



Comparative Study of the Effects of Two High-Carbohydrate Diets on Growth and Hepatic Carbohydrate Metabolic Enzyme Responses in juvenile GIFT tilapia (*Oreochromis niloticus*)

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Abstract

This study aimed to compare the effect of two high-carbohydrates diets on growth, activity and gene expression of hepatic metabolic enzymes in GIFT tilapia juveniles (*Oreochromis niloticus*). Two isonitrogenous (28% crude protein) and isolipidic (5% crude lipids) diets were formulated to contain 40% of wheat starch (WS) or glucose (GLU). Diets were assigned to triplicate groups of 30 fish (initial weight: 11.26g) for 45 days. At the end of trial, SGR, feed intake, feed efficiency, HSI, VI and liver glycogen levels were not significantly influenced by dietary carbohydrates. Plasma glucose was higher in fish fed the GLU diet than in the WS diet. No significant effect of dietary carbohydrates source on GK and G6Pase activities were present, however, these activities were significant higher than the initial levels. In contrast, higher G6PD activities were recorded in GLU group than in the WS group on day 45. Data on gene expression showed that G6Pase and G6PD but not GK was affected by dietary carbohydrates on day 45; the levels of G6Pase and G6PD mRNA in GLU group were significantly higher than in WS group. Overall, dietary high glucose levels was utilized well as energy source by GIFT tilapia juveniles, and seem to be more effective in increasing liver lipogenic activities.

Keywords: *Oreochromis niloticus*; carbohydrate metabolism; enzyme activity; gene expression; growth

Introduction

Tilapia is an important freshwater fish species that is farmed extensively in southern China. When the level of dietary proteins is greater than 40%, the growth of tilapia juveniles is promoted (Al Hafedh, 1999). However, the high protein requirement of fish makes dietary costs higher and contributes to potential high nitrogenous wastes into the environment (Oliva-Teles, 2000; Enes *et al.*, 2008). The use of non-protein digestible energy source such as carbohydrates is a way of minimizing the above mentioned problems. Carbohydrates are a cheap source of energy compared to lipids and proteins in the preparation of a commercial diet (Tran-Duy *et al.*, 2008). Adequate increasing levels of dietary carbohydrates could raise protein-sparing effect in many fish species. Generally, herbivorous or omnivorous fish have a higher ability to utilize dietary carbohydrate than carnivorous fish (Hidalgo *et al.*, 1999), possibly due to higher amylase activity and affinity of insulin receptors in herbivorous or omnivorous fish (Banos *et al.*, 1998). Tilapias are warm-water omnivorous fish, which better utilize digestible carbohydrates as high as 40% in their diets

(Wang *et al.*, 2005).

Fish regulate decomposition and absorption of dietary carbohydrate through glucose metabolism enzymes in liver (Hemre *et al.*, 2002; Enes *et al.*, 2009). Therefore, the study of hepatic carbohydrate metabolic enzymes is an essential step towards understanding the mechanism of carbohydrate utilization and increasing our knowledge of nutritional regulation. Hepatic glucokinase (GK) and glucose-6-phosphatase (G6Pase) play a major role in the regulation of glycolytic and gluconeogenic pathways, respectively (van de Werve *et al.*, 2000). Recent studies have reported an increase in hepatic GK activity as dietary carbohydrate level increases as observed in several fish species such as European sea bass (*Dicentrarchus labrax*) (Moreira *et al.*, 2008), gilthead sea bream (*Sparus aurata*) (Couto *et al.*, 2008), Topmouth culter (*Culter alburnus*) (Liu *et al.*, 2008), rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*) (Panserat *et al.*, 2000a) and southern catfish (*Silurus meridionalis*) (Lin *et al.*, 2006).

Basically, hepatic gluconeogenesis could be inhibited when dietary carbohydrate level is high. However, effects of dietary carbohydrate on

regulation of G6Pase in fish have some ambiguity. Furuichi and Yone (1982) reported that different dietary carbohydrate source (starch, glucose and dextrin) had obvious inhibitive effects on G6Pase activity of common carp (*Cyprinus carpio*). Panserat *et al.* (2002) reported that lower activities and gene expression of hepatic G6Pase in gilthead sea bream fed a diet with 20% digestible carbohydrates comparatively to fish fed a carbohydrate-free diet. Whereas Enes *et al.* (2008a) found, nonsignificant changes in G6Pase activities between fish fed glucose diet and control group. Panserat *et al.* (2000b; 2001) also reported no difference in activities and gene expression of hepatic G6Pase in rainbow trout (*Oncorhynchus mykiss*) fed different dietary carbohydrate levels. Glucose-6-phosphate dehydrogenase (G6PD) is a key carbohydrate metabolic enzyme of the lipogenesis pathway (Towle *et al.*, 1997). Increased dietary starch levels could increase hepatic G6PD activities in European sea bass juveniles at 18 °C, however, the G6PD activities had an opposite change trend at 25 °C (Moreira *et al.*, 2008). Hepatic G6PD activity in gilthead sea head was higher in fish fed glucose diet than that fed the waxy maize starch (Enes *et al.*, 2008b; 2010).

To our knowledge, however, there is little information on the effect of high-carbohydrate diets on activities and gene expression of hepatic GK, G6Pase and G6PD of tilapia. G6Pase activities in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) were not affected by the different dietary carbohydrate source; however, G6PD activities were higher in the fish fed the starch diet than in those fed the glucose diet (Lin and Shiau, 1995). The previous studies took hybrid tilapia as main object. However, currently in China, the increased culture of GIFT (Genetically Improved Farmed Tilapia) strain Nile tilapia is mainly due to its many advantages such as rapid growth rate, high fillet yield, and good disease resistance capability (Qiang *et al.*, 2012). This study takes GIFT tilapia as subject that has great research value and practical significance. The purpose of this study is to elucidate the possible adaptation of hepatic enzymes in juvenile GIFT tilapia, fed two carbohydrate sources, wheat starch and glucose for several weeks. We collected samples on day 0, 27 and 45, and examined the change in regularity of the hepatic GK, G6Pase and G6PD activities. Meanwhile, GK, G6Pase and G6PD genes have been cloned in tilapia, we want to investigate whether gene expression can be modulated by these nutritional statuses.

Materials and Methods

Source of experimental fish

Healthy Nile tilapia juveniles of the 16th generation of the GIFT strain were obtained from Yixing, one of the bases of Freshwater Fisheries Research Center of the Chinese Academy of Fishery

Sciences. Prior to the experiment, the fish were acclimatized for 10 days in indoor concrete tanks at a water temperature 28 ± 0.3 °C, under natural photoperiod and with continuous aeration. During acclimatization, the fish were conditioned to accept submerged food (32% crude protein, 8% crude lipid and 16% wheat starch).

Experimental Diets

Two isonitrogenous (28 % crude protein) and isolipidic (5.0% crude lipids) diets were formulated to contain 40% of either wheat starch (WS diet) or glucose (GLU diet). All dietary ingredients were finely ground, thoroughly mixed and dry-pelleted in a fish-meal laboratory mill using 3-mm diameter dice. Ingredients and proximate composition of the experimental diets are presented in Table 1.

Experimental Procedure

The trial was performed in water recirculation systems, thermoregulated to 28 ± 0.3 °C. A total of 12 plastic tanks (800L each) were used in the experiment, 700L volume of tap water that had been aerated for consecutive 3 days were added into each of the 12 plastic tanks. After 1 weeks of adaptation to the experimental conditions, groups of 30 fish with an initial mean body weight of 11.26g (± 0.05 g) were randomly distributed to each tank. Results of MANOVA showed that there was no significant difference between various experimental groups and between all replicates ($P > 0.05$). Continuous aeration was ensured during the course of entire experiment. Feces at the bottom of plastic tanks were siphoned off on a daily basis. In all tanks, water was constantly replaced by continuous flow at the rate of 2 L min^{-1} to provide oxygen and remove excess nitrogenous wastes. In addition, 1/3 of the water volume was replaced every 3 days, with a temperature difference of less than 0.3 °C. During the experiment water temperature and pH were monitored daily, and dissolved oxygen, ammonia-N and nitrite were measured weekly. Dissolved oxygen never fell below 6 mg L^{-1} , and pH ranged from 7.4 to 7.8. Ammonia-N and nitrite were both maintained at concentrations lower than 0.01 mg L^{-1} , and photoperiod was controlled at a 12-h light/dark cycle.

Experiment One: Effect of Two High Carbohydrate-Enriched Diets on Growth in Juveniles

6 plastic tanks were used in experiment one. Each diet was assigned to triplicate groups of animal. Fish were offered experimental diets presented in Table 1. Fish in all tanks were fed by hand three times daily (7:00 h, 11:30 h and 16:00 h). The fish were fed 6% of their body weight per day. The feeding amount was increased every other week, and the amount was

Table 1. Composition and proximate analyses of the experimental diets

Diets	WS	GLU
Ingredients		
Fish meal	25.00	25.00
Wheat starch	40.00	-
D-Glucose	-	40.00
Fish oil + soybean oil (1:1)	3.00	3.00
Soybean meal	27.80	27.80
Vitamin premix ¹	1.00	1.00
Mineral premix ²	1.00	1.00
Choline chloride	0.50	0.50
Vitamin C phosphate ester	0.20	0.20
Ca(H ₂ PO ₄) ₂	1.50	1.50
Proximate composition (%)		
Dry matter	90.87	90.56
Crude protein	28.23	28.14
Crude lipid	5.11	5.14
Ash	6.21	6.29
Gross energy (kJ/g diet)	20.12	20.79

Note :1) Vitamin premix (mg/kg dry diet) :V_A 10, V_D 0.05, V_E 400, V_K 40, V_{B1} 50, V_{B2} 200, V_{B3} 500, V_{B6} 50, V_{B7} 5, V_{B11} 15, V_{B12} 0.11, V_C 1000, inositol 2000, choline 5000 ; 2) Mineral premix (mg/kg dry diet): FeSO₄·7H₂O 372, CuSO₄·5H₂O 25, ZnSO₄·7H₂O 120, MnSO₄·H₂O 5, MgSO₄ 2475, NaCl 1875, KH₂PO₄ 1000, Ca (H₂ PO₄)₂ 2500.

adjusted according to body weight measurement every 2 weeks. The experiment was conducted for 45 days. The fish were fasted for 24 h prior to sampling. Then, fish were bulk weighed and 5 fish from each tank were sampled for determination of hepatosomatic and visceral indices.

Experiment Two: Effect of Two High Carbohydrate-Enriched Diets on Activities of Hepatic Metabolic Enzymes and Gene Expression

Another 6 plastic tanks were used in experiment two. Feeding and rearing management were same as experiment one. Three fish samples from each tank were collected 6 h after the morning meal on days 0, 27 and 45 during the experiment. The livers samples of 3 fish from each tank were removed, frozen and stored at -80 °C until analysis of enzymatic activities and RNA. To avoid changes of measurement index induced by stress, fish samples were killed with an overdose of tricaine methanesulfonate (2%; MS-222) within 1 min after being captured. One part of the liver sample was used for the determination of enzymes and liver glycogen. The rest was later used for measurement of GK, G6Pase and G6PD gene expression. In addition, the bloods from another 3 fish per tank were sampled on days 0, 27 and 45 during the experiment. Blood was collected from the caudal vein with a heparinised syringe, immediately centrifuged and the plasma frozen at -20 °C until analysis.

Analytical Methods

Proximate Analysis

Diets were analyzed for proximate composition

according to the procedures of Association of Official Analytical Chemists (AOAC, 2000). Moisture was analyzed by drying at 105 °C for 24 h. Crude protein (n*6.25) was analyzed by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Auto-analyzer, Tecator, Höganäs, Sweden). Crude lipid was determined by the ether extraction method using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 h. Gross energy was obtained by means of an adiabatic bomb calorimeter (model WHR-15; Changsha, China, calibrated with benzoic acid).

Hepatic GK, G6Pase and G6PD Activity

The activities of glucokinase (GK, EC 2.7.1.2), glucose-6-phosphatase (G6Pase, EC 3.1.3.9) and glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) were analyzed by the method of Tranulis *et al.* (1996), Panserat *et al.* (2001) and Bautista *et al.* (1988) respectively. Hepatic glycogen contents were determined as described by Plummer (1987). The test kits used were bought from ShangHai Lengton Bioscience Co., LTD (ShangHai, China). The levels of plasma glucose were measured in the automatic biochemical analyzer Roche Cobas C311 (Roche Cobas, Swiss) using kits purchased from ShangHai Lengton Bioscience Co., LTD (ShangHai, China).

Real-Time Quantitative PCR

Gene expression levels were determined by real-time quantitative RT-PCR. Primers for RT-PCR were designed with reference to the known sequences of tilapia as follows Table 1. All primers were

synthesized by ShangHai Genecore, BioScience & Technology Company (ShangHai, China). The PCR products were 100~110 bp long.

We extracted total RNA from the liver using Trizol reagent (Dalian Takara Co. Ltd., China). RNA samples were treated with DEPC water (Dalian Takara Co. Limited, China). We generated cDNA from 350 ng RNA using PrimeScript RT reagent Kit Perfect Real Time kit (Dalian Takara Co. Ltd., China). PCR amplification was conducted using an ABI 7900HT Fast Real-Time PCR System (ABI, USA) and SYBR Green PCR Master Mix (ABI), according to the manufacturer's instructions. RT-PCR was performed as follows: denaturing at 95 °C for 5 min; 40 cycles of denaturing at 95 °C for 15s, annealing at 60 °C for 60s. We calculated the relative quantification of the target gene transcript GK, G6Pase and G6PD with a chosen reference gene transcript (β -actin) using the $2^{-\Delta\Delta C_T}$ method. This mathematical algorithm computes an expression ratio based on real-time PCR efficiency and the crossing point deviation of the sample versus a control. We measured the PCR efficiency by constructing a standard curve using a serial dilution of cDNA. A no template control (NTC) and no reverse transcriptase control (NRT) were used to control for template and genomic contamination, respectively. Values of GK, G6Pase and G6PD mRNA were then expressed relative to fish fed with WS diet on day 0 (assigned an arbitrary value of 1) (Table 2).

Statistical Analysis

Significant differences among means were determined by Tukey's multiple comparison test or paired-samples *t*-tests. All statistical analyses were computed using SPSS 17.0. Values are mean \pm standard error.

Results

Growth of Gift Tilapia

Final body weight were slightly higher in fish fed the WS diet, but no significant differences in specific growth rate, feed intake, feed efficiency and protein efficiency ratio were observed between groups at the end of the trial (Table 3).

Hepatosomatic and visceral indexes, contents of hepatic glycogen and plasma glucose

HSI and VI were not influenced by dietary carbohydrate sources (Tab 3). Plasma glucose levels were however higher in fish fed the GLU diet than the WS diet on days 27 and 45 (Figure 1); the levels of two groups on day 45 were significantly lower than those on day 27 ($P < 0.05$). The contents of liver glycogen first increased with increased rearing time and then decreased for 45 days (Figure 2). The contents of liver glycogen were higher in fish fed the GLU diet than the WS diet, and were not significantly

Table 2. Primer sequences

Target mRNA	Sequence	NCBI Genbank Accession No
GK	F: 5'- GCAGCGAGGAAGCCATGAAGA -3' R: 5'- GAGGTCCCTGACGAC TTTGTGG -3'	XM_003451020
G6Pase	F: 5'-AGCGCGAGCCTGAAGAAGTACT-3' R: 5'-ATGGTCCACAGCAGGTCCACAT-3'	XM_003448671
G6PD	F: 5'-ACAGGAACTGTCAGCCACCTT-3' R: 5'-AGCACCATGAGGTCTGGACCA-3'	XM_003448158
β -actin	F: 5'-CCACACAGTGCCCATCTACGA-3' R: 5'-CCACGCTCTGTCAGGATCTTCA-3'	EU887951.1

Table 3. Growth, hepatosomatic and visceral indexes and plasma glucose of juvenile GIFT tilapia fed two high -carbohydrate enriched diets for 45 days

Diets	WS	GLU
Initial body weight (IBW)(g)	11.28 \pm 0.04	11.22 \pm 0.06
Final body weight (FBW)(g)	57.12 \pm 1.52	55.20 \pm 1.43
Specific growth rate (%/d) ¹	3.61 \pm 0.07	3.54 \pm 0.04
Feed intake (g/fish)	73.11 \pm 1.64	72.48 \pm 1.92
Feed efficiency ²	0.69 \pm 0.05	0.67 \pm 0.04
Protein efficiency ratio ³	2.22 \pm 0.14	2.16 \pm 0.13
HSI ⁴	2.62 \pm 0.70	2.63 \pm 0.58
VI ⁵	10.00 \pm 1.44	10.12 \pm 1.17

Note: Means in the same column which have different letters on the top right have significant difference ($P < 0.05$)

¹ Specific growth rate: $((\ln(\text{FBW}) - \ln(\text{IBW})) / (\text{time in days})) \times 100$.

² Feed efficiency: wet weight gain/dry feed intake.

³ Protein efficiency ratio: wet weight gain/crude protein intake.

⁴ HSI Hepatosomatic index: (liver weight/body weight) \times 100.

⁵ VI Visceral index: (visceral weight/body weight) \times 100.

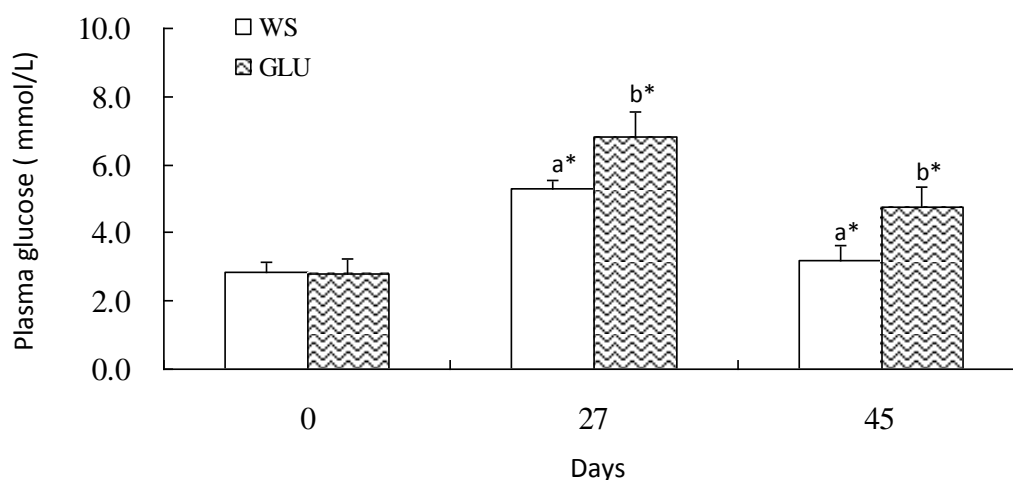


Figure 1. Plasma glucose level of juvenile GIFT tilapia fed two high-carbohydrate diets during 45 days (n=9).

Note: Values are mean \pm standard error. Diverse little letters above histogram bars show significant differences ($P<0.05$) between fish fed two high-carbohydrate diets (paired t-test). Asterisks indicate significant differences ($P<0.05$) from the initial value on Day 0 (Tukey's multiple comparison test). The same as below.

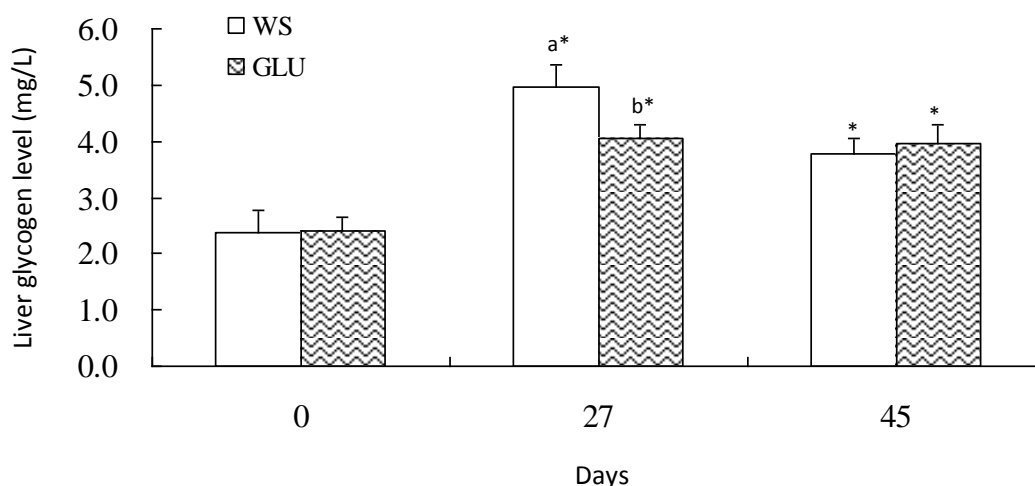


Figure 2. Liver glycogen level of juvenile GIFT tilapia fed two high-carbohydrate diets during 45 days (n=9).

influenced by dietary carbohydrate sources on day 45 ($P>0.05$).

Activities of Hepatic GK, G6Pase and G6PD

Comparing to day 0, hepatic GK activities of GLU and WS groups showed significant higher on day 45 (Figure 3), whereas GK activities were not influenced between two groups on day 45. The GK activities were significantly higher in two groups on day 27 than on days 0 and 45 ($P<0.05$). Hepatic G6Pase activity in two groups tended to increase for 45 days (Figure 4), and G6Pase activity of WS group showed distinctly higher levels on days 27 and 45 than the initial level ($P<0.05$). G6Pase activity was slightly higher in fish fed the GLU diet than those fed the WS diet but it was not significantly affected by dietary carbohydrate sources ($P>0.05$) on day 45. In contrast, higher G6PD activities were recorded in fish

fed the GLU diet than in fish fed the WS diet on day 45 ($P<0.05$) (Figure 5). However, G6PD activities were not different between two groups on day 27, which were also higher than the initial levels.

Levels of hepatic GK, G6Pase and G6PD mRNA

Comparing to day 0, the hepatic GK mRNA levels of GLU and WS groups tended to increase first, reaching peak level on day 27; then two treated groups showed lower levels afterward (Figure 6). The GK mRNA level in fish fed the GLU diet was significantly higher than those fed the WS diet on day 27 ($P<0.05$). However, the GK mRNA levels were not different between two groups on day 45 ($P>0.05$). The levels of hepatic G6Pase mRNA in GLU group increased gradually for 45 days (Figure 7). The G6Pase mRNA levels were significantly higher in fish fed the WS diet on day 27 than those fed the GLU

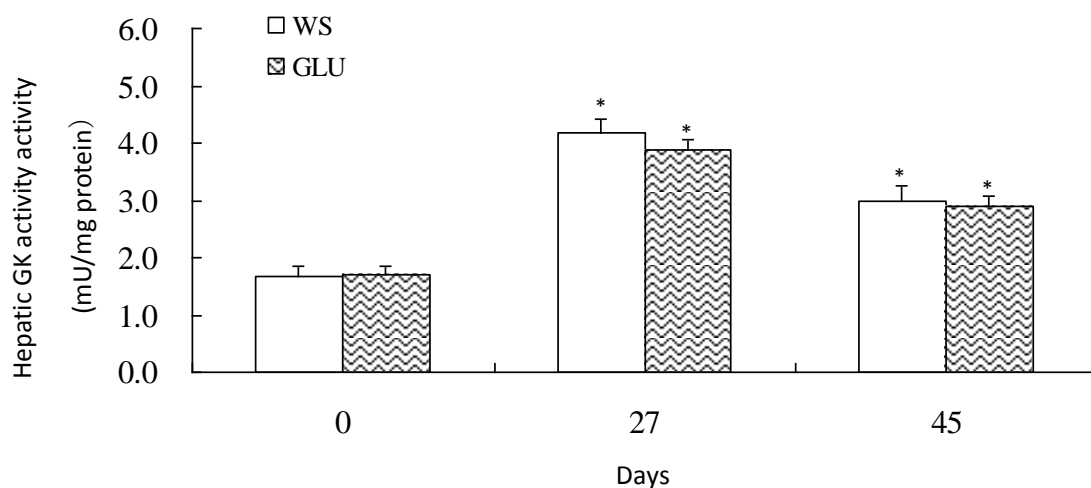


Figure 3. Hepatic GK activity of juvenile GIFT tilapia fed two high-carbohydrate diets during 45 days (n=9).

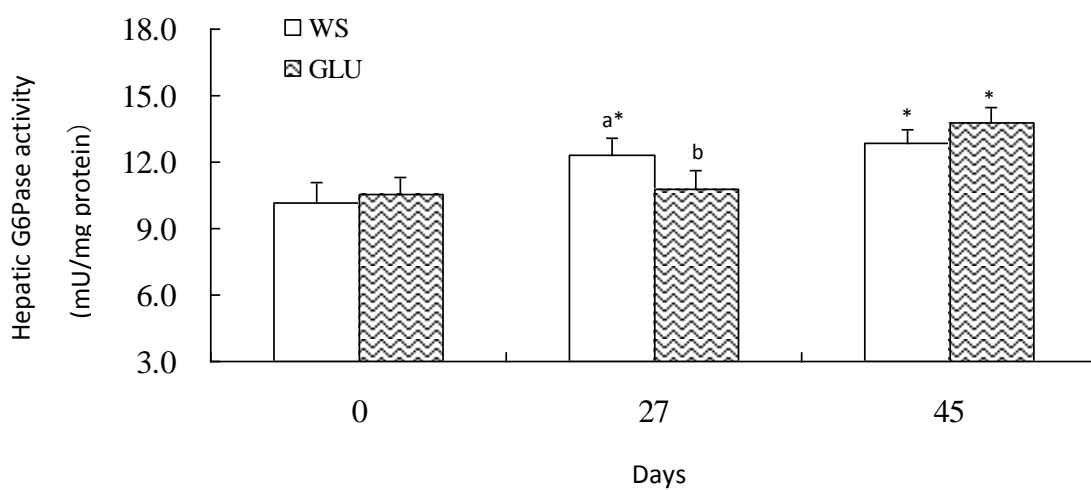


Figure 4. Hepatic G6Pase activity of juvenile GIFT tilapia fed two high-carbohydrate diets during 45 days (n=9).

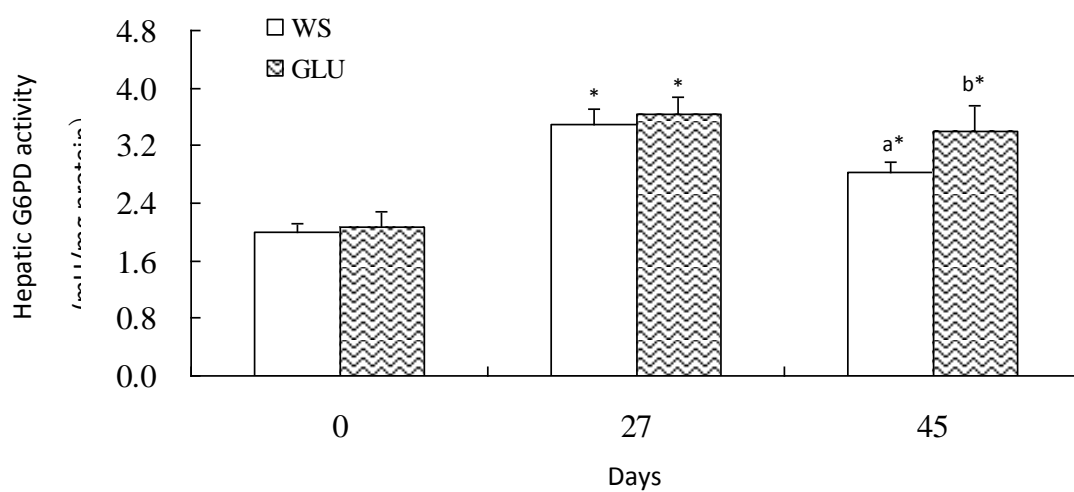


Figure 5. Hepatic G6PD activity of juvenile GIFT tilapia fed two high-carbohydrate diets during 45 days (n=9).

diet. The hepatic G6PD mRNA levels were significantly higher in fish fed the GLU diet on day

45 than those of WS fish ($P < 0.05$) (Figure 8). However, the levels on day 27 showed no significant

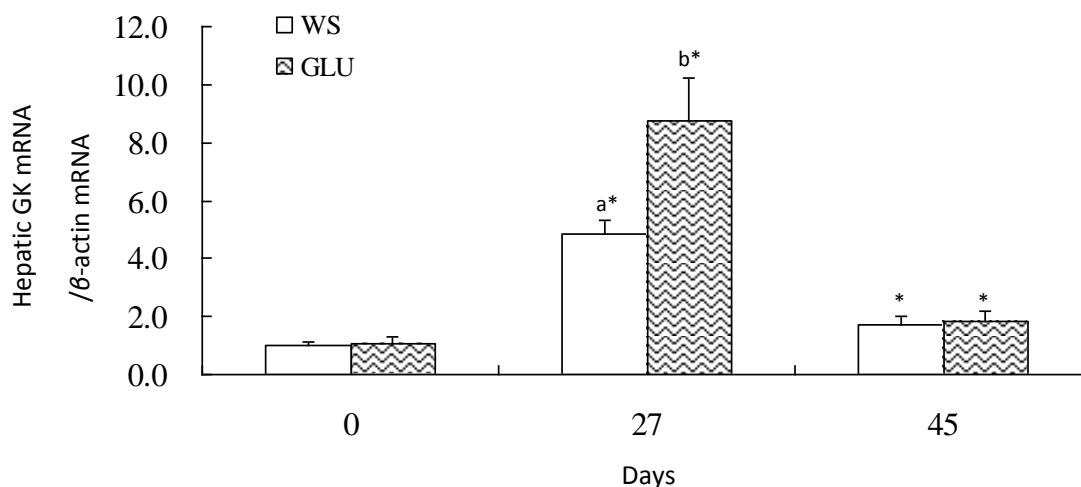


Figure 6. Hepatic GK mRNA level of juvenile GIFT tilapia fed two high-carbohydrate diets during 45 days (n=9)

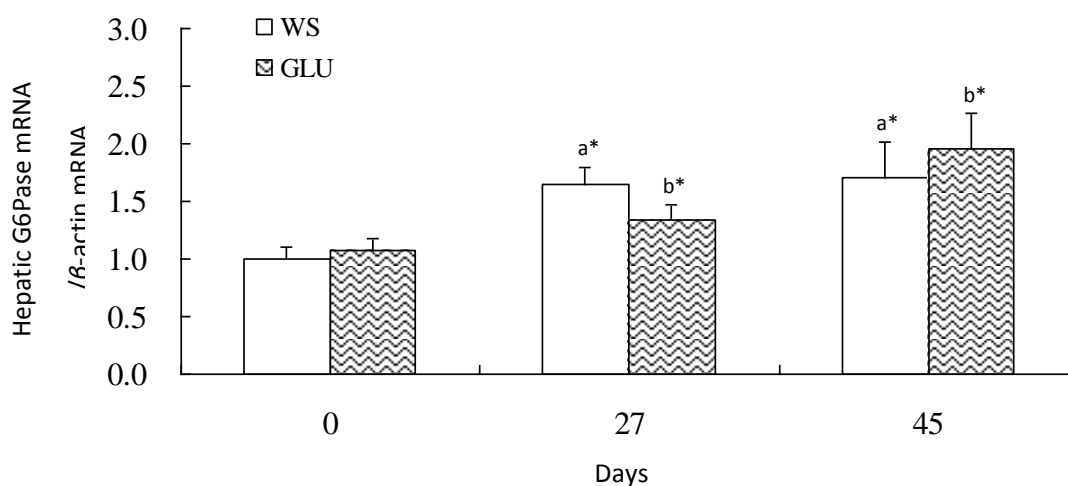


Figure 7. Hepatic G6Pase mRNA level of juvenile GIFT tilapia fed two high-carbohydrate diets during 45 days (n=9)

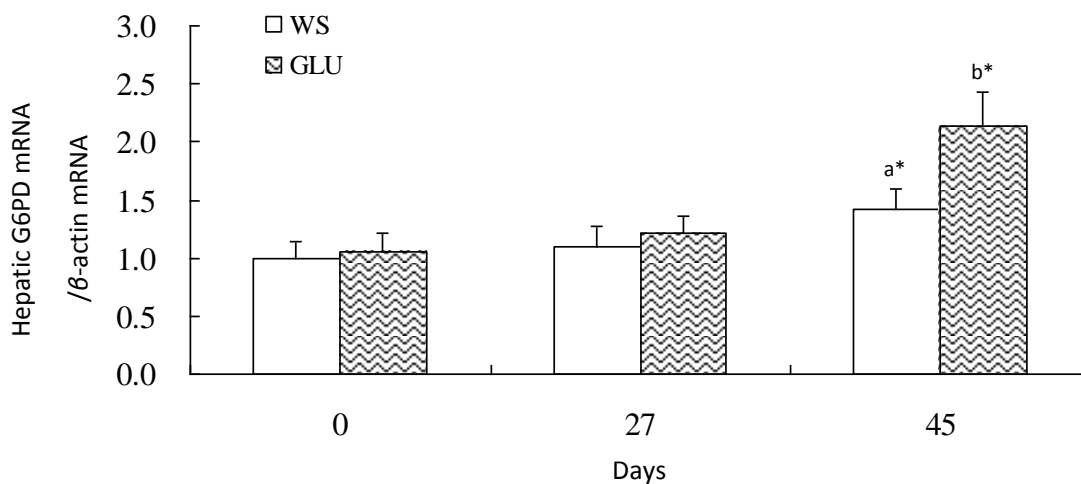


Figure 8. Hepatic G6PD mRNA level of juvenile GIFT tilapia fed two high-carbohydrate diets during 45 days (n=9)

difference between two groups ($P > 0.05$).

Discussion

The relatively poor growth performances of fish fed the glucose have been reported (Lin and Shiau, 1995; Fu, 2005; Cui *et al.*, 2010). The main reason for this could have been the faster absorption of monosaccharide (glucose) than complex carbohydrates (starch), and excess absorbed glucose may have been cleared from circulation before cells can utilize it (Lin *et al.*, 1997). On the other hand, some fish species studied have been reported to have used dietary glucose more efficiently than starch (Hung and Storebakken, 1994; Enes *et al.*, 2008b). Data on our present trial showed that different dietary carbohydrate sources (WS vs. GLU) in GIFT tilapia; final body weight was slightly higher with WS diet, however, there were not obvious differences in specific growth rate between groups. The main reason for this may be the better feed intake of the fish fed the GLU diet. As omnivorous fish fed with a high-glucose diet seem to have a good ability to take up excess glucose. This is different from the result of hybrid tilapia (Tung and Shiau, 1991; Hsieh and Shiau, 2000). The ability to utilize glucose or starch is affected by fish species, dietary formulation, feeding strategies, water temperature and fish size.

Plasma glucose levels were higher in GLU group than WS group at the end of the trial. This finding is consistent with data from other fish species (Enes *et al.* 2006) and can be attributed to the faster glucose absorption from simple sugars as opposed to the complex carbohydrates. In general, fish fed the glucose diet showed poor growth; less muscle accumulation, more fat storage and higher HSI values than those fed the other complex carbohydrate diets (Hutchins *et al.*, 1998; Small and Soares, 1999; Rawles *et al.*, 2008). In the present study, growth and HSI were not significantly affected by the dietary carbohydrate sources. This may be due to the better capacity of juvenile GIFT tilapia to utilize high levels of absorbed glucose. The content of liver glycogen indicates accumulation in the liver as both lipid and glycogen after being converted (Lanari *et al.*, 1999). Kim and Kaushik (1992) demonstrated that live size was directly related to hepatic glycogen level. Glycogen contents in the liver were not significantly affected by the dietary carbohydrate sources in our study. The main reason for this may be not growth inhibition of the fish fed the glucose diet.

GK is a key enzyme for glucose homeostasis in fish (Caseras *et al.*, 2000). The higher GK activities may be related to the higher glycaemia observed in fish fed the glucose diet, which may increase glucose uptake by the liver, as previously observed in rainbow trout (Caseras *et al.*, 2000), and gilthead sea bream (Enes *et al.*, 2008b). However, in our study, the effects of two high-carbohydrate diets in juvenile GIFT tilapia were not evident for GK activities at the end of

the trial. It is interesting to observe that GK expression (an enzymatic and molecular level) both the GLU and WS groups increased distinctly on day 27. Accordingly, hepatic glycogen content was also significantly higher than the initial level. Our results indicated a stimulatory effect on the GK expression after food intake in fish liver for a short time; however, GK expressions of the GLU and WS groups on day 45 were significantly lower than that on day 27. Thereafter, GIFT tilapias fed high-carbohydrate enriched diets possessed regulation capability of carbohydrate metabolic enzymes to some extent. GK expression was rapidly suppressed after glycemia went down to basal values (Fig 1), suggesting that GK expression is important to control glucose homeostasis in fish (Caseras *et al.*, 2000).

With regard to G6Pase activity, a lack of effect of dietary carbohydrate source on G6Pase activity was previously reported in hybrid tilapia (Lin and Shiau, 1995). Similar results were obtained in GIFT tilapia of our results on day 45. Some reports were also found in other fish species. Caseras *et al.* (2002) and Enes *et al.* (2008b), in gilthead sea bream (*Sparus aurata*) juvenile, reported that dietary carbohydrate sources resulted in a lack of regulation of G6Pase activity or gene expression. To our knowledge, this is the first study in GIFT tilapia fed high-carbohydrate diets reporting the effect of different sampling time on G6Pase expression. Data of the present trial showed that fish fed the WS diet has significant effect on G6Pase expression on day 27. Complex molecular carbohydrates structure such as wheat starch, unlike free glucose, are less easily absorbed in the gut lining but may promote the transcription of G6Pase gene. In addition, the fish fed higher carbohydrate level would increase routine metabolic rate (Fu and Xie, 2007). In the present study, we chose lower levels of protein and lipid in diet, and the energy of growth and metabolism in fish was provided by dietary carbohydrate. Thus, gluconeogenesis and hepatic glycogenolysis may be accelerated and G6Pase expression would be activated when sugars were insufficient in blood.

Dietary high carbohydrate level that is not used for energy may accumulate in the liver both as lipid and as fatty acid after being converted (Gumus and Ikiz, 2009). G6PD, a lipogenic related enzyme, is involved in catalyzing the NADPH production which is essential for reductive fatty acid biosynthesis. In our study, higher levels of G6PD were observed in fish fed the GLU diet than those fed the WS diet. This is similarly to what was observed in other fish species (Hung and Storebakken, 1994; Enes *et al.*, 2006). However, contrary to what was observed in hybrid tilapia (Lin and Shiau, 1995). Lin and Shiau (1995) reported that G6PD activity was higher in fish fed the starch diet than those fed the glucose diet. This may be due to three causes: i) we chose wheat starch as a source of dietary carbohydrate, and not corn starch, as in Lin and Shiau (1995), which therefore inform our

result. Degani and Dosoretz (1998) reported that accumulations of fatty acids were the highest in European eels (*Anguilla anguilla*) fed with wheat starch meals, compared with those fed with corn starch meal; in addition, the higher levels of dietary protein and lipid were found on Lin and Shiau (1995) study, and higher protein level may be promoting hepatic gluconeogenesis (Kirchner *et al.*, 2003); ii) in this experiment, the Nile tilapia juveniles used were of the 16th generation of GIFT strain, and furthermore five rounds of mass selection have been carried out on them in Yixing experimental Base, China. The utilization of dietary carbohydrate may have great difference among the tilapia strains; iii) the difference of fish size may affect adaptation of tilapia. It had been reported that carbohydrate utilization was affected by the size of tilapia (Tung and Shiau, 1993). The size of tilapia in this study was bigger (average 11.26 g BW) compared to those (average 0.5-4.5 g BW) tilapia in Lin and Shiau (1995) study.

In conclusion, our data suggest that in GIFT tilapia juveniles' dietary carbohydrate sources are more effective in modulating the response of G6PD than that of GK and G6Pase. For the first time in GIFT tilapia, it was shown that fish fed the GLU diet presented enhanced liver lipogenic capacities compared to fish fed the WS diet, but not hepatic glycolysis and gluconeogenesis.

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