JOURNAL OF THE
WORLD AQUACULTURE SOCIETY

Vol. 47, No. 2
April, 2016
doi: 10.1111/jwas.12259

Effects of Dietary Carbohydrate-to-lipid Ratio on Growth Performance, Body Composition, Digestive Enzyme Activities, and Hepatic Enzyme Activities in Juvenile Large Yellow Croaker, *Larimichthys crocea*

Shujuan Xing

The Key Laboratory of Aquaculture Nutrition and Feeds, Ministry of Agriculture, Qingdao, 266003, China
The Key Laboratory of Mariculture, Ministry of Education, Qingdao, 266003, China
Fisheries College, Ocean University of China, Qingdao, 266003, China

Ruijian Sun

The Key Laboratory of Aquaculture Nutrition and Feeds, Ministry of Agriculture, Qingdao, 266003, China
The Key Laboratory of Mariculture, Ministry of Education, Qingdao, 266003, China
Fisheries College, Ocean University of China, Qingdao, 266003, China
Technology Center of Tongwei Co., Ltd, Chengdu, 610041, China

Xiaoyi Pan, Jun Ma, Wenbing Zhang,[1] and Kangsen Mai

The Key Laboratory of Aquaculture Nutrition and Feeds, Ministry of Agriculture, Qingdao, 266003, China
The Key Laboratory of Mariculture, Ministry of Education, Qingdao, 266003, China
Fisheries College, Ocean University of China, Qingdao, 266003, China

Abstract

An 8-wk feeding trial was conducted to investigate the effects of dietary carbohydrate-to-lipid ratios (CHO : L) on growth performance, body composition, digestive enzyme activities, and hepatic enzyme activities of juvenile large yellow croaker, *Larimichthys crocea*. Six isonitrogenous (45% crude protein) and isoenergetic (18 kJ/g gross energy) diets with varying CHO : L ratios (0.07, 0.48, 1.20, 2.19, 4.81, and 10.48) were fed to triplicate groups of large yellow croaker in floating sea cages. Results showed that the highest specific growth rate (SGR) was found in fish fed diets with CHO : L ratio of 2.19. Fish fed the lower (0.07 and 0.48) CHO : L ratios tended to produce lower growth ($P < 0.05$). The whole-body lipid content significantly decreased, while hepatosomatic index, liver glycogen content, and plasma glucose concentration significantly increased as dietary CHO : L ratios increased ($P < 0.05$). Plasma total cholesterol, triglyceride, and low-density lipoprotein cholesterol concentrations significantly decreased with elevated dietary CHO : L ratios ($P < 0.05$). The increasing dietary CHO : L ratios significantly stimulated the activities of intestinal amylase and hepatic pyruvate kinase and depressed the activity of hepatic phosphoenolpyruvate carboxykinase ($P < 0.05$). Based on a second-order polynomial regression analysis of SGR, 2.38 was determined as the optimal dietary CHO : L ratio for juvenile large yellow croaker.

As a commercially important carnivorous fish species, large yellow croaker, *Larimichthys crocea* has been widely cultured in the south of China, especially in Fujian and Zhejiang provinces. Now it is the third largest mariculture fish species in China, with 105,230 metric tons produced in 2013 (Fishery Bureau, Ministry of Agriculture 2014). The commercial diet for this fish species usually contains approximately 43–48% protein and about 11% lipid

[1] Correspondence to: wzhang@ouc.edu.cn

(Zhang 2012; Lin 2013). Most of the dietary protein is provided by fish meal (above 40%), which results in relatively high feed price. In addition, protein catabolism byproducts are the major source of nitrogen loading to the ambient waters. Therefore, it is desirable to improve the protein utilization by increasing the use of conventional energy sources, such as lipids and carbohydrates in feed.

The current trend of high-lipid diet usage has been shown to induce undesirable increase in fat deposition or even physiological symptoms, such as induction of oxidative stress, and growth retardation (Kjær et al. 2008). Carbohydrates, on the other hand, are more readily available and much cheaper than lipids and the appropriate supplementation of dietary carbohydrate may improve fish growth (NRC 1993; Wilson 1994). However, a prolonged postprandial hyperglycemia and persistent metabolic stress were generally observed after feeding carbohydrate-rich diets (Moon 2001). Therefore, it is imperative to determine the optimum dietary carbohydrate-to-lipid (CHO : L) ratio in fish feeds that produces the best growth.

The aim of this study was to determine the effect of various dietary CHO : L ratios on growth performance, body composition, digestive enzyme, and hepatic enzyme activities in juvenile large yellow croaker.

Materials and Methods

Experimental Diets

Six isonitrogenous (45% crude protein) and isoenergetic (18 kJ/g gross energy) diets with different dietary CHO : L ratios (0.07, 0.48, 1.20, 2.19, 4.81, and 10.48) were formulated (Diets 1–6, respectively; Table 1). Fish protein concentrate, casein, and gelatin were used as the protein sources. Fish oil and wheat starch were used as the lipid and carbohydrate sources, respectively.

Ingredients were ground individually into fine powder through 320 μm mesh. All the ingredients were thoroughly mixed with fish oil, and then the water was added until an adequate consistency was obtained. Pellets (3.0 × 4.0 mm) were made automatically by pelletizer (F-26 (II), South China University of Technology, Guangzhou, China) and dried for about 12 h in a ventilated oven at 60 C. All diets were sealed in polythene bags and stored at −20 C.

Experimental Animals and Procedure

Experimental large yellow croaker juveniles were obtained from a commercial hatchery (Ningbo, Zhejiang province, China). The experimental period was from July 14 to September 9, 2012. Before the initiation of the feeding trial, fish were fed the basal diet (Diet 1) for 2 wk to acclimate to the experimental diets and conditions.

At the beginning of the feeding trial, the fish were fasted for 24 h and weighed after being anesthetized with eugenol (1 : 10,000; 99% purity, Shanghai Reagent, Shanghai, China). Juvenile fish of similar size (mean initial body weight: 7.60 ± 0.10 g) were randomly distributed into 18 floating sea cages (1.0 × 1.0 × 1.5 m) at an equal stocking rate of 60 fish per cage. One treatment (diet) had three replicates (cages). The experimental fish were hand fed to apparent satiation twice daily at 0500 and 1700 h, respectively, for 8 wk. During the experimental period, water temperature ranged from 27 to 30 C and salinity from 25 to 28‰, and dissolved oxygen was approximately 7 mg/L.

Sample Collection and Chemical Analysis

At the termination of the 8-wk feeding trial, the experimental fish in each cage were anesthetized with eugenol (1 : 10,000; 99% purity, Shanghai Reagent) and immediately weighed and sampled after being fasted for 24 h.

Five fish per cage were randomly collected and stored at −20 C for the whole-body composition analysis. Blood samples were collected from the caudal peduncle vein of another 10 fish per cage using heparinized syringes. Plasma samples were obtained after centrifugation (4000 *g* for 10 min) at 4 C and immediately stored at −80 C until analysis. Meanwhile, the liver, intestinal tract, and back muscle were sampled and frozen immediately in liquid nitrogen and stored at −80 C until further assay.

The analysis of diets and fish samples for moisture, crude protein, crude lipid, and ash

TABLE 1. *Formulation and proximate composition of the experimental diets (% dry matter).*

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish protein concentrate[a]	15.00	15.00	15.00	15.00	15.00	15.00
Casein	24.00	24.00	24.00	24.00	24.00	24.00
Gelatin	6.00	6.00	6.00	6.00	6.00	6.00
Fish oil	18.00	15.00	12.00	9.00	6.00	3.00
Wheat starch	0.00	7.60	15.40	23.20	31.00	38.80
Taurine	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[b]	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix[c]	2.00	2.00	2.00	2.00	2.00	2.00
Attractant[d]	1.00	1.00	1.00	1.00	1.00	1.00
Microcrystalline cellulose	31.35	26.75	21.95	17.15	12.35	7.55
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
Mold inhibitor[e]	0.10	0.10	0.10	0.10	0.10	0.10
Proximate composition (%)						
Crude protein	45.51	46.49	45.92	45.60	46.26	45.34
Crude lipid	18.33	15.13	12.00	9.73	5.80	3.23
Ash	2.61	2.53	2.72	2.86	2.79	2.81
Carbohydrate	1.24	7.24	14.43	21.29	27.86	33.87
Gross energy (MJ/kg)	18.77	19.07	18.57	18.54	18.70	18.96
CHO : L ratio	0.07	0.48	1.20	2.19	4.81	10.48

CHO : L = carbohydrate-to-lipid ratio.

[a]Fish protein concentrate is from cod (Qingdao Fulin Biochemical Co., LTD, Qingdao, China).

[b]Mineral premix (mg/kg diet): $MgSO_4 \cdot 7H_2O$, 1200; $CuSO_4 \cdot 5H_2O$, 10; $FeSO_4 \cdot H_2O$, 80; $ZnSO_4 \cdot H_2O$, 50; $MnSO_4 \cdot H_2O$, 45; $CoCl_2 \cdot 6H_2O$(1%), 50; Na_2SeO_3(1%), 20; calcium iodine, 60; zoelite, 18,485.

[c]Vitamin premix (mg/kg diet): retinal palmitate, 32; cholecalciferol, 5; DL-α-tocopherol acetate, 240; menadione, 10; thiamin-HCl, 25; riboflavin, 45; pyridoxine-HCl, 20; cyanocobalamin, 10; D-calcium pantothenate, 60; amine nicotinic acid, 200; folic acid, 20; biotin, 60; mesoinositol, 800; ascorbyl polyphosphate(contained 35% ascorbic acid), 2000; choline chloride, 4000; microcrystalline cellulose, 12,473.

[d]Attractant: dimethyl-propiothetin: glycine: alanine: 5-phosphate inosine = 4 : 2 : 2 : 1 : 1.

[e]Mold inhibitor: Calcium propionate : Fumaric acid = 1 : 1.

content were conducted by the standard methods (AOAC 1995). Moisture content was measured by drying the samples to constant weight at 105 C. Crude protein was determined by the Kjeldahl method (Kjeltec TM 8400, FOSS, Hogana, Sweden) and estimated by multiplying nitrogen by 6.25. Crude lipid was analyzed after diethyl ether extraction using the Soxhlet method (Buchi 36680, Flawil, Switzerland). Ash was examined after combustion in a muffle furnace at 550 C for 16 h. Gross energy was determined with Parr1281 Automatic Bomb Calorimeter (Parr, Moline, IL, USA). Carbohydrate content of diets was analyzed by the 3, 5-dinitro salicylic acid method (Yu et al. 1997).

Biochemical Compositions of Plasma, Liver, and Muscle Glycogen

The concentration of plasma glucose, triglyceride, and the total cholesterol were determined in the affiliated hospital of Qingdao University, Shandong, China.

Analyses of plasma high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) concentration, liver, and muscle glycogen content were carried out by enzymatic-colorimetric methods using commercial kits from Jiancheng Bioengineering Institute, Nanjing, China (High-density lipoprotein cholesterol assay kit, A112-2; Low-density lipoprotein cholesterol assay kit, A113-2; Liver/Muscle glycogen assay kit, A043). All samples were analyzed in triplicate.

Enzyme Analysis

Trypsin and amylase activities in liver and intestinal tract were measured according to the instruction of the commercial kits (Trypsin assay kit, A080-2; Amylase Assay, C016; Jiancheng

Bioengineering Institute). Trypsin activity was defined as: 0.003 variation of absorbance generated per min by trypsin from 1 mg protein at the condition of 37 C, pH 8.0. Amylase activity was defined as: 1 mg tissue protein interacted with substrate for 30 min, hydrolyzed 10 mg starch at 37 C.

The liver samples used for carbohydrate metabolic enzyme analysis were homogenized following the method of Polakof et al. (2008). Hepatic 6-phosphofructo-1-kinase (PFK) and phosphoenolpyruvate carboxykinase (PEPCK) activities were measured following the method of Polakof et al. (2008). Fructose-1, 6-bisphosphatase (FBPase) activity was determined as described by Sangiao-Alvarellos et al. (2003). Enzyme-specific activities were expressed as μmoles of substrate hydrolyzed per minute, per mg of protein (i.e., U/mg protein), 30 C for PFK, PEPCK, and FBPase.

Hepatic hexokinase (HK) and pyruvate kinase (PK) activities were analyzed according to the commercial kits (Pyruvate kinase assay kit, A076-1; Hexokinase assay kit, A077-1; Jiancheng Bioengineering Institute). Activity of the HK was defined as: 1 mmol/L NADPH was generated in the reaction system per minute, per gram of protein (i.e., U/g protein) under the condition of 37 C, pH 7.6. Activity of the PK was defined as: 1 μmol PEP was transformed into pyruvic acid per minute, per gram of protein (i.e., U/g protein) under the condition of 37 C, pH 7.6. Protein concentration in the supernatants was determined following the method of Bradford (1976) using a protein quantitative assay kit (A045-2, Jiancheng Bioengineering Institute) with bovine serum albumin as a standard.

Calculations and Statistical Analyses

The survival of large yellow croaker was expressed as the survival rate (SR), growth performance as the specific growth rate (SGR), feed utilization as the protein efficiency ratio (PER), feed efficiency ratio (FER), and feeding rate (FR). Meanwhile, the condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI) were used as the parameters for body index. The calculation formulae for these parameters were as follows:

$$\mathrm{SR}\% = 100 \times (\text{final fish number}/\text{initial fish number})$$

$$\mathrm{SGR}\% = 100\times (\ln \text{ final body weight} - \ln \text{ initial body weight})/\text{days of feeding trial}$$

$$\mathrm{FR}\%/\text{day} = 100 \times \text{dry feed intake (g)} / \left[(\text{final body weight} + \text{initial body weight})/2\right] /\text{days of feeding trial}$$

$$\mathrm{FER} = \text{wet weight gain (g)} /\text{dry feed intake (g)}$$

$$\mathrm{PER} = \text{wet weight gain (g)} /\text{protein ingested (g)}$$

$$\mathrm{CF}\% = 100\times \text{final body weight}/\text{body length}^3$$

$$\mathrm{HSI}\% = 100\times \text{liver wet weight}/\text{final body weight}$$

$$\mathrm{VSI}\% = 100\times \text{viscera wet weight}/\text{final body weight}$$

The data were subjected to one-way ANOVA. The regression analysis of dietary microcrystalline cellulose and analyzed growth and physiological response parameters was also done by SPSS 17.0 for windows. Data are presented as means ± SEM (standard error of the mean). The level of significance was chosen at $P < 0.05$. The Tukey's test was used to compare the mean values. The second-order polynomial regression model was used to estimate the optimal dietary carbohydrate and lipid levels on the basis of the SGR, respectively, corresponding to the optimal dietary CHO : L ratio of large yellow croaker (Gao et al. 2010).

TABLE 2. *Survival, growth, and feed utilization of juvenile large yellow croaker fed diets with six different carbohydrate-to-lipid ratios.*[a]

Carbohydrate-to-lipid ratios	FBW (g)	SGR[b] (%/d)	FR[c] (%/d)	FER[d]	PER[e]	SR[f] (%)
Diet 1 (0.07)	22.02 ± 1.48^{a}	1.88 ± 0.12^{a}	2.84 ± 0.19	0.50 ± 0.05	1.09 ± 0.12	95.56 ± 0.56
Diet 2 (0.48)	25.95 ± 1.05^{ab}	2.18 ± 0.07^{ab}	2.99 ± 0.13	0.54 ± 0.08	1.17 ± 0.17	97.22 ± 2.78
Diet 3 (1.20)	28.11 ± 1.29^{bc}	2.32 ± 0.08^{bc}	2.38 ± 0.23	0.60 ± 0.09	1.31 ± 0.19	96.11 ± 2.00
Diet 4 (2.19)	33.16 ± 0.82^{c}	2.62 ± 0.04^{c}	2.56 ± 0.08	0.75 ± 0.03	1.65 ± 0.06	97.22 ± 1.11
Diet 5 (4.81)	29.69 ± 0.74^{bc}	2.42 ± 0.04^{bc}	2.43 ± 0.20	0.69 ± 0.07	1.49 ± 0.14	93.33 ± 3.47
Diet 6 (10.48)	26.87 ± 0.62^{ab}	2.24 ± 0.04^{abc}	2.75 ± 0.22	0.56 ± 0.08	1.23 ± 0.17	95.56 ± 2.00
ANOVA (one-way)						
F	12.402	10.614	1.761	1.944	1.957	0.423
P	0.001	0.001	0.196	0.166	0.164	0.824

FBW = final body weight.

[a]Values show with mean ± standard error, $n = 3$. Values in the same column with different superscript letter mean significant difference ($P < 0.05$).

[b]SGR: specific growth rate: 100 × (ln final body weight − ln initial body weight)/days of feeding trial.

[c]FR: feeding rate: 100 × dry feed intake (g)/[(final body weight + initial body weight)/2]/days of feeding trial.

[d]FER: feed efficiency ratio: wet weight gain (g)/ dry feed intake (g).

[e]PER: protein efficiency ratio: wet weight gain (g)/ protein ingested (g).

[f]SR: survival rate: 100 × (final fish number/initial fish number).

Results

Growth and Survival

The survival rates ranging from 93.33 to 97.22% were not significantly influenced by dietary CHO : L ratios ($P > 0.05$). When dietary CHO : L ratios increased from 0.07 to 2.19, the SGR of large yellow croaker also significantly increased from 1.88% per day to 2.62% per day ($P < 0.05$, Table 2). However, higher dietary CHO : L ratios (>2.19) did not result in significantly higher SGR. Dietary CHO : L ratio did not significantly affect FR, FER, and PER of fish ($P > 0.05$, Table 2).

Body Composition

The increasing dietary CHO : L ratio significantly decreased the whole-body lipid content ($P < 0.05$, Table 3), which decreased to the lowest content (5.75%) in fish fed diet with CHO : L ratio of 10.48 (corresponding to 3.23% of lipid). The whole-body moisture, protein, and ash content were not significantly influenced by the dietary CHO : L ratio ($P > 0.05$, Table 3).

HSI, VSI, CF, Liver, and Muscle Glycogen

The liver glycogen content was not significantly influenced by dietary CHO : L ratios from 0.07 to 4.81, while the highest ratio (10.48) led to significantly higher liver glycogen content ($P < 0.05$, Table 4). The value of HSI of large yellow croaker significantly increased from 0.85 to 1.1% as dietary CHO : L ratios increased from 0.07 to 1.20 ($P < 0.05$). Higher ratios (>1.20) did not result in significantly higher HSI. No significant difference was observed in the value of VSI, CF, and muscle glycogen content among dietary treatments ($P > 0.05$, Table 4).

Biochemical Compositions in Plasma

Plasma glucose concentration increased linearly as dietary CHO : L ratio increased. The highest plasma glucose concentration was found in fish fed diet with CHO : L ratio of 10.48, which was not significantly different from those fed diet with CHO : L ratios from 0.48 to 4.81, but apparently higher than that fed diet with CHO : L ratio of 0.07 ($P < 0.05$, Table 5). Both plasma total cholesterol and triglyceride concentrations significantly decreased as the dietary CHO : L ratios increased from 0.07 to 2.19 ($P < 0.05$), while higher dietary CHO : L ratios (>2.19) did not lead to significantly further decreases for both of them.

Plasma LDL-C concentration decreased as CHO : L ratio increased, fish fed diet with CHO : L ratio of 0.48 was significantly higher

TABLE 3. *Whole-body compositions (% wet weight) of juvenile large yellow croaker fed diets with six different carbohydrate-to-lipid ratios.*[a]

Carbohydrate-to-lipid ratios	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
Diet 1 (0.07)	73.13 ± 0.79	16.26 ± 0.10	8.87 ± 0.32 [c]	3.38 ± 0.10
Diet 2 (0.48)	73.25 ± 0.52	16.38 ± 0.11	8.19 ± 0.39 [c]	3.40 ± 0.04
Diet 3 (1.20)	72.90 ± 0.47	16.28 ± 0.23	8.17 ± 0.35 [c]	3.48 ± 0.09
Diet 4 (2.19)	73.25 ± 0.10	16.79 ± 0.14	8.07 ± 0.10 [bc]	3.59 ± 0.02
Diet 5 (4.81)	74.41 ± 0.27	16.72 ± 0.20	6.33 ± 0.41 [ab]	3.57 ± 0.01
Diet 6 (10.48)	75.19 ± 0.64	16.67 ± 0.02	5.75 ± 0.57 [a]	3.62 ± 0.09
ANOVA (one-way)				
F	3.043	2.506	10.229	2.045
P	0.053	0.089	0.001	0.150

[a]Values show with mean ± standard error, $n = 3$. Values in the same column with different superscript letter mean significant difference ($P < 0.05$).

TABLE 4. *Hepatosomatic index (HSI), viscerosomatic index (VSI), condition factor (CF), liver and muscle glycogen of juvenile large yellow croaker fed diets with six different carbohydrate-to-lipid ratios.*[a]

Carbohydrate-to-lipid ratios	HSI (%)[b]	VSI (%)[c]	CF (%)[d]	Liver glycogen (mg/g)	Muscle glycogen (mg/g)
Diet 1 (0.07)	0.85 ± 0.04 [a]	2.67 ± 0.24	1.59 ± 0.03	4.95 ± 1.50 [a]	0.33 ± 0.05
Diet 2 (0.48)	1.03 ± 0.08 [ab]	2.78 ± 0.15	1.60 ± 0.03	5.48 ± 1.35 [a]	0.36 ± 0.03
Diet 3 (1.20)	1.10 ± 0.08 [b]	2.56 ± 0.08	1.58 ± 0.02	6.02 ± 1.38 [ab]	0.30 ± 0.02
Diet 4 (2.19)	1.15 ± 0.03 [b]	2.62 ± 0.10	1.53 ± 0.05	9.69 ± 0.45 [ab]	0.30 ± 0.02
Diet 5 (4.81)	1.20 ± 0.02 [b]	2.52 ± 0.09	1.53 ± 0.07	9.78 ± 0.69 [ab]	0.29 ± 0.03
Diet 6 (10.48)	1.22 ± 0.03 [b]	2.32 ± 0.24	1.51 ± 0.08	11.33 ± 1.48 [b]	0.39 ± 0.08
ANOVA (one-way)					
F	6.291	0.857	0.544	5.038	0.825
P	0.003	0.536	0.740	0.012	0.555

[a]Values show with mean ± standard error, $n = 3$. Values in the same column with different superscript letter mean significant difference ($P < 0.05$).

[b]HSI: 100 × liver wet weight/final body weight.

[c]VSI: 100 × viscera wet weight/final body weight.

[d]CF: 100 × final body weight/body length^3.

than those of fish fed diet with CHO : L ratios of 2.19 and 10.48 ($P < 0.05$, Table 5). Plasma HDL-C concentration was not significantly affected by dietary CHO : L ratio ($P > 0.05$, Table 5).

Digestive Enzyme Activities

The activity of amylase in the intestinal tract of large yellow croaker significantly increased with increasing dietary CHO : L ratio and reached a peak in fish fed diet with CHO : L ratio of 4.81 ($P < 0.05$). No significant difference was observed in the activities of amylase in intestinal tract among fish fed diet with CHO : L ratios from 0.48 to 10.48, and fish fed diet with CHO : L ratio of 4.81 was significantly higher than the fish fed diet with CHO : L ratio of 0.07 ($P < 0.05$, Table 6). The activities of amylase in liver and trypsin in both intestinal tract and liver were independent of dietary treatments ($P > 0.05$).

Carbohydrate Metabolic Enzyme Activities

The activities of hepatic glycometabolism enzyme are showed in Table 7. The activities of PK were significantly up-regulated by elevated contents of dietary carbohydrate. Fish fed diet with CHO : L ratio of 10.48 (corresponding to 33.9% of dietary carbohydrate) resulted in significantly higher PK activity ($P < 0.05$). Meanwhile, the increase of dietary CHO : L ratios significantly down-regulated the activity of PEPCK; fish fed diet with CHO : L ratio of 10.48 was significantly lower than those fed diet with CHO : L ratios of 0.07 and 0.48 ($P < 0.05$). No significant difference was detected in the

TABLE 5. *Plasma parameters of juvenile large yellow croaker fed diets with six different carbohydrate-to-lipid ratios.*[a]

Carbohydrate-to-lipid ratios	Glucose (mmol/L)	Cholesterol (mmol/L)	Triglyceride (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Diet 1(0.07)	2.97 ± 0.14^{a}	2.39 ± 0.18^{b}	3.78 ± 0.38^{b}	1.01 ± 0.01	1.52 ± 0.15^{ab}
Diet 2(0.48)	3.20 ± 0.07^{ab}	2.27 ± 0.08^{ab}	3.07 ± 0.19^{b}	0.97 ± 0.11	1.86 ± 0.20^{b}
Diet 3(1.20)	3.50 ± 0.29^{ab}	2.03 ± 0.30^{ab}	2.81 ± 0.14^{b}	1.01 ± 0.15	1.10 ± 0.21^{ab}
Diet 4(2.19)	3.76 ± 0.27^{ab}	1.53 ± 0.15^{a}	1.55 ± 0.35^{a}	0.99 ± 0.07	0.92 ± 0.09^{a}
Diet 5(4.81)	4.54 ± 0.48^{ab}	1.85 ± 0.19^{ab}	1.33 ± 0.32^{a}	0.95 ± 0.12	1.29 ± 0.24^{ab}
Diet 6(10.48)	4.78 ± 0.56^{b}	1.67 ± 0.12^{ab}	1.26 ± 0.11^{a}	1.03 ± 0.16	0.84 ± 0.12^{a}
ANOVA (one-way)					
F	4.460	3.498	15.638	0.058	4.767
P	0.016	0.035	0.000	0.997	0.012

HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

[a]Values show with mean ± standard error, $n = 3$. Values in the same column with different superscript letter mean significant difference ($P < 0.05$).

TABLE 6. *Digestive enzyme activities in liver and intestinal tract of juvenile large yellow croaker fed diets with six different carbohydrate-to-lipid ratios.*[a]

	Liver		Intestinal tract	
Carbohydrate-to-lipid ratios	Amylase (U/mg prot.)	Trypsin (U/mg prot.)	Amylase (U/mg prot.)	Trypsin (U/mg prot.)
Diet 1 (0.07)	0.32 ± 0.04	1159.66 ± 31.25	0.44 ± 0.02^{a}	2237.84 ± 48.04
Diet 2 (0.48)	0.32 ± 0.05	1199.46 ± 39.95	0.48 ± 0.05^{ab}	2261.56 ± 51.09
Diet 3 (1.20)	0.27 ± 0.03	1278.60 ± 44.42	0.54 ± 0.05^{ab}	2403.31 ± 74.89
Diet 4 (2.19)	0.36 ± 0.05	1152.80 ± 49.38	0.53 ± 0.02^{ab}	2369.78 ± 38.74
Diet 5 (4.81)	0.26 ± 0.05	1203.48 ± 15.26	0.64 ± 0.01^{b}	2336.77 ± 73.80
Diet 6 (10.48)	0.28 ± 0.02	1213.27 ± 55.06	0.63 ± 0.01^{ab}	2384.96 ± 88.82
ANOVA (one-way)				
F	0.654	1.196	3.697	1.096
P	0.666	0.368	0.043	0.411

[a]Values show with mean ± standard error, $n = 3$. Values in the same column with different superscript letter mean significant difference ($P < 0.05$).

activities of HK, PFK, and FBPase of fish among dietary CHO : L ratios ($P > 0.05$).

The Optimal Dietary CHO : L Ratio

The relationship between SGR and dietary carbohydrate and lipid levels were expressed by the polynomial second-order regression model, the regression lines $y = -0.0016x^2 + 0.0676x + 1.7763$, $R^2 = 0.7789$ and $y = -0.0073x^2 + 0.1295x + 1.9221$, $R^2 = 0.7664$ were obtained, respectively. The maximum growth was observed when the dietary carbohydrate and lipid levels were 21.13 and 8.87%, corresponding to a CHO : L ratio of 2.38.

Discussion

In this study, the increasing dietary CHO : L ratios from 0.07 to 2.19 significantly increased the SGR of juvenile large yellow croaker. The highest dietary CHO : L ratio (10.48) with 33.9% of carbohydrate and 3.23% of lipid, however, did not result in significant decline of SGR. It showed that large yellow croaker could tolerate high dietary CHO : L ratio. A similar result was also found in hybrid Clarias catfish, *Clarias microcephalus* × *Clarias gariepinus* (Jantrarotai et al. 1994).

In this study, fish fed either the high lipid–low carbohydrate (Diet 1) or the high carbohydrate–low lipid (Diet 6) diets tended to produce lower SGR to a certain extent. It was suggested that the imbalance of nonprotein energy sources (excessive carbohydrate or lipid content) in the diet would cause growth retardation (Erfanullah and Jafri 1998; Ali and Jauncey 2004). At the same time, fish fed diets

TABLE 7. *Carbohydrate metabolic enzyme activities in liver of juvenile large yellow croaker fed diets with six different carbohydrate-to-lipid ratios.*[a]

	Glycolytic enzyme (U/g prot)			Gluconeogenesis enzyme (U/g prot)	
Carbohydrate-to-lipid ratios	HK	PK	PFK	FBPase	PEPCK
Diet 1 (0.07)	6.04 ± 0.84	17.77 ± 3.71 [a]	5.78 ± 1.82	19.15 ± 1.43	15.10 ± 1.38 [b]
Diet 2 (0.48)	6.31 ± 1.23	16.36 ± 2.94 [a]	5.79 ± 1.49	17.71 ± 1.02	15.04 ± 1.23 [b]
Diet 3 (1.20)	6.93 ± 0.72	19.06 ± 3.71 [ab]	5.96 ± 2.19	16.87 ± 0.83	12.70 ± 1.58 [ab]
Diet 4 (2.19)	6.87 ± 1.65	18.23 ± 2.72 [a]	10.04 ± 0.85	18.58 ± 1.31	12.71 ± 0.52 [ab]
Diet 5 (4.81)	7.53 ± 1.78	22.05 ± 1.17 [ab]	9.62 ± 0.96	17.39 ± 1.50	10.69 ± 2.34 [ab]
Diet 6 (10.48)	6.30 ± 0.91	32.59 ± 2.34 [b]	10.9 ± 0.66	17.92 ± 0.62	7.78 ± 0.54 [a]
ANOVA (one-way)					
F	0.178	4.666	3.305	0.510	4.132
P	0.965	0.016	0.046	0.763	0.023

[a]Values show with mean ± standard error, $n = 3$; values in the same column with different superscript letter mean significant difference ($P < 0.05$).

FBPase = fructose 1, 6-bisphosphatase; HK = hexokinase; PK = pyruvate kinase; PFK = 6-Phosphofructo 1-kinase; PEPCK = phosphoenolpyruvate carboxykinase.

with similar energy content, which was supplied by different energy sources, show distinct utilization of carbohydrate and lipid energy. Similar results have also been observed in grass carp, *Ctenopharyngodon idella* (Gao et al. 2010), yellow sea bream, *Sparus latus* (Hu et al. 2007), and African catfish, *C. gariepinus* (Ali and Jauncey 2004). Buhler and Halver (1961) reported that the highest weight gain was observed when dietary carbohydrate energy equals lipid energy. This equivalence relation could be regarded as a special case for the balance of nonprotein energy sources in the diet. Similar conclusion was also verified in rainbow trout (Hilton et al. 1987). These results were in accordance with this study. When the energy contents from carbohydrate and lipid balanced (Diet 4; carbohydrate and lipid energy are 3.65 and 3.85 kJ/g, respectively, corresponding to the CHO : L ratio of 2.19), the highest value of SGR was recognized. Of course, carbohydrate and lipid also have their own biological functions other than energy sources. The balance of nonprotein energy sources (i.e., carbohydrate and lipid) in diet could be more helpful to dietary carbohydrate and lipid to exert their respective biological functions.

In this study, the treatments of Diet 1 and Diet 2 with higher microcrystalline cellulose inclusion levels (31.35 and 26.75%, respectively) had relative lower SGRs. Because the present diets were designed as isoenergetic, higher lipid contents could be attained only by increasing the cellulose content of the diet. There are several reports indicating that the elevated cellulose content in fish diets may exert a negative effect on the utilization of other nutrients (Kirchgessener et al. 1986). However, this view has been challenged (Jantrarotai et al. 1994). Besides, the influence of cellulose content in fish diets largely depends on the feeding habit and fish species (Gao et al. 2010). Also in some studies, cellulose was used at levels up to 40% for channel catfish, *Ictalurus punctatus* and *Tilapia zillii* without affecting growth rates (Garling and Wilson 1977; El-Sayed and Garling 1988). In previous studies, it was confirmed that high dietary lipid levels reduced the growth of fish, such as channel catfish (Garling and Wilson 1977), red drum (Ellis and Reigh 1991), and walking catfish (Erfanullah and Jafri 1998), which are carnivorous fish species. However, it remains unclear if reduced growth in fish fed the high-lipid diet was due to inefficient lipid utilization by fish, as compared with carbohydrate utilization, or to the negative effects of the high dietary cellulose content (Ali and Jauncey 2004). In this study, the relationships between dietary microcrystalline cellulose levels and parameters significantly influenced by dietary treatments were presented in Table 8. These parameters included final body weight, SGR, body crude lipid, HSI, liver glycogen, plasma glucose, plasma cholesterol, plasma

TABLE 8. *Relationships between dietary microcrystalline cellulose levels and parameters significantly influenced by dietary treatments.*

	Equation	R^2	P
FBW	$y = -0.241x + 32.340$	0.287	0.022
SGR	$y = -0.016x + 2.599$	0.285	0.022
Body crude lipid	$y = 0.127x + 5.081$	0.696	0.000
HSI	$y = -0.015x + 1.380$	0.655	0.000
Liver glycogen	$y = -0.291x + 13.550$	0.657	0.000
Plasma glucose	$y = -0.080x + 5.354$	0.624	0.000
Plasma cholesterol	$y = 0.032x + 1.327$	0.429	0.003
Plasma triglyceride	$y = 0.114x + 0.074$	0.800	0.000
Plasma LDL-C	$y = 0.032x + 0.633$	0.362	0.008
Intestinal tract amylase	$y = -0.009x + 0.709$	0.662	0.001
PK	$y = -0.542x + 31.599$	0.449	0.002
PEPCK	$y = 0.298x + 6.530$	0.596	0.000

FBW = final body weight; HSI = hepatosomatic index; LDL-C = low-density lipoprotein cholesterol; PK = pyruvate kinase; PEPCK = phosphoenolpyruvate carboxykinase; SGR = specific growth rate.

triglyceride, plasma LDL-C, intestinal tract amylase, PK, and PEPCK. Dietary microcrystalline cellulose levels had significant positive correlations with the contents of body crude lipid, and plasma triglyceride, respectively. Meanwhile, significant passive correlations between dietary microcrystalline cellulose levels and HIS, contents of liver glycogen and plasma glucose, and activity of the intestinal tract amylase were found, respectively. It was implied that dietary cellulose could have effects on large yellow croaker in many aspects. Further studies are needed for better understanding of effects of dietary cellulose on large yellow croaker.

Based on the SGR, the optimum dietary CHO : L ratio of juvenile large yellow croaker was estimated to be 2.38. Similar results have been found in rainbow trout, *Oncorhynchus mykiss* (CHO : L ratio of 2.45; Yamamoto et al. 2001). The optimal dietary CHO : L ratio in some fish species are lower than that for large yellow croaker, such as 1.98 for Chinese longsnout catfish (Tan et al. 2007), 1.67 for Asian sea bass, *Lates calcarifer* (Catacutan and Coloso 1997), 1.6 for juvenile rockfish, *Sebastes schlegeli* (Lee and Kim 2009), and 0.62 for juvenile yellowfin sea bream, *S. latus* (Hu et al. 2007). In the meantime, this ratio in some other fish species is much higher, such as 3.39 for walking catfish (Erfanullah and Jafri 1998), 4.7 for grass carp (Gao et al. 2010), 6.2 for juvenile starry flounder, *Platichthys stellatus* (Lee and Lee 2004). These differences could be due to the different species, experimental diet design, environmental conditions or other factors.

A significant decrease in the whole-body lipid content of large yellow croaker with increasing dietary CHO : L ratios was observed in this study. It seems that fish do not readily synthesize body fat from dietary carbohydrates, and the excessive dietary lipid was easier to deposit as body fat in fish (Brauge et al. 1995; Hemre et al. 1995). The digestible energy from carbohydrate and lipid could be different and it would affect the blood glucose concentration and also carbohydrate metabolism. High dietary carbohydrate was found to deposit as hepatic glycogen (Table 4). Plasma glucose increased while cholesterol and triglyceride decreased, indicating that high dietary carbohydrate could not be converted into fish fat. Glyconeogenesis was inhibited (Table 7). Therefore, the dietary lipid other than carbohydrate is regarded as the most important factor influencing the whole-body lipid. Similar results had been documented in Chinese longsnout catfish (Tan et al. 2007), starry flounder (Lee and Lee 2004), barramundi, *L. calcarifer* (Nankervis et al. 2000), and channel catfish (Garling and Wilson 1977). These studies implied that the mechanism of lipid deposition and the source of body lipid are important. Further studies are needed.

The total concentrations of cholesterol and triglyceride in plasma were regarded as the indicator of an active lipid transport in response to the higher dietary lipid levels (Du et al. 2005; Gao et al. 2010). In this study, the plasma total cholesterol and triglyceride concentration significantly decreased as the dietary lipid contents were reduced. This indicates that lipid deposition in juvenile large yellow croaker mainly comes from the ingestion of dietary lipid instead of lipogenesis. Similar results were also reported in turbot, *Psetta maxima* and Chinese longsnout catfish (Regost et al. 2001; Tan et al. 2007). Meanwhile, the increasing dietary carbohydrate contents accompanied by the decrease of

lipid contents significantly lowered the plasma LDL-C concentration. The decrease of plasma LDL-C concentration was in response to the decrease of plasma triglyceride concentration to some extent.

PK and PEPCK are two key enzymes in the carbohydrate metabolism process. In this study, the increment of dietary carbohydrate contents significantly stimulated the activity of hepatic PK. This improvement could also be supported by the significant down-regulation of hepatic PEPCK activity with increasing dietary carbohydrate contents. Previous studies had also reported the stimulation of PK activity by dietary carbohydrates in European sea bass, *Dicentrarchus labrax* (Enes et al. 2006), gilthead sea bream, *Sparus aurata*, L. (Fernández et al. 2007), perch, *Perca fluviatilis* (Borrebaek and Christophersen 2000), and gibel carp, *Carassius auratus gibelio* (Wang 2008). Nevertheless, some studies had revealed that the dietary carbohydrate contents had no effect on PK activity (Suárez et al. 2002; Dias et al. 2004; Enes et al. 2008). It thus appears that large yellow croaker could regulate the activities of key enzymes in glycolysis and gluconeogenesis pathway after ingestion of high-carbohydrate diet.

Conclusion

The results of this study suggested that the diet containing 21.13% carbohydrate and 8.87% lipid, corresponding to a CHO : L ratio of 2.38 was appropriate for juvenile large yellow croaker. Moreover, to some extent, this species can utilize energy from dietary carbohydrate as effectively as lipid.

Acknowledgments

This research was financially supported by a grant from the National Basic Research Program of China (973 Program, 2014CB138600) and the State Spark-Program China (2014GA701001).

Literature Cited

Ali, M. Z. and K. Jauncey. 2004. Optimal dietary carbohydrate to lipid ratio in African catfish *Clarias gariepinus* (Burchell 1822). Aquaculture International 12:169–180.

AOAC (Association of Official Analytical Chemists). 1995. Official methods of analysis of AOAC International, 16th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Borrebaek, B. and B. Christophersen. 2000. Hepatic glucose phosphorylating activities in perch (*Perca fluviatilis*) after different dietary treatments. Comparative Biochemistry and Physiology B 125:387–393.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248–254.

Brauge, C., G. Corraze, and F. Médale. 1995. Effects of dietary levels of carbohydrate and lipid on glucose oxidation and lipogenesis from glucose in rainbow trout, *Oncorhynchus mykiss*, reared in freshwater or in seawater. Comparative Biochemistry and Physiology A 111:117–124.

Buhler, D. R. and J. E. Halver. 1961. Nutrition of salmonoid fishes IX. Carbohydrate requirements of chinook salmon. The Journal of Nutrition 74:307–318.

Catacutan, M. and R. Coloso. 1997. Growth of juvenile Asian seabass, *Lates calcarifer*, fed varying carbohydrate and lipid levels. Aquaculture 149:137–144.

Dias, J., R. Rueda-Jasso, S. Panserat, L. E. da Conceição, E. F. Gomes, and M. T. Dinis. 2004. Effect of dietary carbohydrate-to-lipid ratios on growth, lipid deposition and metabolic hepatic enzymes in juvenile Senegalese sole (*Solea senegalensis*, *Kaup*). Aquaculture Research 35:1122–1130.

Du, Z.-Y., Y.-J. Liu, L.-X. Tian, J.-T. Wang, Y. Wang, and G.-Y. Liang. 2005. Effect of dietary lipid level on growth, feed utilization and body composition by juvenile grass carp (*Ctenopharyngodon idella*). Aquaculture Nutrition 11:139–146.

Ellis, S. C. and R. C. Reigh. 1991. Effect of dietary lipid and carbohydrate levels on growth and body composition of juveniles red drum, *Sciacnopes ocellatus*. Aquaculture 97: 383–394.

El-Sayed, A. M. and D. L. Garling Jr. 1988. Carbohydrate-to-lipid ratios in diets for *Tilapia zillii* fingerlings. Aquaculture 73:157–163.

Enes, P., S. Panserat, S. Kaushik, and A. Oliva-Teles. 2006. Effect of normal and waxy maize starch on growth, food utilization and hepatic glucose metabolism in European sea bass (*Dicentrarchus labrax*) juveniles. Comparative Biochemistry and Physiology A 143:89–96.

Enes, P., S. Panserat, S. Kaushik, and A. Oliva-Teles. 2008. Hepatic glucokinase and glucose-6-phosphatase responses to dietary glucose and starch in gilthead sea bream (*Sparus aurata*) juveniles reared at two temperatures. Comparative Biochemistry and Physiology A 149:80–86.

Erfanullah and A. K. Jafri. 1998. Effect of dietary carbohydrate-to-lipid ratio on growth and body composition of walking catfish(*Clarias batrachus*). Aquaculture 161:159–168.

Fernández, F., A. G. Miquel, M. Córdoba, M. Varas, I. Metón, A. Caseras, and I. V. Baanante. 2007. Effects of diets with distinct protein-to-carbohydrate ratios on nutrient digestibility, growth performance, body composition and liver intermediary enzyme activities in gilthead sea bream (*Sparus aurata*, L.) fingerlings. Journal of Experimental Marine Biology and Ecology 343:1–10.

Fishery Bureau, Ministry of Agriculture. 2014. China fishery statistical yearbook. China Agriculture Press, Beijing, China.

Gao, W., Y. J. Liu, L. X. Tian, K. S. Mai, G. Y. Liang, H. J. Yang, M. Y. Huai, and W. J. Luo. 2010. Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, nutrient utilization and hepatic enzymes activities of herbivorous grass carp (*Ctenopharyngodon idella*). Aquaculture Nutrition 16:327–333.

Garling, D. L. Jr. and R. P. Wilson. 1977. Effects of dietary carbohydrate-to-lipid ratios on growth and body composition of fingerling channel catfish. The Progressive Fish-Culturist 39:43–47.

Hemre, G. I., K. Sandnes, Ø. Lie, O. Torrissen, and R. Waagbø. 1995. Carbohydrate nutrition in Atlantic salmon, *Salmo salar* L.: growth and feed utilization. Aquaculture Research 26:149–154.

Hilton, J., E. Plisetskaya, and J. Leatherland. 1987. Does oral 3, 5, 3′-triiodo-l-thyronine affect dietary glucose utilization and plasma insulin levels in rainbow trout (*Salmo gairdneri*). Fish Physiology and Biochemistry 4:113–120.

Hu, Y. H., Y. J. Liu, L. X. Tian, H. J. Yang, G. Y. Liang, and W. Gao. 2007. Optimal dietary carbohydrate to lipid ratio for juvenile yellowfin seabream (*Sparus latus*). Aquaculture Nutrition 13:291–297.

Jantrarotai, W., P. Sitasit, and S. Rajchapakdee. 1994. The optimum carbohydrate to lipid ratio in hybrid Clarias catfish (*Clarias macrocephalus* × *C. gariepinus*) diets containing raw broken rice. Aquaculture 127:61–68.

Kirchgessener, M., H. Kürzinger, and F. J. Schwarz. 1986. Digestibility of crude nutrients in different feeds and estimation of their energy content for carp (*Cyprinus carpio* L.) Aquaculture 58:185–194.

Kjær, M., M. Todorčević, B. Torstensen, A. Vegusdal, and B. Ruyter. 2008. Dietary n-3 HUFA affects mitochondrial fatty acid β-oxidation capacity and susceptibility to oxidative stress in Atlantic salmon. Lipids 43:813–827.

Lee, S. M. and K. D. Kim. 2009. Effects of dietary carbohydrate to lipid ratios on growth and body composition of juvenile and grower rockfish, *Sebastes schlegeli*. Aquaculture Research 40:1830–1837.

Lee, S. M. and J. H. Lee. 2004. Effect of dietary glucose, dextrin and starch on growth and body composition of juvenile starry flounder *Platichthys stellatus*. Fisheries Science 70:53–58.

Lin, S. 2013. Effects of dietary protein levels and its ratio to energy on growth performances and metabolic responses of large yellow croaker at different stages. Master's thesis. Ocean University of China, China.

Moon, T. W. 2001. Glucose intolerance in teleost fish: fact or fiction? Comparative Biochemistry and Physiology B 129:243–249.

Nankervis, L., S. Matthews, and P. Appleford. 2000. Effect of dietary non-protein energy source on growth, nutrient retention and circulating insulin-like growth factor I and triiodothyronine levels in juvenile barramundi, *Lates calcarifer*. Aquaculture 191:323–335.

NRC (National Research Council). 1993. Nutrient requirements of fish and shrimp. National Academy Press, Washington, District of Columbia, USA.

Polakof, S., J. M. Miguez, and J. L. Soengas. 2008. Dietary carbohydrates induce changes in glucosensing capacity and food intake of rainbow trout. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 295:478–489.

Regost, C., J. Arzel, M. Cardinal, J. Robin, M. Laroche, and S. Kaushik. 2001. Dietary lipid level, hepatic lipogenesis and flesh quality in turbot (*Psetta maxima*). Aquaculture 193:291–309.

Sangiao-Alvarellos, S., R. Laiz-Carrión, J. M. Guzmán, M. P. M. del Río, J. M. Miguez, J. M. Mancera, and J. L. Soengas. 2003. Acclimation of *S. aurata* to various salinities alters energy metabolism of osmoregulatory and nonosmoregulatory organs. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 285:897–907.

Suárez, M., A. Sanz, J. Bazoco, and M. García-Gallego. 2002. Metabolic effects of changes in the dietary protein: carbohydrate ratio in eel (*Angilla anguilla*) and trout (*Oncorhynchus mykiss*). Aquaculture International 10:143–156.

Tan, Q., S. Xie, X. Zhu, W. Lei, and Y. Yang. 2007. Effect of dietary carbohydrate-to-lipid ratios on growth and feed utilization in Chinese longsnout catfish (*Leiocassis longirostris Günther*). Journal of Applied Ichthyology 23:605–610.

Wang, F. 2008. The effect of high levels of dietary starch on growth, metabolism and body composition for gibel carp (*Carassius auratus gibelio*). Master's thesis. Huazhong Agriculture University, China.

Wilson, R. P. 1994. Utilization of dietary carbohydrate by fish. Aquaculture 124:67–80.

Yamamoto, T., K. Konishi, T. Shima, H. Furuita, N. Suzuki, and M. Tabata. 2001. Influence of dietary fat and carbohydrate levels on growth and body composition of rainbow trout *Oncorhynchus mykiss* under self –feeding conditions. Fisheries Science 67:221–227.

Yu, S., C. E. Olsen, and J. Marcussen. 1997. Methods for the assay of 1, 5-anhydro-D-fructose and α-1, 4-glucan lyase. Carbohydrate Research 305:73–82.

Zhang, F. 2012. Lipid requirement and fishmeal replacement in diets of large yellow croaker, *Pseudosciaena crocea* R. Master's thesis. Ocean University of China, Qingdao, Shandong, China.