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Metabolic adaptation to high-starch diet in largemouth bass (*Micropterus salmoides*) was associated with the restoration of metabolic functions via inflammation, bile acid synthesis and energy metabolism

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Abstract

A short-term 2-week (2w) and long-term 8-week (8w) feeding trial was conducted to investigate the effects of low-starch (LS) and high-starch (HS) diets on the growth performance, metabolism and liver health of largemouth bass (*Micropterus salmoides*). Two isonitrogenous and isolipidic diets containing two levels of starch (LS, 9·06 %; HS, 13·56 %) were fed to largemouth bass. The results indicated that HS diet had no significant effects on specific growth rate during 2w, whereas significantly lowered specific growth rate at 8w. HS diet significantly increased hepatic glycolysis and gluconeogenesis at postprandial 24 h in 2w. The hepatosomatic index, plasma alkaline phosphatase, total bile acid (TBA) levels, and hepatic glycogen, TAG, total cholesterol, TBA, and NEFA contents were significantly increased in the HS group at 2w. Moreover, HS diet up-regulated fatty acid and TAG synthesis-related genes and down-regulated TAG hydrolysis and β-oxidation-related genes. Therefore, the glucolipid metabolism disorders resulted in metabolic liver disease induced by HS diet at 2w. However, the up-regulation of bile acid synthesis, inflammation and energy metabolism-related genes in 2w indicated that largemouth bass was still in a state of 'self-repair' response. Interestingly, all the metabolic parameters were returned to homoeostasis, with up-regulation of intestinal glucose uptake and transport-related genes, even hepatic histopathological analysis showed no obvious abnormality in the HS group in 8w. In conclusion, HS feed induced short-term acute metabolic disorder, but long-term metabolic adaptation to HS diet was related to repairing metabolism disorders via improving inflammatory responses, bile acid synthesis and energy metabolism. These results strongly indicated that the largemouth bass owned certain adaptability to HS diet.

Key words: **Largemouth bass: High starch: Glucose metabolism: Lipid metabolism: Metabolic liver disease**

In general, carnivorous fish are characterised by a limited ability to utilise starch as energy source and thus are considered as glucose-intolerant[1]. Long-term intake of excess dietary starch can induce hyperglycaemia, hepatic glycogen and lipid accumulation, and chronic inflammation response, as well as reduce the immune functions and antioxidant capabilities, which may lead to metabolic liver diseases (MLD)[2–5]. Therefore, reducing the starch inclusion in carnivorous fish feed has become a trend to ensure its growth performance and health for many aquatic enterprises. However, the LS inclusion has made a challenge for the processing of floating feeds and product durability, which increases the energy consumption of processing and feed costs[6].

Until now, numerous strategies have been used to improve the starch utilisation in fish, such as nutritional programming, genetic selection, exogenous enzymatic additives, macronutrient balance and the modified gut microbiome affected by tailored diets[7–10]. Several studies showed that carnivorous fish own an adaptive mechanism in response to the fluctuation of dietary starch levels within a certain range in American eel (*Anguilla rostrata*)[11], European sea bass (*Dicentrarchus labrax*)[12], rainbow trout (*Oncorhynchus mykiss*)[13,14], White sturgeon (*Acipenser transmontanus*)[15], Siberian sturgeon (*Acipenser baerii*)[9] and Japanese flounder (*Paralichthys olivaceus*)[16,17]. Generally, fish can improve hepatic glycogen synthesis and glycolysis and reduce gluconeogenesis to alleviate the stress induced by intake of excess starch diets[3,12]. Moreover, excess starch diets provide NADPH or carbon backbones for *de novo* lipogenesis and even induce an increase of lipogenic enzymatic activity and lipid accumulation in the liver[16,18]. Adaptation of

Abbreviations: CAT, catalase; GLUT2, glucose transporter type 2; HS, high starch; LS, low starch; MDA, malondialdehyde; MLD, metabolic liver disease; PAS, periodic acid Schiff; ROS, reactive oxygen species; SOT, superoxide dismutase; TBA, total bile acid; TC, total cholesterol; TP, total protein; 2w, 2weeks; 8w, 8 weeks.

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hepatic enzymes to dietary starch levels has been reported consistently for fish species[19–21]. Moreover, Fu and Xie[22] found that southern catfish (*Silurus meridionalis*) could oxidise unwanted assimilated starch to accelerated energy expenditure by increasing oxygen consumption and fasting metabolic rate during starch overfeeding. Potential flexibility in glucose utilisation also existed in Japanese flounder in the absence of energy[16]. The above results indicated that carnivorous fish had certain adaptability to high starch (HS).

Largemouth bass (*Micropterus salmoides*) is a commercially carnivorous species for freshwater culture with a high economic value in China[23]. MLD is prevalently induced by HS diet in largemouth bass[1]. Previous studies demonstrated the dietary starch level should be less than 10 % to ensure growth performance and liver health[5,24,25], so the starch levels of commercial feed formulations were maintained less than 10 %. To our knowledge, information regarding the changes in intermediate metabolism and molecular adaptation of largemouth bass to HS diet has not been reported. Thus, the objective of the present study was to ascertain if largemouth bass owned certain adaptability to HS. The results of this study would provide insight into glucolipid and energy metabolism in largemouth bass in response to HS diet and shed light on the adaptative mechanism of HS in carnivorous fish.

Material and methods

During the whole experimental period, the fish were maintained in compliance with the Laboratory Animal Welfare Guidelines of China (General Administration of Quality Supervision, Inspection, and Quarantine of the People's Republic of China, Standardization Administration of China, GB/T 35892-2018).

Growth trial and sample collection

Two isonitrogenous and isolipidic experimental diets with 9·06 % (LS) and 13·56 % (HS) starch were prepared, respectively. All ingredients were grinded and passed a 180-μm siever. Stuffs of each diet were well mixed with 13–15 % water for 20 min by a mixer (CH-100, The New Standard Powder Machinery Manufacturing Co., Ltd), then was processed into 3-mm diameter floating pellets under the following extrusion condition: feeding section (90°C/5 s), compression section (130°C/5 s) and metering section (150°C/4 s) using a Twin-screwed extruder (EXT50A, YANGGONG MACHINE, China). All diets were air-dried at room temperature and stored at −20°C until use. The diets formulations and analysed chemical compositions are shown in Table 1.

Largemouth bass was obtained from the commercial Aquafarm. Before the formal feeding trial, fish were acclimatised and fed LS diet with a rate equalling 2 % of wet body weight per d for 2 weeks (2w). Fish (initial body weight was 47·60 ± 0·20 g) were distributed into sixteen cylindrical plastic tanks (capacity: 256 l) with twenty-five fish per tank after 24-h starvation, and each diet was randomly assigned to eight tanks. Fish were fed to apparent satiation twice daily at 08:00 h and 17:00 h. During the experiment, the water temperature was maintained at 21–25°C, pH = 7·2–8·0, dissolved oxygen > 6·0 mg/l and ammonia-N < 0·3 mg/l.

Table 1. Formulation and composition of experimental diets (%)

Ingredients	LS	HS
Fishmeal*	40·0	40·0
Tapioca starch†	5·0	5·0
Wheat flour†	9·1	16·6
Wheat gluten meal‡	4·0	4·0
Cottonseed protein concentrate§	4·0	6·0
Soyabean protein concentrate‖		6·0
Soyabean meal¶	22·0	8·0
Spay-dried blood cell powder	3·0	3·0
Krill meal	1·0	1·0
$Ca(H_2PO_4)_2$	1·0	1·0
Lecithin oil	2·0	2·0
Fish oil*	3·0	3·0
Soyabean oil‖	3·0	3·0
Kelp powder	1·5	
Vitamin and mineral premix**	1·4	1·4
Total	100	100
Analysed chemical composition (DM basis %)		
Moisture	6·25	6·93
Crude ash	10·2	9·7
Crude protein	52·55	51·87
Crude lipid	11·52	11·30
Starch††	9·06	13·56
Gross energy (MJ/kg)	22·09	22·28

LS, low starch; HS, high starch.
Mineral premix (mg/kg diets): $CuSO_4·5H_2O$ 10; $FeSO_4·H_2O$ 300; $ZnSO_4·H_2O$ 200; $MnSO_4·H_2O$ 100; KI (10 %) 80; $CoCl_2·6H_2O$ (10 %Co) 5; Na_2SeO_3 (10 % SE) 10; $MgSO_4·5H_2O$ 2000; NaCl 100; zeolite 4995; antioxidant 200.
* Fishmeal and fish oil were purchased form Triple Nine Fish Protein Co. Ltd.
† Tapioca starch and wheat flour were purchased from Beijing Nankou Flour Mill.
‡ Wheat gluten meal was purchased from Guanxian Xinrui Industrial Co., Ltd.
§ Cottonseed protein concentrate was purchased from Xinjiang Jinlan plant protein Co., Ltd.
‖ Soyabean protein concentrate and soyabean oil were purchased from Yihai Kerry Investment Co. Ltd.
¶ Soyabean meal was purchased from Qingdao Bohai Biotechnology Co., Ltd.
** Vitamin premix (mg/kg diets): vitamin A 20; vitamin D_3 10; vitamin K_3 20; vitamin E 400; vitamin B_1 10; vitamin B_2 15; vitamin B_6 15; vitamin B_{12} (1 %) 8; ascorbic acid (35 %) 1000; calcium pantothenate 40; niacinaminde 100; inositol 200; biotin (2 %) 2; folic acid 10; corn gluten meal 150; choline chloride (50 %) 4000.
†† Starch content was estimated based on the starch content of tapioca starch (72 % starch) and wheat flour (60 % starch).

Fish were anesthetised with 200 mg/l of MS-222 (Sigma) at the end of the 2w or 8 weeks (8w) before sampling. Individual body weight, body length and liver weight of five fish in each tank after starvation for 24 h were recorded to calculate condition factor and hepatosomatic index. Blood was rapidly sampled from the caudal vein, centrifuged (4000 *g*, 10 min, 4°C) to obtain plasma for the analysis of haematological parameters at postprandial 3 h and 24 h. Liver, anterior intestine and muscle samples at postprandial 24 h were dissected and then immediately frozen in liquid N_2 and kept at −80°C for mRNA isolation and tissue homogenate analysis until used. Three liver samples near the bile duct in each tank were fixed in 4 % paraformaldehyde (P1110, Solarbio) for histology determination. Three liver samples near the bile duct in each treatment were fixed in 2·5 % glutaraldehyde (P1126, Solarbio) for ultrastructure analysis. Livers from another three fish in each tank were pooled into ziplock bags and then stored at −20°C for the assay of crude lipid.

Chemical compositions analysis of diets

The crude protein, crude lipid, crude ash, moisture and gross energy contents of experimental diets were analysed according

to standard methods as previously described materials and methods section[26,27].

Plasma and hepatic homogenate parameters

Plasma alanine aminotransferase (C009–2–1), aspartate aminotransferase (C010–2–1), total protein (A045–2–2), albumin (H127–1–2), glucose (361 500), TAG (A110–2–1), total cholesterol (TC, A111–2), alkaline phosphatase (A059–2–2), total bile acid (TBA, E003–2–1), LDL-cholesterol (A113–2), HDL-cholesterol (A112–2) and hepatic TAG, TC, TBA, HDL-cholesterol, glycogen (A043), superoxide dismutase (SOD, A001–3–2), catalase (CAT, A007–1–1), malondialdehyde (MDA, A003–1–2) and intestinal amylase (C016–1–1) were determined by commercial assay kits (Nanjing Jiancheng Co.) following the protocols. The glucagon (MM-3294801), insulin (MM-190901), cyclic-AMP (MM-3259102) and reactive oxygen species (ROS, MM-091101) were determined by the commercial kits (Jiangsu Meimian industrial Co. Ltd). Plasma and hepatic NEFA (KRB0081) were measured by assay kit of Wako Pure Chemical Industries, Ltd (Wako).

Hepatic histopathological and immunofluorescence examination

Liver samples were fixed, dehydrated, embedded, stained for haematoxylin and eosin, periodic acid Schiff (PAS) or sirius red and observed by light microscopy (DM2500, Leica) according to methods of our laboratory as previously described[26,27]. The results of haematoxylin –eosin and PAS staining were observed by light microscopy (DM2500, Leica). The sample treatment and transmission electron microscopy observation were conducted according to the methods described by Lu et al.[28]. The immunofluorescence test for cleaved caspase 3 were following the previously described[26,27]. Anti-cleaved caspase 3 (ab13847, Abcam) was used as the primary antibody. Alexa Flour 555 antibody (A21428, Life Technologies) was used as the secondary antibody. The fluorescent signal was captured using Zeiss LSM700.

Quantitative real-time PCR

Total RNA extraction and cDNA synthesis were carried out as described previously[26,27]. The quantitative PCR analysis was performed using a CFX96™ Real-Time System (Bio-Rad) using iTaq™ Universal SYBR® Green Supermix (1 725 121, Bio-Rad). Each sample was run in triplicate and analysed using the $2^{-\Delta\Delta Ct}$. Elongation factor 1α, *EF1α* (GenBank accession no. KT827794), was used as an endogenous reference gene. The primer sequences are shown in Table 2.

Statistical analysis

All statistical procedures were performed with the aid of the SPSS software version 22.0 for Windows (IBM Inc.). After the homogeneity of the variances was tested, all data means were analysed. An independent *t* test was used to compare the differences between the two groups. Two-way ANOVA was used to analyse the significant differences among treatment means based on postprandial times, starch levels and their interactions for plasma glucose, insulin and glucagon levels. Statistical significance was determined at $P < 0{\cdot}05$. All results were presented as standard error of the mean, and the graphics were drawn by GraphPad Prism Software version 7.0.

Results

Growth performance and morphometric parameters

The results of growth performance and morphometric parameters are presented in Table 3. The SR of largemouth bass in all groups was above 99 %. No significant differences were observed on final body weight, specific growth rate, feed conversion ratio, feeding rate and condition factor between the LS and HS groups at 2w. However, the HS diet improved hepatosomatic index significantly at 2w. During 8w, the final body weight and specific growth rate significantly decreased in the HS group, but the feed conversion ratio, feeding rate, condition factor and hepatosomatic index were not significantly affected.

Haematological parameters and hepatic antioxidant responses

The haematological parameters and hepatic antioxidant responses of largemouth bass are presented in Table 4, 5 and 6. The plasma glucose levels after postprandial 24 h at 2w or 8w in the LS or HS group were significantly lower than that postprandial 3 h, and the glucagon levels were significantly higher than that postprandial 3 h, but no significant differences were observed in insulin levels. HS diet significantly increased plasma glucose levels at 3 h postprandial at 8w compared with the LS diet (Table 4). No significant differences were detected in plasma total protein, albumin, IgM, alanine aminotransferase and aspartate aminotransferase between LS and HS groups at postprandial 24 h at 2w or 8w. Plasma alkaline phosphatase and TBA contents enhanced significantly in the HS group at postprandial 24 h at 2w, but no significant differences were observed at 8w (Table 5). The levels of plasma ROS, hepatic ROS and MDA in the HS group were markedly higher than LS group at 2w, whereas the opposite was true for the SOD and CAT. At 8w, no significant differences were observed in plasma ROS, hepatic ROS and MDA levels between two groups (Table 6).

Hepatic glucose metabolism, intestinal glucose transporter and amylase activity

At 2w, the mRNA levels of both glycolysis (*GK* (glucokinase) and *PK* (pyruvate kinase)) and gluconeogenesis-related genes (*PCK* (phosphoenolpyruvate carboxykinase cytosolic) and *G6Pase* (glucose-6-phosphatase catalytic subunit)) were significantly up-regulated in the HS group, but no significant differences were observed at 8w (Fig. 1(a) and (b)). Meanwhile, enzyme activities of hepatic GK, G6Pase and PCK in the HS group at 2w were also significantly increased, but no significant differences were observed at 8w (Fig. 1(d)–(f)). The mRNA levels of pyruvate dehydrogenase genes (*PDHA* and *PDHB*) related to aerobic oxidation were significantly up-regulated at 2w, while they did not demonstrate any change at 8w (Fig. 1(c)).

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Table 2. Primer sequences used in this study

Genes	Forward primer (5'-3')	Reverse primer (5'-3')	Tm (°C)	E-values (%)
EF1α	TGCTGCTGGTGTTGGTGAGTT	TTCTGGCTGTAAGGGGGCTC	60·4	102·8
GK	ACAGAGTGGTGGACGAGACC	TCGTTCACCAGCTTCATCAG	60	102·6
PK	CCTATCGGAATTGCACTGGA	TTCTTGTAGTCGAGCCAGAG	60·5	99
PCK	TGCTTGACTGGATGTTCAGG	TTCCTCACCTCATCCACCTC	59·3	94·5
G6Pase	GGGAGTCCAGGTGTGTGTCT	CAGCGAAGGAGGTCAAGAAG	56·6	90·9
GLUT2	GAGCCCACGGTACCTTTACA	ACAGAGGAGCGGATCAAAGA	59	96
SGLT1	CTCTGGGGCCATTTTTATCA	GCAAAGGCCATTAGGATGAA	59	92·5
ACC1	ATCCCTCTTTGCCACTGTTG	GAGGTGATGTTGCTCGCATA	57·5	102·2
FASN	TGTGGTGCTGAACTCTCTGG	CATGCCTAGTGGGGAGTTGT	57·5	102·1
LPIN1	TCCTACGTTCCCGAGAGAAA	TACGAGGGAACCACTTCCTG	58·5	98·8
GPAT4	GTGTAAAGGCAGCCATAGCC	GTGCCCACCAGCATTTTACT	58·5	106·9
DGAT1	CACGCCTCTTCTTGGAGAAC	AATGGTACCCACAGCCAGAC	58·5	105·3
ATGL	CCATGATGCTCCCCTACACT	GGCAGATACACTTCGGGAAA	58	99·1
HSL	ATCAGAGCTGGAGCACCCTA	GCAGAGGAGAGCAGAAAGGA	60	99·3
HADH	AACAGGCTGGACAGATTTGG	TCAGGAGGGAATCAAACGTC	59	99·7
ACADM	TGGCTGAGATGGCAATGAAGG	TTGGCGATGGAGGCGTAGTA	60	92
CPT1α	CATGGAAAGCCAGCCTTTAG	GAGCACCAGACACGCTAACA	60·0	98·8
PPARα	CCACCGCAATGGTCGATATG	TGCTGTTGATGGACTGGGAAA	59	104·3
LPL	TTCCTCGACCCTCTGAAAGA	GGAGTCAAGTTTGCCAGGAA	57·5	101·2
PDHA	ACCTACCGCTATCATGGACACA	TCCTTCCTCACCTCCACATCAA	60·5	93
PDHB	TCCAGGTCTGAAGGTTGTGAGT	ATAGTGCCTCCTCCGACATCTC	60	96
HMGCR	GAGGCCACCATACCTGCTAA	AAATCCACCGATGCTACCTG	60	98·9
CYP7A1	CTGGGCTTCACAGGCTAACACC	TTCAGTGTGGGGTCGTTGGG	60·4	102·2
FXR	TAAAAGGCTGCGAAAGAACAC	GGTGGTTGATGTGACCTGTTTGT	60·4	100
CYP8B1	TAGACAGCGGCAACCAGGAG	CCGTGCTTTTGTTTCATCCTATC	60·4	100·8
CS	GCCCTGTATTGCTGCCAAGAT	TGCCTCCTTCATGGTCACTGT	60	96
IDH3a	CCTGTGTGTCTATGGAGGGCTA	CGTTGGCTGGCTTGTTCTGTAA	60·5	90·3
SDHa	GACGGAGTTGACAAGGTGGTTC	TTGTGCTCCTCAGCGATGGT	60	98
SDHb	GCTACTGGTGGAACGGAGACAA	TGGCAGCGATAGAGTGAGAAGG	60	96
CREB	GGAGTCTGTATCGCTCAGCC	ACCAACGTAACTGTGGGACC	60	97
TNFα	CTTCGTCTACAGCCAGGCATCG	TTTGGCACACCGACCTCACC	63	105·7
IL1β	CGTGACTGACAGCAAAAAGAG	GATGCCCAGAGCCACAGTTC	59·4	101·3
TGFβ1	GCTCAAAGAGAGCGAGGATG	TCCTCTACCATTCGCAATCC	59	95·6
IL10	CGGCACAGAAATCCCAGAGC	CAGCAGGCTCACAAAATAAACA	62·1	113·6
Caspase 3	GCTTCATTCGTCTGTGTTC	CGAAAAAGTGATGTGAGGTA	56	94·5
Caspase 8	GAGACAGACAGCAGACAACCA	TTCCATTTCAGCAAACACATC	62·1	103·1
Caspase 9	CTGGAATGCCTTCAGGAGACGG	GGGAGGGGCAAGACAACAGGGT	66	102·2

EF1α, elongation factor-1α; GK, glucokinase; PK, pyruvate kinase; PCK, phosphoenolpyruvate carboxykinase cytosolic; G6Pase, glucose-6-phosphatase catalytic subunit; GLUT2, glucose transporter type 2; SGLT1, sodium/glucose cotransporter 1; ACC1, acetyl-CoA carboxylase 1; FASN, fatty acid synthase; LPIN1, phosphatidate phosphatase1; GPAT4, glycerol-3-phosphate acyltransferase 4; DGAT1, diacylglycerol O-acyltransferase 1; ATGL, adipose triglyceride lipase; HSL, hormone-sensitive lipase; HADH, hydroxyacyl-CoA dehydrogenase; ACADM, acyl-CoA dehydrogenase medium chain; CPT1α, carnitine palmitoyltransferase 1α; PPARα, peroxisome proliferator-activated receptor α; LPL, lipoprotein lipase; PDH, pyruvate dehydrogenase E1 subunit (α or β); HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; CYP7A1, cytochrome P450 family 7 subfamily a member 1; FXR, farnesoid X-activated receptor; CS, citrate synthase; IDH3a, isocitrate dehydrogenase 3 (NAD^+) alpha; SDH, Succinate Dehydrogenase Complex Iron Sulfur Subunit; CREB, cAMP-responsive element binding protein; TGFβ1, transforming growth factor β1.

The mRNA levels of intestinal Na^+/glucose cotransporter type 1 (*SGLT1*) were significantly up-regulated in the HS group at 2w, but no significant differences were observed in glucose transporter type 2 (*GLUT2*) mRNA levels and amylase activity. At 8w, HS diet significantly increased intestinal *GLUT2* and *SGTL1* mRNA levels, and amylase activity (Fig. 1(g) and (h)). At 2w, the hepatic *GLUT2* mRNA levels were significantly up-regulated in the HS group, but no significant differences were observed at 8w (Fig. 1(i)).

Hepatic lipid and total bile acid metabolism

As shown in Fig. 2, hepatic lipid metabolism disorder with the symptoms of TAG and TC accumulation appeared in largemouth bass fed HS diet at 2w. Compared with the LS group, lower plasma TAG and higher hepatic NEFA and TAG levels were observed in the HS group at 2w (Fig. 2(a) and (d)). Meanwhile, the mRNA levels of hepatic fatty acid (*ACC1* (acetyl-CoA carboxylase 1) and fatty acid synthase (*FASN*)) and TAG synthesis (*GPAT4* (glycerol-3-phosphate acyltransferase 4) and *LPIN1* (phosphatidate phosphatase1))-related genes were significantly up-regulated, while TAG hydrolysis (*ATGL* (adipose triglyceride lipase) and *LPL* (lipoprotein lipase)) and β-oxidative (*HADH* (hydroxyacyl-CoA dehydrogenase), *ACADM* (acyl-CoA dehydrogenase medium chain) and *CPTα* (carnitine palmitoyltransferase 1α)) genes were significantly down-regulated (Fig. 2(a)). The accumulation of hepatic TC induced by HS was accompanied with higher plasma HDL-cholesterol levels (Fig. 2(d)) and up-regulated *HMGCR* (3-hydroxy-3-methylglutaryl-CoA reductase) mRNA levels (Fig. 3(a)). Moreover, up-regulated mRNA levels of bile acid synthesis gene *CYP7A1* (cytochrome P450 family 7 subfamily a member 1) and increased contents of TBA in the liver were also induced by HS diet at 2w (Fig. 3).

Interestingly, the symptoms of TAG, TC and TBA accumulation symptoms induced by HS diet could be alleviated or disappear in largemouth bass at 8w (Figs. 2 and 3).

Table 3. Effects of HS diet on the growth performance and morphometric parameters of largemouth bass at 2w and 8w (Mean values with their standard errors of the mean, *n* 4)

	2w					8w				
	LS		HS			LS		HS		
	Mean	SEM	Mean	SEM	*P*	Mean	SEM	Mean	SEM	*P*
IBW*	47·61	0·02	47·61	0·02	–	47·60	0·00	47·60	0·00	–
FBW†	81·71	1·12	80·4	1·32	0·491	170·09	5·82	155·98	1·35	0·043
SR‡	99	1·00	100	0·00	0·391	99	1·00	100	0·00	0·391
SGR§	3·78	0·08	3·74	0·12	0·485	2·09	0·05	1·95	0·01	0·045
FCR‖	0·85	0·05	0·86	0·02	0·868	0·93	0·01	0·95	0·01	0·087
FR¶	0·80	0·07	0·79	0·02	0·757	0·42	0·01	0·42	0·00	0·387
CF**	2·25	0·08	2·12	0·09	0·416	1·86	0·03	1·86	0·03	0·969
HSI††	1·67	0·08	2·24	0·16	0·007	1·73	0·07	1·78	0·09	0·624

HS, high starch; LS, low starch; IBW, initial mean weight; FBW, final body weight; SR, survival rate; SGR, specific growth rate; FCR, feed conversion ratio; FR, feeding rate; CF, condition factor; HIS, hepatosomatic index.
* IBW (g/fish): *n* 4.
† FBW (g/fish): *n* 4.
‡ SR (%) = 100 × final fish number/initial fish number, *n* 4.
§ SGR (%/d) = 100 × (Ln (W_f/W_i))/d, *n* 4.
‖ FCR = feed intake/($W_f + W_d$-W_i), *n* 4.
¶ FR (% bw/d) = 100 × feed intake/[($W_f + W_i + W_d$)/2]/d n 4.
** CF (g/cm^3) = 100 × (body weight, g)/(body length, cm)^3, *n* 20.
†† HSI (%) = 100 × liver weight/whole body weight, *n* 20. W_f is the final total weight, W_d is the total weight of dead fish, W_i is the initial total weight.

Table 4. Effects of HS diet on plasma glucose, insulin and glucagon of largemouth bass at postprandial 3 h or 24 h at 2w and 8w (Mean values with their standard errors of the mean, *n* 8)

			Postprandial time				Two-way ANOVA		
			3 h		24 h				
			Mean	SEM	Mean	SEM	Starch levels	Postprandial times	Interaction
Glucose (mmol/l)	2w	LS	6·88	0·41	5·33	0·39	0·264	< 0·001	0·073
		HS	8·25	0·38	5·01	0·36			
	8w	LS	8·13	0·51	4·94	0·29	0·005	< 0·001	0·154
		HS	10·50	0·63	5·76	0·37			
Insulin (nmol/l)	2w	LS	45·74	2·64	43·96	3·04	0·715	0·582	0·999
		HS	44·57	2·57	42·78	4·25			
	8w	LS	44·60	1·20	42·75	1·77	0·760	0·112	0·621
		HS	45·91	1·56	42·44	1·88			
Glucagon (ng/l)	2w	LS	44·57	2·07	53·16	1·72	0·509	< 0·001	0·859
		HS	43·70	1·90	51·65	1·36			
	8w	LS	41·16	1·95	52·10	1·47	0·236	< 0·001	0·853
		HS	42·71	1·20	54·21	1·35			

HS, high starch; LS, low starch.

Table 5. Effects of HS diet on plasma immune and hepatic function parameters of largemouth bass at postprandial 24 h at 2w and 8w (Mean values with their standard errors of the mean, *n* 8)

	2w					8w				
	LS		HS			LS		HS		
	Mean	SEM	Mean	SEM	*P*	Mean	SEM	Mean	SEM	*P*
Immune parameters										
TP (g/l)	36·63	1·69	34·43	1·09	0·292	35·02	2·05	36·35	1·20	0·584
Albumin (g/l)	11·14	0·57	10·79	0·49	0·582	11·63	0·68	11·36	0·45	0·492
IgM (mg/l)	21·47	2·43	23·35	1·08	0·499	21·42	1·20	21·81	0·94	0·804
Liver functions										
ALT/ (U/L)	20·84	2·83	19·60	2·34	0·740	12·88	1·58	13·01	2·28	0·961
AST/ (U/L)	22·33	3·06	23·36	3·35	0·822	20·03	2·27	20·75	2·25	0·824
AKP (U/L)	185·76	13·18	243·38	17·78	0·018	104·37	5·02	104·82	8·59	0·964
TBA (μmol/l)	8·82	0·37	11·45	1·06	0·035	10·56	0·40	12·14	1·39	0·307

HS, high starch; LS, low starch; TP, total protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AKP, alkaline phosphatase; TBA, total bile acid.

Table 6. Effects of HS diet on antioxidant responses of largemouth bass at postprandial 24 h at 2w and 8w (Mean values with their standard errors of the mean, *n* 8)

	2w					8w				
	LS		HS			LS		HS		
	Mean	SEM	Mean	SEM	*P*	Mean	SEM	Mean	SEM	*P*
Haematological parameters										
ROS (U/ml)	406·09	21·07	477·81	24·85	0·045	474·48	24·25	515·39	17·23	0·191
Hepatic parameters										
ROS (U/mg prot)	5·08	0·13	6·53	0·42	0·013	7·63	0·42	8·17	0·36	0·346
MDA (nM/mg prot)	3·08	0·43	5·53	0·56	0·004	5·13	0·55	5·17	0·22	0·945
SOD (U/mg prot)	187·40	11·13	124·71	11·57	0·002	168·89	9·71	161·83	10·34	0·626
CAT (U/mg prot)	20·12	2·79	13·60	1·01	0·045	19·79	2·17	18·70	2·13	0·725

HS, high starch; LS, low starch; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase.

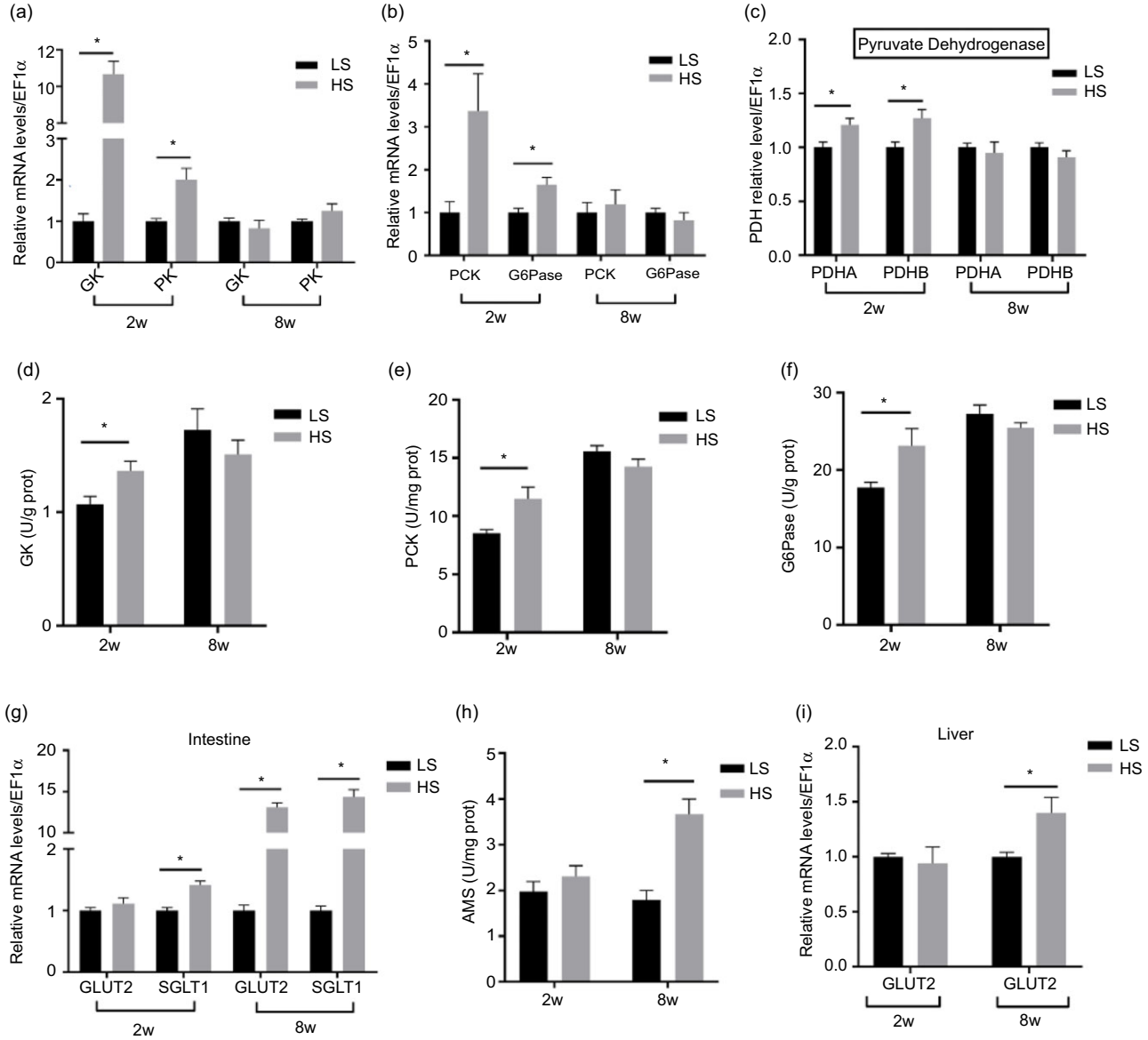

Fig. 1. Effects of HS diet on hepatic glucose metabolism, intestinal glucose transporter and amylase activity of largemouth bass at postprandial 24 h at 2w and 8w. (a) Transcriptional levels of hepatic *GK* and *PK*. (b) Transcriptional levels of hepatic *PCK* and *G6Pase*. (c) Transcriptional levels of pyruvate dehydrogenase-related genes (*PDHA* and *PDHB*). (d) Hepatic GK activity. (e) Hepatic PCK activity. (f) Hepatic G6Pase activity. (g) Transcriptional levels of intestinal *GLUT2* and *SGLT1*. (h) Intestinal amylase activity. (i) Transcriptional levels of hepatic *GLUT2*. Values marked with '*' are significant differences ($P < 0{\cdot}05$) (*n* 8). *GK*, glucokinase; *PK*, pyruvate kinase; *PCK*, phosphoenolpyruvate carboxykinase cytosolic; *G6Pase*, glucose-6-phosphatase catalytic subunit; *PDH*, pyruvate dehydrogenase E1 subunit (α or β); *GLUT2*, glucose transporter type 2; *SGLT1*, sodium/glucose cotransporter 1.

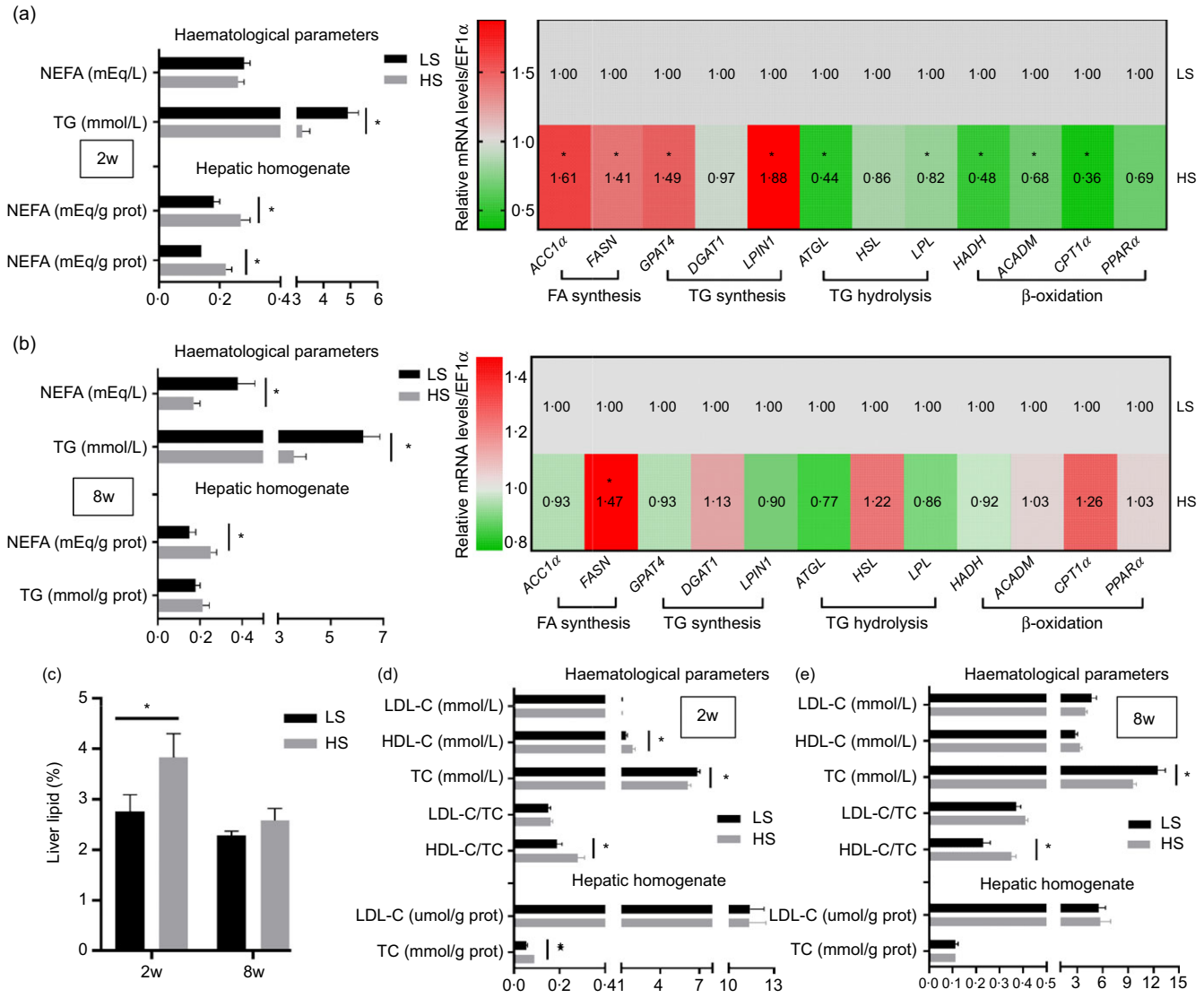

Fig. 2. Effects of HS diet on hepatic lipid metabolism of largemouth bass at postprandial 24 h at 2w and 8w. Plasma NEFA and TAG levels, hepatic NEFA and TAG contents, and transcriptional levels of hepatic FA synthesis (*ACC1* and *FASN*), TAG synthesis (*GPAT4*, *DGAT1* and *LPIN1*), TAG hydrolysis (*ATGL*, *HSL* and *LPL*) and *β*-oxidation (*HADH*, *ACADM*, *CPT1α* and *PPARα*)-related genes at 2w (a) and 8w (b). (c) Liver lipid contents. Plasma LDL-cholesterol, HDL-cholesterol and TC levels, and hepatic LDL-cholesterol and TC contents at 2w (d) and 8w (e). Values marked with '*' are significant differences ($P < 0.05$) (n 8). HS, high starch; *ACC1*, acetyl-CoA carboxylase 1; *FASN*, fatty acid synthase; *GPAT4*, glycerol-3-phosphate acyltransferase 4; *DGAT1*, diacylglycerol O-acyltransferase 1; *LPIN1*, phosphatidate phosphatase1; *ATGL*, adipose triglyceride lipase; *HSL*, hormone-sensitive lipase; *LPL*, lipoprotein lipase.

Hepatic inflammatory and apoptosis responses along with histological analysis

As shown in Fig. 4(a), three typical hepatic phenotypes were observed with symptoms from light to severe by haematoxylin–eosin staining, PAS staining, sirius red staining and apoptosis signals of cleaved caspase 3 in which (I) no obvious abnormality phenotype with shaped hepatocytes and clearly cell nuclei, and with the negative response to cleaved caspase 3; (II) nuclear dense phenotype, with unclear liver cord, which is usually a precursor to liver fibrosis in the clinic with increased micro-vascular collagen and highlighted cleaved caspase 3 signal; and (III) hepatic fibrosis symptoms, along with increased collagen fibres in red by sirius staining and intensive apoptosis (cleaved caspase 3) around the fibrosis tissues. At 2w, two samples were observed nuclear dense, and two samples with serious fibrosis symptoms in the HS group, while eleven samples were no obvious abnormality in the LS group. At 8w, all the sections were no obvious abnormality in the two groups. The PAS staining showed that hepatic glycogen content was increased as the grade of liver disease aggravated. Moreover, HS-induced hepatic glycogen contents significantly increased at 2w, while no significant difference was observed at 8w (Fig. 4b and c). Ultrastructural observation showed that no obvious abnormal hepatocytes exhibited normal euchromatic nucleus, large stacks of the endoplasmic reticulum (ER), abundant mitochondria with intact cristae and uniformly distributed glycogen granules in the cytoplasm; however, hepatocytes of liver fibrosis revealed extensive cellular damage, including irregular nuclear envelope and dense clumped chromatin, a lot of fat droplets, disruption and swelling of ER membranes, mitochondria with broken cristae (Fig. 4(d)).

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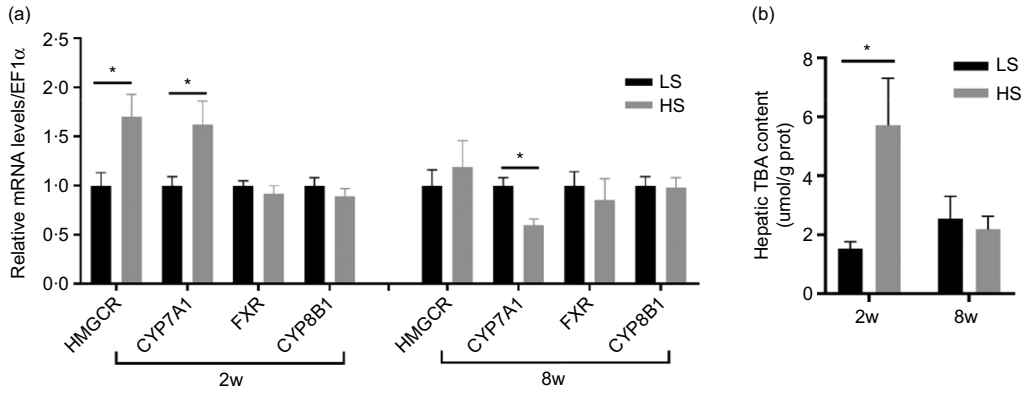

Fig. 3. Effects of HS diet on hepatic TBA metabolism of largemouth bass at postprandial 24 h at 2w and 8w. (a) Transcriptional levels of hepatic cholesterol synthesis (*HMGCR*) and bile acid synthesis (*CYP7A1*, *FXR* and *CYP8B1*)-related gene. (b) The hepatic TBA contents. Values marked with '*' are significant difference ($P < 0.05$) (n 8). LS, low starch; HS, high starch; TBA, total bile acid; *HMGCR*, 3-hydroxy-3-methylglutaryl-CoA reductase; *CYP7A1*, cytochrome P450 family 7 subfamily a member 1; *FXR*, farnesoid X-activated receptor; *CYP8B1*, Cytochrome P450 Family 8 Subfamily B Member 1.

The mRNA levels of both pro- (*TNFα* and *IL1β*) and anti-inflammatory cytokines (*IL10* and *TGFβ1* (transforming growth factor *β*1)) were significantly up-regulated in the HS group at 2w, but no significant differences were observed in *IL1β*, *IL10* and *TGFβ1* mRNA levels at 8w (Fig. 4(e)). Compared with 2w, the mRNA levels of HS-induced *TNFα* were alleviated at 8w, while still significantly higher than the LS group at 8w (Fig. 4(e)).

The mRNA levels of apoptosis-related genes (*Caspase 8* and *Caspase 9*) were significantly up-regulated in the HS group at 2w, while no significant differences were observed between LS and HS groups at 8w. No significant differences were detected on *Caspase 3* mRNA levels in the two groups at 2w or 8w (Fig. 4(f)). These results showed that HS might induce short-term hepatic inflammatory and apoptosis responses.

Energy metabolism

Accelerated energy metabolism rate in largemouth bass fed HS diet was observed at 2w (Fig. 5). The significantly increased plasma and hepatic cyclic-AMP contents (Fig. 5(a) and (b)) and up-regulated mRNA levels of the *CREB* (cAMP-responsive element binding protein) (Fig. 5(c)) promoted the energy metabolism by accelerating the glycolysis and gluconeogenesis processes in the HS group at 2w (Fig. 1(a)). The mRNA levels of tricarboxylic acid cycle-related genes (*IDH3a* (isocitrate dehydrogenase 3 (NAD^+) alpha), *SDHa* and *SDHb* (Succinate Dehydrogenase Complex Iron Sulfur Subunit)) were significantly up-regulated in the HS group at 2w. After 8w feeding, the mRNA levels of HS-induced *IDH3a* were mitigated compared with 2w, while still significantly higher than the LS group, but the mRNA levels of *SDHa* and *SDHb* had no significant differences between the two groups (Fig. 5(d)).

Discussion

Generally, carnivorous fish are known as glucose-intolerant with persistent hyperglycaemia after intake of HS diet[2,29]. Insulin and glucagon are two important endocrine hormones to regulate the glucose homoeostasis of fish by controlling the plasma glucose levels[3]. Increased plasma glucose levels can stimulate insulin or reduce glucagon secretion to relieve hyperglycaemia stress for maintaining glucose homoeostasis[2]. Lin et al.[5] showed that 20 % wheat starch diets induced a significant rise in plasma glucose and insulin levels at postprandial 6 h in largemouth bass. In contrast, the present study investigated HS significantly increased plasma glucose levels at postprandial 3 h, while it did no affect plasma insulin levels. Similar results also were found in eel[30], silver sea bream (*Sparus sarba*)[31] and common dentex (*Dentex dentex*)[32]. Our previous studies showed plasma insulin levels remained unchanged except at 1 h in largemouth bass after a glucose load[33]. However, after bovine insulin injection, the plasma glucose levels of largemouth bass significantly decreased, then restored to the basic values in 6 h, which further confirmed that insufficient secretion of insulin was the main reason for glucose intolerance[33].

The absorption of glucose by the blood from the enteric cavity is facilitated by two key glucose transporters, *SGLT1* and *GLUT2*[34]. In addition, *GLUT2* can affect the capacity of the glucose transfer between liver and blood[8]. Several studies had indicated that dietary starch significantly up-regulated *SGLT1* expression in fish, such as rainbow trout[35] and yellow catfish (*Pelteobagrus fulvidraco*)[36]. The *GLUT2* expression in common carp (*Cyprinus carpio*) foregut was significantly up-regulated after 3 h with a glucose load[37]. HS diet (20 %) significantly up-regulated the expression of hepatic *GLUT2* after postprandial 24 h in rainbow trout[38]. In tilapia (*Oreochromis mossambicus*), glucose injection did not affect hepatic *GLUT2* expression[39]. Until now, the regulatory relationship between starch levels and hepatic *GLUT2* mRNA levels was not clear. Röder et al.[40] and Ghezzi et al.[34] showed that up-regulated mRNA levels of *SGTL1* and *GLUT2* might be critical to the development of glucose tolerance. Increased amounts of glucose transporters implied enhanced capacity of the intestine to transport and absorb glucose[41]. In this study, the intestinal *SGTL1* and *GLUT2* and hepatic *GLUT2* mRNA levels were significantly up-regulated in the HS group at 8w, which strongly indicated that largemouth bass had the adaptability to HS diet. Moreover, the intestinal amylase activity was significantly increased at 8w in response to HS diet. A positive correlation between amylase activity and starch levels was used to evaluate dietary starch

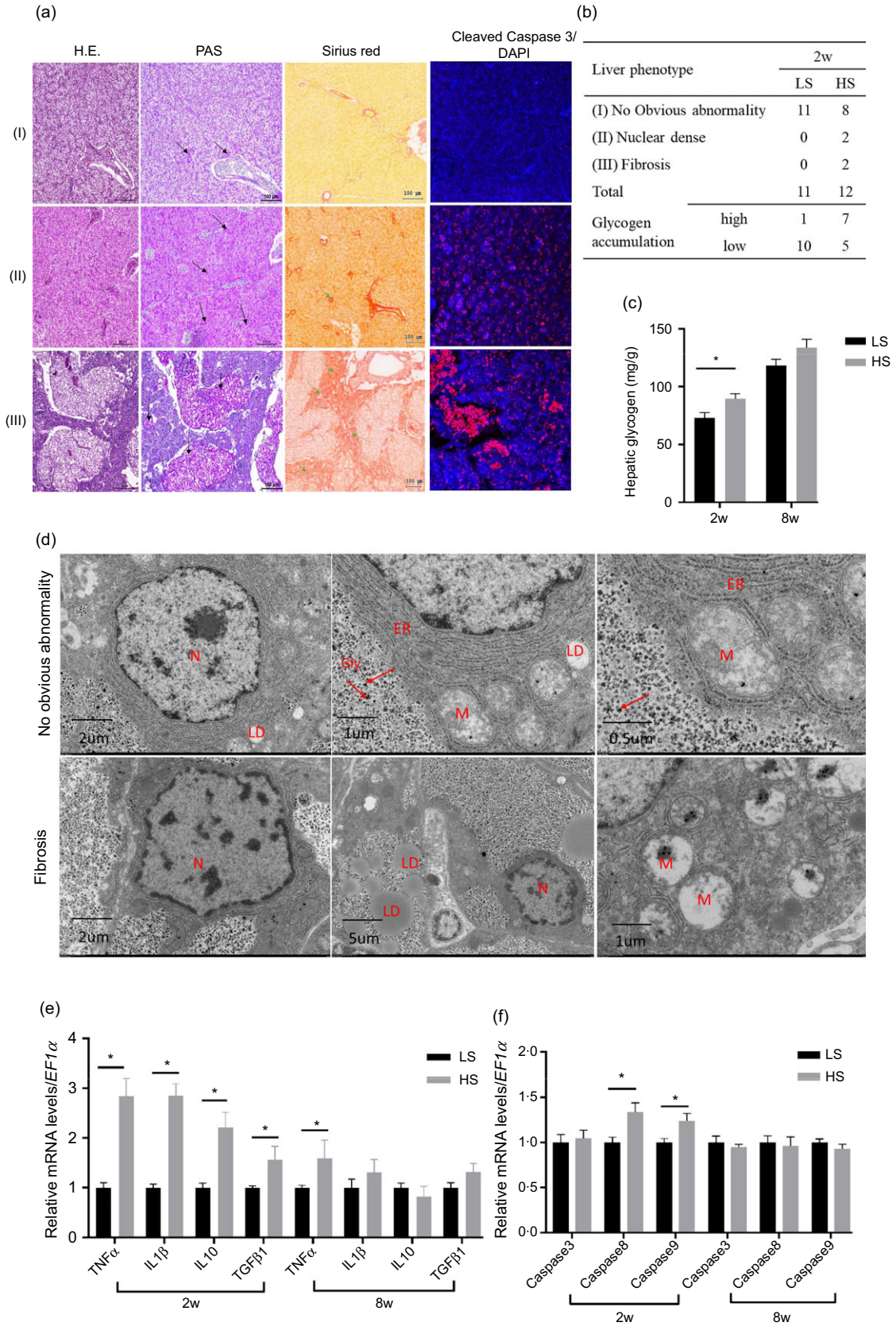

Fig. 4. Effects of HS diet on hepatic histopathological, inflammatory and apoptosis responses of largemouth bass at 2w and 8w. (a) Three phenotypes of hepatic histopathological examination with symptoms from light to heavy by HE staining. PAS staining for glycogen examination (the pink spot represented the glycogen particles marked by black arrows); Sirius red staining for hepatic fibrosis (the red showed collagen fibres marked by green arrow); apoptosis signals of cleaved caspase 3 in red colour, and DAPI for nucleus (bar = 100 μm), in which (I) no obvious abnormality; (II) nuclear dense; and (III) hepatic fibrosis symptoms. (b) Statistical results of liver phenotypes and glycogen accumulation (*n* 12). Since the samples were damaged during the embedding process, the number of slices was less than 12. (c) Hepatic glycogen content (*n* 4). (d) Liver ultrastructure. Note the part of mitochondria (M), nucleus (N), endoplasmic reticulum (ER), glycogen granules (Gly) (red arrows showed) and lipid drop (LD). Fibrosis liver showing severe hepatocyte damage. Observe nucleus with increased heterochromatin patches, irregular nuclear envelope, dense clumped chromatin, disruption and swelling of ER membranes, mitochondria with broken cristae, large lipid droplets. (e) Effects of HS diet on the transcriptional levels of hepatic pro- and anti-inflammation-related genes. (f) Effects of HS diet on the transcriptional levels of hepatic apoptosis-related genes. Values marked with '*' are significant difference ($P < 0.05$) (*n* 8). HE, haematoxylin and eosin; PAS, periodic acid Schiff; DAPI, 4',6-diamidino-2-phenylindole; LS, low starch; HS, high starch.

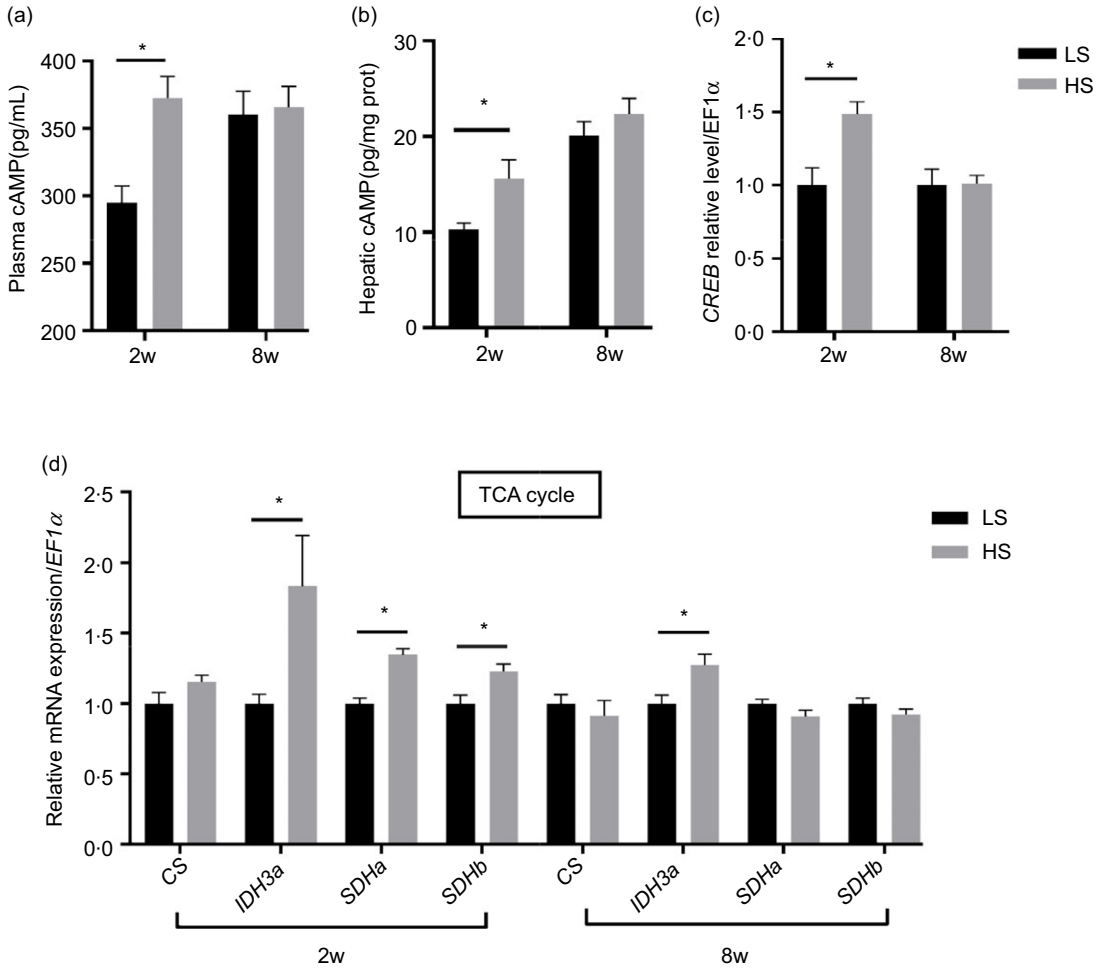

Fig. 5. Effects of HS diet on hepatic energy metabolism of largemouth bass at postprandial 24 h at 2w and 8w. (a) Plasma cAMP contents. (b) Hepatic cAMP contents. (c) Transcriptional levels of hepatic *CREB*. (D) Transcriptional levels of hepatic TCA cycle-related genes (*CS*, *IDH3a*, *SDHa* and *SDHb*). Values marked with '*' are significant difference ($P < 0{\cdot}05$) (n 8). cAMP, cyclic-AMP; CREB, cAMP-responsive element binding protein; TCA, tricarboxylic acid; CS, citrate synthase; *IDH3a*, isocitrate dehydrogenase 3 (NAD^+) alpha; *SDH*, Succinate Dehydrogenase Complex Iron Sulfur Subunit.

adaptation in fish by the genome analysis[42,43], which indicated that increased amylase activity was beneficial to improve the utilisation of starch diet. These results indicated that 13·56 % of starch diet could be effectively absorbed and utilised by the largemouth bass, which further illustrated that largemouth bass had the adaptability to HS diet.

In the present study, HS intake in short-term (2w) induced up-regulated mRNA levels and enzyme activities of glycolysis (GK and PK) and gluconeogenic (PCK and G6Pase)-related genes. Previous studies showed that the expression of hepatic glycolysis-related genes increased significantly with increasing starch levels, whereas the opposite was obtained for hepatic gluconeogenic enzymes in largemouth bass[5,24,25], rainbow trout[38] and golden pompano (*Trachinotus ovatus*)[21]. However, Li et al[44] and Song et al.[45] reported that hepatic gluconeogenic enzyme mRNA levels or activities were closely related to different dietary starch sources. Therefore, the different regulation of hepatic gluconeogenesis by HS diet should be further investigated. In the present study, an abnormally up-regulated gluconeogenic pathway indicated HS diet-induced short-term glucose metabolic disorder. The poor inhibition of gluconeogenesis was considered as one of the reasons for the glucose intolerance of carnivorous fish[38,46,47]. The persistently high level of gluconeogenesis (endogenous glucose production) independent of HS diet may lead to a putative competition between exogenous glucose (feed) and endogenous glucose as the source of energy, which may explain the poor starch utilisation in carnivorous fish[7]. At 8w, no significant differences were observed in the glycolysis and gluconeogenesis enzymes between LS and HS groups, which implied the largemouth bass improved starch utilisation capacity by regulating enzymatic activity. Adaptation of hepatic glucose metabolic enzymes to HS diet had been also reported in gilthead sea bream (*Sparus aurata*)[19], blunt snout bream (*Megalobrama amblycephala*)[20] and golden pompano[21].

In the present study, the glucose metabolism disorder with up-regulated glycolysis and pyruvate aerobic oxidation, which could overproduce acetyl-CoA for energy metabolism, TAG and TC biosynthesis[48]. Significant higher hepatic NEFA and TAG contents but lower plasma TAG were induced by HS diet at 2w, indicating that overproduced acetyl-CoA made a high contribution to TAG synthesis. Meanwhile, the expression of hepatic

fatty acid and TAG synthesis-related genes were significantly up-regulated, while TAG hydrolysis and β-oxidative-related genes were significantly down-regulated, which further confirmed HS induced short-term TAG accumulation in the liver. In the present study, we observed that four of twelve samples in the HS group at 2W showed nuclear dense or fibrosis rather than fatty liver pathological symptom, and this could be related to a fast development of hepatic cell damage. We suppose that a continually sampling, like day by day, could help to get an exact time point for the development from fatty liver (including steatohepatitis) to fibrosis. In general, dietary glucose enters the liver, be stored as glycogen or be converted into lipids if in excess[2,3]. PAS staining is useful for identifying glycogen deposition[49]. In this study, HS diet induced hepatic glycogen accumulation by PAS staining, together with increased content of the hepatic glycogen and lipid at 2w, strongly indicated that HS induced hepatic glycogen and lipid accumulation, then led significantly increased hepatosomatic index. Previous studies investigated that the glycogen and lipid accumulation was positively in response to HS intake, which was an effective way to reduce glucose stress[3,12]. However, consistent with previous studies on largemouth bass[5,24,25], the excess accumulation of glycogen and lipid in the liver may induce liver damage and lead to MLD in this study. Both plasma alanine aminotransferase and aspartate aminotransferase are the main indicators for evaluating liver function. Significant enhancements in the activity of these indicators usually along with liver damage of largemouth bass[26,50]. In this study, no significant differences were observed in plasma alanine aminotransferase and aspartate aminotransferase activities between the LS and HS group at 2w or 8w, but HS induced liver damage (4/12) by histopathological examination at 2w. Therefore, we inferred that HS-induced liver damage of largemouth bass was within the repairable range. These results indicated that the excess accumulation of hepatic glycogen and lipid-induced acute liver damage in largemouth bass, but the recovery of liver health in the long-term remained to be clarified. The significantly higher hepatic TC content, plasma HDL-cholesterol and HDL-cholesterol/TC ratio were also observed in the HS group at 2w, indicating that overproduced acetyl-CoA also induced hepatic TC accumulation. Meanwhile, hepatic TC deposition also resulted in bile acids accumulation with higher plasma alkaline phosphatase levels and hepatic TBA content. Conversion of cholesterol to bile acids is critical for maintaining cholesterol homoeostasis and preventing the accumulation of TC, TAG and toxic metabolites, and injury in the liver[51]. Bile acids are used as metabolic regulators to maintain glucose, lipid and energy metabolism homoeostasis and signalling molecules to protect against inflammation in the liver[52]. In largemouth bass, bile acids supplementation alleviated MLD induced by HS diet via decreasing the hepatic lipid content[26,50]. Therefore, higher hepatic bile acids content induced by HS diet in short term was a positive response to protect against abnormal TAG and TC accumulation in the liver.

Under normal circumstances, the production and elimination of ROS maintain a dynamic balance in the liver. Once the environmental stress occurs (HS stress), ROS levels can increase dramatically and surpass the removal capacity of the antioxidant system, which may result in oxidative damage[53]. MDA production increased is considered as a sign of oxidative stress, and SOD and CAT were able to protect cells or tissues from peroxidation[54]. In this study, HS induced higher hepatic MDA content, whereas SOD and CAT showed the opposite trend at 2w, which was consistent with the previous studies in largemouth bass[5,24,50]. These results suggested that excessive starch intake lowered the hepatic antioxidant abilities of largemouth bass. Growing evidences testified that glycolipid metabolism disorder and abnormal hepatic TAG and TC accumulation could cause strong oxidative stress[53,55,56]. Moreover, metabolic functions in fish also were impaired under strong oxidative stress[5]. Therefore, this acute oxidative stress in largemouth bass was owed to the metabolic disorder induced by HS diet, which led to a reduction in activities of CAT and SOD at 2w. Albumin and IgM are the most widely studied immunoglobulin in fish, which could be a good biomarker for evaluating the immune status of fish[57]. In this study, no significant differences were observed in plasma albumin and IgM levels at 2w or 8w. So, we may infer that although largemouth bass fed HS diet had a risk of oxidative stress, the ROS levels were still under controllable status.

ROS can cause the production of inflammatory responses, ultimately triggering liver apoptosis even necrosis[53,58]. Two stages of inflammation exist, acute and chronic inflammation. Acute inflammation persists only for a short time and is protective response to eliminate the initial cause of cell injury and initiate tissue repair[59]. If the inflammation lasts for a longer time, the chronic inflammation sets in and may predispose the host to various tissues damage, including liver, kidney and intestine[27,60,61]. During the wound healing stage, the significant up-regulated pro-inflammatory cytokine (*IL17*) and anti-inflammatory cytokine (*IL10*) expression were observed in the liver of grass carp (*Ctenopharyngodon idella*), whereas the opposite was shown in the recovery stage[62]. In this study, at 2w, hepatic pro- and anti-inflammatory cytokines mRNA levels were significantly up-regulated in the HS group, while no significant differences were observed at 8w, which indicated HS diet could induce largemouth bass acute inflammation and 'self-repair' response. Similar results also were reported in largemouth bass[26] and Japanese sea bass[27]. If the inflammation is not overcome or treated effectively, prolonged inflammatory responses lead to hepatic fibrosis and apoptosis[60,63]. Cleaved caspase 3 is the most important key executioner in both exogenous and endogenous apoptosis processes activated by promoter-type *Caspase 8* and *Caspase 9*, respectively[64]. Activated caspases trigger compensatory proliferation, referred to as apoptosis-induced proliferation which maintains tissue homoeostasis following massive stress-induced cell death, regenerating lost tissue[26,65]. The significant up-regulated mRNA levels of *Caspase 8* and *Caspase 9* and more cleaved caspase 3 signals (four of twelve samples with apoptosis signals) in the HS group at 2w, but eliminating apoptosis responses at 8w, implied that apoptosis induced by HS might contribute to the liver damage repair.

The cyclic-AMP, which is produced by glycolysis and tricarboxylic acid, has been firmly established as second messenger molecule to regulate energy homoeostasis[66]. This effect appeared to be mediated by activating *CREB* at the mRNA level[67]. In this study, significantly increased plasma and hepatic

cyclic-AMP contents and up-regulated mRNA levels of the *CREB* and tricarboxylic acid cycle-related genes (*IDH3a*, *SDHa* and *SDHb*) were observed at 2w, indicating largemouth bass may up-regulate energy metabolism in response to HS diet. This was similar with previous results, which showed that HS diet could accelerate tricarboxylic acid cycle in largemouth bass[25]. Inflammation could contribute to an increase in energy expenditure in patients with kidney diseases, obesity, anaemia, or haemodialysis[68–71]. The accelerated energy metabolism impeded growth performance and inflammation occurrence in sea bass fed plant protein feed[27]. In addition, southern catfish could oxidise unwanted assimilated starch by accelerating energy expenditure when overfed dietary starch[22], which is a ubiquitous adaptation mechanism in response to HS diet.

According to the above results, accelerated energy metabolism rate and bile acid synthesis were the main ways for largemouth bass to self-repair in response to acute inflammation induced by HS diet. Besides, the lower specific growth rate of largemouth bass in the HS group at 8w could be ascribed to accelerated energy consumption. It was a pity that a longer growth time had not implemented to clarify whether the compensatory growth existed.

Conclusions

In summary, due to insufficient insulin secretion of largemouth bass, intake of HS diet-induced short-term glucose and lipid metabolism disorder along with TAG and TC accumulation symptoms further caused oxidative stress, acute inflammation, apoptosis response and MLD. However, the up-regulated mRNA levels of bile acid synthesis, inflammatory cytokines and energy metabolism-related genes in the acute metabolic disorder stage indicated that the largemouth bass was still in a state of 'self-repair'. After long-term feeding, largemouth bass owned certain adaptability to starch depending on whether metabolic parameters and liver health were returned to homoeostasis. Otherwise, HS-activated apoptosis pathway ultimately led to liver fibrosis and necrosis. Therefore, the largemouth bass could well utilise the HS feed (13·56 %) regardless of poor growth performance. Further studies should pay attention to the molecular mechanism on regulating metabolism disorder under HS diet stress, which was more beneficial to clarify adaptive mechanisms of HS in carnivorous fish.

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We declare that we have no financial and personal relationships with other people or organisations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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