Thesis for the Degree of Master of Science

Dietary substitution effect of *Undaria pinnatifida* with onion extract by-product on growth, chemical composition and air exposure stress of juvenile abalone (*Haliotis discus*, Reeve 1846)

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August 2020

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by

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A dissertation submitted in partial fulfillment of the requirements for the degree of

Master of Science

In the Department of Convergence Study on the Ocean Science and Technology, Ocean Science and Technology School of Korea Maritime and Ocean University

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July 2020

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Abstract

Effect of dietary substitution of *Undaria pinnatifida* with onion extract by-product (OEB) on growth, body composition and air exposure stress of juvenile abalone was determined. One thousand and eighty juvenile abalone were randomly distributed into 18 100 L net cages (sixty per cage). Five formulated diets were prepared. Twenty percent *U. pinnatifida* was included into the control (Con) diet. Twenty five, 50, 75 and 100% *U. pinnatifida* were substituted with an equal amount of OEB, referred to as the OEB25, OEB50, OEB75 and OEB100 diets, respectively. Finally, dry *U. pinnatifida* was prepared to compare formulated diets on growth performance of abalone, referred to as the *Undaria*. Each diet were randomly assigned to triplicate groups of abalone and fed to abalone once a day to satiety with little leftover for 16 weeks. After the 16-week feeding trial, abalone were subjected to air exposure for 24 h and survival was monitored for the next 4 days. Weight gain and specific growth

rate (SGR) of abalone fed all formulated diets were greater than those of abalone fed the *Undaria*. The greatest weight gain and SGR were obtained in abalone fed the OEB50 diet, followed by the OEB75, OEB25, Con and OEB100 diets, in that order. Weight gain and SGR of abalone fed the OEB100 diet were lower than those of abalone fed the Con diet. Shell length, width and height and soft body weight of abalone tended to agree with growth rate of abalone. No difference in the chemical composition of the soft body of abalone was observed. The survival of abalone fed the OEB25, OEB50, OEB75 and OEB100 diets was higher than that of abalone fed the *Undaria* and Con diet at the end of 4-day post observation after 24-h air exposure. In conclusion, *U. pinnatifida* up to 75% could be replaced with OEB in abalone feed when 20% *U. pinnatifida* was included. The greatest growth performance was obtained in abalone fed the OEB50 diet.

KEY WORDS: Abalone (*Haliotis discus*); Dietary substitution; *Undaria pinnatifida*; Onion extract by-product (OEB); Air exposure stressor

배합사료내 양파착즙 부산물의 미역대체에 따른 까막전복 (*Haliotis discus*) 치패의 성장과 체조성 및 공기 노출 스트레스에 미치는 영향

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요 약

까막전복(*Haliotis discus*) 치패용 배합사료내 미역대체원으로서 양파착즙 부산물의 첨가가 전복의 생존율, 성장, 체조성 및 공기노출 스트레스에 미치는 영향을 조사하였다. 전복 치패(평균 체중 ± SE: 6.0 ± 0.00 g) 1,080마리를 18개의 100-L 네트 케이지에 각각 60마리씩 수용하였다. 총 5종류의 실험사료를 준비하였으며, 20%의 미역을 첨가하여 제조한 대조구 사료(Con), Con 사료내 미역을 양파착즙 부산물로 각각 25, 50, 75, 100% 대체한 OEB25, OEB50, OEB75, OEB100 사료와 자연산 먹이인 건조 미역(*Undaria*)을 준비하였으며, 실험구는 3반복을 두었다. 먹이는 전복 전체중의 1.5%-2.5% 수준으로 1일 1회 충분히 공급하였으며, 총 16주간 공급하였다. 16주간의 사육실험 종료 후 전복을 24시간 동안 공기중 노출 시킨 후 4일간 누적 폐사율을 관찰하였다.

16주간의 사육실험 결과, 전복의 생존율은 86.1% 이상으로 나타났다. 증체량(weight gain) 및 일일성장률(specific growth rate, SGR)은 모든 전복용 배합사료 공급구에서 미역 공급구보다 우수하게 나타났고, OEB50 사료 공급구에서 가장 높은 성장률이 나타났으며 다음으로는 OEB75, OBE25,

Con, OEB100 사료 순서로 높은 성장률이 나타났다. 사육실험 종료시 생존한 전복의 각장, 각폭 및 가식부 무게는 전복의 성장 결과와 유사한 경향을 보였다. 전복의 가식부 일반성분 분석 결과 모든 실험구간에 차이가 나타나지 않았다. 전복을 24시간 공기 중 노출 시킨 후 생존율을 관찰한 결과 미역을 양파착즙 부산물로 대체한 모든 배합사료 공급구에서 미역과 Con 사료 공급구 보다 높은 생존율이 나타났다. 이상의 결과를 고려할 때 까막전복용 배합사료내 미역을 20% 첨가시 양파착즙 부산물로 75%까지 대체 가능하며, 배합사료내 미역의 50%를 양파착즙 부산물로 대체시 전복의 성장 향상에 효과적인 것으로 보인다.

KEY WORDS: 까막전복 (*Haliotis discus*); 대체효과; 미역; 양파착즙 부산물; 공기노출 스트레스

1. Introduction

Abalone (*Haliotis* spp.) is considered as one of the most valuable marine gastropods worldwide (FAO, 2017) and commercially important marine shellfish for aquaculture in the Eastern Asia including Korea, Japan and China as well. There has been a dramatic increase in the total aquaculture production of abalone in Korea from 20 metric tons in 2000 to 18436 metric tons in 2019, and their importance is expected to continue in the future (KOSIS, 2020). It has high demand for human consumption for long time due to its high nutritional values, good test and other health benefits (De Zoysa, 2013; Kim et al., 2006a). To meet up the high demand for human consumption, intensive abalone culture techniques are being developed in Korea and China (Cook, 2016; FAO, 2017). Although aquaculture production of abalone is expected to increase worldwide to satisfy the growing global demand, development of sustainable and cost-effective feed for abalone culture is still highly needed. Since abalone farmers are heavily concerning about water quality and easy management of their farms in Korea, they prefer feeding abalone on macroalgae (MA), such as *Undaria pinnatifida* or *Saccharina japonica* as a common feed. However, its market price is sharply increased (> $US 3–4 kg^{-1}) because it is also popular healthy food for human consumption and its seasonal availability is limited (Baek et al., 2019; Jang et al., 2018; Yun et al., 2020a). MA is also one of the highest components (15–30%) in commercial abalone feed and it eventually increases abalone production cost (Hernández et al., 2009; Jang et al., 2018; O'Mahoney et al., 2014). Therefore, feed nutritionists are trying to develop cheap and sustainable alternative feed sources for MA in abalone feed. Inferior growth performance of abalone fed single MA, such as *U. pinnatifida*, *S. japonica* or *Macrocystis pyrifera* (Baek et al., 2019; Choi et al.,

2018; Garcia-esquivel and Felbeck, 2009; Nie et al., 1986; Viana et al., 1993; Yun et al., 2020a) compared to a nutrition-balanced diet has been reported possibly linked with the low protein (amino acids) and lipid (fatty acids) content in the former (Choi et al., 2018; Jang et al., 2018; Mai et al., 1995a, 1995b; Uki et al., 1986; Yun et al., 2020b).

Several feeding trials are conducted to determine suitability of fouling MA, such as *Ulva australis* (Ansary et al., 2019a), *Sargassum horneri* (Ansary et al., 2019b) and their combination (Ansary et al., 2019c) and other MA (*Gracilaria lemaneiformis* and *Hynea spinella*) (Qi et al., 2010; Viera et al., 2005), and agricultural by-product, such as carrot leaf by-product (Baek et al., 2019), citrus peel by-product (Yun et, al., 2020a) and rice bran (Kim et al., 2016) as a replacer for MA (*U. pinnatifida* or *S. japonica*) in abalone feed. Reyes and Fermin (2003) also reported that three terrestrial leaf meals, *Carica papaya*, *Leucaena leucocephala*, *Moringa oliefera* and a freshwater aquatic fern, *Azolla pinnata* as potential ingredients for farmed abalone (*Haliotis asinina*) diet. Utilization of various vegetables including carrot root, cabbage, potato, radish, turnip green, broad bean leaves, squash leaves, and cucumber leaves in aquatic animal diets, such as common carp, *Cyprinus carpio*, Nile tilapia, *Oreochromis nitolicus*, prawn *Macrobrachium rosenberii* and harpacticoid copepods, *Tisbe holothuriae*, *Amphiascella subdebilis* and *Notocra* sp. have been also reported (Baskar et al., 2011; Garces and Heinen, 1993; Kahan, 1979; Magouz et al., 2008).

As onion, *Allium cepa* L., is a major vegetable crop grown in all over the world (Roldán et al., 2008) and contains phenolic, quercetin, vitamins, flavonoids, sulfoxides, peptides, proteins and other bioactive compounds and acts as an important source of therapeutic agents with potential beneficial health effects of human, it is recommended for curing or preventing a wide variety of human diseases (Augusti, 1996; Breu, 1996; Jeong et al., 2009; Ramos et al., 2006). The annual onion production has been increasing from 877514 metric tons in 2000 to

1594450 metric tons in 2019 in Korea (KOSIS, 2020). The popularity and consumption of onion as vegetables for human consumption is steadily increasing all over the world due to its recognition as an important source of natural antioxidants and its importance is expected to continue in the future (Kang et al., 1998; Roldán et al., 2008; Shin et al., 2010).

New antioxidants are generated by the maillard reaction during the heat treatment process before fruit and vegetable extractions (Kim et al., 2013; Robards et al., 1999) and the heat treatment also facilitates the collapse of their tissues and cells. Since physiological activity including antioxidant capacity has been increased due to an increased bioavailability after heat treatment process (Choi et al., 2006; Dewanto et al., 2002; Kim et al., 2006b; Xu et al., 2007), their digestibility in animals can be improved. In addition, many studied have revealed that administration of properly heated soybean meal improved the growth performance, feed intake and feed efficiency of some fish (Arndt et al., 1999; Balogun and Ologhobo, 1989; Viola et al., 1983; Wilson and Poe, 1985).

One of the commonest ways to consume the bioactive compounds in onion is to drink heated-water soluble onion extract. Since demand of onion extract for human consumption to keep healthy body will increase in Korea, several thousand metric tons of onion extract by-product (OEB) is produced every year and regarded as a vegetable by-product. Consequently, OEB can cause an increased disposal cost and potentially create severe agricultural by-product pollution problem. Therefore, OEB, being cheap and supply-stable, can be regarded a novel substitute for MA in abalone feed. Cho (2011) showed that onion extract was the most effective dietary additive to improve growth performance of juvenile olive flounder, *Paralichthys olivaceus* when the fish were fed with one of the diets containing 1% dietary additives (onion extract, *Opuntia ficus-indica* ver. *Saboten*, propolis, lactic acid bacteria, γ-poly-glutamic acid, organic sulfur, Biostone® and fig extract) compared to a control diet (without additive) for 6-week. Akrami et al. (2015) also reported

that dietary supplementation of onion powder improved growth performance of juvenile beluga, *Huso huso* and could modulate some immune parameters of the fish. Cho and Lee (2012) revealed that dietary supplementation of onion powder improved growth performance as well as disease resistance of olive flounder against *Edwardsiella tarda*.

In this study, therefore, dietary substitution effect of *U. pinnatifida* with OEB on growth, body composition and air exposure stress of juvenile abalone (*Haliotis discus,* Reeve 1846) was compared to the *U. pinnatifida*, which is one of commonest feeds in intensive abalone culture in Korea.

2. Materials and methods

2.1 Preparation of OEB

OEB was supplied from Minzi Onion Farm (Muan, Jeollanam-do, Korea), dried at 40℃ in an electrical dry oven (UDS-4522F, Unique Daesung Co. Ltd., Pocheon-si, Gyeonggi-do, Korea) for 48 h, and then grinded as powder.

2.2 Preparation of abalone and rearing condition

Juvenile abalone were purchased from a private hatchery (Daegun abalone farm, Jeju, Korea) and acclimated to the experimental conditions for 2 weeks. Eighteen 100 L (50 cm × 50 cm × 40 cm) net cages were set up for 3 of 3.6 ton concrete raceways (water volume 3.4 ton; 6 net cages per raceway). Underground sand-filtered seawater was supplied throughout the feeding trial at temperature ranging from 18.0 to 18.6 ℃ (mean ± SD 18.2 ± 0.1 ℃), at 108 L min^{-1} per raceway of flow rate. A total of 1080 juvenile abalones averaging 6.0 g were randomly distributed into net cages (60 abalones per net cage). The photoperiod followed natural condition and proper aeration was supplied to each raceway. The experimental diets were fed to abalone once a day (17:00) at a satiation level with a little leftover (about 1.5−2.5% biomass). Dead abalones were removed daily and the cages were cleaned every other day. The feeding trial lasted for 16weeks. At the end of the 16-week feeding trial, abalones were harvested and collectively weighed from each cage.

2.3 Preparation of the experimental diets

The experimental design was completely randomized, consisted of three replications for each diet. Five formulated diets and dry *U. pinnatifida* were prepared (Table 1). Twenty five percent fish meal, 4% soybean meal, 5% casein

Table 1. Ingredient and chemical composition of the experimental diets (%, DM basis)

	Experimental diets					
	Con	OEB25	OEB50	OEB75	OEB100	*Undaria*
Ingredient (%)						
Fish meal	25	25	25	25	25	
Soybean meal	4	4	4	4	4	
Casein	5	5	5	5	5	
Wheat flour	22	22	22	22	22	
Onion extract by-product (CP; 10.7%, CL: 2.2%)	0	5	10	15	20	
Undaria pinnatifida (CP; 21.6%, CL: 3.1%)	20	15	10	5	0	
Squid liver oil	0.5	0.5	0.5	0.5	0.5	
Soybean oil	0.5	0.5	0.5	0.5	0.5	
Sodium alginate	20	20	20	20	20	
Mineral mix[a]	2	2	2	2	2	
Vitamin mix[b]	1	1	1	1	1	
Nutrient (%)						
Dry matter	90.1	91.4	90.5	90.4	90.2	85.4
Crude protein	31.7	30.4	30.0	29.6	29.4	22.9
Crude lipid	4.3	4.3	4.2	4.1	4.1	1.4
Ash	11.7	10.4	9.1	7.9	6.8	22.9
Carbohydrate[c]	52.3	54.9	56.7	58.4	59.7	52.8

[a]Mineral premix contained the following ingredients (g/kg mix): NaCl, 10, $MgSO_4 \cdot 7H_2O$, 150; $NaH_2PO_4 \cdot 2H_2O$, 250; KH_2PO_4, 320; $CaH_4(PO_4)_2 \cdot H_2O$, 200; Ferric citrate, 25; $ZnSO_4 \cdot 7H_2O$,4; Ca-lactate, 38.5; CuCl, 0.3; $AlCl_3 \cdot 6H_2O$, 0.15; KIO_3, 0.03; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2; $CoCl_2 \cdot 6H_2O$, 0.1.

[b]Vitamin premix contained the following amount which were diluted in cellulose (g/ kg mix): L-ascorbic acid, 200; α-tocopheryl acetate, 20; thiamin hydrochloride, 5; riboflavin, 8; pyridoxine, 2; niacin, 40; Ca-D-pantothenate, 12; myo-inositol, 200; D-biotin, 0.4; folic acid, 1.5; p-amino benzoic acid, 20; K_3, 4; A, 1.5; D_3, 0.003; choline chloride, 200; cyanocobalamin, 0.003.

[c]Carbohydrate was calculated by the difference [100 – (% crude protein + % crude lipid + % ash)].

were used as the protein sources in the control (Con) diet. Twenty two percent wheat flour and 0.5% squid liver and 0.5% soybean oils were included in the Con diet as carbohydrate and lipid sources, respectively. Twenty percent *U. pinnatifida* powder was included in the Con diet. Twenty five, 50, 75 and 100% of *U. pinnatifida* powder were substituted with an equal amount of OEB, referred to as the OEB25, OEB50, OEB75 and OEB100, respectively. The experimental diets satisfied for dietary protein and lipid requirements for abalone (Fleming et al., 1996; Mai et al., 1995a, 1995b). In addition, dry *U. pinnatifida* was prepared to compare effect of the formulated diets on growth performance of abalone, referred to as the *Undaria*. All prepared feed ingredients along with 20% sodium alginate for each formulated diet were mixed mechanically and water was added at a ratio of 1:1. A fine paste was made from each of the diets by an electronic mixer (Samwoo Industry Co., Korea) and shaped into 0.15 cm thick sheets, which were then cut into 1 cm^2 flakes by hand. The flakes were then dipped into an aqueous solution of 5% $CaCl_2$ for 1 min. Finally, the flakes were dried naturally for 48 h and stored at –20 ℃ until use.

2.4 Analytical procedures of the diets and carcass of abalone

At the end of the 16-week feeding trial, 30 abalones from each net cage were randomly sampled and frozen at –20 ℃ for the biological measurement and chemical analysis of abalone. Prior to examination, all samples were slightly thawed followed by separation of the shell and soft body tissue. Shell length and shell width were measured in mm with a digital caliper (Mitutoyo Corporation, Kawasaki, Japan), and the ratio of soft body weight to body weight (the soft body weight + the excised shell's weight) was calculated to determine an index of nutritional status for abalone. Specific growth rate (SGR, % body weight gain day^{-1}) was calculated using the formula of Britz (1996): SGR=[(ln(Wf)–ln

(Wi))/days of feeding]×100, where ln(Wf)=natural log of the final mean weight of abalone and ln(Wi)=natural log of the initial mean weight of abalone. The separated soft body tissue from all abalone from each container was then homogenized and used for chemical analysis. Crude protein content was determined by the Kjeldahl method (Auto Kjeldahl System, Buchi B-324/435/412, Switzerland), crude lipid was determined using an ether-extraction method, moisture was determined by oven drying at 105°C for 24-h and ash was determined using a muffle furnace at 550°C for 4 h. All methods were according to standard AOAC (2002) practices.

2.5 Monitoring survival of abalone subjected to air exposure stressor

After the 16-week feeding trial, 20 abalone from each net cage were refed as the same method as the feeding trial for 1 week to minimize handling stress during growth measurement, and then subjected to air exposure stressor based on the slight modification of Cho et al. (2008) and Lee et al. (2016). Twenty abalones from each net cage were completely drained and subjected to air exposure for 24 h. Then, the cages were filled with underground sand-filtered seawater to monitor post mortality for the next 4 days after 24-h air exposure. Dead abalone were removed every 4 h during the 4-day post observation.

2.6 Water stability of the experimental diet

Ten gram of the formulated diets and dry *U. pinnatifida* in 54 laboratory dishes were placed in separate 9, 50 L plastic rectangular containers (51 cm × 36 cm × 30 cm) excluding abalone in triplicate (6 laboratory dishes container^{-1}) at a flow rate of 1.4 L container^{-1} min^{-1} and sampled at 12, 24 and 48 h to evaluate leaching of nutrients in diets to determine their water stability. Nutrient levels in the experimental diets were assessed using the same procedure as the chemical composition of the experimental diets. Water stability of nutrients in the diets was

expressed as the percentage of difference between final dry content and initial dry content for each nutrient based on Mai et al. (1995a).

2.7 Statistical analysis

One-way ANOVA and Duncan`s multiple range test (Duncan, 1955) were used to determine the significant differences among the means of treatments by using SAS version 9.3 program (SAS Institute, Cary, NC, USA). Percentage data was arcsine-transformed prior to statistical analysis. Water stability of the experimental diets was tested by ANOVA with repeated measurement designs (Cody and Smith, 1991). The survival of abalone during 4–day post observation period after 24-h air exposure was analyzed by using Kaplan-Meier survival curve, Log-rank and Wilcoxon tests. In addition, regression analysis was conducted between weight gain, SGR, shell length, shell width, shell height and soft body weight of abalone as the dependent variable and amount of OEB substituted in the experimental diets as the independent variable.

3. Results

3.1 Water stability of the experimental diets over time

Dry matter (Fig. 1), crude protein (Fig. 2), crude lipid (Fig. 3) and ash (Fig. 4) content of the experimental diets were significantly ($p < 0.0001$) changed over all periods of time, and their significant ($p < 0.0001$) interactions (experimental diets × time) were also observed. The retained dry matter in all formulated diets was significantly ($p < 0.05$) higher than that in the *Undaria* at all same period of observations. The highest retention of dry matter content was obtained in the OEB50 diet at 12 h, and the OEB100 diet at 24 and 48 h after seawater immersion.

The retained crude protein content in all formulated diets was also significantly ($p < 0.05$) higher than that in the *Undaria* at all same period of observations. Among the formulated diets, the retention of crude protein content was significantly ($p < 0.05$) higher in the OEB100 diet compared to the OEB25 and OEB75 diets, but not significantly ($p > 0.05$) different from the Con and OEB50 diets at 12 h after seawater immersion. However, there was no significantly ($p > 0.05$) difference among all formulated diets in the retention of crude protein content at 24-h and 48-h after seawater immersion.

The retention of crude lipid content in all formulated diets was significantly ($p < 0.05$) higher than that in the *Undaria* at all same period of observations after seawater immersion. At 12 h after seawater immersion, crude lipid content in the OEB50 diet was significantly ($p < 0.05$) higher than that in the OEB25 and OEB75 diets and *Undaria*, but not significantly ($p > 0.05$) different from that in the Con and OEB100 diets.

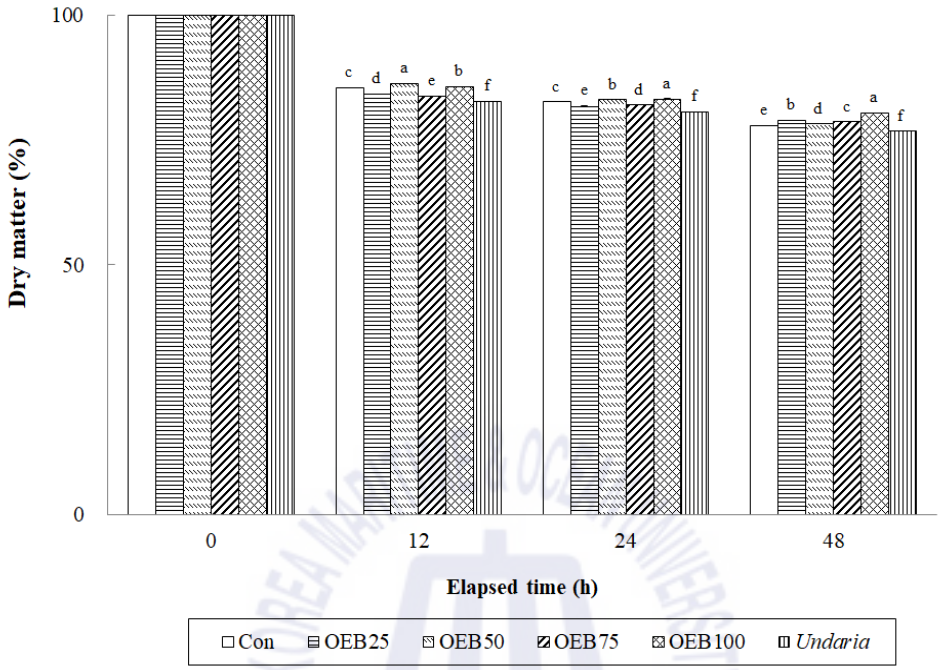

Fig. 1. Changes in dry matter content (%) of the experimental diets at 12, 24 and 48 h after seawater immersion (means of triplicate ± SE). [ANOVA with repeated design: times ($p < 0.0001$) and their interaction (the experimental diets × time) ($p < 0.0001$)]. Different letters in each time point indicates significant ($p < 0.05$) differences between diets within each time point

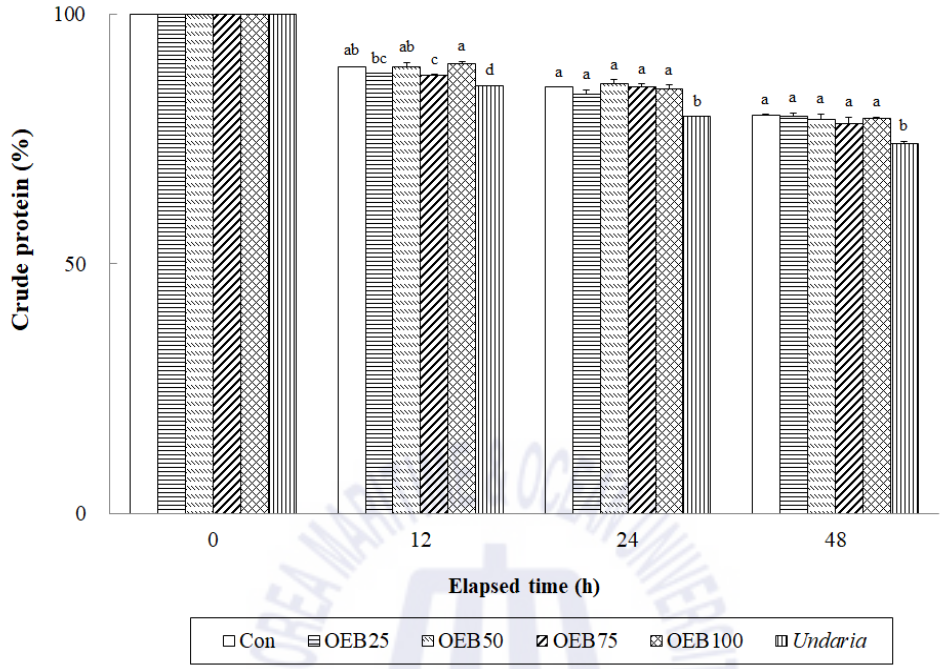

Fig. 2. Changes in crude protein content (%) of the experimental diets at 12, 24 and 48 h after seawater immersion (means of triplicate ± SE). [ANOVA with repeated design: times ($p < 0.0001$) and their interaction (the experimental diets × time) ($p < 0.0001$)]. Different letters in each time point indicates significant ($p < 0.05$) differences between diets within each time point

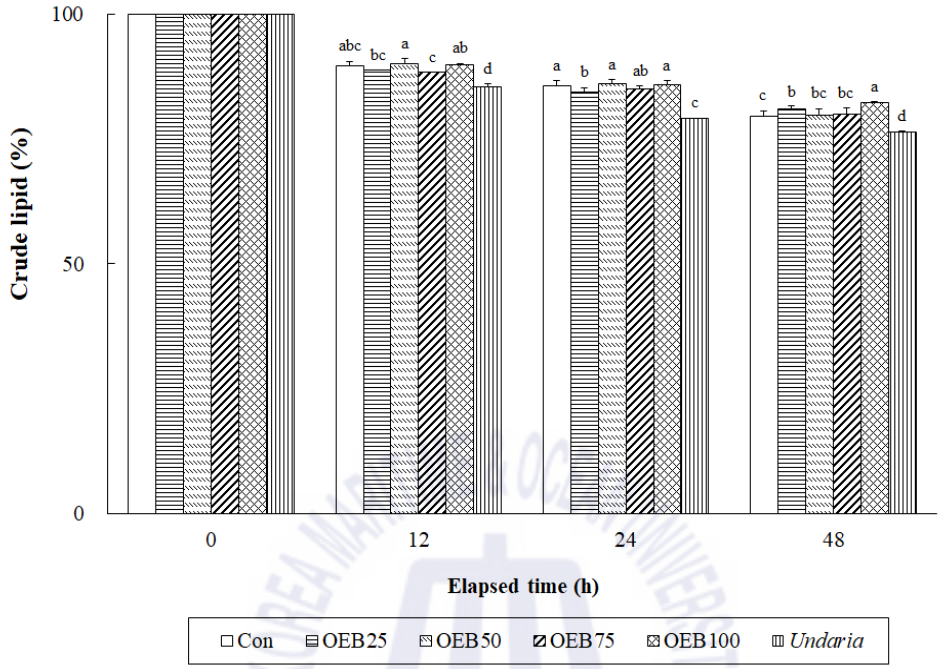

Fig. 3. Changes in crude lipid content (%) of the experimental diets at 12, 24 and 48 h after seawater immersion (means of triplicate ± SE). [ANOVA with repeated design: times ($p < 0.0001$) and their interaction (the experimental diets × time) ($p < 0.0001$)]. Different letters in each time point indicates significant ($p < 0.05$) differences between diets within each time point

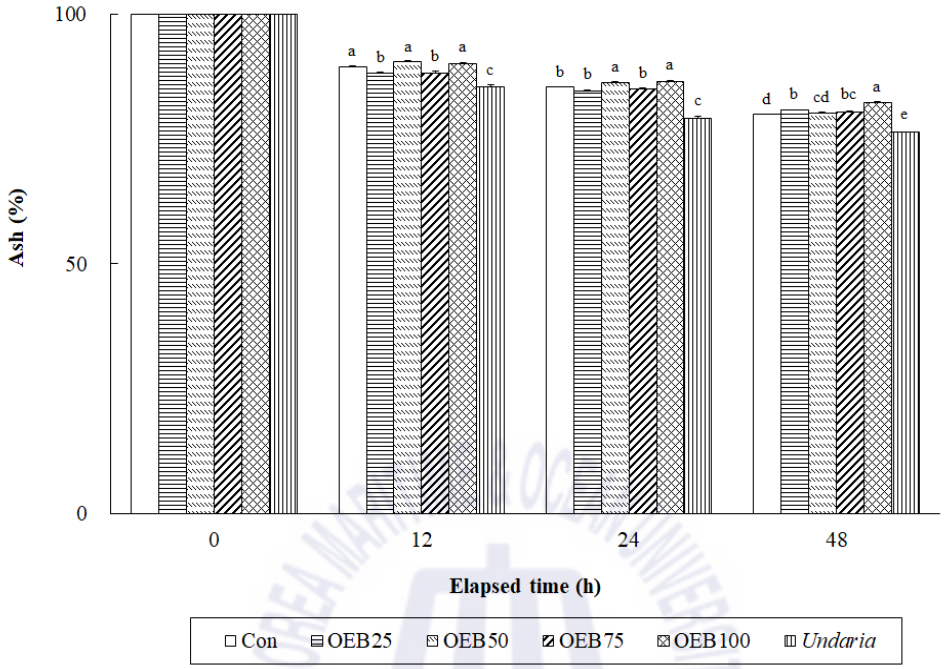

Fig. 4. Changes in crude ash content (%) of the experimental diets at 12, 24 and 48 h after seawater immersion (means of triplicate ± SE). [ANOVA with repeated design: times (p < 0.0001) and their interaction (the experimental diets × time) (p < 0.0001)]. Different letters in each time point indicates significant (p < 0.05) differences between diets within each time point.

The retained ash content in all formulated diets was significantly ($p < 0.05$) higher than that in the *Undaria* at all same period of observations after seawater immersion. At 48-h after seawater immersion, ash content in the OEB100 diet was significantly ($p < 0.05$) higher than that for all other diets.

3.2 Growth performance of abalone

Survival of abalone ranging from 86.1 to 92.8% was not significantly ($p > 0.05$) affected by dietary substitution of *U. pinnatifida* with OEB (Table 2). Abalone fed all formulated diets showed significantly ($p < 0.05$) higher weight gain and SGR than those of abalone fed the *Undaria*. Greatest weight gain and SGR were achieved in abalone fed the OEB50 diet. Weight gain and SGR of abalone fed the OEB25 and OEB75 diets were also significantly ($p < 0.05$) greater than those of abalone fed the Con diet. However, abalone fed the OEB100 diet achieved significantly ($p < 0.05$) lower weight gain and SGR than those of abalone fed the Con diet. Regression analyses revealed quadratic relationship between dietary substitution level of OEB (X) for *U. pinnatifida* in abalone feed and weight gain ($Y= -0.0003X^2 + 0.0317X + 6.7067$, $R^2 = 0.7967$, $p < 0.001$, Y_{max} = X value of 52.83) and SGR ($Y= -0.00002X^2 + 0.0022X + 0.6680$, $R^2 = 0.7967$, $R^2 = 0.8015$, $p < 0.001$, Y_{max} = X value of 55.00), respectively (Table 3).

Table 2 Survival, weight gain and specific growth rate (SGR) of juvenile abalone (*Haliotis discus*) fed the experimental diets substituting *Undaria pinnatifida* with the onion extract by-product for 16-week

Experimental diets	Initial weight (g abalone^{-1})	Final weight (g abalone^{-1})	Survival (%)	Weight gain (g abalone^{-1})	SGR[a] (% day^{-1})
Con	6.0 ± 0.00	12.8 ± 0.04^{c}	92.2 ± 1.47	6.78 ± 0.038^{c}	0.66 ± 0.002^{c}
OEB25	6.0 ± 0.00	13.1 ± 0.11^{b}	91.1 ± 1.47	7.05 ± 0.104^{b}	0.69 ± 0.007^{b}
OEB50	6.0 ± 0.00	13.7 ± 0.09^{a}	92.8 ± 2.22	7.67 ± 0.094^{a}	0.74 ± 0.006^{a}
OEB75	6.0 ± 0.00	13.1 ± 0.03^{b}	91.1 ± 1.47	7.07 ± 0.035^{b}	0.70 ± 0.003^{b}
OEB100	6.0 ± 0.00	12.4 ± 0.06^{d}	92.2 ± 2.42	6.44 ± 0.066^{d}	0.65 ± 0.005^{d}
Undaria	6.0 ± 0.00	12.1 ± 0.09^{e}	86.1 ± 1.11	6.11 ± 0.087^{e}	0.61 ± 0.006^{e}
p-value		$p < 0.001$	$p > 0.2$	$p < 0.001$	$p < 0.001$

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($p > 0.05$).

[a]Specific growth rate (SGR) = [(ln (wf) – ln (wi))/days of feeding] × 100, where ln (wf) = natural log of the final mean weight of abalone and ln (wi) = natural log of the initial mean weight of abalone.

Table 3 Regression analysis of dietary substitution level of *Undaria pinnatifida* with the onion extract by-product (OEB) vs parameters measured [weight gain, specific growth rate (SGR), shell length, shell width, shell height and soft body weight of abalone]

	Regression analysis			
Dependent variables	Equation	*p*-value	R^2	Y_{max}
Weight gain	$Y= -0.0003X^2+0.0317X+6.7067$	< .001	0.7967	X = 52.83
SGR	$Y= -0.00002X^2+0.0022X+0.6680$	< .001	0.8015	X = 55.00
Shell length	$Y= -0.0012X^2+0.0996X+50.9684$	< .001	0.7277	X = 41.50
Shell width	$Y= -0.0007X^2+0.0672X+34.3611$	< .001	0.7603	X = 48.00
Shell height	$Y= -0.0004X^2+0.0286X+12.1790$	< .001	0.8157	X = 35.75
Soft body weight	$Y= -0.0002X^2+0.0218X+8.1130$	< .001	0.7648	X = 54.50

3.3 Biological indices of abalone

Shell length, width and height and soft body weight of abalone fed the Con, OEB25, OEB50 and OEB75 diets were significantly ($p < 0.05$) longer, wider, higher and heavier than those of abalone fed the OEB100 diet and *Undaria*, respectively (Table 4). The longest, widest, highest and heaviest shell length, width, and height and soft body weight were attained in abalone fed the OEB50 diet. However, the ratio of soft body weight to total weight of abalone was not affected by dietary substitution of *U. pinnatifida* with OEB. Regression analyses revealed quadratic relationship between dietary substitution level of OEB (X) for *U. pinnatifida* in abalone feed and biological indices of abalone, shell length ($Y= –0.0012X^2+0.0996X+50.9684$, $R^2 = 0.7277$, $p < 0.001$, Y_{max} = X value of 41.50), shell width ($Y= – 0.0007X^2 + 0.0672X + 34.3611$, $R^2 = 0.7603$, $p < 0.001$, Y_{max} = X value of 48.00), shell height ($Y= –0.0004X^2 + 0.0286X + 12.1790$, $R^2 = 0.8157$, $p < 0.001$, Y_{max} = X value of 35.75) and soft body weight ($Y= –0.0002X^2 + 0.0218X + 8.1130$, $R^2 = 0.7648$, $p < 0.001$, Y_{max} = X value of 54.50), respectively (Table 3).

3.4 Chemical composition of the soft body of abalone

None of the moisture content ranging from 75.2 to 75.5%, crude protein content ranging from 19.2 to 19.7%, crude lipid content ranging from 1.2 to 1.3% and ash content of 2.1% for all diets was altered by the experimental diet (Table 5).

Table 4 Shell length, shell width, shell height, soft body weight and the ratio of soft body weight to total weight of abalone (*Haliotis discus*) at the end of the 16-week feeding trial

Experimental diets	Shell length (mm)	Shell width (mm)	Shell height (mm)	Soft body weight (g)	Soft body weight/total weight
Con	51.1 ± 0.42^{b}	34.5 ± 0.29^{c}	12.3 ± 0.10^{b}	8.2 ± 0.10^{b}	0.64 ± 0.005
OEB25	52.1 ± 0.22^{b}	35.1 ± 0.11^{b}	12.4 ± 0.03^{b}	8.4 ± 0.09^{b}	0.64 ± 0.002
OEB50	53.8 ± 0.99^{a}	36.5 ± 0.04^{a}	13.0 ± 0.08^{a}	8.7 ± 0.11^{a}	0.63 ± 0.003
OEB75	51.3 ± 0.26^{b}	35.2 ± 0.11^{b}	12.2 ± 0.05^{b}	8.3 ± 0.04^{b}	0.63 ± 0.003
OEB100	49.1 ± 0.09^{c}	33.2 ± 0.07^{d}	11.5 ± 0.01^{c}	7.8 ± 0.03^{c}	0.64 ± 0.000
Undaria	48.4 ± 0.25^{c}	32.8 ± 0.17^{d}	11.3 ± 0.06^{c}	7.7 ± 0.13^{c}	0.63 ± 0.007
p-value	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p > 0.5$

Values (means of triplicate ± SE) in the same column sharing a common superscript are not significantly different ($p > 0.05$)

Table 5 Chemical composition (%) of the soft body of abalone at the end of the 16-week feeding trial

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
Con	75.2 ± 0.01	19.2 ± 0.06	1.3 ± 0.06	2.1 ± 0.01
OEB25	75.3 ± 0.08	19.5 ± 0.19	1.2 ± 0.04	2.1 ± 0.03
OEB50	75.2 ± 0.06	19.7 ± 0.18	1.2 ± 0.03	2.1 ± 0.03
OEB75	75.2 ± 0.23	19.4 ± 0.15	1.2 ± 0.04	2.1 ± 0.03
OEB100	75.4 ± 0.14	19.5 ± 0.07	1.3 ± 0.03	2.1 ± 0.04
Undaria	75.5 ± 0.06	19.5 ± 0.07	1.2 ± 0.05	2.1 ± 0.04
p-value	$p > 0.05$	$p > 0.2$	$p > 0.8$	$p > 0.5$

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($p > 0.05$).

3.5 Survival of abalone subjected to 24-h air exposure stressor

Survival of abalone fed the *Undaria* (36.7 ± 1.67%, means of triplicate ± SE) and Con diet (28.3 ± 1.67%) were significantly ($P < 0.001$) lower than that of abalone fed the OEB25 (61.7 ± 1.67%), OEB50 (61.7 ± 1.67%), OEB75 (63.3 ± 1.67%) and OEB100 (63.3 ± 1.67%) diets.

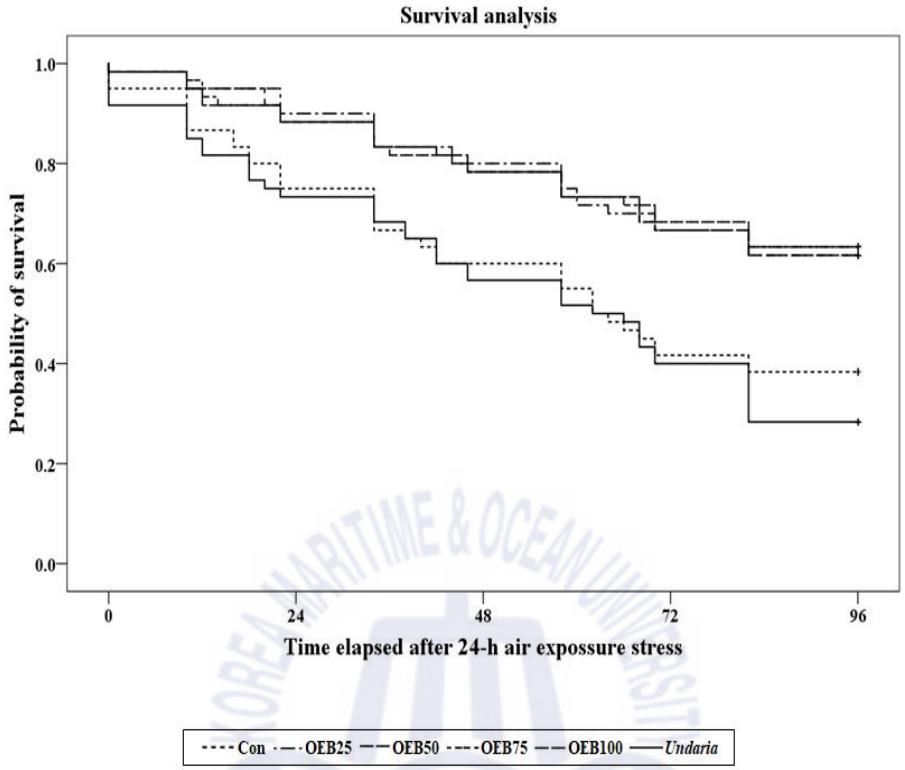

Fig. 5. Survival of abalone fed the experimental diets substituting *Undaria pinnatifida* with onion extract by-product (OEB) for 16 weeks, and then subjected to 24-h air exposure for the next 4 days (means of triplicate ± SE) ($p < 0.001$ for Log Rank and Wilcoxon tests).

4. Discussion

The Superior weight gain and SGR of abalone fed all formulated diets to those of abalone fed the *Undaria* in this study demonstrated that a nutrition-balanced feed can improve the growth performance of abalone over single MA, being consistent with the findings of previous studies (Baek et al., 2019; Bautista-Teruel and Millamena, 1999; Jung et al., 2016; Lee et al., 2018; Viana et al., 1993; Yun et al., 2020a). However, superior weight gain and SGR of abalone fed the Con diet to those of abalone fed the OEB100 diet, but inferior to the OEB75 diet in this study indicated that *U. pinnatifida* up to 75% could be replaced with OEB without retardation of growth performance of abalone when 20% *U. pinnatifida* was included. The greatest weight gain and SGR of abalone fed the OEB50 diet indicated that the growth performance of abalone was most improved, especially when 50% *U. pinnatifida* was substituted with OEB. Lee et al. (2018) stated that white radish by-product and tunic meal of sea squirt was promising alternative for *U. pinnatifida* in diet of abalone (*H. discus*). Similarly, Kim et al. (2016) reported that dietary MA (*S. japonica*) up to 100% can be substituted with rice bran without retarding the growth of abalone (*H. discus*) when 20% *S. japonica* was included. Reyes and Fermin (2003) emphasized that three terrestrial leaf meals of *Carica papaya*, *Leucaena leucocephala* and *Moringa oliefera* and freshwater aquatic fern (*Azolla pinnata*) could be used as novel feed ingredient for farmed abalone (*H. asinina*) diet.

The production of vegetables is increasing with the increased global demand for human consumption since vegetables are essential for human nutrition (Dias, 2011). Onions are one of the most produced vegetables as they are energetic and deliver substantial proportion of vitamins and minerals (FAO, 2017). Recently, by-products of fruit and vegetables have received more attention because they contain possible bioactive compounds and phytochemicals (Coman et al., 2020). Since OEB is much cheaper ingredient than MA (*U. pinnatifida* or *S. japonica*) and contains vitamins,

peptides and minerals (Augusti, 1996; Breu, 1996; Jeong et al., 2009; Ramos et al., 2006), it seems to have high potential as a substitute for MA in abalone feed. Similarly, dietary substitution effect of different sources of agricultural and fishery by-products, such as carrot leaf by-product, radish by-product, rice bran and tunic meal of sea squirt for MA on the growth performance of abalone (*H. discus*) have been reported (Baek et al., 2019; Choi et al., 2018; Jang et al., 2018; Kim et al., 2016; Lee et al., 2018).

Plant materials treated by heat to inactivate protease inhibitors improved their digestibility in animals. This was confirmed by Shipton and Britz (2002), who observed digestibility coefficients in both soya and sunflower meals improved by 45.1% and 7.0%, respectively, by heat and solvent extraction process in South African abalone (*H. midae*). Similarly, physical (heat) treatment improved digestibility of plant seed materials in shrimp (*Penaeus vannamei*) hepatopancreatic extract (García-Carreño et al., 1997). In addition, effect of the intensity of heat treatment on availability of soybean meal was demonstrated in carp (*Cyprinus carpio*) (Abel et al., 1984). Improved digestibility (availability) of plants by heat treatment could explain why abalone fed the diets substituting *U. pinnatifida* up to 75% with OEB in this study improved growth performance of abalone. Therefore, OEB seems to be the environment-friendly and promising alternative source for *U. pinnatifida* in abalone feed. The physiological activity including antioxidant capacity has been also improved due to the increased bioavailability of plants after heat treatment (Choi et al., 2006; Dewanto et al., 2002; Kim et al., 2006b; Xu et al., 2007).

Quadratic relationships between growth performance (weight gain and SGR) of abalone fed all formulated diets and substitution level of OEB for *U. pinnatifida* were explained in this study. The maximum weight gain and SGR of abalone were calculated at 52.83% and 55.75% substitution of *U. pinnatifida* with OEB, respectively. This indicated that about 50% substitution of *U. pinnatifida* with OEB

is the most recommendable ratio in abalone feed when 20% *U. pinnatifida* was included in abalone feed. Similarly, Yun et al. (2020a) reported that MA (*U. pinnatifida*) could be completely (100%) substituted with citrus by-product in abalone (*H. discus*) feed without retardation in growth performance when 20% *U. pinnatifida* was included, and that abalone fed the diet substituting 50% *U. pinnatifida* with citrus by-product achieved the greatest weight gain and SGR. Kim et al. (2016) also reported that 100% *S. japonica* could be substituted with rice bran without retardation of weight gain of abalone (*H. discus*) when 20% *S. japonica* was included in diets and the greatest growth performance was obtained in abalone fed the diet substituting 40% *S. japonica* with rice bran. This could be partially explained by the fact that abalone seemed to utilize carbohydrate more efficiently than lipid as an energy source (Thongrod et al. 2003) unlike other aquatic animals (fish and crustaceans) because they have high levels of the digestive enzymes protease, amylase, cellulase, and alginase, but low levels of lipases (Gomez-Pinchetti and Garcia-Reina, 1993; Britz et al., 1994; Garcia-Esquivel and Felbeck, 2006).

Deterioration of water quality due to nutrient leaching from diets is a primary concern in use of formulated diet for intensive abalone culture. Since abalone are feeding slowly on diets, growth performance of abalone can depend on water stability of diet. Higher dry matter, crude protein, crude lipid and ash contents retained in all formulated diets than the *Undaria* during the 48-h observation after seawater immersion in this study indicated that formulated diets possessed superior water stability to the *Undaria*. This could be another reason that abalone fed all formulated diets achieved superior growth performance to MA (*Undaria*). Then this is supported by results from Lee et al. (2018) and Ansary et al. (2019b)'s studies, in which abalone (*H. discus*) fed formulated diets having greater water stability than MA (*U. pinnatifida*) achieved better growth performance than abalone fed the latter. Mai et al. (1995a) also reported that formulated diets and MA are very

different in their physical properties (texture and water stability), and these properties could affect digestion, absorption and growth performance of abalone (*H. discus hannai*).

Higher shell growth (shell length, width and height) and heavier soft body weight in abalone fed all formulated diets, except for the OEB100 diet than those of abalone fed the *Undaria* in this study demonstrated that biological indices of abalone were relatively well reflected from growth rate of abalone. Similarly, Baek et al. (2019) demonstrated that improved growth performance of abalone (*H. discus*) fed the formulated diets replacing *U. pinnatifida* with carrot leaf by-product over MA (*U. pinnatifida*) was supported by improved shell growth and soft body weight of abalone fed the former. The previous studies also reported that the growth performance of abalone was well reflected from the biological growth indices (shell length, height and width) and soft body weight of abalone (Amin et al., 2020; Lee et al., 2017a, 2017b; Stuart and Brown, 1994).

The composition of the soft body of abalone was not affected by dietary substitution of *U. pinnatifida* with OEB in this study. Similarly, Baek et al. (2019) reported that the chemical composition of the soft body of abalone was unaffected by the diets substituting *U. pinnatifida* with carrot leaf by-product. Rahman et al. (2015) also reported that dietary nutrient content did not influence the chemical composition of the soft body of abalone. Unlike this study, however, dietary nutrient content positively affects the chemical composition of the soft body of abalone (Garcia-Esquivel and Felbeck, 2009; Jang et al., 2018; Lee et al., 2018; Mai et al., 1995a, 1995b; Uki et al., 1986).

Abalone commonly face air exposure stressor during farm activities, such as grading, cleaning and transportation, which could induce mortality and other health complications (Hooper et al., 2011, 2014; Malham et al., 2003; Morash and Alter, 2016). Abalone were subjected to 24-h air exposure stress to examine the stress tolerant capability of abalone fed the diets substituting *U. pinnatifida* with OEB

compared to abalone fed the MA (*U. pinnatifida*) in this study. Higher survival of abalone fed the OEB25, OEB50, OEB75 and OEB100 diets compared to that of abalone fed the *Undaria* and Con diet at the end of 4-day post observation indicated that OEB has the potential to reduce stress and mortality of abalone subjected to air exposure. The highest mortality obtained in abalone fed the *Undaria* indicated that abalone fed the *Undria* is less resistible to 24-h air exposure stress than that of abalone fed the nutritionally balanced diets. The further study to determine dietary inclusion effect of OEB on immune resistance in abalone is needed. Similarly, Cho and Lee (2012) reported that onion powder was an effective immunostimulant to lower cumulative mortality of olive flounder infected with *E. tarda*. Akrami et al. (2015) described that onion might have potential efficacy on immunity of juvenile beluga. Improvements in survival of abalone fed the diets substituting *U. pinnatifida* with OEB subjected to the 24-h air exposure stressor in this study indicated that OEB could be used as a stressor reducer for juvenile abalone. OEB is not only a potentially novel substitute for *U. pinnatifida* in abalone diet to enhance growth, but also a stress reducer to lower mortality of abalone subjected to 24-h air exposure stress.

5. Conclusion

We evaluated the effect of graded substitution level of dietary *U. pinnatifida* with OEB on growth performances, chemical composition and air exposure stressor of juvenile abalone. Superior weight gain and SGR of abalone fed all formulated diets to those of fed the *Undaria* were observed. Shell length, width and height and soft body weight of abalone tended to agree with growth rate. *U. pinnatifida* up to 75% could be replaced with OEB in abalone feed when 20% *U. pinnatifida* was included. The greatest growth and SGR were obtained in abalone fed the OEB50 diet substituting 50% *U. pinnatifida* with OEB.

6. Acknowledgement

I would like to express my sincere appreciation to Prof. Sung Hwoan Cho for his guidance, precious advice and encouragement throughout this study. I wish to express gratitude to my committee, Ph.D. Jin-Hyung Yoo and Prof. Inho Yang for their critical review and comments for my thesis, and to Prof. Cheol Young Choi, In-Seok Park and Ki Hwan Moon for their considerate advices and interests in this dissertation.

Thanks to my lab seniors, Mr. Sang Mok Lee, Bum Hee Park, Sung Choon Ji, In Cheol Choi, Kyoung Tae Kim, Min Su Ha, Gyu Ho Jeon, Sung Hyo Myung, Hee Sung Kim, Dong Gyu Choi, Ye Eun Kim, Won Gwan Jung, Hyeon Jong Kim and Bok Il Jang. Also thanks to lab colleagues and juniors, June Kim, Ah Young Yun, Sung Il Baek, Min Jun Lee Ji Eun Park, So Hyeon Choi, Most. Waheda Rahman Ansary and Dai Qihu. I will never forget the time when we studied together.

Finally, I thank my family, who always give me constant advice and full support and always understand me. Always I appreciate and love you all.

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