

# Effects of Different Dietary Carbohydrate/Lipid Ratios on Growth, Feed Utilization and Body Composition of Early Giant Grouper *Epinephelus Lanceolatus* Juveniles

Weifeng Li<sup>1,2</sup>, Xiaoyi Wu<sup>1,2\*</sup>, Senda Lu<sup>1,2</sup>, Shuntian Jiang<sup>1,2</sup>, Yuan Luo<sup>1,2</sup>, Mingjuan Wu<sup>1,2</sup> and Jun Wang<sup>1,2</sup>

<sup>1</sup>Key Laboratory of Tropical Biological Resources of Ministry of Education, Hainan University, Haikou, China

<sup>2</sup>Department of Aquaculture, Ocean College of Hainan University, Haikou, China

## Abstract

An 8-week growth trial was undertaken to determine effects of different dietary carbohydrate (CHO)/lipid (L) ratios on growth, feed utilization and body composition of early giant grouper *Epinephelus lanceolatus* juveniles. Five isoenergetic (4.1 kcal/g) and isonitrogenous (50% CP, dry-matter basis) experimental diets were formulated to contain different crude lipid (CL) levels (22%, 19.8%, 17.6%, 15.4% or 13.2%, dry-matter basis) and together different corn starch levels (0%, 4.95%, 9.9%, 14.85% or 19.8%), thereby forming different dietary CHO/L ratios. Groups of 41 early giant grouper juveniles (average initial weight of 0.397 g/fish) were stocked into small floating cages (L 120 cm × W 70 cm × H 50 cm). Triplicate groups of fish were fed each dietary treatment three times daily to apparent satiation.

Increasing dietary CHO/L ratio from 0.13 to 1.25 had no significant influences ( $P \geq 0.05$ ) on growth performance of early giant grouper juveniles, but when dietary CHO/L ratio was increased to 1.88, Weight gain (WG) of experimental fish was significantly decreased ( $P < 0.05$ ). Feed conversion ratio (FCR) values among fish fed CHO/L ratios ranging from 0.13 to 1.25 were not significantly different but lower than that of FCR in fish fed the CHO/L ratio of 1.88. No significant differences in protein productive value (PPV) were observed among fish fed CHO/L ratios of 0.13, 0.4, 0.76 and 1.25. Fish fed a CHO/L ratio of 1.88 had significantly lower PPV than fish fed other CHO/L ratios. Hepatosomatic index (HSI) was increased with dietary CHO/L ratio increasing. Fish fed the CHO/L ratio of 0.13 or 0.4 had significantly higher intraperitoneal fat (IPF) ratios than fish fed the CHO/L ratio of 0.76, 1.25 or 1.88. Whole-body lipid content was decreased with the increasing dietary CHO/L ratio. Muscle moisture, protein as well as lipid contents were not influenced by dietary CHO/L ratios. Liver protein and lipid contents had a decreasing trend with dietary CHO/L ratio increasing. Liver glycogen content in fish fed the CHO/L ratio of 1.25 or 1.88 was significantly higher than fish fed the CHO/L ratio of 0.13, 0.4 or 0.76. Based on quadratic broken line model to WG values, 1.30 of CHO/L ratio, corresponding to 14.79% dietary crude lipid and 19.23% dietary CHO, was proved to be optimal for early giant grouper juveniles.

**Keywords:** *Epinephelus lanceolatus*; Carbohydrate; Lipid; Growth

## Introduction

In aquatic feeds, soluble carbohydrate (CHO) and lipid are usually used as energy sources for aquatic animals. Carbohydrate after absorbed can provide fish with equal amounts of energy as protein. Capacities of fish to utilizing CHO depend on the species as reviewed [1,2]. For instance, only 10% of CHO was acceptable for yellowtail kingfish [3], but juvenile humpback grouper were able to efficiently utilize about 20% of readily digestible CHO [4,5], and for juvenile sunshine bass, up to 42% dextrin in the diet could be efficiently utilized [6]. However, excessive supplementations of digestible CHO to diets could compromise fish growth performance [3] and disrupt liver function and increase susceptibility to infectious diseases [2].

Compared to CHO, lipid can provide fish with more energy value per unit and be better utilized by most fish species, and moreover, lipid is the source of essential fatty acids required by fish for normal growth, development and maintaining health [7]. As one of the macronutrients in aquatic feed, lipid has many advantages for fish growth, but in comparison to CHO, it is more expensive and less available, especially so for fish oil. Excess lipids in diets usually increase lipid deposition in fish carcass [8,9], lead to a substantial decline in performance, affect gut health [10] and increase susceptibility to autoxidation and tissue lipid peroxidation, which may also adversely affect the immune response and disease resistance of fish [11].

Optimizing of dietary CHO and lipid levels are beneficial not only to improving fish quality but also to sparing feed cost [12] reported

that dietary lipid could be partially replaced by CHO without reducing productivity or carcass quality of sunshine bass. Based on the best growth performance or health status, suitable dietary CHO/Lipid ratios have been established in some fish species such as walking catfish (3.38) [8] blunt snout bream (3.58) [13], yellow catfish (2.45-5.58) [14] and yellowfin seabream (0.62) [15].

Giant grouper *Epinephelus lanceolatus* has been widely cultured in China in recent years due to its faster growth compared to other grouper species [16]. To date, available information on nutrition of *Epinephelus lanceolatus* is quite limited, with only one published study [16] evaluating effects of choline on lipid metabolism and stress tolerance of juvenile giant grouper. The aim of this study was to determine effects of different dietary CHO/L ratios on growth, feed utilization and body composition of early giant grouper *Epinephelus lanceolatus* juveniles.

**\*Corresponding author:** Xiaoyi Wu, Department of Aquaculture, Ocean College of Hainan University, Haikou, P.R. China, Tel: 01186-898-66279184, E-mail: [wjrk@163.com](mailto:wjrk@163.com)

**Received** December 13, 2015; **Accepted** January 20, 2016; **Published** March 03, 2016

**Citation:** Li W, Wu X, Lu S, Jiang S, Luo Y, et al. (2016) Effects of Different Dietary Carbohydrate/Lipid Ratios on Growth, Feed Utilization and Body Composition of Early Giant Grouper *Epinephelus Lanceolatus* Juveniles. J Aquac Res Development. 7: 415. doi:10.4172/2155-9546.1000415

**Copyright:** © 2016 Li W, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## Materials and Methods

### Experimental diets and designs

In this trial, five isoenergetic (4.1 kcal/g) and isonitrogenous (50% CP, dry-matter basis) experimental diets were formulated to contain different crude lipid (CL) levels (22%, 19.8%, 17.6%, 15.4% or 13.2%, dry-matter basis) and together different corn starch levels (0%, 4.95%, 9.9%, 14.85% or 19.8%), thereby forming different dietary CHO/L ratios of 0.13, 0.40, 0.76, 1.25 and 1.88, respectively (Table 1). Corn starch was used as the main carbohydrate source. The 50% dietary protein level designed in this study was according to the study of dietary digestible energy was calculated or estimated using physiological fuel values of 4.0, 4.0 and 9.0 kcal/g (16.7, 16.7 and 37.7 kJ/g) for carbohydrate, protein [16] and lipid, respectively [17,18]. It was reported that fish larvae utilize dietary phospholipids more efficiently than neutral lipids [19] and have high requirements of phospholipids such as at least 9.5% for pikeperch larvae [20] and 6.95-8.51% for large yellow croaker larvae [21] so in this study, 10% of soy lecithin was supplemented to all experimental diets. The 4.1 kcal/g diet of digestible energy level was closed to those of values in diets for pikeperch larvae [20] and European sea bass larvae [22].

Fishmeal was well ground, and all dry ingredients were weighed and mixed in a Hobart mixer (A-200T Mixer Bench Model unit, Resell Food Equipment Ltd., Ottawa, Canada) for 30 min. Thereafter, oil was gradually added, while mixing constantly. Then, 30-50 mL of water was slowly blended into the mixture for each 100 g of dry matter, resulting

	Dietary CHO/L ratios				
	0.13	0.40	0.76	1.25	1.88
Peruvian fishmeal (Anchovy) <sup>a</sup>	34.03	34.03	34.03	34.03	34.03
Casein	25	25	25	25	25
Yeast meal	2	2	2	2	2
Fish oil (Salmon)	9.42	7.22	5.02	2.82	0.62
Soy lecithin	10	10	10	10	10
Corn starch	0	4.95	9.9	14.85	19.8
Vitamin mixture <sup>b</sup>	4	4	4	4	4
Mineral mixture <sup>c</sup>	2	2	2	2	2
Carboxymethyl cellulose	2.5	2.5	2.5	2.5	2.5
Cellulose	11.05	8.30	5.55	2.80	0.05
Analyzed composition <sup>d</sup>					
Moisture %	9.26	9.17	9.27	9.78	10.32
Crude protein %	49.2	49.8	49.7	49.2	48.9
Crude lipid %	22.6	20.0	17.6	15.1	13.0
Carbohydrate %	3.05	7.96	13.41	18.87	24.43
Ash %	5.66	5.53	5.13	5.28	5.72
Estimated digestible energy <sup>e</sup> (kcal/g)	4.12	4.11	4.11	4.08	4.10
CHO/L (g/g)	0.13	0.40	0.76	1.25	1.88

<sup>a</sup>Yongsheng Feed Corporation, Binzhou, China; proximate composition (% dry matter): moisture: 8.9; crude protein: 73.6; crude lipid: 10.7.

<sup>b</sup>Vitamin mixture contained (as g kg<sup>-1</sup> vitamin mixture): Choline concentrate 50%, 200 g; vitamin E(500 IU/g) 10 g; vitamin D3 (500,000 IU/g) 0.50 g; vitamin B3 1 g; vitamin B5 2 g; vitamin B1 100mg; vitamin B2 0.4 g; vitamin B6 300 mg; vitamin C 20 g; vitamin B9 100 mg; vitamin concentrate B12 (1 g/kg), 1 g; vitamin K3 1 g; meso-inositol 30 g; cellulose, 732.1 g. (from Mazurais et al. 2009).

<sup>c</sup>Mineral mixture contained (as g kg<sup>-1</sup> mineral mixture): 90 g KCl, 40 mg KIO<sub>3</sub>, 500 g CaHPO<sub>4</sub>·2H<sub>2</sub>O, 40 g NaCl, 3 g CuSO<sub>4</sub>·5H<sub>2</sub>O, 4 g ZnSO<sub>4</sub>·7H<sub>2</sub>O, 20 mg CoSO<sub>4</sub>·7H<sub>2</sub>O, 20 g FeSO<sub>4</sub>·7H<sub>2</sub>O, 3 g MnSO<sub>4</sub>·H<sub>2</sub>O, 215 g CaCO<sub>3</sub>, 124 g MgSO<sub>4</sub>·7H<sub>2</sub>O, and 1 g NaF (from Mazurais et al. 2009).

<sup>d</sup>Values represent means of duplicate samples.

<sup>e</sup>By calculation.

**Table 1:** Formulations and analyzed composition of experimental diets.

in suitably textured dough. The diets were pelletized into a noodle-like shape of 1.0-mm diameter using a twin-screw extruder (Institute of Chemical Engineering, South China University of Technology, Guangzhou, PR China) and then all diets were air-dried for 24 hrs, sieved and stored at -20°C until fed.

### Experimental procedures

30-day post hatching (dph) giant grouper juveniles purchased from a commercial marine fish hatchery (Yangpu, Hainan) were put into small floating cages (L 120 cm × W 70 cm × H 50 cm) at a density of 50 fish per cage and acclimated using a commercial grouper micro-diet (Crude protein: 50%, crude lipid: 12%) together with ground muscle from trash fish for 4 days. During the acclimation, fish were hand fed to satiation three times daily (08:00, 12:00 and 17:00) and ground muscle was gradually reduced. After the micro-diet was completely accepted by experimental fish, groups of 41 early giant grouper juveniles (average initial weight of 0.397 g/fish) were randomly distributed into 15 small cages which were labelled and located in five connective 6-m<sup>3</sup> indoor concrete ponds (L 3 m × W 2 m × H 1 m) with 3 cages occurring in each pond. All ponds received flowing sea water (salinity: 33.1 g/L) from the same reservoir at a rate of 3 g/L.

During the experimental period, each dietary treatment had three replicates, and each replicate cage was in different ponds. Water temperature (27-28°C), total ammonia (0-0.20 mg/L) and dissolved oxygen (5.9-6.2 mg/L) were monitored daily. Fish were exposed to a 12 hrs. light: 12 hrs. dark cycle and hand-fed each dietary treatment three times daily (08:00, 12:00 and 17:00) to apparent satiation until pellets were first seen to sink to bottom of the pond. Feed intake was recorded daily, and experimental ponds and cages were cleaned once a week. The growth trial was continued for 8 weeks.

### Sampling and analysis

At the end of the trial, two fish from each cage were collected for whole-body composition analysis. Three fish per cage were individually weighed and dissected to obtain liver, intestine and intraperitoneal fat (IPF) weights for computing body condition indices including hepatosomatic index (HSI) ((liver wt/live wt)\*100) and IPF ratio ((IPF wt/live wt)\*100), respectively. Intraperitoneal fat was obtained by removing and weighing the fat from the abdominal cavity as well as that adhering to the intestine of the fish. Condition factor (CF) also was computed as (bodyweight × 100)/(body length)<sup>3</sup>. Muscle and liver samples for compositional analysis also were taken from these three fish. Livers for glycogen analysis were quickly dissected from another two randomly selected fish which were removed from each replicate cage, and dissected livers were wrapped in aluminium foil, frozen in liquid nitrogen, and stored at 80°C until analyzed.

Crude protein was estimated by measuring total nitrogen by the Dumas method [23] and multiplying by 6.25. Dry matter was determined by heating at 125°C for 3 hrs, and ash was quantified after heating at 650°C for 3 hrs [24]. Crude lipid was determined by chloroform and methanol extraction [25]. Glycogen contents in liver were analyzed [26]. Carbohydrate content of diets was analyzed by the 3'-dinitro salicylic acid method [27,28].

### Statistical analysis

Experimental data obtained for response parameters were tested by subjecting the data to one-way analysis of variance and Tukey's test using SPSS (Version 16.0). Significance was set at P < 0.05.

## Results

### Growth performance and feed utilization

Dietary CHO/L ratios ranging from 0.13 to 1.25 did not significantly affect WG of experimental fish (Table 2), but fish growth performance was significantly reduced as dietary CHO/L ratio was increased to 1.88. The analysis of quadratic broken line model to WG values showed that 1.30 of dietary CHO/L was optimal for early giant grouper juveniles (Figure 1). Fish fed the diet with a CHO/L ratio of 1.88 had a significantly higher FCR than fish fed diets with a CHO/L ratio of 0.13, 0.4, 0.76 or 1.25. No significant differences in FCRs of experimental fish were observed when dietary CHO/L ratio was increased from 0.13 to 1.25. Fish fed diets with a CHO/L ratio of 1.88 had significantly lower PPVs than fish fed diets with a CHO/L ratio of 0.13, 0.4, 0.76 or 1.25.

### Body condition indices

Fish fed the diet with a CHO/L ratio of 0.13 had a significantly lower condition factor (CF) than fish fed a CHO/L ratio of 1.88 (Table 3). Fish fed a CHO/L ratio of 1.88 had significantly higher hepatosomatic index (HSI) than fish fed other CHO/L ratios. Intraperitoneal fat (IPF) ratio of fish fed CHO/L ratios of 0.13 and 0.4 were significantly higher than those of fish fed CHO/L ratios of 0.76, 1.25 and 1.88, while this parameter showed no significant differences between fish fed CHO/L ratios of 0.13 and 0.4 as well as between fish fed CHO/L ratios of 0.76, 1.25 and 1.88.

	Dietary CHO/L ratios				
	0.13	0.40	0.76	1.25	1.88
FBW <sup>2</sup>	36.94 ± 0.65 <sup>a</sup>	36.93 ± 1.29 <sup>a</sup>	36.59 ± 1.36 <sup>a</sup>	37.48 ± 1.83 <sup>a</sup>	31.45 ± 0.15 <sup>b</sup>
Daily FI <sup>3</sup>	1.42 ± 0.01	1.42 ± 0.17	1.48 ± 0.25	1.47 ± 0.12	1.77 ± 0.10
WG% <sup>4</sup>	9204 ± 163 <sup>a</sup>	9202 ± 325 <sup>a</sup>	9115 ± 342 <sup>a</sup>	9340 ± 460 <sup>a</sup>	7822 ± 38 <sup>b</sup>
FCR <sup>5</sup>	0.73 ± 0.00 <sup>a</sup>	0.73 ± 0.05 <sup>b</sup>	0.69 ± 0.03 <sup>b</sup>	0.75 ± 0.04 <sup>b</sup>	0.90 ± 0.03 <sup>a</sup>
PPV <sup>6</sup>	47.05 ± 0.37 <sup>a</sup>	46.21 ± 3.26 <sup>a</sup>	48.67 ± 2.04 <sup>a</sup>	45.89 ± 1.95 <sup>a</sup>	36.90 ± 1.44 <sup>b</sup>
Survival %	64 ± 1	64 ± 4	72 ± 2	62 ± 6	73 ± 5

<sup>1</sup>Treatment means (± SEM) represent the average values of three tanks per treatment, and values within the same row with different letters are significantly (P<0.05) different.

<sup>2</sup>FBW: Final mean body weight (g).

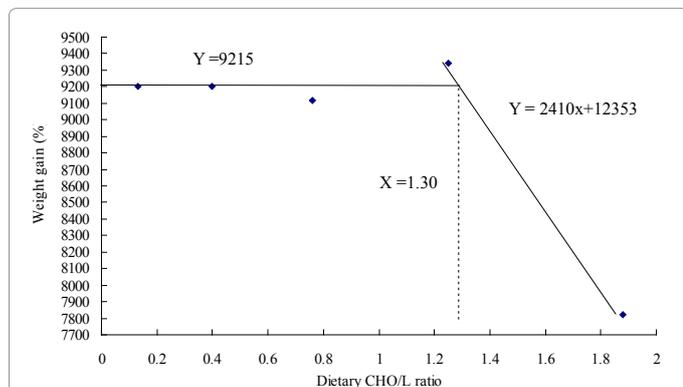
<sup>3</sup>Daily Feed Intake: 100 × feed offered/average total weight/days.

<sup>4</sup>Weight Gain: 100 × (final mean weight - initial mean weight)/initial mean weight.

<sup>5</sup>Feed Conversion Ratio: g dry feed/g weight gain (included the dead fish).

<sup>6</sup>Protein Productive Value: 100 × retained protein (g)/protein fed (g).

**Table 2:** Growth performance and feed utilization of early giant grouper *Epinephelus lanceolatus* juveniles fed diets contained different CHO/L ratios for 8 weeks<sup>1</sup>.



**Figure 1:** The analysis of quadratic broken line model based on WG values showed that 1.30 of dietary CHO/L was optimal for early giant grouper juveniles.

	Dietary CHO/L ratios				
	0.13	0.40	0.76	1.25	1.88
CF <sup>2</sup>	1.83 ± 0.08 <sup>b</sup>	2.07 ± 0.04 <sup>ab</sup>	2.01 ± 0.08 <sup>ab</sup>	1.98 ± 0.00 <sup>ab</sup>	2.13 ± 0.06 <sup>a</sup>
HSI <sup>3</sup>	2.96 ± 0.29 <sup>b</sup>	3.82 ± 0.29 <sup>b</sup>	3.95 ± 0.40 <sup>b</sup>	3.78 ± 0.21 <sup>b</sup>	6.33 ± 0.29 <sup>a</sup>
IPF Ratio <sup>4</sup>	4.61 ± 0.12 <sup>a</sup>	4.89 ± 0.14 <sup>a</sup>	3.61 ± 0.29 <sup>b</sup>	3.73 ± 0.30 <sup>b</sup>	3.63 ± 0.02 <sup>b</sup>

<sup>1</sup>Treatment means (± SEM) represent the average values of three tanks per treatment, and values within the same row with different letters are significantly (P < 0.05) different.

<sup>2</sup>CF: Body weight × 100/(Body length)<sup>3</sup>.

<sup>3</sup>HSI: Liver weight × 100/fish weight.

<sup>4</sup>IPF ratio: Intraperitoneal fat weight × 100/fish weight.

**Table 3:** Body condition indices of early giant grouper *Epinephelus lanceolatus* juveniles fed diets contained different CHO/L ratios for 8 weeks<sup>1</sup>.

	Dietary CHO/L ratios				
	0.13	0.40	0.76	1.25	1.88
<i>Whole-body composition</i>					
Moisture %	69.7 ± 0.4 <sup>b</sup>	69.5 ± 0.1 <sup>b</sup>	70.1 ± 0.2 <sup>b</sup>	70.2 ± 0.4 <sup>b</sup>	71.9 ± 0.3 <sup>a</sup>
Protein %	16.89 ± 0.11 <sup>a</sup>	16.68 ± 0.08 <sup>a</sup>	16.65 ± 0.01 <sup>a</sup>	16.90 ± 0.11 <sup>a</sup>	16.22 ± 0.15 <sup>b</sup>
Lipid %	9.16 ± 0.42 <sup>a</sup>	9.16 ± 0.33 <sup>a</sup>	8.80 ± 0.37 <sup>a</sup>	7.95 ± 0.19 <sup>b</sup>	6.35 ± 0.24 <sup>c</sup>
<i>Muscle composition</i>					
Moisture %	73.97 ± 0.49	75.11 ± 0.94	74.97 ± 0.18	73.93 ± 0.74	75.94 ± 0.32
Protein %	20.80 ± 0.22	20.84 ± 0.71	21.23 ± 0.20	21.60 ± 0.68	20.97 ± 0.20
Lipid %	10.33 ± 1.05	9.06 ± 1.06	8.74 ± 0.52	9.27 ± 0.09	8.67 ± 0.75
<i>Liver composition</i>					
Moisture %	59.18 ± 1.27	62.03 ± 0.63	62.46 ± 1.11	62.84 ± 0.87	62.56 ± 0.46
Protein %	9.48 ± 0.09 <sup>a</sup>	8.56 ± 0.26 <sup>b</sup>	8.14 ± 0.18 <sup>b</sup>	8.29 ± 0.42 <sup>b</sup>	6.42 ± 0.10 <sup>c</sup>
Lipid %	21.04 ± 1.28 <sup>a</sup>	16.41 ± 1.00 <sup>b</sup>	14.80 ± 1.49 <sup>bc</sup>	14.88 ± 0.47 <sup>bc</sup>	13.41 ± 0.35 <sup>c</sup>
Glycogen%	11.61 ± 0.19 <sup>b</sup>	11.95 ± 0.45 <sup>b</sup>	15.76 ± 0.23 <sup>a</sup>	16.76 ± 0.63 <sup>a</sup>	17.29 ± 0.54 <sup>a</sup>

<sup>1</sup>Treatment means (± SEM) represent the average values of three tanks per treatment, and values within the same row with different letters are significantly (P<0.05) different.

**Table 4:** Whole-body, muscle and liver composition (fresh-wt. basis) of early giant grouper *Epinephelus lanceolatus* juveniles fed diets contained different CHO/L ratios for 8 weeks<sup>1</sup>.

### Whole-body, muscle and liver compositions

Values of whole-body lipid content were not significantly different among fish fed CHO/L ratios of 0.13, 0.4 and 0.76, but whole-body lipid contents of fish fed CHO/L ratios of 0.13, 0.4 or 0.76 significantly higher than fish fed CHO/L ratios of 1.25 and 1.88 (Table 4). Fish fed a CHO/L ratio of 1.88 had significantly higher whole-body moisture but lower whole-body protein compared to fish fed a CHO/L ratio of 0.13, 0.4, 0.76 or 1.25. Muscle moisture, protein as well as lipid contents were less affected by dietary CHO/L ratios. Liver moisture showed no significant differences among different experimental treatments. Liver protein and lipid contents of fish fed the diet with a CHO/L ratio of 0.13 were significantly higher than fish fed a CHO/L ratio of 0.4, 0.76, 1.25 or 1.88. Fish fed a CHO/L ratio of 0.76, 1.25 or 1.88 had significantly higher glycogen in liver than fish fed a CHO/L ratio of 0.13 or 0.4.

### Discussion

Results of the present study demonstrated that 1.30 of CHO/L ratio, corresponding to 14.79% dietary crude lipid and 19.23% dietary CHO, was proved to be optimal for early giant grouper juveniles, indicating that reduction in dietary lipid from 22.6% to 14.79%, with concomitant increase in CHO level from 3.05% to 19.23%, corresponding to CHO/L ratios of 0.13 to 1.30, did not negatively affect the growth of early giant grouper *Epinephelus lanceolatus* juveniles, but when fish were fed the

24.32% CHO and 13.0% lipid contained diet with a CHO/L ratio of 1.88, WG was significantly reduced. This indicated that early giant grouper juveniles could effectively utilize 19.23% CHO at 50% dietary CP and 14.79% dietary CL levels, and dietary CHO could replace about 30% lipid without sacrificing fish growth. Previous studies on juvenile humpback grouper initiating ~ 5 g [4] or 8 g [5] of body weight showed that juvenile humpback grouper can efficiently utilize about 20% of readily digestible dietary CHO, which was in line with the results obtained in this study. Successful replacements of partial lipid by digestible CHO have been reported in other fish species such as hybrid striped bass [12], rainbow trout [5], hybrid *Clarias* catfish [29] and yellowfin seabream [15]. In this study, fish fed the low-CHO (3.05%) diet did not display poorer growth performance than fish fed high-CHO (7.96%, 13.41%, 18.87%) diets, meaning that dietary CHO was not essential for growth of early giant grouper juveniles. This disagreed with the results of some other studies [8,29,30].

Dietary lipids are the preferable source of metabolic energy for the development of fish and the main source of essential fatty acids for maintaining rapid growth of larval fish, especially marine fish [31]. The significantly lower WG of fish fed a CHO/L ratio of 1.88 (3.0% fish oil) compared to fish fed CHO/L ratios of 0.13, 0.4, 0.76 and 1.25 (12.6%, 10%, 7.6% and 5.1% fish oil) were perhaps due to the deficiency of essential fatty acids resulted from the decrease of fish oil. Reduced growth and poor feed conversion efficiency in fish fed diets with high CHO/L ratios have also been reported in chinook salmon [32], channel catfish [17], red drum [6,33] and hybrid *Clarias* catfish [29].

Fish fed dietary CHO/L ratios ranging at 0.13-1.25 (15.1-22.6% CL) had similar growth performance, demonstrating that 15.1% of dietary lipid is adequate for fast growth of early giant grouper juveniles. Optimal dietary lipid requirements of several other grouper species have been reported such as juveniles of *E. coioides* (10%) [34,35], *E. malabaricus* (7.9-12.5%) [36,37], *E. aeneus* (10.5-14.7%) [38] and *C. altivelis* (16-26%) [5].

The higher feed conversion ratios (FCRs) and lower PPV of fish fed a CHO/L ratio of 1.88 compared to fish fed a CHO/L ratio of 0.13, 0.4, 0.76 or 1.25 were attributed to their poorer growth compared to fish fed low CHO/L ratios. Similar reduced growth, feed efficiency, and protein retention have also been observed in juvenile yellowfin seabream [15] and red drum [6,33] fed a high carbohydrate and low lipid diet.

In the present study, all experimental diets had similar protein and gross energy contents, so the higher IPF ratios in fish fed diets with low CHO/L ratios (0.13 and 0.4) compared to fish fed diets with high CHO/L ratios (0.76, 1.25 and 1.88) maybe result from their limited ability of utilizing dietary CHO as energy and/ or de novo synthesis of lipid. This is in agreement with the results reported in yellowfin seabream [15] and hybrid striped bass [39]. In the present study, increasing dietary lipid to 17.6% or higher (20.0%, 22.6%) resulted in increased whole-body lipid. Similar results have been reported in rainbow trout [18], red drum [33,40], striped bass [41], channel catfish [42,43], hybrid *Clarias* catfish [29], walking catfish [8], *Tilapia zillii* [43], common carp [44,45] and brown-marbled grouper [46].

It is reported that liver enlargement may result from increased lipid or glycogen deposition [47]. The higher HSI values in fish fed high CHO/L ratios compared to those in fish fed low CHO/L ratios were mainly due to their higher hepatic glycogen contents which resulted from the higher CHO levels contained in the high CHO/L diets. Similar results were also reported in striped bass [48] and Asian seabass [49]. Fish fed the diet with low CHO content (3.05%) had

lower CF value than fish fed the high CHO contained diet, which was possibly due to the lower HSI observed in fish fed the CHO/L ratio of 0.13. No differences in muscle protein and lipid contents among all experimental treatments showed that muscle composition of fish was less influenced by dietary CHO/L ratios. Fish fed high-lipid diets had higher liver lipid content than fish fed low-lipid diets, agreeing with the reports in hybrid striped bass [50,51].

In conclusion, results of this study showed that at 50% dietary crude protein level, 1.30 of CHO/L ratio, corresponding to 14.79% dietary crude lipid and 19.23% dietary CHO, was optimal for growth performance of early giant grouper juveniles, when dietary CHO/L ratio was increased to 1.88 (at 13.0% dietary crude lipid level and 24.32% CHO level), fish growth significantly reduced; Dietary lipid in excess, resulted in increased lipid deposition in the body.

## References

1. Wilson R (1994) Utilization of dietary carbohydrate by fish. *Aquaculture* 124: 67-80.
2. Hemre G, Mommsen T, Kroghdal A (2002) Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. *Aquaculture Nutrition* 8: 175-194.
3. Booth M, Moses M, Allan G (2013) Utilisation of carbohydrate by yellowtail kingfish *Seriola lalandi*. *Aquaculture* 376-379: 151-161.
4. Shiao S, Lin Y (2002) Utilization of glucose and starch by the grouper *Epinephelus malabaricus* at 23°C. *Fish. Sci* 68: 991-995
5. Suwira K, Giri A, Marzuqi M, Trijoko (2004) Utilisation of dietary dextrin by juvenile humpback grouper (*Cromileptes altivelis*). In: Rimmer MA, McBride S, Williams, KC (eds.), *Advances in Grouper Aquaculture*. ACIAR Monograph, vol. 110. Australian Centre for International Agriculture Research, Canberra, Australia, pp. 107-109
6. Serrano J, Nematipour G, Gatlin D (1992) Dietary protein requirement of the red drum (*Scianops ocellatus*) and relative use of dietary carbohydrate and lipid. *Aquaculture* 101: 283-291.
7. Lin X, Shiao S (2003) Dietary lipid requirement of grouper, *Epinephelus malabaricus* and effects on immune responses. *Aquaculture* 225: 243-250.
8. Erfanullah, Jafri A (1998) Effect of dietary carbohydrate-to-lipid ratio on growth and body composition of walking catfish (*Clarias batrachus*). *Aquaculture* 161: 159-168.
9. Hanley F (1991) Effects of feeding supplementary diets containing varying levels of lipid on growth, food conversion, and body composition of Nile tilapia *Oreochromis niloticus* L. *Aquaculture* 93: 323-334.
10. Bonvini E, Parma L, Mandrioli L, Sirri R, Brachelente C, et al. (2015) Feeding common sole (*Solea solea*) juveniles with increasing dietary lipid levels affects growth, feed utilization and gut health. *Aquaculture* 449: 87-93.
11. Dias J, Rueda-Jasso R, Panserat S, Conceição L, Gomes E, et al. (2004) Effect of dietary carbohydrate to lipid ratios on growth, lipid deposition and metabolic hepatic enzymes in juvenile Senegalese sole (*Solea senegalensis*, Kaup). *Aquac Res* 35: 1122-1130.
12. Nematipour G, Brown M, Gatlin D (1992) Effects of dietary carbohydrate:lipid ratio on growth and body composition of hybrid striped bass. *J World Aquacult Soc* 23: 128-132.
13. Li X, Wang Y, Liu W, Jiang G, et al. (2013) Effects of dietary carbohydrate / lipid ratios on growth performance, body composition and glucose metabolism of fingerling blunt snout bream *Megalobrama amblycephala*. *Aquaculture Nutrition* 19: 701-708.
14. Wang L, Liu W, Lu K, Xu W, Cai D, et al. (2014) Effects of dietary carbohydrate/lipid ratios on non-specific immune responses, oxidative status and liver histology of juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture* 426-427: 41-48.
15. Hu Y, Liu Y, Tian L, Yang H, Liang G, et al. (2007) Optimal dietary carbohydrate to lipid ratio for juvenile yellowfin seabream (*Sparus latus*). *Aquaculture Nutrition* 13: 291-297.
16. Yeh S, Shiu P, Guei W, Lin Y, Liu C (2013) Improvement in lipid metabolism and

- stress tolerance of juvenile giant grouper, *Epinephelus lanceolatus* (Bloch), fed supplemental choline. *Aquaculture Research* 46: 1-12.
17. Garling D, Wilson R (1977) Effect of dietary carbohydrate to lipid ratio on growth and body composition of fingerling channel catfish. *Prog Fish-Cult* 39: 43-47
  18. Lee D, Putnam G (1973) The response of rainbow trout to varying protein/energy ratios in a test diet. *J Nutr* 103: 916-922.
  19. Cahu C, Zambonino I, Barbosa V (2003) Effect of dietary phospholipid level and phospholipid: neutral lipid value on the development of sea bass (*Dicentrarchus labrax*) larvae fed a compound diet. *Journal of Nutrition* 90: 21-28.
  20. Hamza N, Mhetti M, Khemis I, Cahu C, Kestemont P, et al. (2008) Effect of dietary phospholipid levels on performance, enzyme activities and fatty acid composition of pikeperch (*Sander lucioperca*) larvae. *Aquaculture* 275: 274-282.
  21. Zhao J, Ai Q, Mai K, Zuo R, Luo Y, et al. (2013) Effects of dietary phospholipids on survival, growth, digestive enzymes and stress resistance of large yellow croaker, *Larimichthys crocea* larvae. *Aquaculture* 410-411: 122-128.
  22. Mazurais D, Glynatsi N, Darias M, Christodouloupoulou S, Cahu C, et al. (2009) Optimal levels of dietary vitamin A for reduced deformity incidence during development of European sea bass larvae (*Dicentrarchus labrax*) depend on malformation type. *Aquaculture* 294: 262-270.
  23. Ebeling M (1968) The Dumas method for nitrogen in feed. *J Assoc Anal Chem* 75: 401-413.
  24. AOAC (1990) Official Methods of Analysis. AOAC (Association of Official Analytical Chemists) 1298.
  25. Folch J, Lees M, Sloane-Stanley G (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 97: 383-394.
  26. Hassidh W, Abraham S (1957) Chemical procedures for analysis of polysaccharides. *Methods of Enzymology* 3: 34-50
  27. Yu S, Olsen C, Marcussen J (1998) Methods for the assay of 1, 5-anhydro-D-fructose and A-1,4-glucanlyase. *Carbohydr Res* 305: 73-82.
  28. Brauge C, Corraze G, Medale F (1993) Combined effects of dietary lipid carbohydrate ratio and environmental factors on growth and nutritional balance in rainbow trout *Aquaculture Research* 21: 220-221.
  29. Jantrarat W, Sitasit P, Rajchapakdee S (1994) The optimum carbohydrate to lipid ratio in hybrid Clarias catfish (*Clarias macrocephalus* × *C. glariepinus*) diets containing raw broken rice. *Aquaculture* 127: 61-68.
  30. McGoogan B (1998) Effects of dietary protein and energy manipulations on growth and aspects of nitrogen metabolism of red drum, *Sciaenops ocellatus*. Ph.D. dissertation, Texas A&M University, College Station, TX 135.
  31. Sargent J, Tocher D, Bell J (2002) The lipids. *Fish Nutrition*. Academic Press, Elsevier, San Diego 181-257.
  32. Buhler D, Halver J (1961) Nutrition of salmonid fishes: IX. Carbohydrate requirements of chinook salmon. *J Nutr* 74: 307-318.
  33. Ellis S, Reigh R (1991) Effects of dietary lipid and carbohydrate levels on growth and body composition of juvenile red drum, *Sciaenops ocellatus*. *Aquaculture* 97: 383-394.
  34. Luo Z, Liu Y, Mai K, Tian L, Liu D, et al. (2004) Optimal dietary protein requirement of grouper *Epinephelus coioides* juveniles fed isoenergetic diets in floating net cages. *Aquac Nutr* 10: 247-252.
  35. Luo Z, Liu Y, Mai K, Tian L, Liu D, et al. (2005) Effect of dietary lipid level on growth performance, feed utilization and body composition of grouper *Epinephelus coioides* juveniles fed isonitrogenous diets in floating net cages. *Aquac Int* 13: 257-269.
  36. Tuan L, Williams K (2007) Optimum dietary protein and lipid specifications for juvenile malabar grouper (*Epinephelus malabaricus*). *Aquaculture* 267: 129-138.
  37. Chen H, Tsai J (1994) Optimal dietary protein level for the growth of juvenile grouper, *Epinephelus malabaricus*, fed semipurified diets. *Aquaculture* 119: 265-271.
  38. Lupatsch I, Kissil G (2005) Feed formulations based on energy and protein demands in white grouper (*Epinephelus aeneus*). *Aquaculture* 248: 83-95.
  39. Gaylord T, Gatlin D (2000) Dietary lipid level but not L-carnitine affects growth performance of hybrid striped bass (*Morone chrysops* ♀ × *M. saxatilis* ♂). *Aquaculture* 190: 237-246.
  40. Williams C, Ronbinson E (1998) Response of red drum to various dietary levels of menhaden oil. *Aquaculture* 70: 107-120.
  41. Millikin M (1983) Interactive effects of dietary protein and lipid on growth and protein utilization of age-O-striped bass. *Trans Am Fish Soc* 122: 185-193.
  42. Page J, Andrews J (1973) Interactions of dietary levels of protein and energy on channel catfish (*Ictalurus punctatus*). *J Nutr* 103: 1339-1346.
  43. El-Sayed A, Garling D (1988) Carbohydrate to lipid ratios in diets for *Tilapia zilli* fingerlings. *Aquaculture* 73: 157-163.
  44. Dabrowski K (1977) Protein requirements of grass carp, *Ctenopharyngodon idella*. *Aquaculture* 12: 63-73.
  45. Takeuchi T, Watanabe T, Ogino C (1979) Availability of carbohydrate and lipid as dietary energy sources for carp. *Bull Jpn Soc Sci Fish* 45: 977-982.
  46. Shapawi R, Ebi I, Yong A, Ng WK (2014) Optimizing the growth performance of brown-marbled grouper, *Epinephelus fuscoguttatus* (Forsk.), by varying the proportion of dietary protein and lipid levels. *Animal Feed Science and Technology* 191: 98-105.
  47. Berger A, Halver J (1987) Effect of dietary protein, lipid and carbohydrate content on the growth, feed efficiency and carcass composition of striped bass, *Morone saxatilis* (Walbaum), fingerlings. *Aquacult Fish Manage* 18: 345-356.
  48. Rawles S, Gatlin D (1998) Carbohydrate utilization in striped bass (*Morone saxatilis*) and sunshine bass (*M. chrysops* ♀ × *M. saxatilis* ♂). *Aquaculture* 161, 201-212.
  49. Catacutan M, Coloso R (1997) Growth of juvenile Asian seabass, *Lates calcarifer*, fed varying carbohydrate and lipid levels. *Aquaculture* 149: 137-144.
  50. Wu X, Castillo S, Rosales M, Burns A, Mendoza M, et al. (2015) Relative use of dietary carbohydrate, non-essential amino acids, and lipids for energy by hybrid striped bass, *Morone chrysops* ♀ × *M. saxatilis* ♂. *Aquaculture* 435: 116-119.
  51. Williams K, Irvin S, Barclay M (2004) Polka dot grouper *Cromileptes altivelis* fingerlings require high protein and moderate lipid diets for optimal growth and nutrient retention. *Aquac Nutr* 10: 125-134.