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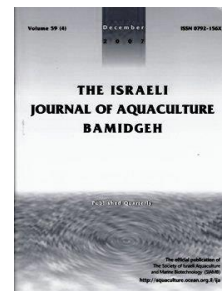
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## Effects of Dietary Corn Gluten Meal on Growth Performance and Cholesterol Metabolism in Juvenile Snakehead (*Ophiocephalus argus*) Fed Practical Diets

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### Abstract

An 8-week growth trial was conducted to investigate the effects of dietary corn gluten meal (CGM) levels on growth performance and cholesterol metabolism of juvenile snakehead (*Ophiocephalus argus*). Six isonitrogenous (crude protein 47%) and isolipidic (crude lipid 11.5%) practical diets were formulated by replacing 0 (D1, control), 6.2 (D2), 12.3 (D3), 18.5 (D4), 22.2 (D5), and 28.4% (D6) fish meal (FM) protein with CGM protein. No significant difference in survival rate was found among dietary treatments ( $P>0.05$ ). No significant differences were observed in feed intake (FI), final body weight (FBW), weight gain rate (WGR), feed efficiency rate (FER) and protein productive value (PPV) among fish fed D1-D3 ( $P>0.05$ ). However, these indices significantly decreased with increasing CGM protein from 18.5%(D4) to 28.4%(D6) of diet ( $P<0.05$ ). Whole-body lipid content of fish fed D6 (28.4 %) was significantly lower than that of fish fed D1 (control) ( $P<0.05$ ). The plasma and liver total cholesterol, free cholesterol, cholesterol esters, LDL-C, and HDL-C levels were affected by dietary CGM protein levels. Broken line analysis based on WGR or PPV indicated that the maximum CGM protein level for the optimal growth of juvenile snakehead was 12.06% or 12.56% respectively.

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## Introduction

Snakehead (*Ophiocephalus argus*) is an important commercial carnivorous fish that is widely farmed in China and south-east Asian countries because of its highly valued flesh, rapid growth, and extreme tolerance of inferior water quality. Snakehead can rely on trash fish, but diets used in snakehead farming are based on high-quality fish meal (FM) as the main protein source for which high demand and limited supply have inflated the price. Replacing FM with sustainable supply and low price plant protein ingredients has become financially important to aquaculture, (Gatlin et al., 2007; Tacon and Metian, 2008). However, when fed high plant protein diets, growth reduction was observed in many carnivorous fish species such as cobia (Chou et al., 2004), yellowtail (Tomas et al., 2005), cuneate drum (Wang et al., 2006), sharpnose seabream (Hernández et al., 2007), and turbot (Yun et al., 2011). Some detrimental effects may be linked to reduced digestion of plant proteins and associated with amino acid imbalance, poor palatability and the presence of anti-nutritional factors (ANFs) (Francis et al., 2001; Gatlin et al., 2007).

Corn gluten meal (CGM) is the residue after starch extraction from corn. It is an important plant protein source with high protein content (60-70% dry matter), low anti-nutritional factors (ANFs) and optimal essential amino acid profile, with the exception of lysine and arginine (Pereira and Oliva-Teles, 2003). Some studies on rainbow trout (Gomes et al., 1995), turbot (Regost et al., 1999), Japanese flounder (Kikuchi, 1999), red sea bream (Takagi et al., 2000), gilthead sea bream (Pereira and Oliva-Teles, 2003), Atlantic salmon (Mente et al., 2003) and Atlantic cod (Hansen et al., 2007) indicated that CGM could potentially replace FM protein. However most of these studies also demonstrated that fish growth performance was inversely related to dietary CGM levels. On the other hand, other feed combinations of soy protein concentrate, wheat gluten or corn gluten meal successfully substituted for FM as the sole source of protein in sea bream feed (Kissil and Lupatsch, 2004).

Plasma total cholesterol is significantly decreased in fish fed plant-based diets compared with those in fish fed FM-based diet. Species studied include rainbow trout (Kaushik et al., 1995), gilthead sea bream (Gomez-Requeni et al., 2004; Sitja-Bobadilla et al., 2005; Venou et al., 2006), Atlantic cod (Hansen et al., 2007), parrot (Lim and Lee, 2009) and turbot (Yun et al., 2011). The hypocholesterolemic effect in plasma might be attributed to fish fed high plant protein diet containing trace amounts of cholesterol (Yun et al., 2011).

The dietary effect of CGM on growth performance and cholesterol metabolism of juvenile snakehead fed practical diets has not been reported. The objective of the present study was, to investigate the effects of dietary corn gluten meal (CGM) levels on growth performance and cholesterol metabolism of juvenile snakehead fed practical diets.

## Materials and Methods

*Feed Ingredients and Diet Formulation.* Fish meal (FM), poultry by-product meal (PBM), soy protein concentration (SPC), and soybean meal (SBM) were used as the primary protein sources (Table 1).

Table 1. Formulation and proximate composition of the experimental diets (%)

Ingredients	Diet no.(protein substitution level)					
	D 1 0%	D 2 6.2%	D 3 12.3%	D 4 18.5%	D 5 22.2%	D 6 28.4%
Fish meal <sup>1</sup>	38.0	35.6	33.3	31.0	29.5	27.2
Poultry by-product meal <sup>2</sup>	5.0	5.0	5.0	5.0	5.0	5.0
SPC <sup>3</sup>	5.0	5.0	5.0	5.0	5.0	5.0
CGM <sup>4</sup>		2.5	5.0	7.5	9.0	11.5
Squid meal	2.0	2.0	2.0	2.0	2.0	2.0
Soybean meal	18.0	18.0	18.0	18.0	18.0	18.0
Wheat flour	23.0	23.0	23.0	23.0	23.0	23.0
Fish oil	3.0	3.1	3.2	3.3	3.4	3.5
Soybean lecithin	1.0	1.0	1.0	1.0	1.0	1.0
Ca(H <sub>2</sub> PO <sub>3</sub> ) <sub>2</sub>	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix <sup>5</sup>	0.2	0.2	0.2	0.2	0.2	0.2
Mineral premix <sup>6</sup>	1.0	1.0	1.0	1.0	1.0	1.0
Ethoxyquin (60%)	0.1	0.1	0.1	0.1	0.1	0.1
Lys-HCl		0.1	0.2	0.3	0.4	0.5
DL-Methionine		0.02	0.03	0.04	0.04	0.05
L-Threonine		0.02	0.04	0.05	0.06	0.07
Rice bran	2.20	1.86	1.43	1.01	0.80	0.38
<i>Analyzed nutrients compositions (dry matter basis)</i>						
Dry matter	93.4	93.1	93.5	93.7	93.4	93.2
Crude protein	47.8	47.4	47.9	47.4	47.6	47.4
Crude lipid	11.5	11.2	11.7	11.9	11.3	11.4
Ash	11.2	11.2	11.3	11.2	11.2	11.1

<sup>1</sup> Fishmeal: steam dried fishmeal, (COPENCA Group. Lima, Peru, dry matter basis), with crude protein: 70.1%, crude lipid: 9.6%;  
<sup>2</sup> Poultry by-product meal composition (dry matter basis): crude protein, 70.0%; crude lipid, 12.0 %;  
<sup>3</sup> SPC: Soy protein concentration (dry matter basis): crude protein, 68.0 %; crude lipid, 1.0 %;  
<sup>4</sup> CGM: Corn gluten meal (dry matter basis): crude protein, 62.0 %; crude lipid, 5.0 %;  
<sup>5</sup> Vitamin premix supplied the diet with (mg/kg diet) the following compounds: retinyl acetate, 32; vitamin D<sub>3</sub>, 5; DL- $\alpha$ -tocopherol acetate, 240; vitamin K<sub>3</sub>, 10; thiamin, 25; riboflavin (80%), 45; pyridoxine hydrochloride, 20; vitamin B<sub>12</sub> (1%), 10; L-ascorbyl-2-monophosphate-Na (35%), 2000; calcium Pantothenate, 60; nicotinic acid, 200; inositol, 800; biotin (2%), 60; folic acid, 20; choline chloride (50%), 2500; cellulose, 2473.  
<sup>6</sup> Mineral premix consisted of (mg/kg diet) of the following ingredients: FeSO<sub>4</sub>·H<sub>2</sub>O, 80; ZnSO<sub>4</sub>·H<sub>2</sub>O, 50; CuSO<sub>4</sub>·5H<sub>2</sub>O, 10; MnSO<sub>4</sub>·H<sub>2</sub>O, 45; KI, 60; CoCl<sub>2</sub>·6H<sub>2</sub>O (1%), 50; Na<sub>2</sub>SeO<sub>3</sub> (1%), 20; MgSO<sub>4</sub>·7H<sub>2</sub>O, 1200; NaCl, 1000; calcium propionate, 1000; zeolite, 1485.

Fish oil and soybean lecithin were used as lipid sources. Wheat flour was used as carbohydrate source. Lysine-HCl, DL-methionine, and L-threonine were supplemented to meet the essential amino acid (EAA) requirements of juvenile snakehead based on the whole body amino acid profile (Table 2).

Table 2. Analyzed amino acid composition of the experimental diets (% dry weight)

Essential amino acid	Diet no.(protein substitution level)						47% whole body protein
	D 1 (0%)	D 2 (6.2%)	D 3 (12.3%)	D 4 (18.5%)	D 5 (22.2%)	D 6 (28.4%)	
Thr	1.92	1.90	1.93	1.91	18.9	1.88	1.82
Val	2.09	2.08	2.07	2.06	20.5	2.03	1.82
Met	1.06	1.07	1.08	1.05	10.7	1.06	1.05
Ile	1.92	1.84	1.87	1.82	18.1	1.81	1.61
Leu	3.45	3.55	3.63	3.74	38.0	3.90	2.95
Phe	2.06	2.08	2.10	2.12	21.4	2.16	1.67
Lys	3.24	3.30	3.37	3.28	33.3	3.33	3.23
His	1.28	1.25	1.27	1.23	12.5	1.25	0.87
Arg	2.73	2.71	2.68	2.66	26.4	2.62	2.59

Six isonitrogenous (crude protein 47%) and isolipidic (crude lipid 11.5%) practical diets were formulated by replacing 0 (D1, control), 6.2(D2),12.3(D3), 18.5(D4), 22.2(D5) and 28.4 % (D6) FM protein with CGM protein. Ingredients were

ground into fine powder through a 246- $\mu$ m mesh. All the ingredients were then thoroughly mixed with fish 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), dried for about 12 h in a ventilated oven at 45°C and kept in a freezer at -20°C.

*Fish, Experimental Conditions and Sample Collection.* Juvenile snakeheads were obtained from Haid fish farm (Panyu, Guangdong, China). They were acclimated to the system and fed with the D1 (control) for 2 weeks before beginning the experiment. Juvenile snakeheads (initial body weight: 11.8±0.1 g) were randomly distributed into 24 flat-bottomed tanks (filled with 3200 l freshwater). Freshwater was continuously pumped from a pond adjacent to the experiment station, passed through sand filters into each tank at a rate of approximately 1.5 l/min. Four replicate tanks were randomly assigned to each diet group and 120 fish were bulk weighed and stocked in each tank. During the 8-week feeding period, fish were fed the experimental diets to apparent satiation twice daily at 07:00 and 17:00 respectively. Uneaten feed was collected 1h after each meal, dried to constant weight at 70°C and reweighed. Leaching loss in the uneaten diet was estimated by leaving five samples of each diet in tanks without fish for 1 h, recovered, dried and reweighed.

At the commencement of the experiment, 10 fish from the same base population were randomly selected to determine their initial whole-body proximate composition. At the end of the experiment, 6 fish of similar weight from each group were sampled and stored frozen -20°C for further whole body composition analysis. Some experimental fish fed D1, D4, and D6 were anaesthetized with eugenol (1:10000) (Shanghai Reagent Corporation, Shanghai, China) before sampling. Blood samples were taken from the caudal vein using heparinized syringes to obtain plasma samples. After centrifugation (4000 g for 10 min) at 4°C they were immediately stored at -20°C until analysis. Liver samples were stored at -80°C for subsequent determination of lipid content. During the 8-week feeding period, water temperature ranged from 26-30°C, pH 7.5-8.0, ammonia nitrogen was lower than 0.1 mg/l, nitrite was lower than 0.1 mg/l, and dissolved oxygen was higher than 6.0 mg/l.

*Chemical Analyses: Dry Matter, Crude Protein, Crude Lipid, and Ash Assays.* Dry matter, crude protein, crude lipid, and ash were analyzed for ingredients, experimental diets, and fish samples (AOAC, 1995). Dry matter was analyzed by drying samples to constant weight at 105°C. Crude protein was determined using the Kjeldahl method and estimated by multiplying nitrogen by 6.25. Crude lipid was measured after diethyl ether extraction using Soxhlet method. Ash was examined by combustion in a muffle furnace at 550°C for 16 h. Triplicate analyses were conducted for each sample.

*Cholesterol Assays* The concentration of total cholesterol (TC), free cholesterol (FC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in plasma were determined by colorimetric enzymatic methods using commercial kits. FC kit was supplied by Shanghai Mingdian bioengineering Co., Shanghai, China. The other kits were supplied by Changchun Huili bio-Tech Co., Changchun, China. After extraction of lipids from 500 mg liver with chloroform:methanol (2:1, v/v) (Folch et al., 1957), the TC and FC content in liver were determined using the same kits as for plasma. The volume of lipid solution was adjusted to 10 ml with chloroform:methanol (2:1, v/v). One milliliter of this lipid solution was sampled, dried under a pure nitrogen stream, and the residue obtained was mixed with 1ml isopropyl alcohol containing 100g Triton X-100/l (Reagent Grade). In plasma and liver, the amounts of cholesterol esters were calculated by subtracting the FC value from the TC value. Triplicate analyses were conducted for each sample.

*Calculations and Statistical Methods.* Growth parameters were calculated as follows: Weight gain rate (WGR) (%) = 100 × [(final body weight – initial body weight) / initial body weight]

Feed intake (FI) (%/d) = 100 × total amount of the feed consumed (g) / [(initial body weight + final body weight) / 2] / days

Feed efficiency rate (FER) = wet weight gain (g) / total amount of the feed consumed (g)

Protein productive value (PPV) (%) = 100 × body wet protein gain (g) / protein intake (g)

Survival rate (SR) (%) = 100 × (final fish number / initial fish number).

The Software SPSS, 11.5 was used for all statistical evaluations. All data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were regarded as significant when  $P < 0.05$ . The broken-line model with Tukey's test was used to analyze the relationship between the WGR or PPV and graded substitution levels of CGM to replace FM. Data are expressed as means  $\pm$  standard error.

### Results

No pathological signs were observed during the 8-week feeding period.

**Survival Rate and Growth Performance.** Survival rate was higher than 95% in all treatments (Table 3), and no significant difference was found among dietary treatments ( $P > 0.05$ ).

Table 3. Growth performance and survival rate of snakehead fed the experimental diets (Means  $\pm$  SE, n = 4)\*

	Diet no. (protein substitution level)					
	D 1 (0 %)	D 2 (6.2 %)	D 3 (12.3 %)	D 4 (18.5 %)	D 5 (22.2 %)	D 6 (28.4 %)
IBW <sup>1</sup>	11.8 $\pm$ 0.1	11.8 $\pm$ 0.1	11.8 $\pm$ 0.1	11.8 $\pm$ 0.1	11.8 $\pm$ 0.1	11.8 $\pm$ 0.1
FBW <sup>2</sup>	55.3 $\pm$ 0.6 <sup>a</sup>	55.0 $\pm$ 0.6 <sup>a</sup>	54.0 $\pm$ 0.8 <sup>a</sup>	42.6 $\pm$ 0.8 <sup>b</sup>	37.3 $\pm$ 0.6 <sup>c</sup>	31.2 $\pm$ 0.9 <sup>d</sup>
WGR <sup>3</sup>	368.5 $\pm$ 6.2 <sup>a</sup>	366.8 $\pm$ 6.1 <sup>a</sup>	357.6 $\pm$ 9.3 <sup>a</sup>	260.7 $\pm$ 8.8 <sup>b</sup>	216.3 $\pm$ 6.4 <sup>c</sup>	164.0 $\pm$ 10.3 <sup>d</sup>
FI <sup>4</sup>	1.15 $\pm$ 0.01 <sup>a</sup>	1.15 $\pm$ 0.01 <sup>a</sup>	1.14 $\pm$ 0.01 <sup>a</sup>	1.10 $\pm$ 0.02 <sup>ab</sup>	0.95 $\pm$ 0.01 <sup>b</sup>	0.83 $\pm$ 0.01 <sup>c</sup>
FER <sup>5</sup>	1.25 $\pm$ 0.02 <sup>b</sup>	1.20 $\pm$ 0.02 <sup>b</sup>	1.18 $\pm$ 0.03 <sup>ab</sup>	1.04 $\pm$ 0.03 <sup>a</sup>	0.96 $\pm$ 0.03 <sup>a</sup>	0.80 $\pm$ 0.04 <sup>a</sup>
PPV <sup>6</sup>	34.3 $\pm$ 0.6 <sup>a</sup>	33.9 $\pm$ 0.6 <sup>a</sup>	33.8 $\pm$ 0.8 <sup>a</sup>	29.5 $\pm$ 0.7 <sup>b</sup>	24.3 $\pm$ 0.6 <sup>c</sup>	19.2 $\pm$ 0.7 <sup>d</sup>
SR <sup>7</sup>	96.3 $\pm$ 0.8	96.2 $\pm$ 0.8	100	100	95.5 $\pm$ 1.2	95.5 $\pm$ 1.0

<sup>1</sup> IBW: initial body weight; <sup>2</sup> FBW: final body weight

<sup>3</sup> Weight gain rate (WGR) (%) =  $100 \times [(\text{FBW} - \text{IBW}) / \text{IBW}]v$

<sup>4</sup> Feed intake (FI) (%/ d) =  $100 \times \text{total amount of the feed consumed} / [(\text{IBW} + \text{FBW}) / 2] / \text{days}$

<sup>5</sup> Feed efficiency rate (FER) = wet weight gain (g) / total amount of the feed consumed (g)

<sup>6</sup> Protein productive value (PPV) (%) =  $100 \times \text{body wet protein gain (g)} / \text{protein intake (g)}$

<sup>7</sup> Survival rate (%) =  $100 \times (\text{final fish number} / \text{initial fish number})$

\*Values in the same row with different superscripts are significantly different ( $P < 0.05$ )

No significant differences were observed in FI, final body weight (FBW), WGR, FER and PPV among fish fed D1-3 ( $P > 0.05$ ). However, these indices decreased significantly with increasing CGM protein from 18.5% to 28.4% of diet ( $P < 0.05$ ). Broken-line analysis based on WGR or PPV indicated that the maximum CGM protein level for the optimal growth of juvenile snakehead was 12.06% or 12.56% (Fig. 1 and 2).

Fig. 1. The relationship between WGR (%) and dietary CGM protein replacement level in juvenile snakehead for 8 weeks.

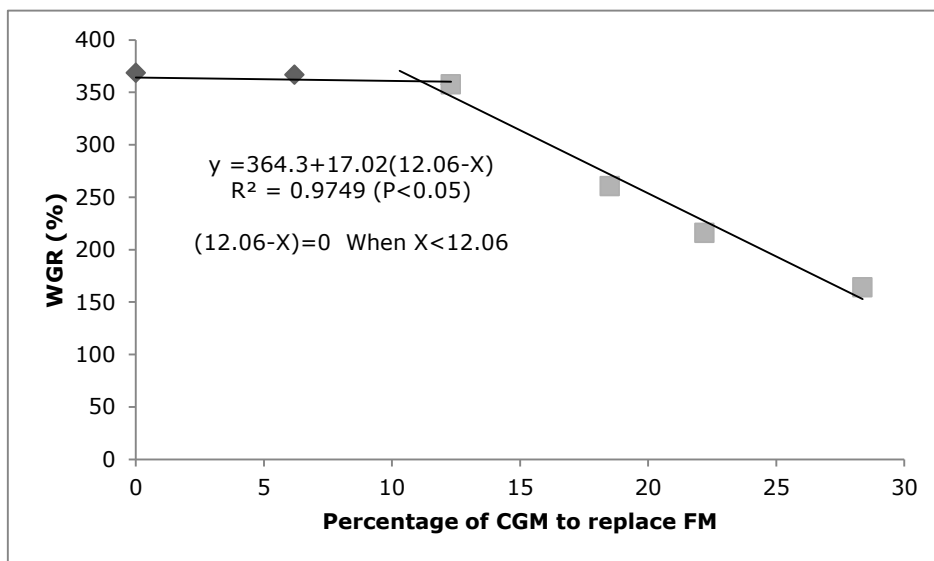
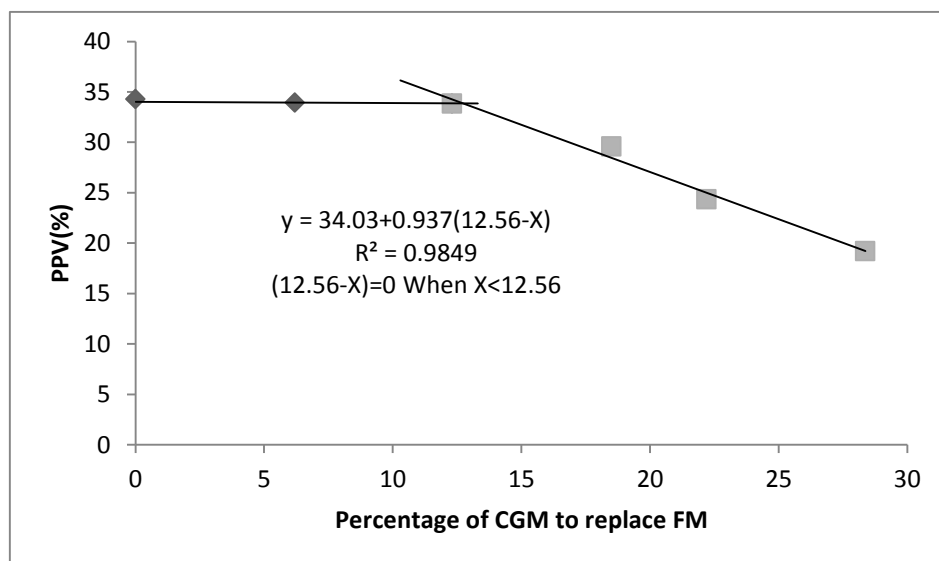


Fig.2. The relationship between PPV (%) and dietary CGM protein replacement level in juvenile snakehead for 8 weeks



**Body Composition** No significant differences were detected among dietary treatments with respect to whole-body moisture (73.8-75.4%), crude protein (14.6-15.0%), and ash (3.5-3.8%) contents of fish (Table 4,  $P > 0.05$ ).

Table 4. Proximate composition in whole body of snakehead fed the experimental diets (% diet in wet basis; means  $\pm$  SE, n = 4)\*

	Diet no. (protein substitution level)					
	D 1 (0%)	D 2 (6.2 %)	D 3 (12.3 %)	D 4 (18.5 %)	D 5 (22.2 %)	D 6 (28.4 %)
Moisture	75.0 $\pm$ 0.4	74.9 $\pm$ 0.8	73.8 $\pm$ 0.1	74.6 $\pm$ 1.1	75.4 $\pm$ 0.8	75.4 $\pm$ 0.8
Crude protein	14.7 $\pm$ 0.2	14.7 $\pm$ 0.2	15.0 $\pm$ 0.9	14.7 $\pm$ 0.6	14.6 $\pm$ 0.6	14.6 $\pm$ 0.6
Crude lipid	7.7 $\pm$ 0.5 <sup>a</sup>	7.9 $\pm$ 0.6 <sup>a</sup>	7.6 $\pm$ 0.5 <sup>a</sup>	7.0 $\pm$ 0.4 <sup>a</sup>	6.7 $\pm$ 0.2 <sup>ab</sup>	5.7 $\pm$ 0.2 <sup>b</sup>
Ash	3.8 $\pm$ 0.2	3.5 $\pm$ 0.4	3.6 $\pm$ 0.4	3.8 $\pm$ 0.2	3.8 $\pm$ 0.2	3.8 $\pm$ 0.2

\*Values in the same row with different superscripts are significantly different ( $P < 0.05$ ).

There was no significant difference in crude lipid among fish fed the D1-5 ( $P > 0.05$ ). However, whole-body lipid content of fish fed D 6 (28.4%) was significantly lower than that of fish fed control diets ( $P < 0.05$ ).

**Plasma and Liver Cholesterol.** No significant differences were detected between D1(control) and D4 (18.5%) with respect to TC, FC, cholesterol esters, HDL-C, LDL-C, and HDL-C/LDL-C (Table 5,  $P > 0.05$ ).

Table 5. Lipid profiles in plasma, and liver of snakehead fed the experimental diet (Means  $\pm$  SE, n = 4)\*

	Diet no. (protein substitution level)		
	D 1 (0 %)	D 4 (18.5 %)	D 6 (28.4 %)
Plasma (mmol/L)			
Total cholesterol	5.10 $\pm$ 0.10 <sup>b</sup>	4.92 $\pm$ 0.31 <sup>b</sup>	3.82 $\pm$ 0.31 <sup>a</sup>
Free cholesterol	2.12 $\pm$ 0.09 <sup>b</sup>	1.92 $\pm$ 0.17 <sup>ab</sup>	1.72 $\pm$ 0.17 <sup>a</sup>
Cholesterol esters	2.98 $\pm$ 0.05 <sup>b</sup>	3.00 $\pm$ 0.09 <sup>b</sup>	2.10 $\pm$ 0.09 <sup>a</sup>
HDL-C <sup>1</sup>	2.62 $\pm$ 0.11 <sup>b</sup>	2.42 $\pm$ 0.37 <sup>b</sup>	1.79 $\pm$ 0.37 <sup>a</sup>
LDL-C <sup>2</sup>	1.96 $\pm$ 0.16 <sup>ab</sup>	1.76 $\pm$ 0.20 <sup>a</sup>	1.65 $\pm$ 0.20 <sup>a</sup>
HDL-C/LDL-C	1.34 $\pm$ 0.06 <sup>b</sup>	1.37 $\pm$ 0.11 <sup>b</sup>	1.08 $\pm$ 0.10 <sup>a</sup>
Liver (g /kg wet liver)			
Total cholesterol	4.25 $\pm$ 0.23 <sup>b</sup>	4.03 $\pm$ 0.31 <sup>b</sup>	3.17 $\pm$ 0.28 <sup>a</sup>
Free cholesterol	1.55 $\pm$ 0.13	1.24 $\pm$ 0.09	1.22 $\pm$ 0.09
Cholesterol esters	2.70 $\pm$ 0.14 <sup>b</sup>	2.79 $\pm$ 0.29 <sup>b</sup>	1.95 $\pm$ 0.29 <sup>a</sup>

<sup>1</sup> HDL-C: high-density lipoprotein cholesterol; <sup>2</sup> LDL-C: low-density lipoprotein cholesterol.

\*Values in the same row with different superscripts are significantly different ( $P < 0.05$ ).

Compared with those fish fed D1 (control), fish fed D6 (28.4%) showed significantly lower plasma and liver TC, FC, cholesterol esters, HDL-C, LDL-C, and HDL-C/LDL-C levels (Table 5,  $P < 0.05$ ).

### Discussion

Limited supply, increasing demand, and high price of FM challenge the sustainable development of the fish culture industry, notably carnivorous fish that are frequently fed feeds with high FM levels (Wang et al., 2006; Glencross et al., 2007). Therefore, cost effectiveness of feed could be improved by replacing FM with more economical protein sources. Numerous studies have focused on assessing the potential to reduce FM levels in fish feed. In the present study, no significant differences in FBW, WGR and FER of snakehead were observed when FM protein was replaced by CGM protein to a level of 12.56 % (the content of CGM in diet was 5.0%). However, when substitution level increased up to 18.5%, the FBW, WGR and FER significantly decreased compared with the control group. The results indicate that snakehead has low tolerance to dietary CGM levels. In the present study, Lysine-HCl, DL-methionine, and L-threonine (crystalline amino acids) were supplemented so that the essential amino acid (EAA) requirements of juvenile snakehead were met based on the whole-body amino acid profiles. This indicated that negative growth was not attributable to the difference in the amino acid profiles. Growth was generally related to protein apparent digestibility coefficients (ADC) for some species. Results of the previous study indicated that CGM had the similar ADC of protein with fish meal for snakehead (Yu et al., 2012).

The limitations in use of alternative plant protein sources may be due to lower FI of fish (Hardy, 2010). In the present study, fish fed the diet with 18.5% or more protein from CGM had significantly lower FI than the control group. Furthermore, WGR was positively correlated with feed intake (Fig. 3,  $P < 0.05$ ,  $R^2 = 0.8868$ ). This suggests that lower FI was one of factors for decreased growth in this study, and to some extent feed palatability was affected when 18.5% or more CGM protein replaced FM protein in snakehead diets. Similar results have also been observed in rainbow trout (Gomes et al., 1995), gilthead sea bream (Pereira and Oliva-Teles, 2003), and sunshine bass (Lewis and Kohler, 2008), which showed that with increasing dietary CGM levels, FI of fish significantly decreased.

Plant protein ingredients, such as soy protein concentrate, and corn gluten meal, are generally low cost, protein sources (Kissil and Lupatsch, 2004). The hypocholesterolemic effect has been found in plasma of a wide range of fish species fed these ingredients alone or together compared with fish fed FM-based diet, such as in rainbow trout (Kaushik et al., 1995), gilthead sea bream (Gomez-Requeni et al., 2004; Sitja-Bobadilla et al., 2005; Venou et al., 2006), Atlantic cod (Hansen et al., 2007), parrot (Lim and Lee, 2009) and turbot (Yun et al., 2011, Yun et al., 2014). In this study, the hypocholesterolemic effect was observed in plasma of fish fed D6 (high plant protein diet) compared with that fish fed D1 (control). The hypocholesterolemic effect in plasma might be attributed to feeding fish a high plant protein diet containing trace amounts of cholesterol (Yun et al., 2011).

Cholesterol metabolism is related to a balance of uptake, biosynthesis, and transport (Maita et al., 2006; Yun et al., 2011). The decreased hepatic TC level found in juvenile snakehead fed the D6 (high plant protein diet) could be expected to occur as a result of decreased cholesterol synthesis and decreased HDL-C/LDL-C ratio. HMG-CoA reductase is a rate-limiting enzyme in hepatic cholesterol synthesis (Maita et al., 2006). Yellowtail fed the plant-based diets showed significantly higher relative expression of HMG-CoA reductase in liver compared with that fish fed the FM-based diet (Maita et al. 2006). This result suggests that the decreased hepatic TC level in fish fed plant-based diets may be not attributed to the reduced ability in fish to synthesize cholesterol. It is well known that LDL carries cholesterol from the liver to peripheral tissues, while HDL carries cholesterol from peripheral tissues to the liver (Chen et al., 2003). Therefore, HDL-C/LDL-C ratio could be used as a cholesterol transport marker. In the present study, fish fed D6 (28.4 %) showed a lower plasma HDL-C/LDL-C ratio compared with fish fed D1 (control), which



indicated that the ability to carry cholesterol from peripheral tissues to the liver was reduced in fish fed D6 (high plant protein diet).

In conclusion, results of the present study showed that: (1) broken line analysis based on WGR or PPV indicated that the maximum corn gluten meal protein level for optimal growth of juvenile snakehead was 12.06% or 12.56%; (2) lower FI was one of the factors for decreased growth in this study, and to some extent feed palatability was affected when 18.5% or more CGM protein replaced FM protein in snakehead diets; (3) the hypocholesterolemic effect in liver might be attributed to feeding fish a high plant protein diet which in turn decreased the ability to carry cholesterol from peripheral tissues to liver.

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