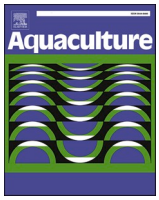
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More simple more worse: Simple carbohydrate diets cause alterations in glucose and lipid metabolism in Nile tilapia (*Oreochromis niloticus*)

Wen-Hao Zhou [a], Chen-Chen Wu [a], Samwel M. Limbu [b,c], Rui-Xin Li [a], Li-Qiao Chen [a], Fang Qiao [a], Yuan Luo [a], Mei-Ling Zhang [a], Tao Han [d,**], Zhen-Yu Du [a,e,*]

[a] Laboratory of Aquaculture Nutrition and Environmental Health (LANEH), School of Life Sciences, East China Normal University, Shanghai, PR China
[b] Department of Aquaculture Technology, School of Aquatic Sciences and Fisheries Technology, University of Dar es Salaam, P.O. Box 60091, Dar es Salaam, Tanzania
[c] UDSM-ECNU Joint Research Center for Aquaculture and Fish Biology (JRCAFB), Dar es Salaam, Tanzania
[d] Department of Aquaculture, Zhejiang Ocean University, Zhoushan 316000, China
[e] ECNU-UDSM Joint Research Center for Aquaculture and Fish Biology (JRCAFB), Shanghai, PR China

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ABSTRACT

Dietary carbohydrates are widely used in aquafeeds as cheap energy source and improve quality of feeds. However, comprehensive assessment of the effects of dietary carbohydrate complexity on fish metabolism and liver health is currently lacking. This study investigated the effect of replacing 50% of starch by using sucrose, fructose and glucose. Four isonitrogenic (400 g/kg protein) and isolipidic (60 g/kg fat) feeds containing starch (polysaccharide), sucrose (disaccharide) and fructose and glucose (monosaccharides) were fed to three replicates of 4.5 ± 0.30 g Nile tilapia (*Oreochromis niloticus*) for eight weeks. Afterwards, growth performance, feed utilization efficiency, glucose and lipid metabolism and liver health were evaluated. The results showed that the Nile tilapia fed on monosaccharide diets had significantly lower growth and feed utilization efficiency than those fed on disaccharide and polysaccharide diets. The Nile tilapia fed on monosaccharide diets reduced significantly lipid deposition, hepatosomatic index (HSI), serum alanine transaminase (ALT) and aspartate aminotransferase (AST) and expression of lipid metabolism genes than those fed on polysaccharide diet. The Nile tilapia fed on disaccharide diet increased significantly the serum insulin, muscle glycogen, and muscle glycogen synthase (*gs*) gene expression compared with those fed on polysaccharide diet. Interestingly, the Nile tilapia fed on monosaccharide diets reduced significantly the glycogen content while they increased insulin, glucose and the expression of liver gluconeogenesis genes such as glucose-6-phosphatase (*g6pase*) and phosphoenolpyruvate carboxykinase (*pepck*) than those fed on polysaccharide diet. Contrary, the Nile tilapia fed on disaccharide diet downregulated liver glucose catabolism and lowered serum triglyceride (TG) levels than those fed on polysaccharide but enhanced muscle peroxisome proliferator activated receptor alpha (*pparα*) and carnitine palmitoyltransferase 1a (*cpt1a*) gene expression, liver gluconeogenesis and pentose phosphate pathways. These results indicate that Nile tilapia utilizes better dietary polysaccharides and disaccharides than monosaccharides. Feeding Nile tilapia on monosaccharide diets caused insulin resistance and glucose metabolism disorders manifested by hyperinsulinism and hyperglycemia, respectively. The carbohydrate complexity affects the nutritional metabolism and liver health of fish.

1. Introduction

Carbohydrate is an essential nutrient for animals. Proper levels of carbohydrates in the diet of farmed fish promote protein sparing effect for energy requirement (Hemer et al., 2002; Stone, 2003) and improve the quality of extruded feed pellets (Honorato et al., 2010). Although carbohydrates are an economical and ideal dietary energy source, the ability of fish to utilize carbohydrates varies among species depending on feeding habits, dietary carbohydrate level and complexity (Kamalam et al., 2017). Carbohydrates are a family of compounds composed of mainly three classes, ranging from monosaccharides and disaccharides to more complex and longer polysaccharide molecules (Niaz et al.,

* Correspondence to: Z. Y. Du; School of Life Sciences, East China Normal University, 500 Dong Chuan Road, Shanghai 200241, PR China.
** Correspondence to: T. Han; Department of Aquaculture, Zhejiang Ocean University, Zhoushan 316000, China.
E-mail addresses: hantao@zjou.edu.cn (T. Han), zydu@bio.ecnu.edu.cn (Z.-Y. Du).

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Table 1
Formulation and proximate composition (dry matter basis) of the experimental diets.

Ingredients (%)	Starch	Sucrose	Fructose	Glucose
Casein	320	320	320	320
Gelatin	80	80	80	80
Soybean oil	60	60	60	60
Corn starch	300	150	150	150
Sucrose		150		
Fructose			150	
Glucose				150
Vitamin premix[1]	12	12	12	12
Mineral premix[2]	12	12	12	12
Ca(H2PO4)2	10	10	10	10
CMC	30	30	30	30
Cellulose	169.75	169.75	169.75	169.75
DMPT	1	1	1	1
Choline chloride	5	5	5	5
BHT	0.25	0.25	0.25	0.25
Total	1000	1000	1000	1000
Proximate composition (g / kg dry matter)				
Crude protein (%)	383	384	388	380
Crude lipid (%)	76	72	74	71
Moisture (%)	89	90	89	88

1 Mineral premix (g/kg): 314.0 g $CaCO_3$; 469.3 KH_2PO_4; 147.4 g $MgSO_4{\cdot}7H_2O$; 49.8 g NaCl; 10.9 g Fe (II) gluconate; 3.12 g $MnSO_4{\cdot}H_2O$; 4.67 g $ZnSO_4{\cdot}7H_2O$; 0.62 g $CuSO_4{\cdot}5H_2O$; 0.16 g KJ; 0.08 g $CoCl_2{\cdot}6H_2O$; 0.06 g NH_4 molybdate; 0.02 g $NaSeO_3$.
2 Vitamin premix (mg or IU/kg): 500,000 I.U. (international units) Vitamin A; 50,000 I.U. Vitamin D3; 2500 mg Vitamin E; 1000 mg Vitamin K3; 5000 mg Vitamin B1; 5000 mg Vitamin B2; 5000 mg Vitamin B6; 5000 μg Vitamin B12; 25,000 mg Inositol; 10,000 mg Pantothenic acid; 100,000 mg Cholin; 25,000 mg Niacin; 1000 mg Folic acid; 250 mg Biotin; 10,000 mg Vitamin C.

2020). Among them, starch is a polysaccharide formed by polymerization of glucose molecules and is currently the most widely used carbohydrate source for fish feeds (Limbu et al., 2020). Sucrose is a disaccharide consisting of two monosaccharides, glucose and fructose, and is also one of the most common carbohydrate sources in aquafeeds (Erfanullah and Jafri, 1995). Feeding fish with diets containing various classes of carbohydrates is expected to affect the nutritional physiology in the body.

Most results show that fish are better at utilizing complex and higher molecular weight carbohydrates than simple ones, especially omnivorous and carnivorous fish such as carp (*Cyprinus carpio*) and red sea bream (*Pagrus major*) (Furuichi and Yone, 1982), channel catfish (*Ictalurus punctatus*) (Wilson and Poe, 1987) and jundiá (*Rhamdia quelen*) (Corrêia et al., 2019). However, some studies in various fish species also found decrease in growth rate with increasing molecular weight of carbohydrates such as in chinook salmon (*Oncorhynchus tshawytscha*) (Buhler and Halver, 1961), rainbow trout (*Oncorhynchus mykiss*) (Bergot, 1979) and gilthead sea bream (*Sparus aurata*) (Enes et al., 2008). This might be due to the fact that, simple carbohydrates are more easily digested and absorbed by the intestinal tract without the need for large amounts of digestive enzymes compared to complex carbohydrates (Wilson, 1994; Krogdahl et al., 2005). Commonly, the apparent digestibility and intestinal uptake decrease with increasing complexities of dietary carbohydrates (glucose > dextrin > starch) in fish (Kamalam et al., 2017), which is the main reason for rapid rise in blood sugar after consumption of simple carbohydrates. Simultaneously, the rapid increase in blood sugar caused by consumption of simple carbohydrates may lead to persistent hyperglycemia if it cannot be used by the body in time (Moon, 2001).

The structural complexity of carbohydrates is closely related to blood sugar, so it is also likely to have an impact on the nutritional metabolism and health of fish. In fact, long-term hyperglycemia usually leads to a series of negative effects (Blaslov et al., 2018). Accordingly, many

Table 2
Primer sequences accession numbers for real-time PCR analysis.

Metabolism Pathway	Gene	Forward primer (5' to 3')	Reverse primer (5' to 3')	GenBank NO
Glycolysis	GK	GACATGAGGACATTGACAAGGGAA	CTTGATGGCGTCTCTGAGTAAACC	XM_003451020.2
	PK	CAGCATAATCTGCACCATCGGT	ATGAGAGAAGTTAAGACGGGCGA	XM_005472621.3
	PFK	AACCTGTGTGTGATTGGAGGTGAT	CGTGATCTTACCGGCTTTAACAAG	XM_003441476.2
Gluconeogenesis	PEPCK	TGGAAGAACAAACCTTGGCG	TGGGTCAATAATGGGACACTGTCT	XM_003448375
	G6Pase	AGACCTTATTGGTGGGTTCACGA	CTGAAGGACTTCCTGGTCCAGTTT	XM_003448671.4
Pentose phosphate pathway	G6PD	ACGGACGAAGAACAGATGGG	CCGTCTCTGAACAACCACCA	XM_003448158.5
Glycogen synthesis	GS	CCTCACTCTGCGCTGTTATTC	CAGCGGCATGCCTTCAGTTT	XM_013276796.3
TCA	PDH	ATGAGCGATCCTGGCGTTAG	AAGGGGTTGGGTGGGTTATG	XM_003446838.5
	IDH	ACGCATCGCTGAGTACGCCTT	AGACCGTCTGACATCCGCATGA	XM_003437590.5
	CS	AGCACCACAGTTTACCAG	AGTGTTGACAAACCCAGA	XM_003438897
Fatty acid oxidation				
	CPT1a	TTTCCAGGCCTCCTTACCCA	TTGTACTGCTCATTGTCCAGCAGA	XM_003440552
	ACO	AGTCCCACTGTGAGCTCCATCAA	CAGACCATGGCAGTTTCCAAGA	KF918710
Fatty acid synthesis	ACCα	TAGCTGAAGAGGAGGGTGCAAGA	AACCTCTGGATTGGCTTGAACA	XM_005471970
	FAS	TCATCCAGCAGTTCACTGGCATT	TGATTAGGTCCACGGCCACA	GU433188
Insulin signal pathway	IR	GTTCGGTGTTGTGTTGTGGG	TCTAGGTCAAACTCCCCCGT	JN967750.1
	GLUT2	CATTGGCATTCTAATCAGCCAGGT	TTGTAATATTGCTGGCGCTCCA	XM_003442884.5
	GLUT4	GCAGGAGGAAAGCCATGCTTATA	ATCATTTCAAAGGAGCGGCAGA	XM_003458705.4
Transcriptional regulation factor	PPARα	GTTCCTCAAGAGTCTCCGCC	AAAGAGCTAGGTCGCTGTCG	KF871430
	SREBP1C	TGCAGCAGAGAGACTGTATCCGA	ACTGCCCTGAATGTGTTCAGACA	XM_005457771
	CHREBP	CTGGAAGTTCTGGATCTATCATCA	CACCTTGGGTTACAGCGAGT	XR_003221896.1
Reference gene	β-actin	AGCCTTCCTTCCTTGGTATGGAAT	TGTTGGCGTACAGGTCCTTACG	KJ126772.1
	EF1a	ATCAAGAAGATCGGCTACAACCCT	ATCCCTTGAACCAGCTCATCTTGT	KJ123689.1

Glycolysis: Glucokinase (GK); Phosphofructokinase (PFK); Pyruvate kinase (PK).
Gluconeogenesis: Phosphoenolpyruvate carboxykinase (PEPCK); Glucose-6-phosphatase (G6Pase).
Pentose phosphate pathway: glucose 6-phosphate dehydrogenase (G6PD).
glycogen synthesis: Glycogen synthase (GS).
Insulin signal pathway: Insulin receptor (IR); Glucose transporter 2 (GLUT2, liver); Glucose transport 4 (GLUT4, muscle).
Fatty acid oxidation: Carnitine palmitoyltransferase Ia (CPTIa); Acyl-coenzyme A oxidase (ACO).
Tricarboxylic acid cycle (TCA): Citrate synthase (CS); Isocitrate dehydrogenase (IDH); pyruvate dehydrogenase (PDH).
Transcriptional regulation factor: Peroxisome proliferator activated receptor alpha (PPARa); Sterol regulatory element binding transcription factor 1 (SREBP1C); Carbohydrate-responsive element-binding protein (CHREBP).
Reference gene: ongationfactor1-alpha (EF1α); β-actin

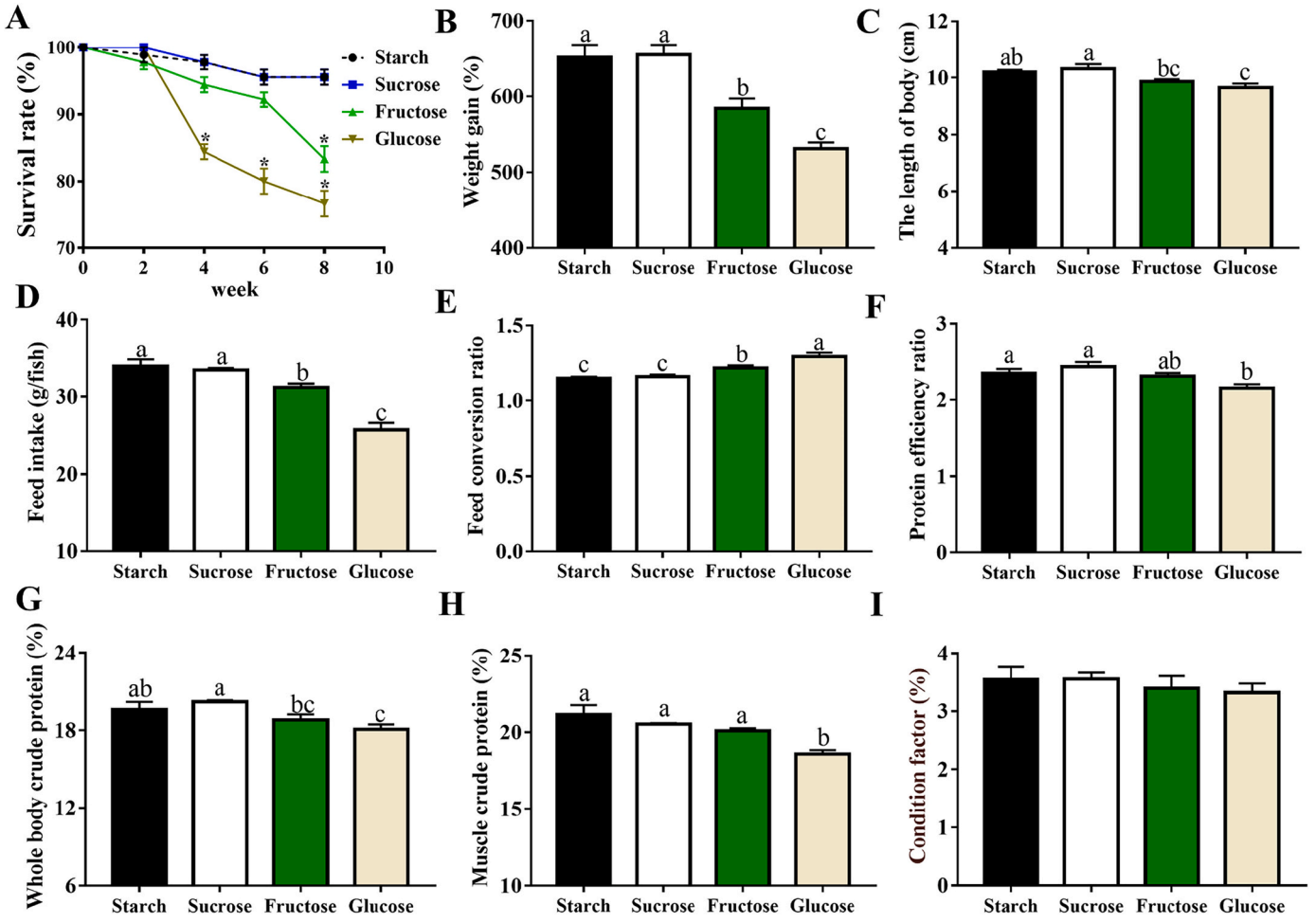


Fig. 1. The growth, feed and protein deposition parameters of Nile tilapia. (A) survival rate, * represents a significant difference from the starch group ($P < 0.05$) (B) weight gain, (C) the length of body, (D) feed intake, (E) feed efficiency ratio, (F) protein efficiency ratio, (G) crude protein content of whole body, (H) crude protein content of muscle, and (I) condition factor. Values are mean ± S.E.M for three replicates (nine fish used for body composition analysis). Bars with different letters show significant difference ($P < 0.05$).

studies have shown that long-term intake of high fructose syrup (glucose and fructose) causes the development of human metabolic syndrome such as obesity, fatty liver and type 2 diabetes (Hodge et al., 2018; Goncalves et al., 2020). Although fish generally have higher plasma insulin levels compared with mammals (Moon, 2001), they have lower insulin sensitivity and are considered to be glucose intolerant (Moon, 2001). Therefore, fish are actually more prone to hyperglycemia than mammals, which is also considered to be related to many fish metabolic diseases (Goessling and Sadler, 2015; Prisingkorn et al., 2017). Consequently, hyperglycemia has been reported to cause lipid deposition in fish (Luo et al., 2020), leading to oxidative stress and increased mortality (Xu et al., 2019). However, until now, most studies on utilization of carbohydrates with different complexity in fish focus mainly on starch and its effect on digestion, growth performance, activities of some glucose metabolism-related enzymes, and insulin regulation(Tian et al., 2000; Tian et al., 2010; Yi et al., 2015; Kamalam et al., 2017). There is still a lack of comprehensive evaluation on the effects of dietary carbohydrate complexity on fish metabolism and liver health.

Nile tilapia is a warm water omnivorous fish, and has been widely cultivated because of its fast growth rate, high fillet yield, and strong disease resistance (Wang et al., 2005; Zhu et al., 2020). Nile tilapia can use up to 40% of digestible dietary carbohydrates (Wang et al., 2005), therefore, it could be used as an ideal fish model to investigate effects of dietary carbohydrate for long-term studies. Starch and sucrose have been widely used as carbohydrate sources in aquatic feeds. Meanwhile, glucose and fructose are the basic components of starch and sucrose. Therefore, in the present study, we used Nile tilapia to explore the effects of carbohydrates with different complexity (starch, sucrose, glucose and fructose) on growth performance, feed utilization efficiency, body composition, liver damage, and glucose and lipid metabolism. This work is important to understand the nutritional metabolic consequences of carbohydrate complexity in fish, and is helpful to improve fish feed formulation.

2. Materials and methods

2.1. Ethical statement

Procedures used in this study were approved by the Committee on the Ethics of Animal Experiments of East China Normal University (F20190101). All experiments were conducted in accordance with the principles of the Laboratory Animal Management guidelines of China.

2.2. Feed ingredients and formulation

To explore the utilization of different types of carbohydrate diets by Nile tilapia, four purified isonitrogen (400 g/kg protein) and isolipidic (60 g/kg fat) feeds were formulated containing starch (polysaccharide), sucrose (disaccharide) and fructose and glucose (monosaccharides). In all diets, casein and gelatin were used as protein sources and soybean oil

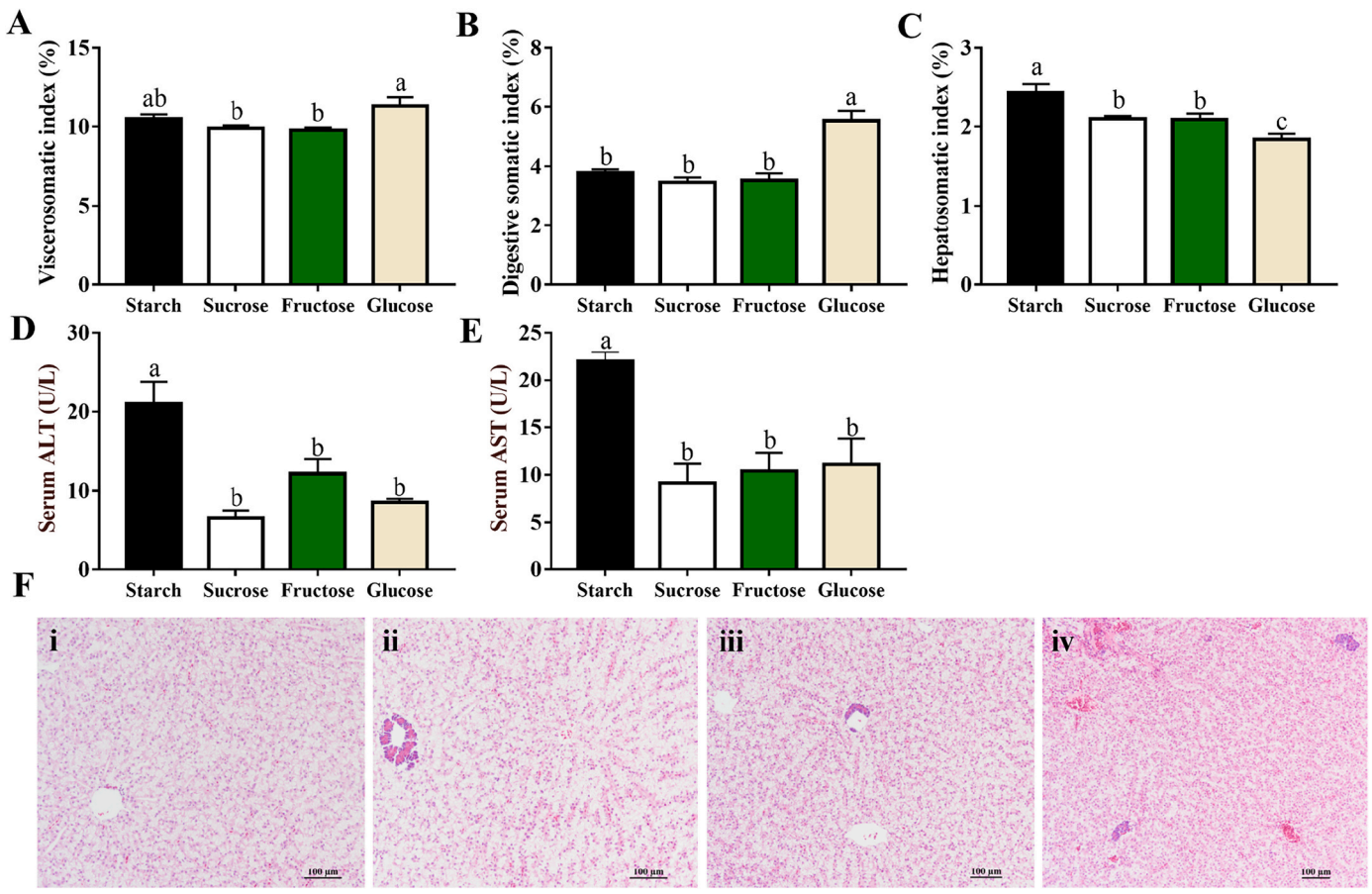


Fig. 2. The somatic parameters and liver damage of Nile tilapia. (A) viscerosomatic index, (B) digestive somatic index, (C) hepatosomatic index, (D) serum alanine transaminase activity (ALT), (E) serum aspartate transaminase activity (AST), and (F) **i**, **ii**, **iii** and **iv** are the liver H & E stained sections for fed on starch, sucrose, fructose and glucose diets, respectively. Values are mean ± SEM for ten fish. Bars with different letters show significant difference ($P < 0.05$).

was used as lipid source. The starch diet (corn starch) was formulated to contain optimal levels of lipid, protein and carbohydrate to satisfy the Nile tilapia nutritional requirements. Afterwards, 50% of corn starch was replaced by using sucrose, glucose and fructose (Table 1). During the feed preparation, individual ingredients were completely mixed by hand followed by feed mixer according to formulations. Subsequent, oil and water were added to form a dough, which was then processed into pellets (2.0 and 2.5 mm diameter) by using a feed pelleting machine (Sun Yat-sen University, Guangzhou, China). The feeds were then dried naturally in the air. After drying, the feeds were stored at −30 °C until used for feeding Nile tilapia during the study.

2.3. Experimental fish management procedures

One thousand Nile tilapia (approximately 2 g) used in this experiment were purchased from Longhu Aquaculture Farm (Guangzhou, China). The fish were acclimated in five 500 L tanks for 15 days under conditions suitable for growth and survival of Nile tilapia. During the acclimatization period, all fish were fed by using a commercial feed (33% protein and 5% lipid, Wuxi, China). After acclimatization, 360 Nile tilapia (4.5 ± 0.30 g) were randomly selected and divided into four treatments (90 fish per treatment, each three replicates). The fish in each tank were reared by using a 200 L tank supplied with clean dechlorinated freshwater. The fish in each tank were fed on the four diets three times per day at a feeding rate of 4% for eight weeks. The fish in each tank were weighted every two weeks and the amount of feed given was adjusted accordingly. The fish were reared on a 12 h:12 h light: dark photoperiod. The water temperature was maintained at 28 ± 1.5 °C while the water pH and total ammonia nitrogen levels ranged from 7.8 to 8.2 and 0.05 to 0.08 mg/L, respectively.

2.4. Determination of growth performance, survival and feed utilization efficiency

At the end of the rearing period, fish were fasted for 24 h prior to sampling. The fish were then anesthetized by using 7 g/L of MS-222 (tricaine methanesulfonate; Sigma, USA). Afterwards, all fish in each treatment were counted to assess survival rate (SR) and then weighed to determine final weight for calculation of weight gain (WG). The amount of feed given and the number of fish were used to calculate feed intake (FI). The feed conversion ratio (FCR) was calculated based on the feed intake (FI) and the biomass of live and dead fish. The weight of fish gained and the amount of protein consumed were used to calculate protein efficiency ratio (PER). The WG, SR, FI, PER and FCR were calculated by using the following formulaes:

$$\text{WG}\ (\%) = \frac{\text{Final fish weight (g)} - \text{Initial fish weight (g)}}{\text{Initial fish weight (g)}} \times 100$$

$$\text{SR}\ (\%) = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$\text{FI (g/fish)} = \frac{\text{Total feed consumption (g)}}{\text{Total number of fish}}$$

$$\text{FCR} = \frac{\text{Total feed intake in each tank (g)}}{[(\text{Final tank biomass} + \text{dead fish}) - \text{Initial tank biomass}]\ (\text{g})}$$

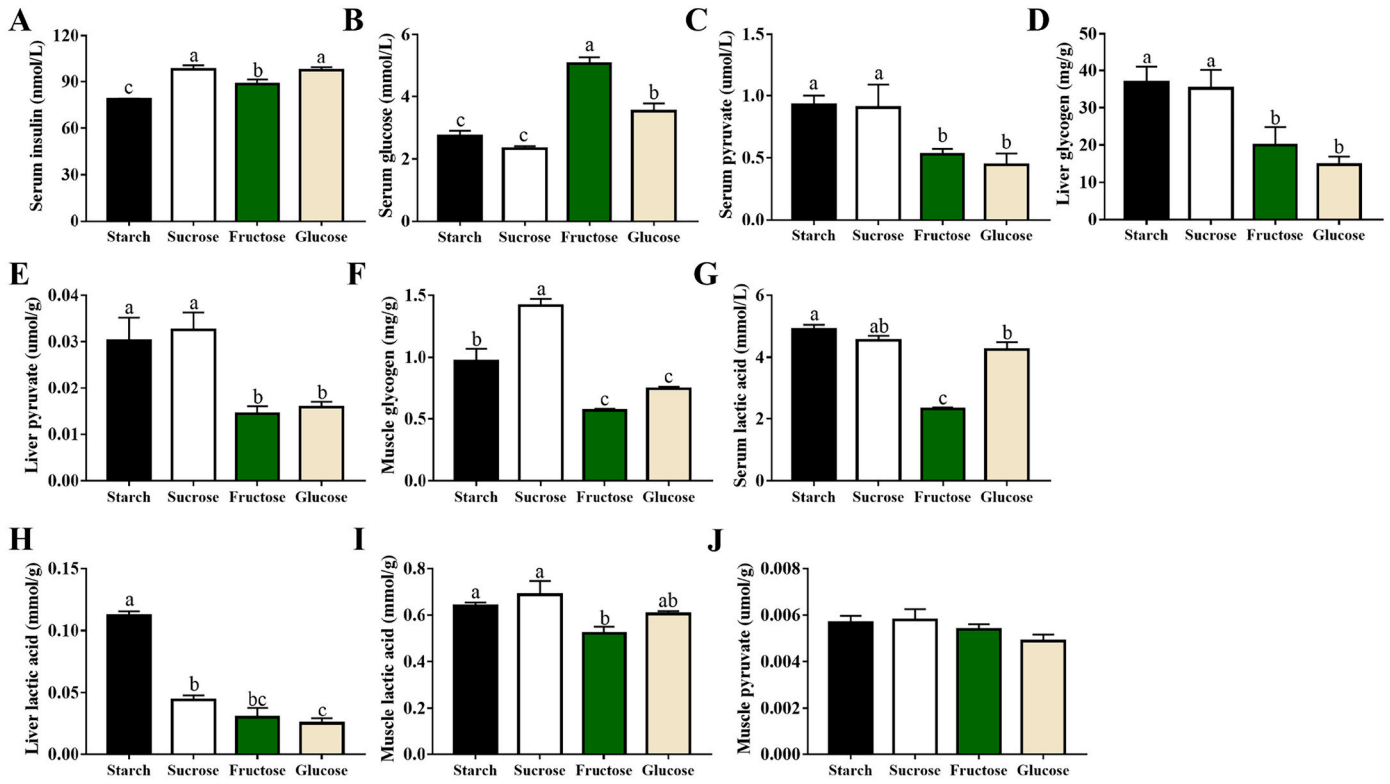


Fig. 3. The glucose metabolism parameters of Nile tilapia. (A) serum insulin, (B) serum glucose, (C) serum pyruvate, (D) serum lactic acid, (E) liver glycogen, (F) liver pyruvate, (G) liver lactic acid, (H) muscle glycogen, (I) muscle pyruvate, and (J) muscle lactic acid. Values are mean ± SEM for nine fish. Bars with different letters show significant difference ($P < 0.05$).

$$\text{PER} = \frac{\text{Total weight gain (g)}}{\text{Total protein intake (g)}}$$

$$\text{CF}\ (\%) = \frac{\text{Final fish weight (g)}}{\text{Final fish length } (cm^3)} \times 100$$

2.5. Collection of blood and calculation of organ indices

A sample of ten fish per treatment were randomly collected and anesthetized as above for blood sampling. Before blood collection, the fish were individually measured to determine their weight and body length for calculation of condition factor (CF). After weight measurement, blood was drawn from the caudal vein by using heparinized 2 mL syringes (Klmedical, China). The blood obtained was immediately centrifuged at 3000 rpm at 4 °C for 10 min. The serum obtained was placed into polypropylene tubes and was immediately stored at −20 °C until needed for analysis. After drawing blood, the fish were individually dissected to obtain viscera, liver, intraperitoneal fat and intestine. The obtained viscera, liver, intraperitoneal fat and intestine were weighted for calculation of viscera somatic index (VSI), hepatosomatic index (HSI), intraperitoneal fat ratio (IPF) and digestive somatic index (DSI) (Du et al., 2006; Xu et al., 2020). The liver and muscle tissues were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis. The VSI, HSI, IPF, DSI and CF were calculated as follows:

$$\text{VSI}\ (\%) = \frac{\text{Viscera weight (g)}}{\text{Fish body weight (g)}} \times 100$$

$$\text{HSI}\ (\%) = \frac{\text{Liver weight (g)}}{\text{Fish body weight (g)}} \times 100$$

$$\text{IPF}\ (\%) = \frac{\text{Intraperitoneal fat weight (g)}}{\text{Fish body weight (g)}} \times 100$$

$$\text{DSI}\ (\%) = \frac{\text{Intestine weight (g)}}{\text{Fish body weight (g)}} \times 100$$

2.6. Tissue and serum biochemical analysis

The liver and muscle tissues from nine fish per treatment were randomly collected for tissue biochemical analysis. The tissues were measured and homogenized by adding saline water (1:9, mg/v) and then centrifuged at 2500 rpm for 10 min. The resulting supernatant was pipetted and placed into polypropylene tubes and stored at −80 °C until needed for biochemical analysis. The triglyceride (TG), non-esterified fatty acid (NFFA) and pyruvate content in serum, liver and muscle were determined by using specific commercial assay kits (Jiancheng Biotech Co., China) according to manufacturer's instructions. The glycogen contents in the liver and muscle were measured by using a specific commercial kit (Jiancheng Biotech Co., China) by following manufacturer's protocols. The serum insulin level was measured by Enzyme Linked Immunosorbent Assay (ELISA) kit (Hengyuan Biotech Co., China) according to manufacturer's instructions. Glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the serum were measured by using specific commercial kits (Jiancheng Biotech Co., China) based on relevant kit protocols.

2.7. Proximate analysis of whole fish and muscle

Nine fish per treatment were randomly selected for proximate analysis. The crude protein of whole fish body and muscle were determined by Kjeltec™ 8200 (FOSS, Sweden) (Zhang et al., 2019). The crude lipid of whole body was analyzed according to the methods of chloroform-methanol (1:2, v:v) extraction (Bustin et al., 2009).

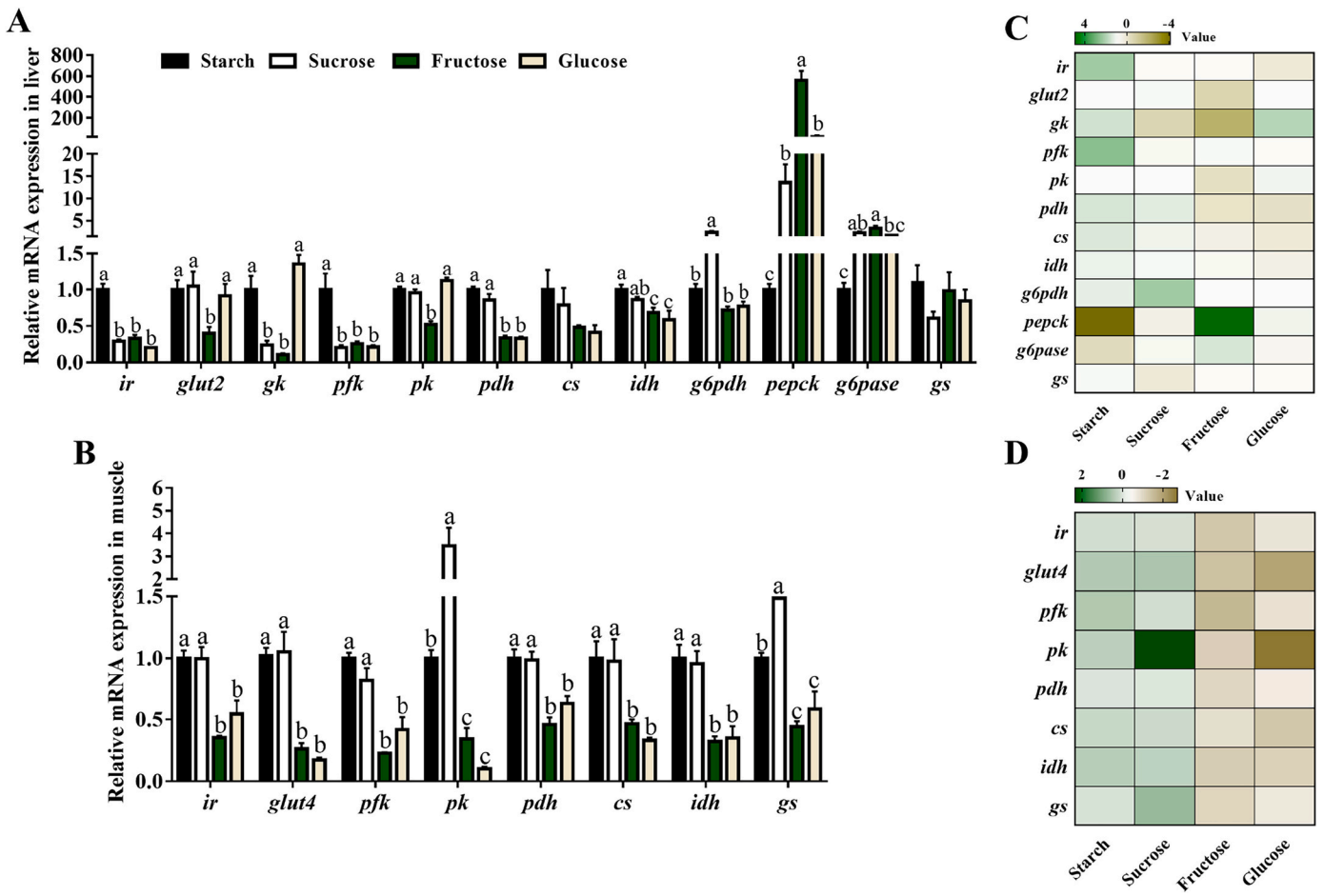


Fig. 4. The mRNA expression of glucose metabolism genes and related heat maps in Nile tilapia. The insulin signal pathway (*ir*), glucose transport (*glut2*), glucose catabolism (*gk*, *pfk* and *pk*), tricarboxylic acid cycle (*pdh*, *cs* and *idh*), pentose phosphate pathway (*g6pdh*), gluconeogenesis (*pepck* and *g6pase*) and glycogen synthesis (*gs*) related genes in the liver (A) and the corresponding heat map (C). The *ir, glut4*, *pfk*, *pk*, *pdh*, *cs*, *idh and gs* genes in the muscle (B) and the corresponding heat map (D). Values are mean ± SEM for nine fish. Bars with different letters show significant difference ($P < 0.05$).

2.8. Histological observation of liver

Six liver tissues from each treatment were randomly sampled and cut into 2 mm pieces. The tissues were dehydrated in a series of ethanol before embedded in paraffin and sliced into 4 μm thick sections. All sections were stained with hematoxylin and eosin (H & E), microscopically examined, and photographed on a Nikon Microscope Eclipse Ni (Nikon Corporation, Tokyo, Japan). In addition, oil red O staining was used to label liver lipid droplets. Six fresh liver tissues (cut into 2 mm thick) were collected from each treatment and immediately frozen in liquid nitrogen. The frozen liver tissues were then sliced into 9 μm thick sections with a cryostat microtome and fixed in 4% formaldehyde for 10 min, then stained with oil red O, and finally prepared for optical microscopy.

2.9. Gene expression analysis by quantitative real time polymerase reaction (qPCR)

Total RNA was extracted from the frozen liver and muscle tissues (approximately 50 to 100 mg) by using Trizol reagent according to the manufacture's protocol. The quantity and quality of the isolated RNA was assessed by the 260/280 nm absorbance ratio by using NANODROP 2000 Spectrophotometer (Thermo, USA). RNA samples were treated by RNase-Free DNase prior to quantitative real-time polymerase reaction (qPCR) (Takara Co. Ltd., Japan) to avoid genomic DNA amplification. The cDNAs of the total RNA were synthesized using a RT reagent Kit (No. R323–01, Vazyme Biotech Co, Nanjing, China). The cDNA was unified based on the concentration determined before qPCR. The mean of the reference genes; elongation factor 1α (EF1α) and β-actin (β-actin) was used to normalize the expression level of the target genes. The primers designed for qPCR are listed in Table 2. The qPCR reaction solution consisted of 5 μL of 2 × ultra SYBR mixture, 0.25 μL of 10 mM forward and reverse primers, 2 μL of cDNA template and 2.5 μL of ddH_2O, under the following reaction conditions: 95 °C for 10 min followed by 40 cycles consisting of 95 °C for 15 s, 60 °C for 10 s and 72 °C for 15 s carried out on a CFX Connect Real-Time System (Bio-Rad, USA). Melting curves were monitored (95 °C for 5 s, 65 °C for 1 min, temperature gradient 0.11 °C/s from 65 °C to 97 °C) to confirm the specificity of the amplification reaction, at the end of the last amplification cycle. The cDNA expression of genes was estimated by using the comparative ($2^{-\Delta\Delta Ct}$) method (Livak and Schmittgen, 2001).

2.10. Statistical analyses

Data were tested for normality by using the Shapiro-Wilk test and homogeneity of variance by using Levene's test. Data were analyzed by using one-way analysis of variance (ANOVA) to compare the measured parameters for the different carbohydrate complexity diets. Duncan's test was used for the multiple comparisons, after ANOVA showed significant differences. All analyses were conducted by using the IBM SPSS Statistics 20 (IBM, Michigan Avenue, Chicago, IL, USA). Results with $P \leq 0.05$ were considered statistically different. All results are presented

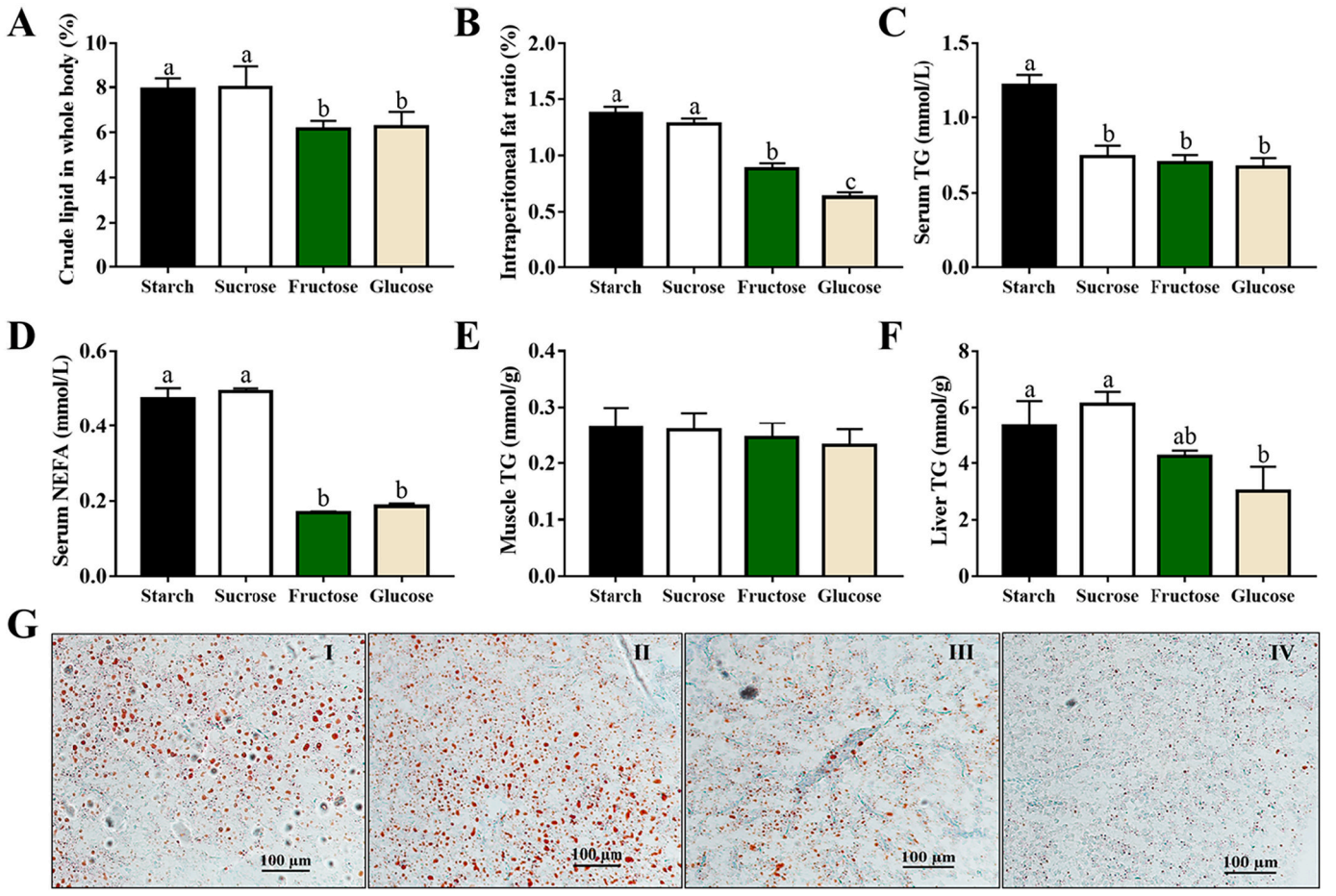


Fig. 5. The lipid deposition in Nile tilapia fed on starch, sucrose, fructose and glucose diets. The crude lipid content of whole body (A), intraperitoneal fat ratio (B), serum TG (C), serum NEFA (D) muscle TG content (E), and liver TG content (F). The liver lipid droplets distribution (G), which is the liver tissue oil red O section of Nile Tilapia fed with the diet of starch group (I), sucrose group (II), fructose group (III) and glucose group (IV), respectively. Values are mean ± SEM for nine fish. Bars with different letters show significant difference ($P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

as means ± standard error of the mean (SEM).

3. Results

3.1. Effects of carbohydrates with different complexity on survival rate, growth and feed performance and protein deposition in Nile tilapia

Feeding the Nile tilapia with fructose and glucose (monosaccharide diet) decreased survival rates compared to those fed on starch (polysaccharide) and sucrose (disaccharide) (Fig. 1A). The Nile tilapia fed on fructose and glucose diets significantly reduced significantly the WG than those fed on starch and sucrose diets ($P < 0.05$; Fig. 1B). Moreover, the Nile tilapia fed on glucose had lower WG than those fed on sucrose diet ($P < 0.05$). However, the Nile tilapia fed on sucrose and starch diet had statistically comparable WG ($P > 0.05$). The Nile tilapia fed on the glucose diet had significantly lower total length (Fig. 1C), FI (Fig. 1D), PER (Fig. 1E) but had higher FCR (Fig. 1F) than those fed on starch and sucrose diets ($P < 0.05$). The fish fed on sucrose and fructose diets had no significant differences in body length (Fig. 1C), PER (Fig. 1G), and crude protein content of whole body (Fig. 1H) and muscles (Fig. 1I) compared to those fed on starch diet ($P > 0.05$). The Nile tilapia fed on fructose and glucose diets had significantly lower FI (Fig. 1E) and higher FCR (Fig. 1F) than those fed on the starch diet ($P < 0.05$). There was no significant difference in CF (Fig. 1I) of Nile tilapia in all treatment groups ($P > 0.05$). Taken together, Nile tilapia fed on monosaccharide diets had lower growth performance, survival rate and feed utilization than those fed on disaccharide and polysaccharide.

3.2. Effects of carbohydrates with different complexity on organ indexes and liver health

The Nile tilapia fed glucose diet had significantly higher VSI (Fig. 2A) and DSI (Fig. 2B) than those fed on fructose, sucrose and starch diets ($P < 0.05$) but not starch diet for VSI ($P > 0.05$). The fish fed on starch, sucrose and fructose diets had statistically similar VSI and DSI ($P > 0.05$). The Nile tilapia fed on glucose diet had significantly lower HSI than those fed of the remain starch, sucrose and fructose (Fig. 2C; $P < 0.05$). However, feeding the fish starch, sucrose and fructose diets did not affect significantly HIS ($P > 0.05$). Moreover, serum AST (Fig. 2E) and ALT (Fig. 2F) activities, which are the classic biomarkers of liver damage, were significantly reduced in Nile tilapia fed on sucrose, fructose and glucose diets compared with those fed on starch diet ($P < 0.05$), with the three diets attaining statistically comparable levels ($P > 0.05$). Similarly, liver H & E stained sections did not show obvious liver injury-related characteristics in all treatments (Fig. 2G). These results indicate that, the monosaccharide and disaccharide carbohydrates relieved the liver damage in Nile tilapia compared with polysaccharide.

3.3. Effects of carbohydrates with different complexity on glucose metabolism related parameters

The Nile tilapia fed on starch diet decreased significantly the serum

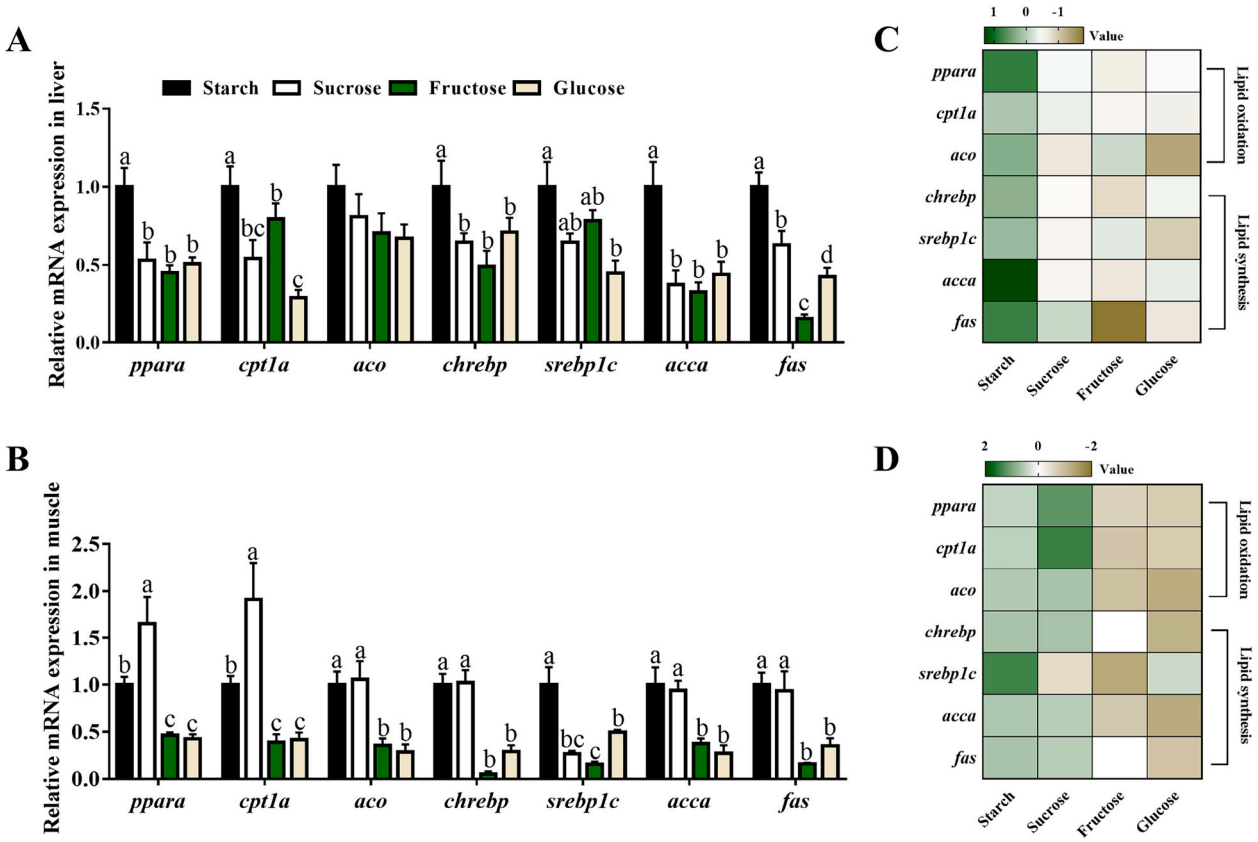


Fig. 6. The mRNA expression of lipid metabolism genes and related heat maps in Nile tilapia. The lipid catabolism-related genes *ppar*α, *cpt1a* and *aco*, and lipid anabolism-related genes *chrebp*, *srebp1c*, *acca* and *fas* in liver (A) or muscle (B), respectively. Heat maps corresponding to the expression of the above genes in liver (C) and muscle (D). Values are mean ± SEM for nine fish. Bars with different letters signify significant difference (P < 0.05).

insulin (Fig. 3A) and glucose (Fig. 3B) levels than those fed on sucrose (except for serum glucose), fructose and glucose diets (P < 0.05). Similarly, fish fed on fructose diet had lower insulin level than those fed on sucrose and glucose diets (Fig. 3A), while those fed on sucrose had lower glucose than those fed on fructose and glucose diets (Fig. 3B). Equally, the fish fed on glucose diet had lower glucose level than those fed on fructose (Fig. 3B). The fish fed on fructose and glucose diets had significantly lower serum pyruvate (Fig. 3C), liver glycogen (Fig. 3D), liver pyruvate (Fig. 3E) and muscle glycogen (Fig. 3F) than those fed on starch and sucrose diets ($P < 0.05$). In most of these parameters, fish fed on fructose and glucose diets and those fed on starch and the sucrose diets had statistically comparable levels ($P > 0.05$) except for a significantly lower level of muscle glycogen for fish fed on starch than sucrose diet (Fig. 3F). The Nile tilapia fed on starch diet had significantly higher lactic acid levels in the serum (Fig. 3G), liver (Fig. 3H) and muscle (Fig. 3I) than those fed on fructose and glucose diets ($P < 0.05$) and sucrose for liver lactic acid. The fish fed on the fructose diet had lower lactic acid level in the muscle (Fig. 3I) than those fed on starch and sucrose diets ($P < 0.05$), while the latter had statistically similar levels similar to those fed on fructose and glucose ($P > 0.05$). Feeding Nile tilapia with the diets with different carbohydrate complexity did not affect muscle pyruvate content (P > 0.05; Fig. 3J). In general, the carbohydrate metabolism between fish fed on sucrose and starch diets were similar, while the fish fed on the two monosaccharides showed lower levels than those fed on starch diet.

3.4. Effects of carbohydrates with different complexity on mRNA expression of genes related to glucose metabolism

The relative mRNA expression of genes involved in insulin signal pathway (*ir*), glucose transport (*glut2/4*), glucose catabolism (*gk*, *pfk* and *pk*), tricarboxylic acid cycle (*pdh*, *cs* and *idh*), pentose phosphate pathway (*g6pdh*), gluconeogenesis (*pepck* and *g6pase*) and glycogen synthesis (*gs*) in the liver are shown in Fig. 4A, while those in the muscle are depicted in Fig. 4B. The Nile tilapia fed on sucrose diet did not affect the mRNA expression of *glut2*, *pk*, *pdh*, *cs*, *idh* and *gs* in the liver compared with those fed on starch diet (Fig. 4A; P > 0.05). However, they reduced the mRNA expression of *ir*, *gk* and *pfk* ($P < 0.05$) and significantly increased the expression of *g6pdh*, *pepck* and *g6pase* in liver ($P < 0.05$) compared with those fed on starch diet. The Nile tilapia fed on fructose diet down-regulated significantly the mRNA expression of *ir*, *glut2*, *gk*, *pfk*, *pk*, *pdh*, and *idh* genes in the liver, while the *pepck* and *g6pase* genes were significantly up-regulated compared with those fed on starch diet (Fig. 4A; $P < 0.05$). The fish fed on glucose diet down-regulated significantly the mRNA expression of *ir*, *pfk*, *pdh*, and *idh* genes, while up-regulated significantly the expression of *pepck* gene than those fed on starch diet (P < 0.05; Fig. 4A). Feeding Nile tilapia with glucose and starch diets did not affect the mRNA expression levels of *glut2*, *gk*, *pk*, *cs*, *g6pdh*, *g6pase* and *gs* genes in the liver (Fig. 4A; $P > 0.05$).

In the muscle, the Nile tilapia fed on fructose and glucose diets down-regulated significantly the mRNA expression of *ir*, *glut4*, *pfk*, *pdh*, *cs*, *idh* and *gs* genes than those fed on starch and sucrose diets (Fig. 4B; P < 0.05). The Nile tilapia fed on sucrose diet did not affect significantly the mRNA expression of most genes (*ir*, *glut4*, *pfk*, *pdh*, *cs* and *idh*) (Fig. 4B; P > 0.05), except *pk* and *gs* genes, which were notably up-regulated compared with those fed on the starch diet (Fig. 4B; $P < 0.05$).

The expression profiles of glucose metabolism genes in liver (Fig. 4C) and muscle (Fig. 4D) are summarized by using heat maps. In general, the Nile tilapia fed on monosaccharide diets suppressed the expression of genes related to glucose catabolism and promoted the expression of gluconeogenic genes compared with those fed on polysaccharide diet,

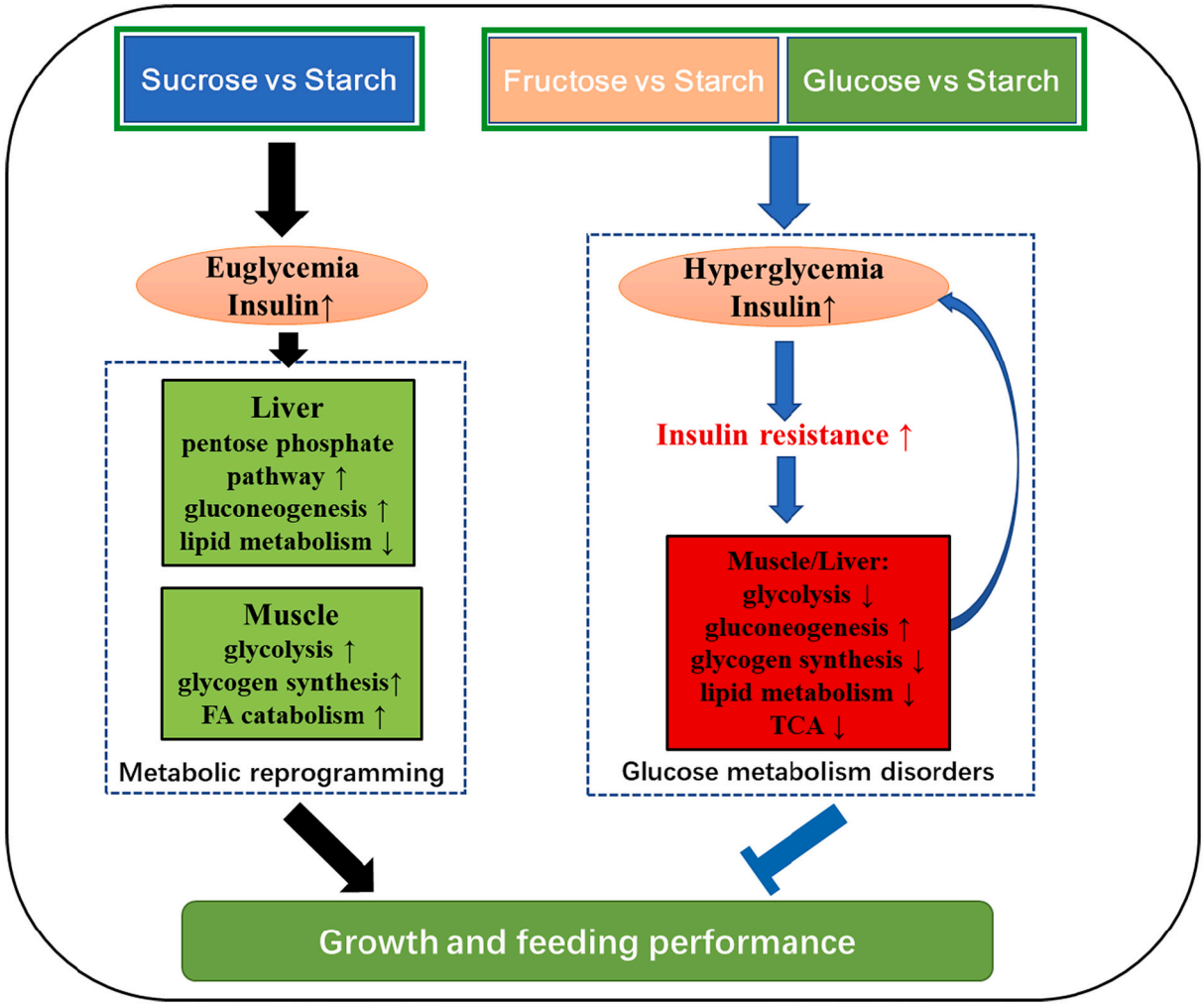


Fig. 7. The alterations in the metabolic and growth characteristics of Nile tilapia caused by diets in the sucrose group, fructose group and glucose group.

whereas the disaccharide diet and the polysaccharide diet had essentially similar glucose catabolic gene expression profiles, the disaccharide diet caused higher expression of hepatic gluconeogenic genes and pentose phosphate pathway genes. (Fig. 4C and Fig. 4D).

3.5. *Effects of carbohydrates with different complexity on lipid metabolism related parameters*

The Nile tilapia fed on sucrose and starch diets had higher whole fish crude lipid (Fig. 5A), IPF (Fig. 5B), serum NEFA (Fig. 5D) and liver TG (Fig. 5F) than those fed on fructose and glucose diets ($P < 0.05$), except for muscle TG in the fructose, which was not significantly different from either the starch or the sucrose. On one hand, most of these parameters were similar between fish fed on sucrose and starch diets, and those fed on fructose and glucose, on the other hand except IPF, which was significantly higher for fish fed on fructose than glucose ($P < 0.05$; Fig. 5B). The Nile tilapia fed on starch diet had significantly higher serum TG than those fed on sucrose, fructose and glucose ($P < 0.05$; Fig. 5C). Feeding Nile tilapia with the diets with different carbohydrate complexity did not affect muscle TG content ($P > 0.05$; Fig. 5E). The Oil red O staining showed smaller volume and reduced number of lipid droplets for Nile tilapia fed on fructose and glucose diets than those fed on starch and sucrose diets consistent to the results on TG content in the liver. Overall, feeding Nile tilapia with starch or sucrose diets did not affect the lipid deposition pattern, while feeding them with the two monosaccharide diets reduced significantly the lipid in whole body and serum.

3.6. *Effects of carbohydrates with different complexity on mRNA expression of lipid metabolism related genes*

The relative mRNA expression of key genes related to lipid oxidation (*pparα*, *cpt1a* and *aco*) and lipid synthesis (*chrebp*, *srebp1c*, *acca* and *fas*) of Nile tilapia are shown in Fig. 6A for liver and Fig. 6B for muscle tissues. The Nile tilapia fed on the starch diet up-regulated the mRNA expression profile of lipid metabolism genes (*pparα*, *cpt1a, chrebp*, *acca* and *fas*) ($P < 0.05$) in the liver than those fed on sucrose, fructose and glucose, except for *aco* gene, which had no statistical difference among the four diets ($P > 0.05$). The fish fed on fructose had higher and lower mRNA expression of *cpt1a* and *fas* genes, respectively than those fed on glucose diet ($P > 0.05$).

Similar to the liver, the Nile tilapia fed on fructose and glucose also reduced significantly the mRNA expression of lipid metabolism-related genes in the muscle compared with those fed on starch and sucrose diets ($P < 0.05$), except *srebp1c* gene, which was not significantly different in fish fed on monosaccharide diets from sucrose diet ($P > 0.05$). In contrast, the expression of lipid oxidation-related genes in the muscle (*pparα* and *cpt1a*) were significantly higher in fish fed on sucrose than starch diet ($P < 0.05$). With exception of *pparα* and *cpt1a*, most genes had statistically comparable expression levels for fish fed on starch and sucrose and those fed on fructose and glucose in the muscle ($P > 0.05$).

The expression profiles of genes related to lipid metabolism in the liver and muscle in Nile tilapia were presented in the form of heat maps (Fig. 6C and D). The expression levels of lipid metabolism genes in the Nile tilapia fed on fructose and glucose diets were substantially lower than those fed on starch and sucrose diets both in the liver and muscle. However, the Nile tilapia fed on sucrose diet significantly increased the mRNA expression of genes related to lipid catabolism in the muscle. These results indicate that, feeding Nile tilapia with monosaccharide diets reduced the lipid metabolism genes expression compared to those fed on disaccharide and polysaccharide diets.

4. Discussion

The results of this study indicate that the dietary carbohydrate complexity significantly affects Nile tilapia growth performance, survival and feed utilization. In the present study, poor weight gain was evident in the Nile tilapia fed on fructose and glucose diets, accompanied by the reduced feed intake and feed efficiency. This was especially apparent in the Nile tilapia fed on glucose diet, which had the lowest protein deposition level. The effect of dietary monosaccharides on the growth performance of Nile tilapia in this study is consistent with the previous results in many fish. In fact, studies in blunt snout bream (*Megalobrama amblycephala*) (Ren et al., 2015), GIFT tilapia (*Oreochromis niloticus*) (Qiang et al., 2016), gibel carp (*Carassius auratus gibelio*) (Tan et al., 2006), gilthead sea bream (*S. aurata*) (Enes et al., 2010), cobia (*Rachycentron canadum*) (Cui et al., 2010), and Chinese long snout catfish (*Leiocassis longirostris*) (Tan et al., 2006) all indicated that dietary monosaccharide can significantly inhibit growth performance compared with dietary polysaccharide. This shows that Nile tilapia has poor utilization ability of dietary monosaccharides like the above fish.

However, the low growth performance, survival rate and feed utilization of Nile tilapia in monosaccharide diets may be related to the glucose metabolism disorder. In the present study, the dietary monosaccharide diets caused a significant increase in serum insulin levels in fish than those fed on starch, accompanied by obvious hyperglycemia, suggesting that dietary monosaccharides induced insulin resistance (Saini, 2010). These results suggest that simple carbohydrates are easier to absorb than complex carbohydrates ones and are more likely to cause hyperglycemia (Luo and Xiaojun., 2010). Many studies have also shown that persistent hyperglycemia can significantly contribute to metabolic disorders and lead to metabolic syndrome (Perseghin, 2009; Wang et al., 2018). Moreover, fish fed on dietary monosaccharides down-regulated most genes related to glucose catabolism than those fed on starch diet, indicating that glucose catabolism was weakened. Furthermore, fish fed on the monosaccharide diets increased the expression of *g6pase* and *pepck* genes than those fed on starch diet, reflecting enhanced gluconeogenesis. Therefore, decreased utilization and increased production of glucose further promoted the occurrence of hyperglycemia (Chen et al., 2019), which aggravated glucose metabolism disorders leading to reduced growth of Nile tilapia.

Unexpectedly, although dietary fructose and glucose caused obvious glucose metabolism disorders, they did not lead to liver damage, but instead reduced the levels of liver damage-related indicators. However, this “damage relief” may not be a normal state. Generally, serum AST and ALT are important and sensitive indicators for evaluating liver injury (Kim et al., 2008; Wu et al., 2017). Moreover, HSI is also an important liver injury diagnosis marker and an indicator of liver health (Saxena et al., 2010; Amaya and Nickell, 2015). Commonly, liver damage is usually accompanied by an increase in liver weight and HSI (Tag, 2015). In present study, the HSI and AST and ALT in the serum were significantly reduced in the Nile tilapia fed on fructose and glucose diets than starch diet. Besides, no obvious damage characteristics were observed in the H & E section of the liver, suggesting that the replacing 50% of starch by using simple carbohydrates does not cause damage to the liver of Nile tilapia. In addition, glycogen and TG contents in the liver were also significantly decreased in fish fed on fructose and glucose diets, which may also be one of the important reasons for the decline in HSI. Consistently, studies in Jade Perch (*Scortum barcoo*) (Alkhafaji et al., 2017), juvenile flounder (*Paralichthys olivaceus*) (Lee et al., 2003) and gibel carp (*C. auratus gibelio*) (Tan et al., 2006) also reported increase in glycogen in the liver accompanied with higher HSI. It is well known that excessive accumulation of TG in the liver causes liver steatosis, leading to severe liver damage (Quiroga and Lehner, 2018). The comprehensive causes of fatty liver in fish have been previously summarized (Du, 2014). Overall, the reduction in liver injury marker levels of Nile tilapia fed monosaccharide diets in this study were mainly related to the reduced accumulation of nutrients (TG and glycogen) in the liver. These results reflect a close relationship between nutrient metabolism and liver damage. At the same time, we hypothesize that the main reason for retarded growth in Nile tilapia fed on monosaccharides is not liver damage.

In fact, studies in mammals have shown that elevated glucose levels suppress appetite, cause satiety and reduce food intake (Chapman et al., 1998; Gielkens et al., 1998). Indeed, the Nile tilapia fed on glucose and fructose diets had elevated glucose levels in the serum and reduced feed intake in the present study. Therefore, the decreased growth performance of Nile tilapia fed on the monosaccharide diets is primarily due to decreased feed intake and glucose metabolism disorders caused by hyperglycemia. The decreased liver damage markers in the Nile tilapia fed on monosaccharide diets were related to the decline in lipid and glycogen deposition in the liver caused by the decreased feed intake.

Similar to dietary monosaccharides, many fish do not utilize dietary sucrose very well. Studies conducted on large yellow croaker (*Pseudosciaena crocea*) (Yi et al., 2015), jundiá (*R. quelen*) (Corrêia et al., 2019) and channel catfish (*I. punctatus*) (Wilson and Poe, 1987), reported that dietary sucrose also reduced significantly WG, specific growth rate (SGR), feed efficiency (FE) and PER compared with dietary polysaccharides. Interestingly, the Nile tilapia fed on sucrose diet had no significant differences in growth performance and feeding compared with those fed on dietary starch, suggesting that Nile tilapia can adapt to dietary sucrose through homeostasis regulation mechanisms.

In the present study, the Nile tilapia fed on dietary sucrose and starch had different glucose and lipid metabolism patterns. The fish fed on sucrose diet reduced the expression of key genes involved in glucose catabolism such as *gk* and *pfk* in the liver than those fed on the starch diet, indicating glycolysis in the liver was decreased. On the other hand, fish fed on dietary sucrose reduced the lactic acid content (a gluconeogenesis substrate) in the liver and up-regulated the expression of key gluconeogenesis genes such as *g6pase* and *pepck* compared to starch diet, suggesting enhanced gluconeogenesis. In fact, dietary sucrose did not cause higher glucose levels in the serum than dietary starch, which may be related to the liver pentose phosphate pathway and muscle glucose metabolism. The pentose phosphate pathway is an important pathway for glucose catabolism, due to its physiological significance for maintaining the redox state, biosynthesis process and proliferation of cells (Tozzi et al., 2006; Riganti et al., 2012; Adams et al., 2019), it is also considered to be one of the important pathways of glucose utilization in fish (Hutchins et al., 1998). In the present study, dietary sucrose up-regulated the expression of g6pdh, a key gene of the pentose phosphate pathway. Studies in GIFT tilapia (*O. niloticus*) also found that *g6pdh* plays a significant role in the maintenance of endocrine status and hepatic glucose metabolism. (Qiang et al., 2014, 2016). It should be noted that dietary sucrose caused more glycogen accumulation, accompanied by a significant up-regulation of *gs* gene in the muscle than dietary starch. Moreover, the fish fed on sucrose diet up-regulated *pk* gene expression than those fed on starch diet in the muscles, indicating promoted glycolysis. Because a previous study at biochemical levels have proved that increased pk activity depends on ongoing mRNA synthesis and de novo protein synthesis mediated by increased enzyme content (Traxinger and Marshall, 1992). Similarly, studies in gilthead sea bream (*S. aurata*) (Couto et al., 2008), blunt snout bream (*M. amblycephala*) (Ren et al., 2015) and southern catfish (*Silurus*

meridionalis) (Luo and Xie, 2010) found that the increase in pk activity plays an important role in promoting fish carbohydrate utilization. Therefore, unlike dietary starch, dietary sucrose may promote liver pentose phosphate pathway, muscle glycogen accumulation and muscle glycolysis, thereby maintaining glucose metabolism balance in Nile tilapia.

Lipid metabolism is an important process for maintaining energy homeostasis of living organisms including Nile tilapia. The metabolites of glucose and lipids are dynamically transported between and within cells, and then converted into other molecules (Chen et al., 2019). In the present study, the Nile tilapia fed on sucrose and starch diets also had different lipid metabolism characteristics. The key genes involved in lipid anabolism and catabolism in the liver of Nile tilapia fed on sucrose diet were down-regulated compared with those fed dietary starch. However, the Nile tilapia fed on sucrose diet significantly up-regulated the expression of *pparα* and *cpt1b*, key genes for lipid catabolism in muscle, but did not alter the expression of the lipid de novo synthesis genes *acca* and *fas*, compared with those fed on starch diet. Actually, except for the significant reduction of TG levels in the serum, the Nile tilapia fed on sucrose and starch diets had no much differences on lipid deposition levels in other tissues. These results suggest that, the fish fed on sucrose diet enhanced muscle lipid utilization compared with those fed on starch diet and these lipids were likely to be derived from serum. Therefore, it is also implied that different lipid storage organs may communicate closely with each other through blood circulation, thus maintaining the dynamic balance of lipid levels in the body. Furthermore, the fish fed on sucrose and starch diets did not affect the expression of key genes (*pdh*, *cs* and *idh*) involved in the tricarboxylic acid cycle in the liver and muscle. In general, although dietary sucrose affected the glucose and lipid metabolism pattern of Nile tilapia compared with dietary starch, it had no effect on body energy supply, suggesting that the glucose and lipid metabolism jointly maintain body energy homeostasis. Accordingly, the Nile tilapia fed on starch and sucrose diets showed similar growth performance. This shows the ability of Nile tilapia to adapt to utilize dietary carbohydrates. Finally, the main metabolic characteristics of Nile tilapia fed on sucrose, fructose and glucose diets versus starch diet were summarized in Fig. 7.

In conclusion, this study found that carbohydrate complexity affects significantly fish metabolism and liver health due to variations in absorption and carbohydrate utilization. Nile tilapia utilizes better polysaccharide and disaccharide than monosaccharides. Nile tilapia farmers can formulate feeds with polysaccharide and disaccharide for improved feed utilization and growth of fish.

Author statement

Wen-Hao Zhou designed and conducted experiment, analyzed data and drafted paper; Chen-Chen Wu conducted experiment; Samwel M. Limbu revised paper; Rui-Xin Li conducted experiment; Li-Qiao Chen revised paper; Fang Qiao revised paper; Yuan Luo conducted experiment; Mei-Ling Zhang revised paper; Tao Han designed study and revised paper; Zhen-Yu Du designed study, analyzed data and revised paper. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors have no competing interests.

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References

Adams, T.L., Latham, G.J., Eisses, M.J., Bender, M.A., Haberkern, C.M., 2019. In: Coté, C. J., Lerman, J., Anderson, B.J. (Eds.), Essentials of hematology. a practice of anesthesia for infants and children (sixth edition). Elsevier, Philadelphia, pp. 217–239.
Alkhafaji, F., Romano, N., Amin, S.M.N., Fadel, A., Ebrahimi, M., Karami, A., Arshad, A. B., 2017. Effects of feeding frequencies on the growth, plasma biochemistry, and liver glycogen of jade perch Scortum barcoo in a recirculating system. N Am J Aquac 79, 216–223.
Amaya, E., Nickell, D., 2015. In: Davis, D.A. (Ed.), Using Feed to Enhance the Color Quality of Fish and Crustaceans. Feed and Feeding Practices in Aquaculture. Woodhead Publishing, Oxford, pp. 269–298.
Bergot, F., 1979. Carbohydrate in rainbow trout diets: effects of the level and source of carbohydrate and the number of meals on growth and body composition. Aquaculture. 18, 157–167.
Blaslov, K., Naranđa, F.S., Kruljac, I., Renar, I.P., 2018. Treatment approach to type 2 diabetes: past, present and future. World J Diabetes 9, 209–219.
Buhler, D.R., Halver, J.E., 1961. Nutrition of salmonid fishes. IX. Carbohydrate requirements of Chinook salmon. J Nutr 74, 307–318.
Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Wittwer, C. T., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55, 611–622.
Chapman, I.M., Goble, E.A., Wittert, G.A., Morley, J.E., Horowitz, M., 1998. Effect of intravenous glucose and euglycemic insulin infusions on short-term appetite and food intake. Am J Phys Regul Integr Comp Phys 274, R596–R603.
Chen, L., Chen, X.-W., Huang, X., Song, B.-L., Wang, Y., Wang, Y., 2019. Regulation of glucose and lipid metabolism in health and disease. Sci China Life Sci 62, 1420–1458.
Corrêia, V., Goulart, F.R., Pianesso, D., Mombach, P.I., Adorian, T.J., de Menezes Lovatto, N., Radünz Neto, J., 2019. Carbohydrate molecule size affects the metabolic and digestive dynamics of jundiá (*Rhamdia quelen*). Aquac Res 50, 3251–3258.
Couto, A., Enes, P., Peres, H., Oliva-Teles, A., 2008. Effect of water temperature and dietary starch on growth and metabolic utilization of diets in gilthead sea bream (*Sparus aurata*) juveniles. Comp Biochem Physiol Part A: Mol Integr Physiol 151, 45–50.
Cui, X.-J., Zhou, Q.-C., Liang, H.-O., Yang, J., Zhao, L.-M., 2010. Effects of dietary carbohydrate sources on the growth performance and hepatic carbohydrate metabolic enzyme activities of juvenile cobia (*Rachycentron canadum Linnaeus*). Aquac Res 42, 99–107.
Du, Z., 2014. Causes of fatty liver in farmed fish: a review and new perspectives. J Fish China 38, 1628–1638.
Du, Z.Y., Clouet, P., Zheng, W.H., Degrace, P., Liu, Y.J., 2006. Biochemical hepatic alterations and body lipid composition in the herbivorous grass carp (*Ctenopharyngodon idella*) fed high-fat diets. Br J Nutr 95, 905–915.
Enes, P., Panserat, S., Kaushik, S., Oliva-Teles, A., 2008. Hepatic glucokinase and glucose-6-phosphatase responses to dietary glucose and starch in gilthead sea bream (*Sparus aurata*) juveniles reared at two temperatures. Comp Biochem Physiol Part A: Mol Integr Physiol 149, 80–86.
Enes, P., Peres, H., Couto, A., Oliva-Teles, A., 2010. Growth performance and metabolic utilization of diets including starch, dextrin, maltose or glucose as carbohydrate source by gilthead sea bream (*Sparus aurata*) juveniles. Fish Physiol Biochem 36, 903–910.
Erfanullah, Jafri, A.K., 1995. Protein-sparing effect of dietary carbohydrate in diets for fingerling *Labeo rohita*. Aquaculture. 136, 331–339.
Furuichi, M., Yone, Y., 1982. Availability of carbohydrate in nutrition of carp and red sea bream. Nippon Suisan Gakkaishi 48, 945–948.
Gielkens, H., Verkijk, M., Lam, W.F., Lamers, C., Masclee, A., 1998. Effects of hyperglycemia and hyperinsulinemia on satiety in humans. Metabolism. 47, 321–324.
Goessling, W., Sadler, K., 2015. Zebrafish: an important tool for liver disease research. Gastroenterology. 149, 1361–1377.
Goncalves, M., Changyuan, L., Jordan, T., Hartman, T.E., Seo-Kyoung, H., Murphy, C.J., Kaitlyn, B., 2020. High-fructose corn syrup enhances intestinal tumor growth in mice. Science. 363, 1345–1349.
Hemer, G.I., Mommsen, T.P., Krogdahl, A., 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. Aquac Nutr 8, 175–194.
Hodge, A.M., Bassett, J.K., Milne, R.L., English, D.R., Giles, G.G., 2018. Consumption of sugar-sweetened and artificially sweetened soft drinks and risk of obesity-related cancers. Public Health Nutr 146, 3329–3334.
Honorato, C.A., Almeida, L.C., Da Silva Nunes, C., Carneiro, D.J., Moraes, G., 2010. Effects of processing on physical characteristics of diets with distinct levels of carbohydrates and lipids: the outcomes on the growth of pacu (*Piaractus mesopotamicus*). Aquac Nutr 16, 91–99.
Hutchins, C.G., Rawles, S.D., Gatlin, D.M., 1998. Effects of dietary carbohydrate kind and level on growth, body composition and glycemic response of juvenile sunshine bass (*Morone chrysops* ♀×*M. saxatilis* ♂). Aquaculture. 161, 187–199.
Kamalam, B.S., Medale, F., Panserat, S., 2017. Utilisation of dietary carbohydrates in farmed fishes: new insights on influencing factors, biological limitations and future strategies. Aquaculture. 467, 3–27.
Kim, W.R., Flamm, S.L., Di Bisceglie, A.M., Bodenheimer, H.C., 2008. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology. 47, 1363–1370.
Krogdahl, A., Hemre, G.-I., Mommsen, T.P., 2005. Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. Aquac Nutr 11, 103–122.

Lee, S.-M., Kim, K.-D., Lall, S.P., 2003. Utilization of glucose, maltose, dextrin and cellulose by juvenile flounder (*Paralichthys olivaceus*). Aquaculture. 221, 427–438.
Limbu, S.M., Zhang, H., Luo, Y., Chen, L.-Q., Zhang, M., Du, Z.-Y., 2020. High carbohydrate diet partially protects Nile tilapia (*Oreochromis niloticus*) from oxytetracycline-induced side effects. Environ Pollut 256, 113508.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 25, 402–408.
Luo, Y.X., Xiaojun., 2010. Progress of carbohydrate utilization in fish. J Fish Sci China 17, 381–390.
Luo, Y., Xie, X., 2010. Effects of high carbohydrate and high lipid diets on growth, body composition and glucose metabolism in southern catfish at two temperatures. Aquac Res 41, e431–e437.
Luo, Y., Hu, C.-T., Qiao, F., Wang, X.-D., Qin, J.G., Du, Z.-Y., Chen, L.-Q., 2020. Gemfibrozil improves lipid metabolism in Nile tilapia *Oreochromis niloticus* fed a high-carbohydrate diet through peroxisome proliferator activated receptor-α activation. Gen Comp Endocrinol 296, 113537.
Moon, T.W., 2001. Glucose intolerance in teleost fish: fact or fiction? Comp Biochem Physiol Part B: Biochem Mol Biol 129, 243–249.
Niaz, K., Khan, F., Shah, M.A., 2020. Chapter 18 - analysis of carbohydrates (monosaccharides, polysaccharides). In: Silva, A. Sanches, Nabavi, S.F., Saeedi, M., Nabavi, S.M. (Eds.), Recent Advances in Natural Products Analysis. Elsevier, pp. 621–633.
Perseghin, G., 2009. Viewpoints on the way to a consensus session: where does insulin resistance start? The liver. Diabetes Care 32, S164–S167.
Prisingkorn, W., Prathomya, P., Jakovlić, I., Liu, H., Zhao, Y.-H., Wang, W.-M., 2017. Transcriptomics, metabolomics and histology indicate that high-carbohydrate diet negatively affects the liver health of blunt snout bream (*Megalobrama amblycephala*). BMC Genomics 18, 1–15.
Qiang, J., Yang, H., He, J., Wang, H., Zhu, Z.X., Xu, P., 2014. Comparative study of the effects of two high-carbohydrate diets on growth and hepatic carbohydrate metabolic enzyme responses in juvenile GIFT tilapia (*Oreochromis niloticus*). Turk J Fish Aquat Sci 14, 515–525.
Qiang, J., Yang, H., Ma, X.Y., He, J., Wang, H., Kpundeh, M.D., Xu, P., 2016. Comparative studies on endocrine status and gene expression of hepatic carbohydrate metabolic enzymes in juvenile GIFT tilapia (*Oreochromis niloticus*) fed high-carbohydrate diets. Aquac Res 47, 758–768.
Quiroga, A.D., Lehner, R., 2018. Pharmacological intervention of liver triacylglycerol lipolysis: the good, the bad and the ugly. Biochem Pharmacol 155, 233–241.
Ren, M., Habte-Tsion, H.-M., Xie, J., Liu, B., Zhou, Q., Ge, X., Chen, R., 2015. Effects of dietary carbohydrate source on growth performance, diet digestibility and liver glucose enzyme activity in blunt snout bream, *Megalobrama amblycephala*. Aquaculture. 438, 75–81.
Riganti, C., Gazzano, E., Polimeni, M., Aldieri, E., Ghigo, D., 2012. The pentose phosphate pathway: an antioxidant defense and a crossroad in tumor cell fate. Free Radic Biol Med 53, 421–436.
Saini, V., 2010. Molecular mechanisms of insulin resistance in type 2 diabetes mellitus. World J Diabetes 1, 68–75.
Saxena, P.N., Rathor, S., Mahour, K., Saxena, N., Bajaj, P., 2010. Alterations in hepatosomatic index and behaviour under stress of arsenic trioxide and their modulation by Curcuma aromatica plant extract in albino rats (Berkenhout). Pharmacologyonline. 1, 243–251.
Stone, D.A.J., 2003. Dietary carbohydrate utilization by fish. Rev Fish Sci 11, 337–369.
Tag, H.M., 2015. Hepatoprotective effect of mulberry (*Morus nigra*) leaves extract against methotrexate induced hepatotoxicity in male albino rat. BMC Complement Altern Med 15, 252.
Tan, Q., Xie, S., Zhu, X., Lei, W., Yang, Y., 2006. Effect of dietary carbohydrate sources on growth performance and utilization for gibel carp (*Carassius auratus gibelio*) and Chinese longsnout catfish (*Leiocassis longirostris Günther*). Aquac Nutr 12, 61–70.
Tian, L.X., Liu, Y.J., Liu, D.H., Liang, G.Y., 2000. Effects of glucose and corn starch on growth and the fat deposition in the mesentery of grass carp. J Fish China 24, 438–441.
Tian, L.X., Liu, Y.J., Hung, S., Deng, D.F., Yang, H.J., Niu, J., Liang, G.Y., 2010. Effect of feeding strategy and carbohydrate source on carbohydrate utilization by grass carp (*Ctenopharyngodon idella*). Am J Agric Biol Sci 5, 135–142.
Tozzi, M.G., Camici, M., Mascia, L., Sgarrella, F., Ipata, P.L., 2006. Pentose phosphates in nucleoside interconversion and catabolism. FEBS J 273, 1089–1101.
Traxinger, R.R., Marshall, S., 1992. Insulin regulation of pyruvate kinase activity in isolated adipocytes. Crucial role of glucose and the hexosamine biosynthesis pathway in the expression of insulin action. J Biol Chem 267, 9718–9723.
Wang, Y., Liu, Y.-J., Tian, L.-X., Du, Z.-Y., Wang, J.-T., Wang, S., Xiao, W.P., 2005. Effects of dietary carbohydrate level on growth and body composition of juvenile tilapia, *Oreochromis niloticus*×*O. aureus*. Aquac Res 36, 1408–1413.
Wang, B., Smyl, C., Chen, C.-Y., Li, X.-Y., Huang, W., Zhang, H.-M., Kang, J.X., 2018. Suppression of postprandial blood glucose fluctuations by a low-carbohydrate, high-protein, and high-Omega-3 diet via inhibition of gluconeogenesis. Int J Mol Sci 19, 1823.
Wilson, R.P., 1994. Utilization of dietary carbohydrate by fish - ScienceDirect. Aquaculture. 124, 67–80.
Wilson, R.P., Poe, W.E., 1987. Apparent inability of channel catfish to utilize dietary mono- and disaccharides as energy sources. J Nutr 117, 280–285.
Wu, C., Chen, L., Lu, Z., Gao, J.E., Chu, Y., Li, L., Ye, J., 2017. The effects of dietary leucine on the growth performances, body composition, metabolic abilities and innate immune responses in black carp *Mylopharyngodon piceus*. Fish Shellfish Immunol 67, 419–428.
Xu, C., Liu, W.-B., Remø, S.C., Wang, B.-K., Shi, H.-J., Zhang, L., Li, X.-F., 2019. Feeding restriction alleviates high carbohydrate diet-induced oxidative stress and inflammation of *Megalobrama amblycephala* by activating the AMPK-SIRT1 pathway. Fish Shellfish Immunol 92, 637–648.
Xu, W., Zhang, Q., Chen, Y., Zhang, Y., Zhou, C., 2020. Effects of dietary carbohydrate types on growth performance, innate immunity, antioxidant ability and glucose metabolism of brook trout *Salvelinus fontinalis*. Aquac Res 51, 4638–4648.
Yi, L.I., Zhou, P., Qiu, H., Hou, Y., Jikang, S., Zhou, Q., 2015. Effects of dietary carbohydrate sources on growth performance,digestive enzyme and carbohydrate metabolic key enzyme activities of large yellow croaker (*Larmichthys crocea Richardson*). Chin J Anim Nutr 27, 3438–3447.
Zhang, H., Luo, Y., Lu, D.-L., Jiao, J.-G., Li, L.-Y., Qin, J.-G., Chen, L.-Q., 2019. Diacylglycerol oil reduces fat accumulation and increases protein content by inducing lipid catabolism and protein metabolism in Nile tilapia (Oreochromis niloticus). Aquaculture. 510, 90–99.
Zhu, H.-J., Qiang, J., Tao, Y.-F., Ngoepe, T.K., Bao, J.-W., Chen, D.-J., Xu, P., 2020. Physiological and gut microbiome changes associated with low dietary protein level in genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*) determined by 16S rRNA sequence analysis. MicrobiologyOpen. 9, e1000.