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Effects of dietary carbohydrate to lipid ratios on growth performance, digestive enzyme and hepatic carbohydrate metabolic enzyme activities of large yellow croaker (*Larmichthys crocea*)

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ABSTRACT

An 8-week feeding trial was conducted to evaluate the effect of dietary carbohydrate to lipid (CHO:L) ratios on growth performance, feed utilization, digestive enzymes and hepatic carbohydrate metabolic enzyme activities of large yellow croaker, *Larmichthys crocea*. Six isonitrogenous and isoenergetic diets (39.0% crude protein and 16.0 MJ kg^{-1} gross energy) were formulated to contain various CHO:L ratios which ranged from 0.39 to 5.97. Triplicate groups of 50 fish (initial weight 7.06 ± 0.48 g) were stocked in 18 floating net cages (1.5 m × 1.5 m × 2.0 m), and were fed twice daily to apparent satiation for 8 weeks. The results indicated that growth performance and feed utilization were significantly influenced by the dietary CHO:L ratios ($P < 0.05$). Maximum weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER) and feed efficiency (FE) occurred at 1.34 dietary CHO:L ratios. There were no significant differences in VSI and HSI among all treatments, however, the lowest condition factor (CF) was observed at 0.39 dietary CHO:L ratios. Moisture, protein, and ash contents of whole body were not significantly affected by the dietary CHO:L ratios ($P > 0.05$), and lipid content in whole body significantly decreased with dietary CHO:L ratios increasing from 0.39 to 1.69 ($P > 0.05$). Hepatic glycogen and muscle glycogen significantly increased with dietary CHO:L ratios from 0.39 to 1.34 then plateaued from 1.34 to 5.97. Fish fed the 0.39 CHO:L ratio diet had higher cholesterol concentration in serum than those fed the diets containing 1.69, 3.00 and 5.97 dietary CHO:L ratios; the lowest glucose concentration in serum was observed at dietary 0.39 CHO:L ratio, however, there were no significant differences in triglyceride and total protein concentrations of serum among all diets. Amylase, pepsin and lipase activities were significantly influenced by the dietary CHO:L ratios, and maximum amylase activity occurred at the 1.34 CHO:L ratios. Glucokinase (GK), pyruvate kinase (PK), and phosphoenolpyruvate carboxykinase (PEPCK) activities significantly increased with dietary CHO:L ratios increasing from 0.39 to 1.34, and then significantly decreased with further increase of dietary CHO:L ratios; no significant difference of phosphofructokinase (PFK), fructose-1,6-biphosphatase (FBPase) and glucose-6-phosphatase (G6Pase) activities was observed among all treatments. Based on weight gain, the optimal CHO:L ratio was determined to be 1.34 (approximately dietary 12.56% starch and 12.15% lipid) of dry diet for juvenile large yellow croaker.

Statement of relevance

The manuscript addresses the effect of dietary CHO:L ratios on growth performance, feed utilization and hepatic carbohydrate metabolic enzymes. Although this study investigated a common nutritional research topic, it still had somehow research originality because such nutritional topic has not been studied in large yellow croaker yet. The results give some valuable data for this marine fish. What's more, the information obtained from the present study would be helpful in developing low-cost diets for this species.

1. Introduction

Development of a cost-effective and nutritionally adequate formulated diet is fundamental to the future viability for marine fish culture. Carbohydrates are the most economical source of energy in formulated aquaculture diets (Rosas et al., 2000). Numerous studies have evaluated the maximum levels of carbohydrates that fish can tolerate without physiological disorders and growth impairment, rather than the optimal level for growth (NRC, 2011). The provision of an appropriate amount of digestible carbohydrates in diets formulated for farmed aquatic species is important to spare the use of lipids and protein as

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sources of energy. However, it is generally accepted that fish utilize less carbohydrate than terrestrial animal, and carnivorous fish species utilize less than herbivorous and omnivorous ones (Wilson, 1994; Hemre et al., 2002; Stone, 2003). The main reason may be due to higher amylase activity in the digestive tracts and metabolic enzyme activity of herbivorous and omnivorous fish species (Hidalgo et al., 1999), and the higher affinity of insulin receptors than those for carnivorous fish (Banos et al., 1998; Stone, 2003). However, some carnivorous fish showed improved growth when fed the diet with an appropriate starch level compared with a non-starch diet (Hemre et al., 2002; Ren et al., 2011). Inadequate carbohydrate in diet may lead to the degradation of protein and lipid, and adequate carbohydrate can spare the consumption of protein and fat for energy (Degani and Viola, 1987; Hidalgo et al., 1993; Wilson, 1994; Catacutan and Coloso, 1997; Shiau, 1997; Peragón et al., 1999; Stone, 2003; Enes et al., 2006). Certainly, excessive carbohydrate in the diet of carnivorous fish depressed the growth performance and impaired some physiological functions (Hutchins et al., 1998; Hemre et al., 2002; Ren et al., 2011). Compared to carbohydrate, lipid can be well utilized by most fish species. Meanwhile, lipid is the source of essential fatty acids, intact phospholipid and cholesterol, and it is required for normal growth, development and maintaining health. However, high levels of dietary lipid could increase susceptibility to autoxidation and tissue lipid peroxidation (NRC, 2011). It is necessary to determine the optimum dietary CHO:L ratio to obtain better growth performance, physiological condition and health status for fish (Wang et al., 2014).

Large yellow croaker (*Larmichthys crocea*) are mainly distributed in the northern South China sea, East China sea and Taiwan strait (Duan et al., 2000). Due to its delicious taste and important commercial value, it is widely cultured in southeastern China coastal area, especially in Fujian and Zhejiang Provinces. However, in the current situation, the grow-out of large yellow croaker heavily depends on trash fish. The development of formulated feeds is still in its infancy. Due to the variable nutrition quality of trash fish and the probability of leading to contagious fish disease associated with pathogens, it prevents the raising of the output of cultured large yellow croaker. Moreover, trash fish also results in environmental contamination due to lower ingestion and soluble in water. Therefore, trash fish replacement with diet is an inevitable trend in the future. No study has been conducted to evaluate the carbohydrate utilization for this fish species. The objectives of this study are to evaluate the effects of dietary CHO:L ratios on growth performance, feed utilization, hematological indexes, digestive enzyme and hepatic carbohydrate metabolic enzyme activities of juvenile large yellow croaker.

2. Materials and methods

2.1. Diet preparation

Six isonitrogenous and isoenergetic diets were formulated to contain six graded levels of CHO:L ratios (Table 1). The experimental diets contained similar gross energy, but the starch contents ranged from 4.40% to 26.13% and lipid contents ranged from 16.56% to 4.86%, the CHO:L ratios ranged from 0.39 to 5.97 (Table 1). All dry ingredients were ground with hammer mill (H-28, South China University of Technology, Guangzhou, China) and sieved through 80-mesh before they were being weighed and mixed thoroughly in a Hobart-type mixer (M-256, South China University of Technology, Guangzhou, China). The oil and water were then added in the above mixture and mixed thoroughly. The resultant dough was cold-extruded into pellets (2.0 and 4.0 mm diameter) using a cold feed extruder (F-26 (II), South China University of Technology, Guangzhou, China) and the pellets were air-dried to about 10% moisture. All experimental diets were packed in vacuum bags, and stored at −20 °C until used. The formulation and proximate composition of the experimental diets are presented in Table 1.

Table 1
Ingredients and proximate composition of the experimental diets (% dry matter).

	Dietary CHO:lipid ratios					
	0.39	0.87	1.34	1.69	3.00	5.97
Ingredients						
Peruvian fish meal	38.00	38.00	38.00	38.00	38.00	38.00
Wheat gluten	20.00	20.00	20.00	20.00	20.00	20.00
Wheat starch	0.00	5.00	10.00	15.00	20.00	25.00
Fish oil	6.55	5.47	4.39	3.30	2.22	1.13
Soybean oil	6.55	5.47	4.39	3.30	2.22	1.13
Soy lecithin	1.50	1.50	1.50	1.50	1.50	1.50
$Ca(H_2PO_4)_2$	1.50	1.50	1.50	1.50	1.50	1.50
Chorine chloride	0.30	0.30	0.30	0.30	0.30	0.30
Mineral premix[a]	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix[a]	3.00	3.00	3.00	3.00	3.00	3.00
Cellulose	20.60	17.76	14.92	12.10	9.26	6.44
Proximate composition (%)						
Moisture	9.18	9.40	9.27	9.92	10.43	11.21
Crude protein	39.25	39.32	39.48	39.22	38.74	39.31
Crude lipid	16.56	13.26	12.15	11.12	8.17	4.86
Ash	9.55	10.07	9.65	10.00	9.99	9.77
Gross energy (MJ kg^{-1})	15.83	15.40	15.85	16.24	15.82	15.27
Crude fiber	18.92	16.38	13.18	10.96	8.17	5.86
Nitrogen-free extract[b]	6.54	11.57	16.27	18.78	24.50	28.99

[a] Mineral and vitamin premix are referred as Mai et al. (2006).
[b] Nitrogen-free extract content = 100 − moisture − crude protein − crude lipid − ash − crude fiber.

2.2. Fish and experimental condition

Experimental fish were obtained from a commercial hatchery farm at Xiangshan bay (Ningbo, P.R. China). Prior to starting the experiment, the fish were conditioned in floating sea cages (3 m × 3 m × 3 m) and were fed a commercial diet (Ningbo Techbank Aquafeed company, Ningbo, P.R. China) for two weeks. The commercial diet contained 45% crude protein and 10% crude lipid. At the end of the acclimation, the fish (initial weight was 7.06 ± 0.48 g) were randomly stocked and sorted into 18 outdoor net cages at outdoor (1.5 m × 1.5 m × 2.0 m) with 50 individuals in each cage. Each diet was randomly allocated to triplicate cages of fish. Fish were hand-fed with experimental diets twice daily to apparent satiation (0500 and 1700 h), and the amount of diet consumption in each net cage was recorded daily. All groups of fish were fed at the same fixed rate, two times daily, initially 6% body weight per day and gradually reduced to 4%. The feeding rate was adjusted every 2 weeks to maintain a level approaching apparent satiation, without overfeeding. The feeding trial lasted for 8 weeks. During the experimental period, water temperature was 26.5–31.5 °C, salinity was 32–36‰, and dissolved oxygen was not less than 7.0 mg L^{-1}.

2.3. Sample collection

At the termination of the 8-week feeding trial, fish in each net cage were individually weighed and sampled for tissue analysis. Hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (CF) were determined from four individual fish per net cage by obtaining tissues (viscera and liver) and expressing ratios as percent of body weight. Four representative fish from each net cage also were anesthetized with tricaine methane sulfonate (MS-222), and approximately 0.5 mL of blood was collected from the caudal vasculature using a 1-mL syringe with a 27-gauge needle, and then clotted at 4 °C overnight. The clot was removed and residual blood cells were separated from the straw-colored serum by centrifugation (836 *g*, 4 °C, 10 min). The serum was frozen in liquid nitrogen and then stored at −80 °C for analysis.

2.4. Proximate composition

All experimental diets and fish samples were analyzed in duplicate for proximate composition following standard methods (AOAC, 1995). Crude protein (N × 6.25) was determined by the Kjeldahl method using Kjeldahl apparatus (Foss Tecator TM 8400, Hoganos, Sweden). Crude lipid was determined by the ether-extraction method using Soxtec System HT (FOSS Tecator HT6, Hoganos, Sweden). Moisture was determined by drying a sample to a constant weight at 105 °C. Ash content was determined by combustion of a sample in a muffle furnace at 550 °C for 8 h. Gross energy was determined using an adiabatic bomb calorimeter.

2.5. Hematology and glycogen analysis

Blood characteristics were determined according to the method described by Kikuchi et al. (1994). The levels of glucose, total protein, triglyceride, cholesterol in a serum sample were determined by automatic Chemistry Analyzer (Hitachi 7600-110, Tokyo, Japan).

The glycogen contents in the liver and muscle were determined according to the method described by Hassid and Abraham (1957), using liver and muscle glycogen assay kits (No. A043) (Jiancheng Bioengineering Ltd., Nanjing, China).

2.6. Digestive enzyme activity

The activities of stomach digestive enzyme (pepsin and amylase) were determined using pepsin assay kit (No. A080-1) and amylase assay kit (No. A016-1) (Jiancheng Bioengineering Ltd., Nanjing, China). Mix sodium phosphate buffer (0.05 M, pH 6.2) and stomach sample (weight:volume = 1:9) into 10% homogenate, and then centrifuged under 2500 rpm for 10 min, extract supernatant for further using. The absorbance was measured at 660 nm.

The activities of lipase were determined using Elisa lipase assay kit for fish (No. A054) (Qiao Du biotechnology Ltd., Shanghai, China).

2.7. Carbohydrate metabolic key enzyme activities

The activities of phosphofructokinase (PFK), fructose-1.6-bisphosphatase (FBPase), glucose-6-phosphatase (G6Pase), glucokinase (GK), pyruvate kinase (PK), and phosphoenolpyruvate carboxykinase (PEPCK) were analyzed according to previous methods (Metón et al., 2003; Zhang et al., 2009). All enzyme activities were expressed per mg of total protein (specific activity). The total protein content in crude extracts was determined at 30 °C using bovine serum albumin as a standard based on the method of Bradford (1976). One unit of enzyme activity was defined as the amount of NADH or NADPH generated by per mg protein per minute at 30 °C.

2.8. Calculations and statistical analysis

$$\text{Weight gain (WG, \%)} = 100 \times (W_t - W_i)/W_i$$

$$\text{Specific growth rate } \left(\text{SGR, \%day}^{-1}\right) = 100 \times (\text{Ln } W_t - \text{Ln } W_i)/t$$

$$\text{Feed efficiency (FE)} = \text{weight gain (g, wet weight)}/\text{feed consumed (g, dry weight)}$$

$$\text{Protein efficiency ratio (PER)} = \text{weight gain (g, wet weight)}/\text{protein intake (g, dry weight)}$$

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

$$\text{Hepatosomatic index (HSI,\%)} = 100 \times \text{liver wet weight (g)}/\text{body wet weight (g)}$$

$$\text{Viscerosomatic index (VSI, \%)} = 100 \times \text{viscerosomatic weight (g)}/\text{body wet weight (g)}$$

$$\text{Condition factor } \left(\text{CF, g} \cdot \text{cm}^{-3}\right) = 100 \times \text{body wet weight (g)}/\text{body length (cm)}^3$$

W_t and W_i presents the final body weight (g) and initial body weight (g) of fish, respectively; t is the experimental duration in days.

All data were presented as mean ± pooled S.E.M. (standard error of the mean) of three replications and analyzed using one-way analysis of variance (ANOVA) and Duncan's multiple-range tests. The level of significance was set at $P < 0.05$. All statistical analyses were performed using SPSS software 15.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA). Regression analysis was also performed using the regression function of SPSS 15.0. The liner or quadratic model was chosen by the Curve Estimation function.

3. Result

3.1. Growth performance, feed utilization and morphologic index

The effects of dietary CHO:L ratios on growth performance, survival, feed utilization and morphologic index are presented in Table 2. There were no significant differences in survival among all treatments, although fish fed the diet containing 0.39 CHO:L ratio had lower survival than those fed the other diets ($P > 0.05$). Fish fed the diet containing 0.39 CHO:L ratio had lower weight gain and SGR than those fed the diets. WG and SGR did not significantly increase with the dietary CHO:L ratios increasing from 1.34 to 5.97. Based on weight gain, the optimal CHO:L ratio for juvenile large yellow croaker was estimated to be 1.34 of dry diet. Fish fed the diet containing 1.34 CHO:L ratios had the highest FE and PER values among all treatments, however, PER significantly decreased when the dietary CHO:L ratios increased from 1.34 to 5.97.

There were no significant differences in VSI and HSI among experimental groups ($P > 0.05$) (Table 2). Fish fed the diet containing 0.39 CHO:L ratio had the lowest condition factor among all treatments, however, there was no significant difference among other treatments which the dietary CHO:L ratios ranged from 0.87 to 5.97.

3.2. Whole body composition and tissue glycogen

Proximate compositions of whole body fed experimental diets are shown in Table 3. No significant differences were observed in moisture, protein and ash content of whole body among all treatments. Lipid content significantly decreased with dietary CHO:L ratios increasing from 0.39 to 1.69 ($P < 0.05$), and did not significantly decrease with dietary CHO:L ratios increasing from 1.34 to 5.97.

Hepatic glycogen content significantly increased with dietary CHO:L ratios increasing from 0.39 to 1.34, thereafter did not significantly increase with dietary CHO:L ratios increasing from 1.34 to 5.97 (Table 4). Muscle glycogen content significantly increased when dietary CHO:L ratios increased from 0.39 to 1.34, and then significantly decreased with dietary CHO:L ratios increasing from 1.69 to 5.97.

3.3. Digestive enzyme activities

Amylase activity significantly increased when the dietary CHO:L ratios increased from 0.39 to 1.34, however, it did not significantly increase with the dietary CHO:L ratios further increasing from 1.34 to 5.97. Pepsin activity significantly increased with the increase of dietary CHO:L ratios, and the lowest pepsin activity was observed in 0.39 CHO:L ratios diet. Lipase activity had an inverse tendency with the amylase activity (Table 5).

Table 2
Growth performance, feed utilization and morphology index of large yellow croaker fed test diets with graded levels of starch for 8 weeks.

CHO:L ratios	Survival (%)	Final weight (g)	Weight gain (%)	SGR[a] (% d^{-1})	FE[a]	PER[a]	HSI[a] (%)	VSI[a] (%)	CF[a] (g cm^{-3})
0.39	79.19 ± 16.02	18.87 ± 3.16	160.54 ± 35.46	4.65 ± 0.63	0.56 ± 0.15	1.85 ± 0.13	0.83 ± 0.05	3.68 ± 0.28	1.43 ± 0.01
0.87	79.12 ± 3.13	21.57 ± 2.68	198.14 ± 45.51	5.25 ± 0.10	0.72 ± 0.04	1.91 ± 0.21	0.80 ± 0.16	4.54 ± 0.48	1.61 ± 0.02
1.34	91.04 ± 3.75	25.03 ± 1.99	235.45 ± 30.05	6.35 ± 0.54	0.79 ± 0.18	2.43 ± 0.09	0.66 ± 0.08	4.14 ± 0.41	1.63 ± 0.02
1.69	89.31 ± 1.46	24.98 ± 4.0	228.60 ± 10.78	5.87 ± 0.19	0.78 ± 0.10	2.00 ± 0.22	0.62 ± 0.14	4.39 ± 0.26	1.69 ± 0.05
3.00	78.65 ± 9.59	23.12 ± 0.69	225.68 ± 56.85	5.81 ± 1.02	0.69 ± 0.12	1.79 ± 0.10	0.66 ± 0.06	4.12 ± 0.04	1.60 ± 0.16
5.97	82.26 ± 7.83	22.90 ± 3.85	223.37 ± 54.67	5.77 ± 0.98	0.74 ± 0.21	1.90 ± 0.09	0.63 ± 0.06	3.66 ± 0.38	1.72 ± 0.11

Regression analysis
Final weight (y) vs. CHO:L ratio (x): $Y = -0.641x^2 + 5.192x + 14.29$ ($R^2 = 0.389$, $P = 0.025$)
Weight gain (y) vs. CHO:L ratio (x): $Y = -6.437x^2 + 56.199x + 112.893$ ($R^2 = 0.337$, $P = 0.046$)
SGR (y) vs. CHO:L ratio (x): $Y = -0.14x^2 + 1.174x + 3.63$ ($R^2 = 0.392$, $P = 0.024$)
FE (y) vs. CHO:L ratio (x): $Y = -0.021x^2 + 0.172x + 0.435$ ($R^2 = 0.219$, $P = 0.036$)
PER (y) vs. CHO:L ratio (x): $Y = -0.48x^2 + 0.318x + 1.589$ ($R^2 = 0.249$, $P = 0.017$)
CF (y) vs. CHO:L ratio (x): $Y = -0.13x^2 + 0.135x + 1.342$ ($R^2 = 0.235$, $P = 0.034$)

Data are means ± S.E.M. (n = 3).
[a] SGR: specific growth rate; FE: feed efficiency; PER: protein efficiency ratio. HSI: hepatosomatic index; VSI: viscerosomatic index; CF: condition factor.

3.4. Hematology characteristics

Total cholesterol and glucose were significantly affected by different dietary starch levels (Table 6). Cholesterol in serum significantly decreased with the increase of dietary starch levels ($P < 0.05$), however, glucose content in serum significantly increased with dietary starch levels increasing from 4.40% to 16.28%. Total protein and triglyceride in serum were not significantly influenced by dietary starch levels.

3.5. Carbohydrate metabolic enzymes

The specific activities of glucokinase (GK), pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK) in the liver were significantly affected by dietary CHO:L ratios (Table 7). Fish fed the diet containing 1.34 CHO:L ratios had higher GK, PK and PEPCK activities than those fed the other diets, and fish fed diets containing 0.39 and 5.97 CHO:L ratios had the lowest GK, PK and PEPCK activities among all treatments. The activities of phosphofructokinase (PFK), fructose-1, 6-biphosphatase (FBPase) and glucose-6-phosphatase (G6Pase) were not significantly influenced by the dietary CHO:L ratios ($P > 0.05$).

4. Discussion

Carbohydrates are considered to supply energy at low cost. The provision of an appropriate amount of digestible carbohydrate in diets for farmed aquatic species is important to spare the use of lipids and protein as sources of energy (Degani and Viola, 1987; Hidalgo et al., 1993; Wilson, 1994; Catacutan and Coloso, 1997; Shiau, 1997; Peragón et al., 1999; Stone, 2003; Enes et al., 2008a). In the present study, the gross energy values are similar, however, it does not mean that they will provide equivalent values of digestible energy. Fish fed the diet containing 0.39 CHO:L ratio had lower WG, SGR, FE and PER than those fed the diets containing higher levels of CHO:L ratios. Based on WG, the optimal CHO:L ratio for juvenile large yellow croaker was determined to be 1.34 of dry weight (approximately dietary 12.56% starch and 12.15% lipid). The results indicated that appropriate CHO:L ratios could improve growth performance and feed utilization, however, lower or higher dietary CHO:L ratios may depress growth performance and feed utilization. The results of present study were lower than those reported by some previous studies, such as rainbow trout (Hilton and Atkinson, 1982), Asian sea bass (Boonyaratpalin, 1991), Atlantic salmon (Helland et al., 1991), Pacific salmon (Hardy, 1991) and Cobia (Ren et al., 2011). Compared with these fish species, large yellow croaker had a lower capacity to utilize starch. Some studies had reported that an appropriate dietary carbohydrate level can improve growth performance in some fish species (Hemre et al., 1995; Peragón et al., 1999; Fraser and Davies, 2009; Ren et al., 2011). High carbohydrate content could depress the growth performance due to the limited capacity to utilize carbohydrate of some fish species (Hilton and Atkinson, 1982; Hu et al., 2007; Ren et al., 2011). However, a protein-sparing effect of digestible starch has been described in many carnivorous species, and differences have been found within the categories (omnivorous and carnivorous) (Hemre et al., 2002; Stone, 2003). It is difficult to define precisely a tolerable level for each species because physical state, molecular complexity, and inclusion level of dietary starch are factors that influence digestibility, glucose tolerance, and ultimately, efficient utilization of carbohydrates (NRC, 2011). Meanwhile, starch sources interact with the other macronutrients (such as protein and lipid) and likely with environmental factors, especially temperature, within a same species (Brauge et al., 1995; Hemre et al., 2002).

Lipid content of whole body significantly decreased with the increase of dietary CHO:L ratios from 0.39 to 1.69; the result of present study was similar to some previous studies, such as hybrid catfish

Table 3
Proximate compositions of the whole body of large yellow croaker fed test diets with graded levels of starch for 8 weeks.

CHO:L ratios	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
0.39	76.72 ± 3.84	15.71 ± 1.46	8.07 ± 0.75	5.03 ± 0.55
0.87	75.24 ± 2.71	16.21 ± 0.47	7.92 ± 0.34	4.11 ± 0.01
1.34	73.69 ± 1.10	16.55 ± 0.48	6.90 ± 0.57	4.65 ± 0.21
1.69	75.82 ± 1.18	16.34 ± 0.61	5.16 ± 0.87	4.84 ± 0.82
3.00	76.28 ± 2.61	16.44 ± 0.76	5.82 ± 0.71	4.28 ± 0.12
5.97	76.17 ± 0.25	16.63 ± 0.13	4.93 ± 0.70	4.68 ± 0.57

Regression analysis
Lipid (y) vs. CHO:L ratio (x): $y = -0.054x^2 - 1.056x + 9.344$ ($R^2 = 0.729$, $P = 0.000$)

Data are means ± S.E.M. (n = 3).

Table 4
Hepatic and muscle glycogen levels of the large yellow croaker fed test diets with graded levels of starch for 8 weeks.

CHO:L ratios	Hepatic glycogen (mg g^{-1})	Muscle glycogen (mg g^{-1})
0.39	18.42 ± 0.61	6.96 ± 2.70
0.87	20.96 ± 0.76	6.13 ± 0.72
1.34	26.06 ± 1.33	10.88 ± 1.95
1.69	27.21 ± 1.55	10.05 ± 2.13
3.00	26.44 ± 0.47	8.72 ± 1.08
5.97	26.91 ± 0.43	7.82 ± 0.97

Regression analysis
Hepatic glycogen (y) vs. CHO:L ratio (x): $Y = -0.604x^2 + 5.944x + 12.691$ ($R^2 = 0.900$, $P = 0.000$)
Muscle glycogen (y) vs. CHO:L ratio (x): $Y = -0.441x^2 + 3.405x + 3.191$ ($R^2 = 0.374$, $P = 0.030$)

Data are means ± S.E.M. (n = 3).

Table 5
The specific activity of stomach digestive enzymes of the large yellow croaker (*Larmichthys crocea*) fed the test diets with graded levels of starch for 8 weeks.

CHO:L ratios	Pepsin (U g^{-1})	Amylase (U g^{-1})	Lipase (U g^{-1})
0.39	80.26 ± 7.31	0.14 ± 0.04	13.27 ± 6.33
0.87	110.55 ± 7.27	0.17 ± 0.01	14.20 ± 4.60
1.34	115.63 ± 13.75	0.21 ± 0.03	11.80 ± 3.28
1.69	115.20 ± 13.21	0.22 ± 0.02	8.44 ± 2.78
3.00	138.83 ± 21.84	0.21 ± 0.01	9.43 ± 5.16
5.97	149.22 ± 11.48	0.22 ± 0.01	8.85 ± 2.82

Regression analysis
Pepsin (y) vs. CHO:L ratio (x): $y = -0.452x^2 + 15.426x + 71.144$ ($R^2 = 0.729$, $P = 0.000$)
Amylase (y) vs. CHO:L ratio (x): $y = -0.005x^2 + 0.53x + 0.092$ ($R^2 = 0.679$, $P = 0.000$
Lipase (y) vs. CHO:L ratio (x): $y = -1.136x + 14.975$ ($R^2 = 0.213$, $P = 0.045$)

Data are means ± S.E.M. (n = 3).

(Jantrarotai et al., 1994), hybrid tilapia (Chou and Shiau, 1996), walking catfish (Erfanullah and Jafri, 1998), African catfish (Ali and Jauncey, 2004) and yellowfin seabream (Hu et al., 2007), however, it is contrary to the results reported for European sea bass and cobia (Moreira et al., 2008; Ren et al., 2011). The differences could be attributed to the different experimental designs; the lipid content of experimental diets was kept at a same level in the study of cobia (Ren et al., 2011), however, in the present study, lipid content reduced from 16.56% to 4.86% of dry diet to maintain same energy levels among all diets. The results also indicated that dietary lipid play a more important role in body lipid deposition compared to dietary carbohydrate for this species. In the present study, HSI decreased from 0.83% to 0.63% when dietary CHO:L ratios increased from 0.39 to 5.97, and is positively correlated to dietary lipid contents and fish fed the diet containing 0.39 CHO:L ratios had lower condition factor than those fed the other diets. The results indicated that large yellow croaker could better use lipid than carbohydrate as hepatic lipid deposition.

Glucose may be stored as glycogen through glycogenesis catalyzed by glycogen synthase (NRC, 2011). In the present study, hepatic glycogen content significantly increased with dietary CHO:L ratios increasing from 0.39 to 1.34, and then did not significantly increase when dietary CHO:L ratios increased from 1.34 to 5.97. However, glycogen in the muscle increased with the dietary CHO:L ratios increasing from 0.39 to 1.34, and then significantly decreased with the increase of dietary CHO:L ratios from 1.69 to 5.97. The results indicated that large yellow croaker could digest starch and accumulate in the liver as glycogen, but it could not digest more dietary carbohydrate (starch content > 12.56% of dry diet). Similar results were observed for some fish species (Moreira et al., 2008; Rosas et al., 2000, 2001; Ren et al., 2011). Most glycogen in fish would be synthesized from glucose formed through gluconeogenesis (i.e., from nonstarch polysaccharides, NSP) (Moon and Foster, 1995; Pereira et al., 1995). Glycolysis is the major route of glucose metabolism in fish as in other animals. Actually, glucose is not a major energy substrate for fish and very little is catabolized via glycolysis. Most of dietary glucose is 1) excreted after digestion, 2) stored in body depots as glycogen or 3) used for fat production, via lipogenesis or production of reducing equivalents for fat production via the pentose phosphate pathway.

It is well known that triglyceride, total protein, cholesterol and glucose contents in blood were related to animal health, and lower or higher plasma tissue will reflect abnormal physical and chemical changes occurring in organisms, and it indicates general metabolism and physiological status (NRC, 2011; Wang et al., 2014). The amount of total protein and triglyceride in serum showed no significant differences in this experiment. However, cholesterol content significantly decreased with the increase of dietary CHO:L ratios; this value may be consistent with dietary lipid content, and it may indicate a more active endogenous lipid transport, in response to a higher dietary lipid level. Our findings are in agreement with previous observation (Wang et al., 2014).

In the present study, blood glucose levels significantly increased with the increase of dietary CHO:L ratios from 0.39 to 1.34 and then plateaued. However, Ren et al. (2011) indicated that glucose content significantly increased with dietary gelatinized cornstarch increasing from 1.3% to 30.4% for juvenile cobia. Absorption of the glucose released by digestion is very efficient and leads to increased blood glucose levels in all fish. The intensity of the blood glucose peak increases with the levels of digestible carbohydrate on the diet (Bergot, 1979; Brauge et al., 1995; Stone et al., 2003a). But the duration of the elevation of blood glucose is very short in many aquatic species, such as rainbow trout (24 h return to basal level, Legate et al., 2001), gilthead sea bream (24 h return to basal level, Peres et al., 1999), hybrid tilapia (6 h return to basal level, Shiau and Chuang, 1995), silver perch (12 h return to basal level, Stone et al., 2003b). In the present study, after 24 h of fasting, glucose content in serum increased with an increase of dietary starch levels. It indicated that large yellow croaker have a relatively lower capacity to metabolize glucose. These results were in agreement with those reported for cobia (Ren et al., 2011).

Some studies have revealed that amylase activity in herbivorous and omnivorous is much greater than carnivorous (Hidalgo et al., 1999), and dietary carbohydrate levels can improve amylase activity (Cahu and Infante, 1994; Lundstedt et al., 2004). In the present study, amylase activity significantly increased with dietary CHO:L ratios increasing from 0.39 to 1.34, however, it didn't significantly increased with dietary CHO:L ratios further increasing from 1.69 to 5.97. These results were inconsistent with those reported for cobia (Ren et al., 2011), and Pintado (Lundstedt et al., 2004). Ren et al. (2011) indicated that amylase activity in the liver was not significantly influenced by the dietary starch levels, however, amylase activity in the intestine tract significantly increased with dietary starch levels increasing from 1.3% to 12.5%, and then did not increase with the dietary starch further increasing from 18.4% to 30.4%. Pepsin activity in the liver significantly increased with the increase of dietary starch level. Our results were similar to those reported by previous studies (Rosas et al., 2000; Mohapatra et al., 2003). The results of present study may indicate that digestible starch could improve the activity of pepsin of this fish. Lipase activity significantly decreased parallel to the increasing of carbohydrate level, which may mainly be due to the decrease of lipid content of experimental diets.

Table 6
Hematological characteristics of the large yellow croaker fed test diets with graded levels of starch for 8 weeks.

Item	Dietary CHO:L ratios					
	0.39	0.87	1.34	1.69	3.00	5.97
Cholesterol (mmol L^{-1})	3.98 ± 0.23	3.2 ± 0.5	3.38 ± 0.87	2.07 ± 0.36	2.93 ± 0.10	2.89 ± 0.43
Triglyceride (mmol L^{-1})	3.03 ± 0.78	2.91 ± 0.39	3.54 ± 0.87	2.98 ± 0.65	3.25 ± 0.3	3.8 ± 0.35
Glucose (mmol L^{-1})	3.08 ± 0.15	4.09 ± 0.53	4.06 ± 0.83	4.48 ± 0.34	4.58 ± 0.56	4.46 ± 0.87
Total protein (g L^{-1})	23.97 ± 3.8	24.27 ± 2.66	24.2 ± 2.21	24.37 ± 2.17	26.9 ± 1.31	24.83 ± 3.07

Regression analysis
Cholesterol (y) vs. CHO:L ratio (x): $y = 0.115x^2 - 1.019x + 4.902$ ($R^2 = 0.449$, $P = 0.011$)
Glucose (y) vs. CHO:L ratio (x): $y = -0.092x^2 + 0.892x + 2.391$ ($R^2 = 0.473$, $P = 0.008$)

Data are means ± S.E.M. (n = 3).

Table 7
Carbohydrate metabolic enzyme activities of the large yellow croaker (*Larmichthys crocea*) fed diets with graded levels of starch.

CHO:L ratios	GK (U g^{-1} protein)	PFK-1 (U g^{-1} protein)	PK (U g^{-1} protein)	PEPCK (U g^{-1} protein)	FBPase (U g^{-1} protein)	G6Pase (U g^{-1} protein)
0.39	9.50 ± 2.38	533.54 ± 16.75	108.24 ± 9.39	1.52 ± 1.19	6.30 ± 1.05	36.56 ± 3.85
0.87	11.81 ± 2.16	546.15 ± 30.19	123.33 ± 20.76	1.45 ± 1.49	6.40 ± 1.07	34.63 ± 4.23
1.34	14.42 ± 2.27	523.89 ± 8.75	180.61 ± 20.83	1.81 ± 0.86	6.30 ± 0.75	42.05 ± 6.02
1.69	12.72 ± 3.00	551.02 ± 27.88	149.43 ± 20.53	1.78 ± 0.39	5.27 ± 0.94	35.61 ± 6.99
3.00	12.42 ± 0.87	561.72 ± 20.39	108.11 ± 11.12	1.64 ± 1.57	6.26 ± 1.29	35.67 ± 5.48
5.97	10.73 ± 2.67	573.44 ± 8.60	84.81 ± 6.06	1.37 ± 1.29	5.47 ± 0.64	32.79 ± 3.18

Regression analysis
GK (y) vs. CHO:L ratio (x): $y = -0.565x^2 + 4.134x + 6.032$ ($R^2 = 0.347$, $P = 0.041$)
PK (y) vs. CHO:L ratio (x): $y = -10.471x^2 + 67.751x + 47.429$ ($R^2 = 0.672$, $P = 0.000$)

Data are means ± S.E.M. (n = 3).

Glucokinase (GK) is one of the four hexokinase (HK) isozymes. The function of glucokinase is to remove glucose from the blood after a meal (NRC, 2011). Lower GK activity was considered to be a main reason why fish can't utilize carbohydrate as well as mammal in some early researches (Tranulis et al., 1991; Moon and Foster, 1995). But some recent studies had proved that GK did exist in fish liver and dietary carbohydrate could promote changes in both GK activity and gene expression (Borrebaek and Christophersen, 2000; Caseras et al., 2000; Panserat et al., 2000a; Borrebaek et al., 2003; Capilla et al., 2003; Metón et al., 2003; Enes et al., 2006, 2008a). In European sea bass and gilthead sea bream, a significant increase in hepatic GK activity was observed with the increase of dietary starch level from 10 to 20% (Enes et al., 2006, 2008a; Moreira et al., 2008). However, GK activity in the liver did not further increase when the dietary starch levels increased from 20 to 30%. It is suggested 20% digestible starch is probably near the tolerance threshold for metabolic utilization of glucose by European sea bass (Moreira et al., 2008). In the present study, a similar result of GK activity in the liver of large yellow croaker was observed; GK activity significantly increased with the dietary CHO:L ratios increasing from 0.39 to 1.34, and then significantly decreased with the increase of dietary CHO:L ratios from 1.69 to 5.97. These results indicated that 1.34 CHO:L ratios are probably near the tolerance threshold for metabolic utilization of glucose by this species; this value is lower than the result of 20% digestible starch for European sea bass (Moreira et al., 2008).

Besides HK, two other enzymes control the regulation of glycolytic pathway: 6-phosphofructo-1-kinase (PFK-1) and pyruvate kinase (PK). PFK-1 catalyzes the phosphorylation of fructose-6-phosphate into fructose-1,6-bisphosphate, and PK catalyzes the last step of glycolysis, the conversion of phosphoenolpyruvate to pyruvate (NRC, 2011). In the present study, PFK activity was not significantly influenced by the dietary CHO:L ratios. There are no significant differences in hepatic PFK activities in hybrid tilapia fed glucose or starch (Lin and Shiau, 1995). However, in common carp, supplementation of the diet with 30% galactose decreased PFK activity (NRC, 2011). There is a significant difference in hepatic PK activity among all treatments; fish fed the diet containing 1.34 CHO:L ratio had higher PK activity than those fed the other diets. In gibel carp, diet supplementation with 30% starch or glucose had no effect on hepatic PK activity (Tan et al., 2006). Some studies found unchanged high PK activity regardless of the dietary carbohydrate level, whereas many other studies revealed an induction of PK activity by dietary carbohydrate levels in different fish species (NRC, 2011).

Phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBPase) and glucose-6-phosphatase (G6Pase) are the key enzymes that control gluconeogenesis (NRC, 2011). In the present study, FBPase and G6Pase activities were not significantly influenced by the dietary CHO:L ratios. Similar results were observed in Atlantic salmon, rainbow trout, gilthead sea bream and European sea bass (Tranulis et al., 1996; Panserat et al., 2000b, 2001; Caseras et al., 2002; Enes et al., 2008a; Moreira et al., 2008). For PEPCK activity, the lowest value was observed in 26.13% starch group, which indicated that high dietary carbohydrate level could down-regulate this enzyme activity as reported in common carp (Panserat et al., 2002). Further investigation is needed to study the metabolism of carbohydrate in future studies for this species.

In conclusion, the results of present study indicated that growth, feed utilization, digestive enzyme and hepatic metabolic enzyme were significantly influenced by dietary CHO:L ratios. High dietary carbohydrate or lipid content can significantly depress growth and feed utilization. On the whole, the suitable dietary CHO:L ratios could improve growth performance and feed utilization, and the optimal CHO:L ratio for maximum weight gain was determined to be 1.34 of dry diet.

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