EFFECT OF SOYBEAN MEAL REPLACEMENT WITH GUT WEED (*Enteromorpha* sp.) AS A PROTEIN SOURCE IN PRACTICAL DIETS FOR BLACK TIGER SHRIMP (*Penaeus monodon*) POST-LARVAE

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ABSTRACT

The study was performed to evaluate the use of gut weed (*Enteromorpha* sp.) as a protein source to substitute soybean meal protein in the diets of post-larval tiger shrimp (*Penaeus monodon*). A diet without gut weed meal, considered as a control, was compared with four experimental diets in which soybean meal protein was replaced by different gut weed protein levels, namely 15, 30, 45 and 60%. All diets were formulated to be equivalent in crude protein (40%) and lipids (7%). The feeding trial was conducted in 100 L plastic tanks filled with water at a salinity of 10 ppt, and provided with continuous aeration. Thirty shrimp post-larvae with a mean initial weight of 0.033g were stocked in each tank and fed the test diets for 45 days. Results showed that survival of the shrimp was not affected by the feeding treatments, and ranged from 84.4 to 88.9%. Overall, the growth rate and feed efficiency of the shrimp fed the gut weed-based diets were comparable to or better than animals fed the control diet. Moreover, the shrimp fed diets containing gut weed meal also showed a better formalin resistance than the ones fed the control diet, although a significant difference was only observed between the 30% replacement treatment and the control group indicating the optimal replacement level for tiger shrimp PL diets.

Keywords: *Enteromorpha* sp., growth, *Penaeus monodon*, Soybean meal, stress resistance.

Ảnh hưởng của việc thay thế protein bột đậu nành bằng protein bột rong bún (*Enteromorpha* Sp.) trong thức ăn cho hậu ấu trùng tôm sú (*Penaeus monodon*)

TÓM TẮT

Nghiên cứu được thực hiện nhằm đánh giá việc sử dụng rong bún (*Enteromorpha* sp.) làm nguồn protein thay thế protein bột đậu nành trong khẩu phần ăn cho hậu ấu trùng tôm sú (*Penaeus monodon*). Thức ăn đối chứng không chứa bột rong bún, được so sánh với 4 thức ăn thí nghiệm, protein bột đậu nành được thay thế bằng protein bột rong bún với các mức khác nhau gồm 15, 30, 45 và 60%. Tất cả thức ăn thí nghiệm có cùng hàm lượng protein thô (40%) và lipid (7%). Thí nghiệm được bố trí trong bể nhựa 100 L ở độ mặn 10‰ và được sục khí liên tục. Khối lượng tôm ban đầu là 0,033g được thả 30 con cho mỗi bể và cho ăn thức ăn thí nghiệm trong 45 ngày. Kết quả cho thấy, tỉ lệ sống của tôm không bị ảnh hưởng bởi các nghiệm thức thức ăn, dao động từ 84,4 đến 88,9%. Nhìn chung, tốc độ tăng trưởng và hiệu quả sử dụng thức ăn của tôm ăn thức ăn có chứa protein rong bún tương đương hoặc tốt hơn so với tôm ăn thức ăn đối chứng.Thêm vào đó, khả năng chịu đựng sốc formol của tôm ở các nghiệm thức có rong bún tốt hơn so với nhóm đối chứng nhưng sự khác biệt có ý nghĩa chỉ được tìm thấy giữa nghiệm thức thay thế 30% protein rong bún và nghiệm thức đối chứng. Điều này được xem là mức thay thế tối ưu cho thức ăn của hậu ấu trùng tôm sú.

Từ khóa: Bột đậu nành, *Enteromorpha* sp., *Penaeus monodon*, tỉ lệ sống, tăng trưởng

1. INTRODUCTION

Aquaculture production is highly dependent on commercial feeds and aquafeeds rely on several common input ingredients such as fishmeal, soybean, corn, fish oil, rice bran and wheat powder, for which they compete in the market place with the animal husbandry sector (FAO, 2013). Currently, their availability is a major concern for their high cost and scarcity of raw materials. Moreover, in shrimp farming, the feed cost is the highest proportion and accounts for more than 50% of the total production costs (Long, 2016). In addition, most feed manufactures are using expensive imported fishmeal and soybean meal as protein sources for aquafeeds resulting in high price. Therefore, assessment of cheaper or more readily available alternative plant protein sources such as seaweed, aquatic plants, or by-products from fisheries may reduce the use of imported ingredients in feeds (Cruz-Suárez *et al.*, 2008; FAO, 2013).

Gut weed (*Enteromorpha* spp.) has a high nutritional value containing 9 - 14% protein, 2 - 3.6% lipids, 32 - 36% ash, and n-3 and n-6 fatty acids at 10.4 and 10.9 g/100 g of total fatty acid, respectively. The protein of this seaweed has a high digestibility up to 98% (Aguilera-Morales *et al.*, 2005). Recent investigations revealed that gut weed belonging to green algae and is distributed abundantly in the extensive shrimp farms and other brackish water bodies of the Mekong delta, Vietnam (ITB-Vietnam, 2011). This indicates that a large quantity of gut weed is available for aquaculture feeds. Moreover, several studies have found that gut weed *Enteromorpha* had the best nutritional and functional properties as an ingredient in shrimp feeds, which improved survival, growth, feed efficiency, and stress resistance of cultured species (Cruz-Suárez *et al.*, 2008; Anh *et al.*, 2014; Mondal *et al.*, 2015).

Black tiger shrimp (*Penaeus monodon*) has a high economic value, and is an important cultured species in the Mekong delta. However, according to the Ministry of Agriculture and Rural Development (2015), shrimp diseases have been observed since 2010, but the most widespread devastation due to early mortality syndrome (EMS) has been reported since 2011 in the Mekong Delta causing serious losses for shrimp farming (Long, 2016). The impact of EMS could be minimized by nursing shrimp post-larvae in tanks for a certain period in order to provide the formulation of well-balanced diets and adequate feeding that are of the utmost importance for successful growth out in ponds. The main objective of the study was to determine the optimal replacement level of soybean protein with gut weed protein in practical diets for the black tiger shrimp *P. monodon* post-larvae.

2. MATERIAL AND METHODS

2.1. Experimental diets

Gut weed (GW) were collected from an abandoned intensive shrimp pond, Bac Lieu province, cleaned under tap water, and shade-dried in thin layers for 3 days. The dried GW was then ground into a powder. Soybean meal and fishmeal were supplied by CATACO Company; other ingredients such as squid oil, gelatin, cassava powder, and rice bran were purchased from commercial suppliers. The dietary ingredients were analyzed for chemical composition (Table 1) prior to the formulation of the diets.

Table 1. Approximate composition (% dry matter) of ingredient

Ingredient	Moisture	Protein	Lipid	Ash	Fiber	NFE
Fish meal	11.08	58.14	9.17	21.36	0.56	10.77
Soybean meal	10.43	44.32	2.23	8.25	1.27	43.93
Gut weed powder	6.19	25.44	2.16	24.17	4.14	44.09
Rice bran	9.86	8.52	8.15	21.32	5.33	56.68
Cassava powder	10.87	5.14	1.77	0.69	2.87	89.53

Table 2. Composition of ingredients (% dry matter) and approximate analysis

Ingredients	0% GW	15% GW	30%GW	45% GW	60% GW
Fishmeal	44.50	44.50	44.50	44.50	44.50
Soybean meal	29.19	24.82	20.44	16.07	11.66
Gut weed powder	-	7.63	15.23	22.88	30.49
Rice bran	3.80	8.18	8.76	6.49	4.25
Cassava powder	16.85	9.51	5.75	4.67	4.10
Squid oil	1.16	1.32	0.82	0.89	0.50
Lecithin	0.50	0.50	0.50	0.50	0.50
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	100	100	100	100
Approximate analysis of experimental feed					
Moisture	10.16	10.68	10.82	10.45	11.02
Protein	40.68	40.06	39.98	39.82	39.88
Lipid	6.98	6.79	6.65	6.72	6.64
Ash	14.28	16.28	16.34	17.26	18.66
Fiber	2.92	3.21	3.85	4.41	5.77
NFE	35.14	33.66	33.18	31.79	29.05
Ca	2.17	2.26	2.59	2.47	2.54
P	1.32	1.26	1.15	1.08	0.92

Five experimental diets were formulated by replacing 0%, 15%, 30%, 45%, and 60% of the soybean (SB) protein in a practical diet with gut weed protein (Table 2). The 0% gut weed treatment was considered as a control. All test diets were formulated to be approximately isonitrogenous and isolipidic (40% and 7% dietary protein and lipid, respectively).

The 'SOLVER' program in Microsoft Excel was used to establish the formulated feeds. In this program, the approximate composition of the ingredients and those of the diets were preset, in which the proportion of SB protein substituted by GW protein must be precise. Based on the composition of SB meal and GW meal it was therefore possible that other ingredients (e.g. amount of cassava powder, rice bran and squid oil) varied as well in order to keep the gross composition of the resulting diets as similar as possible. The diets were made into sinking pellets, which were oven-dried at 60°C, ground, sieved to the desired particle sizes of 300, 500, 700, and 1000μm, and stored at 4°C for later use.

Approximate analysis (moisture, crude protein, total lipid, fiber, and ash) of the ingredients and experimental diets were determined according to the standard methods of AOAC (2000). Nitrogen-free extract (NFE) was estimated on a dry weight basis by subtracting the percentages of crude protein, lipids, crude fiber, and ash from 100% (Table 2).

2.2. Experimental design and management

A feeding trial was conducted for 45 days at the College of Aquaculture and Fisheries, Can Tho University. The test was set up as a completely randomized design with 3 replicates per treatment. The plastic 100-L tanks were filled with 80 L of seawater at a salinity of 10 ppt, and each tank was provided with continuous aeration. *Penaeus monodon* post-larvae (PL) from one single batch were purchased from a commercial hatchery in Can

Tho city and reared in $1m^3$ tank for 3 days before the start of the trial. During this period, shrimp PLs were fed a mixture of experimental feed. 30 PLs (mean initial weight of 0.033 ± 0.005 g) were transferred into each tank. Shrimp post-larvae were fed to satiation four times a day at about 6:00, 11:00, 16:00, and 21:00 hours at about 20-30% of their body weight. The amount of feed given was adjusted according to daily observation. The faecal matter was siphoned out every morning before the first feeding and 50% of the tank volume was exchanged every 7 days.

2.3. Data collection

Daily water temperature and pH were recorded at 7:00 and 14:00 h using a thermo-pH meter (YSI 60 Model pH meter, HANNA instruments, Mauritius). The concentration of NH_4/NH_3 (TAN), NO_2, and alkalinity were monitored weekly using test kits (Sera, Germany). The water samples in the culture tanks were collected prior to water exchange.

At the beginning of the experiment, 30 post-larvae were randomly taken from the conditioning tank to be measured for individual weight and total length. Shrimp sampling was conducted every fifteen days during which 10 shrimp were randomly taken from each tank, weighed as a group using an electronic balance, and mean weights were determined. Shrimp were then returned to their original tanks. The amount of feed given was adjusted according to these weight measurements. At the end of the feeding trial, the survival, mean individual weight, and total length of the shrimp were determined. The feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using the following equations:

FCR = Feed intake (dry weight)/Weight gain (wet weight)

PER = Weight gain/Protein intake

The quality of the experimental shrimp was assessed by studying the resistance of the shrimp to formalin shock following the methods of Samocha *et al.* (1998) and modified by Thanh *et al.* (2002). Ten shrimp from each tank were exposed to a 150-ppm formalin solution in a 10 L-glass bottle for 60 minutes. The same temperature and salinity as in the culture medium was maintained with continuous aeration. Dead shrimp were monitored at 10 minute intervals.

The Cumulative Mortality Index (CMI) was calculated by summing the mortality counts noted at each time interval. CMI= $N_{x1} + N_{x2} + N_{x3} + ... N_{x6}$

Where N is the number of dead individuals at times $x_1, x_2, x_3...x_6$. The higher the numeric value of the index, the less resistant the post-larvae are to stress and vice versa.

2.4. Statistical analysis

Data were analyzed using a one-way ANOVA (SPSS, version 16.0) analysis of variance to find the overall effect of the treatment. Duncan's Multiple Range test was used to identify significant differences among the dietary treatment means at a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Water quality parameters

Table 3 shows that daily mean temperature, pH, and alkalinity fluctuated between 26.2 - 27.8°C, 7.8 - 8.0 and 91- 94 mg $CaCO_3$/L, respectively. Generally, temperature, pH, and alkalinity in each experiment were not much diffent among feeding treatments. The average concentrations of TAN and NO_2^- were not affected by the feeding treatments, varying in the ranges of 0.56 - 0.58 and 0.52 - 0.55 mg/L, respectively (Table 3).

According to Pushparajan and Soundarapandian (2010), maintenance of good water quality in the culture pond is essential for the optimum growth and survival of the black tiger shrimp, *P. monodon*. The levels of physical, chemical, and biological parameters control the quality of pond waters. These authors suggested that the optimum range of temperatures was between 27°C to 30°C, and pH should be maintained from 7.5 to 8.8.

Alkalinity is the buffering capacity of the pond water, and the higher the alkalinity, the better the stabilization of the culture pond is. For successful culture of *P. monodon*, alkalinity is recommended to be in the range of 90 - 126 mg $CaCO_3$/L (Mohanty *et al.*, 2014).

Whetstone (2002) reported that the toxicity of ammonia and nitrite for shrimp is greatly dependent on environmental factors such as pH, dissolved oxygen, salinity, and temperature. For aquaculture purposes, these factors play an important role in the development, growth, and survival of species exposed to ammonia and nitrite. Chen and Lei (1990) reported that the acceptable concentration for juvenile *P. monodon* (0.27 g) was 3.7 mg/L total ammonia-N and 3.8 mg/L nitrite-N in water of 20 ppt.

From the cited published papers above, water quality parameters in the present study were within acceptable ranges for *P. monodon* growth. Therefore, feeding treatments could be the main factor affecting the performances of experimental shrimp.

3.2. Survival, growth rates, and feed efficiency of *P. monodon* post-larvae fed different test diets for 45 days

The effects of replacing soybean meal protein with gut weed *Enteromorpha* protein in the experimental diets on survival, growth performances, and feed efficiency of *P. monodon* are presented in Table 4.

The results showed that the survival of shrimp feeding on the different test diets for 45 days varied from 84.4% to 88.9%. There were no significant differences (P > 0.05) among feeding treatments. This indicated that using gut weed to replace soybean meal protein in the tiger shrimp diet did not affect their survival.

With regards to growth rate, the mean initial weight of shrimp post-larvae was 0.033 ± 0.005 g. After 45 days of the feeding trial, final weight and the specific growth rate (SGR) of experimental shrimp ranged from 0.94 to 0.99 g and 7.34 - 7.53 %/day, respectively, of which the 15% GW and 30% GW treatments had significantly higher values (p <0.05) than those of the control and other substitution treatments. However, for the daily weight gain (DWG) of shrimp, only the 60% GW treatment (0.019 g/day) showed significantly poorer growth (p <0.05) than the remaining feeding treatments (0.020 - 0.021 g/day).

The average feed intake of shrimp was not statistical different among feeding treatments (P > 0.05), ranging from 26.36 to 26.99 mg/shrimp/day. The feed conversion ratio (FCR) was between 1.22 and 1.36, and tended to increase with increasing levels of gut weed protein in the test diets. Statistical results indicated that the 15% GW and 30% GW treatments had significantly lower values (P < 0.05) compared to the control and other feeding treatments.

The protein efficiency ratio (PER) in all feeding treatments was in the range of 1.85-2.04, and showed the opposite trend compared with FCR in which the 15% GW and 30% GW treatments were significantly higher than those in the control and other treatments. Nonetheless, there were not significant differences (P > 0.05) among the control and the 45% GW and the 60% GW treatments.

Table 3. Water quality in the culture tanks during feeding trial

Treatment	Temperature (°C)		pH		Alkalinity ($mgCaCO_3$/L)	TAN (mg/L)	NO_2^- (mg/L)
	7:00 h	14:00 h	7:00 h	14:00 h			
Control	26.8 ± 0.7	29.4 ± 0.9	7.8 ± 0.4	8.2 ± 0.4	91 ± 12	0.56 ± 0.29	0.53 ± 0.47
15% GW	26.9 ± 0.4	29.5 ± 0.8	7.8 ± 0.4	8.1 ± 0.5	93 ± 11	0.58 ± 0.26	0.52 ± 0.43
30% GW	26.7 ± 0.6	29.8 ± 1.0	7.8 ± 0.3	8.3 ± 0.3	94 ± 10	0.60 ± 0.27	0.53 ± 0.44
45% GW	26.8 ± 0.7	29.6 ± 0.9	7.9 ± 0.8	8.2 ± 0.4	92 ± 11	0.58 ± 0.26	0.55 ± 0.43
60% GW	27.02 ± 0.5	29.5 ± 0.8	7.9 ± 0.4	8.3 ± 0.5	93 ± 12	0.57 ± 0.29	0.54 ± 0.48

Table 4. Survival, growth performance and feed efficiency of *P. monodon* post-larvae over a 45-day feeding trial

Treatment	Control	15% GW	30% GW	45% GW	60% GW
Survival (%)	86.7 ± 5.8^{a}	88.9 ± 1.9^{a}	86.7 ± 1.3^{a}	84.4 ± 5.1^{a}	85.6 ± 3.8^{a}
Initial weight (g)	0.033 ± 0.005	0.033 ± 0.005	0.033 ± 0.005	0.033 ± 0.005	0.033 ± 0.005
Final weight (g)	0.94 ± 0.11^{a}	0.99 ± 0.12^{b}	0.97 ± 0.09^{b}	0.94 ± 0.10^{a}	0.91 ± 0.09^{a}
SGR (%/day)	7.43 ± 0.27^{a}	7.53 ± 0.23^{b}	7.51 ± 0.21^{b}	7.42 ± 0.23^{a}	7.34 ± 0.22^{a}
DWG (g/day)	0.020 ± 0.002^{b}	0.021 ± 0.003^{b}	0.021 ± 0.002^{b}	0.020 ± 0.002^{b}	0.019 ± 0.003^{a}
FI (mg/shrimp/day)	26.46 ± 0.80^{a}	26.99 ± 0.27^{a}	26.26 ± 0.47^{a}	26.69 ± 0.73^{a}	26.36 ± 0.54^{a}
FCR	1.31 ± 0.03^{b}	1.22 ± 0.01^{a}	1.25 ± 0.01^{a}	1.33 ± 0.02^{b}	1.36 ± 0.01^{b}
PER	1.88 ± 0.04^{ab}	2.04 ± 0.02^{c}	1.99 ± 0.01^{c}	1.89 ± 0.02^{b}	1.85 ± 0.02^{a}

Note: Values are mean ± standard deviation. Mean values with different superscripts in the same row are significantly different (P < 0.05)

Table 5. Cumulative mortality index (CMI) values measured for *P. monodon* exposed to 250 ppm formalin solution for 60 min

Treatment	Cumulative mortality index
Control	0.83 ± 0.41^{b}
15% GW	0.33 ± 0.52^{ab}
30% GW	0.17 ± 0.41^{a}
45% GW	0.33 ± 0.52^{ab}
60% GW	0.33 ± 0.52^{ab}

Note: Values are mean ± standard deviation. Mean values with different superscripts in the same column are significantly different (P <0.05)

The results in the present study are in agreement with the study of Anh *et al.* (2014) who assessed soybean meal protein substitution with gut weed (*Enteromorpha*) protein or blanket weed (Cladophoraceae) protein at the rates of 20%, 40% and 60% in the practical diets of white leg shrimp (*Litopenaeus vannamei*) post-larvae. The authors found that the survival of shrimp was not affected by the test diets, and ranged from 81.1 to 87.8%. Growth rates of the shrimp fed 20% and 40% replacement levels of gut weed or blanket weed protein in the diets were better or similar to those fed the control diet while at the 60% substitution level, shrimp had poorer growth but there were not any significant differences between the control and the other feeding treatments. Moreover, the feed conversion ratio (FCR) and the protein efficiency ratio (PER) exhibited similar trends as those observed for growth performance, indicating that gut weed and blanket weed could replace up to 40% of soybean meal protein in the diets for *L. vanamei* postlarvae. A similar finding was reported by Cruz-Suarez *et al.* (2006) who compared the potential of *Enteromorpha* meal as an ingredient in shrimp feed formulation with two kelp seaweeds, *Macrocystis* and *Ascophyllum*, by supplementing 3.3% of seaweed in the *Litopenaeus vannamei* diets for 28 days. They found that *L. vannamei* shrimp were larger and had a better feed conversion ratio in the group fed pellets containing *Enteromorpha* than those with *Macrocystis* or *Ascophyllum*.

Another study revealed that red seaweed meal, *Gracilaria* sp., could substitute wheat flour and soybean up to 15% in the shrimp

P. monodon diet while 30% dietary inclusion levels resulted in significantly poorer growth and higher FCR compared to the control (0% seaweed) but survival of shrimp was similar among feeding treatments (Briggs and Funge-Smith, 1996). A parallel confirmation was made by Hafezieh *et al.* (2014) who stated that the survival of *L. vannamei* juveniles fed test diets containing different percentages of brown seaweed (*Sargassum illicifolium*) powder from 5 to 15% were similar to those fed the control diet without seaweed meal (95.2-97.0% survival). The specific growth rate of shrimp ranged from 4.68% to 5.68%, and exhibited no significant differences compared to the control diet while the diets at higher inclusion levels (15% and 10%) of brown seaweed exhibited better FCR (1.15 and 1.17) than the 5% and control diets (1.30 and 1.33). Additionally, Felix and Brindo (2014) found that the substitution of fishmeal with raw and fermented *Kappaphycus alvarezii* at four levels, 0 (control), 10%, 20%, and 30%, in the diet for 45 days did not affect the survival of the giant freshwater prawn *Macrobrachium rosenbergii* juveniles (100% survival). Furthermore, 10% raw *K. alvarezii* powder could be incorporated into the prawn's diet without any compromise in growth performance or feed utilization efficiency (FCR and PER) compared with the control diet. However, higher levels of *K. alvarezii* inclusion (20% and 30%) did not perform well. The authors confirmed that the reduced growth of the prawns fed diets containing higher levels of raw seaweed appeared to be due to the increased fiber content due to seaweed in the diets.

3.4. Cumulative mortality index of *P. monodon* post-larvae fed different diets exposed to a 250 ppm formalin solution for 60 min

The cumulative mortality index (CMI) of the shrimp subjected to formalin stress is shown in Table 5. The results indicated that the stress index in the experimental shrimp fed diets containing gut weed protein was lower than in the shrimps receiving the control diet. However, a significant difference was only observed between the 30% GW treatment and the control group.

Moreover, visual observation found that shrimp mortalities in the control diet happened earlier than other test diets. This means that shrimp fed the control diet were less tolerant to formalin stress than in the animals fed gut weed based-diets.

Previous investigations stated that the formalin stress test can be used as a more flexible tool for diagnosing shrimp quality and to formulate appropriate diets (Samocha *et al.*, 1998; Thanh *et al.*, 2002). In the current study, the effect of the dietary treatments on formalin stress resistance displayed a similar pattern as the growth performance, where *P. monodon* shrimp fed based-gut weed feed had formalin stress test results better than animals fed the control diet. Furthermore, a significant difference in CMI was observed between the 30% GW treatment and the control which was in agreement to the investigations that showed that seaweed meal inclusion in aquafeeds at low levels improved growth performance, feed efficiency, and disease resistance of shrimps (Cruz-Suarez *et al.*, 2008). Similar research results were reported by Peixoto *et al.* (2016) who found that European seabass (*Dicentrarchus labrax*) fed feed supplemented either with *Gracilaria* spp., *Ulva* spp., or *Fucus* spp. at 2.5% or 7.5% levels had improved immune and stress responses without compromising growth performance and feed efficiency.

Earlier studies confirmed that gut weed *Enteromorpha* spp. have high nutritional values. For example, gut weed *Enteromorpha linza* was shown to contain 18 amino acids, and high protein and mineral levels, which was higher than that of other seaweeds (*Laminaria japonica*, *Porphyra haitanensis*, *Hizikia fusiformis*, and *Undaria pinnatifida*), and indispensable amino acids were 53% of the total amino acids (Qing *et al.*, 2006). Additionally, gut weed *Enteromorpha* spp. are rich in high unsaturated fatty acids (LN, ARA and EPA) and amino acids, and protein digestibility of gut

weed is high (98%), especially because it contains high levels of astaxanthin (Aguilera-Morales *et al.,* 2005). Similar findings were reported by Anh (2014), gut weed *Enteromorpha intestinalis* collected in the brackish water bodies form Soc Trang and Bac Lieu provinces contained high levels of essential amino acids and was rich in protein and fatty acids which make it a suitable food for fish and shrimp. Besides, Cruz-Suarez *et al.* (2008) reported that diets supplemented with seaweed meal or their extracts, due to the presence of some bioactive compounds (fucoidan, alginates, laminarins, carrageenans, etc.) can enhance immune resistance and improve survival when shrimp are challenged with bacteria or viruses, and can also help the species to combat a stressful environment. Mondal *et al.* (2015) found that green seaweed *Enteromorpha intestinalis* was a natural high content source of astaxanthin (120.78 ppm) and was included in the formulated diets of farmed tiger shrimp (*Penaeus monodon*) in relation to its quality improvement. Astaxanthin showed strong activity as an inhibitor of lipid peroxidation mediated by active forms of oxygen. Among the functions of astaxanthin in aquaculture, the antioxidant properties can be closely associated with stress resistance (Meyers, 1994). Chien *et al.* (2003) reported that *Penaeus monodon* juveniles fed diets supplemented with a 80 mg astaxanthin/kg diet for 8 weeks showed significantly higher resistance to thermal and osmotic stresses than those fed the control diet. The findings cited above can explain the reason why shrimp in the present study fed based-gut weed diets showed better tolerance to formalin shock than those receiving the control diet.

4. CONCLUSIONS

Survival of the experimental shrimp was similar among the feeding treatments, ranging from 84.4% to 88.9%. The dietary replacement of soybean meal protein with 30% gut weed *Enteromorpha* protein seems to be the optimal level for black tiger shrimp *P. monodon* post-larvae as indicated by a significantly higher growth rate, better feed efficiency, and formalin resistance compared to those in the control diet.

Further research should address the *Penaeus monodon* post-larvae response to biotic or abiotic stressors, clarifying the objective role of dietary gut weed *Enteromorpha* protein replacement as immune and antioxidant stimulating.

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