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Effects of dietary corn gluten meal on growth, digestion and protein metabolism in relation to IGF-I gene expression of Japanese seabass, *Lateolabrax japonicus*

Keke Men, Qinghui Ai *, Kangsen Mai, Wei Xu, Yanjiao Zhang, Huihui Zhou

Key Laboratory of Aquaculture Nutrition and Feed, Ministry of Agriculture, Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, PR China
Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, PR China

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ABSTRACT

A 60-day feeding trial in seawater floating cages (1.5 × 1.5 × 2.0 m) was conducted to investigate the effects of dietary corn gluten meal (CGM) levels on feed intake, growth performance, survival, digestion and protein metabolism in relation to IGF-I gene expression of Japanese seabass (initial body weight 18.09 ± 0.10 g). Six isonitrogenous (crude protein 43%) and isoenergetic (18 kJ g^{-1}) practical diets were formulated by replacing 0 (the control), 15, 30, 45, 60 and 75% of fish meal protein with CGM protein. Each diet was randomly fed to triplicate groups of fish, and each cage was stocked with 30 fish. Fish were fed twice daily (05:30 and 16:30) to apparent satiation. The survival rate ranged from 96 to 100%, and no significant difference was observed among dietary treatments ($P > 0.05$). With increasing dietary CGM levels, feed intake (FI) and specific growth rate (SGR) decreased, however, feed efficiency (FE) showed a contrary changing trend. Fish fed the diet with 75% of protein from CGM had significantly lower SGR than the control group ($P < 0.05$), and FI was significantly lower compared with the control group with a 60% substitution level ($P < 0.05$). Apparent digestibility coefficient (ADC) of protein significantly decreased in fish fed diets with 75% of protein from CGM compared with the control group ($P < 0.05$), but ADCs of lipid and phosphorus both increased with increasing dietary CGM levels, while ADC of dry matter (DM) showed no significant difference among dietary treatments. There were no significant differences in activities of digestive enzymes (protease, alpha-amylase and lipase) among dietary treatments ($P > 0.05$). When the substitution level was equal to or above 15%, the activities of protein metabolism enzymes (alanine aminotransferase, ALT; aspartate aminotransferase, AST) were significantly lower compared with the control group ($P < 0.05$). Hepatic insulin-like growth factor I (IGF-I) gene expression level significantly decreased in fish fed the diet with 60% protein from CGM compared with the control group ($P < 0.05$), but no significant difference was observed in IGF-I gene expression level in dorsal muscle. Results of the present study suggested that protein from CGM could substitute up to 60% of fish meal protein without influencing the growth of Japanese seabass. Dietary CGM level may affect fish growth by regulating digestion, absorption and FI, which partly accounted for the down-regulation of hepatic IGF-I gene expression level.

1. Introduction

Corn gluten meal (CGM) is a high protein ingredient produced as a byproduct during the corn starch processing with protein content between 60% and 62% (Mente et al., 2003). Of the plant-derived protein sources, corn gluten meal has potential to replace fish meal in that it is lack of antinutritional factors, low in fiber and, except for lysine and arginine and to a lesser extent methionine, has an adequate indispensable amino acid profile (Pereira and Oliva-Teles, 2003). Studies on rainbow trout (Gomesa et al., 1995), European seabass (Kaushik et al., 2004), tilapia (Wu et al., 1995), gilthead seabream (Pereira and Oliva-Teles, 2003; Robaina et al., 1997), Japanese flounder (Kikuchi, 1999), turbot (Regost et al., 1999), Atlantic salmon (Mente et al., 2003), Atlantic cod (Hansen et al., 2007a), sunshine bass (Lewis and Kohler, 2008) and *Fugu obscurus* (Zhong et al., 2011b) suggested that corn gluten meal was able to partially replace fish meal without compromising the growth in fish.

Nutritional status highly affects the growth hormone/insulin-like growth factor (IGF) system (Duan, 1998; Moriyama et al., 2000; Thissen et al., 1994, 1999), and insulin-like growth factor I (IGF-I) is the major anabolic agent responsible for tissue growth (Thissen et al., 1999). Of the growth factors, insulin-like growth factor-I (IGF-I), known for its direct role in increasing animal size through the

* Corresponding author at: Key Laboratory of Aquaculture Nutrition and Feed, Ministry of Agriculture, Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, PR China. Tel./fax: +86 532 82031943.
E-mail addresses: qhai@ouc.edu.cn, aiqinghui@163.com (Q. Ai).

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somatotropic axis, has also been shown to stimulate muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases (Sacheck et al., 2004). Studies on fish (Gomez-Requeni et al., 2004; Hevrøy et al., 2007, 2008; Matthews et al., 1997; Pedroso et al., 2006) suggested that food deprivation, high plant protein and low lysine intake could down-regulate hepatic IGF-I expression levels.

Japanese seabass (*Lateolabrax japonicus*) is a carnivorous species widely cultured in China because of its delicious meat and rapid growth. Protein content in diets of Japanese seabass usually accounts for more than 41%, most of which should be supplied by fish meal due to its nutritional value and palatability (Ai et al., 2004). However, as the aquaculture industry developed rapidly, the yield of fish meal is far from satisfied to maintain the growth rate of aquaculture. Studies on replacing fish meal with high-protein plant ingredients (Cheng et al., 2010; Li et al., 2012) have been conducted in diets of Japanese seabass. The objective of the present study was to evaluate CGM as a partial replacement for fish meal in diets of Japanese seabass by examining growth, survival, digestion and protein metabolism in relation to IGF-I gene expression, expecting that the results obtained might be helpful in developing cost effective and sustainable dietary formulations for Japanese seabass.

2. Materials and methods

2.1. Experimental diets

Six isonitrogenous (crude protein 43%) and isoenergetic (18 kJ g^{-1}) practical diets were formulated by replacing 0 (the control), 15, 30, 45, 60 and 75% of protein from white fish meal with corn gluten meal (CGM). 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 Japanese seabass. Monocalcium phosphate was supplemented to meet the phosphorus requirement of Japanese seabass (Zhang et al., 2006). In addition, 500 mg/kg yttrium oxide (Y_2O_3, Fluka Chemicals®) was used as an inert tracer in each diet for determining apparent digestibility of nutrients.

Ingredients were grounded into fine powder through a 246-μm mesh. All the ingredients were mixed with menhaden 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) and dried for about 12 h in a ventilated oven at 45 °C. After drying, the diets were broken and sieved into proper pellet size (1.5 × 3.0 mm and 2.5 × 5.0 mm), and were stored at −20 °C.

2.2. Feeding trial procedures

Japanese seabass (*L. japonicus*) juveniles of the same batch were obtained from a commercial farm in Ningbo, China. The juvenile seabass were fed with the control diet for 10 days to acclimate the experimental diets and conditions. At the start of the experiment, the fish were fasted for 24 h and weighted after anesthetized with eugenol (1:10,000) (Shanghai Reagent Corporation, China). Fish of homogenous size (18.09 ± 0.10 g) were randomly distributed into 18 seawater floating cages (1.5 × 1.5 × 2.0 m), and each cage was stocked with 30 fish. Each diet was randomly assigned to triplicate cages. Fish was hand-fed to apparent satiation twice daily (05:30 and 16:30) for 8 weeks. During the experimental period, rearing water temperature ranged from 18.0 to 24.5 °C, salinity was 26 to 30‰, and dissolved oxygen was approximately 7 mg/L.

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

Ingredients	Diet no. (protein substitution level)					
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	(0)	(15%)	(30%)	(45%)	(60%)	(75%)
Fish meal[1]	52.00	44.20	36.40	28.60	20.80	13.00
CGM[1]	0.00	8.50	17.00	25.30	33.60	42.10
Wheat meal[1]	31.80	30.00	28.30	26.50	24.80	22.90
Fish oil	4.60	5.10	5.60	6.10	6.60	7.10
Soybean oil	1.54	1.23	0.92	0.62	0.31	0.00
Lecithin	2.00	2.00	2.00	2.00	2.00	2.00
Mineral premix[2]	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin premix[2]	2.00	2.00	2.00	2.00	2.00	2.00
Attractants[3]	0.50	0.50	0.50	0.50	0.50	0.50
Antimold	0.10	0.10	0.10	0.10	0.10	0.10
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05
$Ca(H_2PO_4)$[2]	0.00	0.50	1.10	1.70	2.30	2.90
Cellulose	2.00	1.69	1.15	0.83	0.43	0.00
Yttrium oxide	0.05	0.05	0.05	0.05	0.05	0.05
Arginine	0.09	0.29	0.50	0.71	0.92	1.13
Isoleucine	0.17	0.21	0.26	0.30	0.35	0.40
Lysine	0.62	1.06	1.50	1.94	2.37	2.82
Methionine	0.30	0.31	0.32	0.33	0.33	0.34
Valine	0.18	0.21	0.24	0.28	0.32	0.36
Threonine	0.00	0.00	0.01	0.09	0.17	0.25
Proximate composition (% dry matter)[4]						
Crude protein (%)	43.36	43.37	43.43	43.43	43.44	43.31
Crude lipid (%)	11.78	11.77	11.77	11.77	11.76	11.75
Gross energy (%)	18.67	18.67	18.69	18.68	18.68	18.63
Digestible phosphorus (%)	0.94	0.91	0.90	0.89	0.88	0.87
Ash (%)	14.69[a]	13.29[b]	12.02[c]	11.24[d]	10.37[e]	9.72[f]

[1] Fish meal, crude protein 71% dry matter, crude lipid 6% dry matter; corn gluten meal, crude protein 62.4% dry matter, crude lipid 1.8% dry matter; wheat meal, crude protein 16% dry matter, crude lipid 1.4% dry matter.
[2] According to Ai et al. (2004).
[3] Taurine: glycine: betaine = 1:3:3.
[4] Means of three analyses.

Table 2
Essential amino acid (EAA) composition of the ingredients and 43% protein (% dry weight) from Japanese seabass whole body.

EAA	Ingredient (%)			43% whole body protein (%)
	Fish meal	Corn gluten meal	Wheat flour	
Arginine	3.91	1.9	0.58	2.58
Histidine	1.75	1.18	0.27	0.55
Isoleucine	2.68	2.85	0.44	1.78
Leucine	4.99	11.59	0.8	3.01
Lysine	5.22	0.97	0.3	3.36
Phenylalanine	1.71	4.1	0.25	1.51
Threonine	2.71	2.08	0.58	1.60
Valine	2.87	2.98	0.33	2.01
Methionine	3.25	1.42	0.56	1.22

2.3. Analysis and measurement

2.3.1. Sample collection

At the termination of the experiment, fish were fasted for 24 h before harvest. Total number and body weight of fish in each cage were counted and measured. Three fish per cage were randomly collected and stored frozen at −20 °C for whole-body proximate composition analysis. Another four fish from each cage were anesthetized with eugenol (1:10,000), and blood samples were collected from the caudal vasculature using 1 ml heparinized syringes and put still at 4 °C for 4 h. After centrifugation (4000 *g* for 5 min), plasma was collected and stored at −80 °C until analysis. Liver, intestinal tract and dorsal muscle were obtained from four fish per cage, and chyme was removed from the gut using distilled water, then stored frozen at −80 °C.

After the sample collection described above, fecal collection and treatment were conducted according to the method described by Cheng et al. (2010). Remaining fish from the growth trial were fed with the same diet assignments in order to determine the apparent digestibility coefficients (ADCs) of dry matter, protein, lipid and phosphorus. Following a one-week acclimation period, fish from each replicate were anesthetized with eugenol (1:10,000), and manually stripped of feces 6 h after feeding twice a week until sufficient dried feces were collected for analysis (about 2 g). Pooled feces from each replicate were dried for 12 h at 50 °C and stored at −20 °C.

2.3.2. Analysis of diets, feces and fish body composition

Analysis of ingredients, diets, fecal samples and fish body composition were made following the usual procedures (AOAC, 1995): samples were dried to a constant weight at 105 °C to determine the dry matter content; crude protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method (Kjeltec TM 8400, FOSS, Tecator, Sweden); crude lipid by either extraction using Soxhlet (B-801, Switzerland) method; ash by combustion at 550 °C; and energy by an adiabatic bomb calorimeter (PARR1281, USA). Essential amino acids (except for methionine) were determined according to Cheng et al. (2010), feed ingredients were freeze-dried, and then hydrolyzed with 6 N HCl at 110 °C for 24 h, and analyzed by a Biochrom 30 amino acid analyzer (Biochrom Ltd, Cambridge, Science Park, England). Methionine was determined according to the method of Mai et al. (2006) using reverse-phase high-performance liquid chromatography (HPLC, HP1100, USA). Y_2O_3 and phosphorus contents in the diets and feces were determined according to the method of Cheng et al. (2010) using inductively coupled plasma-atomic emission spectrophotometer (ICP-OES, VISTA-MPX, VARIAN, USA) after perchloric acid digestion.

2.3.3. Activities of digestive enzyme assay

Protease activity of the liver and intestine was analyzed according to Kumar et al. (2006). Tyrosine was used as the standard, and one unit of enzyme activity was defined as the amount of enzyme needed to catalyze the formation of 1 μg of tyrosine per minute. Alpha-amylase activity was determined using commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China). Lipase activity was determined according to Cheng et al. (2010) using specific analytical procedures and commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China).

2.3.4. Activities of protein metabolism enzymes

Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method described by Cheng et al. (2010) using commercially available kits (Jiancheng Bioengineering Institute, Nanjing, China).

2.3.5. Real-time quantitative PCR analysis of IGF-I mRNA expression

Total RNA was extracted from the liver and muscle of Japanese seabass using Trizol Reagent (Invitrogen, USA), and electrophoresed on a 1.2% denaturing agarose gel to test the integrity. RNA was treated with RNA-Free DNase (TransGen Biotech, China) to remove DNA contaminant. The quality of RNA was assessed using the Nano Drop® ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA). The 260/280 nm absorbance ratios of all samples ranged from 1.86 to 2.05, indicating a favorable purity of the RNA samples. Purified RNA was reversely transcribed to cDNA using TransScript RT reagent Kit (TransGen Biotech, China) following the instructions. First strand cDNA was diluted 4 times with sterilized double-distilled water. Real-time RT-PCR was conducted in a quantitative thermal cycler (Mastercycler ep realplex, Eppendorf, Germany). The amplification was performed in a total volume of 25 μl, containing 1 μl of each primer (10 mM), 1 μl of the diluted first strand cDNA product, 12.5 μl of 2 × TransStart™ Top Green qPCR SuperMix (TransGen, China) and 9.5 μl of sterilized double-distilled water. The real-time PCR program was as follows: 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s, 57 °C for 10 s, and 72 °C for 20 s. The nucleotide sequences of primers for β-actin and IGF-I were designed following the published sequences of β-actin and IGF-I (GenBank: HE577671.1 and JN596878.2) of Japanese seabass and were listed in Table 3. At the end of each PCR reaction, melting curve analysis was performed to confirm that only one PCR product was present in each of these reactions. Standard curves were made with five different dilutions (in triplicate) of the cDNA samples and amplification efficiency was analyzed

Table 3
Nucleotide sequences of primers used for qPCR amplification.

Target gene	Forward (5′–3′)	Reverse (5′–3′)	GenBank accession no.
IGF-I	TTGTGGACGAGTGCTGCTTC	TTGTCTTGTCTGGCTGCTGTG	JN596878.2
β-Actin	CAACTGGGATGACATGGAGAAG	TTGGCTTTGGGGTTCAGG	HE577671.1

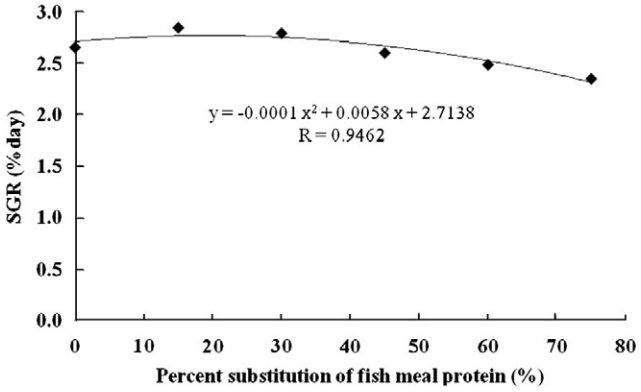

Fig. 1. Regression of dietary substitution of fish meal protein (%) and SGR of Japanese seabass.

according to the following equation $E = 10^{(-1/\text{Slope})} - 1$. The primer amplification efficiency was 1.002 for β-actin and 0.973 for IGF-I, respectively. The absolute ΔC_T value (IGF-I-β-actin) of the slope is 0.073, which is close to zero and indicates that $\Delta\Delta C_T$ calculation can be used for the relative quantification of the target gene. To calculate the expression of IGF-I, the comparative C_T method ($2^{-\Delta\Delta CT}$ method) was used. The relative expression level of IGF-I in the control group was used as calibrator (Livak and Schmittgen, 2001).

2.4. Calculations and statistics methods

The following variables were calculated:

Survival (%) $100 \times$ (final amount of fish) / (initial amount of fish)
Specific growth rate (SGR) $100 \times (\ln W_t - \ln W_0) / t$
Weight gain (WG) $(W_t - W_0) / W_0 \times 100$
Feed intake (FI) $100 \times$ dry feed intake $\times 2 / (W_0 + Wt) / t$
Feed efficiency (FE) $(W_t - W_0)$ / dry feed intake

where W_t and W_0 are final and initial body weights, respectively; and t is duration of experimental days.

All data were subjected to a one-way analysis of variance using SPSS 17.0 for Windows. Differences among the mean values were tested by the Tukey's multiple-range test. The level of significance chosen was at $P < 0.05$ and the results are presented as means ± S.E.M. (standard error of the mean).

3. Results

3.1. Survival and growth performance

Survival rate was all above 96% and there was no significant difference among dietary treatments in feeding trial ($P > 0.05$). FI of fish was significantly affected by the replacement level of FM protein with CGM protein. When 60% of FM protein was substituted by CGM protein, FI was significantly lower than that of the control group (Diet 1) ($P < 0.05$), and no significant difference was observed among other treatments. With increasing dietary CGM levels, SGR showed a second-degree polynomial regression model (Fig. 1), and fish fed the diets with 15% and 30% of protein from CGM showed the best SGR, which had no significant difference compared with the control group ($P > 0.05$), but was significantly higher than that of fish fed the diets with 60% and 75% of protein from CGM ($P < 0.05$). Besides, when the substitution level was 75%, SGR was significantly lower compared with the control group ($P < 0.05$). FE first increased and then decreased with increasing dietary CGM levels, fish fed the diets with 45% and 60% of FM protein substituted with CGM protein had significantly higher FE than the control group ($P < 0.05$), and FE of fish fed the diet with 75% of protein from CGM was significantly lower compared with that of fish fed diets with 30%, 45% and 60% of protein from CGM (Table 4).

3.2. Whole body composition

Fish whole-body composition analysis showed that with increasing dietary CGM levels, whole-body protein, ash and moisture contents showed no significant difference among dietary treatments ($P > 0.05$), but whole-body lipid contents increased. Compared with the control group, fish fed the diet with 75% of protein from CGM had significantly higher lipid content ($P < 0.05$), which was also significantly higher than that of fish fed the diet with 15% of protein from CGM (Table 5).

3.3. The ADC of dry matter and nutrients

The ADC of dry matter showed no significant difference among dietary treatments ($P > 0.05$) (Table 6). When the substitution level was 75%, the ADC value of protein was significantly lower compared with the control group ($P < 0.05$), but no significant difference was observed among other treatments. However, with increasing dietary CGM levels, the ADC values of phosphorus and lipid increased. When the substitution level was above 30%, the ADC values of phosphorus were significantly higher than the control group ($P < 0.05$), which showed the similar trend as the ADC values of lipid.

3.4. Activities of digestive enzymes and protein metabolism enzymes

There were no significant differences in activities of protease, lipase and alpha-amylase in the intestine or liver among dietary treatments ($P > 0.05$) (Table 7).

The activities of AST and ALT in the liver decreased with increasing dietary CGM levels (Table 7). When the substitution level was equal to or above 15%, activities of ALT and AST were significantly lower compared with the control group ($P < 0.05$).

Table 4
Growth performance of Japanese seabass fed diets with different levels of corn gluten meal.

Diets no. (substitution level)	Final body weight	Specific growth rate (% day^{-1})	Feed efficiency	Feed intake (%100 g^{-1} day^{-1})	Survival (%)
Diet 1 (0%)	80.38^{ab}	2.66^{ab}	0.90^{cd}	2.50^{a}	100.00
Diet 2 (15%)	89.15^{a}	2.85^{a}	0.94^{bcd}	2.52^{a}	100.00
Diet 3 (30%)	86.45^{a}	2.79^{a}	0.97^{bc}	2.40^{a}	100.00
Diet 4 (45%)	78.13^{ab}	2.61^{abc}	0.98^{b}	2.26^{ab}	98.67
Diet 5 (60%)	73.07^{b}	2.49^{bc}	1.07^{a}	2.02^{b}	96.00
Diet 6 (75%)	67.72^{b}	2.35^{c}	0.87^{d}	2.22^{ab}	98.67
ANOVA[1]					
Pooled SEM[2]	2.02	0.05	0.02	0.05	0.57
P value	0.001	0.001	<0.001	0.001	0.298

Values are means of three replicates. Means in each column with the same superscripted letters have no significant differences ($P > 0.05$).
[1] ANOVA: one-way analysis of variance.
[2] SEM: standard error of mean.

Table 5
Whole-body composition of Japanese seabass fed diets with different levels of corn gluten meal.

Diets no. (substitution level)	Crude protein (%ww[1])	Crude lipid (%ww[1])	Moisture (%)
Diet 1 (0%)	17.33	7.84[b]	70.49
Diet 2 (15%)	17.15	8.22[b]	70.30
Diet 3 (30%)	17.15	8.95[ab]	69.56
Diet 4 (45%)	17.19	9.06[ab]	69.36
Diet 5 (60%)	16.98	9.30[ab]	69.25
Diet 6 (75%)	16.63	10.17[a]	68.91
ANOVA[2]			
Pooled SEM[3]	0.05	0.23	0.19
P value	0.344	0.019	0.105

Values are means of three replicates. Means in each column with the same superscripted letters have no significant differences ($P > 0.05$).
[1] ww: wet weight.
[2] ANOVA: one-way analysis of variance.
[3] SEM: standard error of mean.

3.5. Expression of IGF-I gene in the liver and dorsal muscle

Relative mRNA expression of IGF-I in the liver and dorsal muscle of Japanese seabass fed diets with increasing dietary CGM levels was presented in Fig. 2. Hepatic IGF-I expression levels showed significant difference among dietary treatments ($P < 0.05$). No significant difference was observed between each treatment and the control group, but fish fed the diet with 30% of protein from CGM had significantly higher hepatic IGF-I mRNA expression compared with fish fed diet with 60% of protein from CGM ($P < 0.05$). IGF-I mRNA expression in dorsal muscle showed an opposite trend, however, no significant difference was observed ($P > 0.05$).

4. Discussion

Partial replacement of fish meal with alternative plant protein sources has been successfully conducted in Japanese seabass, which showed that fish performance has been generally inversely related to inclusion level of test ingredients (Cheng et al., 2010; Li et al., 2012).

The present study showed that there was a second-degree polynomial relationship between the growth of Japanese seabass and dietary CGM levels. No significant difference in SGR was noticed with up to 60% of fish meal replaced by CGM (the content of CGM in diet was up to 33.6%); a higher substitution level induced a significant lower SGR compared with the control group. This observation indicated that, with supplementation of crystalline amino acids, CGM could substitute up to 60% of fish meal in commercial diets of Japanese seabass without influencing the growth. This result was similar with that of Pereira and Oliva-Teles (2003) who indicated that CGM could replace up to 60% fish meal protein in diets for gilthead sea bream juveniles with no significant negative effects on fish performance. However, the result obtained was higher than those reported in juvenile cobia (Luo et al., 2012), puffer (Zhong et al., 2011a), sunshine bass (Lewis and Kohler, 2008), Atlantic cod (Hansen et al., 2007b), Atlantic salmon (Mente et al., 2003), rainbow trout (Morales et al., 1994) and European sea bass (Ballestrazzi et al., 1994), which showed that CGM could replace 15%–52.5% of fish meal with no significant negative effects on growth. These differences could be due to species difference, age, dietary composition, feeding strategy and so on.

Feed intake in the present study showed the minimum value with the substitution level being 60%, and was significantly lower than that of the control group. Similar results have been obtained in cobia (Luo et al., 2012), sunshine bass (Lewis and Kohler, 2008) and gilthead sea bream (Pereira and Oliva-Teles, 2003). This result may be explained by the study of Carr et al. (1996), who indicated that plant-derived protein sources lack the water-soluble biochemical compounds responsible for stimulating feeding behavior in many fish. In the present study, replacing dietary fish meal with CGM resulted in an increase in FE, which was different from that in cobia (Luo et al., 2012), but similar to the finding of gilthead sea bream (Pereira and Oliva-Teles, 2003). This result could be considered as a result of the increase of dietary digestible lipid and phosphorus (Table 6) with increasing CGM levels.

The National Research Council (1993) indicated that protein from CGM is considered to have a good digestibility, and in the present study, ADC values of protein showed no significant difference when the substitution level was no more than 60%. However, higher inclusion of CGM in replacement of fish meal led to a significant decrease in protein digestibility. Regost et al. (1999) pointed out that amino acid availability generally reflected protein digestibility. In the present study, though crystalline amino acids were supplemented to meet the essential amino acid requirements based on the whole-body amino acid composition of Japanese seabass, it seemed that Japanese seabass are incapable to effectively utilize crystalline amino acids when up to 75% of fish meal was replaced by CGM. Several studies (Cho, 1980; Hilton and Slinger, 1981; Windsor and Barlow, 1981) indicated a relation between a high ash content of ingredients with lower digestibility, and ash content of the diets (Table 1) significantly decreased with increasing CGM levels in the present study, both of which may explain

Table 6
Apparent digestibility coefficients of nutrients by Japanese seabass fed diets with different levels of corn gluten meal.

Diets no. (substitution level)	ADC[1] of dry matter (%)	ADC of crude protein (%)	ADC of crude lipid (%)	ADC of phosphorus (%)
Diet 1 (0%)	49.97	81.26[a]	78.57[c]	45.31[b]
Diet 2 (15%)	51.56	81.22[a]	85.66[bc]	48.07[b]
Diet 3 (30%)	55.64	81.35[a]	89.70[a]	68.25[a]
Diet 4 (45%)	50.55	78.57[ab]	85.82[bc]	57.72[ab]
Diet 5 (60%)	55.25	75.35[ab]	86.45[ab]	63.12[a]
Diet 6 (75%)	50.43	74.42[b]	89.00[ab]	68.28[a]
ANOVA[2]				
Pooled SEM[3]	0.72	0.63	0.63	2.44
P value	0.027	0.005	0.001	<0.001

Values are means of three replicates. Means in each column with the same superscripted letters have no significant differences ($P > 0.05$).
[1] ADC: apparent digestibility coefficients.
[2] ANOVA: one-way analysis of variance.
[3] SEM: standard error of mean.

Table 7
The activities of digestive enzymes and protein metabolism enzymes of Japanese seabass fed diets with different levels of corn gluten meal.

Enzyme (U/mg protein)	Tissue	Diets no. (protein substitution level)						ANOVA[1]	
		Diet 1 (0%)	Diet 2 (15%)	Diet 3 (30%)	Diet 4 (45%)	Diet 5 (60%)	Diet 6 (60%)	Pooled SEM[2]	*P* value
Protease	Liver	1.29	1.56	1.34	1.82	1.63	1.54	0.09	0.677
	Intestine	0.56	0.71	0.66	0.68	0.59	0.70	0.02	0.447
Alpha-amylase	Intestine	0.49	0.60	0.58	0.66	0.61	0.64	0.03	0.739
Lipase	Liver	32.87	26.70	27.41	27.35	34.35	31.33	0.98	0.064
	Intestine	22.06	24.99	23.04	23.94	21.88	24.45	0.56	0.626
ALT	Liver	81.99^{a}	68.64^{ab}	64.44^{abc}	57.32^{bcd}	48.84^{cd}	43.91^{d}	3.60	<0.001
AST	Liver	26.55^{a}	18.07^{bc}	20.62^{b}	20.17^{bc}	18.60^{bc}	14.67^{c}	0.99	0.001

Values are means of three replicates. Means in each column with the same superscripted letters have no significant differences ($P > 0.05$).
[1] ANOVA: one-way analysis of variance.
[2] SEM: standard error of mean.

the increase of lipid and phosphorus digestibility with increasing dietary CGM levels. However, in this study, ADC of lipid and phosphorus could not explain the reduced growth performance of Japanese seabass with increasing dietary CGM levels.

The activity of protein metabolism enzymes, such as AST and ALT, decreased when available amino acids are deficient (Cheng et al., 2010). In this study, hepatic activity of AST significantly declined when CGM was incorporated into the diets, and fish fed the diets with 45% or more protein from CGM had significantly lower hepatic ALT activity compared with the control group. This result indicated a decrease of protein utilization and amino acid availability with increasing dietary CGM levels according to Cheng et al. (2010), who referred that AST and ALT catabolize amino acids and transfer amino groups to alpha-keto acids (reversible catalysis), thus the activity of AST and ALT may be reduced as a result of the keto acid reduction caused by essential amino acid deficiency. Lysine and arginine are, respectively, the first and the second limiting amino acids in CGM, and in the present study, despite supplementing crystalline amino acids to meet the essential amino acid requirements based on whole-body amino acid composition of Japanese seabass, unbalanced amino acid absorption would explain, at least partially, the relatively lower growth rate of fish fed high plant protein-based diets.

In the present study, hepatic IGF-I expression decreased in fish fed the diet with high plant protein, which approximately paralleled with the result of growth performance. Fish fed the diet with 60% of protein from CGM showed significantly lower hepatic IGF-I mRNA expression level compared with fish fed the diet with 30% of protein from CGM, but no significant difference was observed in each treatment when compared with the control group. Similar results were also found in gilthead sea bream (Gomez-Requeni et al., 2004), Atlantic salmon (Hevrøy et al., 2008) and juvenile cobia (Luo et al., 2012), which all showed a down-regulation of hepatic IGF-I gene expression in fish fed diet with high plant protein. While IGF-I is the major anabolic agent responsible for tissue growth in mammals and teleost fish, alteration in IGF-I gene expression can partly account for changes in growth rate induced by feed intake (Duan, 1998). Studies on coho salmon (Duan and Plisetskaya, 1993) and grouper (Pedroso et al., 2006) also considered feed restriction a factor responsible for the down-regulation of hepatic IGF-I gene expression. In the present study, both feed intake and hepatic IGF-I expression showed the minimum value when 60% of fish meal was replaced by CGM, from which it could be concluded that feed restriction was one of the factors down-regulating hepatic IGF-I gene expression in Japanese seabass. Study on juvenile cobia (Luo et al., 2012) indicated that imbalance of essential amino acids would be a factor for decreased hepatic IGF-I gene expression, and the same situation appeared in the present study in Japanese seabass. In the present study, all diets were supplemented to contain the same level of lysine, though tissue lysine and FAA levels were not measured in the present study, it could be deducted according to the activity of AST and ALT that most of tissue amino acids were utilized at a lower rate in fish fed high plant protein sources. In mammals, AA's (leucine, glycine, tyrosine, phenylalanine, proline, methionine, tryptophan and histidine in the liver tissue and leucine in the skeletal muscle), together with insulin have been reported to regulate signaling directly and regulate cell growth through protein kinase cascades (Kadowaki and Kanazawa, 2003), and Hevrøy et al. (2007) demonstrated that lysine levels regulated the signal of the GH-IGF system in Atlantic salmon. IGF-I binding protein 1 (IGFBP-1) is found especially to compete and bind with free IGF-I, and protein levels of IGFBP-1 are involved in the regulations of free IGF-I content (Shimizu et al., 2005, 2006). Gomez-Requeni et al. (2004) showed in gilthead sea bream that IGFBP protein levels increase in groups fed diets with plant protein compared to

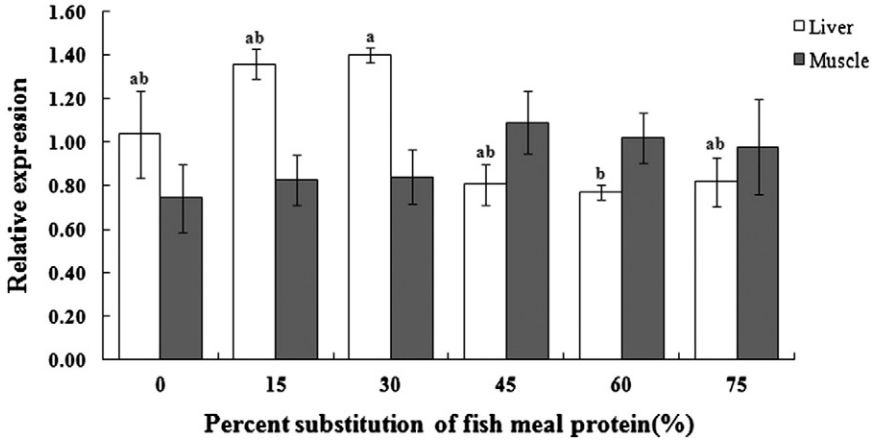

Fig. 2. Relative mRNA expression of IGF-I in the liver and dorsal muscle of Japanese seabass fed diets with graded levels of corn gluten meal for 60 days. Relative mRNA expression was evaluated by real-time quantitative PCR. Values are means ± S.E.M. (n = 3). Bars of the same gene bearing with same letters are not significantly different by Tukey's test ($P > 0.05$).

marine protein. Thus low Lys intake caused by high dietary CGM level, which may induce high IGFBP protein, may be a factor down-regulating IGF-I gene expression levels in Japanese seabass, and further studies are needed to confirm the mechanism.

In conclusion, the results of this study indicate that CGM can replace up to 60% of fish meal protein with no significant negative effects on growth performance of Japanese seabass. Suppression of feed intake and imbalanced amino acid absorption associated with lower hepatic IGF-I gene expression may have restricted the successful incorporation of higher levels of fish meal substituted by CGM in Japanese seabass.

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