

Replacement of fish meal with corn gluten meal in feeds for Asian seabass (*Lates calcarifer*)

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Abstract A 45-day feeding trial was conducted to study the effect of replacing dietary fish meal (FM) with corn gluten meal (CGM) on diet digestibility and growth and proximate body composition of Asian seabass (Lates calcarifer) fingerlings (21.1 \pm 0.95 g). Five isonitrogenous and isocaloric diets, formulated to contain 40% protein and 10% lipid, were prepared with fish meal replacement at 0 (control), 5, 10, 15, and 20% (w/w basis) using CGM. The trial was carried out in 1000-L fiber-reinforced polymer (FRP) tanks with three replicates (each containing 20 fish) for each treatment, and fish were hand fed in excess twice daily at 10.00 and 16.00 h. There was no significant difference in final body weight, average daily gain, and survival for fish fed the CGM0, CGM5, and CGM10 diets, with fish in these treatments performing better than the others. Feed conversion ratios for fish fed CGM0 (1.73 \pm 0.05 g feed /g gain), CGM5 (1.65 \pm 0.06 g feed g⁻¹ gain), and CGM10 $(1.84 \pm 0.07 \text{ g feed g}^{-1} \text{ gain})$ were better than for the other treatments. Protein retention $(28.85 \pm 0.65\%)$ and energy retention $(20.60 \pm 0.39\%)$ were better in the group fed with CGM5 than the other treatments. Apparent digestibility coefficients of dry matter (65.1 \pm 0.3, $64.7 \pm 0.2\%$), protein $(91.6 \pm 0.5, 91.7 \pm 0.3\%)$, and energy $(79.0 \pm 0.27, 8.4 \pm 0.1\%)$ were highest for the CGM5 and CGM10 diets. Highest crude lipid (7.29 ± 0.09%) and gross energy $(7.19 \pm 0.05 \text{ kJ g}^{-1})$ were observed in fish fed the CGM 20 diet. The results indicate that CGM is a potential feed ingredient for seabass and can be included at 10% of the diet without compromising digestibility and growth.

 $\textbf{Keywords} \ \ \text{Barramundi} \cdot \text{Digestibility} \cdot \text{Growth} \cdot \text{Feed formulation} \cdot \text{Plant proteins} \cdot \text{Proximate composition}$

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Introduction

Asian seabass (Lates calcarifer) is a carnivorous, euryhaline fish that is farmed in Southeast Asia and Australia. Aquaculture production of Asian seabass increased from 28,698 t in 2003 to 67,098 t in 2010 (FAO 2013). Seabass is being recognized as an alternate candidate species for commercial aquaculture in India due to its high market demand and excellent growth potential for grow-out culture in ponds and cages (Ravisankar and Thirunavukkarasu 2010). Seabass being a carnivore requires considerable level of quality fish meal in their diet. Fish meal (FM) is an important protein source in the diet of aquatic animals and is well known for its excellent nutrient constituents. However, stagnating production coupled with galloping costs and environmental concerns necessitated the researchers to explore a suitable replacement of FM with alternate protein sources (Tacon and Metian 2008; Hardy 2010; Nandakumar et al. 2013). One of the apt strategies would be the use of potential plant feedstuffs as an alternate protein source for FM to aim at sustainable production of fish feed with optimal cost. Attempts have been made with some of the plant protein sources like soybean meal, canola meal, cotton seed meal, soy protein concentrate, lupin, and pea seed meal, with reasonable success, as replacer of FM in the diet for carnivorous fish species (Gatlin et al. 2007; Tacon et al. 2011; Ayadi et al. 2012). Corn gluten meal (CGM) is a by-product of corn processing industry. Gluten is prepared by centrifugation, filtering, and drying of the slurry received from the primary and secondary stages of corn refining. It consists of insoluble protein in combination with minimal quantity of starch and fiber fractions. The word gluten here is inexact; there is no true gluten in corn, but simply corn proteins. The expression "corn gluten" is colloquial jargon and is one of the abundantly available plant protein source for utilization in agua feeds. CGM is rich in protein (60%) and low in crude fiber content. CGM has been explored as a replacer of fish meal for several fish species (Pereira and Oliva-Teles 2003; Mente et al. 2003; El-Ebiary 2005; Zhong et al. 2011; Lech and Reigh 2012; Jahanbakhshi et al. 2012; Guroy et al. 2013). However, the utility of CGM in the diet of Asian seabass has not been tested so far. Therefore, an attempt has been made to evaluate CGM as a sustainable alternate for fish meal in diets of seabass for its effect on growth, digestibility, body composition, and biological indices under controlled laboratory conditions.

Materials and methods

Diet formulation and preparation

Five isonitrogenous (40%) and isolipidic (10%) diets were used to evaluate the suitability of CGM as a likely ingredient to replace fish meal. Experimental diets were formulated by replacing fish meal with corn gluten meal at 0 (control), 5, 10, 15, and 20% on a weight by weight basis. The ingredient composition of FM and CGM is represented in Table 1 and formulation of the experimental diets and its analyzed constituents are depicted in Tables 2 and 3. Chromic oxide at 0.5% was added as an external marker in all diets for digestibility analysis. The coarse feed ingredients were powdered in a micropulverizer and sieved through a 0.3-mm sieve. The powdered ingredients were mixed along with additives as per the formulation and homogenized thoroughly in an



Table 1 Pr	roximate	composition ((%) and	d essential	amino	acid	composition	(g	16 g^{-1}	N)	of fish	meal	and corn
gluten mea	al												

	Fish meal	Corn gluten meal
Parameters		
Moisture	7.05	8.04
Crude protein	61.92	65.13
Crude lipid	9.36	6.79
Crude fiber	0.35	0.95
Total ash	20.45	1.38
Nitrogen-free extract	0.87	17.71
Essential amino acid composition		
Arginine	5.97	2.55
Histidine	3.23	1.73
Isoleucine	4.35	3.61
Leucine	7.87	13.22
Lysine	8.52	1.46
Methionine	2.93	1.99
Phenylalanine	4.25	6.05
Threonine	4.45	2.45
Valine	5.48	3.43

electrical blender. The diet mix was converted into soft dough by adding the requisite quantity of water and thereafter steam cooked (at atmospheric pressure) for 10 min, cooled, and pelletized through the laboratory scale extruder using a 2.0-mm die. The cold extruded pellet was dried in a hot air oven at 50 °C until the moisture content was reduced below 10%. The oven-dried experimental feeds were packed in airtight containers and stored in a refrigerator for use.

Fish rearing and experimental design

Hatchery-bred and reared Asian seabass fingerlings were procured from the fish hatchery at Muttukadu experimental station, Central Institute of Brackishwater Aquaculture (CIBA), Chennai, India. They were transported to the nutrition wet laboratory and were acclimatized to the laboratory conditions for 3 weeks during which they were fed CIBA Bhetkiahar (a pelleted feed developed at CIBA). The fingerlings (average body weight, 21.1 ± 0.95 g) were randomly distributed into 15 oval 1000-L fiber-reinforced plastic (FRP) tanks with three replicates in each treatment, a replicate containing 20 animals; the experiment being carried out in a completely randomized design. The tanks were supplied with sand-filtered seawater and continuous aeration through air diffuser stones. Throughout the trial, water in the tanks (about 80%) was exchanged twice a day, in the morning, and in the evening. Fishes were hand fed in excess twice daily (10.00 and 16.00 h) and after 30 min, unconsumed feed was siphoned out and dried to determine the actual feed consumption. Animals were weighed individually at the start and end of the experiment, while bulk weighing was carried out at fortnightly intervals to ascertain the increase in weight. The fish that died during the experimental period have been collected and weighed and taken for calculation of growth metrics. Fish were maintained under a natural photoperiodicity (12 h L:12 h D). Water quality parameters viz. temperature, salinity, pH, dissolved oxygen, and total ammonia nitrogen were analyzed on a weekly basis; the values ranging from 26 to 29 °C, 28-31 ppt, 7.4–8.2, 6.0–7.3 mg L^{-1} , and 0.08–0.11 mg L^{-1} were analyzed.



	Control	CGM 5%	CGM 10%	CGM 15%	CGM 20%
Ingredients					
Fish meal ^a	35	30	25	20	15
Shrimp meal	10	10	10	10	10
Corn gluten meal ^b	0	5	10	15	20
Soybean meal	21	21	21	21	21
Groundnut oil cake	8	8	8	8	8
Wheat	6	6	6	6	6
Broken rice	6	6	6	6	6
Maize	5.5	5.5	5.5	5.5	5.5
Fish oil ^a	4	4	4	4	4
Lecithin	1	1	1	1	1
Pegabind ^c	1	1	1	1	1
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Vitamin and minerald	1.8	1.8	1.8	1.8	1.8
Vitamin C ^e	0.1	0.1	0.1	0.1	0.1
Choline chloride	0.1	0.1	0.1	0.1	0.1
Proximate composition					
Moisture	9.36	9.66	9.26	9.4	9.59
Crude protein	40.9	40.91	40.68	40.95	40.86

Table 2 Formulation and proximate composition (%) of experimental diets

10.02

11.23

25.58

18.03

2.6

10.11

10.14

3.05

26.76

18.22

10.28

9.33

2.54

27.5

18.48

10.35

8.43

2.67

28.1

18.59

9.98

12.09

3.03

24.64

17.86

Digestibility trial

Crude lipid

Crude fiber

Nitrogen-free extract

Gross energy (kJ g

Total ash

Three weeks after the start of the experiment, feces collection commenced from all the tanks. For this purpose, the fish were fed in the morning. After 0.5 h, the left over feed was removed

Table 3 Essential amino acid composition of experimental diets (g 16 g⁻¹ N)

Amino acids	Control	CGM 5%	CGM 10%	CGM 15%	CGM 20%
Arginine	5.83	5.61	5.40	5.19	4.97
Histidine	2.52	2.43	2.34	2.25	2.16
Isoleucine	3.78	3.75	3.72	3.69	3.66
Leucine	6.75	7.18	7.61	8.04	8.47
Lysine	6.27	5.80	5.34	4.88	4.42
Methionine	1.98	1.93	1.88	1.83	1.78
Phenylalanine	4.08	4.23	4.38	4.54	4.69
Threonine	3.68	3.56	3.44	3.32	3.20
Valine	4.51	4.39	4.27	4.16	4.04



^a Sardine fish meal and fish oil. Bismifisheries, Mayiladuthurai, Tamil Nadu, India

^b Sukhjit Starch & Chemicals Ltd., Nizamabad, Andhra Pradesh, India

^c BentoliAgrinutrition Asia Pte Ltd., Singapore

^d 100 g of premix contains vitamin A 200,000 IU, vitamin D 40,000 IU, vitamin E 30 U, vitamin K 40 mg, riboflavin 80 mg, Capantothenate 100 mg, nicotinamide 400 mg, vitamin B12 0.24 mg, choline chloride 6 g, Ca 30 g, Mn 1.1 g, I 40 mg, Fe 300 mg, Zn 0.6 g, Cu 80 mg, Co 18 mg. Sarabhai Zydus Animal Health Ltd., Vadodara, Gujarat, India

e Rovimix Stay-C35.DSM Nutritional Products, Turkey

and the bottom of the tank was cleaned. After 2 h, the feces at the bottom was siphoned out onto a bolting cloth, washed gently with distilled water, collected, and dried at 40 °C. The feces collected over a period of 15 days were pooled by tank wise for analysis, and the digestibility of nutrients was calculated as follows:

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ADC of dry matter = (1-\%Cr_2O_3in diet/\%Cr_2O_3in feces) × 100.ADC of nutrient = [1-(\%nutrient in feces × \%Cr_2O_3in diets)/(\%protein in diets × \%Cr_2O_3 in feces)] × 100.
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Chemical analysis

The proximate composition of the ingredients, experimental diets, and whole body composition of initial and final fish samples were analyzed by standard procedures as per (AOAC 2012). At the start of the experiment, 12 fish were used for analysis of initial body composition, and at the termination of the experiment, 4 fish from each tank were collected and used. Fish were killed by over dose of anesthesia to determine the whole body composition. The fish samples were homogenized and dried at 105 °C for 24 h. The dried samples within a tank were pooled and analyzed.

Moisture content was estimated by gravimetric analysis after oven drying at 105 °C for 12 h. Crude protein (CP) was determined by Kjeldahl method (N 9 6.25) after acid hydrolysis (Kjeltec 2100, FOSS, Tecator, Sweden). Crude lipid (CL) was calculated gravimetrically after extraction with petroleum ether in a Soxhlet system SOCS, Pelican, India. Total ash was determined gravimetrically by ignition at 600 °C for 6 h in muffle furnace. Crude fiber was estimated gravimetrically after acid and alkali digestion and loss in mass by combustion at 600 °C for 3 h. Nitrogen-free extract (NFE) was calculated from 1000 – (crude protein + crude lipid + crude fiber + total ash). All the chemical analyses were carried out in triplicate, and the results were expressed in wet weight basis.

Gross energy was determined by semi micro oxygen bomb calorimeter (Parr 1425; Parr Instrument Co., USA). Chromic oxide content of diet and feces was determined using the procedure described by Furukawa and Tsukahara (1966). Amino acids (AA) of ingredients and feed were estimated after acid hydrolysis (6 N HC1; 22 h at 110 °C) following the method of Spackman et al. (1958) and Finlayson (1964). AA were analyzed by post column derivatization method using HPLC (Shimadzu, model LC-1OA; Shimadzu Corp., Japan). The AAs were detected and quantified using a fluorescent detector (FLD-6A) after post column derivatization with 0-pthaladehyde and 2-mercaptoethanol. AA standard solution (Sigma-Aldrich Inc., USA) for fluorescent detection was used as external standard.

Data calculation

On termination of the experiment, fish were anesthetized using 2-phenoxyethanol at a dose of 0.3 mL L⁻¹ and the total length and weight of each fish recorded. Three fish from each tank were randomly selected to measure the biometric indices. Liver and viscera of fish were dissected out and weighed for computation of hepatosomatic index (HSI) and viscerosomatic index (VSI) (Syed Raffic Ali et al. 2015). Growth parameters were calculated as detailed below:



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IBW (g): initial body weight.
FBW (g): final body weight.

ADG (g day<sup>-1</sup>): average daily gain = (FBW (g) – IBW (g) / 45 days.

Survival (%) = (final count of fish / initial count of fish) × 100.

FCR: feed conversion ratio = feed consumed (g, dry weight) / weight gain (g).

PR (%): protein retention = [protein gain (g) / protein intake (g)] × 100.

ER (%): energy retention = [energy gain (kJ) / energy intake (kJ)] × 100.

CF (g (cm<sup>3</sup>)<sup>-1</sup>): condition factor = [(body weight, g) / (length, cm)<sup>3</sup>] × 100.

HSI (%): hepatosomatic index = (liver weight, g / body weight, g) × 100.
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VSI (%): viscerosomatic index = (visceral weight, g / body weight, $g) \times 100$.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) to compare significant differences between treatments, whereas Duncan's multiple range tests was used to compare the means of the treatment. The data were analyzed using SPSS version 16.0 software.

Results

The result of the feeding experiment on growth performance, feed utilization, and nutrient retention of seabass fed experimental diets with varying level of CGM are presented in Table 4. There was no significant difference in final body weight in groups fed with CGM0, CGM5, CGM10, and CGM15 while CGM20 recorded the lowest final body weight of 33.59 g. The lowest ADG was recorded in groups fed with the higher inclusion of CGM (CGM15 and CGM20). The survival obtained in this experiment ranged from 77.78 to 95.67%, and significantly lower survival was recorded in groups fed with CGM15 and GCM20. The group fed with CGM5 diet showed the most favorable value for FCR (1.65) than the other CGM dietfed groups (1.73–2. 24). Similarly, the group fed with CGM5 showed significantly higher values for PR and ER. The CGM5 and CGM10 containing diets showed significantly improved dry matter, protein, and energy digestibility. Higher inclusion of CGM at 20% resulted in significantly (P < 0.05) lower apparent dry matter, protein, and energy digestibility (Table 5). The biological indices such as CF, HSI, and VSI of the post fed experimental animals showed no significant difference among different diets (Table 6).

The whole body composition of seabass fed with the experimental diets showed that as the level of CGM increases, the moisture content decreases whereas the lipid and gross energy content increases (Table 7). The crude protein and ash content showed non-significant difference among different diets.

Discussion

The results of the present study demonstrated that fish meal can be replaced with a considerable amount of CGM in the diet of seabass, without adverse effects on the growth performance, digestibility, body composition, and body indices. Several studies have been attempted to explore the nutritive value of many plant protein sources such as green pea (Ganzon-Naret



Parameters	Control	CGM5 %	CGM 10%	CGM 15%	CGM 20%
IBW (g)	21.6 ± 0.70 $38.93^{a} \pm 1.21$ $0.39^{a} \pm 13.0$ $95.6^{a} \pm 3.8$ $1.73^{ab} \pm 0.05$	21.8 ± 0.95	21.45 ± 1.46	20.71 ± 1.26	20.86 ± 1.29
FBW (g)		$38.69^{a} \pm 1.99$	$38.58^{a} \pm 2.81$	$35.78^{ab} \pm 2.16$	$33.59^{b} \pm 2.09$
ADG (g)		$0.39^{a} \pm 23.6$	$0.38^{a} \pm 30.3$	$0.34^{b} \pm 20.6$	$0.29^{c} \pm 18.9$
Survival (%)		$93.3^{a} \pm 0.0$	$95.6^{a} \pm 3.8$	$77.8^{b} \pm 7.7$	$80.0^{b} \pm 6.7$
FCR		$1.65^{a} \pm 0.06$	$1.84^{b} \pm 0.07$	$2.03^{c} \pm 0.07$	$2.24^{d} \pm 0.14$
PR (%)	$27.71^{a} \pm 0.36$	$28.85^{a} \pm 0.65$ $20.60^{a} \pm 0.39$	$26.90^a \pm 0.49$	$23.90^{b} \pm 0.45$	$21.87^{c} \pm 0.73$
ER (%)	$19.40^{b} \pm 0.20$		$19.70^b \pm 0.29$	$19.22^{b} \pm 0.30$	$19.20^{b} \pm 0.50$

Table 4 Growth performance, feed utilization, and nutrient retention of seabass fed experimental diets containing varying levels of corn gluten meal (CGM) for 45 days

All values are mean \pm SE of three observations. Means with different letters in a row differ significantly (P < 0.05)

2013), canola meal (Plaipetch and Yakupitiyage 2012), lupin concentrate, wheat gluten meal (Glencross et al. 2011), cowpea meal, mung bean meal, cassava leaf meal, and soybean meal (Boonyaratpalin et al. 1998; Eusebio and Coloso 2000; Tantikitti et al. 2005) as alternates for fish meal in seabass with varying degree of success. Based on the result of growth parameters, CGM can be included up to 10% level replacing FM in the diet. However, when CGM increased beyond 10%, growth performance and survival of fish exhibited a significant decline. Guroy et al. (2013) reported a higher value of SGR (1.11) in striped catfish (Pangasianodon hypohthalmus) fingerlings (11.2 g) fed with diet containing CGM at 20% in a 12-week feeding experiment. The results obtained in this study are similar to earlier reports in other species which indicated that when CGM replaced FM at moderate levels, growth was enhanced whereas higher levels led to decreased performance (El-Ebiary 2005; Hansen et al. 2007; Lewis and Kohler 2008; Zhong et al. 2011; Jahanbakhshi et al. 2012). It is worthwhile to remember that lysine is the first limiting amino acid in CGM and inclusion of CGM at higher level could have led to decreased lysine concentration in diets containing CGM15 and CGM20 (4.88 and 4.42 g, 16 g⁻¹ N). Here, it is pertinent to note that in addition to fish meal and CGM, other ingredients in the diet would also be contributing to the amino acid composition of the experimental diets. Similarly, the methionine concentration also decreased at CGM15 and CGM20 diets (1.83 and 1.78 g, $16 \,\mathrm{g}^{-1}$ N). Similar to the results obtained in our study reduction in growth performance was observed when CGM was included at higher levels and the reason attributed was imbalanced amino acid especially lysine for the decreased performance of CGM containing diets (Regost et al. 1999; Pereira and Oliva-Teles 2003; Yigit et al. 2013). Murillo-Gurrea et al. (2001) reported that the lysine and arginine requirement for seabass to be 4.5 and 3.8 g and 16 g⁻¹ N, respectively, for optimal performance of fish based upon the supplementation studies. It is of paramount importance that the ideal protein concept to be applied to diet

Table 5 Apparent digestibility coefficient of seabass fed experimental diets containing varying levels of CGM for 45 days

Parameters	Control	CGM 5%	CGM 10%	CGM 15%	CGM 20%
Dry matter Protein Energy	$63.4^{b} \pm 0.3$ $90.1^{b} \pm 0.5$ $77.3^{b} \pm 0.2$	$65.1^{a} \pm 0.3$ $91.6^{a} \pm 0.5$ $79.0^{a} \pm 0.2$	$64.7^{a} \pm 0.2$ $91.7^{a} \pm 0.3$ $78.4^{a} \pm 0.1$	$63.1^{b} \pm 0.4$ $90.0^{b} \pm 0.1$ $76.9^{b} \pm 0.1$	$62.1^{c} \pm 0.2$ $88.5^{c} \pm 0.4$ $76.0^{c} \pm 0.4$

All values are mean \pm SE of three observations. Means with different letters in a row differ significantly (P < 0.05)



Parameters	Control	CGM 5%	CGM 10%	CGM 15%	CGM 20%
CF HSI	$\begin{array}{c} 1.34 \pm 0.02 \\ 3.73 \pm 0.41 \end{array}$	$\begin{array}{c} 1.36 \pm 0.01 \\ 3.73 \pm 0.27 \end{array}$	$\begin{array}{c} 1.25 \pm 0.09 \\ 2.92 \pm 0.18 \end{array}$	$\begin{array}{c} 1.39 \pm 0.07 \\ 2.43 \pm 0.06 \end{array}$	$1.21 \pm 0.03 \\ 3.48 \pm 0.15$
VSI	8.35 ± 0.55	9.16 ± 0.21	7.23 ± 0.25	7.83 ± 0.69	8.69 ± 0.61

Table 6 Biological indices of seabass fed experimental diets containing varying levels of CGM for 45 days

All values are mean \pm SE of three observations

formulations is to minimize the deficiencies or excess of specific amino acid concentration in the diet and balanced amino acids supplies in the diet to be ensured (Peres and Oliva-Teles 2007). There are reports of evaluation on supplementation of amino acids to the diets of fishes like Atlantic cod (*Gadus morhua*) (Albrektsen et al. 2006), African catfish (*Clarias gariepinus*) (Fasakin et al. 2006), gilthead seabream (*Sparus aurata*) (Kissil and Lupatsch 2004), Japanese flounder (*Paralichthys olivaceus*) (Kikuchi 1999), turbot (*Psetta maxima*) (Regost et al. 1999), and rainbow trout (*Oncorhynchus mykiss*) (Davies and Morris 1997) which indicated that CGM inclusion levels could be enhanced up to 16–29% when supplemented with amino acids. Hence, there does exist a scope for increasing the inclusion level of CGM with supplementation of specific limiting amino acids.

Apparent digestibility coefficients (ADC) of dry matter, protein, and energy of the diets containing various levels of CGM were higher, and the results are comparable to those obtained in other species fed the similar feed (Pereira and Oliva-Teles 2003; Stone et al. 2005). The ADCs for the fish fed CGM5 and CGM10 were significantly higher than those in control (CGM0), while the non-significant differences in ADCs between CGM0 and CGM15 diet infers that CGM could be included up to 15% without any adverse effect on digestibility of nutrients. Fish fed with CGM20 diet showed significantly decreased ADCs for DM, protein, and energy. However, Fournier et al. (2004) reported that the digestibility coefficients of dry matter, protein, and energy increased significantly with increasing level of CGM in the turbot. The reason for the decreased digestibility in CGM20 in the present study is unclear. Decreased digestibility of nutrients at higher level of CGM inclusion could partly be attributed to the quality of the CGM. Plant sources when included at higher levels in the diets of fish may result in decreased digestibility due to imbalanced amino acid profile and presence of anti-nutritional factors like protease inhibitors, and this is dependent on quality and quantity of the plant protein as well as with the type of fish.

Table 7 Whole body composition (% wet weight basis) of seabass fed experimental diets containing varying levels of CGM for 45 days

				Final composition	on	
Parameters	Initial ^a 71.23 ± 0.04 17.77 ± 0.07 5.65 ± 0.03 5.35 ± 0.02 6.56 ± 0.02	Control	CGM 5%	CGM 10%	CGM 15%	CGM 20%
Moisture		$70.34^{a} \pm 0.05$	$70.09^{ab} \pm 0.11$	$69.70^{bc} \pm 0.22$	$69.51^{\circ} \pm 0.14$	$68.88^{d} \pm 0.06$
Crude protein		17.67 ± 0.08	17.65 ± 0.07	17.77 ± 0.10	17.65 ± 0.03	17.73 ± 0.05
Crude lipid		$5.67^{a} \pm 0.03$	$6.14^{b} \pm 0.12$	$6.39^{b} \pm 0.05$	$7.03^{\circ} \pm 0.06$	$7.29^{d} \pm 0.09$
Total ash		5.45 ± 0.03	5.54 ± 0.11	5.45 ± 0.12	5.26 ± 0.09	5.22 ± 0.05
Gross energy		$6.58^{a} \pm 0.03$	$6.69^{a} \pm 0.06$	$6.85^{b} \pm 0.03$	$7.07^{\circ} \pm 0.03$	$7.19^{c} \pm 0.05$

All values are mean \pm SE of three observations. Means with different letters in a row differ significantly (P < 0.05) ^a Not included in statistical analysis





Biological indices such as condition factor (CF), hepatosomatic index (HSI), and viscerosomatic index (VSI) of fish fed with different diets revealed that there was no significant difference between the control and experimental diets containing varying levels of CGM. This observation is congruent to the results obtained on other fish species fed with diets containing CGM or CGM combined with other plant sources (Gomez-Requeni et al. 2004; Kaushik et al. 2004; Guroy et al. 2013). Whole body content of lipid and gross energy of seabass significantly increased with increasing levels of CGM in the diet, whereas the protein and ash content showed non-significant differences among the control and test diets. Similar observation of significant increase in fat content with increasing level of fish meal replacement using plant protein sources was reported in earlier studies, and they attributed the increased lipogenesis with increasing level of fish meal replacement without any effect on nitrogen utilization (Kaushik et al. 2004; Koumi et al. 2009) in the diet of European seabass (Dicentrarchus labrax) and tilapia (Oreochromis niloticus), respectively. Increasing the inclusion level of CGM from 0 to 20% resulted in increased lipid content. This is similar to the earlier reports wherein the lipid content increased linearly with enhanced inclusion of CGM in the catfish and seabream diets (Pereira and Oliva-Teles 2003; Guroy et al. 2013).

The results from this study showed that CGM can be included up to 10% in Asian seabass diet without causing any adverse effect on growth and nutrient utilization. The CGM is a potential alternate protein source for seabass and further investigations are required to maximize the utilization of CGM and to evolve a cost-effective feed for seabass.

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