP-1c and its downstream genes [26,31,63,64], such as *fasn*, *acc1*, *elovl6*, and *scd1*, are up-regulated, thus increasing the accumulation of lipid droplets. High dietary levels of digestible carbohydrate induced lipogenesis and enhanced lipid deposition in the liver of rainbow trout (*Oncorhynchus mykiss*) [12,65,66]. Similar to the aforementioned findings in rainbow trout [65], the expressions of lipogenesis-related genes (*fasn*, *acc1*, *scd1*, *elovl6*, and *me1*) were down-regulated in the grass carp of PO group compared to WH and CO groups (Table 5 and Figure 5). These findings suggested that grass carp fed a diet including wheat or corn starch would show more active lipogenesis in their livers. The results are consistent with the study of gibel carp (*Carassius gibelio*), in which a corn starch diet up-regulated *acc* and *fasn* expressions of hepatopancreas, and down-regulated *cpt1a* expression [67]. However, in yellow catfish (*Pelteobagrus fulvidraco*) [68], corn starch down-regulated the mRNA expression levels of *fasn* and *acc1* compared with glucose, dextrin, and potato starch diets. These inconsistencies might be because of the differences in fasting time prior to final sampling, as well as the feeding habits of fish. Therefore, our results demonstrated that CO diet may be prone to accumulate more hepatic fat via increasing lipogenesis related genes, which has been proved to be an efficient energy conversion pathway in response to glucose intolerance in grass carp and other herbivorous fish species [66].



(**A**) PO vs. WH

Figure 5. *Cont.*



(**B**) PO vs. CO

Figure 5. The signaling network of lipid and carbohydrate metabolism caused by dietary starch sources. (**A**) Significantly differentially expressed genes involved in lipid and carbohydrate metabolism in the comparison of PO vs WH. (**B**) Significantly differentially expressed genes involved in lipid and carbohydrate metabolism in the comparison of PO vs CO. Red color represents up-regulated enzyme, and blue color represents down-regulated enzyme. OAA: oxaloacetate; α-KG: α-ketoglutarate; WH: wheat starch; CO: corn starch; PO: potato starch; and HMG-CoA: hydroxymethylglutaryl-CoA.

4.2.3. Analysis of Steroid Synthesis-Related DEGs

Cholesterol has a variety of physiological functions, including the regulation of membrane function and the biosynthesis of bile acids and steroid hormones [69,70]. The liver is the main organ for cholesterol biosynthesis, and cholesterol is ultimately produced from

mevalonic acid by a series of enzymes such as HMGCR (3-hydroxy-3-methylglutaryl-CoA reductase), SQLE (squalene monooxygenase), LSS (lanosterol synthase), CYP51 (sterol 14-demethylase), MSMO1 (methylsterol monooxygenase 1), and NSDHL (sterol-4alpha-carboxylate 3-dehydrogenase). Cholesterol produces various oxysterols through enzymatic and non-enzymatic routes [71,72], some of which are further metabolized into bile acids by CYP7A1 and cytochrome P450 (CYP) family members [69,71–73]. The hepatic–intestinal circulation of bile acids promotes the emulsification and absorption of nutrients such as lipids and vitamins [73] and enables the biosynthesis of bile acids in the liver and their reabsorption in the small intestine, which act synergistically to maintain the metabolic homeostasis of bile acids and cholesterol [73,74]. The diets supplemented with corn starch increased the plasma glucose levels in gibel carp, in which plasma glucose levels were positively correlated with liver fat contents and negatively correlated with the levels of total cholesterol, amylase, and cholesterol [67]. Meanwhile, probiotic bacteria supplementation in the diet or probiotic fermented starch/other carbohydrates can reduce the systemic levels of blood lipids in multiple fish species, by both transferring cholesterol from plasma to the liver and suppressing hepatic cholesterol biosynthesis [75–78], which could contribute to improve the health status in aquatic animals. In this study, the cholesterol synthesis-related genes (*hmgcs*, *fdft1*, *sqle*, *lss*, *cyp51*, *cyp7a1*, *msmo1*, and *nsdhl*) were all significantly up-regulated in the PO group compared to those in the WH and CO groups (Table 5 and Figure 5), suggesting that the PO diet activated the enzymes related to cholesterol synthesis in the liver of grass carp. These up-regulation of cholesterol synthesis related gene in the PO diet indicated more production of cholesterol, and more cholesterol may be further utilized for lipid metabolism and converted into bile acids thought CYP7A1 [79].

5. Conclusions

In this study, VSI values of grass carp from the CO and WH groups were significantly higher than those of the PO group, while FW and WGR of fish fed with the CO diet were significantly lower than those of the PO diet. Using RNA-seq, 1697 DEGs were screened, functionally annotated, and enriched in multiple signaling pathways related to lipid metabolism. The PO group increased the expressions of steroid synthesis genes while decreasing the gene expressions related to lipid and carbohydrate metabolism. The CO and WH groups increased the expression levels of lipogenesis genes. In conclusion, potato starch is the best starch source, followed by wheat starch, but further studies are required to determine the optimal amounts, whereas corn starch alone may not be suitable as a starch source in grass carp feed. Our work revealed the transcriptomic data of the hepatopancreatic tissues in grass carp fed with different dietary starch types and provided new insights into the mechanisms involved in coping with different dietary starch sources. Meanwhile, muscle quality and other parameters related to growth and nutrition in grass carp fed with different starch source diets need to be further explored, and their application effects in other aquatic animals are also worthy of attention.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8100495/s1, Figure S1: GO classification of up and down-regulated gens in the comparison of Potato-VS-Wheat, Figure S2: GO classification of up and down-regulated gens in the comparison of Potato-VS-Corn, Figure S3: KEGG Pathway functional enrichment result for up/down-regulated gens in the comparison of Potato-VS-Wheat, Figure S4: KEGG Pathway functional enrichment result for up/down-regulated gens in the comparison of Potato-VS-Corn.

Author Contributions: J.Z.: Methodology, Formal analysis, Software, and Writing. X.G. and Z.H.: Methodology, Formal analysis, and Investigation. L.Q.: Provided assistance in sampling. T.X.: Provided assistance in sampling. X.C.: Formal analysis, Reviewing, and Editing. J.X.: Provided assistance in terms of technology. Z.D.: Methodology. C.W.: Provided assistance in terms of technology. H.C.: Writing, Editing, and Funding. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: All experiments were conducted at the Jiangsu Ocean University (Lianyungang, China) in accordance with ethical standards for experimental animals of Jiangsu Ocean University and approved by the Animal Care and Use Committee of Jiangsu Ocean University (Approval Date: 1 September 2019; Approval Code: No. 2020-37). All the grass carp were anesthetized with MS-222 (Sigma, St. Louis, MO, USA) before dissection.

Data Availability Statement: The information on the datasets of the repository and accession number in this work can be found online.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Navarro, D.M.D.L.; Abelilla, J.J.; Stein, H.H. Structures and characteristics of carbohydrates in diets fed to pigs: A review. *J. Anim. Sci. Biotechnol.* **2019**, *10*, 39. [CrossRef] [PubMed]
2. Ansari, F.A.; Guldhe, A.; Gupta, S.K.; Rawat, I.; Bux, F. Improving the feasibility of aquaculture feed by using microalgae. *Environ. Sci. Pollut. Res. Int.* **2021**, *28*, 43234–43257. [CrossRef] [PubMed]
3. Rito, J.; Viegas, I.; Pardal, M.; Metón, I.; Baanante, I.V.; Jones, J.G. Utilization of glycerol for endogenous glucose and glycogen synthesis in seabass (*Dicentrarchus labrax*): A potential mechanism for sparing amino acid catabolism in carnivorous fish. *Aquaculture* **2019**, *498*, 488–495. [CrossRef]
4. Corrêia, V.; Goulart, F.R.; Pianesso, D.; Mombach, P.I.; Adorian, T.J.; Lovatto, N.d.M.; da Silva, L.P.; Neto, J.R. Carbohydrate molecule size affects the metabolic and digestive dynamics of jundiá (*Rhamdia quelen*). *Aquac. Res.* **2019**, *50*, 3251–3258. [CrossRef]
5. Jeong, S.-M.; Khosravi, S.; Lee, S.Y.; Kim, K.-W.; Lee, B.-J. Evaluation of the three different sources of dietary starch in an extruded feed for juvenile olive flounder, *Paralichthys olivaceus*. *Aquaculture* **2021**, *533*, 736242. [CrossRef]
6. Zhao, W.; Xie, J.-J.; Fang, H.-H.; Liu, Y.-J.; Tian, L.-X.; Niu, J. Effects of corn starch level on growth performance, antioxidant capacity, gut morphology and intestinal microflora of juvenile golden pompano, *Trachinotus ovatus*. *Aquaculture* **2020**, *524*, 735197. [CrossRef]
7. Miao, S.; Nie, Q.; Miao, H.; Zhang, W.; Mai, K. Effects of dietary carbohydrate-to-lipid ratio on the growth performance and feed utilization of juvenile turbot (*Scophthalmus maximus*). *J. Ocean Univ. China* **2016**, *15*, 660–666. [CrossRef]
8. Ma, H.-J.; Mou, M.-M.; Pu, D.-C.; Lin, S.-M.; Chen, Y.-J.; Luo, L. Effect of dietary starch level on growth, metabolism enzyme and oxidative status of juvenile largemouth bass, *Micropterus salmoides*. *Aquaculture* **2019**, *498*, 482–487. [CrossRef]
9. Lin, S.-M.; Shi, C.-M.; Mu, M.-M.; Chen, Y.-J.; Luo, L. Effect of high dietary starch levels on growth, hepatic glucose metabolism, oxidative status and immune response of juvenile largemouth bass, Micropterus salmoides. *Fish Shellfish Immunol.* **2018**, *78*, 121–126. [CrossRef]
10. Romano, N.; Kumar, V. Starch gelatinization on the physical characteristics of aquafeeds and subsequent implications to the productivity in farmed aquatic animals. *Rev. Aquac.* **2019**, *11*, 1271–1284. [CrossRef]
11. Roques, S.; Deborde, C.; Richard, N.; Skiba-Cassy, S.; Moing, A.; Fauconneau, B. Metabolomics and fish nutrition: A review in the context of sustainable feed development. *Rev. Aquac.* **2020**, *12*, 261–282. [CrossRef]
12. Li, L.-Y.; Wang, Y.; Limbu, S.M.; Li, J.-M.; Qiao, F.; Chen, L.-Q.; Zhang, M.-L.; Du, Z.-Y. Reduced fatty acid β-oxidation improves glucose catabolism and liver health in Nile tilapia (*Oreochromis niloticus*) juveniles fed a high-starch diet. *Aquaculture* **2021**, *535*, 736392. [CrossRef]
13. Tian, L.-X.; Liu, Y.-J.; Hung, S.S.O. Utilization of Glucose and Cornstarch by Juvenile Grass Carp. *North Am. J. Aquac.* **2004**, *66*, 141–145. [CrossRef]
14. Russell, P.M.; Davies, S.J.; Gouveia, A.; A Tekinay, A. Influence of dietary starch source on liver morphology in juvenile cultured European sea bass (*Dicentrarchus labrax* L.). *Aquac. Res.* **2001**, *32*, 306–314. [CrossRef]
15. Jiang, M.; Zhao, H.; Zhai, S.; Newton, R.J.; Shepherd, B.; Tian, J.; Lofald, A.G.; Teh, S.; Binkowski, F.P.; Deng, D. Nutritional quality of different starches in feed fed to juvenile yellow perch, *Perca flavescens*. *Aquac. Nutr.* **2020**, *26*, 671–682. [CrossRef]
16. Yu, M.; Li, Z.; Rong, T.; Wang, G.; Liu, Z.; Chen, W.; Li, J.; Li, J.; Ma, X. Different dietary starch sources alter the carcass traits, meat quality, and the profile of muscle amino acid and fatty acid in finishing pigs. *J. Anim. Sci. Biotechnol.* **2020**, *11*, 78. [CrossRef] [PubMed]
17. Li, J.; Liu, D.D.; Li, H.; Li, Z.J.; Han, R.L.; Wang, Y.B.; Liu, X.J.; Kang, X.T.; Yan, F.B.; Tian, Y.D. Effects of different starch sources on glucose and fat metabolism in broiler chickens. *Br. Poult. Sci.* **2019**, *60*, 449–456. [CrossRef] [PubMed]
18. Liu, X.-H.; Ye, C.-X.; Ye, J.-D.; Shen, B.-D.; Wang, C.-Y.; Wang, A.-L. Effects of dietary amylose/amylopectin ratio on growth performance, feed utilization, digestive enzymes, and postprandial metabolic responses in juvenile obscure puffer Takifugu obscurus. *Fish Physiol. Biochem.* **2014**, *40*, 1423–1436. [CrossRef]

19. Tian, L.X.; Liu, Y.J.; Yang, H.J.; Liang, G.Y.; Niu, J. Effects of different dietary wheat starch levels on growth, feed efficiency and digestibility in grass carp (*Ctenopharyngodon idella*). *Aquac. Int.* **2011**, *20*, 283–293. [CrossRef]
20. Li, S.; Sang, C.; Turchini, G.M.; Wang, A.; Zhang, J.; Chen, N. Starch in aquafeeds: The benefits of a high amylose to amylopectin ratio and resistant starch content in diets for the carnivorous fish, largemouth bass (*Micropterus salmoides*). *Br. J. Nutr.* **2020**, *124*, 1145–1155. [CrossRef]
21. Tehrany, Z.M.; Amirkolaie, A.K.; Oraji, H. The effects of different grain sources on gut evacuation rate and nutrient digestibility in common carp, *Cyprinus carpio*. *Int. J. Aquat. Biol.* **2018**, *6*, 104–113.
22. Li, S.; Sang, C.; Wang, A.; Zhang, J.; Chen, N. Effects of dietary carbohydrate sources on growth performance, glycogen accumulation, insulin signaling pathway and hepatic glucose metabolism in largemouth bass, Micropterus salmoides. *Aquaculture* **2019**, *513*, 734391. [CrossRef]
23. Gao, Q.; Sun, S.; Han, Z.; Xu, J.; Ding, Z.; Cheng, H. Effects of partial replacement of fishmeal with blood meal and dried porcine soluble on the growth, body composition and intestinal morphology of *Cyprinus carpio*. *Aquac. Res.* **2020**, *51*, 1712–1719. [CrossRef]
24. Liu, Y.; Yan, Y.; Han, Z.; Zheng, Y.; Wang, X.; Zhang, M.; Li, H.; Xu, J.; Chen, X.; Ding, Z.; et al. Comparative effects of dietary soybean oil and fish oil on the growth performance, fatty acid composition and lipid metabolic signaling of grass carp, Ctenopharyngodon idella. *Aquac. Rep.* **2022**, *22*, 101002. [CrossRef]
25. Qu, L.; Xia, T.; Du, X.; Lou, B.; Chen, X.; Xu, J.; Ding, Z.; Wei, C.; Cheng, H. Effects of salinity treatment on muscle quality and off-flavour compounds of grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*). *Aquac. Res.* **2022**, *53*, 4823–4831. [CrossRef]
26. Chen, X.; Xu, Y.; Cui, X.; Zhang, S.; Zhong, X.; Ke, J.; Wu, Y.; Liu, Z.; Wei, C.; Ding, Z.; et al. Starvation Affects the Muscular Morphology, Antioxidant Enzyme Activity, Expression of Lipid Metabolism-Related Genes, and Transcriptomic Profile of Javelin Goby (Synechogobius hasta). *Aquac. Nutr.* **2022**, *2022*, 7057571. [CrossRef] [PubMed]
27. Cock, P.J.A.; Fields, C.J.; Goto, N.; Heuer, M.L.; Rice, P.M. The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants. *Nucleic Acids Res.* **2010**, *38*, 1767–1771. [CrossRef] [PubMed]
28. Kim, D.; Langmead, B.; Salzberg, S.L. HISAT: A fast spliced aligner with low memory requirements. *Nat. Methods* **2015**, *12*, 357–360. [CrossRef]
29. Li, B.; Dewey, C.N. RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinform.* **2011**, *12*, 323. [CrossRef]
30. Wang, L.; Feng, Z.; Wang, X.; Wang, X.; Zhang, X. DEGseq: An R package for identifying differentially expressed genes from RNA-seq data. *Bioinformatics* **2010**, *26*, 136–138. [CrossRef]
31. Zhou, R.; Wu, G.; Qu, L.; Zhong, X.; Gao, Y.; Ding, Z.; Xu, J.; Chen, X.; Cheng, H. Effect of starvation on intestinal morphology, digestive enzyme activity and expression of lipid metabolism-related genes in javelin goby (*Synechogobius hasta*). *Aquacult. Res.* **2022**, *53*, 87–97. [CrossRef]
32. Gatesoupe, F.-J.; Huelvan, C.; Le Bayon, N.; Sévère, A.; Aasen, I.M.; Degnes, K.F.; Mazurais, D.; Panserat, S.; Zambonino-Infante, J.L.; Kaushik, S.J. The effects of dietary carbohydrate sources and forms on metabolic response and intestinal microbiota in sea bass juveniles, Dicentrarchus labrax. *Aquaculture* **2014**, *422–423*, 47–53. [CrossRef]
33. Chen, M.-Y.; Ye, J.-D.; Yang, W.; Wang, K. Growth, Feed Utilization and Blood Metabolic Responses to Different Amylose-amylopectin Ratio Fed Diets in Tilapia (*Oreochromis niloticus*). *Asian-Australas. J. Anim. Sci.* **2013**, *26*, 1160–1171. [CrossRef] [PubMed]
34. Asemani, M.; Sepahdari, A.; Pourkazemi, M.; Hafezieh, M.; Aliyu-Paiko, M.; Dadgar, S. Effect of different sources and forms of dietary carbohydrates on growth performance, body indices and lipogenesis activity of striped catfish Pangasianodon hypophthalmus fingerlings. *Aquacult. Nutr.* **2019**, *25*, 1399–1409. [CrossRef]
35. Ren, M.; Habte-Tsion, H.-M.; Xie, J.; Liu, B.; Zhou, Q.; Ge, X.; Pan, L.; Chen, R. Effects of dietary carbohydrate source on growth performance, diet digestibility and liver glucose enzyme activity in blunt snout bream, Megalobrama amblycephala. *Aquaculture* **2015**, *438*, 75–81. [CrossRef]
36. Nafees, M.S.M.; Kamarudin, M.S.; Karim, M.; Hassan, M.Z.; de Cruz, C.R. Effects of dietary starch sources on growth, nutrient utilization and liver histology of juvenile tinfoil barb (*Barbonymus schwanenfeldii*, Bleeker 1853). *Aquac. Rep.* **2022**, *23*, 101069–101080. [CrossRef]
37. Couto, A.; Peres, H.; Oliva-Teles, A.; Enes, P. Screening of nutrient digestibility, glycaemic response and gut morphology alterations in gilthead seabream (*Sparus aurata*) fed whole cereal meals. *Aquaculture* **2016**, *450*, 31–37. [CrossRef]
38. Zhang, L.; Liu, W.-B.; Brown, P.B.; Xu, C.; Shi, H.-J.; Zheng, X.-C.; Zhang, L.; He, C.; Huang, Y.-Y.; Li, X.-F. Utilization of raw and gelatinized starch by blunt snout bream Megalobrama amblycephala as evidenced by the glycolipid metabolism, glucose tolerance and mitochondrial function. *Aquaculture* **2020**, *529*, 735603. [CrossRef]
39. Chen, Y.; Zhou, Y.; Yang, X.; Cao, Z.; Chen, X.; Qin, Q.; Liu, C.; Sun, Y. Insulin-like growth factor binding protein 3 gene of golden pompano (TroIGFBP3) promotes antimicrobial immune defense. *Fish Shellfish Immunol.* **2020**, *103*, 47–57. [CrossRef]
40. Shrivastav, S.V.; Bhardwaj, A.; Pathak, K.A.; Shrivastav, A. Insulin-Like Growth Factor Binding Protein-3 (IGFBP-3): Unraveling the Role in Mediating IGF-Independent Effects Within the Cell. *Front. Cell Dev. Biol.* **2020**, *8*, 286. [CrossRef]

41. Zhang, X.; Huang, C.; Yang, Y.; Li, X.; Guo, C.; Yang, Z.; Xie, S.; Luo, J.; Zhu, T.; Zhao, W.; et al. Dietary corn starch levels regulated insulin-mediated hlycemic responses and glucose homeostasis in swimming crab (*Portunus trituberculatus*). *Aquacult. Nutr.* **2022**, *2022*, 2355274. [CrossRef] [PubMed]
42. Morigny, P.; Boucher, J.; Arner, P.; Langin, D. Lipid and glucose metabolism in white adipocytes: Pathways, dysfunction and therapeutics. *Nat. Rev. Endocrinol.* **2021**, *17*, 276–295. [CrossRef] [PubMed]
43. Shomali, N.; Mahmoudi, J.; Mahmoodpoor, A.; Zamiri, R.E.; Akbari, M.; Xu, H.; Shotorbani, S.S. Harmful effects of high amounts of glucose on the immune system: An updated review. *Biotechnol. Appl. Biochem.* **2020**, *68*, 404–410. [CrossRef] [PubMed]
44. Garcia, A.A.; Koperniku, A.; Ferreira, J.C.; Mochly-Rosen, D. Treatment strategies for glucose-6-phosphate dehydrogenase deficiency: Past and future perspectives. *Trends Pharmacol. Sci.* **2021**, *42*, 829–844. [CrossRef] [PubMed]
45. Xie, N.; Wen, H.; Xie, S.; Jiang, M.; Yu, L.; Wu, F.; Lu, X.; Meng, X.; Tian, J. Adaptations of hepatic lipid and glucose metabolism in response to high-macronutrient diets in juvenile grass carp. *Aquac. Nutr.* **2021**, *27*, 1738–1749. [CrossRef]
46. Sun, M.; Li, Z.; Xing, Y.; Mu, X.; Cao, Y.; Hao, Y.; Yang, J.; Li, D. Effects of glucose availability on αS1-casein synthesis in bovine mammary epithelial cells. *J. Anim. Sci.* **2022**, *100*, skac330. [CrossRef] [PubMed]
47. Wang, M.; Hu, J.; Yan, L.; Yang, Y.; He, M.; Wu, M.; Li, Q.; Gong, W.; Yang, Y.; Wang, Y.; et al. High glucose–induced ubiquitination of G6PD leads to the injury of podocytes. *FASEB J.* **2019**, *33*, 6296–6310. [CrossRef]
48. Cai, W.; Liang, X.-F.; Yuan, X.; Liu, L.; He, S.; Li, J.; Li, B.; Xue, M. Different strategies of grass carp (*Ctenopharyngodon idella*) responding to insufficient or excessive dietary carbohydrate. *Aquaculture* **2018**, *497*, 292–298. [CrossRef]
49. Li, R.X.; Liu, H.Y.; Chen, Q.; Tan, B.-P.; Dong, X.-H.; Chi, S.-Y.; Yang, Q.-H. Glucose tolerance of grass carp Ctenopharyngodon idellus after a long-term adaptation to carbohydrate-to-lipid ratio diets. *Aquacult. Res.* **2018**, *49*, 3881–3888. [CrossRef]
50. Liang, H.; Mokrani, A.; Chisomo-Kasiya, H.; Wilson-Arop, O.-M.; Mi, H.; Ji, K.; Ge, X.; Ren, M. Molecular characterization and identification of facilitative glucose transporter 2 (GLUT2) and its expression and of the related glycometabolism enzymes in response to different starch levels in blunt snout bream (*Megalobrama amblycephala*). *Fish Physiol. Biochem.* **2018**, *44*, 869–883. [CrossRef]
51. Liu, D.; Regenstein, J.M.; Diao, Y.; Qiu, J.; Zhang, H.; Li, J.; Zhao, H.; Wang, Z. Antidiabetic effects of water-soluble Korean pine nut protein on type 2 diabetic mice. *BioMedicine* **2019**, *117*, 108989. [CrossRef] [PubMed]
52. Cai, W.-J.; Liang, X.-F.; Yuan, X.-C.; Li, A.-X.; He, S. Changes of DNA Methylation Pattern in Metabolic Pathways Induced by High-Carbohydrate Diet Contribute to Hyperglycemia and Fat Deposition in Grass Carp (Ctenopharyngodon idellus). *Front. Endocrinol.* **2020**, *11*, 398. [CrossRef] [PubMed]
53. Li, S.L.; Li, Z.Q.; Zhang, J.C.; Sang, C.; Chen, N. The impacts of dietary carbohydrate levels on growth performance, feed utilization, glycogen accumulation and hepatic glucose metabolism in hybrid grouper (*Epinephelus fuscoguttatus* ♀× *E. lanceolatus* ♂). *Aquaculture* **2019**, *512*, 734351. [CrossRef]
54. Sari, D.N.; Ekasari, J.; Nasrullah, H.; Suprayudi, M.A.; Alimuddin, A. High carbohydrate increases amylase, plasma glucose, and gene expression related to glycolysis in giant gourami Osphronemus goramy. *Fish Physiol. Biochem.* **2022**, *48*, 1495–1505. [CrossRef] [PubMed]
55. Shi, Y.; Zhong, L.; Zhong, H.; Zhang, J.; Liu, X.; Peng, M.; Fu, G.; Hu, Y. Taurine supplements in high-carbohydrate diets increase growth performance of Monopterus albus by improving carbohydrate and lipid metabolism, reducing liver damage, and regulating intestinal microbiota. *Aquaculture* **2022**, *554*, 738150. [CrossRef]
56. Gao, Z.; Liu, C.; Mai, K.; He, G. Nutrient Sensing for the Future of Land-Farmed Animal and Aquaculture Nutrition. *Engineering* **2023**, *23*, 112–117. [CrossRef]
57. Trefts, E.; Shaw, R.J. AMPK: Restoring metabolic homeostasis over space and time. *Mol. Cell* **2021**, *81*, 3677–3690. [CrossRef] [PubMed]
58. Zhang, Y.; Lu, R.; Qin, C.; Nie, G. Precision nutritional regulation and aquaculture. *Aquac. Rep.* **2020**, *18*, 100496. [CrossRef]
59. Wallace, M.; Metallo, C.M. Tracing insights into de novo lipogenesis in liver and adipose tissues. *Semin. Cell Dev. Biol.* **2020**, *108*, 65–71. [CrossRef]
60. Wu, W.; Sun, J.; Ji, H.; Yu, H.; Zhou, J. AMP-activated protein kinase in the grass carp Ctenopharyngodon idellus: Molecular characterization, tissue distribution and mRNA expression in response to overwinter starvation stress. *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* **2020**, *246–247*, 110457. [CrossRef]
61. Xu, C.; Liu, W.-B.; Remø, S.C.; Wang, B.-K.; Shi, H.-J.; Zhang, L.; Liu, J.-D.; Li, X.-F. Feeding restriction alleviates high carbohydrate diet-induced oxidative stress and inflammation of Megalobrama amblycephala by activating the AMPK-SIRT1 pathway. *Fish Shellfish Immunol.* **2019**, *92*, 637–648. [CrossRef] [PubMed]
62. Jiang, P.; Ren, L.; Zhi, L.; Yu, Z.; Lv, F.; Xu, F.; Peng, W.; Bai, X.; Cheng, K.; Quan, L.; et al. Negative regulation of AMPK signaling by high glucose via E3 ubiquitin ligase MG53. *Mol. Cell* **2021**, *81*, 629–637.e5. [CrossRef] [PubMed]
63. Xu, J.; Li, X.; Yao, X.; Xie, S.; Chi, S.; Zhang, S.; Cao, J.; Tan, B. Protective Effects of Bile Acids Against Hepatic Lipid Accumulation in Hybrid Grouper Fed a High-Lipid Diet. *Front. Nutr.* **2022**, *9*, 813249. [CrossRef] [PubMed]
64. Tan, X.-Y.; Xu, Y.-C.; Zhao, T.; Zhang, L.-H.; Luo, Z. Novel insights for SREBP-1 as a key transcription factor in regulating lipogenesis in a freshwater teleost, grass carp *Ctenopharyngodon idella*. *Br. J. Nutr.* **2019**, *122*, 1201–1211. [CrossRef]
65. Luo, Y.; Hu, C.-T.; Qiao, F.; Wang, X.-D.; Qin, J.G.; Du, Z.-Y.; Chen, L.-Q. Gemfibrozil improves lipid metabolism in Nile tilapia *Oreochromis niloticus* fed a high-carbohydrate diet through peroxisome proliferator activated receptor-α activation. *Gen. Comp. Endocrinol.* **2020**, *296*, 113537. [CrossRef] [PubMed]

66. Li, X.; Han, T.; Zheng, S.; Wu, G. Hepatic glucose metabolism and its disorders in fish. In *Advances in Experimental Medicine and Biology, Recent Advances in Animal Nutrition and Metabolism*; Wu, G., Ed.; Springer: Cham, Switzerland, 2022; Volume 1354, pp. 207–236.
67. Song, X.; Liu, H.; Jin, J.; Han, D.; Zhu, X.; Yang, Y.; Xie, S. Data Mining Evidences Variabilities in Glucose and Lipid Metabolism among Fish Strains: A Case Study on Three Genotypes of Gibel Carp Fed by Different Carbohydrate Sources. *Aquac. Nutr.* **2023**, *2023*, 7589827. [CrossRef] [PubMed]
68. Yang, S.-B.; Zhao, T.; Wu, L.-X.; Xu, Y.-C.; Tan, X.-Y. Effects of dietary carbohydrate sources on lipid metabolism and SUMOylation modification in the liver tissues of yellow catfish. *Br. J. Nutr.* **2020**, *124*, 1241–1250. [CrossRef]
69. Luo, J.; Yang, H.; Song, B.-L. Mechanisms and regulation of cholesterol homeostasis. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 225–245. [CrossRef]
70. Lu, X.Y.; Shi, X.J.; Hu, A.; Wang, J.Q.; Ding, Y.; Jiang, W.; Sun, M.; Zhao, X.; Luo, J.; Qi, W.; et al. Feeding induces cholesterol biosynthesis via the mTORC1–USP20–HMGCR axis. *Nature* **2020**, *588*, 479–484. [CrossRef]
71. Samadi, A.; Sabuncuoglu, S.; Samadi, M.; Isikhan, S.Y.; Chirumbolo, S.; Peana, M.; Lay, I.; Yalcinkaya, A.; Bjørklund, G. A Comprehensive Review on Oxysterols and Related Diseases. *Curr. Med. Chem.* **2020**, *28*, 110–136. [CrossRef]
72. Lefort, C.; Cani, P.D. The Liver under the Spotlight: Bile Acids and Oxysterols as Pivotal Actors Controlling Metabolism. *Cells* **2021**, *10*, 400. [CrossRef] [PubMed]
73. Jiao, T.-Y.; Ma, Y.-D.; Guo, X.-Z.; Ye, Y.-F.; Xie, C. Bile acid and receptors: Biology and drug discovery for nonalcoholic fatty liver disease. *Acta Pharmacol. Sin.* **2022**, *43*, 1103–1119. [CrossRef] [PubMed]
74. Wang, L.; Sagada, G.; Wang, C.; Liu, R.; Li, Q.; Zhang, C.; Yan, Y. Exogenous bile acids regulate energy metabolism and improve the health condition of farmed fish. *Aquaculture* **2023**, *562*, 738852. [CrossRef]
75. Ringø, E.; Harikrishnan, R.; Soltani, M.; Ghosh, K. The Effect of Gut Microbiota and Probiotics on Metabolism in Fish and Shrimp. *Animals* **2022**, *12*, 3016. [CrossRef] [PubMed]
76. Dawood, M.A.O.; Koshio, S. Application of fermentation strategy in aquafeed for sustainable aquaculture. *Rev. Aquac.* **2020**, *12*, 987–1002. [CrossRef]
77. Romano, N.; Dauda, A.B.; Ikhsan, N.; Karim, M.; Kamarudin, M.S. Fermenting rice bran as a carbon source for biofloc technology improved the water quality, growth, feeding efficiencies, and biochemical composition of African catfish *Clarias gariepinus* juveniles. *Aquac. Res.* **2018**, *49*, 3691–3701. [CrossRef]
78. Mohammady, E.Y.; Aboseif, A.M.; Soaudy, M.R.; Ramadan, E.A.; Hassaan, M.S. Appraisal of fermented wheat bran by Saccharomyces cerevisiae on growth, feed utilization, blood indices, intestinal and liver histology of Nile tilapia, *Oreochromis niloticus*. *Aquaculture* **2023**, *575*, 739755. [CrossRef]
79. Adam, A.H.; Verdegem, M.; Soliman, A.A.; Zaki, M.; Khalil, R.H.; Nour, A.-E.M.; Khaled, A.A.; El Basuini, M.F.; Khalil, H.S. Effect of dietary bile acids: Growth performance, immune response, genes expression of fatty acid metabolism, intestinal, and liver morphology of striped catfish (*Pangasianodon hypophthalmus*). *Aquac. Rep.* **2023**, *29*, 101510. [CrossRef]