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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*

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Abstract
Two experiments were conducted to investigate the effects of *Ulva lactuca* and *Laminaria japonica* algae in diets on growth, survival, fatty acid compositions and interleukin-10 productions of sea cucumber. In the first experiment, 56 days feeding trail was conducted to evaluate the growth performance, survival and fatty acid compositions of the sea cucumber fed one of the four experimental diets containing diet 1 (Control diet), diet 2 (*Ulva lactuca*), diet 3 (*Laminaria japonica*) and diet 4 (*Ulva lactuca* + *Laminaria japonica*) in recirculating aquaculture system. Results showed that survival was not significantly different among the dietary treatments and specific growth rate (SGR) of sea cucumber fed the diet 3 (1.82 % d^{-1}) was significantly higher than that of sea cucumber fed the other diets ($P < 0.05$). Fatty acids content of sea cucumber were significantly different among the diet groups. The highest amount of eicosapentaenoic acid, docosahexaenoic acid, palmitoleic acid and branched chain fatty acids were found when sea cucumber fed diet 3. The second experiment was conducted to determine IL-10 gene expression where mice splenocytes were stimulated with 10 µg/ml of each diet fed sea cucumber extracts for 2 hours. The result showed that IL-10 gene expression levels were significantly higher in diet 3 fed sea cucumber extracts compared to other experimental diets. The results of two experiments suggest that dietary inclusion with 14% *Laminaria japonica* algae may improve growth, n-3 polyunsaturated fatty acids, branched chain fatty acids of juvenile sea cucumber and could up-regulate of IL-10 gene expression.

Keywords: Sea cucumber (*Apostichopus japonicus*), algae, growth**,** fatty acids, Interleukin (IL)-10

1. Introduction
Among echinoderms, sea cucumber *Apostichopus japonicus* is the commercially important mariculture species in Asia [17]. Market demand for this species has increased because of its aphrodisiac and curative properties [23]. However, wild production of sea cucumbers has declined due to overexploitation and pollution [7]. Depletion of wild production together with high commercial value has encouraged the people to develop aquaculture methods for holothurians, especially *A. japonicus* [7, 55].
Successful culture of juvenile sea cucumbers requires proper knowledge about feed intake behavior and dietary requirements [46]. However, little is known regarding which artificial diets are capable of inducing rapid growth and healthy conditions of commercially important sea cucumbers [46, 55, 57].
Sea cucumbers have the ability to synthesize long-chain polyunsaturated fatty acids in diets. Fatty acids of sea cucumber play essential roles in the metabolic activities of organisms [40, 47]. 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 [14, 38] and arachidonic acid is responsible for blood clotting in wound healing [18]. It was well known that fatty acids compositions of consumers tended to be flexible due to the shift in the fatty acids compositions of diets [26].
Sea cucumber is deposit-feeders that ingest sediment with organic matter [31, 29] and their gut contents have lots of macroalgae and seaweed, shell fragments from mollusks, crustaceans and barnacles, echinoderm ossicles, many pelagic and benthic foraminifera and diatoms [15, 56].

Traditionally, sea cucumbers are cultured in ponds without artificial feeds. But recently more and more farmers have started to feed the sea cucumbers with formulated diets to increase production [44]. Formulated diets for sea cucumbers are commonly made of macroalgal powder and sea mud. As one of the high quality feeds for sea cucumber, *Sargassum thunbergii* is widely used to make artificial feed. More and more *S. thunbergii* has been harvested in recent years with the rapid expansion of sea cucumber farming scale, which results in severe damage to *S. thunbergii* resource [54, 48]. Meanwhile, sea cucumber contained high n-6 fatty acids, low n-3 fatty acids and lower ratio of n-3/n-6 fatty acids when fed with commercial feed [10]. 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 good quality sea cucumber. Several researchers reported that juvenile sea cucumbers fed commercially available dried powdered macroalgae (*Ulva lactuca*, *Laminaria japonica*, *Sargassum thunbergii*, *Sargassum polycystum*) and sea mud exhibited significant growth [3, 25, 58]. In our study, we used *Ulva lactuca*, *Laminaria japonica* as a partial alternative source of *Sargassum thunbergii* to produce significant growth and good quality sea cucumber. *Ulva lactuca*, *Laminaria japonica* are popular and cheaper algae in Korea and widely used in the culture of sea urchins and abalone [2, 35].

Sea cucumbers have many therapeutic effects against various diseases [5, 11]. Moreover, sea cucumber extracts have potent biological effects and have antiviral, anti-cancer, antibacterial, anti-oxidant, anti-inflammation effects [8, 9, 20, 50]. In China and Malaysia, sea cucumbers have been traditionally used for the remedy of different inflammatory diseases like asthma. Interleukin (IL)-10 is a potent anti-inflammatory cytokine that down regulates the synthesis of Th1 (T helper 1) and Th2 (T helper 2) associated cytokines, chemokines, and inflammatory enzymes. It plays a vital role for the mitigation of allergic responses. But till now, there are no reports demonstrating the effect of different algae in sea cucumber on IL-10 production.

In the present study, the effects of *Ulva lactuca* and *Laminaria japonica* algae in prepared feeds on growth, survival, fatty acid compositions and Interleukin (IL)-10 productions of the sea cucumber were examined.

2. Materials and Methods

2.1 Experiment 1

2.1.1 Animal source and acclimation

The experiment was carried out for 56 days in the wet laboratory of Marine Biology and Aquaculture, Gyeongsang National University, Republic of Korea. Sea cucumbers used in this experiment were collected from the Goseong Sea cucumber farm. Prior to the experiment, sea cucumbers were transferred to the laboratory in fiberglass aquaria and acclimated for 7 days at 19 °C. During acclimation period, sea cucumbers were fed with algal powder (*S. thunbergii*) and sea mud.

2.1.2 Experimental diets

Four experimental diets designed as diet 1 (control), diet 2, diet 3 and diet 4 were prepared. Ingredients and proximate compositions of experimental diets were presented in Table 1. Diet 1 was used as the control diet where 15% *Sargassum thunbergii* and 20% wheat flour were used. Wheat flour was replaced by 14% *Ulva lactuca* and 6% *Nannochloropsis oculata* for diet 2, 14% *Laminaria japonica* and 6% *Nannochloropsis oculata* for diet 3 and 7% *Ulva lactuca*, 7% *Laminaria japonica* and 6% *Nannochloropsis oculata* for diet 4. All ingredients were ground into fine powder through a 200 μm mesh, thoroughly mixed and stored at −20 °C.

Table 1: Ingredients and composition of experimental diets for sea cucumber (% dry matter basis)

Ingredients	Diet 1 (control)	Diet 2	Diet 3	Diet 4
Ulva lactuca powder	0	14	0	7
Laminaria japonica powder	0	0	14	7
Nannochloropsis oculata powder	0	6	6	6
Wheat flour	20	0	0	0
Seaweed powder	10	10	10	10
Soybean meal	8	8	8	8
Shell fish powder	8	8	8	8
Shell powder	2	2	2	2
Calcium phosphate	2	2	2	2
Yeast protein	5	5	5	5
Soyabean lecithin	4	4	4	4
Mineral premix[a]	0.5	0.5	0.5	0.5
Vitamin premix[b]	0.5	0.5	0.5	0.5
Sea mud	40	40	40	40
Proximate composition (%)				
Crude protein	16.43	17.23	16.94	17.09
Crude lipid	1.76	2.97	2.59	2.78
Ash	40.89	44.61	45.59	45.1

[a] Mineral premix (g kg-1 premix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 200.0; KH_2PO_4, 130.0; Ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 10; Ca-lactate, 25.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KIO_3, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0

[b] Vitamin premix (g kg-1 premix): ascorbic acid, 92.7; α-tocopheryl acetate, 14.5; thiamine hydrochloride, 7; riboflavin, 7.0; pyridoxine hydrochloride, 1.4; niacin, 27.8; Ca-D-pantothenate, 9.7; myo-inositol, 139.1; D-biotin, 0.5; folic acid, 0.5; p-amino benzoic acid, 13.9; menadione, 4.2; retinyl acetate, 0.65; cholecalciferol, 0.8; cyanocobalamin, 0.004.

2.1.3 Experimental design

After 2days starvation, 80 sea cucumbers with initial wet body weights of 10.19 ± 0.09 g were randomly selected from acclimatized sea cucumbers and placed in equal number into 16 fiberglass aquaria (45×60×50 cm^3) to form 4 groups in tetraplicate. The initial body weights of sea cucumber were measured individually as described in Battaglene *et al*. (1999) [3]. The 4 groups were fed once daily (17:00 h) with different experimental diets such as Diet 1, Diet 2, Diet 3, and Diet 4 respectively.

2.1.4 Rearing conditions

During the experiment, aeration was provided continuously and to ensure water quality 2/3 volume of the water in each aquarium was exchanged every day. Seawater temperature was controlled at 18 ± 1.0 °C. Temperature of water bath was regulated by a thermostat, which controlled the on / off switch of a 2000-W electric heater. Dissolved oxygen was maintained above 5.0-7.0 mg/ L, the levels of ammonia in the water of aquaria were less than 0.25 mg/ L. Other conditions were salinity 32 ± 1 psu; pH 7.7–8.3; photoperiod 24 h dark. The aquaria were wrapped in black carbon paper to maintain continuous dark period. The longer and darker light conditions are better for a population of *A. japonicus* to

induce sea cucumbers to feed continuously.

2.1.5 Procedure and sample collection

Sixteen 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. During the experiment, sea cucumbers were fed once per day (at about 17:00 h). Uneaten feed was collected from aquaria by siphon at 24h later and dried at 65°C to constant weight. Sea cucumbers feces were also collected by siphon once per day (16:00h). The feces were dried at 65°C to constant weight and those from each aquarium were pooled for further analysis. At the end of 8 weeks experiment, all the experimental sea cucumbers were deprived of food to clear their guts for 2 days, weighed and then dried at 65°C until constant weight was achieved.

2.1.6 Data calculation

Survival rate (SR), specific growth rate (SGR), ingestion rate (IR), feces 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 5 sea cucumbers in each aquarium; T is the experimental period; I is the dry weight of the total feed ingested and F is the dry weight of feces.

2.1.7 Fatty acids analysis

The sea cucumbers were cleaned to remove the visceral organs and body fluid before homogenization. Total lipids of sea cucumber were extracted according to the Bligh and Dyer method (1959) [4] by using solvent mixture consisting of chloroform and methanol (2:1, v/v). After phase equilibration, the lower chloroform layer was removed and total lipids were extracted by removing solvent using a rotary evaporator (R-114, BUCHI, Swiss) at 38°C. 100 mg of extracted total lipid were put into a capped tube and added 1.5 ml 0.5 N NaOH-methanol solution. The sample was mixed by vortex and heated 100°C for 8 minutes for saponification. After cooling, methylation was done by using a fatty acid methyl ester (FAME) with BF_3-methanol. Then the sample was dissolved into 2 ml iso-octane and fatty acids were analyzed via GC technique using gas chromatography (Clarus 600, Perkin Elmer, USA) equipped with capillary column (Omegawax-320, 30 m × 0.25 mm I.D., Supelco Co., Bellefonte, PA, USA). The operating parameters were as follows: carrier gas =helium; detector (FID) temperature =270°C; injection temperature = 250°C; column temperature =180°C for 8 min, programmed to increase at 3°C/min up to 230°C with a final holding time of 10 min; split injection at 1:50 ratio. Menhaden oil was used as standard. Each of the specific fatty acid methyl ester peaks was identified by determining its equivalent chain length with reference to the known standard [1].

2.2 Experiment 2

2.2.1 Preparation of sea cucumber extract

At first, ***our*** experimental sea cucumbers were cleaned and removed the visceral organs. After that, sea cucumbers were cut into small pieces and homogenized. 180 g of samples were boiled in 360 ml distilled water for 20 min. After removing solid materials from the water, the boiled water was vaporized using a microwave until the mixture was reduced by 50%. 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 that, the supernatant was discarded. The extract pellet was washed with 70% ethyl alcohol and centrifuged under the same conditions. The supernatant was discarded and the pellet was evaporated under a vacuum. The final extracts were prepared by re-suspending the pellet in 20mL distilled water [22].

2.2.2 IL-10 gene expression

To evaluate IL-10 gene expression, mice splenocytes were stimulated with 10 µg/ml of each experimental diet fed sea cucumber extracts for 2 h. After mouse experiments, the lung was obtained from sacrificed each mice and used for RNA isolation. The total RNAs were isolated by Qiazol reagent (Qiagen Scienece, USA) according to the manufacturer's protocols. 2 µg of total RNAs were transcribed using M-MLV reverse transcriptase (Promega, USA), according to the manufacturer's protocols. IL-10 mRNA expression levels were synthesis by real-time PCR using the iCyclerTM (Bio-Rad Laboratories, Hercules, CA, USA). GAPDH was used for reference gene. IL-10 and GAPDH primer sequence are previously described [22].

2.3 Statistical analysis

Statistical analysis was performed using software SPSS 18.0 with possible differences among diet treatments being tested by one-way ANOVA. Duncan's multiple range tests were used to analyze the differences among treatments. Differences were considered significant at a probability level of 0.05.

3. Results

3.1 Experiment 1

3.1.1 Growth and Survival

The growth performance and survival of sea cucumber are shown in Table 2. Survival of sea cucumber was not affected by different experimental diets. The sea cucumbers in the different treatments showed high survival rates (100%). For all treatments during this period, no sea cucumbers were died at the end 8-week feeding trial. There was a significant difference in growth performance of sea cucumber fed the different experimental diets. At the end of the experiment, final wet and dry body weights of sea cucumbers showed the highest value for the diet 3 group and the lowest value for the diet 1 group ($P<0.05$).

The highest SGR (1.82% d^{-1}) was observed in sea cucumber fed the diet 3. SGR of sea cucumbers fed the diet 1 was significantly ($P < 0.05$) lower than other experimental diets (Fig. 1).

Table 2: Initial and Final wet weight (WW), dry weight (DW) of sea cucumber fed different experimental diets (mean±SE)

Experimental Diets	Initial WW (g)	Initial DW (g)	Final WW (g)	Final DW (g)	Survival (%)
Diet 1	10.19±0.10	1.07	17.71±0.98	1.86	100
Diet 2	10.29±0.08	1.08	27.29±0.89	2.87	100
Diet 3	10.10±0.12	1.06	28.17±0.78	2.96	100
Diet 4	10.19±0.09	1.07	24.22±0.60	2.54	100

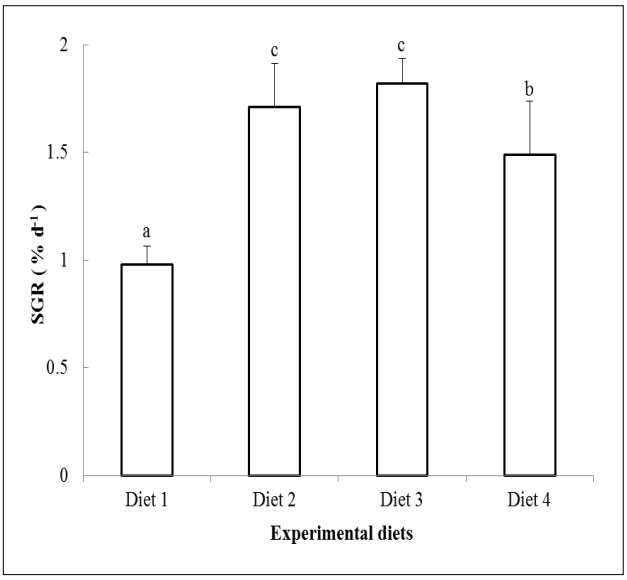

Fig 1: Specific growth rate of sea cucumber fed different experimental diets. Different letters indicate significant differences ($P < 0.05$) between treatments within the same group, and bars represent standard errors.

3.1.2 Ingestion rate and feces production rate

Both ingestion rates (Fig. 2) and feces production rates (Fig. 3) of the sea cucumbers showed significant differences among different diet treatments. The highest IR (0.71 g g^{-1} d^{-1}) and FPR (0.65 g g^{-1} d^{-1}) was observed in sea cucumber fed the diet 1. Sea cucumbers fed with diet 2 and diet 4 showed significantly lower IR (0.45 and 0.49 g g^{-1} d^{-1} respectively) and FPR (0.39 and 0.42 g g^{-1} d^{-1} respectively) than those fed other diets ($P < 0.05$).

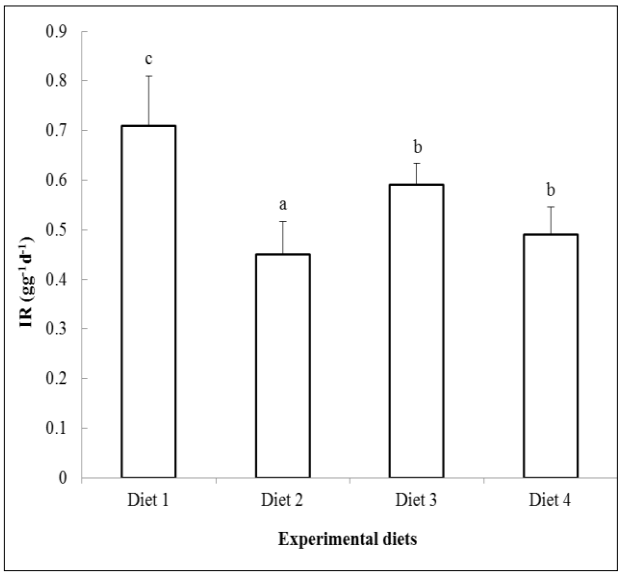

Fig 2: Ingestion rate (IR) of sea cucumber fed different experimental diets. Different letters indicate significant differences ($P < 0.05$) between treatments within the same group, and bars represent standard errors.

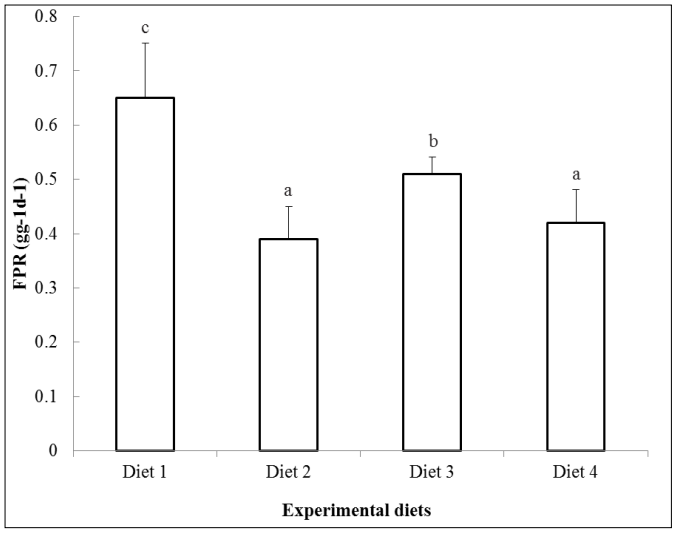

Fig 3: Feces production rate (FPR) of sea cucumber fed different experimental diets. Different letters indicate significant differences ($P < 0.05$) between treatments within the same group, and bars represent standard errors.

3.1.3 Food conversion efficiency

Food conversion efficiency (FCE) is presented in Figure 4. FCE was significantly different among different diet treatments. FCE of the sea cucumbers fed with diet 2 was 3.56%, which was significantly higher than those fed with other diets ($P < 0.05$). Sea cucumbers fed diet 1 showed the lowest FCE (1.38%).

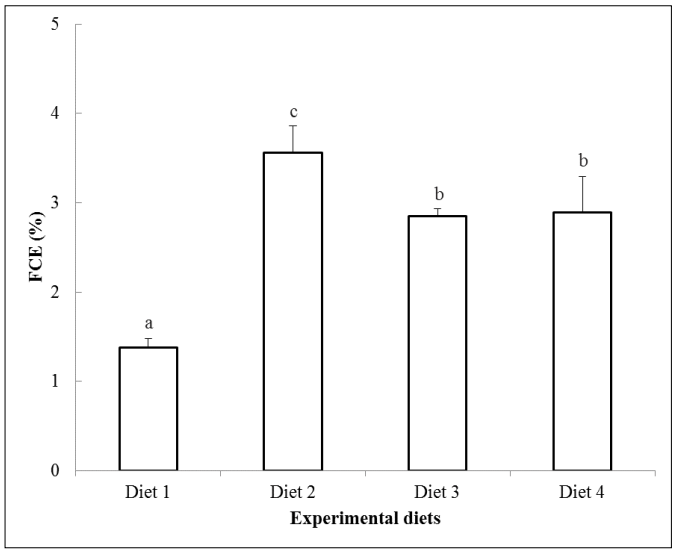

Fig 4: Food conversion efficiency (FCE) of sea cucumber fed different experimental diets. Different letters indicate significant differences ($P < 0.05$) between treatments within the same group, and bars represent standard errors.

3.1.4 Fatty acid compositions

Table 3 shows the fatty acid compositions of sea cucumbers. Fatty acid compositions of sea cucumbers were affected by different experimental diets. Fatty acids contained in the sea

cucumber *Apostichopus japonicus* at 3% or more of the total fatty acids were 16:1n-7, 16:4n-3, 18:0, 18:1n-7, 18:1n-9, 18:2n-6, 20:1n-11, 20:4n-6, 20:5n-3 and 23:1n-9 in all diet groups. The highest amount of eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and palmitoleic acid (16:1n-7) were found when sea cucumber fed diet 3. A considerable amount of branched chain fatty acids (BCFA) of sea cucumbers were found in all diet groups.

Table 3: Fatty acids compositions of sea cucumber fed different experimental diets (%) (mean ± SD)

Fatty acids	Diet 1	Diet 2	Diet 3	Diet 4
12:00	0.02±0.02	0.12±0.03	0.04±0.01	0.06±0.03
iso- 13:00	0.04±0.01	0.15±0.02	0.11±0.03	0.08±0.02
13:00	0.02±0.01	0.08±0.01	0.06±0.02	0.04±0.00
iso-14:0	0.18±0.04	0.13±0.05	0.30±0.03	0.22±0.07
14:00	0.81±0.03	0.92±0.03	1.38±0.05	1.36±0.09
iso-15:0	0.22±0.03	0.44±0.01	0.68±0.03	0.44±0.03
anteiso-15:0	0.78±0.02	0.74±0.02	0.87±0.04	0.52±0.04
15:00	0.39±0.02	0.22±0.05	0.39±0.01	0.37±0.02
iso-16:0	0.12±0.01	0.24±0.00	0.33±0.02	0.21±0.01
PRISTANE	0.02±0.00	0.59±0.04	0.63±0.04	0.35±0.04
16:00	5.15±0.09	3.53±0.10	2.39±0.08	4.98±0.06
16:1n-9	0.72±0.04	0.50±0.05	0.80±0.03	0.81±0.09
16:1n-7	3.45±0.08	8.49±0.10	9.93±0.15	4.53±0.12
TME-16:0	0.89±0.01	0.45±0.04	1.01±0.07	0.05±0.00
16:1n-5	0.32±0.02	0.20±0.03	0.31±0.04	0.31±0.02
16:2n-9	0.12±0.03	0.09±0.01	0.15±0.03	0.15±0.01
16:2n-4	0.33±0.05	0.12±0.03	0.13±0.01	0.13±0.01
17:00 + PHYTANE	0.05±0.01	0.48±0.05	0.71±0.06	0.66±0.03
16:3n-4	0.05±0.00	0.05±0.02	0.03±0.00	0.04±0.01
16:3n-3	0.37±0.06	0.33±0.06	0.37±0.03	0.41±0.08
16:3n-1	0.07±0.02	0.08±0.00	0.08±0.02	0.08±0.03
16:4n-3	5.30±0.08	5.98±0.11	4.93±0.10	4.87±0.08
16:4n-1	0.22±0.02	0.16±0.01	0.09±0.010	0.14±0.04
18:00	4.32±0.04	4.12±0.09	4.30±0.11	4.75±0.15
18:1n-9	5.92±0.09	6.15±0.14	8.33±0.16	7.27±0.18
18:1n-7	4.70±0.07	3.82±0.09	4.30±0.08	4.77±0.05
18:1n-5	0.13±0.01	0.19±0.03	0.19±0.01	0.16±0.02
18:2n-7	0.90±0.02	0.96±0.02	0.55±0.03	0.66±0.03
18:2n-6	5.19±0.05	4.76±0.08	5.63±0.10	5.58±0.14
18:2n-4	0.22±0.02	0.17±0.01	0.17±0.02	0.19±0.02
18:3n-6	1.02±0.02	0.71±0.04	0.81±0.05	0.94±0.07
18:3n-4	0.03±0.00	0.24±0.02	0.19±0.03	0.36±0.02
18:3n-3	0.30±0.02	0.47±0.05	0.43±0.04	0.13±0.03
18:4n-3	0.18±0.04	0.17±0.01	0.19±0.03	0.10±0.05
18:4n-1	0.10±0.02	0.18±0.03	0.12±0.01	0.06±0.02
20:00	1.98±0.05	1.89±0.04	1.87±0.03	2.04±0.05
20:1n-11	6.80±0.09	6.29±0.11	4.65±0.08	5.86±0.13
20:1n-9	2.48±0.05	2.51±0.07	3.14±0.06	2.98±0.09
20:1n-7	0.72±0.03	0.57±0.02	0.60±0.04	0.71±0.05
20:1NMID	0.27±0.02	0.34±0.03	0.18±0.02	0.25±0.02
20:2n-6	2.54±0.04	2.33±0.09	1.93±0.05	2.34±0.08
20:3n-6	1.60±0.01	1.36±0.05	0.74±0.01	1.46±0.05
20:4n-6	16.71±0.16	12.79±0.18	10.21±0.13	15.62±0.14
20:3n-3	0.15±0.03	0.17±0.02	0.22±0.03	0.27±0.01
20:4n-3	0.11±0.01	0.13±0.01	0.11±0.02	0.10±0.02
20:5n-3	3.48±0.04	6.82±0.08	8.16±0.11	4.42±0.07
22:00	1.67±0.02	1.63±0.05	1.33±0.03	1.45±0.07
22:1n-11, 13	1.28±0.03	1.08±0.01	0.86±0.04	0.98±0.03
22:1n-9	1.47±0.04	1.27±0.04	1.35±0.06	1.51±0.06
22:1n-7	2.26±0.05	1.84±0.03	1.41±0.03	1.93±0.05
21:5n-3	0.32±0.03	0.31±0.02	0.42±0.02	0.34±0.02
23:1n-9	9.32±0.17	7.71±0.14	5.77±0.13	7.56±0.18
22:5n-6	1.11±0.03	0.82±0.03	0.66±0.04	0.89±0.02
22:5n-3	0.34±0.05	0.49±0.02	0.61±0.03	0.48±0.01
22:6n-3	2.73±0.07	3.64±0.09	4.87±0.08	3.05±0.10
Σ BCFA	2.58	3.57	4.82	2.78
Σ n-3 PUFA	12.99	18.51	19.69	14.18
Σ n-6 PUFA	28.18	22.77	19.98	26.83
n-3/n-6	0.46	0.81	0.99	0.53

Σ BCFA, Sum of the branched chain fatty acids; Σ n-3 PUFA, Sum of the n-3 polyunsaturated fatty acids; Σ n-6 PUFA, Sum of the n-6 polyunsaturated fatty acids

3.2 Experiment 2

3.2.1 Interleukin (IL)-10 expression level

In order to establish proper algae for sea cucumber diet, we synthesize IL-10 gene expression levels. Splenocytes were stimulated with each experimental diet fed sea cucumber extracts for 2 hours. Results showed that IL-10 gene expression levels were significantly increased in diet 2 and diet 3 compared to other experimental diet (Fig. 5). The highest IL-10 gene expression levels were found when sea cucumber fed 14% *L. japonica* containing diet. However, IL-10 gene expression levels were not increased by diet 1 and diet 4 and have no significant differences. These results suggest that 14% *L. japonica* algae could up-regulate of IL-10 gene expression.

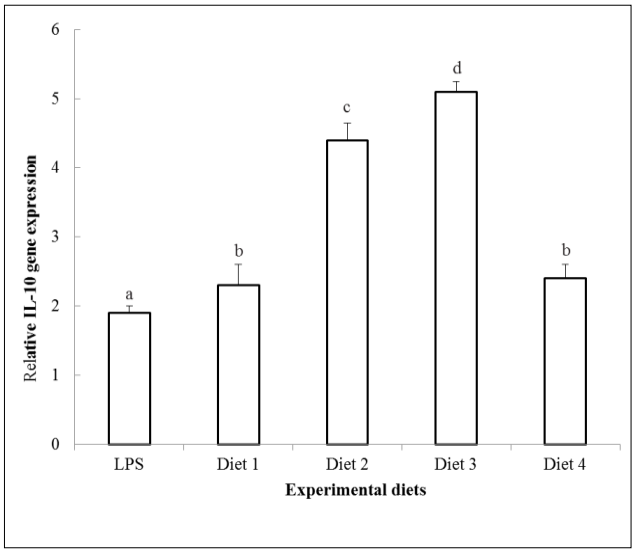

Fig 5: IL-10 gene expression. IL-10 gene expressions were significantly increased by administration of diet 3.

4. Discussion

In all treatments, all sea cucumber were alive and survival rates of sea cucumbers were excellent (100%) and were higher than the rates reported in previous similar studies [13, 45, 57]. The results showed that sea cucumber might have the ability to tolerate the different algae such as *Ulva lactuca*, *Laminaria japonica*, *Nannochloropsis oculata* in diet.

Different types of algae such as *Sargassum thunbergii*, *Sargassum polycystum*, *Laminaria japonica*, *Spirulina platensis*, *Ulva lactuca*, *Undaria pinnatifida* and *Pyropia spheroplasts* have been used to study the nutritional requirements of sea cucumber [24, 41, 42, 43, 46, 51, 55]. Most researchers have used *S. thunbergii* or *S. polycystum* as main feed ingredient in land-based intensive culture systems [3, 58]. However, in our study of various experimental diets, the SGR was much higher in sea cucumbers fed 14% *L. japonica* containing diets compared to other diets (Fig. 1). Zhu *et al*. (2007) [58] reported that the SGR of sea cucumbers decreased when they were fed *L. japonica*. Smaller (0.49 g) size sea cucumbers were used in their experiment compared to ours (10.19 g) and this might reasons for different results. Yanagisawa (1998) [52] and Yingst (1976) [53] observed that different size of sea cucumber may have different diet choice and nutrition requirement. Some of our results agreed with many researchers. Seo *et al*. (2011) [42] reported that sea cucumbers fed 20% *L. japonica* and 20% *S. thunbergii* containing diet grew much better than that only eating *S. thunbergii* (40%) diet. Xia *et al*. (2012) [51] also found that similar results when sea cucumber fed *L. japonica* or *U. lactuca* algae containing diet.

Algae are an important food source for sea cucumbers. In our studies the protein content of diet 3 were lower than that of diet 2, diet 4 but the results showed that SGR of the sea cucumbers fed diet 3 were significantly higher than that of those fed other experimental diets. The results indicate that some other factors responsible for the nutrient effects of the algae besides the protein, lipid and energy contents. Holothurians like sea cucumbers have no specialized organ for grinding or gland for chemical digestion [27], digestive enzyme activities are very low and have very little cellulose activity [49]. Therefore, sea cucumbers are able to assimilate a specific amount of cellulose content.

In the present studies, the higher SGR of sea cucumber was observed in the treatments fed with 14% *L. japonica* containing diet though protein and lipid contents of these diet was comparatively low. *L. japonica* is multicellular, filamentous, cell walls are easy to broken and have comparatively lower cellulose content [6, 30]. So, sea cucumber could easily digest and took full advantages of nutrients in *L. japonica* algae.

Ingestion rates (IR) of sea cucumber were significantly affected by different experimental diets. There was a negative relationship between IR and the protein level. In natural ecosystem, low nutritional value of sediment consumed by deposit feeders means those animals need to consume large amounts of sediment in order to obtain a net input of energy [39, 16]. Vise versa, when food quality becomes better, in certain season for example, internal appetite regulation would work actively to decrease food ingestion. 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 *et al*. (1998) [28] reported that sea urchin (*Strongylocentrotus franciscanus*), prepared diets of different protein levels resulted in different ingestion rate. Otero-Villanueva *et al*. (2004) [33] also found in *Psammechinus miliaris* that lowest ingestion rate was related to high energetic diet.

In this study, the highest amount of branched chain fatty acids (BCFA) and palmitoleic acid (16:1n-7) were found in sea cucumbers fed diet 3. BCFA play a vital role to increase the expression of anti-inflammatory cytokine IL-10 and protect against necrotizing enterocolitis (NEC) in the rat pup model [37]. Nishimura *et al*. (2016) [32] reported that palmitoleic acid might have anti-allergic and anti-inflammation activity, this activity will be use to production of medicine or healthy improve food.

n-3 PUFA is very important to protect the inflammatory diseases. Long-chain polyunsaturated fatty acids especially eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) can reduce the inflammation, cancer, and arthritis [14, 38]. According to Jingjing Li *et al*. (2013) [19] intakes of long chain n-3 PUFAs are inversely co-related with the incidence of inflammatory disease like asthma in American young adults. Our results showed that long-chain polyunsaturated fatty acids especially EPA and DHA were significantly higher in diet 3 group sea cucumbers.

Regulatory T cells (Treg cells), known as suppressor T cells, are subpopulation of T cells and modulate the immune systems [21]. IL-10 is one of the Treg cells and known as a key regulator of immunity to many infection or inflammatory disease [12]. For instance, high levels of IL-10 have protective effect against asthma disease [36]. Conversely, lack of IL-10

promotes cell apoptosis during virus infection in small intestine [34]. In previous study, we already investigated that administration of sea cumber total extract can up-regulate of IL-10 and ameliorate asthma disease [22]. Here, we suggested 14% *L. japonica* algae to increase Interleukin (IL)-10 gene expression.

5. Conclusions

In conclusion, the present study showed that dietary inclusion with 14% *Laminaria japonica* algae may improve growth, branched chain fatty acids, n-3 polyunsaturated fatty acids of juvenile sea cucumber and could up-regulate of IL-10 gene expression.

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