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# Effects of dietary rapeseed meal on growth performance, digestion and protein metabolism in relation to gene expression of juvenile cobia (*Rachycentron canadum*)

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ABSTRACT

A 60-day feeding trial in seawater floating cages (1.5×1.5×2.5 m) was conducted to investigate the effects of dietary rapeseed meal (RM) levels on feed intake, growth, survival, digestion and protein metabolism in relation to gene expression of juvenile cobia (initial body weight 94.6 g). Five isonitrogenous (crude protein 450 g $kg^{-1}$ of dry matter) and isoenergetic (20 kJ $g^{-1}$) practical diets were formulated by replacing 0 (the control), 125, 250, 375 and 500 g $kg^{-1}$ fish meal protein with RM protein. Each diet was randomly fed to triplicate groups of fish, and each cage was stocked with 20 fish. Fish were fed twice daily (06:00 and 18:00) to apparent satiation. The survival ranged from 96.7 to 98.3%, and no significant difference was observed among dietary treatments ($P>0.05$). With increasing dietary RM levels, feed intake (FI), specific growth rate (SGR) and feed efficiency (FE) decreased. Fish fed the diet with 250 g $kg^{-1}$ or more protein from RM had significantly lower SGR and FE than the control group ($P<0.05$), but there was no significant difference in FI at this level compared with the control group ($P>0.05$). Apparent digestibility coefficients (ADCs) of dry matter (DM), crude protein and energy significantly decreased with increasing dietary RM levels ($P<0.05$). Fish fed the diet with 250 g $kg^{-1}$ or more protein from RM had significantly lower ADC values of crude protein and energy compared with the control group ($P<0.05$). Whole-body crude protein and crude lipid decreased with increasing dietary RM levels. Fish fed the diet with 500 g $kg^{-1}$ protein from RM had significantly lower whole-body crude protein and crude lipid compared with the control group ($P<0.05$). However, whole-body moisture and ash showed opposite trends with crude protein and crude lipid. Moisture, crude protein and crude lipid contents in cobia muscle showed similar trends with those in whole body. There were no significant differences in plasma ammonia, urea, cholesterol and amino acids among fish fed the experimental diets ($P>0.05$). Fish fed the diet with 500 g $kg^{-1}$ protein from RM had significantly lower aspartate aminotransferase (AST) activity in liver than the control group ($P<0.05$). Hepatic insulin-like growth factor I (IGF-I) gene expression level was significantly decreased in fish fed the diet with 500 g $kg^{-1}$ protein from RM compared with the control group ($P<0.05$). However, IGF-I gene expression level in dorsal muscle was significantly increased in fish fed this diet compared with the control group ($P<0.05$). No significant differences were observed in target of rapamycin (TOR) expression levels in cobia liver and dorsal muscle at different RM levels ($P>0.05$). Results of the present study indicated that protein from RM could substitute 125 g $kg^{-1}$ fish meal protein without influencing the growth, feed utilization and protein metabolism in cobia. The higher substitution levels of RM induced negative influences on feed intake, growth and hepatic IGF-I expression level.



## 1. Introduction

Rapeseed meal (RM) is one of important protein sources with protein content varying between 320 and 450 g $kg^{-1}$ of dry matter (Burel et al., 2000). Compared with some other commercially available plant proteins, RM has a relatively favorable amino acid profile (Friedman, 1996), and is also the source of minerals, vitamins and other microelements. However, as most plant protein source, RM also contains many anti-nutritional factors (ANFs) such as fiber, oligosaccharides, sinapine, tannins, phytic acid and glucosinolates (GLS) which limit its utilization. Many studies reported that with increasing dietary RM levels, fish growth performance decreased (Cheng et al., 2010; Satoh et al., 1998; Webster et al., 1997).

Protein deposition is the main determinant of live weight (biomass) gain in fish (Dumas et al., 2007). Hence, the lower growth of fish could be attributed to the lower protein deposition. Protein deposition is determined by the balance between the processes of protein synthesis and degradation which are regulated by interactions among hormonal, nutritional, and other influences through cellular signaling pathways (Liu and Barrett, 2002). Recent studies have suggested that the target of rapamycin (TOR) signaling pathway plays

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an important role in protein synthesis and degradation in mammalian animals, and TOR protein was the central in TOR signaling pathway. TOR protein is a conserved Ser/Thr kinase, and can relay a permissive nutritional signal to downstream targets which modulate the initiation and elongation phases of translation (Wullschleger et al., 2006). Some studies on rainbow trout (Lansard et al., 2009, 2010; Seiliez et al., 2008) and Jian carp (Chen et al., 2011; Wu et al., 2011) indicated that nutrition status could regulate the TOR signaling pathway in fish as in mammals. Insulin-like growth factors (IGFs), which regulate the activity of TOR protein via IRS-PI3K-Akt pathway, are important upstream regulators of the TOR signaling pathway (Wullschleger et al., 2006). Among IGFs, IGF-I, which was principally synthesized in the liver, was the major anabolic agent responsible for tissue growth (Thissen et al., 1999). Nutritional status had a profound effect on IGF-expression in fish (Duan et al., 2010), and some studies have demonstrated that food deprivation, high plant protein and lower lysine intake could decrease hepatic IGF-I expression levels of fish (Gómez-Requeni et al., 2004; Hevrøy et al., 2007, 2008; Matthews et al., 1997; Pedroso et al., 2006).

Cobia (*Rachycentron canadum*) is a carnivorous marine fish and can grow from fingerling to 4–6 kg marketable size in 1 year with high feed efficiency (Chou et al., 2001). With the success of artificial propagation and larval production, the culture of cobia has become widely distributed in the southern coastal provinces of China (Zhou et al., 2005). Some studies on replacing FM with dietary plant protein in diets of cobia have been reported (Chou et al., 2004; Lunger et al., 2006, 2007a,b; Romarheim et al., 2008; Salze et al., 2010; Zhou et al., 2005). As far as we know, currently there is no published information on the use of RM in the diets of cobia. Therefore, the present study was to evaluate RM as a partial replacement for fish meal in diets of cobia by examining feed intake, growth, survival, digestion, and protein metabolism in relation to gene expression.

## 2. Materials and methods

### 2.1. Experimental diets

Five isonitrogenous (crude protein 450 g $kg^{-1}$ of dry matter) and isoenergetic (20 kJ $g^{-1}$) experimental diets were formulated replacing 0 (the control), 125, 250, 375 and 500 g $kg^{-1}$ of protein from Peruvian fish meal (FM) with roasted rapeseed meal (RM; *Brassica napus*, China). The ingredients, proximate composition and amino acid profile of ingredients are given in Tables 1 and 2. Crystalline amino acids were supplemented to meet the essential amino acid requirements based on the whole-body amino acid composition of cobia. And monocalcium phosphate was supplemented to meet the phosphorous requirement of cobia (Zhou et al., 2004a). In addition, 1 g $kg^{-1}$ yttrium oxide ($Y_2O_3$, Fluka Chemicals®) was used as an inert tracer in each diet for determining apparent digestibility of nutrients.

Ingredients were ground into fine powder through a 246-μm mesh. All the ingredients were thoroughly mixed with oil, and water was added to produce stiff dough. The dough was then pelleted with an experimental feed mill (F-26 (II), South China University of Technology, China). Cold-pressed pellets (4.0 mm diameter) were air-dried to about 10% moisture, and were stored at −20 °C prior to use in the feeding trial.

### 2.2. Feeding trial procedures

Disease-free cobia juveniles (*R. canadum*) were obtained from Jiufu Fish Hatchery in Sanya (Hainan, China). The control diet was fed to all fish during a 1-week conditioning period. At the start of the experiment, fish were fasted for 24 h and weighed after being anesthetized with eugenol (1:10,000) (Shanghai Reagent Corporation, China). Fish of homogenous size (94.6 g) were randomly distributed into 15 seawater floating cages (1.5×1.5×2.5 m), and each cage was stocked with 20 fish. Each diet was randomly assigned to three replicate cages. Fish was hand-fed to apparent satiation twice (06:00 and 18:00) daily for 60 days. During the experimental period, rearing water temperature ranged from 29.5 to 32.0 °C, salinity was 24 to 26‰, and dissolved oxygen was approximately 7 mg $l^{-1}$.

**Table 1**
Formulation and proximate composition of the experimental diets (g $kg^{-1}$ dry matter).

| Ingredients | Diet no. (protein substitution level, g $kg^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Diet 1 (0) | Diet 2 (125) | Diet 3 (250) | Diet 4 (375) | Diet 5 (500) |
| Fish meal (Peruvian)[a] | 560 | 490 | 420 | 350 | 280 |
| Rapeseed meal[a] | 0 | 130 | 260 | 390 | 520 |
| Wheat meal[a] | 285 | 230 | 175 | 120 | 65 |
| Fish oil | 6.4 | 14.8 | 23.2 | 31.6 | 40 |
| Soybean oil | 7.2 | 5.4 | 3.6 | 1.8 | 0 |
| Soybean lecithin oil | 20 | 20 | 20 | 20 | 20 |
| α-cellulose | 64.7 | 48.6 | 32.5 | 16.4 | 0. 3 |
| Mineral premix[b] | 20 | 20 | 20 | 20 | 20 |
| Vitamin premix[b] | 20 | 20 | 20 | 20 | 20 |
| Ethoxyquin | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Sodium alginate | 10 | 10 | 10 | 10 | 10 |
| Betaine | 3 | 3 | 3 | 3 | 3 |
| DL-methionine | 0 | 0.8 | 1.6 | 2.4 | 3.2 |
| L-lysine (78%) | 2.2 | 3.4 | 4.6 | 5.8 | 7 |
| Monocalcium phosphate | 0 | 2.5 | 5 | 7.5 | 10 |
| $Y_2O_3$ | 1 | 1 | 1 | 1 | 1 |
| *Proximate composition (dry matter)*[c] | | | | | |
| Crude protein (g $kg^{-1}$) | 449 | 451 | 453 | 454 | 456 |
| Crude lipid (g $kg^{-1}$) | 95 | 94 | 95 | 95 | 96 |
| Gross energy (kJ $g^{-1}$) | 19.5 | 19.5 | 19.7 | 19.9 | 20.0 |
| Digestible phosphorus (g $kg^{-1}$) | 9.1 | 8.9 | 8.5 | 8.4 | 8.2 |
| Ash (g $kg^{-1}$) | 125 | 122 | 120 | 118 | 115 |
| Tannins (g $kg^{-1}$) | 0.2 | 1.7 | 3.1 | 4.6 | 5.8 |
| Phytic acid (g $kg^{-1}$) | 2.2 | 7.8 | 11.4 | 15.5 | 18.7 |

[a] Guangdong Yuehai Feed Group Co. Ltd., Guangdong, China. Fish meal composition (dry matter basis): crude protein, 706 g $kg^{-1}$; crude lipid, 119 g $kg^{-1}$. Rapeseed meal composition (dry matter basis): crude protein, 451 g $kg^{-1}$; crude lipid, 14 g $kg^{-1}$; crude fiber, 121 g $kg^{-1}$; tannins, 12 g $kg^{-1}$; phytic acid, 31 g $kg^{-1}$. Wheat meal composition (dry matter basis): crude protein, 152 g $kg^{-1}$; crude lipid, 10 g $kg^{-1}$.
[b] According to Ren et al. (2011).
[c] Means of three analyses.

### 2.3. Analyses and measurement

#### 2.3.1. Sample collection

Before the experiment, five fish from the same population were randomly selected for determination of initial whole-body proximate composition. At the termination of the experiment, the fish were fasted for 24 h before harvest. Total number and body weight of fish in each cage were counted and measured. Four fish per cage were

**Table 2**
Amino acid composition of the ingredients and 450 g $kg^{-1}$ protein (g $kg^{-1}$ dry weight) from cobia whole body.

| Essential amino acids | Ingredient (g $kg^{-1}$) | | | 45 g $kg^{-1}$ whole body protein (g $kg^{-1}$) |
|---|---|---|---|---|
| | Fish meal | Rapeseed meal | Wheat meal | |
| Arginine | 40.8 | 25.4 | 6.1 | 27.2 |
| Histidine | 17.4 | 10.6 | 3.0 | 11.0 |
| Isoleucine | 34.2 | 18.6 | 4.9 | 19.1 |
| Leucine | 54.4 | 30.1 | 8.9 | 34.7 |
| Lysine | 54.3 | 22.8 | 3.6 | 35.0 |
| Phenylalanine | 29.7 | 17.2 | 6.3 | 18.8 |
| Threonine | 30.4 | 19.2 | 3.7 | 21.4 |
| Valine | 37.9 | 32.6 | 5.5 | 19.9 |
| Methionine | 21.4 | 7.5 | 1.9 | 12.3 |