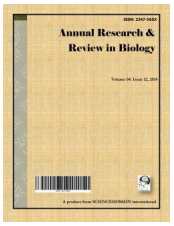

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Effects of Diets Containing Different Concentration of *Saccharina japonica* Algae on Growth and Interleukin (IL)-10 Gene Expression of Juvenile Sea Cucumber *Apostichopus japonicus*

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Authors' contributions

This work was carried out in collaboration between all authors. Author KK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AM, UCJ and DIL managed the analyses of the study. Authors HSY and SJK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The effects of diets containing different concentration of *Saccharina japonica* algae (0%, 5%, 10%, 15%, 20% and 25%) on growth and Interleukin (IL)-10 gene expression of juvenile sea cucumber *Apostichopus japonicus* were studied. At first, 08 weeks feeding trail was conducted to evaluate the growth performance of sea cucumbers fed with one of the six experimental diets. Result showed that sea cucumbers fed 15% *Saccharina japonica* algae diet had higher specific growth rate (SGR) and food conversion efficiency (FCE) than the other experimental diets ($P<0.05$). Secondly,

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Interleukin (IL)-10 gene expression was determined where mice splenocytes were treated with different experimental diets fed sea cucumber extracts for two hours. The highest Interleukin (IL)-10 gene expressions was found in 15% *Saccharina japonica* algae diets fed sea cucumbers extract compared to other diets except 10% *Saccharina japonica* algae diet. Results of this experiment suggest that 15% *Saccharina japonica* containing diet perform better growth and could elevate IL-10 gene expression. This information might be useful in the further development of more appropriate diets for the culture of sea cucumbers.

Keywords: Sea cucumber; Apostichopus japonicus; Saccharina japonica; growth; interleukin (IL)-10.

1. INTRODUCTION

Sea cucumber *Apostichopus japonicus* belongs to Echinodermata, Holothuroidea, is a commercially important species in China, Japan and Republic of Korea [1,2]. Its market demand has increased with increasing interest as a healthy food for human. Sea cucumber production from natural resources has decreased due to pollution and over-exploitation [3,4]. Recently sea cucumber *Apostichopus japonicus* has been widely cultured in Asia especially Korea, China, Malaysia, Japan for its high nutritive and economic value [5,6,7,2]. Like other holothurians, sea cucumbers are deposit-feeders that ingest sediment containing organic matter [8,9,10,11] and their gut contents are dominated by different algae, diatoms, detritus, crustaceans and barnacles, echinoderm ossicles, bacteria and protozoa [12,13,2,14].

Generally, *A. japonicus* are cultured in earthen ponds without supplement feeds, but to increase yield many farmers have begun to feed sea cucumbers with supplementary feeds [15]. Supplement feeds of sea cucumbers are mainly composed of different algae powder and sea mud. The algae *Sargassum thunbergii* belonging to Sargassaceae is widely used as best ingredients for sea cucumber feed in land-based intensive culture system [16,17]. Rapid expansion of sea cucumber farming, natural resources of this algal species has decreased because they are overexploited, not produced commercially, and its price is increasing rapidly [18,19,20,2,14]. Meanwhile, sea cucumber have high content of n-6 fatty acids, low n-3 fatty acids and lower n-3/n-6 ratio by feeding commercial feed where mostly used *S. thunbergii*. But n-3 fatty acids and of n-3/n-6 ratio is very important for inflammatory and allergic diseases like asthma [21,14,22]. So, decreasing the amount of *S. thunbergii* algae in sea cucumber feed will be one strategy to increase the sustainability of the sea cucumber culture. Therefore, it is necessary to find cheaper and optimal substitutes for *S. thunbergii* algae to maintain sustainable development of sea cucumber farming. *Saccharina japonica* algae may be a good alternative choice.

Saccharina japonica is abundant algae in coastal areas and also inexpensive. This algal species is mostly used in abalone culture [23]. It is a a marine species of Phaeophyceae and is widely cultured in China, Japan and Korea and suppresses thyroid function [24].

Sea cucumber is commonly known as "sea ginseng" and widely utilised as folk medicine in Asia and Middle East countries. It contains many bioactive compounds such as brain glycosides, chondroitin sulfates, triterpene glycosides, sulfated polysaccharides, glycosaminoglycan, phenolics, sterols, peptides, cerberosides etc [25] and has pharmacological activities such as anti-angiogenic, antiviral, anti-cancer, anti-hypertension, antibacterial, anti-oxidant, anti-inflammatory effects [26,27,28,29]. In Malaysia and China, it has been used as tonic and traditional remedy of different allergic and inflammatory diseases like asthma. Interleukin (IL)-10 is an anti-inflammatory cytokine that plays a vital role for the mitigation of inflammatory responses [2,14,22]. IL-10 is an anti-inflammatory cytokine that down regulates the synthesis of Th1 (T helper 1) and Th2 (T helper 2)-associated cytokines, chemokines, and inflammatory enzymes. There is no information about the effects of diets containing different concentration of *Saccharina japonica* algae in sea cucumber on IL-10 gene expression.

Therefore, two experiments were conducted in this study. The first experiment was to investigate the effects of diets containing different concentration of *Saccharina japonica* on growth of juvenile sea cucumber and then the second experiment was to examine Interleukin (IL)-10 gene expressions of mice splenocytes by different experimental diets fed sea cucumber extracts.

2. MATERIALS AND METHODS

2.1 Experiment 1

2.1.1 Experimental sea cucumber collection and acclimation

The experiment was carried out for 08 weeks in the aquaculture and fish nutrition laboratory, Gyeongsang National University, Korea. Experimental sea cucumbers were collected from the local sea cucumber farm (SJ Bio Sea Cucumber farm, Tongyeong, Korea). Before start the experiment, sea cucumbers were transferred to the laboratory in fiberglass aquaria and acclimatised for 7 days at 18.5°C. By using a thermostat, temperature of water bath was maintained.

2.1.2 Experimental diets

Six experimental diets designed as Diet 0 (control), Diet 5, Diet 10, Diet 15, Diet 20 and Diet 25 were prepared. Ingredients and chemical compositions of different experimental diets were listed in Table 1. Diet 0 was used as the standard diet where *Saccharina japonica* powder was not included. For diets 5, 10, 15, 20 and 25, *Saccharina japonica* powder were used at the rate of 5%, 10%, 15%, 20% and 25%, respectively. All the raw materials were dried, ground into fine powder through 0.15-mm mesh, then thoroughly mixed and stored at 4°C until used.

2.1.3 Experimental design

After 48 hrs starvation, 240 juvenile sea cucumbers (average initial body weight 4.04±0.07 g) were randomly selected and placed in equal number into 24 rectangular plastic tanks (45 L of water volume each) to form 6 groups in tetraplicate. The initial body weights of sea cucumber were measured individually as described in Battaglene et al. [17] by using AND HT-120 equipment. Four replicate groups of sea were fed one of the six experimental diets such as Diet 0, Diet 5, Diet 10, Diet 15, Diet 20 and Diet 25 respectively.

In whole experimental period, aeration was provided continuously into each tank and filtered sea water was continuously supplied at a flow rate of 1 L min^{-1} every day to ensure water quality. Seawater temperature was controlled at 19.5 ± 2.0°C. Dissolved oxygen > 5.0 mgL^{-1}, ammonia < 0.25 mgL^{-1}, salinity 30 ± 2 ppt, pH 7.9 to 8.4, photoperiod 24 h dark. The longer darker period are better for a population of sea cucumber to induce sea cucumber to feed continuously.

2.1.4 Procedure and sample collection

Twenty five sea cucumbers were sampled from the acclimated sea cucumbers simultaneously while experimental sea cucumbers were selected to determine the initial body weight of the experimental sea cucumbers. Sea cucumbers were fed once daily (17:00 h) at the rate of 5% of the body weight for 8 weeks. Twenty four hours

Table 1. Ingredients and proximate composition of experimental diets for sea cucumber (%)

Ingredients	D 0 (control)	D 5	D 10	D 15	D 20	D 25
Saccharina japonica powder	0	5	10	15	20	25
Wheat flour	25	20	15	10	5	0
Seaweed powder	5	5	5	5	5	5
Corn meal	7	7	7	7	7	7
Fish meal	8	8	8	8	8	8
Calcium phosphate	2	2	2	2	2	2
Shell powder	3	3	3	3	3	3
Yeast protein	4	4	4	4	4	4
Lecithin	4	4	4	4	4	4
Mineral	1	1	1	1	1	1
Vitamin	1	1	1	1	1	1
Sea mud	40	40	40	40	40	40
Proximate composition (%)						
Protein	16.14	16.40	16.68	16.95	17.21	17.48
Lipid	3.24	3.27	3.29	3.32	3.34	3.37
Ash	40.60	42.15	43.70	45.25	46.80	48.35

Table 2. Primers used for real-time PCR

Primer	Sequence
GAPDH- for*	5′-TAC CCC CAA TGT GTC CGT C-3′
GAPDH-rev†	5′-AAG AGT GGG AGT TGC TGT TGA AG-3′
IL-10-for	5′-GCT ATG CTG CCT GGT CTT ACT G-3′
IL-10-rev	5′-TCC AGC TGG TCC TTT GTT TG-3′

for; forward; rev†; reverse*

later uneaten feed and faeces were removed through syphon from aquaria. After that, uneaten feed and faeces were desalted with fresh water and then dried at 65°C to constant weight for calculation use. Sea cucumbers were starved for 2 days at the end of 08 weeks experiment, weighed and then dried at 65°C until constant weight was achieved.

2.1.5 Data calculation

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

$SGR\ (\%\ d^{-1}) = 100\ (\ln W_2 - \ln W_1)/T$
$IR\ (g\ g^{-1}\ d^{-1}) = C/[T\ (W_2 + W_1)/2]$
$FPR\ (g\ g^{-1}\ d^{-1}) = F/[T\ (W_2 + W_1)/2]$
$FCE\ (\%) = 100\ (W_2 - W_1)/C$

where W_1 and W_2 are initial and final dry body weights of all 10 sea cucumbers in each aquarium; T is the duration of the experiment (56 d); C is the dry weight of feed consumed and F is the dry weight of feces.

2.2 Experiment 2

2.2.1 Sea cucumber extract

Sea cucumbers were cleaned, then the internal organs and body fluid were removed before homogenisation. One hundred ninety gram of sea cucumber samples were boiled in 380 ml distilled water for 20 min. After that, solid materials removed from the water and boiled water was reduced by 50% using a microwave. After centrifugation of the extracts at 500 × g for 10 min, a 5-fold volume of 100% ethyl alcohol was added to the supernatant and incubated at 20°C for 24 h. After centrifugation, supernatant was discarded and extract pellet was washed with 70% ethyl alcohol and centrifuged at 500 × g for 10 min. After that, supernatant liquid was removed and pellet was evaporated. The final extracts were prepared by re-suspending the pellet in 25 mL distilled water [30,2,14, 22].

2.2.2 IL-10 gene expression

To evaluate IL-10 gene expression, 10 µg/ml of experimental diet fed sea cucumber extracts to mice splenocytes for 2 h. After mouse experiments, the spleen was obtained from each sacrificed mice and used for RNA isolation. Total RNA was isolated using Qiazol reagent protocols (Qiagen Scienece, USA). According to the manufacturer's protocols, 2 µg of total RNAs were transcribed using M-MLV reverse transcriptase (Promega, USA). The expression of IL-10 was determined by quantitative PCR using the iCyclerTM (Bio-Rad Laboratories, Hercules, CA, USA). GAPDH was used as reference gene. Primer sequence was shown in Table 2 [2].

2.3 Statistical Analysis

Statistical analysis was performed using software SPSS 16.0. Inter-treatment differences among diet treatments were analysed using one-way ANOVA followed by Duncan's multiple range tests and differences were considered significant at a probability level of 0.05.

3. RESULTS

3.1 Experiment 1

3.1.1 Growth

Table 3 showed the initial and final body weights (wet and dry) of sea cucumbers for the six experimental diet treatments. The growth performance of sea cucumbers fed with D 15 diet was significantly higher than the other dietary treatments ($P<0.05$).

SGR of the sea cucumbers were significantly different among diet treatments and showed a descending order of D 15>D 10>D 20>D 25>D 5>D 0. The maximum value of SGR (1.67 % d^{-1}) was found in sea cucumbers fed D 15 diet, which was significantly higher than other experimental diet treatments and D_0 diet showed significantly lower SGR (0.63% d^{-1}) than those fed other diets ($P<0.05$) (Fig. 1).

Table 3. Initial and Final wet weight (WW), dry weight (DW) of sea cucumbers in different experimental diet groups

Experimental diets	Initial WW (g)	Final WW (g)	Initial DW (g)	Final DW (g)
D 0	4.17±0.06	6.23±0.20	0.38	0.57
D 5	4.06±0.08	7.36±0.42	0.3	0.67
D 10	3.95±0.09	10.24±0.95	0.36	0.93
D 15	4.07±0.07	11.73±1.08	0.37	1.07
D 20	3.96±0.06	8.71±0.88	0.36	0.79
D 25	4.05±0.09	7.69±0.74	0.37	0.70

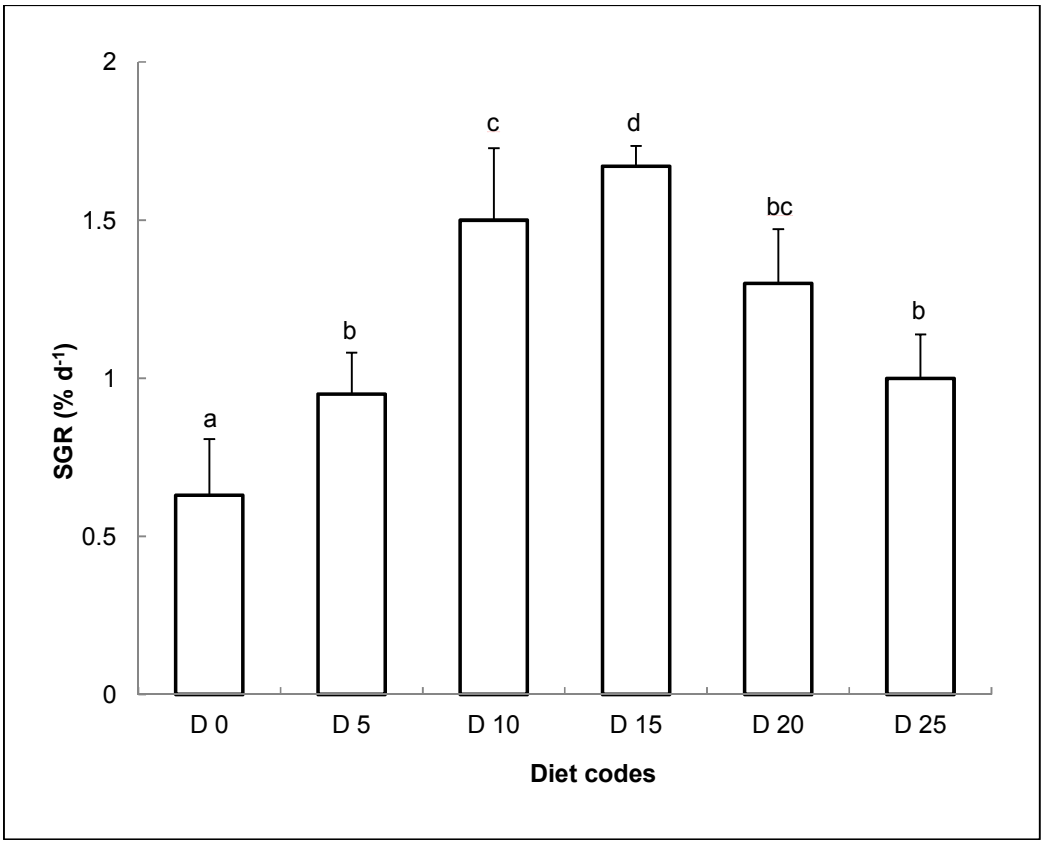

Fig. 1. Specific growth rate (SGR) of sea cucumbers (*Apostichopus japonicus*) fed different experimental diets

3.1.2 Ingestion rate (IR) and faeces production rate (FPR)

Both IR (Fig. 2) and FPR (Fig. 3) of the sea cucumbers were significantly varied in different dietary treatments and showed the same ascending order of D 25<D 20<D 15<D 10<D 5<D 0. The IR and FPR of sea cucumbers fed diet D_0 was 0.72 g g^{-1} d^{-1} and 0.63 g g^{-1} d^{-1} respectively was significantly higher than the other experimental diet treatments (P<0.05).

3.1.3 Food conversion efficiency

Fig. 4 showed the food conversion efficiency (FCE) of sea cucumber in different diet treatments. There were significant differences in FCE among different diet treatments. FCE value of diet D_{15} (3.25%) was significantly higher than other diets treatments (P < 0.05). The lowest FCE (0.81%) was observed when sea cucumbers fed D_0.

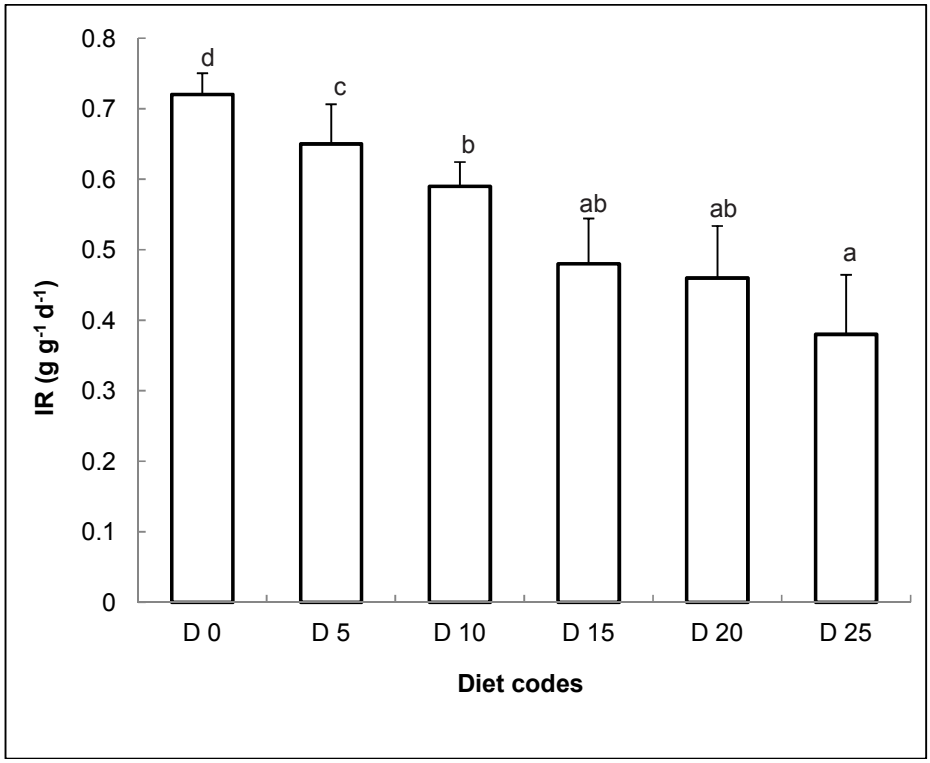

Fig. 2. Ingestion rate (IR) of sea cucumbers (*Apostichopus japonicus*) fed different experimental diets

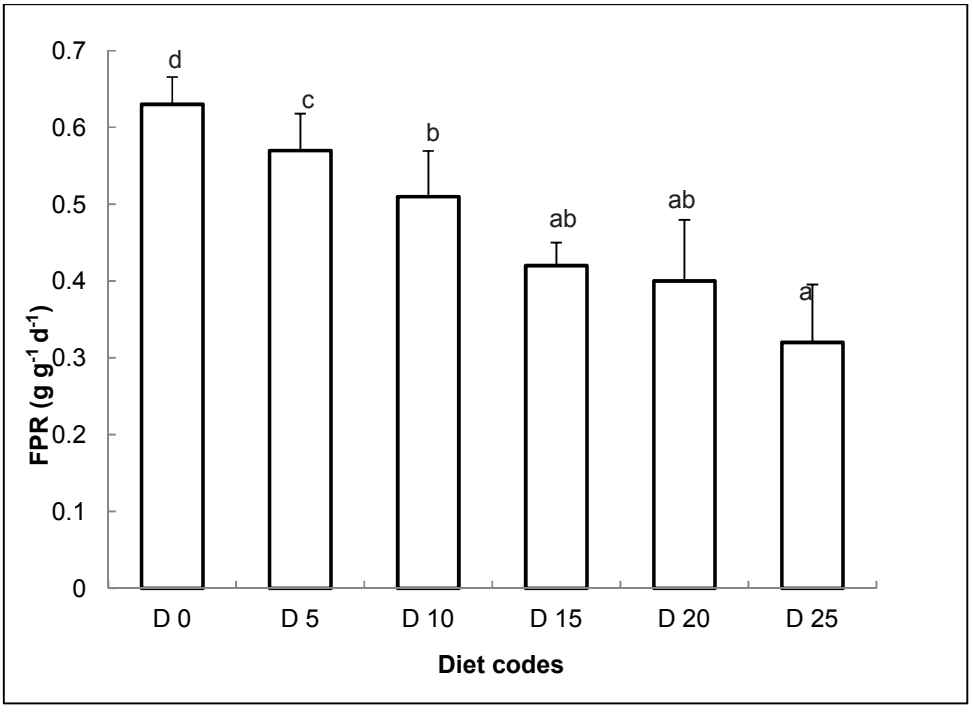

Fig. 3. Faeces production rate (FPR) of sea cucumbers (*Apostichopus japonicus*) fed different experimental diets

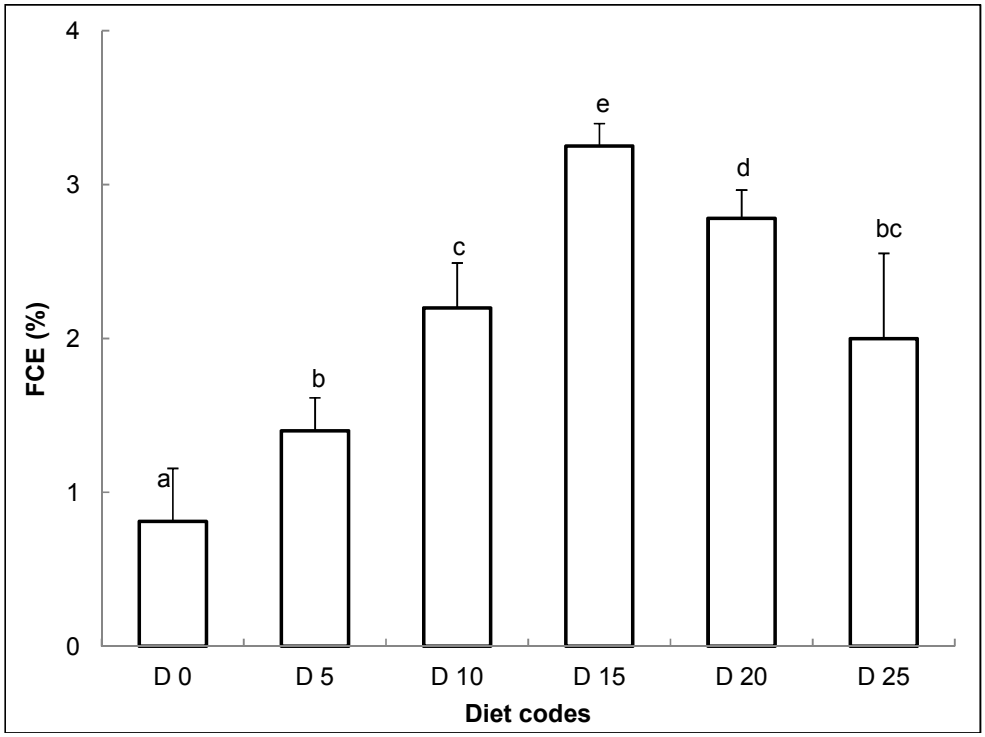

Fig. 4. Food conversion efficiency (FCE) of sea cucumbers (*Apostichopus japonicus*) fed different experimental diets

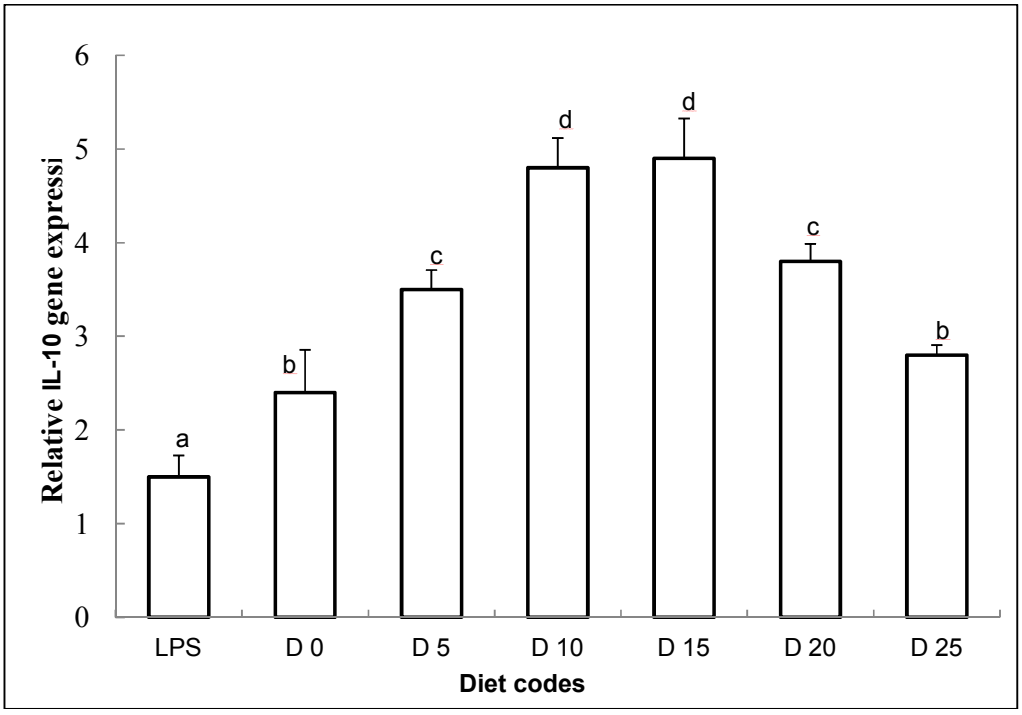

Fig. 5. IL-10 gene expression fed with different diets fed sea cucumbers extract

3.2 Experiment 2

3.2.1 Interleukin (IL)-10 expression level

In order to develop proper concentration of *Saccharina japonica* algae for *Apostichopus japonicus* diet, we evaluated IL-10 gene expression levels by stimulating mice splenocytes with different diet fed sea cucumber extracts. Splenocytes treated with sea cucumber extracts showed a significant increase of IL-10 gene expression in D_{10} and D_{15} diets than the other dietary treatments (Fig. 5). The highest IL-10 gene expression levels were found when the sea cucumbers were fed D_{15} diet.

4. DISCUSSION

Currently, *S. thunbergii* is usually used as main ingredients for sea cucumber *A. japonicus* feed, but a proper substitute of it is desiderated due to severe damage of wild *S. thunbergii* resource [19,20]. Naturally, gut contents of sea cucumber *A. japonicus* have lots of algae [12]. Arachidonic acid, eicosapentaenoic acid, the polyunsaturated fatty acids biomarker of sea weeds, *Saccharina japonica*, represents the higher mass fraction among polyunsaturated fatty acids in the body wall of sea cucumbers [31,32,33,34,2] which indicate that sea weeds, *Saccharina japonica* might have an excellent contribution to sea cucumber food source. The present study showed that specific growth rate (SGR) of sea cucumber *A. japonicus* fed with 15% *Saccharina japonica* containing diet was as high as those fed with D 0 (Control) diet (Fig. 1). From this result, we recommend that *Saccharina japonica* algae containing diet could perform better than the commercial feed (mainly used *S. thunbergii* algae).

The present results showed that among different experimental diets, SGR was much higher in sea cucumbers fed with D 15 (1.67% d^{-1}) diet compared to other diet. Liu [35] found that SGR of sea cucumbers were 0.83% d^{-1} and 0.72% d^{-1} when sea cucumbers were fed with diet of 70% *S. thunbergii* algae, 20% yellow soil,10% fish meal and 80% *S. thunbergii*, 20% sea mud respectively. Ying et al. [36] also found that SGR of *A. japonicus* was 1.40% d^{-1} when they were fed with 60% *S. thunbergii* algae and 40% sea mud. Sea cucumber perform better growth fed with *Saccharina japonica* algae containing diet [14,22]. Moreover, *Saccharina japonica* is widely used in abalone culture because of high growth rate [23]. Therefore, it is clear that the necessary proportion of *Saccharina japonica* (dry matter) with *S. thunbergii* algae was good for sea cucumber culture.

SGR showed significant differences among the different experimental diets treatments. In the present study, SGR of the sea cucumbers were gradually decreased when sea cucumber fed diets containing more than 15% *Saccharina japonica.* A prerequisite to utilise microalgal cell contents is to break the cell wall by one of the three mechanisms, i.e., acid hydrolysis, enzymatic digestion or mechanical trituration [37]. Tilapia is the species which rely on acid hydrolysis. They are particularly well adapted to disrupt cyanobacterium cell walls because pH values of their stomach fluid are as low as 1.25 or even 1.0 during active digestion [38,10,39]. Takeuchi et al. [40] reported that tilapia *Oreochromis niloticus* even showed normal growth rate and body composition when fed solely raw Spirulina. As echinoderm and depositor feeder, the structure and environment of the digestive tract of the sea cucumber *A. japonicus* are quite different from those of tilapia. Holothurians have no specialised organ for grinding or gland for chemical digestion, digestive enzyme activities are very low and have very little cellulose activity [41,42,43, 44,2,14]. So, sea cucumbers are capable to digest certain amount of cellulose content.

Ingestion rates of *A. japonicus* were greatly influenced by different dietary treatments. Santos et al. [45] and Hudson et al. [46] reported that a negative relationship was observed between protein content of diet and ingestion rate. In natural ecosystem, low nutrient and energy content of diets ingested by holothurians like sea cucumbers require to ingest large amount of diets in order to get net input of energy and vise versa [2,14]. In our study, IR of *A. japonicus* increased with decreasing protein level of different experimental diets. Anisuzzaman *et al.*, (2018) [2] also found same phenomenon in sea cucumbers that lower IR was related to higher protein content diets.

Interleukin (IL)-10 gene expression level were significantly affected by different experimental diets fed sea cucumbers extract. IL-10 is an anti-inflammatory cytokine, secreted by Treg cells, suppress immune responses and also can inhibit the synthesis of pro--inflammatory cytokines, protect against asthma disease [47]. In the present study, we found that (IL)-10 gene expression level significantly increased in the

spleen of mice induced by 10 and 15% *Saccharina japonica* diets fed sea cucumbers extracts. This result suggests that anti-inflammatory cytokine (IL-10) production significantly elevate by the administration of optimal concentration of *Saccharina japonica* fed sea cucumbers extract.

5. CONCLUSION

Results of this study suggest that diet containing 15% *Saccharina japonica* algae could increase the growth of juvenile sea cucumber *A. japonicus* and also elevate of IL-10 gene expression.

ETHICAL APPROVAL

All experimental protocols followed the guidelines of the Institutional animal Care and Use Committee of the Pusan National University and Gyeongsang National University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sloan NA. Echinorderm fisheries of the world: A review. Echinodermata (Proceedings of the fifth international echinoderm conference). A. A. Balkema Publishers, Rotterdam, Netherlands. 1984; 109–124.
2. Anisuzzaman M, Feng J, U-Cheol Jeong, Jong-Kuk C, Hak-Sun Y, Da-In L, Seok-Joong K. Effects of *Nannochloropsis* concentration in diet on growth, survival and anti-inflammatory cytokine (Interleukin-10) production of the sea cucumber *Apostichopus japonicus.* Turk J Fish Aquat Sci. 2018;18:567-575. Available:http://dx.doi.org/10.4194/1303-2712-v18_4_08
3. Conand C. Present status of world sea cucumber resources and utilization: An international overview. Advances in sea cucumber aquaculture and management (Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A. eds), FAO, Rome, Italy. 2004;13–23.
4. Uthicke C. Overfishing of holothurians: Lessons from the Great Barrier Reef. In: Advances in sea cucumber aquaculture and management (Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel JF, Mercier A. eds), FAO, Rome, Italy. 2004;163–171.
5. Chen JX. Present status and prospects of sea cucumber industry in China. Workshop on advances in sea cucumber aquaculture and management (ASCAM). FAO, Rome, Italy. 2004;463.
6. Sun HL, Liang MQ. Nutrient requirements and growth of the sea cucumber, *Apostichopus japonicus*. In: Lovatelli A, Conand C, Purcell S, Uthicke S, Hamel J, Mercier A. (Eds.), Advances in sea cucumber aquaculture and management. FAO Fisheries Technical 463, FAO, Rome, Italy. 2004;327–331.
7. Yuan X, Yang H, Zhou Y, Mao Y, Zhang T, Liu Y. The influence of diets containing dried bivalve feces and/or powdered algae on growth and energy distribution in sea cucumber *Apostichopus japonicus* (Selenka) (*Echinodermata*: *Holothuroidea*). Aquaculture. 2006;256:457–467. Available:http://dx.doi.org/10.1016/j.aquaculture.2006.01.029
8. Choe S. Study of sea cucumber: Morphology, ecology and propagation of sea cucumber. Kaibundou Publishing House, Tokyo, Japan. 1963;219.
9. Yingst JY. The utilization of organic matter in shallow marine sediments by an epibenthic deposit-feeding holothurian. J Exp Mar Biol Ecol. 1976;23:55–69.
10. Moriarty DJW. Feeding of *Holothuria atra* and *Stichopus chloronotus* on bacteria, organic carbon and organic nitrogen in sediments of the Great Barrier Reef. Aus J Mar Fresh Res. 1982;33:255–263. DOI: 10.1071/MF9820255
11. Michio K, Kengo K, Yasunori K, Hitoshi M, Takayuki Y, Hideaki Y, Hiroshi S. Effects of deposit feeder *Stichopus japonicus* on algal bloom and organic matter contents of bottom sediments of the enclosed sea. Marine Pollution Bulletin. 2003;47:118–125. DOI: 10.1016/S0025-326X(02)00411-3
12. Hauksson E. Feeding biology of *Stichopus tremulus*, a deposit feeding holothurian. Arsia 64, 155–160. Italy. 1979;25–38. Available:http://dx.doi.org/10.1080/00364827.1979.10411376
13. Zhang BL, Sun DL, Wu YQ. Preliminary analysis on the feeding habit of *Apostichopus japonicus* in the rocky coast waters off Lingshan Island. Mar Sci. 1995; 3:11–13.
14. Anisuzzaman M, U-Cheol Jeong, Feng J, Jong-Kuk C, Kamrunnahar K, Da-In L,

Hak-Sun Y, Seok-Joong K. Effects of different algae in diet on growth and interleukin (IL)-10 production of juvenile sea cucumber *Apostichopus japonicus.* Fish & Aqua Sci. 2017;20:1-8.
Available:http://dx.doi.org/10.1186/s41240-017-0069-5

15. Shi C, Dong SL, Pei SR, Wang F, Tian XL, Gao QF. Effects of diatom concentration in prepared feeds on growth and energy budget of the sea cucumber *Apostichopus japonicus* (Selenka). Aquacult Res. 2013; 46:609-617.
DOI: 10.1111/are.12206
16. Sui X. The main factors influencing the larval development and survival rate of the sea cucumber *Apostichopus japonicus*. Oceanolo et Limnolo Sinica. 1989;20:314–321.
17. Battaglene SC, Seymour EJ, Ramofafia C. Survival and growth of cultured juvenile sea cucumbers *Holothuria scabra*. Aquacult. 1999;178:293–322.
Available:http://dx.doi.org/10.1016/S0044-8486(99)00130-1
18. Lobban CS, Harrison PJ. Seaweed ecology and physiology. New York, USA: Cambridge University Press; 1994.
19. Yuan CY. Current status and development of feed in sea cucumber. Fish Sci. 2005; 24:54–56.
20. Wang FJ, Sun XT, Li F. Studies on sexual reproduction and seedling-rearing of *Sargassum thunbergii*. Mar Fish Res. 2006; 27:1–6.
21. Feng J, Anisuzzaman M, U-Cheol Jeong, Jong-Kuk C, Hak-Sun Y, Seung-Wan K, Seok-Joong K. Comparison of fatty acid composition of wild and cultured sea cucumber *Apostichopus japonicus*. Korean J Fish Aqua Sci. 2016;49:474-485.
Available:http://dx.doi.org/10.5657/KFAS.2016.0474
22. Anisuzzaman M, U-Cheol Jeong, Feng J, Kamrunnahar K, Jong-Kuk C, Da-In L, Hak-Sun Y, Seok-Joong K. Effects of *Ulva lactuca* and *Laminaria japonica* algae in prepared feeds on growth, survival, fatty acid compositions and Interleukin (IL)-10 production of sea cucumber *Apostichopus japonicus.* Int J Fish & Aqua Stu. 2018; 6(2):387-395.
23. Agatsuma Y. Food consumption and growth of the juvenile sea urchin *Strongylocentrotus intermedius*. Fish Sci. 2000;66:467–472.
24. Miyai K, Tokushige T, Kondo M. Suppression of thyroid function during ingestion of seaweed "Kombu" (*Laminaria japonoca*) in normal Japanese adults. Endocrine J. 2008;55(6):1103–1108.
DOI: 10.1507/endocrj.k08e-125
25. Bordbar S, Anwar F, Saari N. High-value components and bioactives from sea cucumbers for functional foods: A review Mar Drugs. 2011;9:1761–805.
26. Esmat AY, Said MM, Soliman AA, El-Masry KS, Badiea EA. Bioactive compounds, antioxidant potential, and hepatoprotective activity of sea cucumber (*Holothuria atra*) against thioacetamide intoxication in rats. Nutrition. 2013;29: 258–267.
DOI: 10.1016/j.nut.2012.06.004
27. Farshadpour F, Gharibi S, Taherzadeh M, Amirinejad R, Taherkhani R, Habibian A, Zandi K. Antiviral activity of *Holothuria* sp. a sea cucumber against herpes simplex virus type 1 (HSV-1). Eur Rev Med Pharmacol Sci. 2014;18:333–337.
28. Kiani N, Heidari B, Rassa M, Kadkhodazadeh M, Heidari B. Antibacterial activity of the body wall extracts of sea cucumber (*Invertebrata*; *Echinodermata*) on infectious oral streptococci. J Bas Clin Physiol Pharmacol. 2014;1:1–7.
DOI: 10.1515/jbcpp-2013-0010
29. Wijesinghe WA, Jeon YJ, Ramasamy P, Wahid ME, Vairappan CS. Anticancer activity and mediation of apoptosis in human HL-60 leukaemia cells by edible sea cucumber (*Holothuria edulis*) extract. Food Chem. 2013;139:326–331.
DOI: 10.1016/j.foodchem.2013.01.058
30. Lee DI, Park MK, Kang SA, Choi JH, Kang SJ, Lee JY, Yu HS. Preventive intra oral treatments of sea cucumber ameliorate ova-induced allergic airway inflammation. Am J Chin Med. 2016;44:1663-1674.
DOI: 10.1142/S0192415X16500932
31. Kharlamenko VI, Zhukova NV, Khotimchenko SV, Svetashev VI, Kamenev GM. Fatty-acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). Mar Ecolo Pros Seri. 1995;120:231–241.
32. Budge SM, Parrish CC, Mckenzie CH. Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. Mar Chem. 2001;76:285–303.

Available:http://dx.doi.org/10.1016/S0304-4203(01)00068-8

33. Gao F, Yang HS, Xu Q. Seasonal variations of fatty acid composition in *Apostichopus japonicus* body wall. Mar Sci. 2009;33:15–19.
DOI: 10.1007/s00343-011-0041-7
34. Ce S, Shuanglin D, Surui P, Fang W, Xiangli T, Qinfeng G. Effects of diatom concentration in prepared feeds on growth and energy budget of the sea cucumber *Apostichopus japonicus* (Selenka). Aquacult Res. 2015;46:609–617.
DOI: 10.1111/are.12206
35. Liu Y. Effects of dietary diets on growth and energy budget of sea cucumber, *Apostichopus japonicus* Selenka. Doctor dissertation of Ocean University of China; 2010.
Available:http:// www.cnki.net. (in Chinese, with English abstract)
36. Ying L, Shuanglin D, Xiangli T, Fang W, Qinfeng G. Effects of dietary sea mud and yellow soil on growth and energy budget of the sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture. 2009;286:266–270.
Available:http://dx.doi.org/10.1016/j.aquaculture.2008.09.029
37. Bitterlich G. Digestive process in silver carp *Hypophthalmichthys molitrix* studied in vitro. Aquacult. 1985;50:123–131.
38. Caulton MS. The importance of pre-digestive food preparation to *Tilapia rendalli* Boulanger when feeding on aquatic macrophytes. Trans Rhod Sci Assoc. 1976;57:22–28.
39. Payne AI. Gut pH and digestive strategies in estuarine grey mullet Mugilidae and tilapia Cichlidae. J Fish Res Board Can. 1978;13:627–629.
40. Takeuchi T, Lu J, Yoshizaki G, Satoh S. Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw Spirulina. Fish Sci. 2002;68:34–40.
41. Massin C. Food and feeding mechanisms: Holothuroidea. In: Echinoderm Jangoux, M, Lawrence, JM, editors. A. A. Balkema Publishers, Rotterdam, Netherlands. 1982; 115–120.
42. Cui LB, Dong ZN, Lu YH. Histological and histochemical studies on the digestive system of *Apostichopus japonicus*. Chin J Zool. 2000;35:2–4.
43. Fu XY. Study of proteases from digestive tract of sea cucumber (*Stichopus japonicus*). (Master Thesis of Ocean University of China (in Chinese with English abstract). 40. Jiang LX, Yang N, Li J, Wang WQ, Wang RJ, Liu, J. Effect of temperature and pH on the activities of digestive enzymes in *Apostichopus japonicus*. Oceanol. Limnol Sin. 2007;38: 476–480.
44. Wang JQ, Tang L, Xu C, Cheng JC. Histological observation of alimentary tract and annual changes of four digestive enzymes in sea cucumber (*Apostichopus japonicus*). Fish Sci. 2007;26:481–484.
45. Santos V, Billett DSM, Rice, AL, Wolff GA. Organic matter in deep-sea sediments from the porcupine abyssal plain in the North-east Atlantic Ocean: 1. Lipids. Deep-sea Res. 1994;41:789–819.
Available:https://doi.org/10.1016/0967-0637(94)90077-9
46. Hudson IR, Wigham BD, Tyler PA. The feeding behavior of a deep-sea holothurian, *Stichopus tremulus* (Gunnerus) based on *in situ* observation and experiments using a remotely operated vehicle. J Exp Mar Biol Ecol. 2004;301:75–91.
Available:http://dx.doi.org/10.1016/j.jembe.2003.09.015
47. Lee DI, Park MK, Kang SA, Anisuzzaman M, U-Cheol J, Feng J, Seok-Jeong K, Lee JY, Yu HS. Sea cucumber lipid-soluble extra fraction prevents ovalbumin-induced allergic airway inflammation. J Med Food. 2018;21(1):21–29.

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