Effects of dietary lipid-to-carbohydrate ratio on growth and carbohydrate metabolism in juvenile cobia (Rachycentron canadum)

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1. Introduction

The cobia, Rachycentron canadum, is widely distributed in tropical and sub-tropical waters, and it has been cultured as a recreational fish species (Dirty and Shaw, 1992). Excellent flesh quality, rapid growth, and adaptability to culture conditions, confer highly desirable characteristics for global commercial aquaculture.

on cobia (Holt et al., 2007). The cobia is extensively farmed in cages in China, Vietnam, and Philippines and its production has been initiated in European Union, Brazil, and other Latin American and Caribbean countries (Holt et al., 2007; Chi et al., 2014). It is produced mainly in Asian-Pacific coast with a world production above 40,000 t per year (FAO, 2018; Tveteras, 2016).

Dietary requirements of cobia for macronutrients include crude protein (Chou et al., 2001; Lunger et al., 2006), lipid (Chou et al., 2001; Wang et al., 2005), carbohydrate (Cui et al., 2010; Ren et al., 2011), methionine (Zhou et al., 2006; Wang et al., 2016), lysine (Zhou et al., 2007) and choline (Mai et al., 2009). Lipid and carbohydrate have been given priority in nutritional studies for their protein-sparing effect because they are the principal energetic components and have lower relative costs than protein (Wang et al., 2005; Stone et al., 2003). Higher dietary lipid levels (above 15%) produced little practical benefit because of higher fat accretion in cobia (Wang et al., 2005). Dextrin and wheat starch were the optimal carbohydrate sources for juvenile cobia (Cui et al., 2010).
The effect of dietary carbohydrate level on the growth performance of juvenile cobia with 6 isonitrogenous and isolipidic diets was reported and the results showed that the supplementation of gelatinized corn starch should not exceed 24.2% (Ren et al., 2011). However, nothing was reported about effects of lipid-to-carbohydrate ratio in diets on cobia. Therefore, the aim of this study was to find the suitable ratio of lipid to carbohydrate in diets for juvenile cobia.

2. Materials and methods

All animal care and experimental procedures in the present study were approved by the Guangdong Academy of Agricultural Sciences and conducted in accordance with the Guidelines for Experimental Animals.

2.1. Experimental diets

Six isonitrogenous (44% crude protein) semi-purified diets were prepared to vary in the ratio of lipid to carbohydrate as shown in Table 1. White fish meal, fish oil, corn oil, and dextrin were used respectively as the protein, lipid, and carbohydrate sources. Diet ingredients were ground through a 60-mesh sieve. Lipid and distilled water (40%) were added to the premixed dry ingredients and thoroughly mixed until homogenous in a Hobart-type mixer. The 4 mm diameter pellets were wet extruded, and then air dried, sealed in plastic bags and stored at – 20 °C until used.

2.2. Experimental fish

The experimental fish were obtained from a commercial farm in Zhanjiang, Guangdong, China. Before initiation of the feeding trials, fish were acclimated to the experimental conditions for a 28-d period by feeding an experimental diet (diet 1, D1) with 2 meals/d in outdoor ocean cages. The feeding trial was conducted in 18 outdoor ocean nylon cages (2.0 m × 1.0 m × 2.5 m) in Zhanjiang, Guangdong, China. In the beginning of the experiment, fish (average initial weight was 14.72 ± 0.43 g) with the same size were weighed and sorted into cages with 15 fish for each cage. Three replicates (cages) of fish were used for testing each diet. The feeding experiment lasted for 8 weeks. During the 56-d feeding period, fish were hand-fed to apparent satiation (initially 5% to 6% of body weight per day and then gradually increased) twice daily (09:00 and 16:00). The amount of diet consumed by fish in each cage was recorded daily, and the amount offered was adjusted according to amount consumed the day before. A 12 h light:12 h dark photoperiod was used during the feeding trial. During the experimental period, temperatures ranged from 27 to 30 °C, salinity was 30% to 34%, and dissolved oxygen was not less than 5.0 mg/L.

2.3. Sample collection and analysis

At the termination of the 8-week feeding trial, the fish were made to undergo fasting for 24 h, and all fish were counted and weighed to determine weight gain (WG), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention efficiency (PR) and survival rate (SR). Fish in each cage were anesthetized with tricaine methane sulfonate (MS-222) at 120 mg/L. Three fish from each cage were used for proximate composition analysis of whole body, and 5 fish per cage were individually weighed and the livers were weighed for calculation of hepatosomatic index (HSI). The livers of 5 fish were sampled, sealed in plastic bags and frozen at −80 °C until the analysis of enzymes involved in carbohydrate metabolism. Blood samples were collected from the caudal vein of 5 fish with similar sizes from each group approximately 24 h after the last feeding, and the plasma was separated by centrifugation and stored at −80 °C until analysis.

Crude protein, crude lipid, moisture and ash content in diets and whole body were determined by standard methods (Association of Official Analytical Chemists (AOAC), 1995). Moisture was determined by oven-drying at 105 °C until constant weight. Crude protein (nitrogen × 6.25) was determined by the Kjeldahl method after acid digestion using an Auto Kjeldahl System (1030-Autoanalyzer, Tecator, Hoganas, Sweden). Crude lipid was determined by the ether-extraction method using a Soxtec System (Soxtec System HT6, Tecator, Sweden). Ash was determined by muffle furnace at 550 °C for 24 h. Blood glucose (GLU), triglyceride (TG), cholesterol (CHOL), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were determined by using an automatic biochemical analyzer (Hitachi 7170A, Japan) provided by a clinical laboratory (The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China). Fructose-1,6-diphosphatase (FDPase) activity was assayed by the method of McGilvery (1955). Malic enzyme (ME) activity was assayed by the method of Wise and Ball (1964). Phosphofructokinase (PFK) and hexokinase (HK) activities were assayed by the methods of Bergmeyer et al. (1983). All enzyme activities were expressed per mg of total protein (specific activity). 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 nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) generated by per mg protein per minute at 30 °C.

2.4. Calculations

The following variables were calculated:

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WG (\%) = 100 \times \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}}
\]

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FCR = \frac{\text{Feed fed (g DM)}}{\text{WG (g)}}
\]
PER = WG (g)/Protein intake (g),
PRE (%) = [(Final body nitrogen – Initial body nitrogen) × 100]/Total nitrogen fed,
SR (%) = 100 × (Final amount of fish)/(Initial amount of fish),
HSI (%) = 100 × (Liver weight/Whole body weight).

2.5. Statistical analysis

Data from each treatment were subjected to one-way ANOVA. Tukey’s test was used to compare the mean values between individual treatments when overall differences were significant at a level that was less than 0.05 (P < 0.05). Statistical analysis was performed using SPSS 11.5 package (SPSS, IL, USA).

3. Results

3.1. Growth performance

The WG, FCR, PER, PRE and SR of cobia fed the experimental diets are presented in Table 2. Dietary lipid-to-carbohydrate ratio significantly affected WG, FCR, PER and PRE of juvenile cobia in this trial (P < 0.05). The WG and PER in D1 group were significantly lower than those in other groups (P < 0.05). The FCR in D1 group was significantly higher than that in other groups (P < 0.05). In terms of PRE, D4 and D6 were significantly higher than D1 group (P < 0.05). The SR of fish among all treatments ranged from 75.7% to 91.1%. The SR in D4 group was the highest among all groups and was significantly higher than that in D1, D2 and D5 groups (P < 0.05).

3.2. Whole body composition and hepatosomatic index

Whole body composition and HSI of cobia fed the experimental diets are presented in Table 3. The HSI, whole body moisture and crude protein were significantly affected by dietary lipid-to-carbohydrate ratio (P < 0.05). The HSI in D3 and D4 was significantly lower than that in D1 (P < 0.05). Whole body moisture was significantly lower in D2 than in D1 (P < 0.05), and was significantly higher in D4 and D5 than in D1 (P < 0.05). Whole body crude lipid and ash were not affected by dietary lipid-to-carbohydrate ratio.

3.3. Blood metabolites

The TG, GLU, CHOL, HDL-C and LDL-C concentrations in the blood of cobia fed the experimental diets are presented in Table 4. As dietary lipid-to-carbohydrate ratio decreased, serum TG concentration decreased. It was the lowest in D6 among all groups, and was significantly lower than that in D1 and D2 (P < 0.05). The CHOL, HDL-C, LDL-C and GLU concentrations were not significantly affected by dietary lipid-to-carbohydrate ratio.

3.4. Carbohydrate metabolism

The activities of FDPase, PFK, ME and HK in the liver of cobia fed the experimental diets are showed in Table 5. Dietary lipid-to-carbohydrate ratio significantly affected activities of FDPase, ME and PFK of cobia (P < 0.05). The FDPase in D4 was the highest among all groups, and was significantly higher than that in D1 and D3 (P < 0.05). A significant higher PFK activity was observed in D3 and D4 compared to other groups (P < 0.05). The ME activity in D4 and D5 was significantly higher than that in other groups (P < 0.05). No significant difference was observed in liver HK activity among the groups.

4. Discussion

In the present experiment, the value of WG was higher than those reported in previous studies on cobia (Chou et al., 2001, 2004), but was lower than those observed in lately studies on cobia (Cui et al., 2010; Ren et al., 2011). This is probably due to differences in initial weight of the fish in these experiments. However, the value of SR was lower than those observed in studies on cobia (Cui et al., 2010; Ren et al., 2011), which is probably due to outdoor ocean cages used in the present study. In previous experiments, indoor cylindrical fiberglass tanks were used for testing and were provided with maintain optimal water quality throughout the studies for the fish (Cui et al., 2010; Ren et al., 2011).

To our knowledge, this is the first report describing the effect of lipid-to-carbohydrate ratio in diets. Moreover, for this species, optimum dietary lipid and carbohydrate levels still are somewhat contradictory. Cobia has a limited lipid tolerance, recommending a moderate dietary lipid level (5% to 15% of diet) for maximum performances (Wang et al., 2005). Previously, a dietary lipid level was recommended between 6% and 18% in diets to obtain fish maximum growth (Chou et al., 2001). Interestingly, in the present study, the increase in dietary lipid-to-carbohydrate ratio, from 1.31 to 2.26 in diets led to a significant decrease in growth performance, feed and protein utilization efficiency. For other fish species, both lipids and gelatinized starch have been reported to be good digestible energy sources for carnivorous fish (Peres and Oliva-Teles, 2002; Fernández et al., 2007; Oliva-Teles, 2012). However, lipids were found to be utilized more efficiently and to be more tolerated than carbohydrates, as high dietary levels of carbohydrate may reduce digestibility (Peres and Oliva-Teles, 2002) and may impair metabolic utilization due to prolonged post-prandial hyperglycaemic levels (Enes et al., 2011). Nevertheless, an appropriate dietary carbohydrate level may improve growth performance and spare dietary protein and lipids, even in carnivorous fish (Higgs et al., 2009; Leggatt et al., 2009).

Together, the present results on growth, feed and protein utilization suggest that carbohydrate is a better digestible energy

Table 2

| Group | IBW, g | WG, % | FCR | PER | PR, % | SR, % |
|-------|--------|-------|-----|-----|-------|-------|
| D1    | 14.7 ± 0.62 | 381 ± 2.20<sup>b</sup> | 1.59 ± 0.49<sup>a</sup> | 1.47 ± 0.45<sup>b</sup> | 21.8 ± 7.14<sup>a</sup> | 75.7 ± 5.42<sup>b</sup> |
| D2    | 14.7 ± 0.52 | 526 ± 7.94<sup>a</sup> | 1.29 ± 0.08<sup>b</sup> | 1.76 ± 0.11<sup>a</sup> | 272 ± 2.04<sup>a</sup> | 80 ± 6.74<sup>b</sup> |
| D3    | 14.7 ± 0.57 | 549 ± 14.5<sup>a</sup> | 1.27 ± 0.19<sup>b</sup> | 1.84 ± 0.27<sup>a</sup> | 287 ± 3.43<sup>a</sup> | 85.7 ± 13.9<sup>a,b</sup> |
| D4    | 14.7 ± 0.38 | 577 ± 43.2<sup>a</sup> | 1.22 ± 0.09<sup>b</sup> | 1.85 ± 0.14<sup>a</sup> | 297 ± 1.57<sup>a</sup> | 91.1 ± 3.86<sup>a</sup> |
| D5    | 14.6 ± 0.38 | 621 ± 17.7<sup>a</sup> | 1.19 ± 0.20<sup>b</sup> | 1.98 ± 0.28<sup>a</sup> | 295 ± 6.26<sup>a,b</sup> | 78.3 ± 12.1<sup>b</sup> |
| D6    | 15.1 ± 0.14 | 553 ± 23.2<sup>a</sup> | 1.19 ± 0.06<sup>b</sup> | 1.90 ± 0.10<sup>a</sup> | 29 ± 0.55<sup>b</sup> | 84.4 ± 10.2<sup>a,b</sup> |

IBW – initial body weight; WG – weight gain; FCR – feed conversion ratio; PER – protein efficiency ratio; PR – protein retention efficiency; SR – survival rate.

<sup>a</sup> Values (mean ± standard deviations of three replications) in the same column with different superscripts are significantly different (P < 0.05).

Lipid-to-carbohydrate ratio of diets of D1, D2, D3, D4, D5 and D6 groups was 2.26, 1.31, 0.78, 0.47, 0.34 and 0.23, respectively.
source than lipids for cobia, promoting a higher protein-sparing effect. For other lean species, such as sole, turbot and halibut, it was reported a decline in growth rate and feed utilization with effect. For other lean species, such as sole, turbot and halibut, it was reported a decline in growth rate and feed utilization with increasing lipids as energy source more efficiently than it utilizes lipids. The optimal lipid-to-carbohydrate ratio in juvenile cobia diets was from 14.3% to 19.1% in the diets (Ren et al., 2011). However, to keep energy variable in all treatments, significantly lower HSI was observed in cobia fed the diets containing 0.78 and 0.47 of lipid-to-carbohydrate ratio, indicating that dietary digestible lipid-to-carbohydrate ratio and energy levels significantly influence HSI. High dietary lipid levels above 15% produced little practical benefit because of higher fat accretion in cobia (Wang et al., 2005). Significantly higher whole body lipid content was observed in cobia fed the diets containing high starch above 12.5% (Ren et al., 2011).

Though there was an increase of whole body lipid content with increasing dietary digestible carbohydrate or lipid, the dietary digestible lipid-to-carbohydrate ratio did not significantly influence whole body lipid of cobia. Significantly higher whole body protein content was observed in cobia fed the diets containing 0.47 and 0.34 of lipid-to-carbohydrate ratio in the present study. Thus, as compared with control, the higher whole body protein content accompanied with the better PRE in 0.47 of lipid-to-carbohydrate ratio group suggests that the moderate lipid-to-carbohydrate ratio could improve utilization of dietary protein. In the present study, serum TG significantly decreased with decreasing dietary lipid levels from 19.2% to 7.8%. The decrease of the TG due to the decrease in dietary lipid was reported for grass carp (Du et al., 2005) and Atlantic salmon (Hamre et al., 2003).

The FDPase, PFK, HK and ME of cobia were involved in the synthesis and degradation of carbohydrates and lipids. Significantly higher activities of liver FDPase and PFK were observed in cobia fed the diet containing 0.47 of lipid-to-carbohydrate ratio in this study. This is in agreement with the results reported for some species (Borrebaek and Christophersen, 2000; Enes et al., 2008). Higher activities of the glycogenolytic and gluconeogenesis enzymes may suggest that cobia have metabolic ability to adapt to moderate carbohydrate levels (about 15% to 20% carbohydrate). The activities of lipogenic enzyme ME significantly increased in cobia fed the diets containing 0.47 and 0.34 of lipid-to-carbohydrate ratio. This is in agreement with a study that the ME activity was stimulated by elevated levels of dietary carbohydrate in cobia (Wang et al., 2005). It has been confirmed in many fish species that lipogenesis is stimulated by high-carbohydrate diets and conversely suppressed by high-lipid dietary (Brauge et al., 1995; Catacutan and Coloso, 1997). The HK activity was not significantly affected by dietary treatments, confirming that this enzyme is not under nutritional regulation as already observed in European sea bass, rainbow trout, gilthead seabream, common carp and mirror carp (Panserat et al., 2000; Kirchner et al., 2005; Enes et al., 2006; Li et al., 2016, 2018).

5. Conclusion

This study provides some insight into the carbohydrate and lipid nutrition of juvenile cobia and indicates that cobia utilizes carbohydrates as energy source more efficiently than it utilizes lipids.

Table 3
Whole body composition (g/kg wet weight) and hepatosomatic index (HSI, %) of cobia fed experimental diets for 8 weeks.

| Group 1 | Moisture | Crude protein | Crude lipid | Ash | HSI |
|---------|----------|---------------|-------------|-----|-----|
| D1      | 754 ± 1.89 a | 145 ± 2.61 ab | 718 ± 0.63 | 44.3 ± 5.32 | 2.19 ± 0.54 a |
| D2      | 737 ± 3.56 ab | 151 ± 4.56 ab | 709 ± 3.89 | 39.3 ± 3.74 | 1.73 ± 0.32 ab |
| D3      | 752 ± 10.4 ab | 153 ± 3.67 ab | 667.3 ± 3.34 | 47.2 ± 7.56 | 1.97 ± 0.27 ab |
| D4      | 747 ± 4.61 ab | 156 ± 2.82 ab | 610 ± 6.12 | 48.9 ± 6.12 | 1.38 ± 0.09 b |
| D5      | 743 ± 7.09 ab | 156 ± 10.5 b | 621 ± 5.01 | 48.5 ± 1.89 | 1.65 ± 0.21 ab |
| D6      | 751 ± 13.5 ab | 154 ± 4.89 ab | 661 ± 0.44 | 469 ± 4.22 | 1.67 ± 0.28 ab |

a, b Values (mean ± standard deviations of 3 replications) in the same column with different superscripts are significantly different (P < 0.05).

Table 4
Blood GLU, TG, CHOL, HDL-C and LDL-C of cobia fed experimental diets for 8 weeks (mmol/L).

| Group 1 | GLU | TG | CHOL | HDL-C | LDL-C |
|---------|-----|----|------|-------|-------|
| D1      | 4.63 ± 1.00 | 1.39 ± 0.28 ab | 2.11 ± 0.09 | 0.32 ± 0.02 | 0.17 ± 0.02 |
| D2      | 4.00 ± 0.95 | 1.38 ± 0.15 ab | 2.75 ± 1.24 | 0.34 ± 0.09 | 0.25 ± 0.19 |
| D3      | 3.97 ± 0.76 | 1.18 ± 0.13 ab | 2.74 ± 1.68 | 0.32 ± 0.08 | 0.15 ± 0.12 |
| D4      | 4.17 ± 0.97 | 1.12 ± 0.38 ab | 1.85 ± 0.18 | 0.32 ± 0.03 | 0.12 ± 0.07 |
| D5      | 5.17 ± 1.04 | 1.12 ± 0.49 ab | 1.85 ± 0.24 | 0.34 ± 0.03 | 0.12 ± 0.03 |
| D6      | 4.60 ± 0.66 | 0.96 ± 0.09 ab | 1.91 ± 0.13 | 0.33 ± 0.02 | 0.09 ± 0.04 |

GLU – glucose; TG – triglyceride; CHOL – cholesterol; HDL-C – high density lipoprotein-cholesterol; LDL-C – low density lipoprotein cholesterol.

a, b Values (mean ± standard deviations of 3 replications) in the same column with different superscripts are significantly different (P < 0.05).
0.47, which was helpful to enhance glycolytic, gluconeogenesis and lipogenesis pathways.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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