Animal Nutrition 10 (2022) 86–98

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

New insights into the influence of *myo*-inositol on carbohydrate metabolism during osmoregulation in Nile tilapia (*Oreochromis niloticus*)

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ARTICLE INFO

Article history:
Received 21 December 2021
Received in revised form
8 March 2022
Accepted 20 April 2022
Available online 27 April 2022

Keywords:
Myo-inositol
Carbohydrate metabolism
Osmoregulation
Oreochromis niloticus

ABSTRACT

A two-factor (2 × 3) orthogonal test was conducted to investigate the effects of dietary *myo*-inositol (MI) on the osmoregulation and carbohydrate metabolism of euryhaline fish tilapia (*Oreochromis niloticus*) under sustained hypertonic stress (20 practical salinity units [psu]). 6 diets containing either normal carbohydrate (NC, 30%) or high carbohydrate (HC, 45%) levels, with 3 levels (0, 400 and 1,200 mg/kg diet) of MI, respectively, were fed to 540 fish under 20 psu for 8 weeks. Dietary MI supplementation significantly improved growth performance and crude protein content of whole fish, and decreased the content of crude lipid of whole fish ($P < 0.05$). Curled, disordered gill lamella and cracked gill filament cartilage were observed in the gill of fish fed diets without MI supplementation. The ion transport capacity in gill was significantly improved in the 1,200 mg/kg MI supplementation groups compared with the 0 mg/kg MI groups ($P < 0.05$). Moreover, the contents of Na^+, K^+, Cl^- in serum were markedly reduced with the dietary MI supplementation ($P < 0.05$). The fish fed 1,200 mg/kg MI supplementation had the highest MI content in the gills and the lowest MI content in the serum ($P < 0.05$). Additionally, the fish fed with 1,200 mg/kg MI supplementation had the highest MI synthesis capacity in gills and brain ($P < 0.05$). Dietary MI markedly promoted the ability of carbohydrate metabolism in liver ($P < 0.05$). Moreover, fish in the 1,200 mg/kg MI groups had the highest antioxidant capacity ($P < 0.05$). This study indicated that high dietary carbohydrate would intensify stress, and impair the ability of osmoregulation in tilapia under a long-term hypersaline exposure. The supplementation of MI at 1,200 mg/kg in the high carbohydrate diet could promote carbohydrate utilization and improve the osmoregulation capacity of tilapia under long-term hypertonic stress.

1. Introduction

As the global aquaculture industry continues to grow, aquaculture faces various problems such as freshwater shortage, water quality deterioration, and disease outbreaks (Botta et al., 2020; Merino et al., 2010; Willer and Aldridge, 2019). Increasing numbers of researchers have proposed seawater or saline water aquaculture of freshwater fish to improve the fish food quality and achieve better economic benefits (Deutsch et al., 2007; Ton Nu Hai and Speelman, 2020). However, long-term high salinity stress can

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2022.04.006

adversely affect the growth and health of fish; such as low survival rate, nutritional metabolism disorders, tissue structure disarrangement, reduced antioxidant capacity and non-specific immunity (Li et al., 2020; Mohamed et al., 2021; Mozanzadeh et al., 2021; Wu et al., 2021). Therefore, the mechanism of osmotic regulation must be explored in fish, which is essential to develop different strategies to improve the salinity tolerance of fish.

Osmoregulation is an energy-costing process in aquatic organisms. Numerous prior studies have shown that 10% to 50% of the energy consumption will be used for osmotic regulation in fish during salinity adaptation (Islam et al., 2020; Mozanzadeh et al., 2021; Singha et al., 2021; Shukry et al., 2021). Some previous studies have shown that carbohydrate catabolism and glucose transportation were significantly improved for energy supply in euryhaline fish during the process of salinity acclimation (Guo et al., 2020; Islam et al., 2020; Moniruzzaman et al., 2020). For example, more glucose in the liver was transferred to the blood in response to the increased energy demands of Mozambique tilapia (*Oreochromis mossambicus*) under acute high salinity stress (Fiess et al., 2007). Other studies have shown that salinity stress can increase gluconeogenesis and glycolysis activities in the liver, and promote the transfer of glucose to osmotic regulatory tissues for the energy demand of osmotic regulation (Makaras et al., 2020; Zhu et al., 2021). Appropriate supplementary carbohydrate in the diet can satisfy the energy requirement of the body to cope with stress over time (Javed and Usmani, 2015; Souza et al., 2018). However, excessive carbohydrate intake may cause inhibited growth, lipid accumulation in fish, liver damage and metabolic disorders (Li et al., 2020). Therefore, the balance between the function and metabolism of carbohydrate is important to improve the osmotic capacity of fish (Xu et al., 2017, 2020; Zhan et al., 2020).

The main function of *myo*-inositol (MI) is as a precursor of the second messenger involved in a variety of intracellular metabolism regulatory pathways (Bu et al., 2021; Chen et al., 2019; Cui and Ma, 2020; Cui et al., 2020). In addition, MI can act as compatible osmolyte to protect cells from hypertonic challenge (Bu et al., 2021; Cui and Ma, 2020; Cui et al., 2020). Zhu et al. (2021) found that the *myo*-inositol biosynthesis (MIB) was enhanced in the *O. mossambicus* during salinity adaptation (Zhu et al., 2021). Moreover, MI can promote the utilization of lipid and carbohydrate in animals by participating in signal transduction and affecting the glucose metabolism and insulin regulation (Gonzalez-Uarquin et al., 2020). Therefore, the purpose of this study was to investigate whether the addition of MI in high-carbohydrate diets can increase the osmotic capacity of fish, and alleviate the adverse effects of high dietary carbohydrate by promoting the utilization of carbohydrate.

The tilapia *Oreochromis niloticus* are an important aquaculture fish due to its rapid growth and reproductive capacity (Root et al., 2021). Tilapia is a euryhaline fish which can tolerate a salinity range between 0 and 40 psu (Rairat et al., 2020). Therefore, it was an ideal species to investigate the osmoregulation mechanism of fish (Tang et al., 2020; Wang et al., 2018). Therefore, this study was carried out in tilapia to investigate the influence of MI on carbohydrate metabolism during osmoregulation. The results of the research could provide a theoretical support for the salinity domestication of euryhaline fish.

2. Materials and methods

Animal care and treatment procedures were carried out in strict accordance with the ethical requirements of Animal Experiment of East China Normal University (permit number: E20120101).

2.1. Experiment animals, feed formula and experimental design

Six semi-purified diets were formulated with a different carbohydrate percentage: 30% (normal concentration [NC]) and 45% (high carbohydrate [HC]), and concentration of MI (0, 400, and 1,200 mg/kg diet) (Shiau and Su 2005; Bu et al., 2021). NC-0, NC-400, NC-1,200 are normal carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively. HC-0, HC-400, HC-1,200 are high carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively. Corn starch was the main source of carbohydrates. The composition of 6 experimental diets is shown in Table 1. All powder ingredients were sieved twice with a 60-mesh strainer, and then mixed thoroughly following the formula. The MI of each group was dissolved in water first and added into the mixed ingredients. Subsequently, machine F-26 II (South China University of Technology, Guangdong, China) was used to process particles with a diameter of 2 mm. The diets were air-dried and stored at −20 °C until use.

Fish used in this trial were obtained from Yiqian Fish Farm (Guangzhou, China). The fish were maintained in five 500 L tanks at 26 ± 1 °C in the Biological Experimental Station of East China Normal University for 2 weeks. During the temporary feeding period, fish were fed with a commercial puffed diet (protein 33%, fat 5% and carbohydrate 20%) twice a day. After the temporary culture stage, 540 healthy fish with similar weights (1.30 ± 0.05 g) were randomly assigned to eighteen 300 L aquaculture tanks. The 18 tanks were randomly divided into 6 groups with three replicates in each group. Before the formal experiment, salty water was added to the freshwater to increase the water salinity. The salt used in the trial was purchased from Tangjie Haisheng Crystal Factory in Tianjin. The water salinity was increased by 3 to 4 psu per day until it reached 20 psu (Zhu et al., 2018; Yu et al., 2021; Shukry et al., 2021). During the experiment, all fish were fed twice a day (08:30 and 17:30) with the amount of 4% of their body weight. The feeding amount was adjusted according to the fish weight recorded every week. During the trial, two-thirds of the water in each tank was replaced daily. Continuous aeration was supplied to maintain sufficient oxygen and the photoperiod was maintained on a 12 h:12 h (light/dark) cycle. The water temperature, pH and salinity were maintained at 27 ± 1 °C, 7.4 to 7.6 and 20.0 ± 0.2 psu, respectively. All the water indexes were measured every morning and evening.

2.2. Sampling collection

At the end of the trial, all fish were fasted for 24 h. Fish from all groups were weighed, measured and counted to calculate the final weight, weight gain rate (WG), survival rate (SR) and feed conversion ratio (FCR). Four fish were randomly selected and anesthetized by 20 mg/L of tricaine methane sulfonate (Western Chemicals, Inc., Ferndale, Washington) from each tank. The blood collected from the caudal vein was divided into 2 parts, and put in a 4 °C refrigerator overnight. One allotment was added with heparin sodium to detect the serum osmotic pressure, and the other part was centrifuged at 2,500 g at 4 °C for 10 min. The supernatant was taken and stored at −80 °C for biochemical indicator detection. Then, the liver, kidney, gills, and brain were collected in turn and immediately stored at −80 °C. The liver and visceral mass was weighed to calculate the hepatosomatic index (HSI), visceral index (VSI) and condition factor (CF). The entire sampling process was carried out on ice. Three fish per tank were randomly selected and kept at −20 °C for whole fish body composition.

Table 1
Formulation and chemical composition of experimental diets.

Item	Diets[1]					
	NC-0	HC-0	NC-400	HC-400	NC-1,200	HC-1,200
Ingredients, g/kg dry basis						
Casein (vitamin-free)	320	320	320	320	320	320
Gelatin	80	80	80	80	80	80
Soybean oil	70	70	70	70	70	70
Corn starch	300	450	300	450	300	450
Myo-inositol[2], mg/kg diet	0	0	0.4	0.4	1.2	1.2
Vitamin premix[3]	5	5	5	5	5	5
Mineral premix[4]	5	5	5	5	5	5
$Ca(H_2PO_4)_2$	15	15	15	15	15	15
Carboxy methyl cellulose	25	25	25	25	25	25
Cellulose	175.75	27.75	175.35	27.35	176.55	26.55
Phagostimulant	2	2	2	2	2	2
Butylated hydroxytoluene	0.25	0.25	0.25	0.25	0.25	0.25
Total	1,000	1,000	1,000	1,000	1,000	1,000
Chemical composition, %						
Moisture	10.05	10.56	10.03	10.23	10.68	10.39
Crude protein	37.22	37.98	37.55	37.74	37.65	37.84
Crude lipid	6.95	6.96	6.83	6.87	6.85	6.93
Ash	2.88	2.88	2.87	3.02	3.02	2.91
GE, KJ/g	15.99	15.91	15.97	15.92	15.84	15.92
P/E, mg/KJ	22.17	22.71	22.38	22.56	22.62	22.61
NPE, KJ/g	9.78	9.57	9.70	9.62	9.56	9.61
Myo-inositol, mg/kg	–	–	402	409	1,210	1,206

GE = gross energy; P/E = protein-to-energy ratio; NPE = non-protein energy.
[1] NC-0, NC-400, NC-1,200 are normal carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively. HC-0, HC-400, HC-1,200 are high carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively.
[2] Supplied by Sangong Biotech, Ltd., Shanghai, China.
[3] Vitamin premix, mg/kg diet: retinal palmitate (500,000 IU/g), 8; cholecalciferol (1,000,000 IU/g), 2; menadione, 10; DL-α-tocopherol acetate, 200; thiamin-HCl, 10; riboflavin, 12; pyridoxine-HCl, 10; D-calcium pantothenate, 32; amine nicotinic acid, 80; folic acid, 2; cyanocobalamin, 0.01; biotin, 0.2; choline chloride, 400; ascorbic acid, 60; α-cellulose, 4,173.79.
[4] Mineral premix, mg/kg diet: $ZnSO_4 \cdot H_2O$, 150; $FeSO_4 \cdot H_2O$, 40; $MnSO_4 \cdot H_2O$, 15.3; $CuSO_4 \cdot 5H_2O$, 8.3; potassium iodide, 5; $CoCl_2 \cdot 6H_2O$, 0.05; Na_2SeO_3, 0.09; α-cellulose, 4,785.76.

2.3. Growth performance statistical method

Weight gain (WG, %) = 100 × (final body weight − initial body weight)/initial body weight;

Survival rate (SR, %) = 100 × (final fish number/initial fish number);

Feed conversion ratio (FCR) = feed consumption/(final biomass − initial biomass + dead fish weight);

Condition factor (CF, %) = 100 × (wet body weight, g)/(body length, cm)^3;

Hepatosomatic index (HSI, %) = 100 × wet hepatopancreas weight/wet body weight;

Visceral index (VSI, %) = 100 × wet visceral weight/wet body weight.

2.4. Whole-body composition detection

The detection of whole fish body components was taken based on the previous test methods in our laboratory. The specific experimental operation is supplied in Supplementary material.

2.5. Histological analysis

Gills on the same side of three fish in each tank were randomly selected and fixed in 4% paraformaldehyde solution for 48 h. The subsequent processes were carried out according to the methods in the previous articles of our laboratory, and the specific experimental operation was in Supplementary material.

2.6. Detection of biochemical indicators

The kits used for the determination of glucose content (F006-1-1), Na^+ (C002-1-1), K^+ (C001-2-1) and Cl^- (C003-2-1) content in serum were purchased from Nanjing Jiancheng Bioengineering Institute. The activity of SOD (A001-3-2) and GSH-Px (A005-1-2) and the content of MDA (A003-1-2) in liver and liver glycogen (A043-1-1) and muscle glycogen (A043-1-1) content were also detected by the kits purchased from Nanjing Jiancheng Bioengineering Institute. The content of MI in different tissues (liver, gill, kidney and serum) was determined by the method previously published. The serum osmotic pressure was detected by the freezing point osmotic pressure detector (Fiske Micro-Osmometer Model 210).

2.7. Gene expression analysis

According to the manufacturer's protocol, total RNA was extracted from the liver, gills, kidney and brain with Trizol reagent (Takara, Dalian, China). After the quantity and quality control of the total RNA, reverse transcription was performed using the kit (RR047, Takara, Japan). All operations were carried out according to the manufacturer's procedures. Gene expression was detected by quantitative real-time polymerase chain reaction (qRT-PCR) with *β-actin* as the housekeeping gene. The primer sequences of each gene were listed in Table 2. The efficiency of qRT-PCR was between 85% and 105%, and the correlation coefficients of different genes was above 0.98. Expression of related genes were calculated to *β-actin* using the $2^{-\Delta\Delta ct}$ method.

2.8. Experimental data analysis

All statistical analyses were performed using SPSS Statistics 19.0 software. All data meet the normal distribution and variance

Table 2
Primer pair sequences and product size of the genes used for real-time PCR (qPCR).

Gene	Position	Primer sequence	Length	Tm	Product Size, bp	GenBank
gk	Forward	GTCATCAACCTGATGCGGGA	20	60.18	163	XM_003451020.5
	Reverse	ACCTGTCACGGAAACATGGG	20	59.75		
g6pase	Forward	GGATGCTAATGGGCCTGGTC	20	59.78	169	AY963627.1
	Reverse	CAGCTACCAGTGTGCCTGTAA	21	59.60		
g6pdh	Forward	TCCAGAACCTCATGGTGCTT	20	60.18	312	XM_005478106.4
	Reverse	GGCTCCTTGAAGGTAAGGACG	21	59.69		
mips	Forward	CGTCCTACGAGGGAACCTCT	20	60.39	179	XM_005477233.3
	Reverse	GCAGAGTCTTTGCACGGAATA	21	58.65		
impa1	Forward	ATAAGCCGGGAAGCAGTCTC	20	59.53	132	XM_025910145.1
	Reverse	GTGTTTGGTCGTTCGATGGTG	21	60.07		
glut	Forward	GTTGGAACTGCGGTGATTGGCT	22	59.98	167	FJ914655.1
	Reverse	ATAGCAACAGCGATGGACCACAC	23	60.01		
nka	Forward	CGTGCTGAATTTAAGGCAGGTCA	23	58.73	103	LC556924.1
	Reverse	GCAAAGCTGATTCAGAAGCGTCAC	24	59.01		
nhe	Forward	ATGAAGCGTCAGCCTAGGAA	20	63.81	99	XM_003447282.5
	Reverse	TCCCAGAGCCTGGATCATAC	19	63.98		
cftr	Forward	TCACCAGCATCGCTGTAGATG	20	66.30	135	XM_013273808.3
	Reverse	GGTTGTCGATGACGATATCAGG	21	65.40		
β-actin	Forward	GGATTCACTCTGAGCGCCG	19	58.43	203	KJ126772.1
	Reverse	CCGTCTCCTTACCTTTGGGTG	21	59.12		

gk = glucokinase; *g6pase* = glucose-6-phosphatase; *g6pdh* = glucose-6-phosphate dehydrogenase; *mips* = *myo*-inositol-1-phosphate synthase; *impa1* = *myo*-inositol monophosphatase; *glut* = glucose transporters; *nka* = Na^+/K^+-ATPase; *nhe* = Na^+/H^+ exchanger; *cftr* = cystic fibrosis transmembrane conductance regulator.

homogeneity test. Two-factor analysis of variance was used to analyze the main effect and interaction of the 2 factors. Then, One-Way Analysis of Variance followed by Duncan's multiple comparison test was used to determine all data. $P < 0.05$ means the difference is statistically significant. All data are on average ± standard error (means ± SEM).

3. Results

3.1. Growth and physiological parameters

Growth and physiological parameters of tilapia are shown in Table 3. No significant difference was found in FCR and CF among all groups ($P > 0.05$). The final weight, WG, SR, HSI and VSI were markedly influenced by the MI concentrations ($P < 0.05$). The WG and HSI were markedly influenced by the carbohydrate levels ($P < 0.05$). Fish fed the diet with 400 mg/kg MI supplementation had the highest final weight, WG and SR ($P < 0.05$). Compared with the fish in NC groups, the WG was significantly higher in the HC groups ($P < 0.05$). Compared with the fish in HC groups, the HSI were markedly reduced in NC levels ($P < 0.05$).

3.2. Whole fish body composition

Crude protein content was significantly affected by MI supplementation ($P < 0.05$). The crude lipid content was greatly affected by carbohydrate levels, MI content and their interaction ($P < 0.05$). The lowest crude protein was found in 0 mg/kg MI supplementation groups ($P < 0.05$). The highest crude lipid was monitored in HC-0 and HC-400 group ($P < 0.05$) (Table 4). No remarkable differences were observed in moisture and ash between different treatment groups ($P > 0.05$).

3.3. Serum glucose content and glycogen content in liver and muscle

The content of muscle glycogen was influenced by the interaction between carbohydrate levels and MI supplementation ($P < 0.05$). The MI supplementation affected the contents of serum glucose, liver glycogen and muscle glycogen ($P < 0.05$). The lowest serum glucose content was found in 0 mg/kg MI supplementation groups ($P < 0.05$). The lowest liver glycogen content was found in 1,200 mg/kg MI supplementation groups ($P < 0.05$). NC-0 group had the highest muscle glycogen content ($P < 0.05$) (Table 5).

3.4. Observation of the gill histological

Tilapia gills are composed of gill arch, gill rake and gill filament, with hyaline cartilage tissue running through the middle of the gill filament, and many gill lamellae arrange in parallel on both sides of the gill filament. In the groups fed the diet without MI supplementation, the gill lamellas were severely deformed and curled under both carbohydrate levels. In addition, the basal of the outer epithelial layer was thickened, with shortened gill lamella and cracked gill filament (Fig. 1A, E, C and G). In HC-0 group, the distribution of red blood cells on the gill lamella was disordered and there was partial accumulation of red blood cells (Fig. 1C and G). However, in the MI supplementation groups, the gill lamella arrangement was closely ordered, symmetrical and complete, and no abnormality was observed (Fig. 1B, F, G and H).

3.5. The expression of ion transporter in gills

The gene expression of Na^+/K^+-ATPase (*nka*) was affected by carbohydrate levels, MI concentrations and the interaction among carbohydrate levels and MI supplementation ($P < 0.05$). The expression of Na^+/H^+ exchanger (*nhe*) was pronouncedly affected by MI concentrations ($P < 0.05$). The cystic fibrosis transmembrane conductance (*cftr*) was prominently influenced by carbohydrate levels and the interaction among carbohydrate levels and MI supplementation ($P < 0.05$). The fish in NC-1,200 group had highest *nka* and *cftr* gene expression level in the gills ($P < 0.05$, Fig. 2A and C). The *nhe* gene expression level was up-regulated in 400 mg/kg and 1,200 mg/kg MI supplementation groups ($P < 0.05$, Fig. 2B).

3.6. Content of serum ions and serum osmolarity parameters

The content of serum Na^+, K^+ and Cl^- were markedly affected by carbohydrate levels and MI supplementation ($P < 0.05$). Serum osmolarity was influenced by carbohydrate levels ($P < 0.05$, Fig. 3D). The lowest serum Na^+, K^+ and Cl^- were found in 1,200 mg/kg MI supplementation groups ($P < 0.05$, Fig. 3A, B and C). HC diet markedly decreased serum Na^+, K^+ and Cl^- content. ($P < 0.05$,

Table 3
Growth performance and physiological parameters of *O. niloticus* fed different experiment diets.

Diets[1]	Initial weight, g	Final Weight, g	WG, %	FCR	CF, %	HIS, %	VSI, %	SR, %
NC-0	1.30 ± 3.01	266.91 ± 62.11	768.11 ± 41.30	1.22 ± 0.02	2.95 ± 0.07	1.78 ± 0.10	11.57 ± 0.47	78.33 ± 1.66
NC-400	1.29 ± 2.01	326.90 ± 6.36	904.82 ± 36.04	1.19 ± 0.01	3.06 ± 0.06	1.69 ± 0.08	11.42 ± 0.18	83.33 ± 0.00
NC-1,200	1.30 ± 3.01	294.46 ± 4.66	867.03 ± 12.63	1.23 ± 0.01	3.01 ± 0.06	1.52 ± 0.11	9.83 ± 0.42	77.78 ± 1.11
HC-0	1.32 ± 3.01	260.01 ± 07.10	831.19 ± 90.45	1.19 ± 0.02	3.04 ± 0.15	1.52 ± 0.11	10.56 ± 0.27	70.00 ± 3.33
HC-400	1.32 ± 3.02	326.82 ± 6.97	954.36 ± 81.19	1.22 ± 0.03	3.15 ± 0.08	2.23 ± 0.09	9.91 ± 0.34	78.33 ± 1.66
HC-1,200	1.33 ± 3.02	335.21 ± 5.45	946.86 ± 7.12	1.19 ± 0.02	2.96 ± 0.02	1.96 ± 0.13	9.47 ± 0.16	80.00 ± 3.33
Carbohydrate level, g/kg								
NC	–	296.09 ± 9.68	846.63 ± 24.17^{X}	1.21 ± 0.01	3.00 ± 0.04	1.61 ± 0.08^{X}	11.16 ± 0.37	79.85 ± 1.02
HC	–	307.38 ± 13.35	910.68 ± 26.35^{Y}	1.20 ± 0.01	3.07 ± 0.06	1.92 ± 0.07^{Y}	10.23 ± 0.29	76.07 ± 1.75
Dietary MI, mg/kg								
0	–	263.51 ± 9.49^{A}	799.59 ± 24.19^{A}	1.21 ± 0.01	2.99 ± 0.08	2.06 ± 0.08^{B}	12.20 ± 0.31^{C}	74.11 ± 2.07^{A}
400	–	326.86 ± 5.16^{B}	929.58 ± 28.67^{B}	1.21 ± 0.02	3.10 ± 0.05	1.67 ± 0.05^{A}	10.27 ± 0.30^{B}	80.88 ± 1.27^{B}
1,200	–	314.84 ± 10.1^{B}	906.00 ± 18.70^{B}	1.21 ± 0.01	3.00 ± 0.03	1.59 ± 0.07^{A}	9.33 ± 0.23^{A}	78.88 ± 1.11^{A}
Two-way ANOVA (*P* value)								
MI	–	0.000	0.002	0.978	0.341	0.007	0.000	0.040
Carbohydrates	–	0.215	0.025	0.351	0.395	0.000	0.083	0.064
Interaction	–	0.090	0.887	0.240	0.701	0.621	0.259	0.084

NC = 300 g/kg carbohydrate level; HC = 450 g/kg carbohydrate level.
MI = *myo*-inositol; WG = weight gain rate; SR = survival rate; FCR = feed conversion ratio; HSI = hepatosomatic index; VSI = visceral index; CF = condition factor.
Data were expressed as mean ± SEM (standard error of the mean) ($n = 3$, replicate tanks). Means in the same column with different superscripts (A, B, C or X, Y) are significantly different ($P < 0.05$). Dietary MI = A, B, C; dietary carbohydrate level = X, Y.
[1] NC-0, NC-400, NC-1,200 are normal carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively. HC-0, HC-400, HC-1,200 are high carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively.

Table 4
Proximate composition of *O. niloticus* fed different experiment diets.[1]

Diets[1]	Crude protein, %	Crude lipid, %	Moisture, %	Ash, %
NC-0	14.04 ± 0.30	7.04 ± 0.13^{b}	75.05 ± 0.447	3.23 ± 2.07
NC-400	15.99 ± 0.42	5.98 ± 9.11^{a}	75.70 ± 0.11	3.24 ± 2.25
NC-1,200	15.09 ± 0.13	6.06 ± 0.08^{a}	74.38 ± 0.47	2.74 ± 7.12
HC-0	14.02 ± 0.39	8.12 ± 1.03^{c}	74.61 ± 0.75	3.03 ± 0.01
HC-400	15.77 ± 0.48	7.84 ± 8.23^{c}	74.65 ± 0.36	3.00 ± 0.29
HC-1,200	14.42 ± 0.39	6.04 ± 0.10^{a}	74.65 ± 0.43	2.88 ± 8.02
Carbohydrate level, g/kg				
NC	15.04 ± 0.32	6.36 ± 0.70^{X}	74.69 ± 0.24	3.07 ± 0.11
HC	14.74 ± 0.34	7.33 ± 0.33^{Y}	74.99 ± 0.31	2.97 ± 0.08
Dietary MI, mg/kg				
0	14.03 ± 0.22^{A}	7.58 ± 0.24^{B}	75.38 ± 0.26	3.13 ± 0.05
400	15.88 ± 0.29^{B}	6.91 ± 0.43^{AB}	74.50 ± 0.40	3.12 ± 0.18
1,200	14.75 ± 0.23^{B}	6.05 ± 0.06^{A}	74.65 ± 0.25	2.81 ± 0.06
Two-way ANOVA (*P*-value)				
MI	0.001	0.000	0.414	0.145
Carbohydrates	0.338	0.000	0.316	0.489
Interaction	0.671	0.000	0.409	0.481

NC = 300 g/kg carbohydrate level; HC = 450 g/kg carbohydrate level; MI = *myo*-inositol.
Data were expressed as mean ± SEM (standard error of the mean) ($n = 3$, replicate tanks). Means in the same column with different superscripts (A, B, C for dietary MI; a, b, c for dietary treatment; or X, Y for dietary carbohydrate level) are significantly different ($P < 0.05$).
[1] NC-0, NC-400, NC-1,200 are normal carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively. HC-0, HC-400, HC-1,200 are high carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively.

Fig. 3A, B and C). The higher serum osmolarity were found in HC groups than NC groups ($P < 0.05$, Fig. 3D).

3.7. MI content in the gills, serum, kidney and liver

The content of MI in serum and gill were significantly affected by MI concentrations ($P < 0.05$, Fig. 4A and C). No obvious difference was discovered in the kidney and liver among the groups ($P > 0.05$, Fig. 4B and D). The lowest MI content in serum was observed in 1,200 mg/kg MI groups and 400 mg/kg MI groups ($P < 0.05$, Fig. 4A). The MI content in the gill was significantly increased with the increased MI concentrations ($P < 0.05$, Fig. 4C).

3.8. MI-synthesis-related genes expression in the different tissues

MI supplementation affected the expressions of *myo*-inositol monophosphatase (*impa1*) in liver, *myo*-inositol-1-phosphate synthase (*mips*) and *impa1* in the gill and the brain ($P < 0.05$, Fig. 5B, D, E, F and H). The liver *impa1* genes expression was affected by carbohydrate levels ($P < 0.05$, Fig. 5E). There was no significant difference of *mips* genes expression in the liver and *mips* and *impa1* in kidney between different treatment groups ($P > 0.05$, Fig. 5A, C and G). Fish fed 1,200 mg/kg MI groups had highest expression levels of *impa1* in the liver, *mips* and *impa1* in the gill and brain ($P < 0.05$, Fig. 5B, D, E, F and H). HC groups had higher *impa1* expression levels in liver than NC groups ($P < 0.05$, Fig. 5E).

3.9. Expression of glucose metabolism related genes in liver

The glucose-6-phosphatase (*g6pase*) and glucose transporter (*glut*) gene expression levels in the liver were markedly affected by MI concentrations ($P < 0.05$, Fig. 6A and D). The glucose-6-phosphate dehydrogenase (*g6pdh*) expression level was

Table 5
Serum glucose levels and liver and muscle carbohydrate contents of *O. niloticus* fed different experiment diets.

Diets[1]	Serum glucose, mmol/L	Liver glycogen, mg/g	Muscle glycogen, mg/g
NC-0	4.14 ± 0.14	28.01 ± 2.78	3.52 ± 0.56^{b}
NC-400	4.44 ± 0.13	23.70 ± 2.28	2.03 ± 0.21^{a}
NC-1,200	5.74 ± 0.25	22.13 ± 2.55	1.76 ± 0.22^{a}
HC-0	4.28 ± 0.27	32.77 ± 2.26	1.99 ± 0.29^{a}
HC-400	5.04 ± 0.33	22.70 ± 1.99	2.21 ± 0.20^{a}
HC-1,200	5.82 ± 0.11	18.14 ± 4.75	2.01 ± 0.15^{a}
Carbohydrate level, g/kg			
NC	4.69 ± 0.15	24.83 ± 1.54	2.47 ± 0.28
HC	5.01 ± 0.18	24.04 ± 2.59	2.07 ± 0.49
Dietary MI, mg/kg			
0	4.21 ± 0.15^{A}	29.91 ± 1.96^{C}	29.91 ± 1.96^{B}
400	4.73 ± 0.18^{B}	23.26 ± 1.46^{B}	23.26 ± 1.46^{A}
1,200	5.78 ± 0.12^{C}	20.13 ± 2.62^{A}	20.13 ± 2.62^{A}
Two-way ANOVA (*P* value)			
MI	0.000	0.008	0.030
Carbohydrates	0.138	0.976	0.163
Interaction	0.450	0.358	0.014

NC = 300 g/kg carbohydrate level; HC = 450 g/kg carbohydrate level; MI = *myo*-inositol.
Data were expressed as mean ± SEM (standard error of the mean) (n = 3, replicate tanks). Means in the same column with different superscripts (A, B, C or a, b, c) are significantly different ($P < 0.05$). Dietary MI = A, B, C; dietary treatment = a, b, c.
[1] NC-0, NC-400, NC-1,200 are normal carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively. HC-0, HC-400, HC-1,200 are high carbohydrate addition with 0, 400, and 1,200 mg/kg MI, respectively.

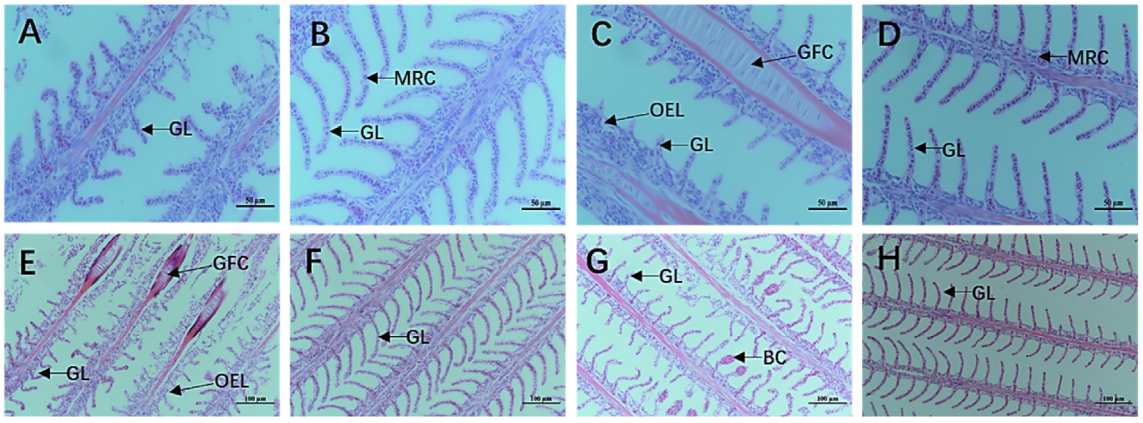

Fig. 1. Effects of *myo*-inositol at different carbohydrate levels on gills structure parameters of *O. niloticus*. (A, E) NC-0 group staining section of gills structure; (B, F) NC-1,200 group staining section of gills structure; (C, G) HC-0 group staining section of gills structure; (D, H) HC-1,200 group staining section of gills structure. (A) to (D), scale bar = 100 μm. (E) to (H), scale bar = 100 μm. GL = gill lamella; MRC = mitochondria-rich cell; OEL = outer epithelial layer; BC = blood cell; GFC = gill filaments cartilage; NC-0 = normal carbohydrate addition with 0 mg/kg MI; NC-1,200 = normal carbohydrate addition with 1,200 mg/kg MI; HC-0 = high carbohydrate addition with 0 mg/kg MI; HC-1,200 = high carbohydrate addition with 1,200 mg/kg MI.

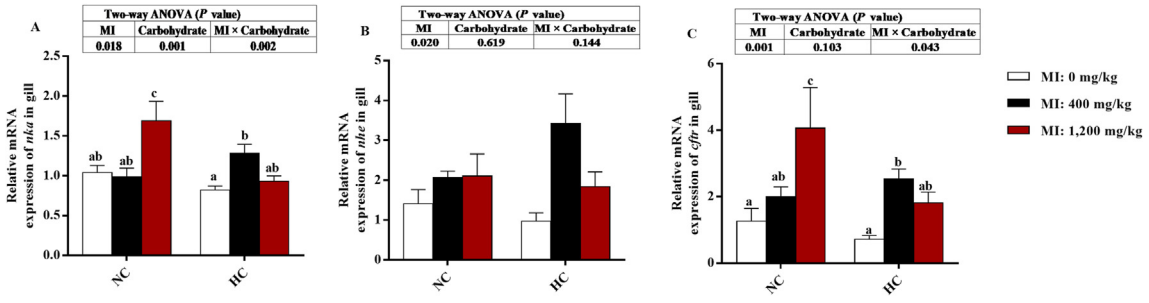

Fig. 2. Effects of *myo*-inositol at different carbohydrate levels on mRNA levels of ion transporter in gill of *O. niloticus*. Data were expressed as mean ± SEM (standard error of the mean) (n = 3, replicate tanks). Bars with different superscripts ($^{a, b, c}$) are significantly different ($P < 0.05$). NC = 300 g/kg carbohydrate level; HC = 450 g/kg carbohydrate level; *nka* = Na^+/K^+-ATPase; *nhe* = Na^+/H^+ exchanger; *cftr* = cystic fibrosis transmembrane conductance.

significantly affected by carbohydrate levels, MI contents and their interaction ($P < 0.05$, Fig. 6B). The expression of glucokinase (*gk*) gene was significantly influenced by carbohydrate levels and MI concentrations ($P < 0.05$, Fig. 6C). The expression levels of *g6pase*, *gk* and *glut* were significantly up-regulated with the MI supplementation increased in all groups ($P < 0.05$, Fig. 6A, C and D). The *g6pdh* expression level was evidently up-regulated in the NC-0 group ($P < 0.05$, Fig. 6B).

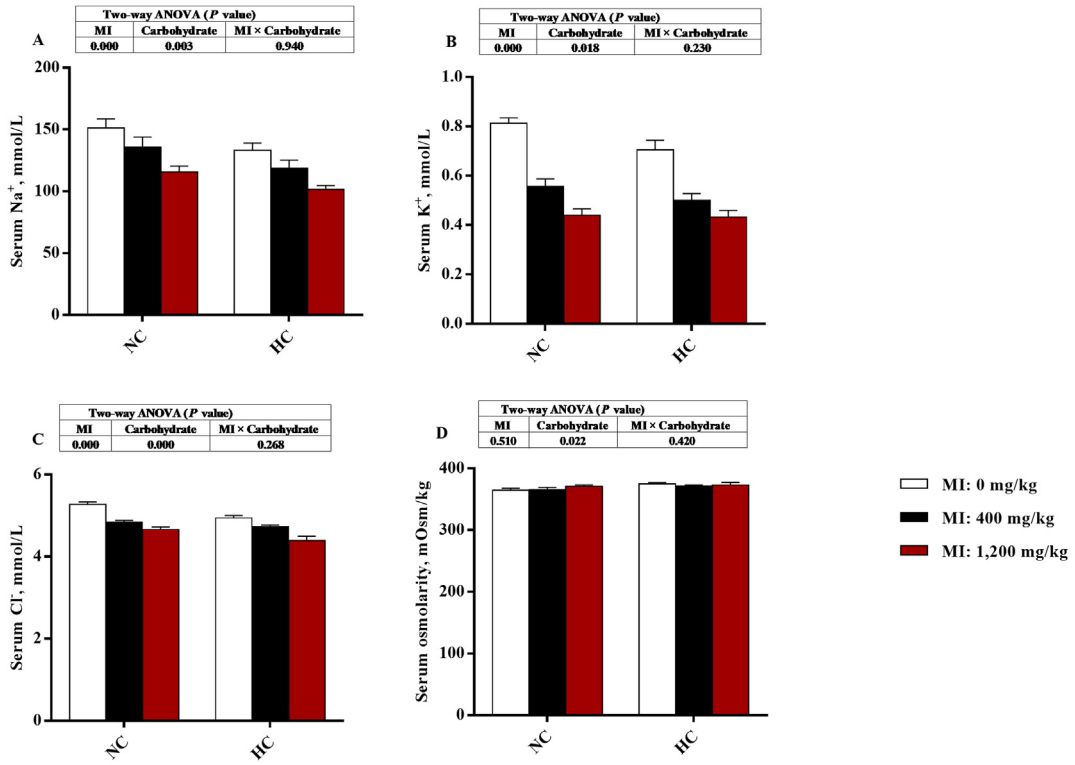

Fig. 3. Effects of *myo*-inositol at different carbohydrate levels on serum ions content and serum osmolarity parameters of *O. niloticus*. Data were expressed as mean ± SEM (standard error of the mean) (n = 3, replicate tanks). NC = 300 g/kg carbohydrate level; HC = 450 g/kg carbohydrate level.

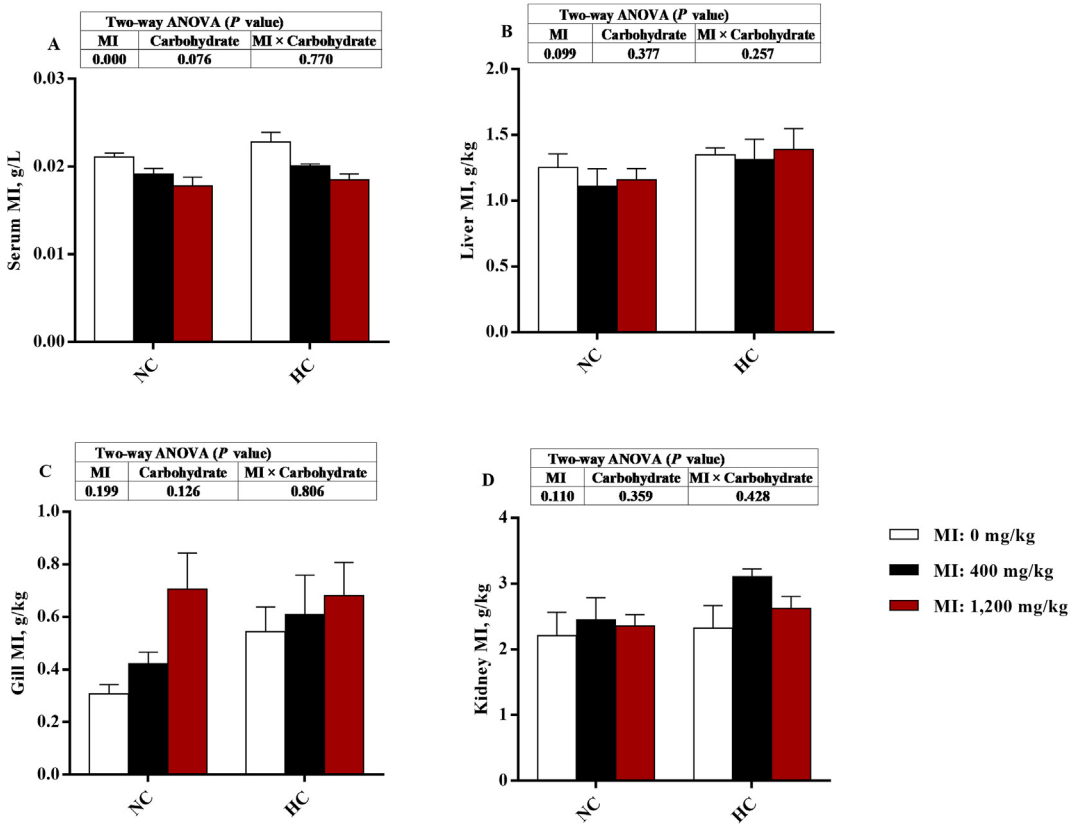

Fig. 4. Effects of *myo*-inositol at different carbohydrate levels on *myo*-inositol content in the different tissue parameters of *O. niloticus*. Data were expressed as mean ± SEM (standard error of the mean) (n = 3, replicate tanks). NC = 300 g/kg carbohydrate level; HC = 450 g/kg carbohydrate level; MI = *myo*-inositol.

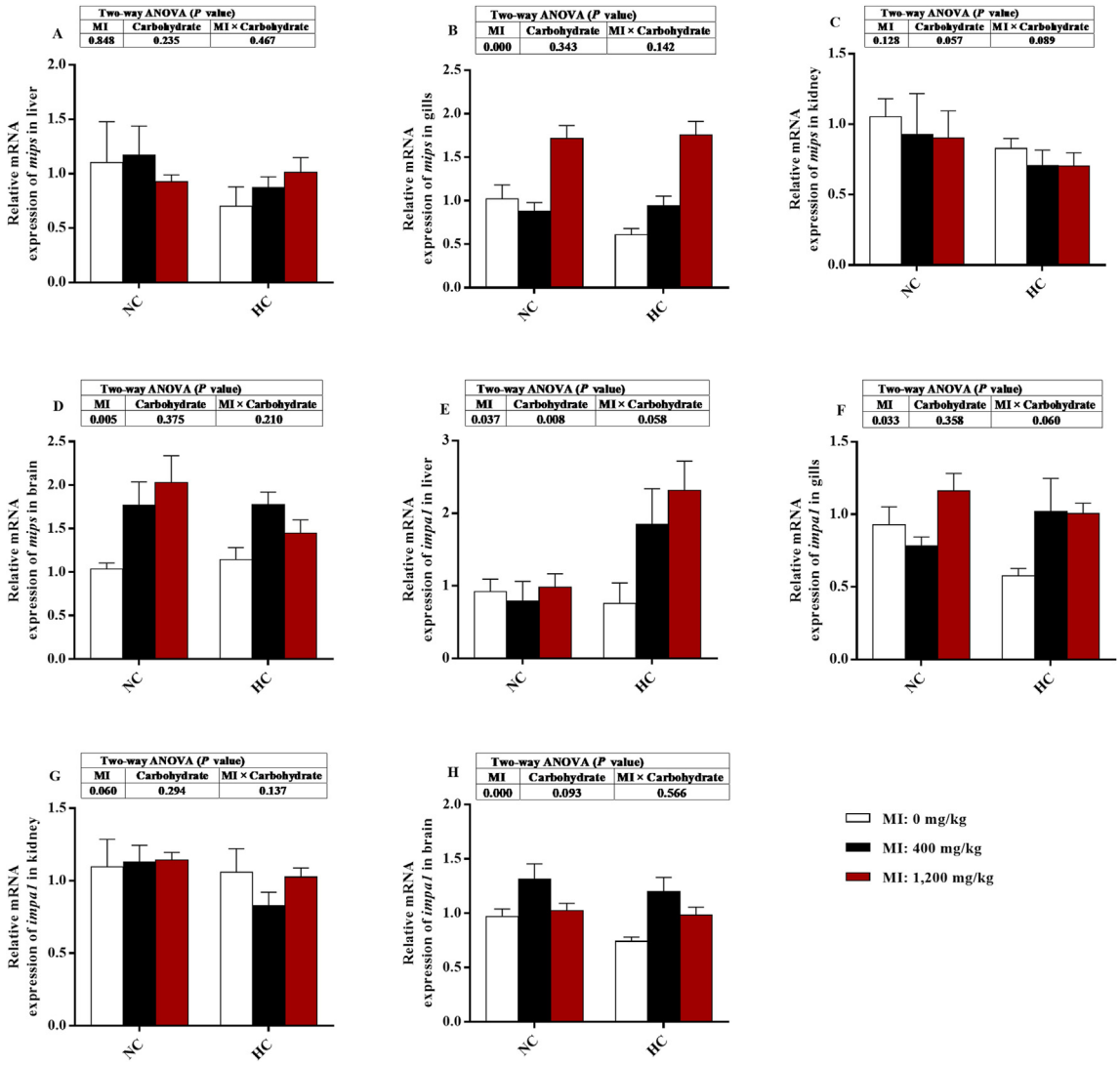

Fig. 5. Effects of *myo*-inositol at different carbohydrate levels on the mRNA levels of genes related to *myo*-inositol synthesis in the different tissue of *O. niloticus*. Data were expressed as mean ± SEM (standard error of the mean) (n = 3, replicate tanks). NC = 300 g/kg carbohydrate level; HC = 450 g/kg carbohydrate level; *mips* = *myo*-inositol-1-phosphate synthase; *impa1* = *myo*-inositol monophosphatase.

3.10. Antioxidant related parameters in liver

The SOD and GSH-Px activities and the content of MDA in the liver were greatly affected by MI concentrations ($P < 0.05$). The activity of SOD and GSH-Px were markedly increased in fish fed 400 mg/kg and 1,200 mg/kg MI supplementation groups ($P < 0.05$, Fig. 7A and B). Furthermore, the content of MDA was significantly higher in 0 mg/kg MI supplementation than other MI groups ($P < 0.05$, Fig. 7C).

4. Discussion

Although carbohydrates can meet the high energy demand for osmoregulation during salinity stress, high carbohydrate diets may lead to lipid deposition in fish, increasing the risk of fatty liver and disrupting the function of antioxidant systems (Li et al., 2018; Limbu et al., 2020; Luo et al., 2020). In addition, persistent hyperglycemia usually occurs after high carbohydrate intake in fish, which would lead to liver glycogen deposition, liver cell damage and metabolic disorders (Li et al., 2020; Vinosha et al., 2020; Wu et al., 2021). The results of this study showed that high dietary carbohydrate increased WG, HSI, and the crude lipid content in tilapia, indicating that high carbohydrates could cause abnormal obesity in tilapia. In addition, high carbohydrate in diets may not only cause abnormal accumulation of fat in fish, but may also cause ROS production (Zhang et al., 2021). Therefore, long-term high carbohydrate stress may disturb energy metabolism and physiological function and affect the antioxidant defense system in fish (Ding et al., 2022; Li et al., 2020; Peng et al., 2020). At the same time, long-term hypertonic stress could cause ROS accumulation (Chang et al., 2021). However, the antioxidant enzyme system and non-enzyme system in the body could eliminate ROS (Li et al., 2020). SOD and GSH-Px are antioxidant enzymes, which can reduce oxidative stress from free radicals (Moniruzzaman et al., 2021; Paital et al., 2019). SOD and GSH-Px indirectly reflect the state of collective antioxidant capacity (Liu et al., 2021a). MDA is one of the peroxidation products in the process of material metabolism. The increase of MDA content was an indicator for oxidative damage (Flohr et al., 2012; Tsikas, 2017). The results showed that long-term salinity stress leads to the decrease of SOD activity and the increase

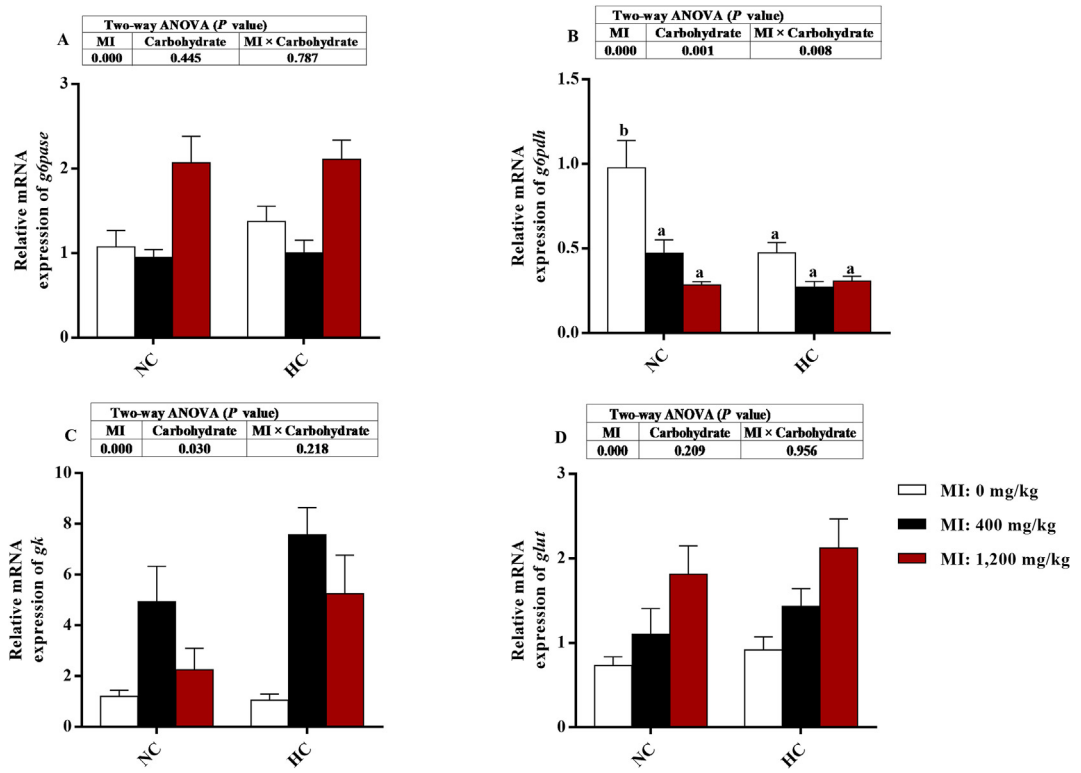

Fig. 6. Effects of *myo*-inositol at different carbohydrate levels on the mRNA levels of genes related to carbohydrate metabolism in the liver of *O. niloticus*. Data were expressed as mean ± SEM (standard error of the mean) (n = 3, replicate tanks). Bars with different superscripts ($^{a, b}$) are significantly different ($P < 0.05$). NC = 300 g/kg carbohydrate level; HC = 450 g/kg carbohydrate level; *g6pase* = glucose-6-phosphatase; *g6pdh* = glucose-6-phosphate dehydrogenase; *gk* = glucokinase; *glut* = glucose transporters.

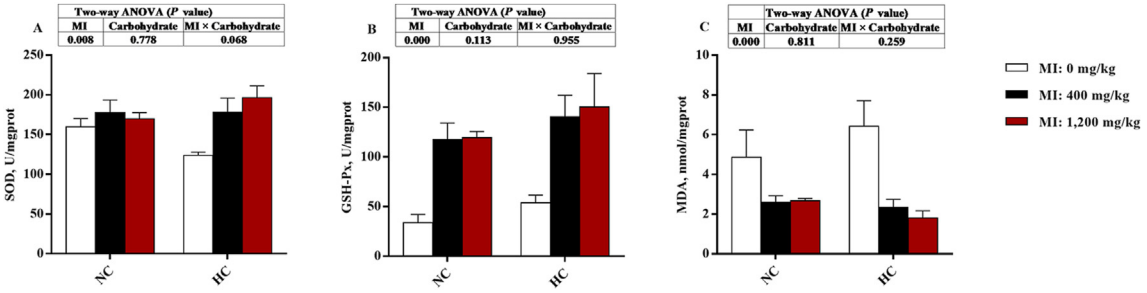

Fig. 7. Effects of *myo*-inositol at different carbohydrate levels on immune-related parameters in the liver of *O. niloticus*. Data were expressed as mean ± SEM (standard error of the mean) (n = 3, replicate tanks). NC = 300 g/kg carbohydrate level; HC = 450 g/kg carbohydrate level; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; MDA = malonaldehyde.

of MDA content in liver, indicating decreased antioxidant capacity in the liver. Similar with our results, the study on the Jian carp (*Cyprinus carpio* var. Jian) showed that exogenous MI could reduce the production of free radicals, aggrandize the activity of antioxidant enzymes, avoid oxidative stress and alleviate apoptosis (Wang et al., 2021). With MI supplementation, the activities of the antioxidant enzyme were increased in the liver. Therefore, under long-term hypertonic stress, high carbohydrate diets showed adverse effects on the growth and the function of antioxidant system in tilapia. However, appropriate amounts of MI in high carbohydrate diets could increase WG and SR, reduce the accumulation of body fat, and improve the antioxidant performance of tilapia, which would be beneficial to improve adaptation of fish exposed to long-term hypertonic stress.

Although euryhaline fish have strong adaptability to salinity change, a series of changes in the metabolism often occur to compensate for the increased energy demand under long-term hyperosmotic stress (Fiess et al., 2007; Mankiewicz et al., 2021; Zhu et al., 2021). In addition, high carbohydrate feed could also provide the extra energy required by euryhaline fish for the osmoregulation (Kumkhong et al., 2021; Xu et al., 2017). Nevertheless, a high carbohydrate feed often caused hyperglycemia and would lead to a huge accumulation of liver glycogen which would impair the function of liver in fish (Oliveira-Júnior et al., 2021; Rodrigues et al., 2018; Sousa et al., 2020). Relevant studies showed that liver glycogen is preferentially decomposed and utilized when fish are under osmotic stress (Guo et al., 2020; Islam et al., 2020). However, a high carbohydrate diet could provide energy for the osmotic regulation in fish and the glycogen may be used in priority. Glycogen accumulation was observed in the liver of fish under a long-term salinity stress in the current study; this was not conducive to the transport of carbohydrates to osmoregulation

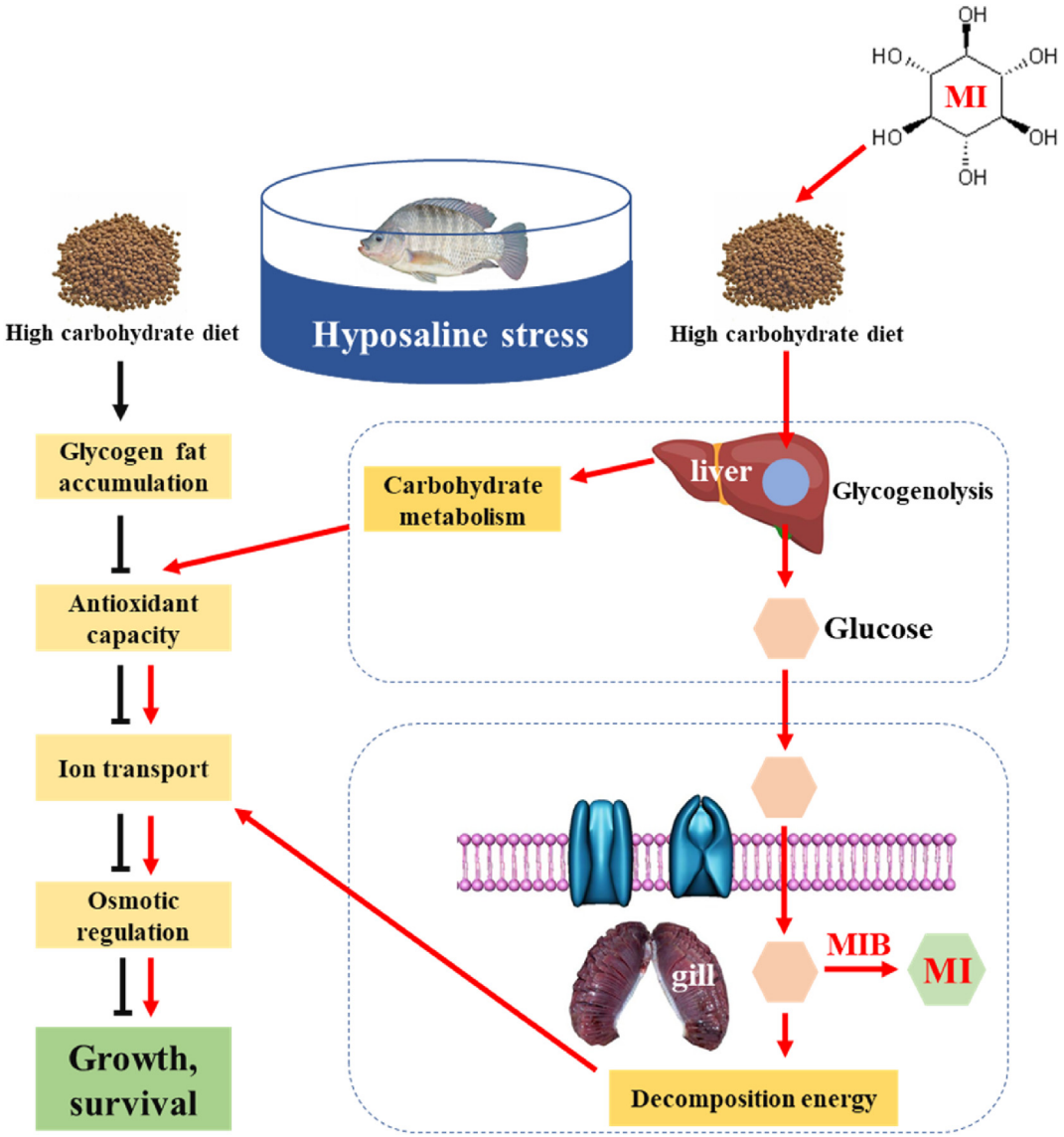

Fig. 8. Metabolic pathway map of the overall article. MI = *myo*-inositol; MIB = *myo*-inositol biosynthesis.

tissues under salinity stress. Therefore, the accumulation of liver glycogen is not only detrimental to osmotic regulation of the whole fish, but also interferes with energy supply under salinity stress. However, the fish fed diets with MI in the current study showed lower glycogen content. These results indicated that MI can mobilize the utilization of liver glycogen under salinity stress and alleviate the accumulation of liver glycogen caused by a high carbohydrate diet (Chen et al., 2019; Khosravi et al., 2015; Zhu et al., 2020, 2021). Dietary MI also enhanced the ability of gluconeogenesis, and increased the glucose transport capacity in the liver in this study. This might be the reason for the elevated blood glucose in fish. Because it is an important metabolic organ, the liver can produce a large amount of glucose by decomposing liver glycogen and gluconeogenesis, which would be then transported to osmotic regulation tissues through blood, to provide energy demanded by osmotic regulation (Liu et al., 2021b; Zhou et al., 2020). Therefore, the adverse effects caused by high dietary carbohydrate would be alleviated by dietary MI in fish. In the meantime, the utilization of carbohydrates can be promoted by dietary MI in fish, which would effectively supply more energy for the long-term adaptation of fish to salinity stress.

Several researches suggested that the gill of euryhaline fish has a great capacity to regulate osmotic balance during osmotic stress (Mozanzadeh et al., 2021; Vargas-Chacoff et al., 2021). Hypersaline stress will lead to the accumulation of inorganic ions in cells, which will destroy the structure of intracellular functional proteins and disturbed the ion transport of cells (Nogueira and Bianchini, 2018; Shui et al., 2018; Wood and Eom, 2021). The ion homeostasis in the gills of euryhaline fish depends on the interaction of multiple ion pumps, such as; *nka*, *nhe*, and *cftr*, which can create an electrochemical gradient for the transport of ions across the lateral and apical membranes in the gill base (Islam et al., 2020; Lin et al., 2021; Nakamura et al., 2021; Yang et al., 2009). In this experiment, a high-carbohydrate diet is detrimental to *nka* transporter activity, MI supplementation could improve the activities of ion transporters. The possible mechanism is that MI, as an osmotic effector, could balance the cell osmotic pressure instead of ions, reducing the inorganic ions accumulation and guarantee the normal ion transport (Fougere et al., 2020; Vargas-Chacoff et al., 2021). Meanwhile, in this research, the contents of Cl^-, Na^+ and K^+ in the serum decreased with the addition of MI. Mitochondria-rich cells in the gill filament contain various ion transporters (Carmo et al., 2018;

Fernandes et al., 2013; Furukawa et al., 2011). Therefore, these cells are mainly responsible for the ion uptake and excretion in gill, and the number of mitochondria-rich cell are the determining factor of the ion transport efficiency (Nogueira and Bianchini, 2018). In the present study, histological analysis of gills showed that impairment, e.g. curved and deformed gill lamella, abnormal blood cell accumulation and cracked gill filament cartilage, occurred in gills in the group without MI under long-term hypersaline stress. These results showed that exogenous MI could protect the function and structure of gill from ion hazard caused by hypersaline stress, and maintain the osmotic balance.

More compatible organic osmolytes are needed to maintain osmotic balance when hypertonic stress persists in the body (Dawood et al., 2020; Vargas-Chacoff et al., 2021). With the addition of MI, the content of MI in gills increased significantly, while there was no evident change of MI content in the liver and kidney. This phenomenon has also been found in the studies about turbot (*Scophthalmus maximus*) (Cui and Ma, 2020; Cui et al., 2020). The possible reason is that MI plays a more important role in osmotic regulation in the gill than in the kidney and liver (Zhu et al., 2021). Though carbohydrate metabolism could supply the reaction substrate of MIB pathway, high dietary carbohydrate did not affect the expression of genes involved in MIB pathway in the current study. The possible mechanism is that appropriate glucose may also act as osmolyte and keep the balance of osmotic pressure in the cells (Asaro et al., 2018; Strbak et al., 2015). Moreover, some metabolites of glucose, such as alcohols, can also act as osmolytes (Anni et al., 2016). Except for gill, the activity of MIB pathway was also induced by dietary MI in the brain. The brain may be an essential organ of osmoregulation and neurohormonal regulation. The MI metabolites can provide a substrate to synthesize phosphatidylinositol, which is essential for signal transduction in response to hyperosmotic stress (Bu et al., 2021; Con et al., 2021; Upton and Riley, 2013). The changes of MIB pathway were consistent with the with the results of the MI content in the gill. This may be that the osmolytes supplied by glucose could not satisfy the requirement of cells in the gill. Therefore, MI was synthesized by MIB pathway and accumulated in the gill cells maintaining the structural integrity and guaranteeing the efficient ion transport (Hu et al., 2018; Ma et al., 2020).

5. Conclusion

Although high carbohydrate diets may adversely affect the health of tilapia, they would provide energy for osmotic regulation under long-term hypertonic stress. Dietary MI could regulate carbohydrate metabolism pattern to provide energy support for long-term salinity culture. Exogenous MI could protect the function and structure of gill from ion poisoning caused by hypertonic stress, ensure efficient ion transport and maintain osmotic balance in tilapia. Dietary 1,200 mg/kg MI could significantly improve the antioxidant capacity and improve the utilization of carbohydrates during osmoregulation, and thus promote the growth performance of tilapia in long-term salinity culture (Fig. 8).

Authors contributions

Jiahua Zhu, **Xiaodan Wang** and **Liqiao Chen** conceived this research and designed the experiments; **Jiahua Zhu**, **Fan Zhang**, **Jingyu Pan** and **Yuxing Huang** performed experiments; **Jiahua Zhu** analyzed data and drafted the manuscript; **Jianguang Qin**, **Xiaodan Wang**, **Chuanjie Qin**, **Erchao Li** and **Liqiao Chen** polished the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations 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.

Acknowledgment

This work was sponsored by grants from the National Natural Science Foundation of China (No. 32172946), China Postdoctoral Science Foundation (2018 M630418), the Fundamental Research Funds for the Central Universities, ECNU and China Agriculture Research System-46 (CARS-46).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2022.04.006.

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