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ORIGINAL RESEARCH

Co-culture with *Chaetomorpha* sp. enhanced growth performance and reduced feed conversion ratio of the giant tiger prawn, *Penaeus monodon*

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Abstract The increase in global demand for fishery products has led to a fivefold increase in aquaculture production since 1990. Commercial feed is the highest production cost in intensive aquaculture, and residual commercial feed leads to eutrophication; hence it is important to find a low-cost alternative that has less environmental impact. We investigate the use of the filamentous green algae, *Chaetomorpha* sp., as a raw feed for giant tiger prawns. The giant tiger prawn, *Penaeus monodon* was grown in monoculture, and in co-culture with *Chaetomorpha* sp. to investigate the potential benefits of co-culturing. Five 20-day-old giant tiger prawn juveniles were released in 70-L monoculture and co-culture tanks, and the specific growth rate (SGR) and feed conversion ratio (FCR) were measured after 10 weeks. The final mean body weight of co-cultured prawns was approximately 50 % heavier than that of monocultured prawns. The SGR in co-culture tanks was 4.79 ± 0.08 % day^{-1}, which was higher than that in monoculture tanks (4.14 ± 0.27 % day^{-1}). The FCR was 38.9 % lower in co-culture than in monoculture tanks. The protein content of *Chaetomorpha* sp. obtained from proximate analysis was almost the same or a little lower than other filamentous green seaweeds; however, the *Chaetomorpha* sp. has higher fiber and gross energy. These results show that *Chaetomorpha* sp. has potential for reducing feed costs in prawn intensive aquaculture through co-culturing.

Keywords Cladophoraceae · Feed conversion ratio · Growth rate · Intensive aquaculture · Penaeidae · Proximate analysis

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Introduction

Aquaculture has risen in importance worldwide over the past two decades because of increasing global demand for fisheries products. World aquaculture production has rapidly increased from approximately 13 million tons in 1990 to approximately 66 million tons in 2012 (FAO 2014). In 2012, the total world aquaculture production reached almost 42 % of total fishery production (FAO 2014).

Reduction of the amount of commercial feed used in intensive aquaculture is useful both from an environmental aspect and for cost management. Residual commercial feed causes eutrophication in intensive aquaculture; therefore, reduction of commercial feed helps prevent eutrophication inside and outside of aquaculture environments. From another point of view, commercial feed represents about 43–68 % of the total culture cost, which makes it the highest production cost in intensive aquaculture (Chandra et al. 2010; Hung and Quy 2013). However, it is first necessary to find a no or low-cost alternative to enable reduction of commercial feed usage.

Trials using several species of filamentous green algae (*Ulva*, *Chaetomorpha*, and *Cladophora*) as raw or dried feed for aquacultured crustaceans and fish have recently been successfully conducted. Integrated aquaculture of brackish water prawns or freshwater herbivorous fish from tropical zones with species from the genus *Ulva* has resulted in increased growth of aquaculture species (Limsuwan et al. 2008; Cruz-Suárez et al. 2010; Gamboa-Delgado et al. 2011; Siddik and Anh 2015). Although other filamentous green seaweeds including *Chaetomorpha* and *Cladophora* may be as easily applied in intensive aquaculture systems as *Ulva* species because of their euryhalinity, so far only few studies have investigated their application as raw feed for aquaculture species (Tsutsui et al. 2010).

An unidentified species of *Chaetomorpha* grows abundantly in the central Thailand. This alga has extremely wide euryhalinity and eurythermy (Tsutsui et al. 2015), making it of potentially higher benefit to intensive shrimp aquaculture ponds than *Ulva* species; however, the use of *Chaetomorpha* sp. in aquaculture has not yet been tested. This study aims to investigate the effect of *Chaetomorpha* sp. as raw feed on the growth of giant tiger prawns, and assess *Chaetomorpha* sp. as a potential alternative for reducing feeding cost in intensive shrimp culture. This study will also detail the proximate analysis of *Chaetomorpha*, which has not yet been reported.

Materials and methods

All research activities in this study were permitted by the National Research Council of Thailand (NRCT) (Project ID 2011/005). Care and handling of the experimental animals were conducted in accordance with institute regulations and Japanese law (Kurosawa 2008). The experiment was performed in a semi-outdoor experimental facility with a transparent roof and natural temperature at the Shrimp Co-culture Research Laboratory (SCORL), King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand.

Experimental shrimp and seaweed

A batch of 15-day-old giant tiger prawn post-larvae (PL_{15}) was purchased from a commercial nursery in Chon Buri Province, Thailand. After transportation to SCORL, the PL_{15} were acclimated in a 2000-L fiberglass tank with seawater of salinity 20 PSU and gentle aeration until becoming 20-day-old juveniles (J_{20}). Commercial grow-out pellets with >40 % crude protein (Vital prawn pellets, Higashimaru Co. Ltd., Kagoshima, Japan) were provided three times a day.

The *Chaetomorpha* sp. used in this study have been allocated accession numbers AB759541 and AB819998 in the DNA Data Bank of Japan (DDBJ) (Tsutsui et al. 2015). It was originally collected at a salt swamp in Samut Sakhon Province, Thailand, and cultured at SCORL in 70-L plastic tanks at a seawater salinity of 20 PSU.

Experimental design

Three replications each were made of *Chaetomorpha* sp. in monoculture (control) and in co-culture of giant tiger prawns and *Chaetomorpha* sp. (experimental treatment). The 70-L plastic control and treatment tanks

(40 cm long × 57 cm wide × 30 cm high) were randomly arranged, and connected to each other by polyvinyl chloride pipes. The water was circulated through all tanks with a flow rate of about 30 L h^{-1} to maintain the same water conditions, and was passed through an external filter to improve water quality. Water was adjusted to an approximate salinity of 20–25 PSU. Water temperature was recorded but not adjusted, and varied from 26.7 to 31.5 °C during the experiment.

Five J_{20} individuals (body weight 0.39 ± 0.03 g) were released in each tank, creating a density of about 20 individuals per m^2. Commercial pelleted feed (vital prawn) was fed three times daily in each tank. Feeding quantity was recorded every time for each tank; quantity was adjusted according to the appetite of the juveniles, which was shown as feed remaining on the bottom of the tank. Excreta and remaining shrimp feed were removed from the tanks every morning. *Chaetomorpha* sp. was maintained to cover about 30–50 % of the water surface or bottom of the co-culture tanks. Seaweed was freely grazed by shrimp in co-culture tanks. Any seaweed, including diatoms, growing in the monoculture tanks (controls) was inhibited and scraped off from the walls with a brush every week.

Measurement of growth and feed conversion ratio (FCR)

The individual body weight (wet weight) of each juvenile in each tank after 10 weeks was measured and recorded using a digital balance (PL602-S, Mettler-Toledo, Schwerzenbach, Switzerland). Specific growth rate (SGR) and FCR were calculated by the following formulae:

$$\mathrm{SGR}\ (\%\ \mathrm{d}^{-1}) = (\ln W_\mathrm{t} - \ln W_\mathrm{i}) \times D^{-1} \times 100, \quad (1)$$

where W_t is terminal wet weight, W_i is initial wet weight, and D is culture duration

$$\mathrm{FCR} = F/(W_\mathrm{t} - W_\mathrm{i}), \quad (2)$$

where F is feed given, W_t is terminal wet weight, and W_i is initial wet weight

Analysis of proximate composition and energy

Gross energy and proximate composition of *Chaetomorpha* sp. cultured at SCORL were analyzed by standard methods based on AOAC (2012) by Central Laboratory (Thailand) Co. Ltd., Bangkok, Thailand. Protein content was measured by Kjeldahl method on AOAC official method 981.10 using a factor of 6.25. Ash was measured after incineration at 600 °C for 2 h based on AOAC official method 942.05. Fat was extracted with ether using a Soxhlet apparatus and weighed following AOAC official method 920.39. Crude fiber was measured by the loss on ignition of the residue after digestion with 0.313 N sulfuric acid and 0.255 N sodium hydroxide based on AOAC official method 978.10. Carbohydrates were calculated by subtracting the protein, lipid and ash levels from 100 %. Gross energy was initially calculated as calories (protein, 4 kcal g^{-1}; fat, 9 kcal g^{-1}; carbohydrate, 4 kcal g^{-1}), and then converted to joules.

Statistics analysis

Data were shown as mean ± standard deviation (SD). Differences in SGR and FCR between control and treatment tanks were tested via the Tukey–Kramer honestly significant difference (HSD) test using JMP 10 software (SAS Institute, Cary, NC, USA); $P < 0.05$ was considered to denote significant difference.

Results

Growth

Mean body weight of shrimp from co-culture treatment tanks at the end of 10 weeks was 11.20 ± 0.652 g, which was significantly higher and approximately 50 % heavier than shrimp from monoculture tanks

Table 1 Growth performance, SGR, and FCR of giant tiger prawn juveniles in monoculture and in co-culture with *Chaetomorpha* sp.

	Monoculture	Co-culture
Mean initial weight (g) of individual shrimp	0.39 ± 0.026[a]	0.39 ± 0.034[a]
Mean terminal weight (g) of individual shrimp	7.15 ± 1.278[a]	11.20 ± 0.652[b]
Final weight difference compared with control (%)	NA	156.6
SGR (% day^{-1})	4.14 ± 0.27[a]	4.79 ± 0.08[b]
FCR	2.39 ± 0.28[a]	1.46 ± 0.62[b]
FCR reduction rate compared with control (%)	NA	38.9

Values are shown as mean value ± standard deviation from triplicate data

Different superscript labels within the same row indicate significant difference between means (Tukey–Kramer HSD test, $P < 0.05$)

NA not applicable, *SGR* specific growth rate, *FCR* feed conversion ratio

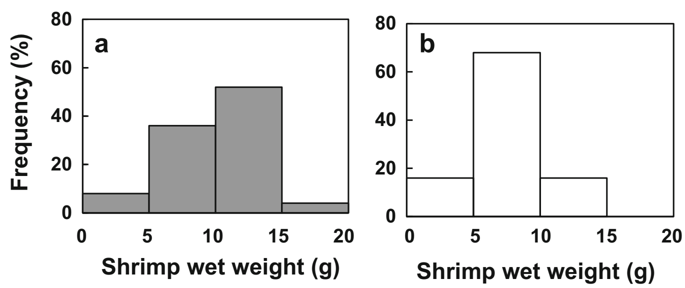

Fig. 1 Size variation of giant tiger prawns at 10 weeks in (**a**) co-culture with *Chaetomorpha* sp. and in (**b**) monoculture

(7.15 ± 1.278 g) (Table 1). The size frequency of shrimp in the co-culture treatment tanks showed that the ratio of larger sizes was higher than that in the monoculture tanks at the end of the experimental period (Fig. 1). SGR in the co-culture treatment tanks was 4.79 ± 0.08 % day^{-1}, which was also significantly higher than that in control tanks (4.14 ± 0.27 % day^{-1}) (Table 1).

FCR

FCR was significantly lower in co-culture (1.46 ± 0.62) than in monoculture tanks (2.39 ± 0.28) (Table 1). FCR reduction rate in co-culture tanks was 38.9 % compared with control tanks (Table 1).

Proximate composition

The proximate composition of wind-dried *Chaetomorpha* sp. per 100 g dry weight (DW) was: crude protein 20.4 ± 0.16 g, fat 0.8 ± 0.03 g, ash 14.0 ± 0.05 g, carbohydrate 64.8 g (including crude fiber 21.8 ± 0.06 g) (Table 2). Gross energy in 100 g DW of *Chaetomorpha* sp. was 1455.0 ± 0.35 kJ (Table 2).

Discussion

Research into improving the growth and/or feed efficiency of various aquaculture species using filamentous green seaweeds as raw or dried feed has been increasing since around 2010 (Tsutsui et al. 2007; Limsuwan et al. 2008; Ergün et al. 2009; Cruz-Suárez et al. 2010; Tsutsui et al. 2010; Gamboa-Delgado et al. 2011; Siddik and Anh 2015; Siddik et al. 2014). For species related to the seaweed investigated in the present study, *Ulva clathrata* improved growth of whiteleg shrimp by 107–164 % (Cruz-Suárez et al. 2010; Gamboa-Delgado et al. 2011), *U. intestinalis* improved growth of whiteleg shrimp and giant tiger prawns (without

Table 2 Gross energy and proximate composition of *Chaetomorpha* sp. and related species (per 100 g DW)

Species	Present species (*Chaetomorpha* sp.)	*Ulva clathrata*	*Ulva prolifera*	*Ulva* sp.
Energy (kJ)	1455.0 ± 0.35	1045.6[a]	1381.3[a]	1312.1[a]
Protein (g)	20.4 ± 0.16	20.7 ± 3.1	20.0	26.6
Fat (g)	0.8 ± 0.03	1.5 ± 0.3	0.3	0.7
Ash (g)	14.0 ± 0.05	38.4 ± 5.5	17.9	22.5
Carbohydrate[b] (g)	64.8	39.4	61.8	50.8
Crude fiber[c] (g)	21.8 ± 0.06	5.6 ± 0.5		
Reference	Present study	Cruz-Suárez et al. (2010)	MEXT (2010)	MEXT (2010)

Values are shown as mean value ± standard deviation from triplicate data. Proximate composition was re-calculated minus moisture content from original data

DW dry weight, *MEXT* Ministry of Education, Culture, Sports, Science and Technology, Japan

[a] For comparison, these values were re-calculated from the original data by applying the same gross energy coefficient (protein, 4 kcal g^{-1}; fat, 9 kcal g^{-1}; carbohydrate, 4 kcal g^{-1})

[b] Carbohydrate = 100 − (protein + fat + ash); therefore, no SD applied

[c] Crude fiber is treated as a component of carbohydrate

specific data) (Limsuwan et al. 2008), and *Chaetomorpha ligustica* increased SGR of giant tiger prawns by up to 157 % at the early juvenile stage (Tsutsui et al. 2010). Although direct comparisons with the studies cited above cannot be made because of differences in co-cultured species, our results suggest that the *Chaetomorpha* sp. is able to improve shrimp growth by almost the same amount as that of the other filamentous green seaweeds under co-culture conditions.

Previous studies have found that the filamentous green seaweed *Ulva* spp. can reduce FCR for shrimp and herbivorous fish (Cruz-Suárez et al. 2010; Siddik and Anh 2015). For example, *U. clathrata* reduced the FCR for whiteleg shrimp by approximately 47.5–62.1 % (Cruz-Suárez et al. 2010), and *U. intestinalis* reduced the FCR for spotted scat, red tilapia, and giant gourami by 26–58 % (Siddik and Anh 2015). Our study found that *Chaetomorpha* sp. as a raw feed reduced the FCR for giant tiger prawns by approximately 40 % under co-culture conditions, indicating that *Chaetomorpha* sp. can reduce artificial feed expenses as efficiently as *Ulva* species. This is the first report of FCR reduction for a farmed species by the genus *Chaetomorpha* under co-culture conditions, as previous reports have only described improvement in shrimp growth (Tsutsui et al. 2010).

In Table 2, the extremely high content of ash in *U. clathrata* (38.4 %) is believed to be salt contamination on the surface of the thalli. Considering the difference in ash content, proximate composition analysis showed that the protein content of *Chaetomorpha* sp. was the same or a little less than the closely related *Ulva* species. Conversely, *Chaetomorpha* sp. had higher energy and fiber content, but lower ash level (Table 2). Artificial commercial feed containing about 30–60 % protein is generally used in intensive shrimp aquaculture (Shigeno et al. 1972; Cruz-Suárez et al. 2010; Tsutsui et al. 2010; Hung and Quy 2013). The protein content of *Chaetomorpha* sp. and several species of *Ulva* are much lower than that of artificial feeds. However, despite its lower protein content, the enhancement of shrimp growth and reduced FCR showed that the *Chaetomorpha* sp. contributed as effectively as commercial feed to shrimp growth.

It has previously been reported that mysids and *Acetes* shrimp living in mangrove estuaries have cellulose-degrading capabilities (Niiyama et al. 2012). Although it has never been reported whether Penaeidae species have similar cellulose-degrading abilities, there is a high probability of this, as Penaeidae belong to the same taxonomic class (Malacostraca) as mysids and *Acetes* shrimp, and, like mysids and *Acetes* shrimp, are omnivorous. If the giant tiger prawn does have cellulose-degrading abilities, the *Chaetomorpha* sp. seaweed is more likely to contribute to the growth of this shrimp, as this alga has a higher fiber content than *Ulva* species. In addition, *Chaetomorpha* sp. as a supplemental raw feed may be more advantageous to growth enhancement and FCR reduction of giant tiger prawns owing to its higher gross energy content compared with *Ulva* species. However, the possible contribution of cellulose to growth enhancement and FCR reduction in giant tiger prawn requires future, detailed dietary studies.

Almost all filamentous green seaweeds currently used for growth enhancement of aquaculture species as raw or dried dietary feed are from the genus *Ulva*; species used for this application include *U. clathrata* for whiteleg shrimp, (Cruz-Suárez et al. 2010; Gamboa-Delgado et al. 2011), *U. intestinalis* for whiteleg shrimp (Limsuwan et al. 2008), *U. intestinalis* for spotted scat, red tilapia, and giant gourami (Siddik and Anh 2015), *U. rigida* for Nile tilapia (Ergün et al. 2009), and *Ulva* sp. for Nile tilapia (Siddik et al. 2014). The frequent use of *Ulva* spp. may be due to their euryhalinity and eurythermy (Msuya and Neori 2002; Kakinuma et al. 2004; McAvoy and Klug 2005; Tsutsui et al. 2012), which facilitates their application to variable aquaculture environments with a wide range of salinities, and to their widespread availability. The *Chaetomorpha* sp. investigated in this study has a wider salinity tolerance range than *Ulva* species; *Chaetomorpha* sp. can grow in salinities of 0.2–80 PSU and has a high growth rate maximum of $<60\ \%\ \text{day}^{-1}$ (Tsutsui et al. 2015). Therefore, *Chaetomorpha* sp. may be better and more suitable than *Ulva* species in intensive shrimp aquaculture ponds.

Conclusion

The *Chaetomorpha* sp. that grows abundantly in the central Thailand enhanced the growth of giant tiger prawns and reduced the FCR when used as a raw supplemental feed under co-culture conditions. The protein content of this species is almost the same or a little less than other filamentous green seaweeds, such as *Ulva* species; however, it has the advantage of higher fiber and gross energy levels. Thus, *Chaetomorpha* sp. has the potential to reduce commercial feed costs in the shrimp aquaculture industry through co-culturing. This species of *Chaetomorpha* may be more suitable and better for shrimp co-culture systems worldwide than *Ulva* species, which are currently used as raw or dried supplemental feeds.

Author's contribution The work presented here was carried out in collaboration among the authors. IT and KH conceived the study. The methods were designed by IT, JS, and KH. The experiment was performed by IT, JS, CM, DA, HS, PP, and SK. The manuscript was drafted by IT, KH, and HS. JS carried out data analysis. MG and HS participated in experimental coordination. All authors read and approved the final manuscript.

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Conflict of interest The authors declare that they have no competing interests.

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