The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB)

&

University of Hawai'i at Mānoa

&

AquacultureHub

http://www.aquaculturehub.org

ISSN 0792 - 156X

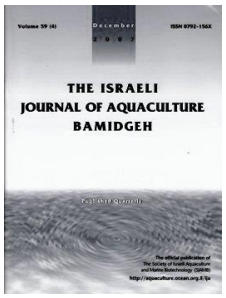
PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH - Kibbutz Ein Hamifratz, Mobile Post 25210, ISRAEL

Phone: + 972 52 3965809

http://siamb.org.il

The Israeli Journal of Aquaculture - Bamidgeh, IJA_68.2016.1259, 10 pages



Utilization and Metabolism of α-Starch by Black Carp (*Mylopharyngodon piceus*) Juveniles

Miao Ling-Hong [1,2], Ren Ming-Chun [2], Ge Xian-Ping [1,2*], Xie Jun [1,2], Zhu Jian [1,2], Liu Bo [1,2*], Zhou Qun-Lan [1,2], Pan Liang-Kun [2]

[1] *Fishery College, Nanjing Agriculture University, Wuxi 214081, China*

[2] *Key Laboratory of Genetic Breeding and Aquaculture Biology of Freshwater Fishes, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Jiangsu Wuxi 214081, China*

Key words: *Mylopharyngodon piceus*; carbohydrate; growth; immunity; transmission electron microscopy

Abstract

The ability of black Carp *Mylopharyngodon piceus*, to utilize high levels of dietary carbohydrate was assessed using artificial purified feed, containing normal (20%, NS group) and high (40%, HS group) levels of digestible carbohydrate. After a 10 week feeding trial, specific growth rate, weight gain rate, and protein efficiency ratio of the HS group were significantly lower ($P<0.05$). The 40% carbohydrate diet significantly influenced the crude protein and crude fat levels in the muscle tissue ($P<0.05$). Plasma cortisol of the HS group was significantly lower ($P<0.05$), and levels of plasma triglycerides and total cholesterol were highly significantly lower ($P<0.01$). Hepatic superoxide dismutase concentrations of the HS group were lower ($P<0.05$), and contents of the carbohydrate metabolic enzyme, hepatic pyruvate kinase were highly significantly lower ($P<0.01$). Hepatic ultrastructure results show that the high carbohydrate diet induced sedimentation of glucose and fat in the hepatic cells, and ultrastructural damage was observed. Although *Mylopharyngodon piceus* are able to tolerate long-term feeding of the 40% carbohydrate diet, effects on growth and immunity were negative resulting in ultrastructural damage of the hepatic cells.

* Corresponding author: Xian-Ping Ge. Tel: 0086-510-85557892, fax: 86-510-85553304, E-mail: gexp@ffrc.cn; Bo Liu. Tel: 0086-510-85556101.

Introduction

Carbohydrates are the cheapest energy source in fish feeds and are efficiently used by fish. Inclusion of carbohydrates in artificial fish diets can improve growth performance, decrease ammonia excretion, and spare proteins resulting from use as an energy source in many fish species (Azaza et al., 2013). These effects may in turn help to reduce fish feed costs. Developments in nutritional physiology, aquaculture technology, and economic constraints have triggered the use of cheaper feed ingredients, containing higher carbohydrate levels. Excess dietary carbohydrates which cannot be efficiently utilized for energy by fish negatively affect fish health. They can cause metabolic disturbances (Polakof et al., 2012), lead to physiological alterations, such as prolonged hyperglycemia (Hatlen et al., 2005) trigger hepatic anti-oxidative response (Azaza et al., 2013), cause high fat deposition in the whole body and the liver (Hemre et al., 2002), lead to low red blood cell and hemoglobin levels (Abdel-Tawwab et al., 2010), increase liver histopathology (Russell et al., 2001), and impair bone development (Tan et al., 2009).

Nutrient levels in feed are important determinants of disease resistance in fish. Both overnutrition and malnutrition cause stress in fish, in turn affecting immune response and disease resistance (Chandra, 1996). Aquaculture studies have found that a high intake of glucose slows down growth rate, decreases efficiency of feed utilization, causes accumulation of hepatic glycogen, metabolic disorders (Hemre et al., 2002), and long term hyperglycemia (Moon, 2001). In addition, the intake of excess glucose has been shown to cause metabolic stress and decrease immunity (Pieper and Pfeffer, 1980). Excessive deposition of glycogen causes damage to somatic cells in rainbow trout and decreases the detoxification capacity of the liver through its influence on mineral metabolism (Stone, 2003a). Although carbohydrates are a high quality energy source and are relatively inexpensive, research into whether high carbohydrate diets cause stress to fish, influence fish growth, or affect disease resistance is inconclusive.

Fish species differ in their ability to digest carbohydrates. Even within fish species the carbohydrate content of natural diets varies. This variability reflects anatomical and functional differences in the gastrointestinal tract and associated organs. Fish, particularly those considered to be carnivorous, are limited in their use of carbohydrates for energy (Moon, 2001; Stone, 2003a). The physiological basis for this apparent glucose intolerance is not fully understood (Hemre et al., 2002; Enes et al., 2009). Black carp, *Mylopharyngodon piceus* (Richardson), is one of four major Chinese cyprinids and a typical carnivorous fish. Its demand for digestible carbohydrate is about 20% (Wang et al, 1984). This study examines differences in glucose utilization and tolerance in *Mylopharyngodon piceus* by comparing the effect of 20% and 40% digestible carbohydrate levels in feed on growth performance, antioxidant capacity, non-specific immunity and ultrastructure of the liver cells in the fish. The study provides a theoretical analysis of utilization of dietary carbohydrates in carnivorous fish.

Ethics statement. This study was approved by the Animal Care and Use Committee of the Centre for Applied Aquatic Genomics at Chinese Academy of Fishery Sciences.

Materials and Methods

Diets. In this experiment α-starch was used as the source of digestible carbohydrate. Two isonitrogenous and isolipidic feed formulas were designed (Table 1). In the control formula (with normal starch levels; NS), and the high glucose formula (HS), 20% and 40% α-starch was added respectively. In the lower carbohydrate content formula (NS), α-starch content was replaced with microcrystalline cellulose.

The content of digestible carbohydrates in each group was 20.5% and 40.5%, respectively. All experimental feeds were thoroughly mixed and an SLP-45 granulator (Chinese Academy of Fishery Sciences, Fishery Machinery Instrument Research Institution) was used to prepare the 3mm diameter pelleted feeds.

Table 1. Composition and proximate analysis of the normal starch (NS; control) and high starch (HS) experimental diets used in this study.

	Diets	
	NS	*HS*
Ingredients (%)		
fish meal [a]	48.0	48.0
α-starch [b]	20.0	40.0
microcrystalline cellulose	27.0	7.0
Fish oil	1.0	1.0
1% premix feed [c]	1.0	1.0
carboxyl methyl cellulose	2.0	2.0
calcium dihydrogen phosphate	1.0	1.0
Total	100.0	100.0
Proximate analysis (% dry weight)		
dry matter	90.7	91.0
crude protein	30.3	31.2
crude fat	5.32	5.30
digestible carbohydrate [d]	20.5	40.5
gross energy (KJ·g-1)	17.0	18.5
calcium	2.1	2.1
total phosphorous	1.6	1.6

[a] Fish meal: protein 68%., Tongwei Co., Ltd. China
[b]α-starch: bought from Jing Lingta Co., Ltd. China
[c] Premix feed (kg^{-1} diet): $CuSO_4 \bullet 5H_2O$, 2.0 g; $FeSO_4 \bullet 7H_2O$, 25 g; $ZnSO_4 \bullet 7H_2O$, 22 g; $MnSO_4 \bullet 4H_2O$, 7 g; Na_2SeO_3, 0.04 g; KI, 0.026 g; $CoCl_2 \bullet 6H_2O$, 0.1 g; VA, 900000 IU; VD, 200000 IU; VE, 4500 mg; VK_3, 220 mg; VB_1, 320 mg; VB_2, 1090 mg; VB_5, 2000 mg; VB_6, 500 mg; VB_{12}, 1.6 mg; VC, 10000 mg; Pantothenate, 1000 mg; Folicacid, 165 mg.
[d] Dietary digestible carbohydrate determined according to Wang (2008)

Feeding and management. Mylopharyngodon piceus specimens were obtained from Changyang Lake Fish Farm, Liyang City, Jiangsu Province, China. Healthy fish of similar size (initial body weight: 33.80 ± 1.11 g; mean ± SE) were selected and allocated into ten tanks, at a stocking density of 25 fish per tank. Tanks were randomly divided into two groups, NS (control), and HS (high starch), with five tanks per group. Fish were handfed to near satiation three times a day (08:30, 12:30, and 16:30) for 70 days.

The feeding trial was conducted in an indoor freshwater recirculating system composed of ten fiberglass tanks (300 L each) and their associated mechanical filtration units. All tanks were supplied with equal aeration and water flow rates (approximately 3 L/min). During the experimental period, water temperature was monitored using a data logger and various water quality parameters were recorded (temperature 26.0 ± 1.5°C; DO ≥ 6 mg/L; NH_3 ≤ 0.1 mg/L; pH 6.8–7.0).

Sampling and analysis. At the end of the 70-day feeding trial, the fish were fasted for 24 h before sampling. Total weight and number of the fish per tank were recorded. Weight gain rate (WGR), specific growth rate (SGR), feed conversion ratio (FCR), feeding rate (FR), and protein efficiency ratio (PER) were calculated. Three fish from each tank (15 per group) were then sampled. The fish were immediately euthanized with MS-222 (200 mg/L). Blood samples were obtained from the caudal vein and centrifuged at 4000 rpm for 10 min at 4°C. Serum was collected and stored at -20°C until analysis. The liver was stripped immediately after blood samples were taken. Part of the liver was used to prepare a 10% liver homogenate and stored at −20°C to determine the hepatic antioxidant indices. The remaining part of the liver tissue blocks were washed with physiological saline solution and fixed with 2.5% glutaraldehyde solution for 24 h, to prepare sections for transmission electron microscopy (TEM). Fish were scaled, and muscle from the back of the fish was taken for muscle composition analysis.

Serum total protein (TP), triglyceride (TG), total cholesterol (CHOL), lactic acid (LA), glucose (GLU), alkaline phosphatase (ALP), and aspartate transarninase (AST) levels were determined by colorimetric method (Beckman Cx-4 Automatic Biochemical

Analyzer, Fullerton, CA, US) and kits purchased from Shanghai Mingdian Bioengineering Co., Ltd. (China). Serum cortisol B (COR) and insulin (INS) levels were determined using a radioimmunoassay (RIA; Beijing North Institute of Biological Technology, China). In the liver, pyruvic acid (PA), pyruvate kinase (PK), superoxide dismutase (SOD), methane dicarboxylic aldehyde (MDA) and total antioxidant capacity (T-AOC) levels were determined using commercial kits provided by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Dry matter, crude protein (CP), crude fat (CF) and ash in the muscle tissue were determined according to the established methods of the Association of Official Analytical Chemists (AOAC, 2003). Dry matter was determined after drying in an oven at 105°C until constant weight; crude protein (N × 6.25) was analyzed with the Kjeldahl method after acid digestion; lipid levels were assessed by Soxhlet extraction with ether; ash was determined after drying in a muffle furnace at 550°C until constant weight.

Statistical analysis. All data are expressed as mean ± standard error (mean ± SE). Independent t-tests were conducted to compare the means between the groups using SPSS (version 16.0). The levels of significance and high significance were set at $P<0.05$ and $P<0.01$, respectively.

Results

Growth and feed utilization rates. After 70 days of consuming the two diets with different levels of carbohydrates, SGR, WGR, and PER were significantly lower ($P<0.05$) in the HS group compared to the NS group (Table 2). FR and FCR of fish in both groups were not significantly different ($P>0.05$).

Table 2. The effects of the two experimental diets used in this study on growth and feed utilization in *Mylopharyngodon piceus.*

	NS	*HS*
Initial body weight	34.71 ± 0.13	34.64 ± 0.10
final body weight	84.91 ± 3.90	69.68 ± 3.02*
SGR[a]	1.28 ± 0.07	0.93 ± 0.01*
WGR[b]	144.74 ± 0.09	92.24 ± 0.07*
FCR[c]	1.11 ± 0.01	1.14 ± 0.02
FR[d]	2.12 ± 0.05	2.15 ± 0.02
PER[e]	1.88 ± 0.04	1.45 ± 0.09*

Values presented are mean ± S.E., n = 5; NS = normal starch (control) diet; HS = high starch (glucose rich) diet.
*treatments significantly different ($P < 0.05$; T-test).
[a] Specific growth rate (SGR, %) = 100 × [(Ln final body weight − Ln initial body weight)/test days].
[b] Weight gain rate (WGR, %) = 100 × (average final body weight − average initial body weight)/average initial body weight.
[c] Feed conversion rate (FCR) = feed intake/weight gain.
[d] Feeding rate (FR, % weight/day) = (100 × dry feed intake)/[days × (terminal body weight + initial body weight)/2].
[e] Protein efficiency ratio (PER, %) = 100 × (wet weight gain)/(crude protein intake).

Muscle composition. There was no significant difference ($P>0.05$) in dry matter and ash content of muscle in fish from both groups (Table 3). CP content in the HS group was significantly lower ($P<0.05$), than the NS group, while CF content was significantly higher ($P<0.05$).

Table 3. The effects of the two experimental diets used in this study on the muscle composition of *Mylopharyngodon piceus*

Muscle component	*NS*	*HS*
DM (%)	24.23 ± 0.69	24.61 ± 0.31
CP (%) DM	81.27 ± 0.21	78.43 ± 0.78*
CF (%) DM	8.81 ± 0.44	11.82 ± 0.91*
Ash (%) DM	7.59 ± 0.32	7.83 ± 0.46

Values are mean ± S.E., n = 15; NS = normal starch (control) diet; HS = high starch (glucose rich) diet; DM = dry matter; CP = crude protein; CF = crude fat.
*treatments significantly different ($P < 0.05$; T-test).

Plasma biochemical indicators. CHOL, TG and COR levels in plasma of fish in the HS group were significantly higher than in the NS group (Table 4); the differences were highly significant ($P < 0.01$) for CHOL and TG levels. TP content of fish plasma in both groups was not significantly different ($P > 0.05$).

Table 4. The effects of the two experimental diets used in this study on the plasma biochemical indicators in *Mylopharyngodon piceus.*

Plasma indexes	*NS*	*HS*
TP (g/L)	20.36±1.63	21.81±1.04
CHOL (mmol/L)	4.47±0.38	6.4±0.3**
TG (mmol/L)	1.52±0.22	2.61±0.26**
COR	56.73±7.15	98.96±12.04*

Values are mean ± S.E., n = 15; NS = normal starch (control) diet; HS = high starch (glucose rich) diet; TP = total protein; CHOL = total cholesterol; TG = tryglyceride and COR = cortisol B.
*treatments significantly different ($P < 0.05$; T-test).
**treatments highly significantly different ($P < 0.01$; T-test).

Carbohydrate metabolism. Hepatic PK content was highly significantly lower ($P<0.01$) in the HS group (Table 5). In contrast, plasma INS, GLU, LA, and hepatic PA levels in both groups were not significantly different ($P > 0.05$).

Table 5. The effects of the two different experimental diets used in this study on carbohydrate metabolism in *Mylopharyngodon piceus.*

		NS	*HS*
Plasma indexes	INS (mmol/L)	1.20±0.3	1.11±0.26
	GLU (mmol/L)	37.57±2.01	35.49±0.90
	LA (mmmol/L)	3.35±0.27	4.16±0.25
Hepatic indexes	PK (U/gprot)	250.90±9.86	159.15±13.52**
	PA (umol/mgprot)	0.56±0.06	0.44±0.04

Values are mean ± S.E., n = 15; NS = normal starch (control) diet; HS = high starch (glucose rich) diet; INS = insulin; GLU = glucose; LA = lactic acid; PK = pyruvate kinase; PA = pyruvic acid.
*treatments significantly different ($P < 0.05$; T-test).
**treatments highly significantly different ($P < 0.01$; T-test).

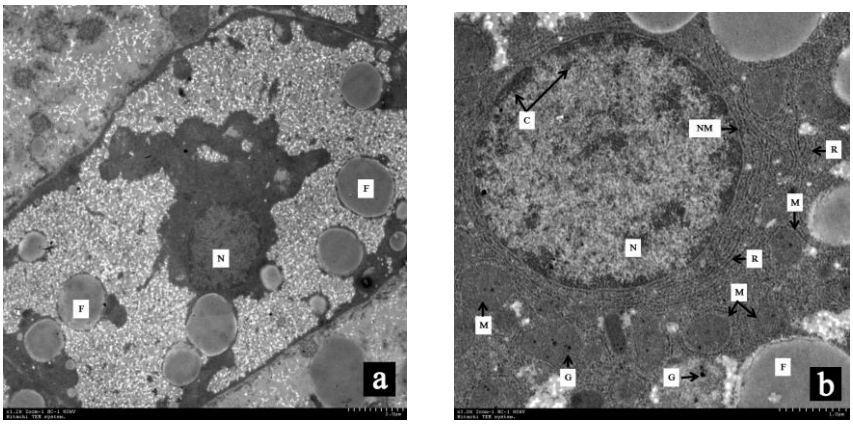
Immunity. SOD content of the liver in the HS group was highly significantly lower ($P<0.01$) than in the NS group (Table 6). In contrast, the MDA and T-AOC in the liver, and plasma AST and AKP levels were not significantly different ($P > 0.05$).

Table 6. The effects of the two different experimental diets used in this study on immunity of *Mylopharyngodon piceus*.

		NS	*HS*
Plasma indexes	ALP(U/L)	117.43±6.08	154.71±18.18
	AST (U/L)	73.86±6.33	70.14±3.36
Hepatic indexes	SOD(U/mgpro)	659.43±54.71	454.75±35.38*
	T-AOC (U/mgpro)	12.28±3.72	12.30±2.73
	MDA (nmol/gpro)	10.08±1.04	11.45±3.65

[1] Values are mean ± S.E., n = 15. NS = normal starch (control) diet; HS = high starch (glucose rich) diet.ALP= alkaline phosphatase; AST= aspartate transarninase; SOD= superoxide dismutase; T-AOC= total antioxidant capacity; MDA= methane dicarboxylic aldehyde.
* treatments significantly different ($P < 0.05$; T-test).

The hepatic ultrastructure. The images from TEM clearly show that the liver cells of fish fed the NS diet had round cell nuclei, and the heterochromatin in the nucleus was close to the intima with high electron density (Figures 1a, b). Cytoplasm was also homogeneous in fish fed the NS diet. The endoplasmic reticulum and mitochondria were distinct; rough endoplasmic reticulum was dominant, arranged around the cell nuclei and mitochondria. The mitochondria were numerous, with a clear structure, rich matrix, a short and tubular shaped ridge, with a striped appearance when sectioned longitudinally, and circular in transverse sections. Electron density of the matrix was homogeneous and glycogen particles were rare. There were fewer, small, lipid droplets in the cells.



In the HS group, although the nuclei in the liver cells were round, there was more hetrerochromatin and the nuclear membranes were uneven. (Figures 1c, d, e, f). Rough endoplasmic reticula in the cells were low, smooth and smooth, disorderly around the mitochondria and nuclei; most of the smooth endoplasmic reticulum was arranged close to the cell membrane and around the mitochondria. Mitochondrial shape in liver cells of fish fed the HS diet was also distorted. Electron density was low, the double membrane structure was damaged or destroyed, there was a crowded and irregular arrangement of organelles scattered through the cytoplasm and the end of the ridge of the mitochondria was enlarged and bubbly. The number of glycogen granules in the nuclei and cytoplasm of the fish fed the HS diet was higher than the NS diet and they were distributed in different organelle-like plaque structures. There were many different sized lipid droplets in the cytoplasm, showing light gray colored balls with the homogeneous dye; parts of the fat droplets extruded the cell nucleus.

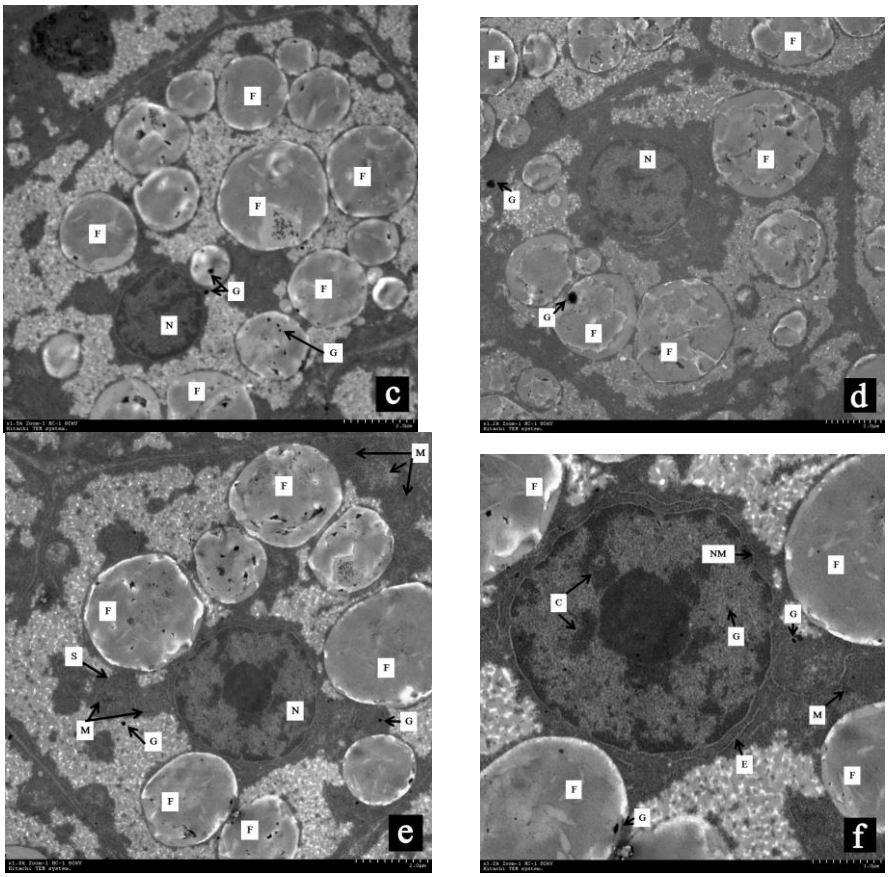


Fig. 1. Ultrastructure of the hepatic cells in the fish subjected to diets with different levels of carbohydrate. (a) The hepatic cell ultrastructure and (b) the hepatic cell nucleus ultrastructure of the fish fed with the NS diet (×1200 and ×3000, respectively). (c–e) The hepatic cell ultrastructure and (f) the hepatic cell nucleus ultrastructure of the fish fed with the HS diet (c, ×1500; d, ×1200; e, ×1500; f, ×3000). Key: C, chromatin; M, mitochondria; N, nucleus; NM, nuclear membrane; R, rough endoplasmic reticulum; S, smooth endoplasmic reticulum; G, glycogen; F, fat; E, endoplasmic reticulum.

Discussion

Studies of cultured fish species investigating high carbohydrate content in the diet have focused on effects of the origin of the starch and effects of its inclusion at different levels in the diet on the nutrient digestibility and retention efficiency, as well as growth performance (Capilla, 2003; 2004). In fish diets some digestible carbohydrate is often used to partially replace protein thereby saving protein for other functions (Erfanullah and Jafri, 1995).

In this experiment when digestible carbohydrate was 40%, specific growth rate, weight gain rate, and utilization efficiency of protein were all significantly ($P< 0.05$) lower than when it was 20%. Starch content of 30% in the feed of *Bidyanus* sp. improved the protein sparing effect, but when increased to 45%, growth decreased significantly (Stone et al., 2003b). When *Cyprinus carpio* were fed diets containing 10%, 20% and 30% dextrin, their growth was not significantly affected, but in diets containing 40% dextrin, growth decreased (Furuichi and Yone, 1980).

Excessive use of carbohydrate in feed does not affect growth and feed utilization in fish (Lee and Lee, 2004). Body protein and ash contents of *Dicentrarchus labrax* were not different after feeding fish with 13.6%, 22.5% and 31.1% starch (Moreira et al. 2008). In our experiment, muscle crude protein content was significantly lower in the HS group than that in the NS group, and crude fat content was significantly higher.

The liver is an important metabolic organ in the body, and nutrient metabolism in the livers of fish can change with different levels of carbohydrate in the feed. Plasma total protein, triglyceride and total cholesterol levels reflect the physiological and metabolic functioning of the liver. Liver function impairment decreases protein synthesis, and quantity and quality of protein levels in the plasma change; this can lead to a decrease of serum total protein and albumin levels. In our experiment, the plasma triglyceride content was significantly higher in the HS group impairing ability to metabolize excessive intake of sugar that accumulated in the form of fat and large amounts of triglycerides during the process of fat metabolism (Panserat et al. 2002). Elevated triglyceride levels cause formation of fatty liver. In mammals, damaged liver cells resulted in lowered serum cholesterol levels (Yan 2006). However in our study total cholesterol content in the HS group was significantly higher. Studies have shown that when fish are in situations of stress (captivity, fear, high density, or fluctuation in temperature and salinity) serum cortisol levels tend to increase (Fevolden et al., 2003; Liu et al., 2011). Cortisol levels are often used as a reliable indicator of fish stress. Serum cortisol content of the fish in the HS group was significantly higher than in the control group (NS), indicating that long-term exposure to a high carbohydrate diet caused stress in *Mylopharyngodon piceus*.

In fish, during glycol metabolism, blood glucose and blood insulin levels directly reflect the utilization and metabolism of carbohydrates. In this study 20% and 40% carbohydrate in the diets did not lead to differences in blood glucose and insulin levels, but the high carbohydrate diet significantly lowered the level of hepatic pyruvate kinase. Pyruvate kinase, which turns phospho-enol-pyruvic acid and ADP into ATP and pyruvic acid, is one of the major rate-limiting enzymes in the process of glycolysis suggesting that excessive carbohydrate in the diet also inhibits the glycolytic function of the liver in *Mylopharyngodon piceus*.

In this experiment, plasma alkaline phosphatase levels and aspartic acid transaminase were not affected by the different carbohydrate diets. In animals, alkaline phosphatase is an important regulatory enzyme for metabolism, and also an important detoxification system.

In *M. piceus,* liver superoxide dismutase levels were significantly lowered by the 40% carbohydrate diet compared to the 20% carbohydrate diet. The methane dicarboxylic aldehyde levels and total antioxidant capacity were similar. This indicates that, when fed daily, the 40% carbohydrate diet has a negative influence on the hepatic anti-oxidative capacity, and may even induce liver damage. These results differ from those of Zhao (2009) in *M. piceus.* In normal hepatic cells the rough endoplasmic reticulum is arranged around the nucleus as a lamellar structure, with many ribosomes, the sites of protein

synthesis, attached. In fish the rough endoplasmic reticulum is widely distributed in the cytoplasm, especially around the nucleus and there are smaller amounts of smooth endoplasmic reticulum, distributed in the dieldrin cavity and near bile canaliculi. The smooth endoplasmic reticulum suggests cellular detoxification, bile salt formation and the glucoside acid connection of the bile duct wall (Bonates and Ferri, 1980). Under normal circumstances, mitochondria are round or oval in fish hepatic cells; the ridge is a short tubular shape, arranged sparsely and irregularly. In specimens with damaged liver cells, the structure of the inner ridge is often destroyed, and the intra-membranous space is generally obvious, electron staining is usually very light, and the electrons in the intra-membranous space are deep, with particulate matter. Transmission electron microscopy showed that the cell nucleus, mitochondria and other organelles of the liver cells in HS group had distorted shapes, random arrangements, swelling, local collapse and disintegration of the cristae in the mitochondria, which may block protein synthesis compared with the NS group. A large number of ribosomes were detached from the rough endoplasmic reticulum, and the size of smooth endoplasmic reticulum was greater. These results suggest that the HS diet caused irritation and injury to the hepatic cells in *M. piceus*.

Fish fed the HS diet had greater amounts of glycogen and lipid droplets in their hepatic entocytes compared to the NS group. High glucose induced many lipid droplets and glycogen granules in the hepatic cells during research into glucose metabolism in *Erythroculter ilishaeformis* (Wang 2009). Accumulation of hepatocyte glycogen was found to be the result of pathological changes in the decomposition pathway, and could result from long-term exposure to a high carbohydrate diet (Gonzalez et al. 1993). This study has shown that the liver in *M. piceus* has a certain capacity for glucose catabolism, and transforms some excess glycogen into fat, storing it as a source of energy in the liver.

Conclusions

Although our results have shown that *M. piceus* can tolerate 40% dietary carbohydrate, at this level of carbohydrate in the daily diet, growth tests showed that weight gain rate, specific growth rate, and protein efficiency, were negatively affected. In addition, blood and liver physiology, immune index, and ultrastructure of hepatic cells show adverse effects on liver cells, and excess carbohydrate inhibits growth, causes inhibition of metabolism in hepatic cells and affects the immune function of carnivorous *M. piceus*.

Acknowledgements

We are grateful to the graduate students and those from the Fish Disease and Nutrition Department, Freshwater Fisheries Research Center (FFRC), Chinese Academy of Fishery Sciences (CAFS) for their help. This work was funded by the China Agriculture Research System Special Project of National Conventional Freshwater Fishery Industry (CARS-46), the National Natural Science Foundation of China (31472289).

References

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A., et al., 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 298 (3-4): 267-274.

AOAC, 2003. Official Methods of Analysis of the Association of Official Analytical Chemists 15th ed. *Association of Official Analytical Chemists*, Inc., Arlington, VA.

Azaza, M.S., Khiari, N., Dhraief, M.N., et al., 2013. Growth performance, oxidative stress indices and hepatic carbohydrate metabolic enzymes activities of juvenile Nile tilapia, *Oreochromis niloticus* L., in response to dietary starch to protein ratios. *Aquac Res*, 46(1):14-27.

Bonates, A.B., Ferri, S., 1980. Fine structure of a freshwater teleost (*Pimelodus maculatus*) hepatocytes revealed by ultrathin sections. *Anatomischer Anzeiger*, 148 (2):132-44.

Capilla, E., Médale, F., Navarro, I., et al., 2003. Muscle insulin binding and plasma levels in relation to liver glucokinase activity, glucose metabolism and dietary carbohydrates in rainbow trout. *Regul Peptides*, 110 (2): 123-132.

Capilla, E., Médale, F., Panserat S., et al., 2004. Response of hexokinase enzymes and the insulin system to dietary carbohydrates in the common carp, *Cyprinus carpio*. *Reprod Nutr dev*, 44 (3), 233-242.

Chandra, R.K., 1996. Nutrition immunity and infection: from basic knowledge of dietary manipulation of immune responses to practical application of ameliorating suffering and improving survival. *Proceeding National Academic Society*, 93 (25): 14304-14307.

Enes, P., Panserat, S., Kaushik, S.,et al., 2009. Nutritional regulation of hepatic glucose metabolism in fish. *Fish Physiol Biochem*, 35(3): 519-539.

Erfanullah, Jafri, A.K., 1995. Protein-sparing effect of dietary carbohydrate in diets for fingerling *Labeo rohita*. *Aquaculture*, 136 (3-4) :331-339.

Fevolden, S.E., Roed, K.H., Fjalestad, K.T., 2003. A combined salt and confinement stress enhances mortality in rainbow trout (*Oncorhynchus mykiss*) selected for high stress responsiveness. *Aquaculture*, 216 (1): 67-76.

Furuichi, M., Yone, Y. 1980. Effects of dietary dextrin levels on growth and fed efficiency, the chemical composition of liver and dorsal muscle, and absorption of dietary protein and dextrin in fishes. *Bull Chem Soc Japan*, 46 (2):225-229.

Gonzalez, S.,Crespo, S., Brusle, J., 1993. Histo-cytological study of the liver of the cabrilla sea bass, *Serranus cabrilla* (Teleostei, Serranidae), an available model for marine fish experimental studies. *J Fish Biol*, 43 (3):363-373.

Hatlen, B., Grisdale-Helland, B., Helland, S.J., 2005. Growth, feed utilization and body composition in two size groups of Atlantic halibut (*Hippoglossus hippoglossus*) fed diets differing in protein and carbohydrate content. *Aquaculture* , 249 (1-4): 401-408.

Hemre, G.I., Mommsen, T., Krogdahl, Å., 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquac Nutr*, 8 (3): 175–194.

Lee, S.M., Lee, J.H., 2004. Effect of dietary glucose, dextrin and starch on growth and body composition of juvenile starry flounder *Platichthys stellatus*. *Fish Sci*, 70 (1):53-58.

Liu, B., Wang, M.Y., Xie, J., et al., 2011. Effects of acute cold stress on the biochemical, immune parameter and level of HSP70 gene expression in liver of GILT strain of Nile tilapia (*Oreochromis niloticus*). *J Ecol*, 31(14): 1-9.

Moon, T.W., 2001. Glucose intolerance in teleost fish: fact or fiction. *Comp Biochem and Phys B*, 129 (2): 243-244.

Moreira, I.S., Peres, H., Couto, A., et al., 2008. Temperature and dietary carbohydrate level effects on performance and metabolic utilisation of diets in European sea bass (*Dicentrarchus labrax*) juveniles. *Aquaculture,* 274 (1) :153-160.

Panserat, S., Perrin, A., Kaushik, S., 2002. High dietary lipids induce liver glucose-6-phosphatase expression in rainbow trout (*Oncorhynchus mykiss*). *J Nutr*, 132 (2):137-141.

Pieper, A., Pfeffe,r E., 1980. Studies on the effect of increasing proportions of sucrose or gelatinized maize starch in diets for rainbow trout (*Salmo gairdneri,* R.) on the utilization of dietary energy and protein. *Aquaculture*, 20 (4): 333-342.

Polakof, S., Pansera,t S., Soengas, J.L., et al., 2012. Glucose metabolism in fish: a review. *J Comp Physiol B.* 182 (8): 1015-1045.

Russell, P., Davies, S., Gouveia, A., et al., 2001. Influence of dietary starch source on liver morphology in juvenile cultured European sea bass (*Dicentrarchus labrax* L.). *Aquac Resh*, 32 (Suppl 1), 306-314.

Stone, D.A.J., 2003a. Dietary carbohydrate utilization by fish. *Rev Fish Sci*, 11(4): 337-369.

Stone, D.A.J, Allan, G.L., Anderson, A.J., 2003b. Carbohydrate utilization by juvenile silver perch, Bidyanus *bidyanus* (Mitchell). III. The protein-sparing effect of wheat starch-based carbohydrates. *Aquac Res*, 34 (2):123-134.

Tan, Q.,Wang, F., Xie, S., et al., 2009. Effect of high dietary starch levels on the growth performance, blood chemistry and body composition of gibel carp (*Carassius auratus* var. gibelio). *Aquac Res*, 40 (9), 1011-1018.
Wang, D.Z., Song, T.F., Du, H.B., et al., 1984. The effect of protein and carbohydrate content in feeds on the growth of Black Carp fingernlings. *J Fish China*, 1984,8(1):9-17.
Wang, F., 2008. The effect of high levels of dietary starch on growth, metabolism and body composition for gibel carp (*Carassius auratus gibelio*) Huazhong Agricultural University, Wuhan, Hubei.
Wang, G.Y., 2009. Influence of carbohydrate levels in daily ration on growth, blood indexs and expression of GK, G6Pase and Hsc70 gene of erythroculter ilishaeformis. Nanjing Agricultural University, Nanjing, Jiangsu.
Yan, H.W., Zhang, X., Dang, S.G., 2006. Application of serum total cholesterol in liver diseases. *Gastroenterology,* 2:1-3.
Zhao, Y.Z., 2009. Advanced in the study of utilization of carbohydrate by black carp (*Mylopharyngodln piceus* Richardson). Suzhou University, Suzhou, Jiangsu.