Co-Culture of Red Seaweed (*Gracilaria tenuistipitata*) and Black Tiger Shrimp (*Penaeus monodon*) with Different Feeding Rations

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Abstract - The study was performed to assess the effects of different feeding rations on growth and feed efficiency of the black tiger shrimp (*Peneaus monodon*) co-cultured with red seaweed (*Gracilaria tenuistipitata*) in tanks. Shrimp was mono-cultured and fed commercial feed (CF) to satiation considered as a control and the five other treatments, shrimps were co-cultured with red seaweed (1 kg m^{-3}) and received CF at 100%, 75%, 50% and 25% and 0% feed ration of the control. Juvenile shrimps with mean initial weight of 0.46-0.51 g were run in triplicate and stocked at a density of 100 ind. m^{-3}, and at salinity of 15 ppt. After 60 days of culture, water quality parameters in terms of nitrogen compounds (TAN, NO_2^- and PO_4^{3-}) in co-culture tanks were significantly ($p<0.05$) lower than those in the monoculture. Survival of shrimps varied in the range of 45.00-98.33%, of which the treatment without feeding (0% feed ration) was lowest and significantly different ($P<0.05$) from the other treatments. Growth rate and productivity of shrimps in co-culture fed from 50% to 100% satiation were statistically higher ($p<0.05$) than those in the control group, and the feed cost could be reduced from 40.89% to 69.94%. Proximate composition of shrimp meat such as moisture, protein and ash were similar among treatments except lipid content was significantly lower and red coloration of shrimp after boiling was markedly darker in co-culture than those in the control. These results indicated that co-culture of *P. monodon* and *G. tenuistipitata* with reduction of feeding ration up to 50% satiation resulted in improvement of growth, feed cost, and shrimp color as well as maintain better water quality in the culture tanks.

***Index Terms* –** Co-culture, *Penaeus monodon*, *Gracilaria tenuistipitata*, feed cost, growth rate, water quality

I. INTRODUCTION

The black tiger shrimp (*Penaeus monodon*) has high economic value, and is one of the important cultured species in the Mekong delta, Vietnam. However, intensification of shrimp farming has caused soil and water pollution (Lan, 2013). Moreover, feed cost occupies large proportion in the intensive shrimp farming system, accounted for 58% of the total production cost (Hung and Quy, 2013). Previous studies found that integrated/co-culture system of seaweed with fish/shrimp gave several benefits such as reducing the risk of ecological impacts, improving the yield of both primary and secondary production, and maintaining optimum water quality (Troell, *et al*., 2009; Jaspe *et al*., 2011; Susilowati, *et al*., 2014; Samocha, *et al*., 2015; Tsutsui, *et al*., 2015, Anh and Ngan, 2017). It also effectively utilizes the different natural food present in the production area, thus improving efficiency of converting available resources to the product.

Red seaweed *Gracilaria* is a genus with a large number of species, they are euryhaline and found in brackish water with wide salinity range. These species have played an important role in the production of agar as well as high absorption of nutrient in water body (FAO, 2003; Hau and Dai, 2010). Other study reported that *Gracilaria cervicornis* could partially substitute for the industrial feeds used in shrimp (*Litopenaeus vannamei*) farming (Marinho-Soriano *et al*., 2007), and survival, growth and production of *L. vannamei* were improved in polyculture with *Gracilaria verucosa* (Susilowati, *et al*., 2011). Moreover, *Gracilaria tikvahiae* cultured with the shrimp *L. vannamei*, this seaweed absorbed nearly 35% of the nitrogen input by shrimps within 18 days of experiment (Samocha, *et al*., 2015). Recently, red seaweed (*Gracilaria tenuistipitata*) has been found abundantly with other seaweed species in the improved extensive shrimp farms from Bac Lieu and Ca Mau province, and are believed to be good for shrimps (Anh, *et al*., 2017). Therefore, the aim of this study was to assess the effects of different feeding rations on growth and feed efficiency of the black tiger shrimp (*Peneaus monodon*) co-cultured with red seaweed (*Gracilaria tenuistipitata*) in tanks. Theses works could provide useful information for further research in field conditions that contribute to develop sustainable shrimp farming in Mekong delta of Vietnam.

II. MATERIALS AND METHODS

Source of experimental shrimp, red seaweed and commercial feed

The black tiger shrimp postlarvae from one single batch were purchased from a commercial hatchery in Bac Lieu province and reared in 2 m^3 tank for 3 weeks to obtain juvenile stage with individual weight of about 0.5 g.

Red seaweed (*Gracilaria tenuistipitata*) was collected in the improved-extensive shrimp farm from Bac Lieu province, Vietnam and then red seaweed was separated from other seaweeds and acclimated to adapt experimental salinity for 5 days. Commercial feed (GROBEST LANDFOUND No. 2 and No. 3) is produced by Grobest Company, Dong Nai province, Vietnam.

Table 1 Proximate composition of *Gracilaria tenuistipitata* (% dry matter) and commercial feed

Feed type	Moisture	Protein	Lipid	Ash	Fiber
Gracilaria sp.	85.44	12.34	1.36	28.47	10.26
Grobest feed*	≤11	≥42	5-7	≤15	≤3

** Information based on label from Grobest Company*

Experimental design

The experiment was carried out for a period of 60 days at the College of Aquaculture and Fisheries, Can Tho University, Vietnam. Six feeding treatments were randomly designed in triplicate. Shrimp was mono-cultured and fed commercial feed (CF) to satiation considered as a control, the other five treatments; shrimps were co-cultured with red seaweed (*Gracilaria tenuistipitata*) and fed at the rates of 100%; 75%, 50%, 25% and 0% (without receiving CF) feed ration of the control. These treatments were as follow:

- Mono-culture shrimp _feeding to satiation (Control)
- Shrimp+ *G.tenuistipitata* _ 100% feeding rate of control (G+100%C)
- Shrimp+ *G.tenuistipitata* _ 75% feeding rate of control (G+75%C)
- Shrimp+ *G.tenuistipitata* _ 50% feeding rate of control (G+50%C)
- Shrimp+ *G.tenuistipitata* _ 25% feeding rate of control (G+25%C)
- Shrimp+ *G.tenuistipitata* _ 0% feeding rate of control (G+0%C)

Experimental system and management

The experimental system was set up under transparent roof, juvenile shrimps with mean initial weight of 0.46-0.51g were stocked at density of 100 ind. m^{-3} in the 150-L tanks at salinity of 10 g L^{-1}, and all culture tanks were provided continuous aeration. For co-culture, the red seaweed *G. tenuistipitata* (1 kg m^{-3}) was distributed in each tank. Shrimps were fed four times a day at 7:00, 11:00, 15:00 and 19:00 h. The feeding ration were based on the allocated treatments and the commercial feed (Growbest) was used for the black tiger shrimp at different developmental stages as recommended by the manufacturer. Water exchange was done every 10 days, about 30% of the tank volume.

Water quality

Daily water temperature and pH in the culture tanks were recorded at 7:00 and 14:00 hours using a thermo-pH meter (YSI 60 Model pH meter, HANNA instruments). Alkalinity was measured by test kit (Sera, Germany). The concentrations of TAN (NH_3/NH_4^+), NO_2^- and PO_4^{3-} were determined at a 20-day interval using a spectrophotometer according to American Public Health Association (APHA, 1998).

Growth rate and feed utilization

To determine the growth performance during the feeding trial, initial and final as well as intermediate samples were taken to measure average individual shrimp weight. Sampling was done at a 20-day interval. Ten shrimps in each tank were randomly taken and weighed in groups using an electronic balance with an accuracy of 0.01 g and then the shrimps were returned to the original tanks. At the end of experiment, all shrimp was individually weighed and the survival was calculated.

Growth data of experimental shrimps consisting of weight gain (WG), daily weight gain (DWG), specific growth rate (SGR) and survival; feed conversion ratio (FCR) calculated using the following equations:

Weight gain (g) = Final weight - Initial weight
DWG (g/day) = (final weight - initial weight)/cultured days x 100
SGR (%/day) = ((ln final weight)–(ln initial weight))/cultured days x 100
Survival (%) = Final number of shrimp/Initial number of shrimp x 100
FCR = Feed provided (dry weight)/Weight gain (wet weight)

Criteria for evaluating shrimp quality

Texture and proximate composition of shrimp meat, and color of boiled shrimps were determined at the end of experiment. Experimental shrimps in each treatment were cooked for 5 minutes and shrimp sample was taken in one photo for comparing the color among treatments. Proximate analysis (moisture, crude protein, lipid, fiber and ash) of red seaweed and shrimp meat were carried out according to the standard methods of Association of Official Analytical Chemists, AOAC (2000). Texture of shrimp meat was measured by the TA.XT plus texture analyzer (Stable micro system YL, UK).

Statistical analysis

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

III. RESULTS

Water quality parameters

During culture period, the average water temperature and pH were in the ranges of 26.2-29.3^{o}C and 8.1-8.4, respectively. The mean alkalinity fluctuated from 119.3 to 133.7mgCaCO$_3$L^{-1} (Table 2). Generally, the temperature, pH and alkalinity were similar among treatments.

Table 2 Average temperature, pH and alkalinity during culture period

Treatments	Temperature (^{o}C)		pH		Alkalinity (mgCaCO$_3$L^{-1})
	7:00	14:00	7:00	14:00	
Control	26.3±0.6	29.1±0.7	8.1±0.3	8.4±0.2	123.3±10.8
G+100%C	26.2±0.6	29.1±0.6	8.1±0.2	8.4±0.2	125.3±12.7
G+75%C	26.2±0.7	29.3±0.8	8.1±0.2	8.4±0.2	123.8±14.7
G+50%C	26.3±0.8	29.1±0.7	8.1±0.2	8.3±0.2	133.7±13.4
G+25%C	26.3±0.7	29.0±0.6	8.1±0.2	8.4±0.2	119.3±12.7
G+0%C	26.3±0.7	29.2±0.7	8.1±0.3	8.4±0.2	125.3±12.7

Previous study reported that the black tiger shrimp could tolerate a wide range of temperature from 23^{o}C to 34^{o}C, the optimal range was 26- 29^{o}C, and pH range to maintain the good performance of shrimp was 7.5- 8.5 (Pushparajan and Soundarapandian, 2010). In the present study, temperature and pH were in the suitable range for normal growth of tiger shrimp. According to Tao (2015), the appropriate range of alkalinity for postlarvae of tiger shrimp was 110-120 mgL^{-1}. The alkalinity in the experiment was slightly higher than the recommended values that might not cause negative effects on shrimp performances.

The mean levels of TAN and NO_2^- in the culture tanks varied in the ranges of 0.05-1.09 mgL^{-1} and 0.03-4.24 mgL^{-1}, respectively (Table 3). These parameters tended to decrease with reducing feeding ration, of which the highest concentration was found in the control with satiation feeding regime, followed by the co-culture with 100%, 75%, 50% and 25% feed ration of the control and the lowest value was observed for the co-culture without feeding. There were significantly different among treatments (p<0,05).

Table 3 The concentrations of TAN, NO_2^- and PO_4^{3-} during culture period

Treatments	TAN (mgL^{-1})	NO_2^- (mgL^{-1})	PO_4^{3-} (mgL^{-1})
Control	1.09±0.63^{a}	4.24±2.24^{a}	0.49±0.15^{a}
G+100%C	0.51±0.31^{b}	2.16±1.21^{b}	0.29±0.12^{b}
G+75%C	0.38±0.20^{c}	2.03±0.97^{b}	0.22±0.03^{bc}
G+50%C	0.27±0.14^{cd}	1.12±0.59^{c}	0.19±0.07^{cd}
G+25%C	0.18±0.10^{d}	0.88±0.59^{c}	0.17±0.07^{cd}
G+0%C	0.05±0.01^{e}	0.03±0.02^{d}	0.07±0.03^{e}

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

Previous studies revealed that the toxicity of ammonia and nitrite for shrimp is greatly dependent on environmental factors such as pH, dissolved oxygen, salinity, and temperature (Chen and Lei, 1990; Whetstone, *et al.*, 2002). In aquaculture, these factors play an important role in the development, growth, and survival of species exposed to ammonia and nitrite. Appropriate levels of TAN and nitrite for culturing juvenile of *P. monodon* (0.27 g) were 3.7 mg L^{-1} and 3.8 mg L^{-1}, respectively (Chen and Lei, 1990). Therefore, the concentration of TAN and NO_2^- in the control treatment was still in the tolerant level of *P. monodon*. Moreover, the contents of PO_4^{3-} in the culture tanks followed similar pattern as observed for TAN and NO_2^-. The highest PO_4^{3-} level was also found in the control and then gradually reduced with decreasing feed ration, and the lowest level was observed in the co-culture with no feeding. Statistical results revealed that mono-culture tanks had significantly higher value of PO_4^{3-} compared to the co-culture treatments. The study of Kasnir *et al.* (2014) reported that the criteria concentration for PO_4^{3-} in shrimp pond was 0.05 - 0.5 mg L^{-1}, so the amount of phosphate in the control was still in the acceptable range to experimental shrimps.

It is well known that *Gracilaria* species have been used as biofilter to improve water quality in aquaculture system (FAO, 2003; Susilowati, *et al*., 20). The study of Marinho-Soriano *et al.* (2009) revealed that red seaweed (*Gracilaria birdiae*) had high biofiltration capacity which help to significantly reduced concentrations of the three nutrients analyzed (PO_4^{3-}, NH_4^+ and NO_3^-) over the study period. The concentration of PO_4^{3-} decreased by 93.5%, NH_4^+ by 34% and NO_3^- by 100% after the 4-week experimental period. Additionally, Hau and Dai (2010) stated that red seaweed *Gracilaria* species have ability to absorb nutrient excessive their requirement. Abreu *et al*. (2011) also found that *Gracilaria* sp. was the most efficient biofilter and very useful in ecological engineering application.

The results of the current study are in agreement with the results from Anh, *et al*. (2014), authors stated that the contents of TAN and NO_2^- in the co-culture of the white leg shrimp (*Litopenaeus vannamei*) with gut weed (*Enteromorpha* sp.) or green seaweed (Cladophoracea) were considerably lower than those in the mono-culture. Similar finding showed that the macroalga *Gracilaria tikvahiae* cultured with the shrimp *L. vannamei* in an integrated multi-trophic Aquaculture (IMTA) system. This species uptake nearly 35% of the nitrogen input by shrimp within 18 days of experiment that helped to improve water quality (Samocha, *et al.*, 2015).

Growth rate

Results showed that growth in weight of experimental shrimps was affected by different feeding regimes from day 20 onwards. At day 20, the mean weight of shrimps attained 0.86-2.63 g, of which the lowest and highest values were observed for the G+0%C treatment (co-culture without feeding) and the G+50%C treatments (co-culture with 50% feed ration of the control), respectively. The control treatment showed intermediate value, this tendency was found at day 40 (1.82-4.68 g) and day 60 (Figure 1).

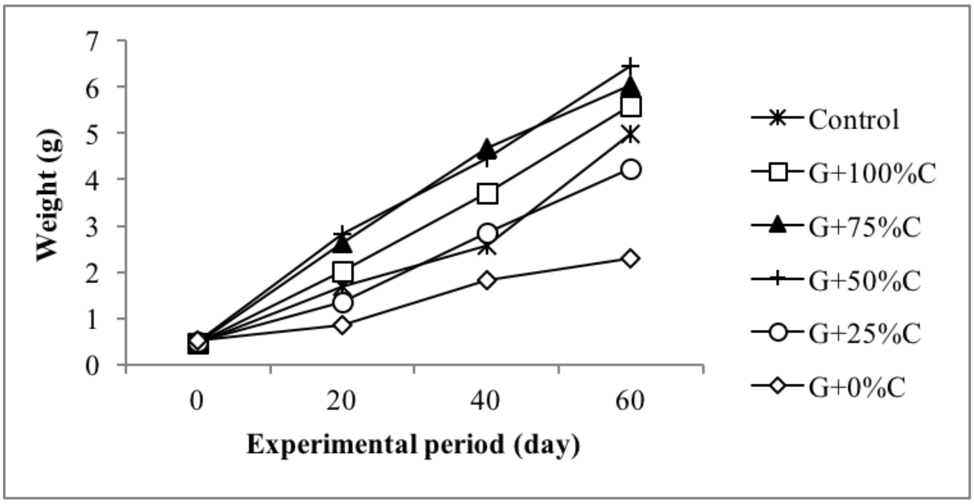

Figure 1 Variation in weight of shrimp during culture duration

Table 4 Shrimp performances after 60 days of culture

Treatment	Control	G+100%C	G+75%C	G+50%C	G+25%C	G+0%C
IW (g)	0.47 ± 0.03^{a}	0.46 ± 0.03^{a}	0.48 ± 0.02^{a}	0.49 ± 0.04^{a}	0.49 ± 0.03^{a}	0.51 ± 0.02^{a}
FW (g)	4.97 ± 0.19^{a}	5.58 ± 0.08^{b}	6.03 ± 0.40^{bc}	6.44 ± 0.21^{c}	4.22 ± 0.12^{d}	2.30 ± 0.1^{e}
WG (g)	4.51 ± 0.19^{a}	5.12 ± 0.08^{ab}	5.55 ± 0.40^{bc}	5.95 ± 0.21^{c}	3.73 ± 0.12^{d}	1.94 ± 0.2^{e}
DWG (g day^{-1})	0.075 ± 0.003^{a}	0.086 ± 0.002^{ab}	0.092 ± 0.007^{bc}	0.099 ± 0.003^{c}	0.062 ± 0.002^{d}	0.032 ± 0.004^{e}
SGR (% day^{-1})	3.92 ± 0.06^{a}	4.13 ± 0.02^{b}	4.19 ± 0.11^{b}	4.27 ± 0.06^{b}	3.54 ± 0.04^{c}	2.39 ± 0.10^{d}
IL (cm)	3.41 ± 0.2	3.31 ± 0.18	3.44 ± 0.19	3.32 ± 0.17	3.34 ± 0.21	3.30 ± 0.20
FL (cm)	7.77 ± 0.94^{a}	8.06 ± 0.98^{ab}	9.23 ± 1.01^{3}	9.35 ± 1.01^{b}	7.16 ± 0.88^{ab}	4.96 ± 0.96^{c}
Survival (%)	88.33 ± 7.64^{b}	95.00 ± 5.00^{b}	98.33 ± 2.89^{b}	91.67 ± 5.77^{b}	86.67 ± 2.89^{b}	45.00 ± 13.23^{a}

IW: Initial weight, FW: Final weight; IL: Initial length, FL: Final length. Values are mean ± standard deviation.

Mean values with different superscripts in the same row are significantly different (P<0.05)

Table 4 showed that average initial weight of *P. monodon* shrimps were 0.46-0.51 g, and similar among treatments (p>0.05). After 60 days of culture, the final weight (FW) of experimental shrimps varied in the range of 2.30- 6.44 g, in which larger weight of shrimps compared to those in the control were observed at feeding rates from 50% to 100% feed ration of the control while the co-culture without feeding showed the poorest growth. Additionally, growth rate of shrimps in terms of weight gain (WG) and daily weight gain (DWG) and specific growth rate (SGR) followed the same pattern as observed for the final weight. Statistical analysis for FW, WG and DWG showed that the G+50%C treatment had the highest values and significant differences from the control and the G+100%C; G+25%C and G+0%C treatments while it was insignificant difference (p>0.05) from those in the G+75%C treatment. However, for specific growth rate (SGR) the there were not statistical differences (p>0.05) among the G+100%C; G+75%C and G+50%C treatments.

Growth in length of shrimp followed similar trend as growth in weight. The final length of shrimps were in the ranges of 5.23-9.19 cm, in which the shortest length was in the G+0%C followed by the control, and highest values was found for the G+75%C and G+50%C treatments. There were significant differences (p<0.05) among the control and the G+75%C; G+50%C and G+0%C treatments.

These findings was similar to the study of Anh *et al*. (2014), growth rate of shrimp *L. vannamei* in co-culture with green seaweed fed 50% and 75% satiation were equal to or significantly higher than shrimp fed to satiation in mono-culture. Anh and Ngan (2017) also revealed that growth performance of shrimp (*P. monodon*) in co-cultured with sea grape (*Caulerpa lentillifera*) fed 50% or 75% *ad libitum* were considerably higher than those in the mono-culture fed at *libitum*. Similarly, the study of Tsuisui *et al.* (2015) found that the enhancement of growth performance in co-culturing black tiger shrimp (*P. monodon*) with green seaweed (*Chaetomorpha* sp.). After 10 weeks of cultivation, the final mean weight of co-cultured shrimp was 50% higher than the mono-cultured one (control), and the SGR of shrimp in the integrated system (4.79% day^{-1}) was also obviously higher than the control (4.14% day^{-1}).

Izzati (2011) reported that co-culture tiger shrimp-*Gracilaria*, this seaweed was better in supporting the growth of shrimp because *Gracilaria* was the most efficient bio-filter, which was capable in reducing excess of nutrient resulting in better water quality and served as food source for shrimp. Additionally, *Gracilaria* sp. was found to be rich in essential amino acids such as valine, leucine, isoleucine, methionine, phenylanin (Wen, *et al*., 2006). According to FAO (2003); Hau and Dai (2010), *Gracilaria* species have high nutritional value; they contain high indispensable amino acids and fatty acids, pigmentation, antioxidant substance that is a good source of food for fish and shrimps.

In the current experiment, shrimp integrated *Gracilaria* and applied reduction of feed ration between 50% and 75% of satiation indicating shortage of feed for their requirement so these animals could ingest *Gracilaria* present in the culture tank as feed supplement for them. In this case, shrimps were lived in the good environment and good supplemental feed that favored growth of shrimps. However, when application of feed regimes at 25% amount of control feed or without supplying commercial feed to shrimp that caused considerably poorer growth performance, especially the treatment without feeding had both lowest survival and growth rate. It was noted that differences in nutritional composition between commercial feed and red seaweed *G. tenuistipitata* used in this study could be the reason for the observed differences in shrimp performance. The commercial feeds contain ≥ 42% protein and 5-6% lipid, which are within recommended levels for the growth of *P. monodon* shrimp produced by manufacturer (Table 1). Previous studies reported that the range of 35-45 % protein level in the diet produces the maximum weight gain of *P. monodon* shrimps. Wu (1986) also pointed out that 6% lipid level contained in the diet would bring about the best growth in *P. monodon*. In contrast, *G. tenuistipitata* used this study had lower protein (12.34%) and lipid contents (1.36%) (Table 1). This might be another cause of the poorest growth rate and reduced survival of shrimps consuming only this seaweed.

Shrimp survival

Survival of experimental shrimps after 60 days of culture was in the range of 45.00-98.33%, of which the co-culture of shrimp-*Gracilaria* without feeding had the lowest value and significant difference (p<0.05) compared to the remaining treatments. Furthermore, co-culture with feeding regimes from 25% to 100% feed ration of the control that improved shrimp survival but these treatments were not statistical difference (p>0.05) from the control- monoculture (Table 4). It indicated that reducing feeding rate up to 25% feed ration in the co-culture treatments did not considerably affect survival of shrimps. However, the co-culture of shrimp-*Gracilaria* without receiving commercial feed resulting in the lowest survival. It could explain that the phenomenon of conspecific cannibalism in the culture tanks was clearly observed at the later period of the experiment, when pieces of dead shrimps were observed in these culture tanks and most likely constitute shrimps that were eaten by the bigger ones during molting.

The current experiment was comparable to the study of Anh *et al*. (2014), who reported that the survival of shrimp in mono-culture and co-culture of *L. vannamei* with green seaweed (*Enteromorpha* and Cladophoraceae) combined with feed reduction up to 25% feed supply of the control was not significant difference, and obtained survival of 85-95%. However, Susilowati *et al.* (2011) reported that in polyculture of white leg shrimps (*L. vannamei*) and seaweeds (*G. verucosa*) helped increase in the survival of shrimp from 45.2% to 94.6%. Similar finding was found by Anh and Ngan (2017) who revealed that shrimps (*P. monodon*) co-cultured with sea grape (*C. lentillifera*) attained significantly higher survival (88.3-96.7%) compared to the control (78.3%). Furthermore, it was noted that the experimental shrimps in the present study were challenged with pathogenic bacteria, it was found that integrating red seaweed (*Gracilaria* sp.) with black tiger shrimp (*P. monodon*) could induce the immune response of haemocytes and improve the survival rate of shrimp with *Vibrio parahaemoticus*. After 14 days of artificial infection, the mortality of the shrimp in the co-culture treatments (23.3%) was much lower than the mono-culture one (63.3%) (Hoa *et al*., 2016).

Biomass of red seaweed *Gracilaria tenuistipitata*

Variation of red seaweed *G. tenuistipitata* biomass in the co-culture treatments during 60 days of experiment was presented Figure 2. It was found that in the first week of culture, the weight of red seaweed slightly increased in all treatments (initial weight of 200 g and 209-216 g biomass at the first week), and then tended to decline during experimental period were seen in all reducing feeding treatments. The more reduction of feed rations the more of red seaweed biomass decrease *i.e.* at day 60, red seaweed biomass in the G+100%C treatment was 132 g and only 85 g seaweed was obtained in the co-culture without feeding treatment (G+0%C). In contract, the co-culture treatment with 100% feeding regime of the control (G+100%C), biomass of red seaweed tended to increase during culture period (initial weight of 200 g and 315 g at day 60).

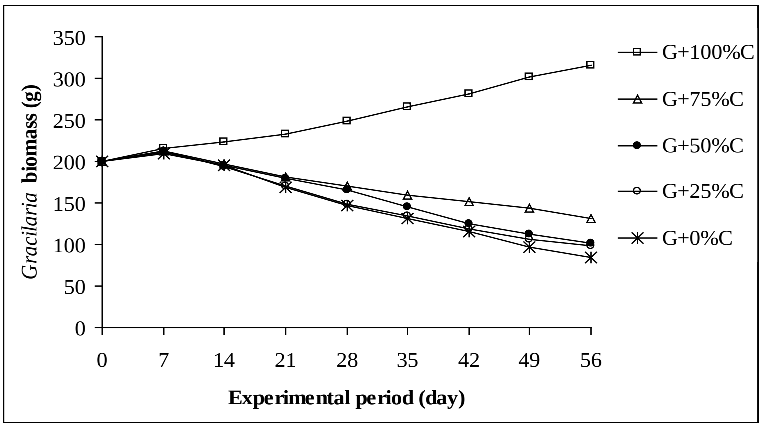

Figure 2 Biomass of red seaweed *Gracilaria tenuistipitata* during experiment

These results indicated that during the first week, the tiger shrimps have not consumed seaweed yet, and when the pellet feed was not supplied enough at later stage, shrimp might use the red seaweed available in the culture tanks to compensate for food shortage resulting in decreased in biomass for all co-culture treatments with reducing feed supply. For co-culture of shrimp-red seaweed fed to satiation, they might not need extra food so in this case red seaweed only served as bio-filter, absorbed nutrient (N and P compounds) released from shrimps and dissolved substances from the pellet feed for their growth and development as a consequence biomass of red seaweed increased. Similar result was found by the study of Anh *et al.* (2014), biomass of green seaweed in co-culture decreased with reduction of feed ration from 25% to 75% satiation during culture period.

Shrimp production, feed conversion ratio (FCR) and feed cost

Table 5 Production of shrimp, FCR and commercial feed (CF) cost after 60 days of culture

Treatment	Shrimp production ($g\ m^{-3}$)	FCR	CF cost for shrimp growth ($USD\ kg^{-1}$)	Reduction ratio of CF compared to control treatment (%)
Control	730.5±36.9^{b}	1.46±0.12^{c}	2.37±0.20	-
G+100%C	883.6±44.9^{c}	1.25±0.09^{c}	2.04±0.14	-14.19±6.05
G+75%C	987.6±48.1^{c}	0.87±0.08^{b}	1.41±0.13	-40.41±5.70
G+50%C	983.3±58.1^{c}	0.54±0.02^{a}	0.88±0.03	-62.96±1.38
G+25%C	609.9±30.7^{b}	0.44±0.02^{a}	0.71±0.03	-69.94±1.23
G+0%C	173.5±55.2^{a}	-	-	-

Commercial feed price: 1.63 USD kg^{-1}

Mean values with different superscripts in the same column are significantly different ($p<0.05$)

After 60 days of culture, shrimp production in the G+100%C, G+75%C and G+50%C treatments were similar (883.6-987.6 g m^{-3}), and significantly higher ($p<0.05$) than those in the control (730.5 g m^{-3}) and the G+25%C (609.9 g m^{-3}) and the G+0%C treatments (172.5 g m^{-3}).

Feed conversion ratio (FCR) of pellet feed was 0.44-1.46 of which the highest FCR was observed in the control and progressively reduced with decreasing feed ration supplied to the culture tanks. Statistical analysis indicated that the control treatment had significantly higher value (p<0,05) than other treatments, except the G+100%C treatment which was provided the same feed ration as the control. Furthermore, the G+25%C treatment was lowest but it not statistical difference ($p>0.05$) from the G+50%C treatment (Table 5).

The commercial feed cost for 1 kg of weight gain of shrimp has the same pattern as FCR, the highest cost (2.37 USD kg^{-1}) was found in the control and steadily declined with reduction of feeding rate provided to the culture tanks and the lowest feed cost was seen in the G+25%C treatment (0.71 USD kg^{-1}). Besides, when applying co-culture of shrimp-red seaweed with 100% feed ration of the control, the feed cost reduced only small proportion (14.19%). When co-culture system combined with feed reduction from 75% to 25% feed ration of the control, the feed cost could be reduced form 40.41% to 69.94% (Table 5). Especially, reduction of feed cost in the G+50%C treatment was 62.96% and growth rate and survival as well as production of shrimps in this treatment were considerably higher than those in the control. This reduction level could be considered the optimal feeding ration in co-culture of black tiger shrimp-red seaweed *G. tenuistipitata*.

The present results are in accordance with the study of Susilowati *et al.* (2011), who found that in polyculture of shrimps (*L. vannamei*) and red seaweeds (*G. verucosa*) helped increase in the survival of shrimp from 45.2% to 94.6%, the absolute growth had a weight increased from 9.57 g to 12.97 g, the specific growth rates increased from 4.75% to 5.07%, and biomass productions also increased from 181.56 g m^{-2} to 883.95 g/m^{-2}. Similar findings reported by Anh (2014), co-culture of shrimp-green seaweed, shrimp yield increased at 50% and 75% feed ration of the control while FCR tended to decrease with reduction of feeding rate compared to the control fed *ad libitum*, and helped to reduce up to 50% feed supply. Anh and Ngan (2017) also found that growth performance and yield of *P. monodon* in co-cultured fed 50 or 75% satiation were significantly higher than those in the control as well as feed cost could be reduced from 44.1% to 71.8%. Another study from Tsuisui *et al.* (2015) revealed that the enhancement of growth performance and FCR in co-culturing black tiger shrimp (*P. monodon*) with green seaweed (*Chaetomorpha* sp.). After 10 weeks of cultivation, the final mean weight of co-cultured shrimp was 50% higher than the mono-cultured one. The SGR in integrated shrimp (4.79±0.08% day^{-1}) also obviously higher than the control (4.14±0.27% day^{-1}). In addition, FCR in co-cultured tanks was 38.9% lower than the mono-culture tanks.

Proximate composition of fresh shrimp meat after 60 days of culture

Table 6 showed that the texture of fresh shrimp meat were high (138.7-182.8 gxcm) in the co-culture combined with different feeding regimes while these values were lower in the control (92.7 g*cm) and the G+0%C treatment and they significantly differed from the remaining treatments.

Table 6 Proximate composition of fresh shrimp meat (% wet weight)

Treatments	Texture (gxcm)	Moisture	Protein	Lipid	Ash
Control	$92.7±19.2^{a}$	$78.5±0.5^{b}$	$14.48±0.38^{bc}$	$0.83±0.05^{b}$	$1.41±0.08^{ab}$
G+100%C	$138.7±38.4^{b}$	$78.7±0.4^{b}$	$15.68±0.23^{c}$	$0.70±0.06^{ab}$	$1.52±0.16^{ab}$
G+75%C	$182.8±32.6^{b}$	$79.0±0.3^{b}$	$15.21±0.47^{bc}$	$0.72±0.05^{ab}$	$1.63±0.12^{ab}$
G+50%C	$156.8±34.4^{b}$	$79.1±0.7^{b}$	$15.20±0.34^{bc}$	$0.73±0.04^{ab}$	$1.83±0.13^{b}$
G+25%C	$149.6±24.8^{b}$	$79.6±0.5^{b}$	$14.02±0.13^{ab}$	$0.71±0.04^{ab}$	$1.50±0.11^{ab}$
G+0%C	$57.6±6.7^{a}$	$84.3±0.4^{a}$	$13.11±0.34^{a}$	$0.62±0.05^{a}$	$1.32±0.06^{a}$

Values are mean ± standard deviation.

Mean values with different superscripts in the same column are significantly different (P<0.05)

The moisture content of shrimp meat in the co-culture without feeding (84.3%) was considerably higher than the control (78.5%) and other treatments (78.7-79.6%). The protein contents in the G+100%C; G+75%C and G+50%C were higher compared to the control but not significant differences (p>0.05) among these treatments, and the G+0%C treatment had a lowest value and significantly differed from the other treatments. The lipid content of shrimp meat in the control treatment was highest (0.83%), and significantly different (p<0.05) from those in the G+0%C (0.62%) but not significant difference (p>0.05) with other treatments. The level of ash was in the range of 1.32-1.83%, of which the G+50%C treatment was significantly higher than the G+0%C treatment (Table 6).

Proximate composition of shrimp meat in the current study was comparable to the results of Kasuppasamy *et al.* (2014), who reported that the black tiger shrimp contained 11.41 mg g^{-1} of protein, 1.06 mg g^{-1} of lipid and 80.89% of moisture. Ash content was also compared to the result in the study of Nguyen Tien Luc (2014), who reported that there was 1.98% of ash in the proximate composition of black tiger shrimp.

Color of boiled shrimps

Figure 3 showed that the boiled shrimps had red coloration was markedly darker in the co-culture than in the mono-culture. Comparing among co-culture treatments, the G+ 0%C treatment had darkest orange red color followed by the G+75%C. The color of shrimp after cooking in the G+100%C, G+50%C and G+25%C treatments showed no clear differences among treatments.

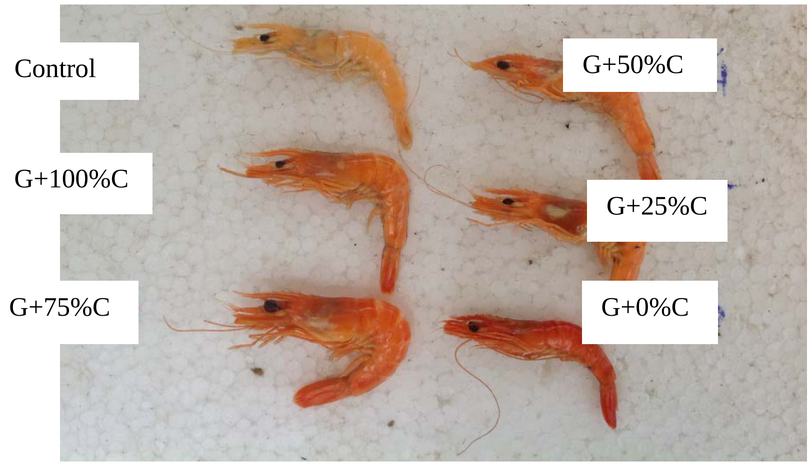

Figure 3 Color of the experimental shrimps after boiling at 100^{o}C in 5 minutes

Yu *et al.* (2003) stated that pale red color was observed in intensive shrimp culture that caused by the lack of astaxanthin. Norziah, and Ching (2000) found that red seaweed (*Gracilaria changgi*) contained high amount of β-carotene and other pigmentation. According to Chanda *et al.* (2010), seaweed contained a lot of antioxidant molecules, such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites, including carotenoids (α- and β-carotene, fucoxanthin, astaxanthin) which was very helpful to build the high pigmentation for shrimp. Report of Lorenz (1998) stated that red seaweeds contained high concentration of astaxanthin which would explain the high pigmentation of the black tiger shrimp in the integrated treatments with *Gracilaria*. There was an agreement with the study of Anh *et al.* (2014), when integrating shrimp with *Enteromorpha* sp. and Cladophoraceae, shrimps had darker red colour than those in the mono-culture treatments. Similarly, according to information from AQUA Culture Asia Pacific (2007), the tiger shrimp was much healthier and greater growth performance in the extensive ponds with seaweed presence. Shrimps in those ponds had higher pigmentation, better texture, and good flavor compared to those cultured in the intensive farming system.

In summary, co-culture of the black tiger shrimp (*P. monodon*)-red seaweed (*Gracilaria tenuistipitata*) and applied feeding rate from 50% to 75% satiation that improved water quality, survival, growth, feed efficiency and quality of shrimp compared to those in the monoculture.

Future research is needed to apply the best result of the present study in field conditions in order to demonstrate the practical approach and financial efficiency for further development of co-culture system shrimp-red seaweed in Vietnam and other countries.

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