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Effects of partial replacement of fishmeal protein by protein extracted from green seaweed (*Cladophoraceae*) in mudskipper (*Pseudapocryptes elongatus*) diets

Nguyen Thi Ngoc Anh* and Tran Ngoc Hai

College of Aquaculture and Fisheries, Can Tho University, Vietnam

*Correspondence: Nguyen Thi Ngoc Anh (email: ntnanh@ctu.edu.vn)

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ABSTRACT

The study was conducted to assess the possible use of protein extracted from green seaweed (*Cladophoraceae*) in mudskipper (*Pseudapocryptes elongatus*) diets. Five isoenergetic and isonitrogenous test diets (approximately 30% crude protein and 7% crude lipid) were formulated through replacing different levels of the fishmeal (FM) protein (0, 15, 30, 45 and 60%) by the protein extracted from green seaweed, and triplicate per diet treatment was done. The 0% extracted green seaweed (EGW) was considered as control diet. Thirty fish with mean individual initial weight of 0.43 g were reared in the 100-L tank at salinity of 10 ppt, and fed *ad libitum* twice a day. After 45 days, the survival rates of fish were not significantly different among feeding treatments, varying from 91.1 to 94.4%. FM protein replaced from 15% to 45% treatments showed better or similar growth rates to that of the control diet. Although feed conversion ratio (FCR) increased, and protein efficiency ratio decreased with increasing levels of FM substitution from 30% upwards, statistical differences were not found among feeding treatments. The proximate composition of fish fillet such as the moisture, lipid, and ash contents tended to decrease with increasing level of EGW protein while the protein contents slightly increased at higher inclusion of EGW protein in the diet from 15% to 45%. The present study indicated that protein extracted from green seaweed (*Cladophoraceae*) could replace fishmeal protein up to level of 45% in formulated feed for the mud skipper (*P. elongatus*) diets.

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1 INTRODUCTION

In practice, aquaculture production is highly dependent on commercial feeds whose basic ingredient is fishmeal as main protein source for carnivorous species. However, the decrease in the availability and the increase in the price of fishmeal have encouraged the findings for alternative sustainable aquaculture feed ingredients that are locally available with equivalent nutritional value

(FAO, 2011). In this regard, seaweeds have been considered a good dietary source of proteins, carbohydrates, vitamin, trace minerals and other bioactive compounds for fish and shrimps (FAO, 2003). Especially, protein extracts from seaweeds are higher in protein than raw seaweed meals (Cruz-Suarez *et al.*, 2008; Kumar *et al.*, 2008). Numerous studies reported that seaweeds could be used as protein source in partial replacement of fishmeal in spotted scat fish, *Scatophagus argus* (Nguyen Thi

Ty Ni *et al.*, 2013), Asian seabass *Lates calcarifer* (Udayasoundari *et al.*, 2016) and shrimp diets (Cruz-Suarez *et al.*, 2008).

Previous study has found that green seaweed (Cladophoraceae) is abundant and available year-round in brackish water bodies in the Mekong Delta of Vietnam (ITB-Vietnam, 2011). This seaweed has protein contents between 10-20% with balanced amino acid profiles (Khuantrairong and Traichaiyaporn, 2011); especially protein extracted from this seaweed can be obtained as high as up to levels of 40-60% protein (ITB-Vietnam, 2011). This high protein could be a good source for aqua-feeds, particularly for brackish herbivorous species. Recently, the mudskipper, *Pseudapocryptes elongatus* is commonly cultured in the coastal areas of the Mekong Delta. It is a high valuable fish for domestic consumption as well as for export. Mudskipper has good tolerance to a wide range of salinities. It has become an important candidate for coastal aquaculture development and an alternative culture species to shrimp farming in the Mekong Delta (Minh *et al.*, 2010). Furthermore, the semi-intensive and intensive culture of mudskipper can deliver high profits, with limited risk of disease and low inputs if compared to shrimp culture (Truong Hoang Minh and Nguyen Thanh Phuong, 2011). Therefore, assessing the high concentrated protein extracted from green seaweed (EGW), a by-product from bio-fuel extraction as protein source in the *P. elongatus* feed is necessary. The aim of this study is to explicate of replacing fishmeal by different percentages of EGW in mudskipper fingerlings on their growth, feed efficiency and proximate composition.

2 MATERIALS AND METHODS

2.1 Experimental system

Feeding trial was carried out at College of Aquaculture and Fisheries, Can Tho University. The experiment was set up as a completely randomized design with 3 replicates. The 100-L plastic tanks were filled with 80 L water with salinity of 10 ppt, and provided with continuous aeration. Feeding tray

was distributed in each tank for collecting uneaten feed. Fifty percent of tank water was exchanged weekly.

2.2 Experimental fish

Wild fingerlings of mudskipper were purchased from a provider in Bac Lieu province. Fish were stocked in a 1 m³ tank for one week in order to acclimate the fish to the tank conditions and to acquaint with the feeding method (feed on a feeding tray). After acclimation, 30 uniformly sized fish with mean individual weight of 0.43 g were stocked in each tank. Fish were fed twice a day at 7:00 and 17:00. The initial feed ration was 8% of the biomass, but this was adjusted daily based on the presence or absence of residual feed to ensure fish fed *ad libitum*. About 90 minutes after feeding, unconsumed feed was removed, transferred to aluminum cups and dried to a constant weight. Culture period lasted for 45 days.

2.3 Feeds and experimental design

Kien Giang fishmeal (manufactured in Kien Giang province, Vietnam) was purchased from CATACO company in Can Tho city. Other ingredients such as soybean meal, rice bran, squid oil, gelatin, cassava meal, etc. were purchased from commercial suppliers. The concentrated protein extracted green seaweed was supplied by the Institute of Tropical Biology, Ho Chi Minh City. The dietary ingredients were analyzed for their proximate composition (Table 1) prior to the formulation of the diets.

Five test diets were formulated by replacing 0, 15, 30, 45 and 60% of the fishmeal protein in a standard diet with protein extracted green seaweed (Table 2). In the 0% EGW (control treatment), Kien Giang fishmeal was the main protein source. All diets were formulated to be approximately isonitrogenous (30% crude protein), and isolipidic (7% crude lipid). The experimental diets were formulated by the 'SOLVER' in Microsoft Excel. These diets were made into sinking pellets (1000 µm) using a pellet hand-machine, oven-dried at 60°C, and stored at 4°C before use.

Table 1: Proximate composition (% of dry matter) of feed ingredients

Ingredients	Moisture	Protein	Lipid	Ash	Fiber	NFE***
Fishmeal*	10.12	59.06	8.15	28.74	0.92	3.13
Soybean meal	10.43	44.32	2.23	8.25	1.27	43.93
Protein EGW**	9.14	44.55	4.28	15.58	0.82	34.77
Rice bran	9.86	8.52	8.15	21.32	4.36	57.65
Cassava meal	10.87	3.73	1.77	0.69	3.87	89.94

*Kien Giang fishmeal

** EGW: Protein extracted green seaweed

***NFE: Nitrogen-free extract

Table 2: Composition of feed ingredients and proximate composition in experimental feed (% dry matter)

Ingredients	Treatments				
	Control	15%EGW	30%EGW	45%EGW	60%EGW
Fishmeal	31.00	26.35	21.71	17.05	12.40
Soybean meal	20.65	20.65	20.65	20.65	20.65
Protein extracted (EGW)	0.00	6.17	12.32	18.49	24.67
Rice bran	23.70	24.43	25.20	25.96	26.68
Cassava powder	19.06	16.69	14.29	11.90	9.53
Squid oil + soybean oil*	1.59	1.71	1.83	1.95	2.07
Premix - vitamin	2.00	2.00	2.00	2.00	2.00
Gelatin	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00
Proximate analysis of experimental feeds					
Moisture	10.34	11.09	10.86	10.96	10.91
Protein	31.04	30.74	30.65	30.33	30.39
Lipid	6.96	6.48	6.82	6.84	6.78
Ash	15.47	16.6	17.73	17.81	18.28
Fiber	4.24	4.69	4.55	4.47	4.61
NFE	42.29	41.49	40.25	40.55	39.94
Gross energy (kcal/g)	4.19	4.09	4.07	4.06	4.04

Gross energy was calculated based on protein = 5.65; lipid = 9.45 and NFE = 4.20 (kcal/g)

*Ratio of squid oil/soybean oil = 1:1

2.4 Data collection

Water quality: daily water temperature and pH were recorded at 7:00 and 14:00 using a thermo-pH meter (YSI 60 Model pH meter). The concentrations of NO₂⁻ and NH₄/NH₃ (TAN) were monitored weekly using test kits (Sera, Germany).

Growth performance and feed utilization: initial, final and intermediate samples were taken to measure average individual fish weight. Sampling was conducted at a 15-day interval. Ten fish in each tank were randomly sampled and weighed in groups of 10 using an electronic balance with an accuracy of 0.01 g, and mean weights were determined.

Growth performance of experimental fish consisting of weight gain (WG), daily weight gain (DWG) and specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate were calculate using the following equations:

$$\text{WG (g)} = \text{final weight} - \text{initial weight}$$

$$\text{DWG (g/day)} = 100 \times (\text{final weight} - \text{initial weight}) / \text{days of culture}$$

$$\text{SGR (\%/day)} = 100 \times (\text{final weight} - \text{initial weight}) / \text{days of culture}$$

$$\text{FCR} = \text{feed intake (dry weight)} / \text{weight gain (wet weight)}$$

$$\text{PER} = \text{WG} / \text{protein intake}$$

$$\text{Survival rate (\%)} = 100 \times (\text{number of fish harvested} / \text{number of fish stocked})$$

Proximate composition of experimental fish: five fish was randomly taken in each tank at the termination of the experiment and only fish fillet was used for proximate analysis (moisture, crude protein, lipid and ash).

2.5 Chemical analysis

Proximate analysis (moisture, crude protein, crude lipid, fiber and ash) of the ingredients, experimental diets and fish fillet were carried out according to the standard methods of AOAC (2000). Nitrogen-free extract was estimated on a dry weight basis by subtracting the percentages of crude protein, lipids, crude fiber and ash from 100%.

2.6 Statistical analysis

The percentage data are normalized through arcsine transformation before statistical analysis. For all treatments, the results were analyzed statistically with one-way ANOVA analysis of variance to find the overall effect of the treatment (SPSS, version 16.0). Tukey test was used to identify significant differences among the mean values at a significant level of $p < 0.05$.

3 RESULTS

3.1 Water quality parameters

Water temperature fluctuated between 26.4 and 29.0°C and pH values were between 7.4 and 7.9. Variations in TAN and NO₂⁻ concentrations during the culture period were in the ranges of 0.16-0.24 and 1.04 -1.15 mg/L, respectively (Table 3).

For aquaculture pond, the temperature and pH ranges for tropical fish were 25-30°C and 6.5-8.5, and suitable level of TAN and NO₂⁻ should be less than 0.5 mg/L and 0.2-2 mg/L, respectively (Boyd,

1998). Generally, the water quality was not much different among feeding treatments and remained within the suitable range for the normal growth of mudskipper.

Table 3: Water quality parameters in the culture tanks

Treatment	Temperature		pH		TAN (mg/L)	NO ₂ ⁻ (mg/L)
	7:00	14:00	7:00	14:00		
Control	26.5±0.6	29.0±1.2	7.5±0.2	7.8±0.2	0.18±0.12	1.09±0.87
15% EGW	26.4±0.6	28.8±1.1	7.4±0.2	7.9±0.2	0.16±0.11	1.04±0.78
30% EGW	26.4±0.7	28.8±1.2	7.4±0.2	7.8±0.1	0.18±0.12	1.15±0.84
45% EGW	26.5±0.7	28.7±1.1	7.4±0.2	7.9±0.1	0.23±0.19	1.06±0.88
60% EGW	26.5±0.6	28.7±1.1	7.4±0.2	7.8±0.1	0.24±0.21	1.18±0.93

3.2 Growth performance and survival of mudskipper

Survival and growth rates of fish over 45 days of culture are given in Table 4. The survival rates of fish were not significantly different (p>0.05) among feeding treatments, ranging from 88.9% to 94.4%.

The final individual weight of fish varied from 2.28 to 3.11 g, of which fish fed 15% and 30% replacement levels of FM protein by protein extracted green

seaweed (15% EGW and 30% EGW) were larger than those fed the control diet. Moreover, fish fed diets with higher substitution of FM protein (45% EGW and 60% EGW) resulted in smaller weight. Nevertheless, the statistical results showed that significant differences (p<0.05) were only found between the control and the 60% EGW treatment.

Table 4: Survival and growth performance of *P. elongatus* after 45 days of study

Treatment	Control	15% EGW	30% EGW	45% EGW	60% EGW
Survival (%)	93.30±6.67 ^a	88.89±5.09 ^a	91.11±7.70 ^a	94.44±5.09 ^a	94.44±1.92 ^a
Initial weight (g)	0.43±0.10	0.43±0.10	0.43±0.10	0.43±0.10	0.43±0.10
Final weight (g)	2.89±0.90 ^{bc}	3.11±0.73 ^c	2.95±0.71 ^{bc}	2.68±0.53 ^b	2.28±0.41 ^a
Weight gain (g)	2.46 ± 0.9 ^{bc}	2.68 ± 0.73 ^c	2.52 ± 0.71 ^{bc}	2.25 ± 0.53 ^b	1.85 ± 0.41 ^a
DWG (g/day)	0.055±0.020 ^{bc}	0.060±0.016 ^c	0.056±0.016 ^{bc}	0.050±0.012 ^b	0.041±0.009 ^a
SGR (%/day)	4.14±0.62 ^{bc}	4.34±0.48 ^c	4.22±0.48 ^{bc}	4.03±0.41 ^b	3.67±0.37 ^a
Initial length (cm)	4.37±0.35	4.37±0.35	4.37±0.35	4.37±0.35	4.37±0.35
Final length (cm)	8.79±0.86 ^b	8.85±0.70 ^b	8.79±0.63 ^b	8.60±0.61 ^{ab}	8.36±0.50 ^a

Means with different superscripts in the same row are significantly different (p<0.05)

Growth rates of fish (WG, DWG and SGR) followed the same pattern as observed for final weight. At lower percentage of protein EGW substitution (15% and 30% EGW) in the diets, the growth of fish was improved and then tended to decrease with increasing dietary inclusion of protein EGW from 45% onwards. Although the absolute values in the 15% EGW and 30% EGW treatments were higher, and the 45% EGW treatments was lower compared to those in the control group, there were no significant differences (p>0.05) among these feeding treatments. Fish fed 60% protein substitution showed the

poorest growth performance and significant differences (p<0.05) from the control and the other groups.

The mean final length of fish varied from 8.36 to 8.85 cm. In general, growth in length of fish showed similar effects as found for weight data. Fish fed the 60% EGW diet showed significantly lower values (p<0.05) compared to other groups, except the 45% EGW treatment.

Table 5: Feed utilization of *P. elongatus* after 45 days of feeding trial

Treatment	Control	15% EGW	30% EGW	45% EGW	60% EGW
FI (mg/fish/day)	86.2±2.3 ^b	84.5±2.5 ^{ab}	91.1±2.9 ^b	85.9±2.5 ^b	77.6±3.29 ^a
FCR	1.59±0.12 ^{ab}	1.42±0.13 ^a	1.64±0.16 ^{ab}	1.72±0.07 ^{ab}	1.90±0.14 ^b
PER	2.04±0.15 ^{ab}	2.30±0.20 ^b	2.06±0.19 ^{ab}	1.92±0.09 ^{ab}	1.74±0.13 ^a

Means with different superscripts in the same row are significantly different (p<0.05)

The average feed intake (FI) was between 77.6 and 91.1 mg/fish/day with the highest and lowest values found in the 30% EGW and 60% EGW treatments, respectively. The statistical results indicated that FI in the 60% EGW treatment was significantly lower than that in the other test diets ($p < 0.05$) except the 15% EGW treatment.

The FCR and PER in fish fed the 15% EGW diet were lower than those of the control group, but statistical differences were not observed ($p > 0.05$) between these two treatments. Furthermore, from the 30% fishmeal replacement onwards, a gradual increase in FCR and decrease in PER occurred with increasing dietary inclusion of green seaweed protein. However, the 60% EGW treatment was not significantly different ($p > 0.05$) from the control group (Table 5).

Table 6: Proximate composition of fish fillet (% dry matter) over 45 days feeding trial

Treatment	Control	15% EGW	30% EGW	45% EGW	60% EGW
Moisture	80.2±1.17 ^a	80.4±1.32 ^a	80.1±1.28 ^a	79.9±0.93 ^a	79.9±1.69 ^a
Crude protein	65.3±0.16 ^b	66.6±0.27 ^c	66.3±0.24 ^c	65.9±0.30 ^{bc}	63.9±0.19 ^a
Total lipid	14.7±0.18 ^c	13.3±0.23 ^b	12.3±0.25 ^{ab}	11.8±0.18 ^a	11.6±0.15 ^a
Ash	14.7±0.14 ^c	11.4±0.29 ^a	12.6±0.40 ^b	12.2±0.23 ^{ab}	12.4±0.30 ^{ab}

Means with different superscripts in the same row are significantly different ($p < 0.05$)

Moisture contents of fish fillet were not significant differences ($p > 0.05$) among feeding treatments, ranging from 79.9% to 80.4%. The concentrations of crude protein (63.9-66.6%) increase with increasing levels of EWG in the diet up to 45%, but diet included at higher proportion of EGW (60% GW) caused the lowest protein content. The statistical analysis of protein indicated that the values in the 15% EGW and 30% EGW treatments were significantly higher than those in the control and the 60% EGW treatment. Additionally, a gradual decrease in the total lipid content of the fish fillet found with increasing dietary inclusion of EGW, and the value in the control treatment was significantly higher than that in the experimental diets. Similarly, the contents of ash in fish fillet varied between 11.4% and 14.7% of which the control treatment was considerably higher level ($p < 0.05$) if compared to all other diets (Table 6).

The statements mentioned above indicated that the growth performance and the feed utilization of mudskipper fed diets up to 45% protein fishmeal replaced by protein extracted from green seaweed (Cladophoraceae) were similar to those received the control diet without containing EGW protein.

4 DISCUSSION

The survival rates of fish in the present study is in accordance with previous researchers who evaluated the substitution of fishmeal protein with gut weed (*Enteromorpha*) protein for the spotted scat (*Scatophagus argus*) (Nguyen Thi Ty Ni *et al.*, 2013), mudskipper (*Pseudapocryptes elongates*) (Nguyen Thi Ngoc Anh *et al.*, 2012) and tilapia, (*Oreochromis niloticus*) (Siddik *et al.*, 2015). These

authors reported that gut weed protein replaced fishmeal protein up to 50% in the practical diets did not affect the survival of the experimental fish.

It was found that from 15% to 45% of substitution levels, the proportions of EGW protein in the feed formulation were from 6.17 to 18.5%. These diets gave the growth rate and the feed utilization of fish was better or comparable to the control group. At 60% replacement (EGW protein in the diets was 24.67%) (Table 2), the growth performances and the feed efficiency of fish were significantly reduced. These results are in line with the study of Valente *et al.* (2006), diet containing 10% *Ulva* sp. enhanced the growth in sea bass (*Dicentrarchus labrax*) juveniles. El-Tawil (2010) reported the reduced growth rate of red tilapia fed a diet replacing 15% fishmeal with *Ulva* sp. meal. Nguyen Thi Ngoc Anh *et al.* (2012) found that the growth rate and the feed efficiency of mudskipper fed the 10% gut weed protein substitution for fishmeal protein showed slightly higher growth, and at 40% replacement level gave comparable performance to those received the control diet. Similar observation was in the case of the giant gouramy (*Osphronemus goramy*) fingerlings; fishmeal protein could be replaced by up to 30% Cladophoraceae protein or 45% *Enteromorpha* protein in the test diet that were not significant difference from the control group. Particularly, fish received the 15% *Enteromorpha* protein diet attained better growth than those in the control group (Nguyen Thi Ngoc Anh *et al.*, 2013). Similar finding was reported by Abdel-Warith *et al.* (2016), African catfish fed a diet with *U. lactuca* included at 20% and 30% levels showed poorer growth and feed utilization than the control group and fish fed diets containing 10% of *U. lactuca*.

The present experiment was managed to successfully replace 45% of fishmeal with EGW protein for mudskipper juvenile growth and feed utilization. According to the review of Cruz-Suarez *et al.* (2008), most nutritional researchers investigated seaweed meals or seaweed extracts at low inclusion rates (less than 10%) to establish their possible usefulness as functional (binder effect), nutritional, and nutraceutical (health protective effect) supplement in shrimp and fish feeds. However, the optimum inclusion level varies depending on seaweed types and feeding habit of consumer species. Cruz-Suarez *et al.* (2008) also reported that the supplement of seaweed at appropriate proportion in feed formulation has resulted in improved pellet quality, higher feed intake, improved feed efficiency, better growth rate, and higher animal product quality (higher pigmentation, low cholesterol content).

Moreover, using balanced feed formulations based on alternative protein sources, primarily of plant origin, has resulted in an improvement in the overall nutritional quality of practical diet formulations as well as considerable reduction in formulation costs (Samocho *et al.*, 2004; Udayasoundari, *et al.*, 2016). Other studies stated that the advantages of partial inclusion of seaweed supplement can be attributed to the balance of dietary fibers, lipid, carbohydrates, minerals, and carotenoid together with basic nutritional requirements in fish diet in comparison to commercial diet (Ergun *et al.*, 2013; Udayasoundari, *et al.*, 2016).

The FCR and PER values of mudskipper in the present study varied in the ranges of 1.42-1.90 and 1.74-2.30, respectively, which are better than those in the results of Nguyen Thi Ngoc Anh *et al.* (2012), who reported FCR and PER values of 1.93-2.48 and 1.34-1.73 when feeding mudskipper with 10% to 50% *Enteromorpha* protein for 60 days. Another research of Nguyen Thi Ngoc Anh *et al.* (2013) observed FCR values were 1.86-2.34 and PER values of 1.44-1.78 in the giant gouramy on the diets of 0% to 45% *Enteromorpha* or Cladophoraceae protein replacement for fishmeal within 56 days. In case of tilapia (*O. niloticus*) fed diets with 0% to 50% *Enteromorpha* protein substitution for fish meal obtained FCR values of 1.45 to 1.66 and PER values of 2.01 to 2.29 (Siddik *et al.*, 2015), which are similar to the present study.

Substituting fishmeal protein with seaweed protein in fish feed generally influenced the body composition of fish (Shapawi and Zamry, 2016; Udayasoundari, *et al.*, 2016). In the current experiment, moisture contents of fish fillet were 79.9 to 80.4%. Similar trend of moisture contents was also obtained by Nguyen Thi Ngoc Anh *et al.* (2012); mudskipper

fed with diets containing from 10% to 50% *Enteromorpha* protein replacement levels were 79.7 to 80.2%. The lipid content of mudskipper fillet considerably decreased with increasing EGW level in the diets, similar to the findings of Nguyen Thi Ngoc Anh *et al.* (2012) for mudskipper, Nguyen Thi Ngoc Anh *et al.* (2013) for giant gouramy, and Siddik *et al.* (2015) for tilapia. On the contrary, the content of protein was highest in fish included at 15% EGW and 30% EGW in the diets. These results are in agreement with the previous study; protein content was highest in the tilapia fed at 10% and 20% *Enteromorpha* protein replacement for fishmeal in the diets (Siddik *et al.*, 2015). Furthermore, the study of Shapawi and Zamry (2016) found that the whole-body proximate composition of Asian seabass (*Lates calcarifer*) was significantly affected by the different seaweeds incorporated in the experimental diets. The diets with seaweed inclusion resulted in higher body moisture and ash, and lower crude protein and lipid than those fed with the control diet.

5 CONCLUSIONS

The protein extracted from green seaweed Cladophoraceae could replace up to 45% fishmeal protein in the practical diet for the mudskipper (*Pseudocryptes elongatus*) fingerlings.

The green seaweed protein-based feed should be further improved by the inclusion of some essential amino acids and fatty acid supplements as well attractant substances in order to meet the nutritional requirements of *P. elongatus*.

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