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Research Article

Two Strains of Gibel Carp (*Carassius gibelio*) Exhibit Diverse Responses to Carbohydrates in a Low-Lipid Diet

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An 8-week feeding trial was conducted to investigate carbohydrate use in a low-lipid diet between different strains of gibel carp (*Carassius gibelio*). The wild-type Dongting strain and artificially selected CASIII strain were fed three levels of carbohydrate: 15%, 30%, and 45%, in a low-lipid formulation (4%). The results showed that low-lipid supplementation with high carbohydrate seems to improve the feeding rate (FR) and specific growth rate (SGR), while it did not result in hyperglycaemia in gibel carp. Transcriptional regulation was slightly affected by carbohydrate levels; only hepatic *pfk* expression was more increased in the 30% and 45% carbohydrate diets than in the 15% carbohydrate diet ($p < 0.05$). Regarding the strains, CASIII obtained higher growth performance by having higher FR and SGR than Dongting ($p < 0.05$). Relatively higher glycaemia was found in CASIII, and it was coupled with increased plasma triglycerides and insulin ($p > 0.05$). Enhanced gene expression of *pk* involved in glycolysis and decreased mRNA levels of *pepck* in gluconeogenesis were also observed in CASIII ($p < 0.05$). There were strong strain ∗ diet interactions of gene expression involved in lipogenesis, and Dongting had higher mRNA levels of *srebp-1*, *acly*, and *fas* when fed carbohydrate at 45% ($p < 0.05$). In conclusion, the tolerant level of carbohydrates in a low-lipid diet could be up to 45% for gibel carp by an improvement of growth and noninducement of postprandial plasma glucose. CASIII had a better ability to use dietary carbohydrates for growth with active glycolysis and restrained gluconeogenesis, and Dongting had more potential in lipid metabolism in response to carbohydrate intake.

1. Introduction

Numerous studies have confirmed the protein-sparing effect of dietary carbohydrates [1–3], and they are considered environmentally friendly and cost-effective for the aquaculture industry. However, fish are regarded as poor carbohydrate users because of atypical regulation of postprandial hyperglycaemia after inappropriate carbohydrate intake [4] due to the poor molecular regulation of glucose-related metabolic pathways: relatively low efficiency of glycogen synthesis [5], inactive induction of glycolysis [6], inability to restrain endogenous glucose production through gluconeogenesis [7, 8], and limited conversion from glucose to lipid metabolites [4, 9, 10].

Glucose in fish mainly comes from the digestion of dietary carbohydrate sources and the conversion of nonsugar substances. Dietary carbohydrates, usually starches, are digested into glucose in the presence of amylase and then transported by blood with the assistance of sodium-dependent glucose transporter (*sglt1*) in the enterocyte membrane and facilitative glucose transporter (*glut2*) in the basolateral membrane [11]. Previous studies have dem-

onstrated that *sglt1* and *glut2* are regulated as glucose sensor and are strongly upregulated by carbohydrate intake [11]. After transporting, glucose is enriched in blood, which is supposed to be used by three classic pathways: glycogen synthesis, glycolysis, and glucose-induced lipogenesis. Glycogen storage in tissues is characterized as a quick response to glucose load, while in rainbow trout, glycogen production and decomposition are activated at the same time [5], which is recognized as one of the key reasons for persistent hyperglycaemia in fish. Regarding glycolysis, there are three key enzymes, glucokinase (GK), 6-phosphofructokinase (6-PFK), and pyruvate kinase (PK), which determine the efficiency of blood glucose clearance [12]. However, GK is reported to be inactive in most fish, and PK is not affected by dietary carbohydrate levels [12, 13]. Thus, inefficient glycolysis is also known as one of the key reasons for postprandial hyperglycaemia in fish. Another important pathway in glucose homeostasis is lipogenesis, although it is not directly linked to glucose metabolism, which could transfer excessive glucose into fatty acids and thus alleviate glucose overload [4, 10]. However, the lipogenic pathway is poorly induced by carbohydrate intake in fish, which also explains the poor ability of carbohydrate utilization in fish. Unlike mammals, the gluconeogenetic pathway remains active regardless of food intake in fish. For example, the three key enzymes phosphoenolpyruvate carboxykinase (PEPCK), fructose 1,6-bisphosphatase (FBP), and glucose-6-phosphatase (G6P) are not strongly inhibited in fish fed a carbohydrate-enriched diet, especially G6P, which is stimulated in response to high glucose levels [7, 14, 15]. The atypical control of gluconeogenesis is widely identified as the key factor for the limited use of carbohydrates in fish.

Many strategies have been explored to promote dietary carbohydrate utilization in fish. Previous studies found that the changes in dietary lipid contents affected carbohydrate uptake and glucose metabolism [11, 16, 17]. Lipid and glucose metabolism are closely interrelated, sharing intermediate metabolites and receptor signalling [18]. Gibel carp growth and glucose homeostasis were not significantly affected by feeding high carbohydrates in a low-fat diet in comparison with feeding optimal dietary carbohydrates and lipids [19]. Thus, it seems that the reduction of lipid content could be beneficial for maximizing carbohydrate utilization in aquafeed. Genotype selection is also acknowledged as a promising strategy to improve the use of dietary nutrients. Mazur et al. [20] revealed that Chinook salmon (*Oncorhynchus tshawytscha*) strains displayed different capacities of clearing glucose load. Recently, numerous selections have been made for rainbow trout according to specific objectives. One typical example was the case of two rainbow trout lines divergently selected for their muscle lipid content [21], showing that the fat line had a higher ability to use glucose than the lean line [22–25]. In three decades, a series of gibel carp clones have also been successively bred, and several advanced varieties such as gibel carp "CASIII" are widely cultured in mainland China [26–28]. Our previous studies have found the existence of genotype-diet interactions between CASIII and CASV in plant protein source utilization [29]. However, there is no clear understanding of carbohydrate use between gibel carp clones, and the mechanism underlying glucose metabolism in response to carbohydrate intake for the omnivorous fish gibel carp has not been investigated.

Therefore, the present study designed three carbohydrate levels, especially in a low-fat diet, to study the effects of dietary carbohydrates on fish growth and glucose metabolism. Artificially selected CASIII and wild-type Dongting were compared to study the variousness and/or uniqueness in carbohydrate utilization between genotypes to provide scientific data for future generation selection of gibel carp. We analyzed growth performance, whole body composition, plasma metabolites, and the mRNA levels of the selected genes in glucose-associated pathways, such as glycolysis, gluconeogenesis, lipogenesis, and lipid oxidation, both at the phenotype level and molecular level to evaluate carbohydrate use between different genotypes of gibel carp.

2. Materials and Methods

2.1. Experimental Diets. Three experimental diets (15CHO, 30CHO, and 45CHO) were designed with the same protein and lipid content but varying carbohydrate contents (15%, 30%, and 45%). Table 1 shows the nutrient formulation and chemical content of the experimental diets. Fishmeal and casein were used as protein source for 36%. Fish oil and soybean oil (1 : 1) were used as fat source, and the total lipid content was designed as 4%. Corn starch was used as the digestible carbohydrate source, and it was supplemented by three levels as 15%, 30%, and 45%, respectively. At first, all materials were thoroughly crushed and filtered through a 40-mesh sieve (mesh size: 0.25 mm) and then well mixed, manufactured into pellets (2 mm diameter) using a laboratory feed machine (SLP-45, Fishery Mechanical Facility Research Institute, Shanghai, China); finally, the pellet feed was dried at 70°C in the oven and then kept at 4°C.

2.2. Feeding Experiment, Experimental Fish, and Sampling Process. The two strains of gibel carp were from the hatchery of the Chinese Academy of Sciences at the Institute of Hydrobiology in Wuhan, Hubei, China. They were fed the mixed experimental diets for four weeks prior to the trial to acclimatize to the conditions. After 24 h of food deprivation, all fish were pooled at the start of the experiment. Each strain of fish was batch-weighed and distributed at a density of 20 fish per tank in round fibreglass tanks (diameter: 100 cm, water volume: 300 L). Each duplicate was fed in triplicate with 15CHO, 30CHO, and 45CHO diets (2 strains $*$ 3 diets in three replicates, total of 18 tanks). At 8:30 a.m. and 14:30 p.m., all fish were fed until being apparently full. Daily feed consumption was recorded for each tank. The water flow rate was approximately 1500 mL min^{-1} during the trial, and the water temperature was monitored daily at 28.5 ± 1°C. The light intensity at the water surface was approximately 3 $mols^{-1}m^{-2}$, and the light period was from 08:00 a.m. to 22:00 p.m. Water quality tests were weekly analyzed (dissolved oxygen > 5 mg L^{-1}, pH was 7.0-7.5, ammonia nitrogen was 0.5 mg L^{-1}, and residual chloride was 0.01 mg L^{-1}).

Table 1: Formulation and chemical composition of the three experimental diets.

Diets	15CHO	30CHO	45CHO
Ingredients (% DM)			
White fish meal[1]	10.00	10.00	10.00
Casein[2]	30.20	30.20	30.20
Fish oil[3]	1.00	1.00	1.00
Soybean oil[4]	1.00	1.00	1.00
Corn starch[5]	15.00	30.00	45.00
Choline chloride	0.11	0.11	0.11
Cellulose	34.30	19.30	4.30
Vitamin premix[6]	0.39	0.39	0.39
Mineral premix[7]	5.00	5.00	5.00
Carboxymethyl cellulose	3.00	3.00	3.00
Chemical composition			
Crude protein (% DM)	36.24	36.40	37.10
Crude lipid (% DM)	4.38	3.87	4.07
Gross energy (kJ/g DM)	20.60	20.60	20.70

15CHO: 15% corn starch diet; 30CHO: 30% corn starch diet; 45CHO: 45% corn starch diet; DM: dry matter. [1]American Seafood Company, Seattle, Washington, USA. [2]Sigma Chemical Co., St. Louis, MO, USA. [3]Anchovy oil from Peru, purchased from Coland Feed, Wuhan, Hubei, China. [4]Yihai Kerry Y Co., Ltd., purchased from Wuhan, Hubei, China. [5]Fuchen Chemical Reagent Plant, Tianjin, China. [6]Vitamin premix (mg/kg diet): thiamin, 20; riboflavin, 20; pyridoxine, 20; cyanocobalamine, 0.02; folic acid, 5; calcium pantothenate, 50; inositol, 100; niacin, 100; biotin, 0.1; starch, 645.2; ascorbic acid, 100; vitamin A, 110; vitamin D, 20; vitamin E, 50; vitamin K, 10. [7]Mineral premix (mg/kg diet): NaCl, 500; $MgSO_4 \cdot 7H_2O$, 4575.0; $NaH_2PO_4 \cdot 2H_2O$, 12500.0; KH_2PO_4, 16000.0; $Ca(H_2PO_4)_2 \cdot H_2O$, 6850.0; $FeSO_4$, 1250.0; $C_6H_{10}CaO_6 \cdot 5H_2O$, 1750.0; $ZnSO_4 \cdot 7H_2O$, 111.0; $MnSO_4 \cdot 4H_2O$, 61.4; $CuSO_4 \cdot 5H_2O$, 15.5; $CoSO_4 \cdot 6H_2O$, 19.02; KI, 178.33; starch, 6253.33.

After the feeding trial, the fish in each tank were weighed after 24 h of food deprivation to determine growth performance. Feeding rate (FR, %BW d^{-1}) = 100 × (feed intake in dry matter, g)/[days × (initial body weight, g + final body weight, g)/2]. Specific growth rate (SGR, %/d) = 100 × [Ln (final body weight, g) − Ln (initial body weight, g)]/days. Feed efficiency (FE, %) = 100 × (wet weight gain, g)/(feed intake in dry matter, g). Protein retention efficiency (PRE, %) = 100 × (body protein gain, g)/(protein intake, g). Four fish from each tank were sampled and frozen at -20°C for whole body composition analysis. The remaining fish were refed for one more week, and then, two fish per tank were randomly sampled at 2 h, 6 h, and 24 h, respectively, after their last meal. MS-222 (100 mg L^{-1} tricaine methane sulfonate, Argent Chemical Laboratories, Inc., Redmond, WA, USA) was used to anaesthetize the fish, and blood was drawn from the caudal vein with heparinized syringes and centrifuged (3000 *g*, 15 min, 4°C). The collected plasma was immediately frozen and stored at -20°C for further examination. Meanwhile, the fish's liver was separated at 6 h after the last meal, immediately frozen in liquid nitrogen, and then stored at -80°C.

2.3. Chemical Analysis of Dietary and Whole Body Composition. The AOAC-recommended methodologies for proximate composition analysis of diets and fish bodies were used (2003). Moisture was measured by drying to a consistent weight in a 105°C oven. After acid digestion with a Kjeltec Analyzer Unit, the crude protein content (N 6.25) was calculated using the Kjeldahl technique (4800 FOSS Tecator, Haganas, Sweden). Diethyl ether was used as the extraction liquid in a Soxtec system (Soxtec System HT6, Tecator, Haganas, Sweden) to assess crude lipid content. Dietary ash was measured after burning in a muffle furnace at 550°C.

2.4. Plasma Metabolites. Commercial kits (Wako, Chuo-Ku, Japan) were applied according to the manufacturer's instructions to quantify plasma glucose and triglycerides. The mutarotase-GOD method was used to measure glucose levels in plasma: α-D-glucose is converted to β-D-glucose by mutarotase, then β-D-glucose reacts with glucose oxidase (GOD) to generate hydrogen peroxide, which is then oxidatively condensed with phenol and 4-aminoantipyrine, and the concentration of glucose in samples is measured by the absorbance of a red color generated by the oxidative condensation reaction. Lipoprotein lipase and glycerol kinase are used to convert triglycerides to glycerol-3-phosphate, which is used to measure plasma triglycerides. The oxidative condensation of N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline sodium salt (DAOS) with 4-aminoantipyrine is aided by hydrogen peroxide, which is formed through a reaction between glycerol-3-phosphate and glycerol-3-phosphate oxidase (GPO). Finally, the absorbance of a blue color produced by the oxidative condensation reaction might be used to determine the triglyceride concentration in samples. We used a commercially available ELISA kit from Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China, to analyze plasma insulin.

2.5. mRNA Level Analysis. Total RNA samples were conducted on the liver. Samples were extracted using TRIzol regent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and then quantified by spectrophotometry at the absorbance of 260 nm. The integrity of the samples was assessed using agarose gel electrophoresis. Total RNAs with high RNA ratios (A260/A280 = 1.8 – 2.0) and clear ribosomal bands were used for further analysis. One microgramme total RNA per sample was reverse transcribed using an Invitrogen™ M-MLV First-Strand Synthesis Kit (Invitrogen, Carlsbad, CA, USA). No adding reverse transcriptase or total RNA replacing by sterile double-distilled water was performed as two negative controls for detecting genomic DNA contamination and reagent pollution.

Target gene expression was determined by quantitative real-time RT-PCR. Elongation factor-1 alpha (*ef1α*) was regarded as reference gene, and the specific primers of the target genes had already been published in the previous studies [30] shown in Table 2. Quantitative RT-PCR was carried out on a LightCycler 480 II (Roche Diagnostics, Neuilly-sur-Seine, France) using SYBR Green I Master (Roche Diagnostics GmbH, Mannheim, Germany). PCR were performed using 2 μL of the diluted cDNA (60 times) mixed with 0.24 μL of each primer (10 μM), 3 μL of Light Cycle 480 SYBR Green I Master (Roche Diagnostics), and 0.52 μL of the DNase/RNase/protease-free water (Invitrogen,

Table 2: Primer sequences and accession numbers for qPCR analysis.

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)	GenBank accession number
Reference gene			
ef1α	GTTGGAGTCAACAAGATGGACTCCAC	CTTCCATCCCTTGAACCAGCCCAT	AB056104
Glucose transport			
glut2	CTCGTGGATGAGCTACCTCAGCAT	CCCTGACTGAAGATCTCCGCCA	KX898504
Glycolysis			
gck	GAGGAGATGCGTAAGGTGGAGCT	TTCTCATACAGCTGATGTCCAGGGTT	KX898498
pfk	ACACCGGATGCCGCAGAAGCA	TCGATCTCTCCGGTCACATACTCG	KX898500
pk	GCATCTGTGTCTGCTGGACATCGA	TGAGAGCCGTGAGAGAAGTTCAGTC	KX898502
Gluconeogenesis			
pepck	AGACAAACCCTCATGCCATGGCAAC	GGGTCTATGATGGGGCACTGG	KX898506
fpb	CACAAATGTTACAGGTGACCAGGTGAA	AATGTTTGAAGAGCCATCCAGAGGGT	GU593002
g6p	CCTTACTGGTGGGTCCATGAGACT	TGGGCCGGTCTCACAGGTCAT	KX898505
Lipogenesis			
srebp-1	GGCCCTCTACTGCGTGGCACA	ACCACCATTTGGAGTGAGGGTCAC	KX898507
acly	AGTTTGGCCACGCTGGAGCTTGT	CCCAGCTCATCGAAGCTCTTGG	KX898508
fas	CCACACCATGGACCCACAGCT	CTGGGTCTTTACTGAAGGCCTCT	KF511494
Fatty acid oxidation			
cpt1a	GAAGCTCATCAGGCTGTGGCCTT	TTCCAGGAGTGAAGTCCGGAGAG	KX898509
aco3	TGTGGAGGACACGGTTACCTTGC	AGTTGCTGGTCTGCTGCAGAAGG	KX898510

Reference gene: *ef1α*: elongation factor-1 alpha. Glucose transporter: *glut2*: glucose transporter 2. Glycolysis: *gck*: glucokinase; *pfk*: 6-phosphofructokinase; *pk*: pyruvate kinase. Gluconeogenesis: *pepck*: phosphoenolpyruvate carboxykinase; *fbp*: fructose 1,6-bisphosphatase; *g6p*: glucose-6-phosphatase. Lipogenesis: *srebp-1*: sterol regulatory element binding protein 1; *acly*: ATP citrate lyase; *fas*: fatty acid synthase. Fatty acid oxidation: *cpt1a*: carnitine palmitoyl transferase 1 isoform a; *aco3*: acyl-CoA oxidase 3.

Carlsbad, CA, USA) in a total volume of 6 μL. The qPCR was initiated at 95°C for 10 min, followed by 45 cycles of a three-step amplification program (15 s at 95°C, 30 s at the specific primer annealing temperature, and 30 s at 72°C). A melt curve was used to confirm the amplification of single product after amplification. Replicate samples and negative controls were included in each PCR experiment. The Pfaffl method was used to obtain relative quantification of target gene expression [31]. The relative expression ratio (R) of a target gene was calculated on the basis of real-time PCR efficiency (E) and the CT deviation (ΔCT) of the unknown sample versus a control sample and expressed in comparison to the *ef1α*, the reference gene by the LightCycler 480 software (Version SW 1.5; Roche Diagnostics). The slope of a standard curve using serial dilution of cDNA was used to calculate PCR efficiencies, which ranged between 1.8 and 2.0.

2.6. Statistical Analysis. Normality of distributions was assessed by the Shapiro-Wilk test. Data were analyzed by two-way *ANOVA* to assess the differences between diets, strains, and interactions. If interactions between diets and strains were statistically significant, *post hoc* Tukey test would be used to compare all the groups. Data were analyzed with SPSS 19.0, SPSS Inc., Chicago, IL, USA. Treatment effects and interactions were considered statistically significant at $p < 0.05$. Results were presented as means ± s.d. ($n = 3$ tanks per group).

3. Results

3.1. Survival Rate and Growth Performance. The fish were uniformly selected prior to the trial, and there was no difference of initial body weight between strains or diets (Table 3, $p < 0.05$). After 8 weeks of feeding trial, the zootechnical parameters were firstly determined. There was no significant difference of survival rate between strains or diets (Table 3, $p < 0.05$), while there were many significant differences of growth parameters between strains and diets (Table 3, $p < 0.05$): CASIII had higher final body weight, feeding rate (FR), and specific growth rate (SGR) than Dongting ($p < 0.05$); FR and SGR were also higher in the fish fed with 45CHO diet ($p < 0.05$); no significant difference was found in feed efficiency (FE) irrespective of strains and diets ($p > 0.05$); fish fed with 45CHO diet had higher protein retention efficiency (PRE) than 30CHO diet then followed by 15CHO diet ($p < 0.05$).

3.2. Whole Body Composition. The whole body composition including moisture content, crude protein content, crude lipid content, and ash content was analyzed, and the results are shown in Table 4. CASIII had higher moisture content than Dongting, irrespective of diets ($p < 0.05$). There were no significant differences of crude protein between different strains or diets ($p > 0.05$). Higher body lipid content was found in Dongting than in CASIII, irrespective of diets ($p < 0.05$), but no significant effects of whole body lipid

Table 3: Zootechnical parameters in two strains of gibel carp fed different carbohydrate diets (15CHO diet, 30CHO diet, and 45CHO diet).

Zootechnical parameters	15CHO		30CHO		45CHO		p value		
	Dongting	CASIII	Dongting	CASIII	Dongting	CASIII	Diet	Strain	Diet ∗ strain
Survival rate (%)	83.33 ± 2.89	81.67 ± 10.41	85.00 ± 13.23	77.50 ± 31.82	80.00 ± 20.00	86.67 ± 11.55	0.9892	0.9633	0.7577
Initial body weight (g)	2.00 ± 0.04	2.03 ± 0.02	2.01 ± 0.03	2.00 ± 0.03	2.02 ± 0.02	2.41 ± 0.66	0.4094	0.3141	0.4632
Final body weight (g)	$5.53 \pm 0.70_{A}$	10.37 ± 0.72^{B}	6.00 ± 1.05^{A}	7.70 ± 1.84^{B}	5.47 ± 1.30^{A}	8.23 ± 0.35^{B}	0.1757	**<0.0001**	0.0705
FR (% BW/d)	$2.68 \pm 1.49_{Aa}$	4.46 ± 0.65^{Ba}	4.35 ± 1.13^{Aa}	5.33 ± 0.11^{Ba}	5.48 ± 0.51^{Ab}	6.84 ± 1.84^{Bb}	**0.0094**	**0.0362**	0.8542
SGR (%/d)	$1.71 \pm 0.22_{Aa}$	2.67 ± 0.16^{Ba}	$2.17 \pm 0.36_{Aab}$	$2.88 \pm 0.21_{Bab}$	2.02 ± 0.25^{Ab}	3.26 ± 0.15^{Bb}	**0.0196**	**<0.0001**	0.2269
FE (%)	34.00 ± 1.00	41.00 ± 1.58	46.33 ± 5.51	42.00 ± 7.07	40.67 ± 6.51	52.00 ± 1.53	0.1614	0.1960	0.2960
PRE (%)	$16.33 \pm 2.08_{a}$	21.00 ± 1.00^{a}	23.00 ± 3.61^{b}	26.00 ± 1.41^{b}	32.00 ± 5.29^{c}	32.33 ± 4.16^{c}	**0.00001**	0.1425	0.5617

15CHO: 15% corn starch diet; 30CHO: 30% corn starch diet; 45CHO: 45% corn starch diet. Data were presented as mean ± s.d. ($n = 3$ tanks); statistical differences were evaluated by two-way *ANOVA* ($p < 0.05$, values in bold). ^{A,B}Different capital letters indicate significant differences between strains ($p < 0.05$). ^{a,b}Different lowercase letters indicate significant differences between diets ($p < 0.05$). Feeding rate (FR, %BW d^{-1}) = 100 × (feed intake in dry matter, g)/[days × (initial body weight, g + final body weight, g)/2]. Specific growth rate (SGR, %/d) = 100 × [Ln (final body weight, g) − Ln (initial body weight, g)]/days. Feed efficiency (FE, %) = 100 × (wet weight gain, g)/(feed intake in dry matter, g). Protein retention efficiency (PRE, %) = 100 × (body protein gain, g)/(protein intake, g).

Table 4: Whole body composition in two strains of gibel carp fed different carbohydrate diets (15CHO diet, 30CHO diet, and 45CHO diet).

Whole body composition	15CHO		30CHO		45CHO		p value		
	Dongting	CASIII	Dongting	CASIII	Dongting	CASIII	Diet	Strain	Diet ∗ strain
Moisture (%)	$71.67 \pm 0.54_{A}$	$74.10 \pm 0.20_{B}$	$71.12 \pm 1.58_{A}$	$75.78 \pm 1.18_{B}$	$70.67 \pm 0.90_{A}$	$73.58 \pm 1.56_{B}$	0.2547	**0.0001**	0.2931
Crude protein (%)	15.22 ± 0.31	14.95 ± 0.75	15.29 ± 0.81	15.11 ± 0.57	15.52 ± 0.57	14.92 ± 0.44	0.9140	0.2646	0.8332
Crude lipid (%)	8.49 ± 0.11^{B}	$5.90 \pm 0.43_{A}$	9.29 ± 0.72^{B}	$5.84 \pm 0.01_{A}$	9.53 ± 0.84^{B}	$6.47 \pm 0.52_{A}$	0.0807	**<0.0001**	0.4707
Ash (%)	3.71 ± 0.12^{c}	3.73 ± 0.08^{c}	3.53 ± 0.08^{b}	3.48 ± 0.12^{b}	3.37 ± 0.18^{a}	3.30 ± 0.06^{a}	**0.0004**	0.6030	0.7933

15CHO: 15% corn starch diet; 30CHO: 30% corn starch diet; 45CHO: 45% corn starch diet. Data were presented as mean ± s.d. ($n = 3$ tanks); statistical differences were evaluated by two-way *ANOVA* ($p < 0.05$, values in bold). ^{A,B}Different capital letters indicate significant differences between strains ($p < 0.05$). ^{a,b}Different lowercase letters indicate significant differences between diets ($p < 0.05$).

between diets were observed ($p > 0.05$). Fish fed with 45CHO diet had higher ash content than those with 30CHO diet, then followed by those with 15CHO diet ($p < 0.05$).

3.3. Postprandial Plasma Metabolite Content. Postprandial plasma metabolites related to glucose metabolism such as plasma glucose, plasma triglyceride, and plasma insulin were examined, and the results at 2 h, 6 h, and 24 h after the last meal are shown in Figure 1. There were no significant differences in plasma glucose, triglycerides, and insulin between carbohydrate levels ($p > 0.05$). While the differences between fish strains were significant ($p < 0.05$), higher plasma glucose was observed in CASIII than in Dongting (2 h, $p < 0.05$), higher plasma triglyceride was found in CASIII than in Dongting (2 h and 6 h, $p < 0.05$), and plasma insulin was also higher in CASIII than in Dongting (6 h, $p < 0.05$).

3.4. Hepatic Glucose Metabolism at 6 h after the Last Meal. Regarding the glucose metabolism in the liver, the mRNA level of hepatic glucose transporter 2 (*glut2*) is shown in Figure 2. No significant difference of *glut2* was observed between diets and strains ($p > 0.05$).

The mRNA levels of target genes encoding key enzymes involved in glycolysis were measured (Figure 3). There was no significant difference in *gck* mRNA levels, irrespective of strain and diet ($p > 0.05$). *pfk* was more highly expressed in fish fed the 30CHO diet and 45CHO diet than in those fed the 15CHO diet ($p < 0.05$). CASIII showed higher *pk* mRNA levels than Dongting, irrespective of the diets ($p < 0.05$).

We also studied the expression of the target genes involved in the gluconeogenic pathway (Figure 4). Dongting had significantly higher mRNA levels of *pepck* than CASIII ($p < 0.05$), and no difference in *pepck* mRNA levels was observed between three diets ($p > 0.05$). There were no

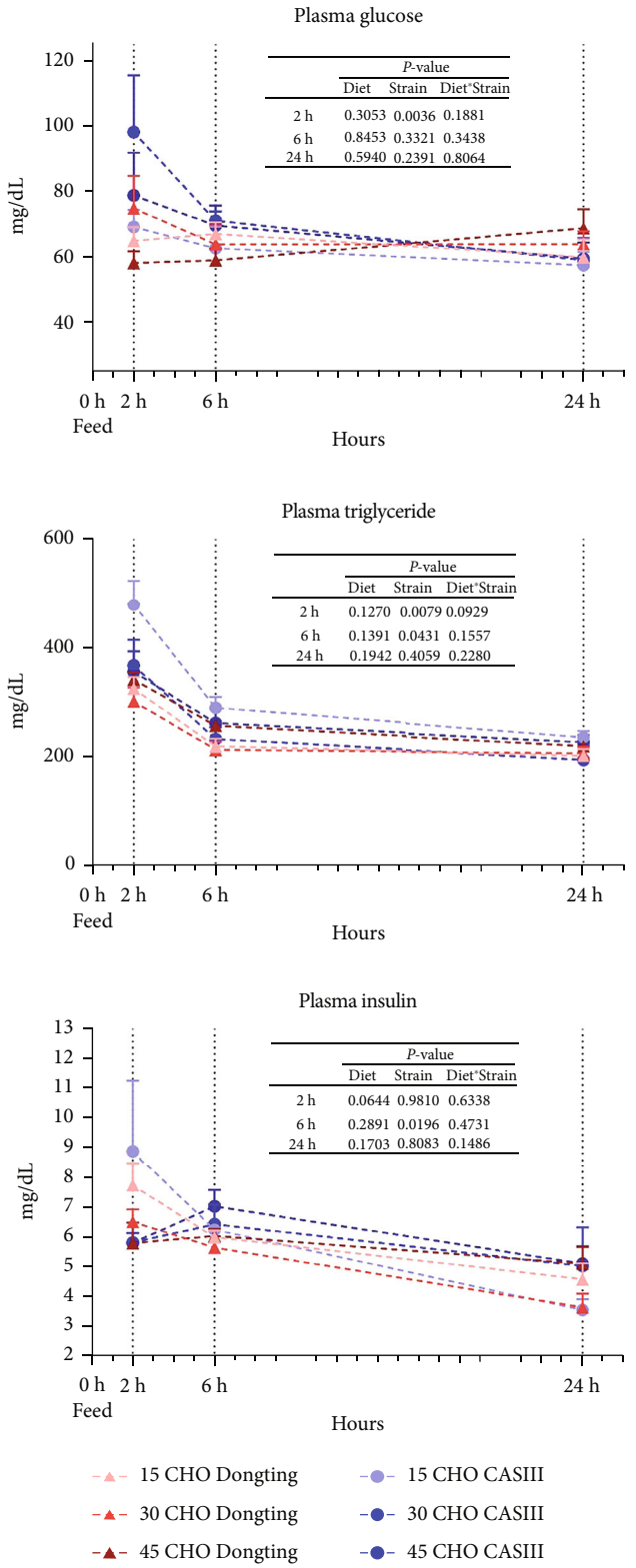

Figure 1: Plasma metabolites in two strains of gibel carp fed different carbohydrate diets (15CHO diet, 30CHO diet, and 45CHO diet) at 2 h, 6 h, and 24 h after the last meal. 15CHO: 15% corn starch diet; 30CHO: 30% corn starch diet; 45CHO: 45% corn starch diet. Data were presented as mean ± s.d. ($n = 3$ tanks); statistical differences were evaluated by two-way *ANOVA* ($p < 0.05$, values in bold).

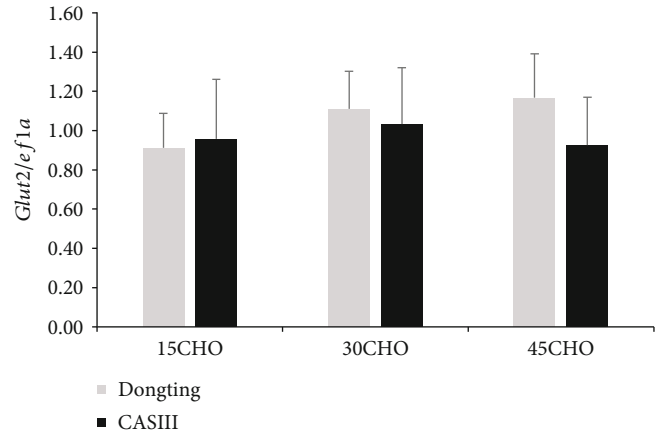

Figure 2: mRNA level of hepatic glucose transporter in two strains of gibel carp fed with different carbohydrate diets (15CHO diet, 30CHO diet, and 45CHO diet) at 6 h after the last meal. *glut2*: glucose transporter 2. Data were presented as mean ± s.d. ($n = 3$ tanks); statistical differences of *glut2* were evaluated by two-way *ANOVA* ($p < 0.05$, values in bold).

significant differences of *fbp* and *g6p* gene expression between strains and diets ($p > 0.05$).

3.5. Lipid Metabolism at 6 h after the Last Meal. We further analyzed lipid metabolism in the liver for it was reported closely related to glucose use. The gene expression involved in the lipogenic pathway are shown in Figure 5. Significant diet ∗ strain interactions in the mRNA levels of *srebp-1*, *acly*, and *fas* were detected ($p < 0.05$), and Dongting fed the 45CHO diet had higher expression levels of these genes than other groups ($p < 0.05$).

No significant differences were found in the mRNA levels of *cpt1a* and *aco3*, the selected enzymes involved in fatty acid oxidation, between strains and diets (Figure 6, $p > 0.05$).

4. Discussion

Regarding the diets, different carbohydrate levels were compensated by cellulose and were associated with different body ash contents in the present study, and the results were in accordance with previous observations in gibel carp and grass carp (*Ctenopharyngodon idella*) that increasing the replacement of starch by cellulose decreased body ash content [32]. For fish growth, the inclusion of digestible carbohydrates increased FR, especially when the level of carbohydrates reached to 45%. Although it failed to improve FE, a high-carbohydrate diet showed a protein-sparing effect by a higher PRE in the 45CHO diet, which was also observed in medium-sized gibel carp [32]. And, a carbohydrate-enriched diet also improved fish growth by a significantly higher SGR in the 45CHO group, whereas a previous study found that 38% carbohydrate decreased the growth of juvenile gibel carp [32]. The different performance attributed by high carbohydrate intake may be due to the low-lipid content in the present study (4%, half-optimum dietary lipid level of juvenile gibel carp), and a similar positive impact on

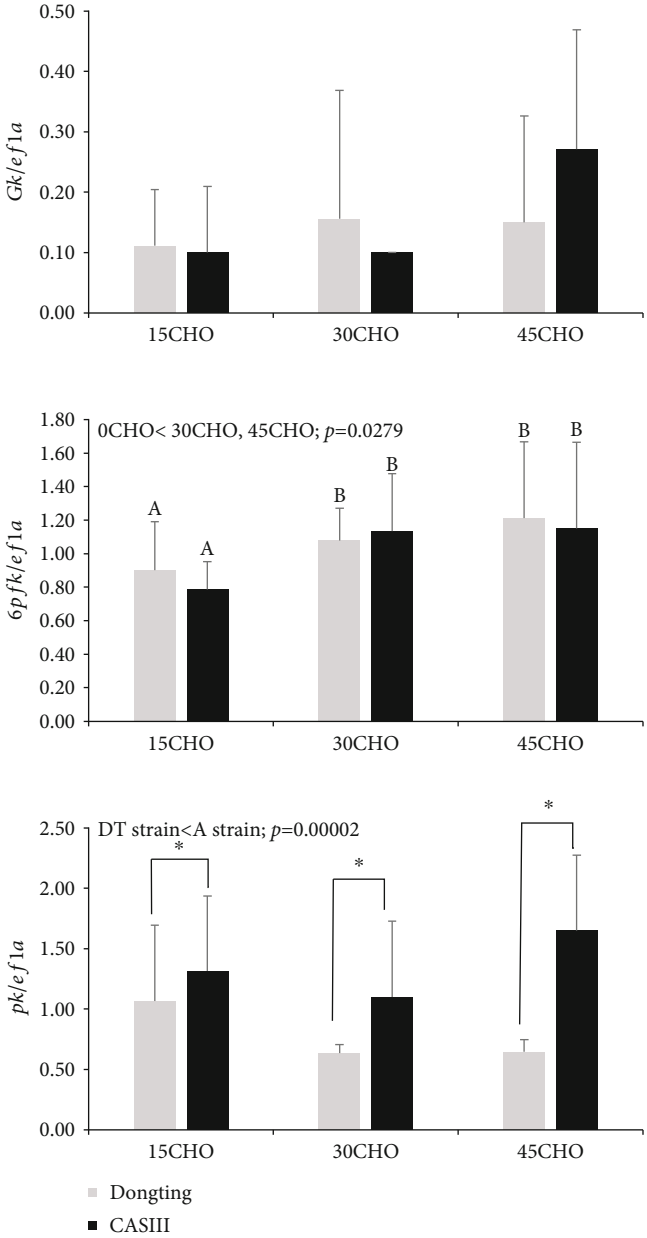

Figure 3: mRNA levels of hepatic glycolytic enzymes in two strains of gibel carp fed different carbohydrate diets (15CHO diet, 30CHO diet, and 45CHO diet) at 6 h after the last meal. *gck*: glucokinase paralogs; *pfk:* 6-phosphofructokinase; *pk:* pyruvate kinase. Data were presented as mean ± s.d. ($n = 3$ tanks); statistical differences of *gck*, *pfk*, and *pk* were evaluated by two-way *ANOVA* ($p < 0.05$, values in bold). [a,b]Different lowercase letters indicate significant differences between diets ($p < 0.05$) for 6*pfk* expression. The line with ∗ indicates significant differences between strains ($p < 0.05$) for *pk* expression.

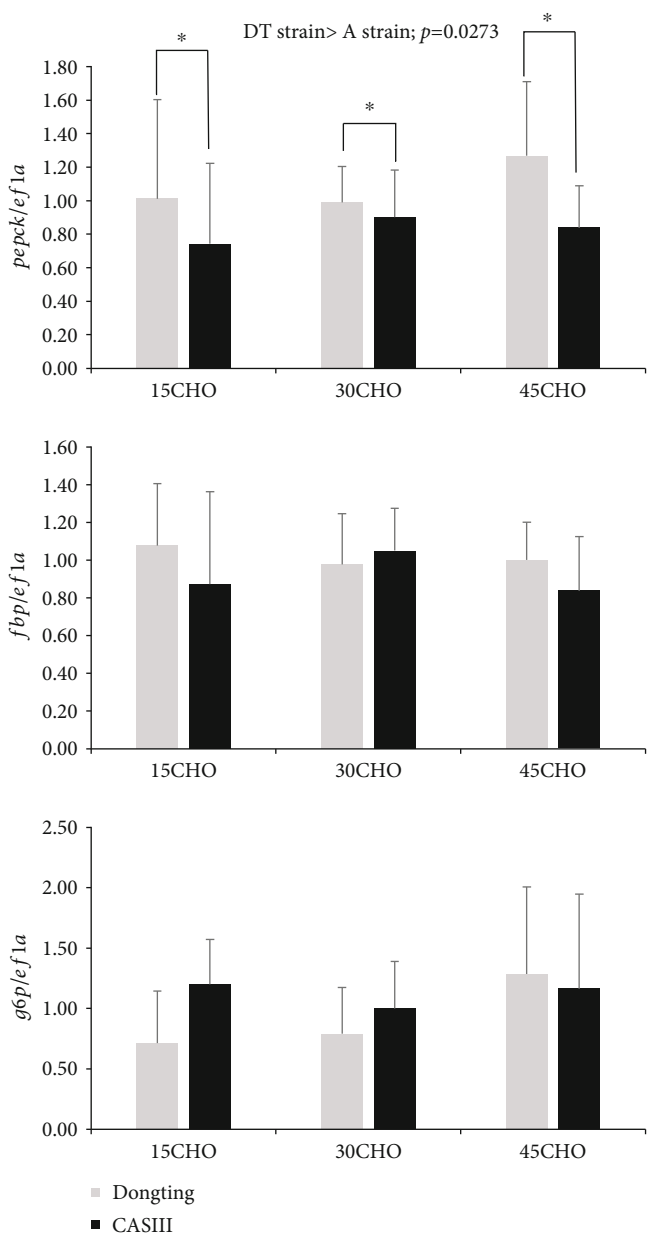

Figure 4: mRNA levels of hepatic gluconeogenic enzymes in two strains of gibel carp fed different carbohydrate diets (15CHO diet, 30CHO diet, and 45CHO diet) at 6 h after the last meal. *pepck*: phosphoenolpyruvate carboxykinase; *fbp*: fructose 1,6-bisphosphatase; *g6p*: glucose 6-phosphatase. Data were presented as mean ± s.d. ($n = 3$ tanks); statistical differences of *pepck*, *fbp*, and *g6p* were evaluated by two-way *ANOVA* ($p < 0.05$, values in bold). The line with ∗ indicates significant differences between strains ($p < 0.05$) for *pepeck* expression.

growth by high carbohydrates was also detected in blunt snout bream (*Megalobrama amblycephala*) when fed low-lipid diet. This could be explained by that alleviating the stress caused by dietary lipids consequently leads to more carbohydrate utilization [33]. In addition, the present study found that 45% dietary carbohydrate did not induce hyperglycaemia and hypertriglyceridaemia in two strains of gibel carp even at 2 h after the last meal. The relatively low level of plasma glucose in the present study could also be related to the lower level of dietary lipid. Compared to high-carbohydrate intake alone, the interaction between dietary macronutrients was more likely to induce postprandial hyperglycaemia [34]. From the above results, we speculated that low dietary lipid may promote carbohydrate use in gibel carp.

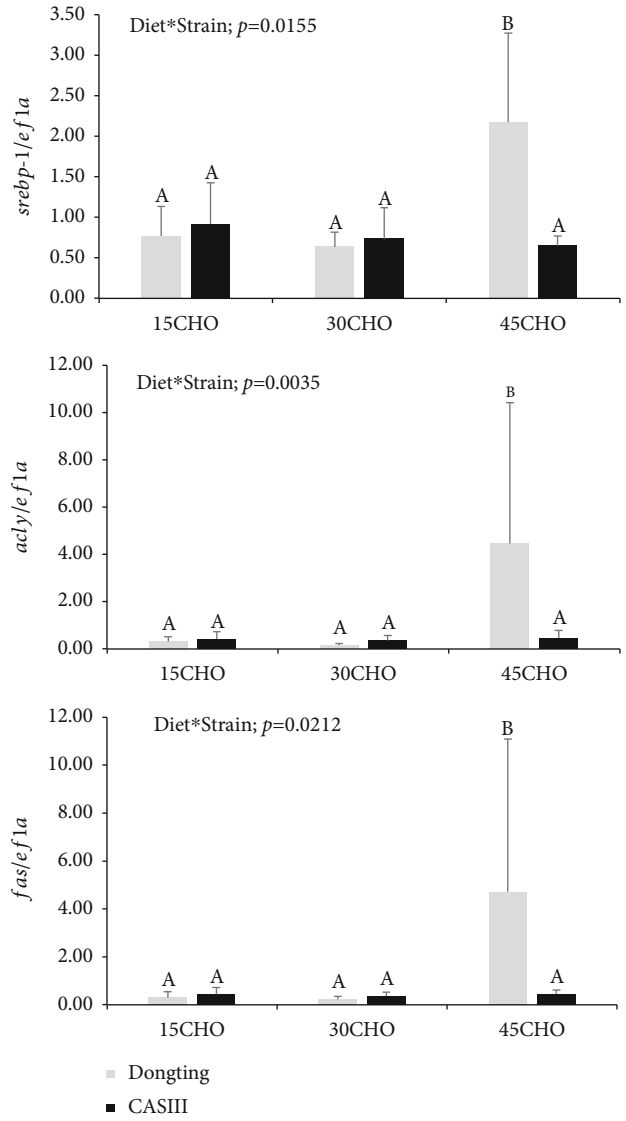

Figure 5: mRNA levels of hepatic lipogenic enzymes in two strains of gibel carp fed carbohydrate diets (15CHO diet, 30CHO diet, and 45CHO diet) at 6 h after the last meal. *srebp-1*: sterol regulatory element binding protein 1; *acly*: adenosine triphosphate citrate lyase; *fas*: fatty acid synthase. Data were presented as mean ± s.d. ($n = 3$ tanks). Because of interaction, a *post hoc* Tukey's test was performed for *srebp-1*, *acly*, and *fas* ($p < 0.05$, values in bold). Different superscripts indicated significant differences between treatments. [a,b]Different superscripts indicated significant differences between treatments for the expressions of *srebp-1*, *acly*, and *fas*.

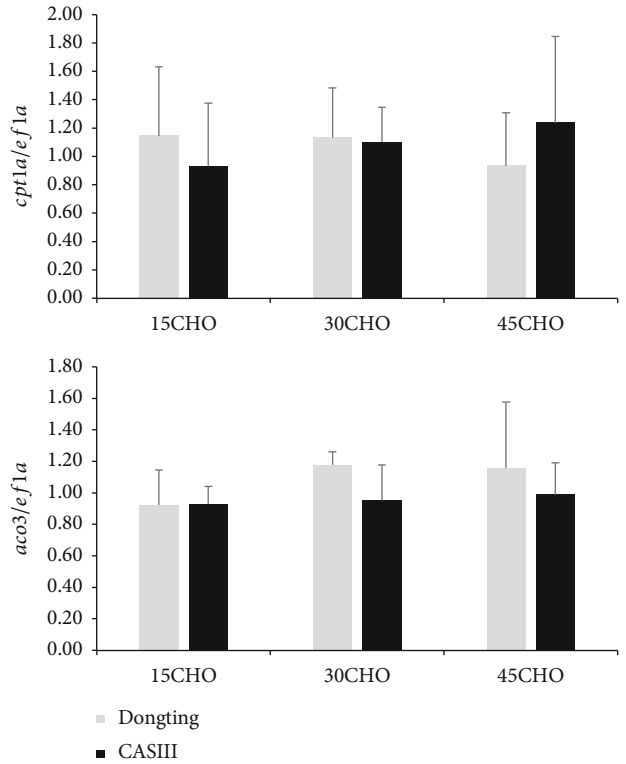

Figure 6: mRNA levels of hepatic fatty acid oxidation enzymes in two strains of gibel carp fed different carbohydrate diets (15CHO diet, 30CHO diet, and 45CHO diet) at 6 h after the last meal. *cpt1a*: carnitine palmitoyl transferase 1 isoform a; *aco3*: acyl-CoA oxidase 3. Data were presented as mean ± s.d. ($n = 3$ tanks); statistical differences of *cpt1a* and *aco3* were evaluated by two-way *ANOVA* ($p < 0.05$, values in bold).

4.1. Dietary Carbohydrates Slightly Affected Glucose Metabolism at the Molecular Level. Regarding glucose metabolic pathways, the first step was glucose transporting conducted by *glut2* in the liver. In the experiment, the *glut2* mRNA level was not affected by dietary carbohydrate levels. These results were not surprising, because *glut2* gene expression was reported not to be strongly regulated by dietary glucose, while it was more likely to be regulated by amino acids in fish [22, 35]. After transporting into the cells, glucose could be utilized by glycolysis to maintain glucose homeostasis, and in most carnivorous fish, endogenous glucose production was simultaneously induced by high-carbohydrate intake [4]. Recently, atypical regulation of glucose metabolism was observed in rainbow trout: the last two key glycolytic enzymes, *pfk* and *pk*, were inhibited by carbohydrate treatment [15, 36], whereas some of the genes encoding gluconeogenic enzymes, such as *g6pcb1b*, *g6pcb2a*, and *fbp1a,* were all induced, resulting in a carbohydrate-intolerant phenotype [15, 36]. In the present experiment, high-carbohydrate diets increased *pfk* mRNA levels encoding glycolytic enzymes, and the three key genes, *pepeck*, *fbp*, and *g6p*, encoding gluconeogenic enzymes were not affected by dietary carbohydrate levels. Moreover, a glucose administration test also conducted on Dongting and CASIII showed that the expression of genes involved in gluconeogenesis was significantly downregulated after glucose loading [37]. Therefore, compared with rainbow trout, the existence of efficient regulation of glycolysis and gluconeogenesis in gibel carp could partly explain its better ability to use dietary carbohydrates at the molecular level.

4.2. Genotype-Related Differences in Carbohydrate Utilization. The present study found that genotype did affect feeding and growth. The selected CASIII displayed a higher FR and SGR than wild-type Dongting. Better growth

performance of CASIII was persistently reported by our previous evaluations [38, 39]. For whole body composition, CASIII had higher body moisture and lower lipid content than Dongting. Interestingly, similar results were also found in the previous studies that higher growth performance was always accompanied by higher body moisture [29, 40–42]. There were no clear reasons for these results, and further studies are needed to better explain this phenomenon. In the present study, CASIII showed a higher level of plasma glucose at 2 h after the last meal in contrast to what was observed in the same two strains [37]. The difference may be associated with a higher FR in CASIII, which led to more glucose release during the early digestion period. In response to excess of plasma glucose, a significant increase in plasma triglycerides was accordingly detected in CASIII. These results were consistent with a previous study showing that selected strains had higher plasma lipid metabolites than unselected Dongting when fed a carbohydrate-enriched diet [41], suggesting an efficient conversion from glucose into triglycerides in CASIII. Insulin is a key hormone secreted by the hepatopancreas and is responsible for glucose homeostasis [43]. Insulin acts as a primary regulator of plasma glucose levels by increasing glucose uptake in muscle and inhibiting hepatic glucose production [44]. Presently, in response to glycaemia, a higher plasma insulin concentration was measured in CASIII, which correlated with the restrained gluconeogenesis observed in CASIII. But, it was inconsistent with the results in a comparison between the CASIII and CASV strains: there was an absence of genotype effects on postprandial insulin [19]. The difference could be attributed to different sampling time, because after 24 h of starvation, plasma glucose recovered to the baseline level, and thus, the changes in insulin levels would rarely be observed [19]. In the present study, higher triglyceride production and insulin secretion may suggest a higher capacity of CASIII for regulating glucose homeostasis.

Studies on transcriptional levels showed that *glut2* was not affected by the genotypes of rainbow trout [15, 22, 36], and it was similarly expressed in different strains of gibel carp as revealed by Jin et al. [37]. The same expression pattern of *glut2* was also observed in the present study: the *glut2* mRNA level was not affected by the diets or strains. At 2 h after the last meal, the elevated mRNA level of *pk*, a glycolytic enzyme, was observed in CASIII, showing enhanced glycolysis in response to carbohydrate intake compared to Dongting. On the other hand, a lower mRNA abundance of the gluconeogenic enzyme *pepck* was also detected in CASIII, suggesting restrained gluconeogenesis in CASIII than in Dongting. Jin et al. [37] also showed the same effects on *pk* and *pepck* expression in these two strains by glucose administration. The efficient regulation of glycolysis and gluconeogenesis in CASIII may partly explain its higher potential in dietary glucose use as the previous observation [41].

4.3. Interactions of Genotype and Diet in Lipid Metabolism. The main interactions of genotype and diet were particularly observed in the gene expression involved in lipogenesis. Dongting showed higher mRNA levels of *srebp-1*, *acly*, and *fas* when fed the 45CHO diet. As in many fish, the activities of lipogenic enzymes are increased by dietary carbohydrates, which are linked to a decrease in hyperglycaemia [4, 22, 23]. The fat rainbow trout line also showed an induction of hepatic lipogenesis by dietary carbohydrates, which was reported to permit more efficient control of postprandial glycaemia [24, 25]. In the present study, a higher lipogenic capacity was observed in Dongting, which led to higher whole body lipid and was related to lower plasma glucose in Dongting. Moreover, a previous study also found a special capacity of lipid production in Dongting when fed with plant protein sources [42]. The results were in accordance with a comparison of fat rainbow trout line (F line) and lean rainbow trout line (L line) in response to carbohydrate diets: F line had a higher lipogenic ability to store excess glucose, but which could not improve its growth performance compared with the L line [9, 23]. In the present study, although Dongting was speculated to have a higher ability to metabolize lipids in a high-carbohydrate diet, it had relatively lower growth than CASIII.

5. Conclusion

In summary, our experiment studied the effects of dietary carbohydrates on glucose and lipid metabolism in the liver of gibel carp. These findings suggested that gibel carp can use 45% carbohydrate in a low-fat diet without significant negative effects. CASIII and Dongting showed diverse responses to carbohydrate diets: CASIII had better growth performance and typical regulation of glucose homeostasis by upregulating *pk* in glycolysis and downregulating *pepck* in gluconeogenesis. Interestingly, lipid-related metabolism was strongly stimulated by high-carbohydrate levels in Dongting by enhancing hepatic lipogenesis, which then caused a higher lipid deposition in the whole body. Our data indicated that low-lipid formulation may be a promoter of carbohydrate use, at least for the growth of gibel carp, and confirmed that the strains of gibel carp represented specific genetic backgrounds associated with a particular use of dietary nutrients.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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