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The characteristics of glucose homoeostasis in grass carp and Chinese longsnout catfish after oral starch administration: a comparative study between herbivorous and carnivorous species of fish

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Abstract

An oral starch administration trial was used to evaluate glucose homoeostasis in grass carp (*Ctenopharyngodon idella*) and Chinese longsnout catfish (*Leiocassis longirostris* Günther). Fish were administered with 3 g of a water and starch mixture (with 3:2 ratio) per 100 g body weight after fasting for 48 h. Fish were sampled at 0, 1, 3, 6, 12, 24 and 48 h after oral starch administration. In grass carp, plasma levels of glucose peaked at 3 h but returned to baseline at 6 h. However, in Chinese longsnout catfish, plasma glucose levels peaked at 6 h and returned to baseline at 48 h. The activity of intestinal amylase was increased in grass carp at 1 and 3 h, but no significant change in Chinese longsnout catfish was observed. The activity of hepatic glucose-6-phosphatase fell significantly in grass carp but change was not evident in Chinese longsnout catfish. The expression levels and enzymic activity of hepatic pyruvate kinase increased in grass carp, but no significant changes were observed in the Chinese longsnout catfish. Glycogen synthase (*gys*) and glycogen phosphorylase (*gp*) were induced in grass carp. However, there was no significant change in *gys* and a clear down-regulation of *gp* in Chinese longsnout catfish. In brief, compared with Chinese longsnout catfish, grass carp exhibited a rapid increase and faster clearance rate of plasma glucose. This effect was closely related to significantly enhanced levels of digestion, glycolysis, glycogen metabolism and glucose-induced lipogenesis in grass carp, as well as the inhibition of gluconeogenesis.

Key words: **Glucose homoeostasis: Amylase: Glycolysis: Gluconeogenesis: Lipogenesis**

It is usually considered that a herbivorous fish could utilise carbohydrate much better than a carnivorous fish. The suitable dietary carbohydrate level is about 45 % for rohu (*Labeo rohita*), a herbivorous fish[1], but is only 17 % for golden pompano (*Trachinotus ovatus*), a carnivorous fish[2]. Considering the high cost of feeds, it is necessary to improve carbohydrate utilisation in fish. However, much more still needs to be done about the complete characteristics and regulation of glucose homoeostasis in fish with different feeding habits.

Fish possess a range of enzymes for digesting and absorbing simple or complex forms of carbohydrates[3]. Previous research reported that omnivorous fish, such as common carp (*Cyprinus carpio*) and goldfish (*Carassius auratus*), exhibit higher levels of amylase activity than carnivorous fish such as rainbow trout (*Oncorhynchus mykiss*) and gilthead seabream (*Sparus aurata*)[4]. The transmembrane transportation of glucose requires the assistance of Na-dependent GLUT 1 (SGLT1) in the enterocyte membrane and facilitative GLUT

Abbreviations: acc, acetyl-CoA carboxylase; acca, acetyl-CoA carboxylase α; chrebp, carbohydrate-responsive element binding protein; fas, fatty acid synthase; FBP, fructose 1,6-bisphosphatase; G6Pase, glucose-6-phosphatase; g6pc, glucose-6-phosphatase catalytic subunit; G6PDH, glucose-6-phosphate dehydrogenase; GK, glucokinase; gp, glycogen phosphorylase; gys, glycogen synthase; pck, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PK, pyruvate kinase; SGLT1, Na-dependent GLUT 1; srebp1, sterol regulatory element-binding protein.

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type 2 (GLUT2) in the basolateral membrane[5]. Previous work suggested that levels of SGLT1 mRNA were significantly up-regulated following a glucose load, but oral glucose did not up-regulate GLUT2 mRNA levels in rainbow trout[6]. To the contrary, subsequent research showed that high levels of dietary starch significantly increased the transcriptional level of GLUT2 in blunt snout bream (*Megalobrama amblycephala*)[7]. However, few studies have paid attention to the role of carbohydrate digestion and absorption in maintaining glucose homoeostasis of different species of fish, particularly with regard to the role of amylase.

After glucose loading, the clearance rate of blood glucose is thought to be much faster in herbivorous fish than in carnivorous species[5,8,9]. Previous research has reported that the activity of pyruvate kinase (PK) in grass carp[10] and gilthead seabream[11] can be elevated by high levels of dietary carbohydrate. However, other studies have reported that dietary carbohydrate has no effect on the activity or expression of PK in Senegalese sole (*Solea senegalensis*)[12] or rainbow trout[13]. In general, after glucose loading, the relative expression or the activity of key enzymes in gluconeogenesis significantly decreased, for example, the mRNA levels of glucose-6-phosphatase (G6Pase) significantly decreased in gibel carp (*Carassius gibelio*)[14]. However, a failed regulation of G6Pase was reported in European seabass (*Dicentrarchus labrax*)[15] and gilthead seabream[15,16]. In fish, excess ingested glucose could be stored as glycogen and this depends on feeding habits and nutritional status[5]. In largemouth bass (*Micropterus salmoides*)[17] and golden pompano (*T. ovatus*)[2], levels of hepatic glycogen have been reported to increase with the increase of dietary carbohydrate. In some fish, excessive amounts of ingested glucose can also be transferred to body lipid storage. Sterol regulatory element-binding protein (*Srebp1*) and carbohydrate-responsive element binding protein (*chrebp*) are two key regulatory factors of glucose-induced lipogenesis. Experiments involving rainbow trout and blunt snout bream showed significant changes in the expression of these two factors in response to dietary carbohydrate[18,19]. The expression of fatty acid synthase (*fas*) has also been shown to be up-regulated in rainbow trout fed with a high carbohydrate diet[13]. Despite fish, particularly carnivorous fish, display a low ability to utilise carbohydrates and maintain glucose homoeostasis, some studies have reported that carnivorous fish, such as rainbow trout[20,21] and gilthead seabream[22], can adapt their metabolic response to glucose loading, although the ability to adjust is limited. Therefore, more attention should be paid on the different metabolic reactions to glucose loading within species in order to have a better understanding glucose metabolism in fish with different feeding habits.

Grass carp (*Ctenopharyngodon idella*), a herbivorous fish, and Chinese longsnout catfish (*Leiocassis longirostris* Günther), a carnivorous fish, are two commonly cultured species in China. Previous studies reported that the optimal level of dietary starch for grass carp was 33 % which was much higher than that in Chinese longsnout catfish (10 %)[23,24]. After a glucose load, grass carp had an efficient clearance of glucose by increasing the activity of hepatic hexokinase[25]. Compared with grass carp, Chinese longsnout catfish has a lower ability of utilising dietary carbohydrates. Furthermore, the difference between grass carp and Chinese longsnout catfish in glucose homoeostasis and metabolism is still unclear. Therefore, in the present study, we characterised glucose homoeostasis in grass carp and Chinese longsnout catfish following the oral administration of starch and compared the pathway related to carbohydrate metabolism such as, digestion, transportation, gluconeogenesis, glycolysis, glycogen synthesis and glucose-induced lipogenesis. The data of the models in Chinese longsnout catfish and grass carp contribute to improve the present understanding of glucose intolerance.

Materials and methods

Experimental animals

Grass carp (64·12 (SE 9·50) g) were obtained from a commercial fish farm (Hanshou, Changde, Hunan, China). Chinese longsnout catfish (19·63 (SE 3·08) g) were obtained from another fish farm (Shishou, Jingzhou, Hubei, China). Prior to the oral administration of starch, both grass carp and Chinese longsnout catfish were cultured in an indoor recirculating system for 2 weeks to acclimatise. During this period, all fish were fed to apparent satiety twice per d with experimental feeds which had been formulated specially for these two species (Table 1); we also ensured that carbohydrate levels were appropriate for each species. During both the acclimation period and the experimental period, water quality was maintained as follows: temperature 22 (SE 2)°C, ammonia–N content 0·15 (SE 0·03) mg/l, dissolved O 6·71 (SE 0·10) mg/l and pH 6·81 (SE 0·12). Photoperiod was 12 h dark–12 h light; the period of daylight was 08.00–20.00 hours.

Oral starch administration

The administration of starch was performed by gavage using a lavage tube (outer diameter 1·00 mm). Prior to the oral administration of starch, all fish underwent fasting for a period of 48 h in order to ensure that their digestive tract was empty and that they had a basal level of plasma glucose. Then, fish were randomly selected, anaesthetised with MS-222 (80 mg/l; Sigma), immediately weighed and administered with 3 g of a mixture of water and maize starch (water–starch at a ratio of 3:2) per 100 g body weight. Maize starch (gelatinisation degree of starch is 4·15 % and performed according to Holm *et al.*[26]; the total digestibility of starch *in vitro* is 49·79 % and performed according to

Table 1. Proximate composition of diets used to feed grass carp and Chinese longsnout catfish

Diets	Grass carp	Chinese longsnout catfish
Maize starch* (g 100/g diet)	30	8
Proximate composition (g 100/g DM)		
Moisture	6·78	4·45
Total protein	33·25	41·28
Total lipid	6·69	15·06
Gross energy (MJ/kg)	17·45	19·17

* Maize starch was obtained from Yufeng industrial group.

Table 2. Primers used in the PCR analysis for grass carp

Gene	GenBank accession no.	Forward primer (5′–3′)	Reverse primer (5′–3′)	Amplicon size (bp)
β-Actin	M25013	CGTGACATCAAGGAGAAG	GAGTTGAAGGTGGTCTCAT	299
pkl	JQ951928	GGGGTTTCTTCAGTTCGGGT	AGAAGTGCAGCACAAGCAGA	194
pfk	KP262352.1	ATCAACACACCCGACTGCC	TGGTACACACTGCATCAGTCC	164
pck	JQ898294.1	ATCGTCACGGAGAACCAA	CCTGAACACCAAACTTAGCA	202
*g6pc**	ESTs	AAAGACAGCAGGTAGAAGAGG	ACGGAAAACAAGAAGAGCAG	131
fbp	EW688169.1	TGAGATGCCACCAGATGAGC	GGCAGGTACGGCATCAGATTA	272
sglt1	MH539755	CGTGCTGTCCCTGTTTCTC	GACCACCTGTTACTGTAT	152
glut2	KY763986.1	TGGGTGTTGCAACTGGACAT	GTTGCCACAAACTAACGGTCC	287
srebp1	KJ162572.1	GTGGAGCTTCTGGTCTGTGACC	GAACAGCTTGTGCTGGGCCTGT	180
chrebp	MK953928	GCAGTCGTGGAGGATCACAGAT	GATGAGAAGTGGCGCTTGTTGC	170
g6pd	KP148257.1	GACAAGATGAGCACCCTTCCT	AGATCTCCCGAAGCACCCAT	131
acc	GU908475	TGGCTGCACTGCACTCTCACT	GGTCCAGCTTCCCTGCGGTC	81
fas	HM802556	GCCTAACGTGGGAAGAATGCA	GAGCGAGGTCTTGGGCTCTTTAT	256
gp	JQ782458	AGGAAGCGGATGATTGGT	ATTGAAGTCGTTGGGAGC	233
gys	JQ792167	CCTCCAGTAACAACTCACAACA	CAGATAGATTGGTGGTTACGC	277

pkl, Pyruvate kinase (liver type); *pfk*, phosphofructokinase; *pck*, phosphoenolpyruvate carboxykinase; *g6pc*, glucose-6-phosphatase catalytic subunit; *fbp*, fructose 1,6-bisphosphatase; *sglt1*, Na-dependent GLUT 1; *srebp1*, sterol regulatory element-binding protein; *chrebp*, carbohydrate-responsive element binding protein; *g6pd*, glucose 6-phosphate dehydrogenase; *acc*, acetyl-CoA carboxylase; *fas*, fatty acid synthase; *gp*, glycogen phosphorylase; *gys*, glycogen synthase.
* According to Li *et al.*[30].

Tang *et al.*[27]) was obtained from Yufeng industrial group. Another group of fish was administered with saline (0·9 %) as a sham treatment in order to assess the effects of oral administration on plasma glucose levels. Following the administration of starch, six fish were sampled immediately; these samples were designated as time 0 h. And, time 0 h was used as the control group. Further samples were collected at 1, 3, 6, 12, 24 and 48 h. One aquarium (150 litres) was used for each sampling time in order to minimise the stress of sampling. A total of eighty-four grass carp and eighty-four Chinese longsnout catfish were sampled.

At each time point, six fish from each tank were anaesthetised with MS-222 (80 mg/l) and samples of blood, liver and anterior intestine were acquired. Blood was taken from dorsal vessels using a syringe and then centrifuged at 3000 ***g*** (4°C) for 10 min to obtain plasma samples. Samples of liver and anterior intestine were collected immediately after blood sampling and were quickly frozen in liquid N_2 and stored at −80°C for further analysis. All experiments were conducted in accordance with the Guiding Principles for Care and Use of Laboratory Animals approved by the Institute of Hydrobiology, Chinese Academy of Science (approval ID: IHB20140724).

Biochemical assays

The proximate composition of feeds was analysed following Association of Official Analytical Chemists methods[28]. Total protein content (N × 6·25) was determined after acid digestion with the Kjeldahl method, using a 4800 Kjeltec Analyzer Unit (FOSS Tecator). Total lipid content was determined using ether extraction in a Soxtec system (Soxtec System HT6; Tecator), with diethyl ether as the extraction liquid. Gross energy was determined by an Oxygen bomb calorimeter (Parr 6200; Parr Instrument Company). Glucose, TAG, cholesterol levels in plasma and glycogen contents in liver were measured with commercial assay kits (Nanjing Jiancheng Bioengineering Institute). The activity of amylase in intestine and PK was measured by commercial assay kits (Nanjing Jiancheng Bioengineering Institute). The activity of phosphofructokinase (PFK), fructose 1,6-bisphosphatase (FBP) and glucose-6-phosphate dehydrogenase (G6PDH) was measured using commercial assay kits (Beijing Solarbio Science and Technology Co. Ltd). The activity of G6Pase in liver was determined by spectrophotometric assay using commercial assay kits (Suzhou Comin Biotechnology Co. Ltd). One unit of enzyme activity is defined as the amount of enzyme that will generate 1 nmol/ml NADH per min at 25°C. The activity of glucokinase (GK) in liver was determined by the methods described by Panserat *et al.*[29]. The protein concentration of liver homogenate was determined by the Coomassie Brilliant Blue G250, using bovine serum albumin as the standard.

Quantitative PCR analysis

Total RNA was extracted from liver samples using TRIzol Reagent (Ambion, Life Technologies) in accordance with the manufacturer's instructions. RNA quality was assessed by agarose gel electrophoresis. Total RNA was then reverse-transcribed using an M-MLV First-Strand Synthesis Kit (Invitrogen). The primers for PCR were designed according to published sequences for grass carp and Chinese longsnout catfish in GenBank (Tables 2 and 3). The primers used for glucose-6-phosphatase catalytic subunit (*g6pc*) in grass carp were based on those reported previously by Li *et al.*[30].

Real-time PCR analysis was carried out using LightCycle 480 SYBR Green I Master Mix with a LightCycle® 480 II system (Roche). All reactions were performed in a 6 μl volume including 2 μl cDNA template, 0·24 μl forward and reverse primer, 0·52 μl ddH_2O and 3 μl LightCycle 480 SYBR Green 1 Master. Negative controls (in which the template was replaced by ddH_2O) were amplified in the same plate. PCR conditions were as follows: pre-incubation 95°C for 5 min, followed by forty cycles of amplification (95°C for 10 s, 60°C for 20 s, 72°C for 10 s). A melting curve was then created (0·5°C increments from 65 to 95°C) to

Table 3. Primers used in the PCR analysis for Chinese longsnout catfish

Gene	GenBank accession no.	Forward primer (5′–3′)	Reverse primer (5′–3′)	Amplicon size (bp)
β-Actin	JN833583.1	TCCTCCGTCTGGATTTGG	TCCGTCAGGCAGCTCATA	212
Pkl	MH626447	TGCTTCCAGAGGGTAGTCTC	GATGATGATTGGACGCTGCA	190
Pfk	MN183276	ACATTGTAGGTCTTGTGGGTT	AGACCCAGTCAGCTCCAGAT	216
Pck	MH703585	CTATTGGCACTCCTTCGTCAC	GGTAAACTCAGAGCCATCAAC	322
g6pc	MH570216	TCTCTTGAAGGCTGTGGGAG	TAGAGTGGCGAGTGCAGG	165
fbp	MN183277	AACAGGACCCATAAACGTGCT	GTTGTCGGTCATGGATCACT	139
sglt1	MH667317	GGAGGATTTGAGTGGAATGC	TGTCCGCAGAGATCTTAGTG	190
glut2	MH748105	TGGGTGGAATGTGCTGCTGTG	GATGAAGTTACTGGTCCAGTTGC	230
srebp1	MK934525	CTATGGCTAATGCTGAGAAG	TGGCTCCCACACATCAGAGACG	121
chrebp	MK953927	TTCGGTCCTACATCTCCAAC	GCTCTGTCTTGGTTATTCCG	120
g6pd	MN183278	CACATGAAGGTGGCAGACACTG	CACCCCTTGGTGCTCAGACATTG	233
acc	MH570215	GTGCTGGAGAACCTGATGAAC	GAACAGAGGTGATGTTGCTGG	232
fas	MH779479	CTGAGGCTTATGCTAGAGAT	GAACATGCTGTGTCGATGGC	251
gp	MH643794	GGAGTCGCTATGATTCACTCC	GCAGTTGAGGAGCTGTCGTTTG	381
gys	MH555092	CCACTCACAACATGCTGGACG	GTGCACTCGCCTGGTGTGTATC	229

pkl, Pyruvate kinase (liver type); *pfk*, phosphofructokinase; *pck*, phosphoenolpyruvate carboxykinase; *g6pc*, glucose-6-phosphatase catalytic subunit; *fbp*, fructose 1,6-bisphosphatase; *sglt1*, Na-dependent GLUT 1; *glut2*, glucose transporter 2; *srebp1*, sterol regulatory element-binding protein; *chrebp*, carbohydrate-responsive element binding protein; *g6pd*, glucose 6-phosphate dehydrogenase; *acc*, acetyl-CoA carboxylase; *fas*, fatty acid synthase; *gp*, glycogen phosphorylase; *gys*, glycogen synthase.

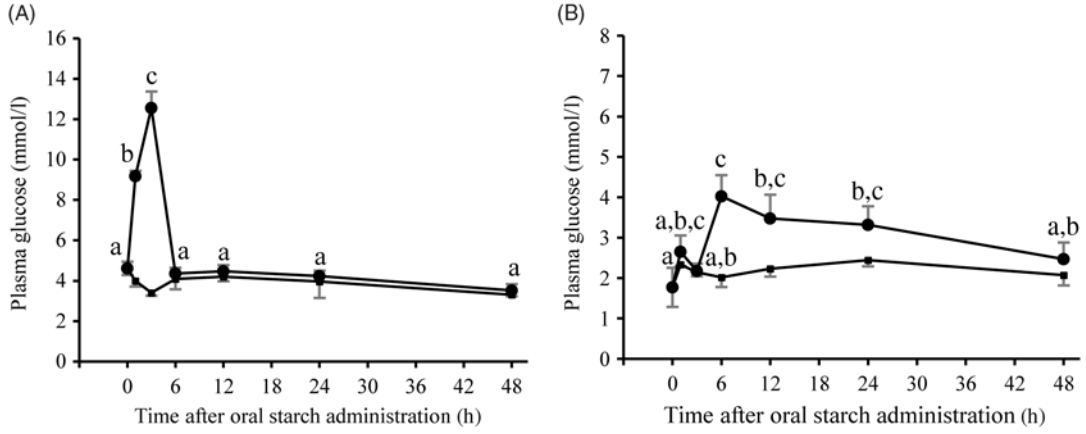

Fig. 1. Changes in the plasma glucose levels of (A) grass carp and (B) Chinese longsnout catfish following the oral administration of starch. Each point represents the mean of six replicates. [a,b,c] Unlike letters indicate significant differences ($P < 0{\cdot}05$) between different sampling times. —●—, Starch; —■—, saline.

confirm the amplification of a single product. *β-Actin* was chosen as an internal reference for normalisation. In the pretrial of our experiment, it has been found that the gene expression of *β-actin* was very stable in the target tissues of grass carp and Chinese longsnout catfish. The amplification efficiency of the genes analysed in the present study varied from 1·95 to 2·05. Final data were calculated according to the method described by Vandesompele *et al.*[31].

Statistical analysis

All data were analysed using SPSS 20 for Windows. Duncan's multiple range test was used to detect the significance of differences between groups followed by one-way ANOVA. Before all analyses, all data were tested for the normality of distribution (one-sample Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test) among different time points. Statistical significance was set to $P < 0{\cdot}05$, and data are presented as mean values with their standard errors (n 6).

Results

Plasma glucose

Plasma glucose levels did not change significantly following the administration of oral saline ($P > 0{\cdot}05$). In grass carp, plasma glucose rose from 4·61 (SE 0·33) to 12·56 (SE 0·81) mmol/l over the first 3 h after starch administration and returned to baseline by 6 h; there was no significant difference in plasma glucose level when compared between 0 and 6 h (Fig. 1(A)). In Chinese longsnout catfish, the plasma glucose levels at 6, 12 and 24 h were significantly higher than those at 0 h ($P < 0{\cdot}05$). There was no significant difference in plasma glucose levels when compared between 48 and 0 h ($P > 0{\cdot}05$).

Digestion and transportation

Following the oral administration of starch, we detected significantly higher levels of amylase activity at 1 and 3 h compared with 0 h ($P < 0{\cdot}05$) in the anterior intestine of grass

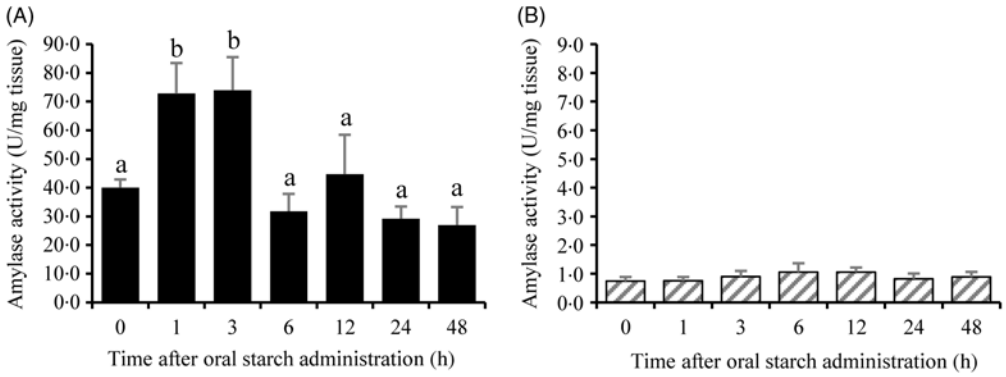

Fig. 2. Amylase activity in the anterior intestine of (A) grass carp and (B) Chinese longsnout catfish following the oral administration of starch. Each bar represents the mean of six replicates. [a,b] Mean values with unlike letters are significantly different ($P < 0·05$).

carp (Fig. 2(A)). However, there were no significant differences in terms of amylase activity in the anterior intestine of Chinese longsnout catfish following the oral administration of starch (Fig. 2(B)).

In grass carp, the highest levels of mRNA expression for *sglt1* were detected 1 h after the administration of starch; expression levels then decreased steadily from 3 to 12 h (Fig. 3(A)). In Chinese longsnout catfish, the mRNA levels of *sglt1* at 3 h were significantly higher than at 12, 24 and 48 h (Fig. 3(B)). There was no significant change in the transcriptional levels of *glut2* in the intestine of grass carp ($P > 0·05$) (Fig. 3(C)), although the mRNA levels of *glut2* increased significantly ($P < 0·05$) at 6 and 12 h in the liver of grass carp, reaching a peak at 12 h (Fig. 3(E)). The expression of *glut2* in the livers of Chinese longsnout catfish was significantly higher ($P < 0·05$) at 12 h (Fig. 3(F)).

Hepatic gluconeogenesis

In grass carp, the activity of G6Pase, which plays a key role in catalysing the final step of gluconeogenesis, decreased significantly until 12 h after the oral administration of starch ($P < 0·05$) but then returned to baseline by 24 h (Fig. 4(A)). Similarly, the expression of *g6pc*, which encodes G6Pase, decreased significantly ($P < 0·05$) until 12 h after administration but then increased significantly ($P < 0·05$) at 48 h (Fig. 4(C)). However, in Chinese longsnout catfish, both liver G6Pase activity and *g6pc* expression remained unchanged after starch treatment (Fig. 4(B) and (D)). FBP and phosphoenolpyruvate carboxykinase (PEPCK) are both rate-limiting enzymes for catalysing gluconeogenesis. The enzymes activity and mRNA levels of FBP and the mRNA levels of *pck*, which encode PEPCK, showed no significant changes in either grass carp or Chinese longsnout catfish following the administration of starch ($P > 0·05$).

Hepatic glycolysis

A significant increase in hepatic GK activity was detected in grass carp ($P < 0·05$) at 3 and 6 h after the oral administration of starch (Fig. 5(A)). We also detected a significant increase in the hepatic activity of GK ($P < 0·05$) in Chinese longsnout catfish (Fig. 5(B)) at these same time points. PK plays a key role in catalysing the final step of glycolysis. The activity of hepatic PK in grass carp increased slowly following the administration of starch and peaked at 12 h; at this time point, activity was significantly higher ($P < 0·05$) than that at 0 h (Fig. 5(C)). Furthermore, the mRNA levels of *pk* were significantly increased ($P < 0·05$) at 12 h after the administration of starch (Fig. 5(E)). There was no significant change in the activity and transcription of PK at any of the sampling time points in the livers of Chinese longsnout catfish (Fig. 5(D) and (F)). Furthermore, there were no significant changes in the activity of enzymes and expression levels of *pfk* at any of the sampling time points following the administration of starch; this was the case for both grass carp and Chinese longsnout catfish ($P > 0·05$) (Fig. 5(G)–(J)).

Hepatic glycogen

In grass carp, the expression levels of hepatic glycogen synthase (*gys*) and glycogen phosphorylase (*gp*) were significantly higher ($P < 0·05$) at 6 h than at 0 h. However, there was no significant change in terms of glycogen content ($P > 0·05$) in the livers of grass carp following the administration of starch (Fig. 6(A), (C) and (E)). In contrast to grass carp, there was a significant increase in glycogen content in the livers of Chinese longsnout catfish from 12 to 48 h ($P < 0·05$). Furthermore, the expression levels of hepatic *gp* were significantly reduced ($P < 0·05$) in Chinese longsnout catfish but there was no significant change in the mRNA expression of *gys* (Fig. 6(B), (D) and (F)).

Glucose-induced lipogenesis

For all sampling time points, plasma TAG and cholesterol levels were consistently higher in grass carp than in Chinese longsnout catfish (Fig. 7). Plasma cholesterol in grass carp increased significantly ($P < 0·05$) 1–3 h after the administration of starch, compared with 0 h. In Chinese longsnout catfish, levels of plasma cholesterol increased significantly ($P < 0·05$) 12–24 h after administration (Fig. 7(B)).

The expression levels of hepatic *chrebp* were significantly up-regulated ($P < 0·05$) in grass carp 6 h after the oral administration of starch (Fig. 8(A)). However, there were no significant changes in the levels of hepatic *chrebp* in Chinese longsnout catfish (Fig. 8(B)). In grass carp, the relative expression of hepatic *srebp1* increased significantly ($P < 0·05$) at 3 and 6 h (Fig. 8(C)). The activity of glucose 6-phosphate dehydrogenase (*g6pd*) in grass carp significantly elevated at 3 and 6 h (Fig. 9(A)).

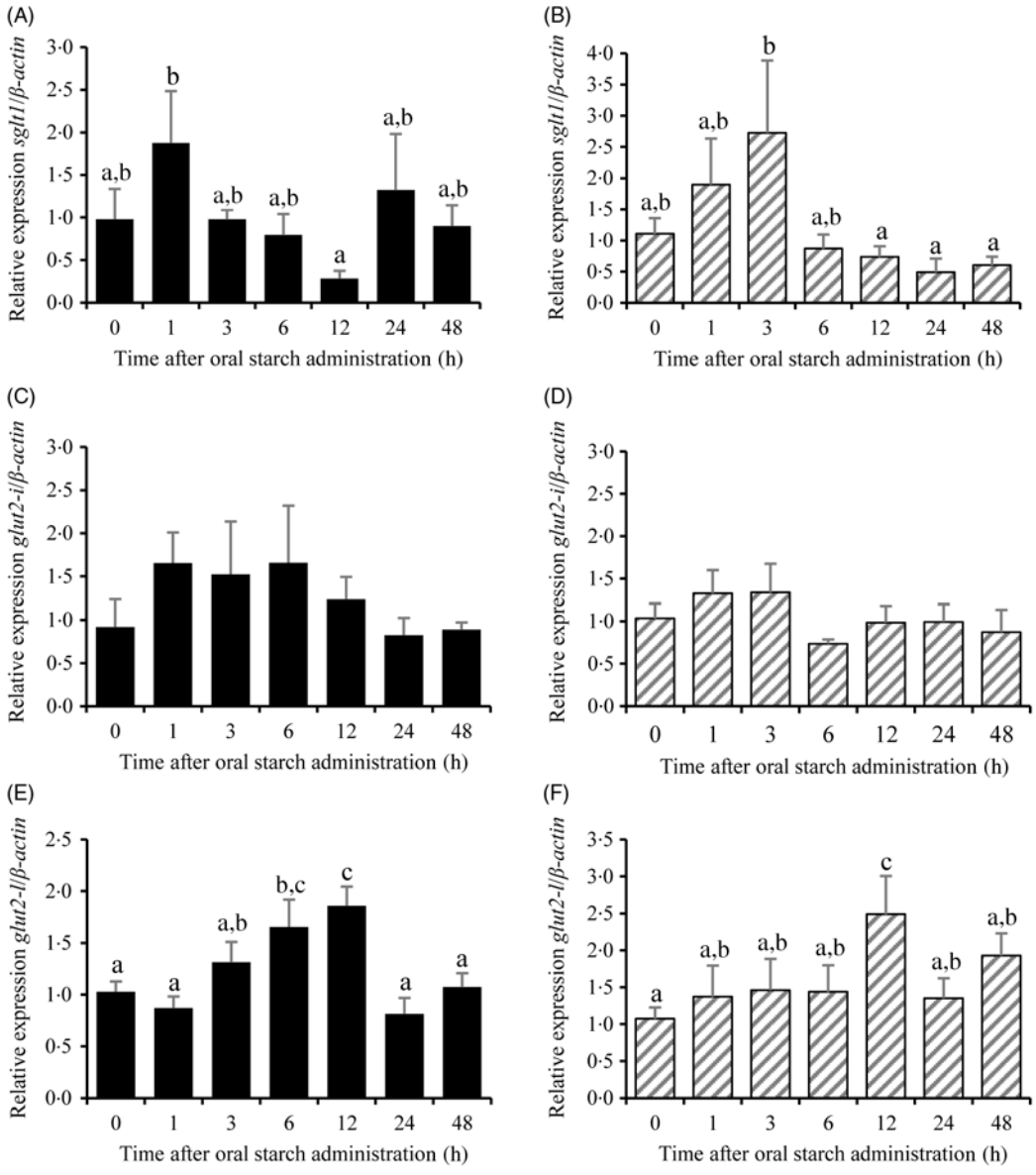

Fig. 3. Relative expression of GLUT in grass carp and Chinese longsnout catfish following the oral administration of starch: (A) Na-dependent GLUT 1 (*sglt1*) in the intestine of grass carp; (B) *sglt1* in the intestine of Chinese longsnout catfish; (C) GLUT type 2 (*glut2*) in the intestine of grass carp; (D) *glut2* in the intestine of Chinese longsnout catfish; (E) *glut2* in the liver of grass carp; and (F) *glut2* in the liver of Chinese longsnout catfish. Each bar represents the mean of six replicates. [a,b,c] Mean values with unlike letters are significantly different ($P < 0{\cdot}05$).

Compared with 0 h, the relative expression of hepatic *g6pd* increased significantly in the Chinese longsnout catfish at 24 and 48 h ($P < 0{\cdot}05$) (Fig. 9(D)). The mRNA levels of acetyl-CoA carboxylase (*acc*) increased at 1–6 h, peaked at 6 h and then returned to baseline at 12 h in the livers of grass carp (Fig. 8(E)). Compared with 0 h, the expression levels of *acc* were significantly higher ($P < 0{\cdot}05$) at 24 and 48 h in the livers of Chinese longsnout catfish (Fig. 8(F)). The expression levels of hepatic *fas* in grass carp increased significantly at 1–6 h ($P < 0{\cdot}05$).

Discussion

Our analysis revealed an interesting difference in the carbohydrate metabolism of fish in that the oral administration of starch led to an increase in plasma glucose levels; glucose peaked at 3 h in grass carp and 6 h in Chinese longsnout catfish. The time taken to reach a glucose peak following the oral administration of starch was previously reported to be 3 h in tilapia (*Oreochromis niloticus*)[32] and 6 h in white sturgeon (*Acipenser transmontanus*)[33]. However, after intraperitoneal injection of glucose, plasma glucose concentrations peaked at 1 h in both tilapia[34] and blunt snout bream[35]. Differences in the timing required to reach a glucose peak between present study and those published previously are not only related to species differences but also related to differences in the methods used to administer carbohydrates. In addition, the peak levels of plasma glucose in grass carp were almost three times higher than that in Chinese longsnout catfish. This difference could be due to a range of reasons. Firstly, digestion and absorption play a key role in the initial steps of glucose

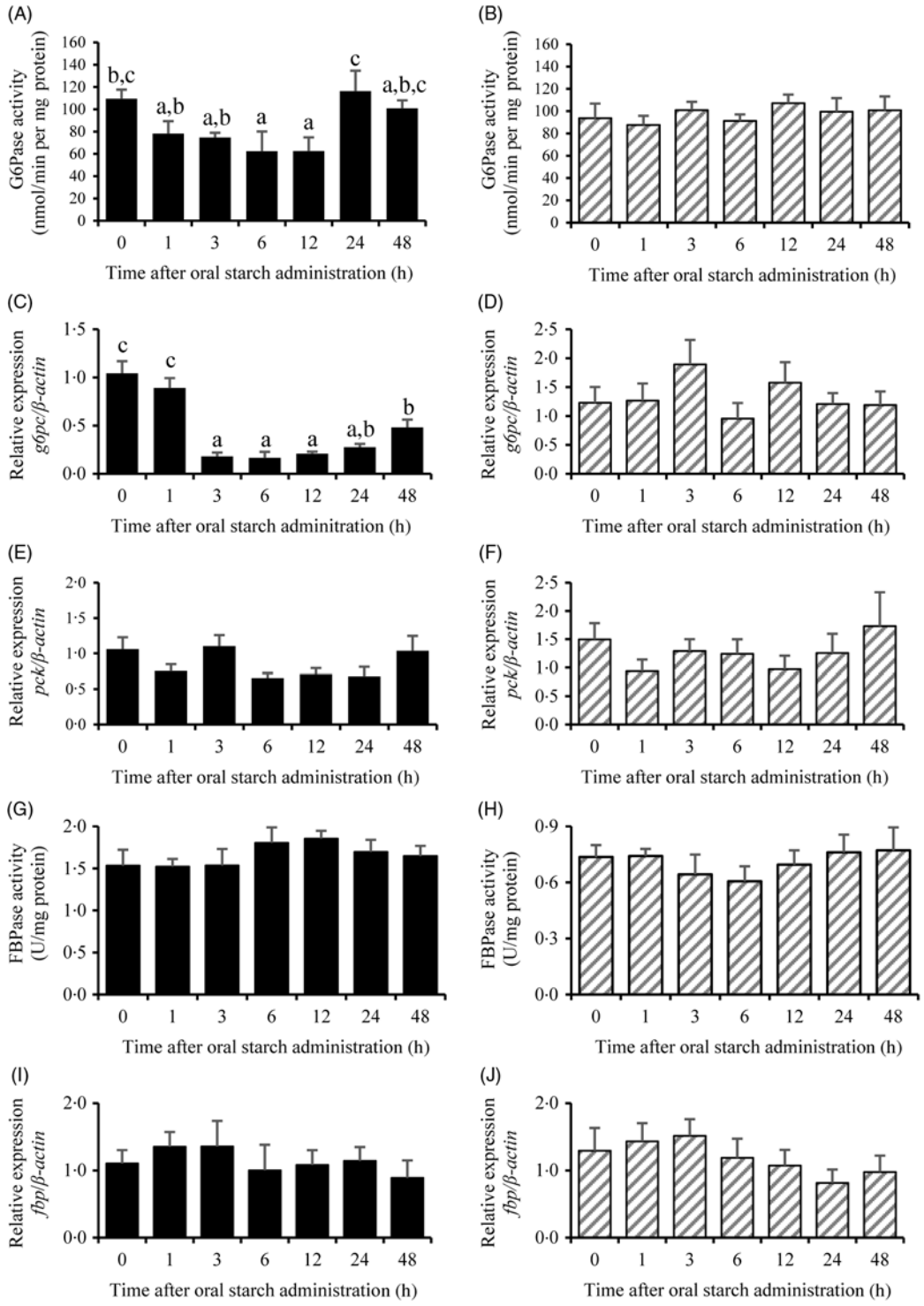

Fig. 4. Enzymic activity and expression levels of hepatic gluconeogenesis in grass carp and Chinese longsnout catfish following the oral administration of starch. Enzymic activity of glucose-6-phosphatase (G6Pase) in (A) grass carp and (B) Chinese longsnout catfish; expression levels of glucose-6-phosphatase catalytic subunit (*g6pc*) in (C) grass carp and (D) Chinese longsnout catfish; expression levels of phosphoenolpyruvate carboxykinase (*pck*) in (E) grass carp and (F) Chinese longsnout catfish; enzymic activity of FBPase in (G) grass carp and (H) Chinese longsnout catfish; expression levels of fructose 1,6-bisphosphatase (*fbp*) in (I) grass carp and (J) Chinese longsnout catfish. Each bar represents the mean of six replicates. [a,b,c] Mean values with unlike letters are significantly different ($P < 0{\cdot}05$).

metabolism; amylase plays a critical role during these processes. A previous comparative study, featuring species with different food habits, indicated that the amylase activity of goldfish was almost fifty-eight times higher than that of rainbow trout[4]. We observed a similar result in the present study that the amylase activity of grass carp was 54- to 82-fold higher than Chinese longsnout catfish following the administration of starch. The activity of amylase had a close relationship with the utilisation of

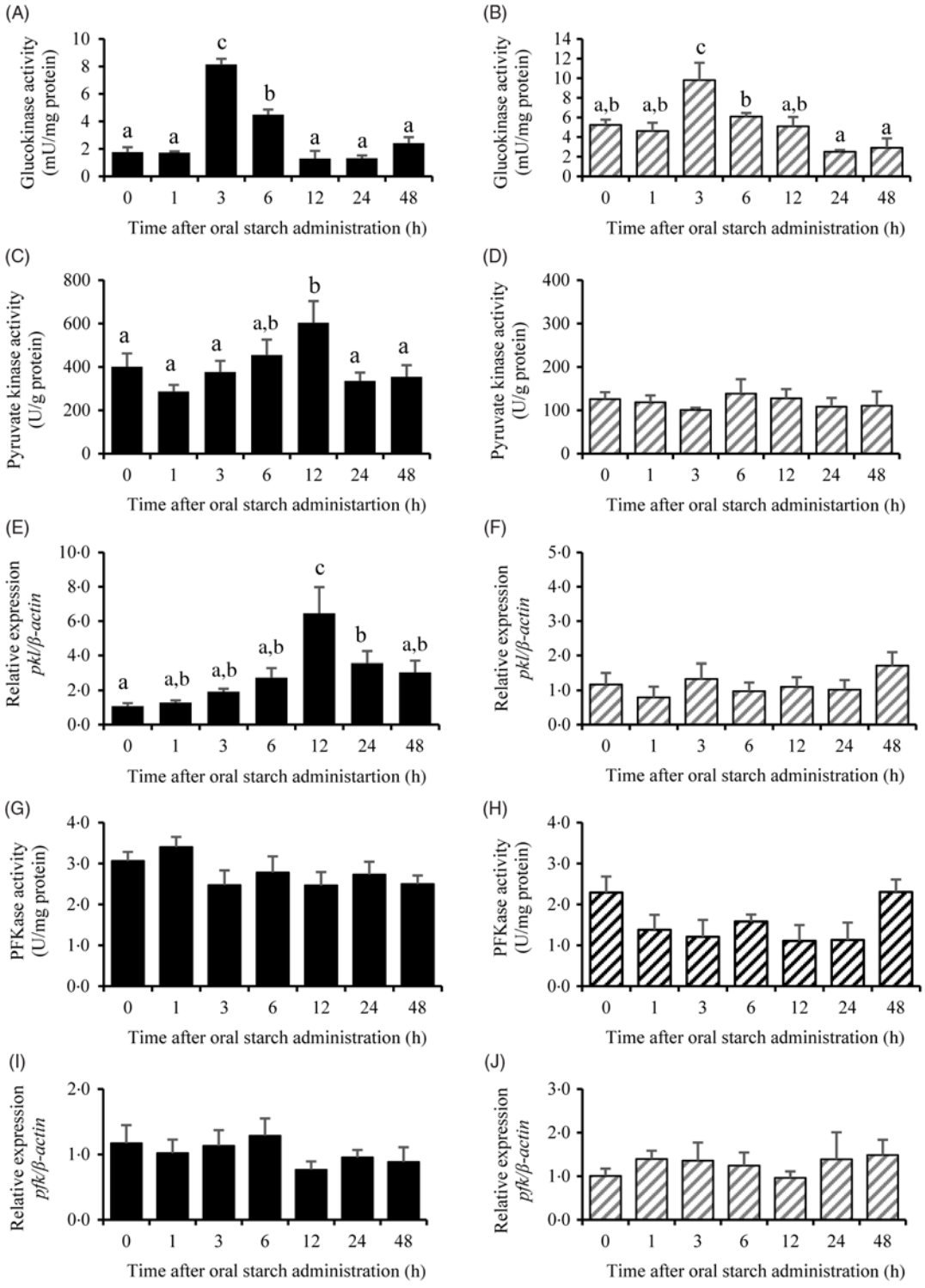

Fig. 5. Enzymic activity and expression levels of hepatic glycolysis in grass carp and Chinese longsnout catfish following the oral administration of starch. Enzymic activity of (A) glucokinase in grass carp; (B) glucokinase in Chinese longsnout catfish; (C) pyruvate kinase (PK) in grass carp; (D) PK in Chinese longsnout catfish; expression level of (E) pyruvate kinase (liver type) (*pkl*) in grass carp; (F) *pkl* in Chinese longsnout catfish; enzymic activity of phosphofructokinase (PFKase) in (G) grass carp and (H) Chinese longsnout catfish; expression level of *pfk* in (I) grass carp and (J) Chinese longsnout catfish. Each bar represents the mean of six replicates. [a,b,c] Mean values with unlike letters are significantly different ($P < 0{\cdot}05$).

dietary carbohydrates[36]. Dietary supplementation of α-amylase enhanced the digestibility of starch and G6PDH activity in the liver of Rohu carp (*L. rohita*)[37,38]. After a meal with high digestibility (97–99 %) of starch, gluconeogenesis was not particularly depressed, while lipogenic pathways were enhanced in gilthead seabream[39]. Interestingly, after the administration of starch, the amylase activity of grass carp increased significantly at 1 and 3 h and then returned to baseline at 6 h; Chinese longsnout

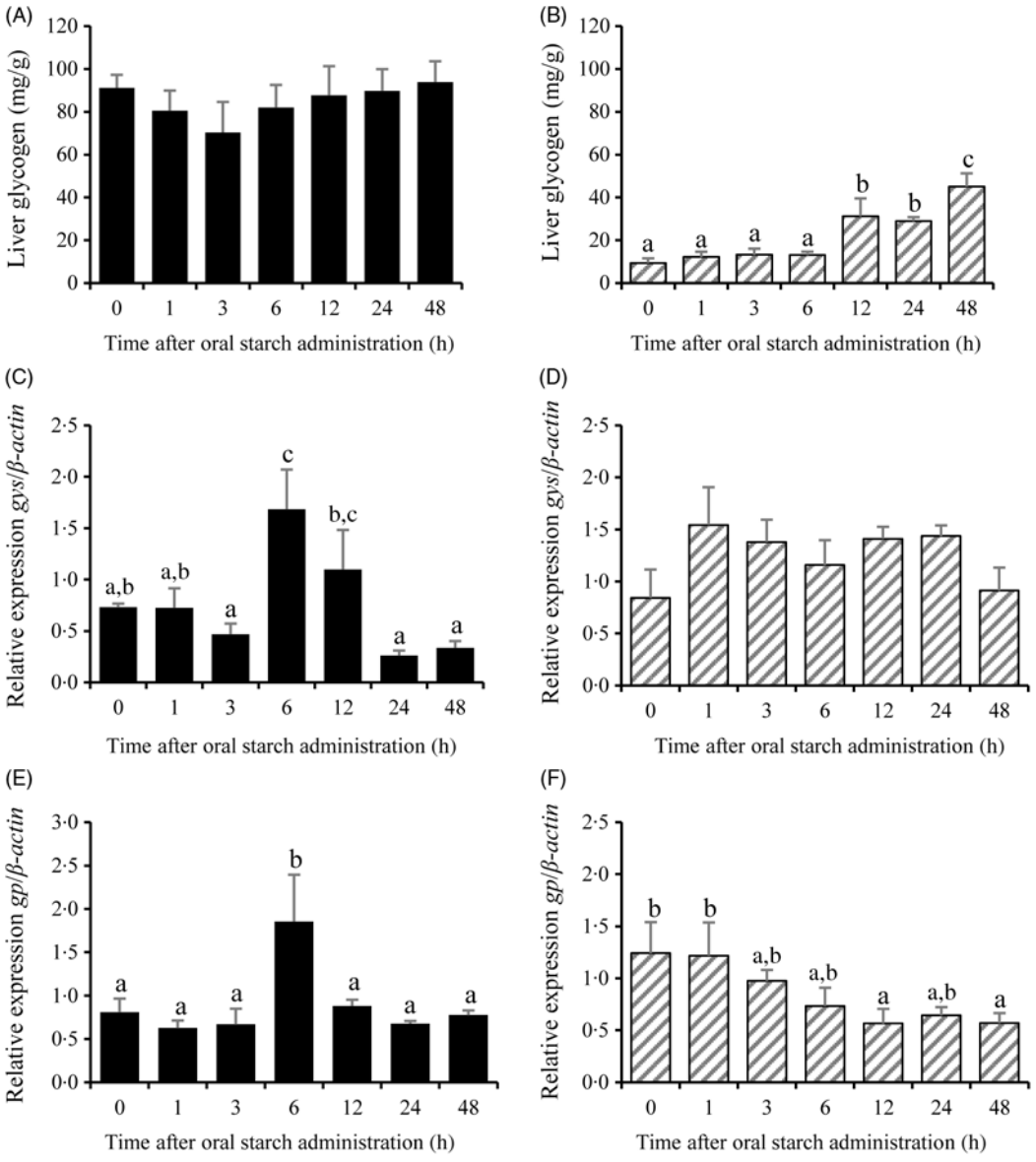

Fig. 6. Hepatic glycogen content in (A) grass carp and (B) Chinese longsnout catfish; relative expression of hepatic glycogen synthase (*gys*) in (C) grass carp and (D) Chinese longsnout catfish; relative expression of hepatic glycogen phosphorylase (*gp*) in (E) grass carp and (F) Chinese longsnout catfish following the oral administration of starch. Each bar represents the mean of six replicates. [a,b,c] Mean values with unlike letters are significantly different ($P < 0{\cdot}05$).

catfish, however, showed no changes in the activity of amylase. Our results relating to amylase were supported by the fact that intestinal amylase activity was previously reported to be induced significantly with increased levels of dietary carbohydrate in common carp (*C. carpio*)[40] and golden pompano (*T. ovatus*)[2], although no changes were reported for southern catfish (*Silurus meridionalis* Chen)[41]. Differences in the activity of amylase in grass carp and Chinese longsnout catfish could potentially explain the differences in the way that plasma glucose increases in these fish following exposure to an excess of carbohydrate.

The absorption of glucose by the blood from the enteric cavity is facilitated by two key GLUT, Na-dependent GLUT 1 (SGLT1) and the facilitative GLUT2[42]. *Sglt1* expression is directly regulated by dietary carbohydrate[43,44], and the mRNA levels of *glut2* in zebrafish intestine were previously shown to be regulated by refeeding[45]. In the present study, the mRNA levels of *sglt1* in grass carp and Chinese longsnout catfish increased in a manner similar to plasma glucose and then decreased at a time point when plasma glucose levels peaked. A previous study involving rainbow trout showed that the expression levels of *sglt1* increased following the oral administration of glucose[46]. These data imply that SGLT1, located in the brush border/apical membrane, could play a role in rising plasma glucose levels in fish. However, in the present study, the expression of *glut2* in the intestine of grass carp and Chinese longsnout catfish did not show any significant change following the oral administration of starch, as also shown previously in the rainbow trout[46,47].

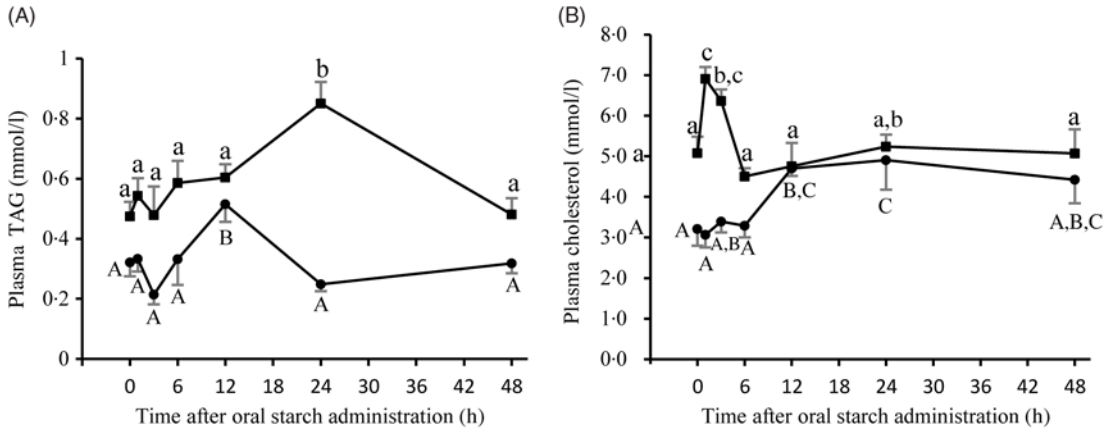

Fig. 7. Changes in (A) plasma TAG and (B) plasma cholesterol in grass carp and Chinese longsnout catfish following the oral administration of starch. Each point represents the mean of six replicates. [a,b,c] Unlike letters indicate significant differences ($P < 0{\cdot}05$) between sampling times for grass carp. [A,B,C] Unlike letters indicate significant differences ($P < 0{\cdot}05$) between sampling times for Chinese longsnout catfish. —●—, Chinese longsnout catfish; —■—, grass carp.

Gluconeogenesis is a main pathway to produce glucose in order to raise glycaemia in fish. Phosphoenolpyruvate carboxykinase, FBP and G6Pase are the key enzymes in regulation of hepatic gluconeogenesis. Previous studies have reported that the expression of *pck* was not affected by glucose injection in the liver of tilapia[34] and the transcription level of *fbp* was not influenced in common carp after a high carbohydrate meal[48]. In line with the previous studies, we found that the expression levels of *pck* and *fbp* were not affected by the oral administration of starch in either grass carp or Chinese longsnout catfish. However, reduced expression levels of *pck* and *fbp* were previously reported in gibel carp after a glucose load[14]. Our data showed that the transcriptional expression and enzymic activity of G6Pase were inhibited in grass carp but not in Chinese longsnout catfish. This was supported by previously published results which stated that the effect of dietary carbohydrate on G6Pase in fish is species-specific[5,16,48]. A failure to inhibit the gluconeogenic pathway was previously reported to be responsible for postprandial hyperglycaemia in rainbow trout[20,49]; this appears to also be the case for Chinese longsnout catfish. However, similar to our present observations in grass carp, a previous study involving roho labeo (*L. rohita*) reported that the activity of G6Pase was significantly reduced after a high carbohydrate meal[50]. Another study reported that the expression of G6Pase was inhibited in gibel carp following the injection of glucose[14]. Therefore, compared with herbivorous fish, carnivorous fish appear to exhibit lower abilities to inhibit gluconeogenesis for the maintenance of glucose homoeostasis.

Another interesting result was that plasma glucose levels in grass carp returned to baseline at 6 h after the oral administration of starch; in Chinese longsnout catfish, glucose levels did not return to baseline until the 48 h time point. The time returned to baseline after oral starch administration was previously reported to be 8 h in tilapia[32] and 15 h in white sturgeon (*A. transmontanus*)[33]. These results indicated that Chinese longsnout catfish, a carnivorous fish, has a poor capacity for clearing blood glucose. Glycolysis is the primary pathway for utilising glucose and converting glucose into pyruvate. The transcriptional expression and activity of hepatic GK, a rate-limiting enzyme of glycolysis, are strongly induced by dietary carbohydrate[51,52]. Previous research in gilthead seabream proved that the activity of GK increased significantly 6–8 h after feeding[53]. In line with gilthead seabream, the activity of GK in grass carp and Chinese longsnout catfish was highly induced by the oral administration of starch. Significantly increased activity of GK was also reported in blunt snout bream after a glucose load[35]. The expression level of *pfk* is considered to represent an effective indicator for postprandial hepatic glycolysis in tilapia[54]; however, our data showed that the activity and transcription levels of hepatic *pfk* were not stimulated by the oral administration of starch in grass carp and Chinese longsnout catfish. Similar results were previously published for gibel carp[14] and tilapia[34]. High levels of dietary carbohydrate are also known to significantly affect PK in fish, another rate-limiting enzyme in glycolysis, although this effect is species-dependent[5]. In the present study, the activity and relative expression of hepatic PK increased and peaked at 12 h in grass carp but then decreased; there were no significant changes in the expression of hepatic PK in Chinese longsnout catfish. The present results concurred with previous studies reporting significant changes of hepatic PK expression in grass carp[10] but no significant changes in rainbow trout[13,55]. Therefore, in Chinese longsnout catfish, it appears that PK, not GK, is responsible for the prolonged postprandial period of hyperglycaemia.

In order to maintain glucose homoeostasis, excessive amounts of glucose can be stored as glycogen. Previous studies reported a significant increase in hepatic glycogen content following glucose loading in hybrid grouper[56] and tilapia[34]. After intraperitoneal injection of glucose, the expression of hepatic *gys1* increased significantly and liver glycogen accumulated in tilapia[34]. However, in the present study, hepatic glycogen synthesis and catabolysis were significantly induced by the administration of oral starch but did not affect the hepatic glycogen content in grass carp. However, in Chinese longsnout catfish, there was no change in hepatic glycogen synthesis and reduced levels of hepatic glycogen catalysis; collectively, this resulted in a significant increase in hepatic glycogen

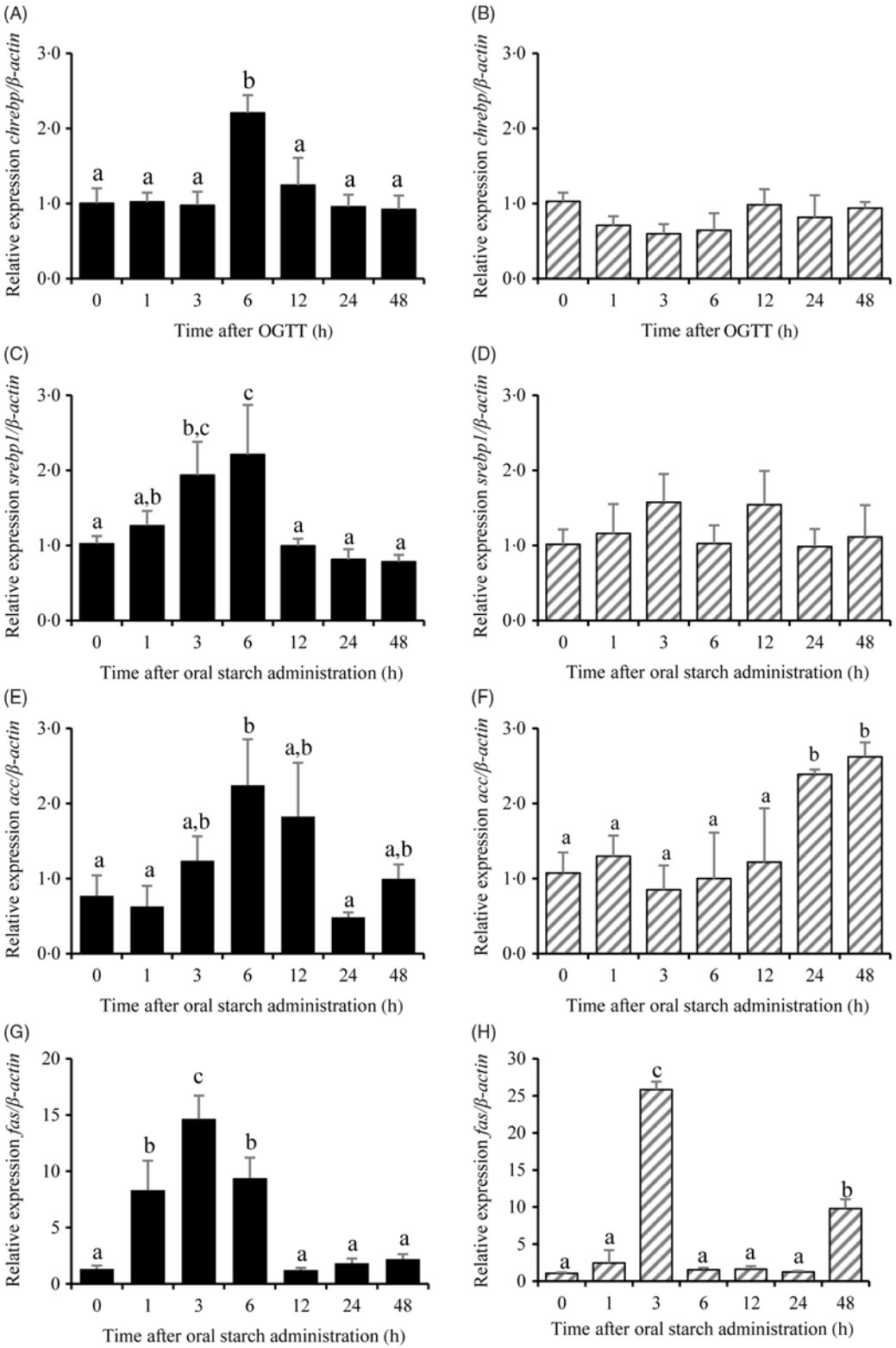

Fig. 8. Relative expression of hepatic carbohydrate-responsive element binding protein (*chrebp*), sterol regulatory element-binding protein (*srebp1*), acetyl-CoA carboxylase (*acc*) and fatty acid synthase (*fas*) in grass carp and Chinese longsnout catfish following the oral administration of starch. (A) *chrebp* in grass carp; (B) *chrebp* in Chinese longsnout catfish; (C) *srebp1* in grass carp; (D) *srebp1* in Chinese longsnout catfish; (E) *acc* in grass carp; (F) *acc* in Chinese longsnout catfish; (G) *fas* in grass carp; (H) *fas* in Chinese longsnout catfish. Each bar represents the mean of six replicates. [a,b,c] Mean values with unlike letters are significantly different ($P < 0{\cdot}05$).

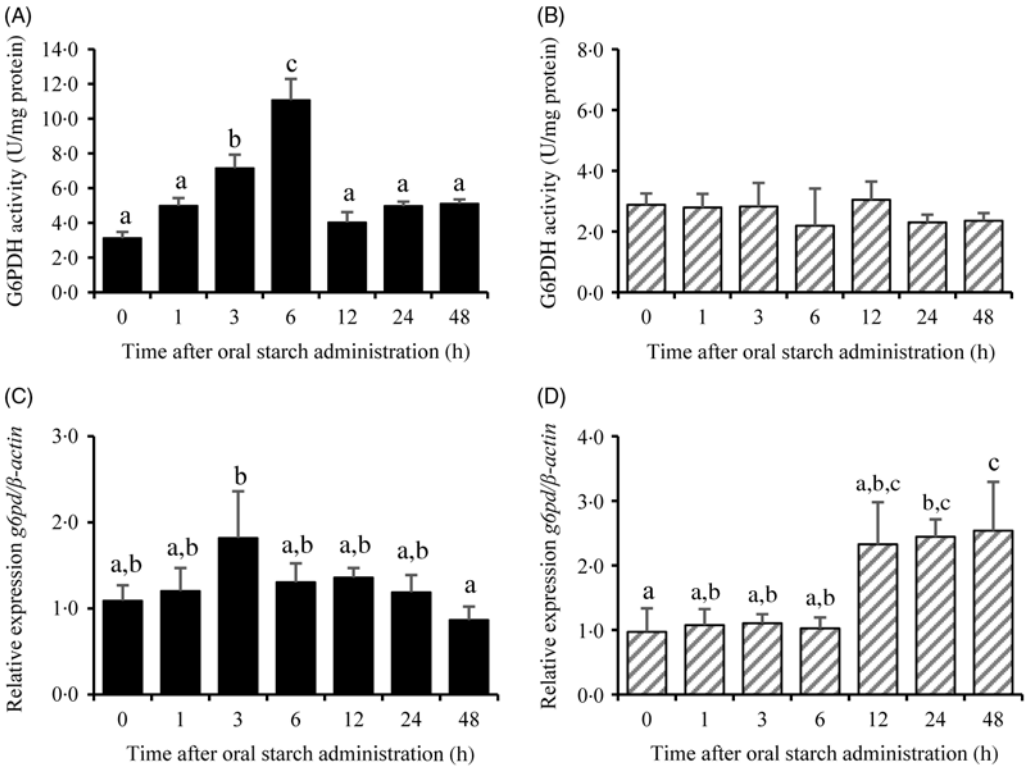

Fig. 9. Enzymic activity and expression of hepatic glucose-6-phosphate dehydrogenase (G6PDH) in grass carp and Chinese longsnout catfish following the oral administration of starch. Enzymic activity of G6PDH in (A) grass carp and (B) Chinese longsnout catfish; relative expression of *g6pd* in (C) grass carp and (D) Chinese longsnout catfish. Each bar represents the mean of six replicates. [a,b,c] Mean values with unlike letters are significantly different ($P < 0{\cdot}05$).

content. This indicated that the increased hepatic glycogen content in Chinese longsnout catfish was not the result of increased levels of glycogen synthesis but rather the reduced catabolism of glycogen. In addition, our present data showed that hepatic glycogen content in grass carp was almost 9·7 times higher than that in Chinese longsnout catfish at 0 h. Therefore, the storage and metabolism of hepatic glycogen after oral starch administration in grass carp and Chinese longsnout catfish appear to be very different.

Lipogenesis plays an important role in glucose metabolism because excess ingested glucose can be stored in the form of lipid[5]. Increased plasma TAG levels have been reported after a glucose load in both tilapia[34] and European seabass[22]. Similar results were evident in the present study; while plasma levels of TAGs and total cholesterol were increased in both species, grass carp possessed markedly higher levels than Chinese longsnout catfish following the oral administration of starch. This indicated that grass carp may possess better ability to convert carbohydrate to lipid than Chinese longsnout catfish. Carbohydrates could provide NADPH for lipogenesis and G6PDH is one of the key enzymes in this process. In line with previous studies[39,57,58], elevated activities and expressions of G6PDH induced by carbohydrates were reported in grass carp. However, the absence of a clear effect of oral starch administration on the activity of G6PDH was found in Chinese longsnout catfish. SREBP and ChREBP directly activate the expression of genes related to the synthesis of TAGs and cholesterol[59]. The present study found that the oral administration of starch induced high expression levels of *srebp1* and *chrebp* in grass carp but not in Chinese longsnout catfish. A previous study showed that the expression of *chrebp* was induced in blunt snout bream in response to high dietary levels of carbohydrate[19]. Another study reported that the increased expression of *chrebp* enhanced the expression of target genes involved in glycolysis, glycogen storage and lipogenesis[60]. In the present study, the up-regulated expression of *srebp1* (3 and 6 h), *chrebp* (6 h), *acc* (6 h), *fas* (1, 3 and 6 h) in the livers of grass carp following the oral administration of starch indicated that the process of lipogenesis had been activated. In a previous study, increased levels of glucose led to the increased expression of *srebp1* but not evident in *fas*[14]. In rainbow trout, *srebp1* was up-regulated after the injection of insulin but the expression levels of *g6pd*, *acc* and *fas* remained unchanged[61]. In another study with rainbow trout, the transcription level of *srebp1* did not increase with dietary carbohydrates but the enhanced activity of FAS and mRNA level of *g6pd* were observed[57]. In the present study, although the expression levels of *srebp1* and *chrebp* did not change in the livers of Chinese longsnout catfish, we still observed the up-regulation of *g6pd* (24 h), *acc* (24 and 48 h) and *fas* (3 and 48 h) following the oral administration of starch. Increased relative expression levels of *acca* (acetyl-CoA carboxylase α) and *fas* have also been reported in tilapia following glucose injection and feeding[34,54]. These results indicated that glucose-induced lipogenesis plays an important role in glucose metabolism but still needs more attention in different fish.

Conclusions

The ongoing investigations into the multiple ways of carbohydrate utilisation emphasise the need to understand the difference in glucose homoeostasis between different fish. In the present study, grass carp, but not Chinese longsnout catfish, exhibited a rapid and significant increase in plasma glucose following the oral administration of starch. This was further supported by the induction of amylase activity in this species. The present findings also suggested that grass carp exhibited a faster clearance rate of plasma glucose than Chinese longsnout catfish. This finding was related to the significant inhibition of gluconeogenesis and enhanced levels of glycolysis, hepatic glycogen metabolism and glucose-induced lipogenesis in grass carp. The present study indicated that grass carp and Chinese longsnout catfish, species of fish with significantly different requirements for dietary carbohydrate, exhibited clear differences in glucose homoeostasis and carbohydrate metabolism and with regard to digestion, transportation, gluconeogenesis, glycolysis, glycogen synthesis and lipogenesis. The present study provides a good reference for glucose homoeostasis, especially with the model of Chinese longsnout catfish.

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D. H. designed the experiment. J. S. performed the experiment and prepared the manuscript under the supervision of D. H. Y. G., L. M. and L. X. contributed to data analysis. Y. Y. helped with chemical analysis. S. C., J. J., H. L., X. Z. and S. X. provided suggestions on the experimental design and the manuscript.

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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