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Effects of Marine Microalgae (*Schizochytrium sp.*) in Prepared Feeds on Growth and Survival Rate of Juvenile Sea Cucumber *Apostichopus japoncus*

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Abstract

A 60 day feeding experiment was conducted to evaluate the growth performance and survival rate of the sea cucumber *Apostichopus japonicus* fed on six experimental diets containing different inclusion level of *Schizochytrium* algae (0%, 2%, 4%, 6%, 8% and 10%) in a recirculating aquaculture system (RAS). After the feeding trial, survival was not significantly different among the dietary treatments. Results showed that diets affected the specific growth rate (SGR), ingestion rate (IR), faeces production rate (FPR) and food conversion efficiency (FCE) of sea cucumber. SGR of sea cucumber fed diet containing 6% *Schizochytrium sp* algae was significantly higher than that of sea cucumber fed the other diets ($P < 0.05$). The lowest IR and FPR was found when sea cucumber fed diets containing 10% *Schizochytrium sp*. Results of the experiment suggest that dietary inclusion with 6% *Schizochytrium sp* algae may improve growth of juvenile sea cucumber. Such detailed information could be helpful in further development of more appropriate diets for culture of sea cucumber.

Keywords: Sea cucumber; *Apostichopus japonicas*; *Schizochytrium*; Growth.

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1. Introduction

Sea cucumber have long been exploited as an important fishery resource in Russia, China, Japan and North and South Korea and *Apostichopus japonicus* is considered to be the most valuable species in many parts of Asia [1,2]. Market demand for this species has increased because of its high nutritive value. However, over the last two decades the production of sea cucumbers from the wild sources has been declining due to overexploitation and pollution [3]. To meet the demand of consumers, sea cucumber *Apostichopus japonicus* has been widely cultured in Asia in recent years [4,5]. *Apostichopus japonicus* is deposit-feeders that ingest sediment with organic matter including bacteria, prozotoa, diatoms and detritus of plants or animals [6,7,8,9,10].

Sea cucumbers have the ability to synthesize long-chain polyunsaturated fatty acids in diets. Lipids of sea cucumber play essential roles in the metabolic activities of organisms [11,12]. In particular, long-chain polyunsaturated fatty acids especially eicosapentaenoic acid and docosahexaenoic acid may reduce the risk of coronary heart disease, cancer, inflammation and arthritis [13,14] and arachidonic acid is responsible for blood clotting in wound healing [15]. However, most animals cannot synthesize longer chain polyunsaturated fatty acids such as eicosapentaenoic acid, arachidonic acid and docosahexaenoic acid. Instead, these are formed by Phytoplankton and some bacteria are responsible to form these polyunsaturated fatty acids and transferred through the food web [16,17].

Traditionally, sea cucumber, *A. japonicus* are cultured in coastal pond. Most sea cucumber culture ponds are man-made and earthen. Usually the sea cucumbers are cultured in ponds without supplement feeds. But recently more and more farmers have started to feed the sea cucumbers with macroalgae to improve the production [18]. Formulated diets for sea cucumbers are commonly made of macroalgal powder. Among macroalgae, brown algal *Sargassum thunbergii* is widely distributed over shallow coastal area in Korea, Japan and China and commonly used as a main feed ingredient in sea cucumber culture [19,20].

However, it is difficult to satisfy demand for sea cucumber culture because this algal species is not produced commercially and its use as feed ingredients is also expensive [21]. Moreover, in recent years, more and more *S. thunbergii* has been harvested with the rapid expansion of sea cucumber farming scale, which results in severe damage to *S. thunbergii* resource [22].

In other important things, by feeding commercial feed where mostly used *S. thunbergii*, sea cucumber have high level of n-6 fatty acids and low n-3 fatty acids and balance of n-3/n-6 ratio is not good [23]. But for many allergic and inflammatory diseases like asthma, n-3 fatty acids and good balance of n-3/n-6 ratio is very important. So, Reducing the *S. thunbergii* content of sea cucumber feed will be one strategy to increase the sustainability of the sea cucumber culture.

Therefore, it is critical to find good substitutes for *S. thunbergii* to relieve the pressure on natural *S. thunbergii* resource and produce more n-3 fatty acids containing sea cucumber. *Schizochytrium sp* algae might be an important choice.

Schizochytrium sp is one of the main groups of seawater phytoplankton. *Schizochytrium sp* is a genus of unicellular protists found in coastal marine habitats in the family Thraustochytriaceae. As an important source of food for a variety of economic aquatic animals, *Schizochytrium sp* has already been widely used in aquatic animal production. *Schizochytrium sp* offers high levels of lipids and polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid (DHA, 22:6n-3)[24].

In the present study, the effects of different concentration of *Schizochytrium sp* algae in prepared feeds on growth and survival rate of the sea cucumber *Apostichopus japonicus* were examined.

2. Materials and methods

2.1 Animal source and acclimation

The experiment was carried out for 8 weeks in the laboratory of Marine Biology and Aquaculture, Gyeongsang National University, Republic of Korea. Juvenile sea cucumbers were collected from the Goseong Sea Cucumber farm. Prior to the experiment, they were acclimated to the experimental conditions for 2 weeks.

2.2 Experimental diets

Six experimental diets designed as Diet 1 (control), Diet 2, Diet 3, Diet 4, Diet 5 and Diet 6 were prepared. Proximate compositions and ingredients used in the experimental diets were presented in **Table 1.** Diet 1 was used as the control diet where no used in *Schizochytrium* powder.

For diet 2, diet 3, diet 4, diet 5 and diet 6, *Schizochytrium* powder were used with the percentages of 2%, 4%, 6%, 8% and 10%, respectively.

All ingredients were ground into fine powder through a 200 μm mesh, thoroughly mixed and stored at −20 °C before use.

2.3 Experimental design

After 24 h starvation, the initial body weight of the sea cucumbers was measured individually. 240 sea cucumbers with initial wet body weights of 3.14±0.06g (mean±SE) were randomly selected from acclimatized animals and placed in equal number into 24 fiberglass aquaria (45×60×50 cm^3) to form 6 groups in tetraplicate. The 6 groups were fed with different experimental diets such as Diet 1, Diet 2, Diet 3, Diet 4, Diet 5 and Diet 6 respectively. A complete randomized block design was used to arrange the 24 aquaria of 6 treatment groups.

2.4 Rearing conditions

During the experiment, aeration was provided continuously into each tank and filtered sea water was continuously supply at a flow rate of 1 L min^{-1} every day to ensure water quality.

Water temperature was maintained at 19.5 ± 2.0 $^{\circ}$C and the levels of ammonia in the water of aquaria were less than 0.25 mg/ L. Other conditions were salinity 32 ± 1 psu; pH 7.8–8.2; photoperiod 24 h dark.

Table 1: Composition of experimental diets for *Apostichopus japonicus* (% dry matter basis)

Ingredients	Diet 1 (Control)	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Schizochytrium powder*	0	2	4	6	8	10
Wheat flour*	10	8	6	4	2	0
Seaweed powder*	20	20	20	20	20	20
Soybean meal*	8	8	8	8	8	8
Shell fish powder*	8	8	8	8	8	8
Shell powder	2	2	2	2	2	2
Calcium phosphate	2	2	2	2	2	2
Yeast protein*	5	5	5	5	5	5
Soyabean lecithin	4	4	4	4	4	4
Mineral	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin	0.5	0.5	0.5	0.5	0.5	0.5
Sea mud*	40	40	40	40	40	40
Proximate composition (%)						
Crude protein	17.91	18.24	18.56	19.89	19.21	19.51
Crude lipid	3.34	4.14	4.94	5.70	6.55	7.23
Ash	43.50	43.56	43.62	43.68	43.74	43.80

**Schizochytrium* powder (dry matter, %): crude protein 16.3, crude lipid: 40; wheat flour (dry matter, %): crude protein 17.19, crude lipid: 3.0; seaweed powder (dry matter, %): crude protein 19.4, crude lipid: 2.0; soybean meal (dry matter, %): crude protein 48.0, crude lipid: 3.5; shell fish powder (dry matter, %): crude protein 15.2, crude lipid: 2.0; Yeast protein (dry matter, %): crude protein 51.0, crude lipid: 2.5; Sea mud (dry matter, %): crude protein 2.74, crude lipid: 0.90

2.5 Procedure and sample collection

Before experiment Twenty four sea cucumbers were collected from the acclimated sea cucumbers selected to determine the initial body weight of the experimental sea cucumbers. During the experiment, sea cucumbers were fed once a day at about 16:00 h, faeces and uneaten feed were collected by siphon after 22 h and then dried at 65°C to constant weight for calculation use. At the end of experiment, all the sea cucumbers were starved to clear their guts for 48 h, weighed, and then dried at 65°C until constant weight was achieved.

2.6 Data calculation

Survival rate (SR), specific growth rate (SGR), ingestion rate (IR), faeces production rate (FPR) and food conversion efficiency (FCE) were calculated as follows:

$SR (\%) = 100 \times (N_2/N_1)$

$SGR (\% d^{-1}) = 100 (\ln W_2 - \ln W_1)/T$

$IR (g\ g^{-1}\ d^{-1}) = I/[T (W_2 + W_1)/2]$

$FPR (g\ g^{-1}\ d^{-1}) = F/[T (W_2 + W_1)/2]$

$FCE (\%) = 100 (W_2 - W_1)/I$

where N_1 is the number of individuals alive at start of experiment and N_2 is the number of individuals alive at end of experiment; W_1 and W_2 are initial and final combined dry weights of all 10 sea cucumbers in each aquarium; T is the duration of the experiment; I is the dry weight of feed ingested and F is the dry weight of faeces.

2.7 Statistical analysis

Statistics was performed using software SPSS 16.0 with possible differences among diet treatments being tested by one-way ANOVA. Duncan's multiple range tests were used to test the differences among treatments. Differences were considered significant at a probability level of 0.05.

3. Results

3.1 Survival and Growth

The sea cucumbers showed high survival rates (100%) in the all treatments. During the experimental period, no sea cucumbers were lost.

There were no significant differences in wet and dry body weights of sea cucumbers assigned to each treatment at the beginning of the experiment among diet treatments ($P>0.05$) **(Table2)**. At the end of the experiment, final wet and dry body weights of experimental sea cucumbers fed with diet 4 was significantly higher than those fed with other diets ($P<0.05$) **(Table 2)**.

Table 2: Initial and Final wet weight (WW), dry weight (DW) of *Apostichopus japonicus* fed different test diets (mean±SE)

Experimental Diets	Initial WW (g)	Final WW (g)	Initial DW (g)	Final DW (g)
Diet 1	3.20±0.05	4.60±0.10	0.27±0.00	0.39±0.01
Diet 2	3.10±0.10	5.20±0.33	0.26±0.01	0.45±0.03
Diet 3	3.11±0.06	7.50±0.84	0.27±0.00	0.64±0.06
Diet 4	3.23±0.08	8.60±1.18	0.28±0.01	0.73±.08
Diet 5	3.22±0.04	7.05±0.78	0.28±0.00	0.60±.05
Diet 6	2.99±0.09	5.10±0.94	0.26±0.01	0.44±.07

SGR of the test sea cucumbers varied in different diet treatments and showed a descending order of diet 4>diet 5>diet 3>diet 6>diet 2>diet 1. The value of SGR in diet 4 (1.46% d^{-1}) was significantly higher than those fed other experimental diets (Fig. 1) ($P < 0.05$). Significantly lowest SGR was observed in sea cucumber fed diet 1(0.53% d^{-1}) ($P < 0.05$).

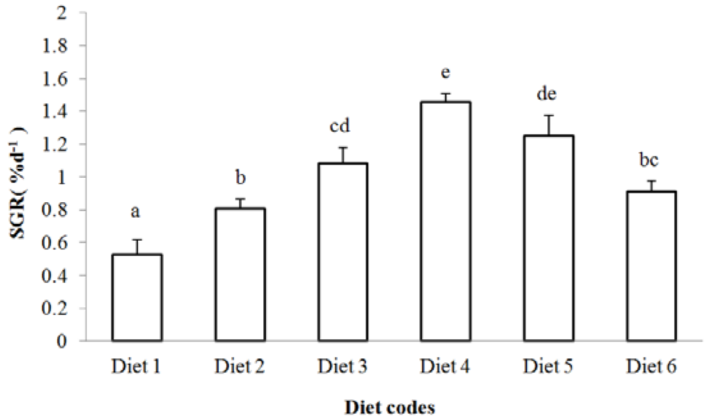

Figure 1: Specific growth rate of *Apostichopus japonicus* fed different test diets. Different letters indicate significant differences ($P < 0.05$) between treatments within the same group, and bars represent standard errors.

3.2 Ingestion rate and faeces production rate

Both ingestion rates (IR, see **Figure 2**) and faeces production rates (FPR, see **Figure 3**) of the sea cucumbers showed significant differences among different dietary treatments ($P < 0.05$). IR and FPR decreased with increasing *Schizochytrium* proportion. Sea cucumbers fed with diet 1 showed significantly higher IR (0.64 g g^{-1} d^{-1}) and FPR (0.56 g g^{-1} d^{-1}) than other treatments ($P < 0.05$) except diet 2. Sea cucumber fed with diet 1 showed the lowest IR (0.31 g g^{-1} d^{-1}) ($P < 0.05$) and FPR (0.21 g g^{-1} d^{-1}) ($P < 0.05$) among all treatments.

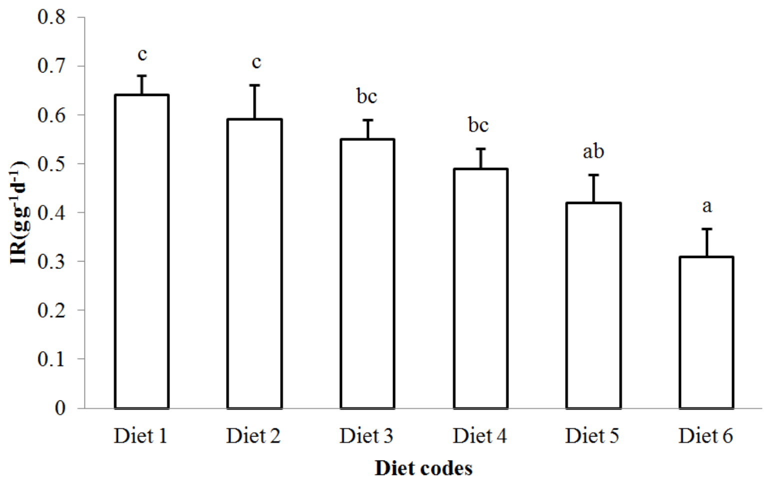

Figure 2: Ingestion rate (IR) of *Apostichopus japonicus* fed different test diets. Different letters indicate significant differences ($P < 0.05$) between treatments within the same group, and bars represent standard errors.

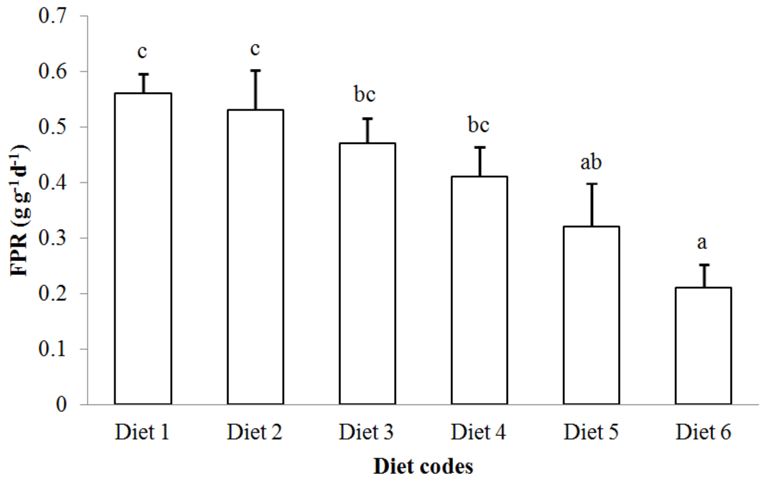

Figure 3: Faeces production rate (FPR) of *Apostichopus japonicus* fed different test diets. Different letters indicate significant differences ($P < 0.05$) between treatments within the same group, and bars represent standard errors.

3.2 Food conversion efficiency

Figure 4 showing the Food conversion efficiency (FCE) of the sea cucumbers fed different experir l diets.

Food conversion efficiency (FCE) of the sea cucumbers fed diet 1 showed significantly lower than those other treatments ($P < 0.05$). FCE of the sea cucumbers fed with diet 6 was 2.99%, which was significantly higher than those fed with other diets except diet 4, diet 5 ($P < 0.05$).

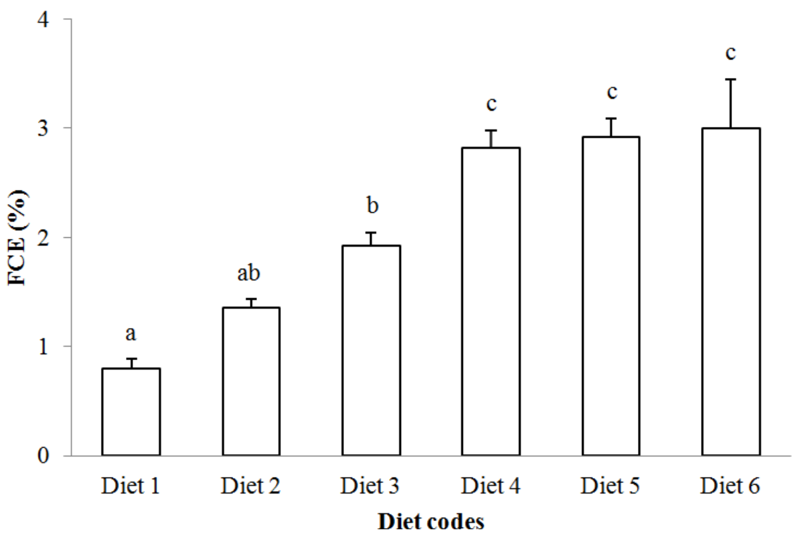

Figure 4: Food conversion efficiency (FCE) of *Apostichopus japonicus* fed different test diets. Different letters indicate significant differences ($P < 0.05$) between treatments within the same group, and bars represent standard errors.

4. Discussion

In our experiment, survival rates of sea cucumbers in all treatments were very good (100%) and were higher than the rates reported in earlier similar studies [25,26]. This result illustrated that sea cucumber, *A. japonicus* might have the ability to tolerate the different proportion of *Schizochytrium* algae in diet.

Several research studies have used different seaweeds, such as *S. thunbergii*, *U. lactuca*, *Spirulina platensis*, *S. polycystum*, *L. japonica*, *Undaria pinnatifida* etc to study about the nutritional requirements of sea cucumber [27,28,29,30,31]. Most researchers have used *S. thunbergii* as a main feed ingredient in land-based intensive culture systems. Currently, *S. thunbergii* is deemed to be the most commonly used for sea cucumber *A. japonicus* food, but a substitution of it is desiderated due to severe exhaustion of natural *S. thunbergii* resource [32]. There are lots of different microalgae in gut contents of sea cucumber in nature [33]. Arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid, the fatty acids biomarker of diatom, *nannochloropsis*, *schizochytrium*, accounts for the higher mass fraction among polyunsaturated fatty acids in the body wall of *A. japonicas* which means that these microalgae may have a great contribution to *A. japonicus* food source [34,35,36]. This study showed that the SGR of *A. japonicus* fed with 6% *schizochytrium sp* containing prepared diet was as high as those fed with other diets. These result suggested that the prepared diet containing *schizochytrium sp* algae may perform better than the traditional feed where only used *S. thunbergii* algae.

Our experimental results showed that among the six prepared diet treatments, SGR of the sea cucumbers fed with Diet 4 (1.46 % d^{-1}) was higher than the other diets (Figure 1).

Liu and his colleagues (2010) reported that SGR of the sea cucumbers *A. japonicus* was 0.83% d^{-1} when fed 70% *S. thunbergii* algae, 10% fish meal and 20% yellow soil containing diet [37]. Ce Shi and his colleagues (2015) reported that SGR of the sea cucumbers *A. japonicus* was 1.36% d^{-1} when fed 70% *S. thunbergii* algae, 20% sea mud and 10% white fish meal containing diet [38]. So, it is clear that the certain proportion of *schizochytrium sp* (dry matter) with *S. thunbergii* algae was good for sea cucumber culture.

In this study, SGR of the sea cucumbers were increased with increasing level of *schizochytrium* until 6% after that gradually decreased. There are three mechanisms such as acid hydrolysis, enzymatic digestion or mechanical trituration required to break the cell wall of microalgae [39]. Many fishes like tilapia are the species which deem on acid hydrolysis. They are able to disrupt cell walls because pH values of their stomach fluid are as low as 1.25 [40,41,42]. Takeuchi and his colleagues (2002) reported that the tilapia *Oreochromis niloticus* even showed normal growth rate when fed solely raw Spirulina [43]. As echinodermand deposit feeder, the structure and environment of the digestive tract of the sea cucumber, *A. japonicus* are quite different from those of tilapia fish. Sea cucumber have no specialized organ for grinding or gland for chemical digestion, the pH values of digestive tract are generally higher than 6 [44,45,46,47] and the cellulose activities are quite low [48]. Therefore, sea cucumber *A. japonicas* are able to digest certain amount of cellulose content.

The present result showed that IR of the sea cucumbers was decreased with increasing level of *schizochytrium sp* algae in diet. There was a negative relationship between IR and the protein level i.e ingestion rate gradually decreased with increasing protein level. Thus in this study, ingestion rate of sea cucumbers decreased when protein content of the diets increased. The same phenomenon was also found in other echinoderms. McBride and his colleagues (1998) reported that sea urchin (*Strongylocentrotus franciscanus*), prepared diets of different protein levels resulted in different ingestion rate [49].

5. Conclusion

The results of the present studies suggest that growths of sea cucumber *A. japonicus* are greatly influenced by different inclusion level of *Schizochytrium sp* algae in prepared feeds. Dietary inclusion with 6% *Schizochytrium sp* algae may improve growth of juvenile sea cucumber.

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