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Effects of the formulated diet on growth performance and resistance of juvenile abalone [*Haliotis discus* (Reeve, 1846)] subjected to various stress condition

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A dissertation

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CONTENTS

List of Tables

List of Figures

전복용 배합사료의 공급에 따른 전복 치패의 성장 및 다양한 스트레스에 대한 내성 평가

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요 약

본 연구에서는 전복용 배합사료내 어분 및 해조류 대체원으로서 각각 발효대두박 및 생미강 사용에 따른 전복의 성장 및 다양한 스트레스에 대한 내성을 평가하였다. 마리당 평균 4.3 g의 전복 84,000마리를 무작위로 선별하여 12개의 5 ton 유수식 수조에 7,000마리씩 각각 수용하였다. 총 4종류의 실험사료를 준비하였다. 대조구(Standard) 사료는 주요 단백질원으로 어분 14% 및 발효대두박 25%를 첨가하였으며, 주요 탄수화물원으로 소맥분 20%를 첨가하였고, 25%의 해조류를 첨가하였다. 어분 50%를 발효대두박으로 대체한 실험사료(FM50), 어분 50%와 해조류 50%를 각각 발효대두박과 생미강으로 대체한 실험사료(FM50 + MA50) 및 어분 50%와 해조류 100%를 각각 발효대두박과 생미강으로 대체한 실험사료(FM50 + MA100)를 준비하였다. 실험사료의 효능을 평가하기 위하여 자연산 먹이인 미역(*Undaria pinnatifida*)과 다시마(*Laminaria japonica*)를 공급하는 실험구를 두었으며, 모든 사료는 2반복구를 두었다. 16주간의 사육실험 종료후 생존한 전복을 대상으로 하여 다양한 스트레스 조건(공기 노출, 염분 급변화, 수온 급변화)에 노출시 전복의 누적폐사율을 측정하였다. 전복의 체중증가와 일일성장률은 배합사료를 공급한 모든 실험구(Standard, FM50, FM50 + MA50, FM50 + MA100)에서 자연산 먹이를 공급한 실험구(*Undaria pinnatifida*,

Laminaria japonica)보다 유의적으로 높게 나타났다. 염분 급변화와 수온 급변화에 대한 전복의 누적폐사율은 자연산 먹이를 공급한 실험구에서 배합사료를 공급한 모든 실험구에 비하여 유의적으로 높게 나타났다. 결론적으로 전복의 성장에 있어서 배합사료를 공급한 실험구에서 자연산 먹이를 공급한 실험구보다 우수하게 나타났으며, 또한 공기 노출을 제외한 다양한 스트레스에 대한 내성도 자연산먹이를 공급한 실험구보다 배합사료를 공급한 실험구에서 높게 나타났다.

Keywords: 전복(*Haliotis discus*); 배합사료; 어분; 해조류; 스트레스 내성

I. Experiment

Effect of the formulated diets on performance and resistance of juvenile abalone [*Haliotis discus* (Reeve, 1846)] subjected to various stress conditions

Abstract

Performance and stress resistance of juvenile abalone (*Haliotis discus*) fed formulated diets substituting fish meal (FM) and macroalgae (MA) with soybean meal and rice bran, respectively, was compared with the MA under various stress conditions. Four experimental diets (Standard, FM50, FM50 + MA50 and FM50 + MA100 diets) were prepared in duplicate. The dry MA (*Undaria pinnatifida* and *Laminaria japonica*) were also prepared to compare with the effects of formulated diets on the performance of abalone to achieve the industry standard. Eighty four thousand juvenile abalone were distributed into twelve 5 ton tanks (7,000 abalone per tank). The diets were fed to abalone once a day to satiation. At the end of the 16-week feeding trial, abalone were subjected to the various stress (air exposure, sudden salinity and temperature changes) conditions and cumulative mortality was monitored. Weight gain and specific growth rate (SGR) of abalone fed the all formulated diets were higher than the dry MA. The cumulative mortality of abalone fed the MA was higher than that of abalone fed the all formulated diets at the end of observation after sudden salinity and temperature changes. In conclusion, the well-formulated diets produced better growth performance of abalone over the dry MA. Abalone subjected to the various stress conditions after being fed with all

formulated diets, except for abalone fed with the Standard, FM50 and FM50 + MA50 diets after air exposure, were more resistant than those fed the MA.

Keywords: abalone (*Haliotis discus*), formulated diets, fish meal, macroalgae, stress resistance

1. Introduction

Annual world aquaculture production of abalone increased from 2502 to 119017 metric tons in 2000 and 2013 over forty seven times and in Korea increased from 20 to 8982 metric tons in 2000 and 2014 about four hundred fifty times (FishStatJ 2015). This trend of increased aquaculture production of abalone will continue into the future due to high demand for human consumption, and the expansion of abalone farms.

In Korea, abalone farmers prefer feeding abalone on macroalgae (MA), such as *Undaria pinnatifida* Harvey or *Laminaria japonica* Areschoug, over formulated feed due to perceptions that this natural diet is easier to manage. Farmers are likely to misunderstand that supplying the artificially formulated feed to abalone causes water pollution in farming, because of poor water stability, as well as producing weaker abalone against a variety of stressors subjected to year-around abalone culture, such as air, high temperature and low salinity exposures than abalone fed MA. In Australia, however, all abalone farms routinely feed using a formulated diet despite similar stressors (Bansemer et al. 2016). Abalone (*Haliotis discus* Reeve) is a marine warm-water gastropod mollusc and endemic to the waters off Japan and Eastern Asia including Jeju island in Korea (Han 1998). Abalone is produced throughout year-round culture. A stress of air exposure to abalone commonly occurs during size grading and transporting of abalone, and cleaning of shelters. A stress of exposure to higher than average water temperature also occurs during the summer season. A stress of low salinity exposure to abalone occurs at outdoor farm during the raining season as well. These stressors can depress immunity of abalone and increase their susceptibility to disease (Edwards et al. 2000, Malham et al. 2003, Cheng et al. 2004b & c, Hooper et al. 2007, Song et al. 2007, Travers et al. 2008, Vosloo et al. 2013, Hooper et al. 2014). Mortality of abalone also can occur in the wild due to high ambient temperature and salinity changes (Takami et al. 2008, Park et al. 2013).

Seasonal availability of MA commonly used as abalone feed is limited during winter season in wild in Korea. In addition, abalone farmers are at a transition point where they are used to feeding MA, but are now being forced to look at other options. To improve abalone aquaculture production, therefore, development of low-cost, but nutritionally-balanced feed for abalone must be undertaken. Well-formulated feed can produce improved growth performance of abalone compared to single macroalgal diet (Lee 1998, Kim et al. 1998, Cho et al. 2006, Cho et al. 2008a & b, Garcia-Esquivel & Felbeck 2009, Dang et al. 2011, Kim et al. 2016a, Myung et al. 2016).

A few comparative data on the stability of formulated feed in water and stress resistance, growth or survivorship of abalone fed on MA and formulated feeds are available (Cho & Kim 2012, David et al. 2014). Development of the alternative feed ingredients for fish meal (FM) and MA, which are the most expensive components in abalone feed, is of primary researcher interest. The most promising alternative feed ingredients for FM and MA are soybean meal (Uki et al. 1985, Lee et al. 1998, Cho et al. 2008a, Cho 2010) and rice bran, which are agriculture byproducts rich in nutrients, such as crude protein and vitamins (Kim et al. 2016a). In addition, fermentation of soybean meal increases the protein content, eliminates trypsin inhibitors, and reduces peptide size (Hong et al. 2004). Oral administration of fermented soybean meal produced a promising effect on performance of fish (Lim & Lee 2011, Zhou et al. 2011). Therefore, performance and stress resistance of juvenile abalone fed the formulated diets substituting FM and MA with soybean meal and rice bran was compared with MA against various stress conditions in this study. Water stability of the formulated diets was also compared with the MA.

2. Materials and Methods

2.1. Preparation of Abalone and Rearing Conditions

One hundred thousand juvenile abalone (*H. discus*) were purchased from a private hatchery and transferred to Cheil abalone farm (Jeju Special Self-Governing Province, Korea). Before initiation of the feeding trial, abalone were acclimated to the experimental conditions for 2 weeks and fed with dry *U. pinnatifida* once a day at a ratio of 3 - 5% total biomass.

Twelve 5 ton concrete flow-through raceway indoor tanks (water volume: 3 ton) with a flow rate of 45.6 L/min were used for this experiment (n = 2 per diet, Table 1). Juvenile abalone [4.3 ± 0.01 g (mean ± SD), n = 84000] were randomly distributed into tanks (n = 7000 per tank). Sand-filtered seawater, at a temperature ranging from 17.3 to 21.2°C (mean ± SD: 19.5 ± 0.03°C), was supplied throughout feeding trial. Aeration was supplied into each raceway and the photoperiod followed natural conditions. The experimental diets were fed to abalone once a day (17:00h) to satiation with a little leftover (about 2 – 3% total biomass). Dead abalone were removed daily and the bottoms of the tanks were hose-cleaned twice a week. The feeding trial lasted for 16weeks. At the end of the feeding trial, two hundred abalone were randomly chosen from each tank and then collectively weighed to evaluate weight gain.

2.2. Preparation of the Experimental Diets

Four experimental diets (Standard, FM50, FM50 + MA50 and FM50 + MA100 diets) were prepared in duplicate (Table 1). The Standard diet was formulated to satisfy dietary nutrient requirements of abalone (Uki et al. 1986, Mai et al. 1995a & b). The four experimental diets were pelletized by an extruded pelleter (Jyoda, Japan) at Ewha Oil and Fat Industry Co. Ltd. (Busan, Korea). Finally, dry *U. pinnatifida* and *L. japonica* were prepared to compare with the effects of extruded diets on the performance of abalone.

Table 1. Feed formulation of the experimental diets (%, DM basis)

	Experimental diets					
	Standard	FM50	FM50 + MA50	FM50 + MA100	*U. pinnatifida*	*L. japonica*
Ingredients (%, DM)						
Fish meal	14	7	7	7		
Fermented soybean meal	25	32	32	32		
Wheat flour	20	20	20	20		
Macroalgae (MA)[1]	25	25	12.5			
Rice bran			12.5	25		
Others	13.5	13.5	13.5	13.5		
Vitamin premix[2]	0.5	0.5	0.5	0.5		
Mineral premix[3]	2	2	2	2		
Nutrients (%, DM)						
Dry matter	98.7	98.8	97.8	98.1	86.0	88.7
Crude protein	36.3	36.1	38.0	38.3	20.7	9.0
Crude lipid	1.4	1.4	3.7	6.8	0.8	0.4
Carbohydrate[4]	42.4	43.5	46.4	46.7	42.4	58.1
Ash	19.9	19.0	11.9	8.2	36.1	32.5

[1]Macroalgae (MA) is the mixture of *Undaria pinnatifida* and *Hizikia fusiforme* at a ratio of 1:1.
[2]Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): excipient, 317; riboflavin, 23.8; pyridoxine, 4.7; niacin, 95.2; Ca-pantothenate, 33.3; inositol, 476.9; folic acid, 1.5; p-amino benzoic acid, 47.6.
[3]Mineral premix contained the following ingredients (g/kg mix): Excipient, 45.5; $MgSO_4$, 140.8; NaH_2PO_4, 92.4; KH_2PO_4, 246; $Ca(H_2PO_4)_2$, 139.5; $ZnSO_4$, 22.5; Ca-lactate, 310; $AlCl_3$, 0.15; KI, 0.15; $MnSO_4$, 2; $CoCl_2$, 1.
[4]Carbohydrate was calculated by the difference between 100 and sum of crude protein, crude lipid and ash contents.

2.3. Growth measurements

One hundred abalone at the start and fifty abalone from each tank at the termination of the feeding trial were sampled and frozen for analysis. Prior to examination, all samples were slightly thawed, followed by separation of the shell and soft-body tissue. Shell length, width and height were measured to a precision of 1.0 mm with a digital caliper (Mitutoyo Corporation, Kawasaki, Japan), and the ratio of soft body weight to whole body weight (the soft body weight + shell) was calculated to determine a condition index for abalone. Specific growth rate (SGR, %/day) was calculated using the formula of Britz (1996): SGR = [(ln(Wf) - In(Wi))/days of feeding] × 100, where In(Wf) = natural log of the final mean weight of abalone and In(Wi) = natural log of the initial mean weight of abalone.

2.4. Proximate Analysis of Abalone Flesh and Water Stability of Nutrients in the Diets

The pooled separated soft body tissue from the sampled abalone from each tank was then homogenized and used for proximate analysis. Crude protein content was estimated from total nitrogen using 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 AOAC (1990) practices.

The four extruded diets, dry *U. pinnatifida* and *L. japonica* were placed in separate 70 L plastic rectangular containers (120 cm × 36 cm) without abalone in duplicate. These containers were then placed within a, 5 ton concrete flow-through raceway indoor tank at a flow rate of 45.6 L/min and subsampled at 12, 24, 48 and 72 h to evaluate leaching of nutrients in the diets to determine their water stability. Nutrient levels in the diets were assed using the same procedure described above for the abalone flesh. Water stability of nutrients in the diets was expressed

as the percentage of final dry content to initial dry content for each nutrient based on Mai et al. (1995a)'s study.

2.5. Stress Resistance of Abalone Subjected to the Various Stress Conditions

The various stress conditions of abalone was modified based on Cho et al. (2008b)'s study. At the end of the 16-week feeding trial, sixty randomly chosen abalone from 12, 5 ton tank were distributed into 3, 70 L plastic rectangular containers (120 cm × 36 cm) (twenty abalone per container). Twelve, 70 L plastic rectangular containers from each tank were randomly placed into 1 ton indoor fiber reinforced plastic (FRP) tank and 3, 1 ton FRP tanks were used for stress resistance of abalone subjected to the various stress conditions (air exposure, sudden low salinity and high temperature changes). The designated diets were fed to abalone for two weeks to minimize stress of abalone resulted from handling before being subjected to their stress resistance test.

Seawater in twelve containers with abalone in a 1 ton indoor FRP tank was completely drained and subjected to air exposure for 30 h at room temperature. After 30 h air exposure, the 1 ton FRP tank was filled with natural seawater. Dead abalone were eliminated every two hours during 30 h for air exposure and 70 h for post stress monitoring.

Twelve containers with abalone in another 1 ton indoor FRP tank at salinity 31 in natural seawater was moved to the other 1 ton FRP tank adjusted at salinity 25 by mixing with tap and seawater. Salinity was measured by using YSI 6-Series Multi-Parameter (YSI, Yellow Springs, OH, USA). Salinity of twenty five maintained in the tank for the whole period of observation. Dead abalone were removed every 2 h for 96 h and 4 h for the next 72 h.

Twelve containers with abalone in the other 1 ton indoor FRP tank at 21°C in seawater was moved to the other 1 ton FRP tank adjusted at 30°C by submerged titanium heater (TH-2000) with automatic thermostat 413-H (A-MI Corporation,

Incheon, Korea). 30°C maintained in the tank for the whole period of observation. Dead abalone were removed every 2 h for 80 h when mortality of abalone fed the MA reached 100%.

2.6. Statistical Analysis

One-way ANOVA and Duncan`s multiple range test (Duncan 1955) were used to determine the significance of the differences among the means of treatments by using SAS version 9.3 program (SAS Institute, Cary, NC, USA). Water stability of the experimental diets was tested by ANOVA with repeated measurement designs (Cody & Smith 1991). Percentage data was arcsine-transformed prior to statistical analysis.

3. Results

3.1. Water Stability of the Experimental Diets

Dry matter, crude protein, crude lipid and ash content of the experimental diets was significantly changed over all period of time ($P < 0.0001$), except for 12 h ($P < 0.0004$) and 72 h ($P < 0.0006$) of crude lipid content (Figs. 1, 2, 3 and 4), and their significant ($P < 0.0001$) interactions (experimental diets × time) were also observed. After 12 h immersion in seawater, the retention of dry matter (Fig. 1) and crude protein (Fig. 2) content in the *L. japonica* was significantly ($P < 0.05$) higher than those in the all other diets and lowest for *U. pinnatifida*. After 24 h, the amount of dry matter content retained in the all formulated (Standard, FM50, FM50 + MA50 and FM50 + MA100) diets was significantly ($P < 0.05$) higher than that in the *U. pinnatifida* and *L. japonica*. The proportion of crude protein content retained in the all formulated diets was significantly ($P < 0.05$) higher than that in the *U. pinnatifida*, but not significantly different from that in the *L. japonica* at 24 h after seawater immersion. The amount of crude protein retained in the all formulated diets was significantly ($P < 0.05$) higher than in the *U. pinnatifida* and *L. japonica* at 48 and 72 h after seawater immersion, except for that in the FM50 + MA50 diet at 48 h.

The amount of crude lipid retained in the FM50 + MA100 diet was significantly ($P < 0.05$) higher than that in all other diets, followed by the FM50 and Standard diets, *U. pinnatifida*, FM50 + MA100 diet and *L. japonica* at 12 h after seawater immersion (Fig. 3). After 24 h, the percent of crude lipid content retained in the all formulated diets was significantly ($P < 0.05$) higher than that in the *U. pinnatifida* and *L. japonica*.

The proportion of the ash content retained in the all formulated diets was significantly ($P < 0.05$) higher than that in the *U. pinnatifida* and *L. japonica* at 12, 24, 48 and 72 h after seawater immersion (Fig. 4). The highest ash content was observed in the FM50 + MA100 diet, but lowest for the *U. pinnatifida* throughout 72 h observation.

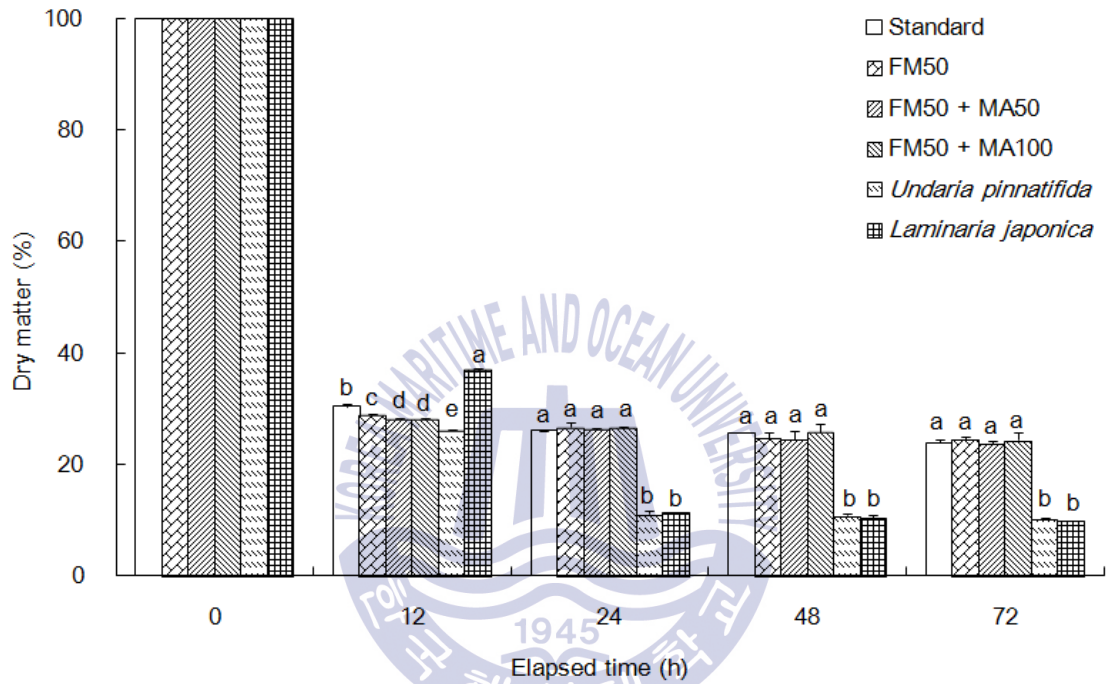

Figure 1. Changes in dry matter content (%) of the experimental diets at 12, 24, 48 and 72 h after seawater immersion (means of duplicate ± SE). [ANOVA with repeated design: times (P < 0.0001) and their interaction (experimental diets × time) (P < 0.0001)]. Different letters in each time point indicates difference between diets within each time point.

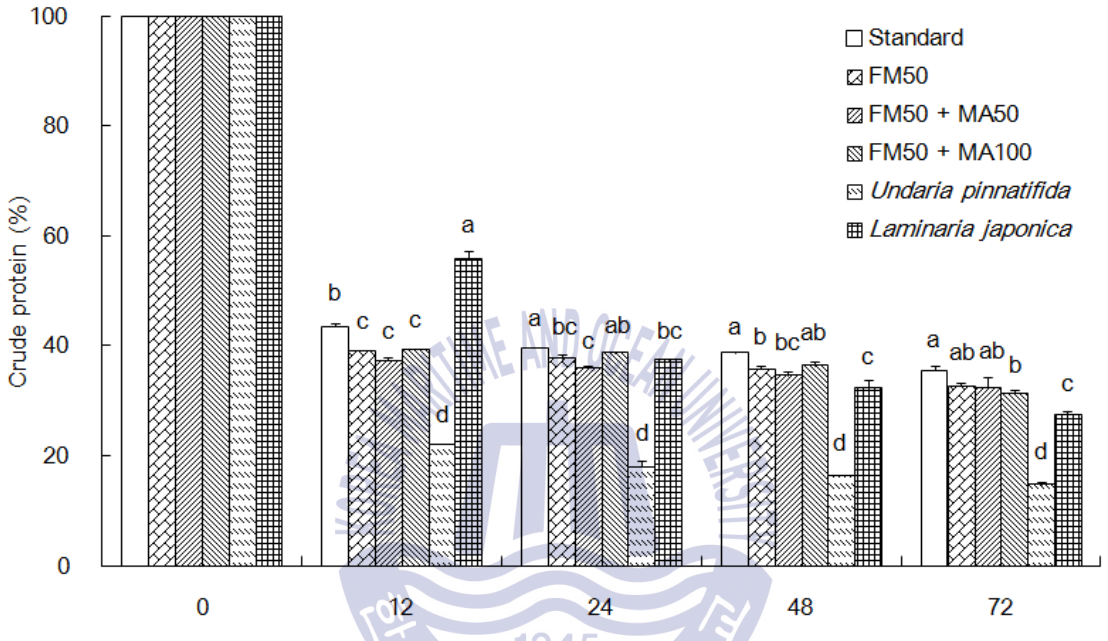

Figure 2. Changes in crude protein content (%) of the experimental diets at 12, 24, 48 and 72 h after seawater immersion (means of duplicate ± SE). [ANOVA with repeated design: times (P < 0.0001) and their interaction (experimental diets × time) (P < 0.0001)]. Different letters in each time point indicates difference between diets within each time point.

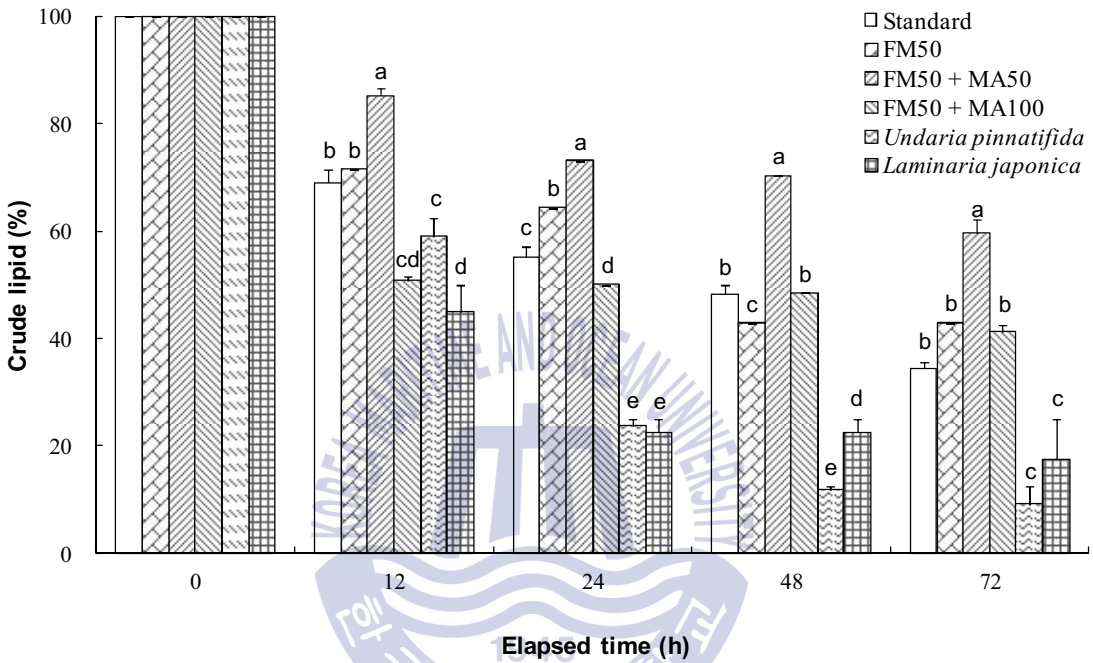

Figure 3. Changes in crude lipid content (%) of the experimental diets at 12, 24, 48 and 72 h after seawater immersion (means of duplicate ± SE). [ANOVA with repeated design: 12 h (P < 0.0004), 24 and 48 h (P < 0.0001), 72 h (P < 0.0006), and their interaction (experimental diets × time) (P < 0.0001)]. Different letters in each time point indicates difference between diets within each time point.

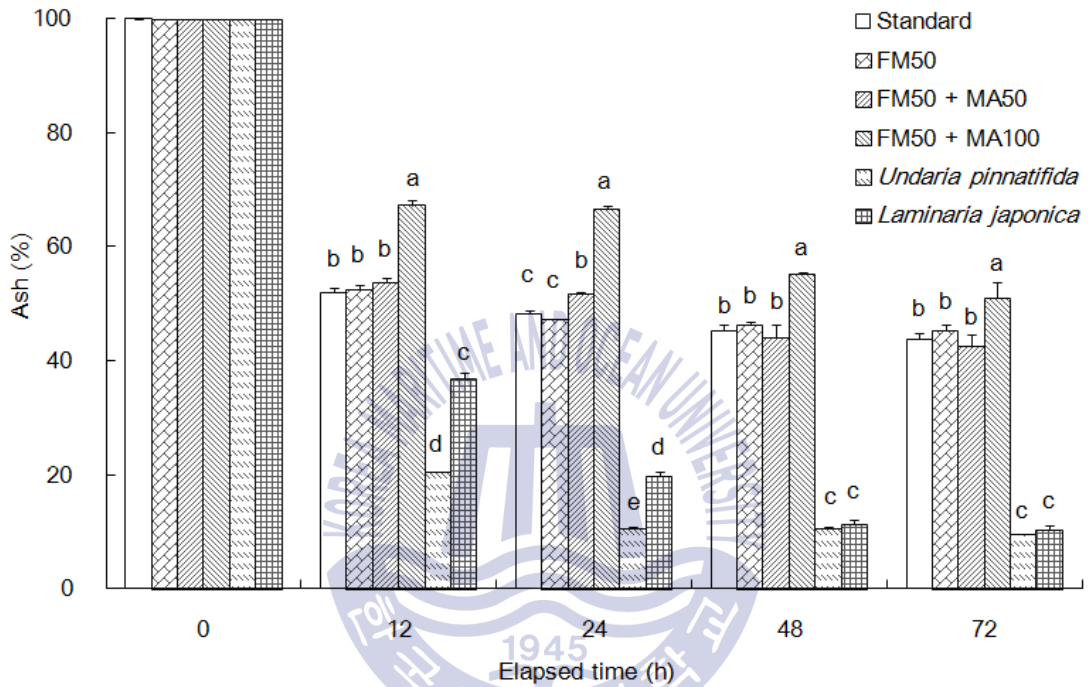

Figure 4. Changes in ash content (%) of the experimental diets at 12, 24, 48 and 72 h after seawater immersion (means of duplicate ± SE). [ANOVA with repeated design: times (P < 0.0001) and their interaction (experimental diets × time) (P < 0.0001)]. Different letters in each time point indicates difference between diets within each time point.

3.2. Growth Performance of Abalone

Survival of abalone fed the all formulated (Standard, FM50, FM50 + MA50 and FM50 + MA100) diets was significantly (F-value: 258.7, df=5; $P < 0.0001$) higher than that of abalone fed the MA (Table 2). Weight gain and SGR of abalone fed the all formulated diets were also significantly ($P < 0.05$) higher than those of abalone fed the MA. In addition, weight gain and SGR of abalone fed the *L. japonica* were significantly ($P < 0.05$) higher than those of abalone fed the *U. pinnatifida*.

The longest shell length was obtained in abalone fed the Standard diet, followed by the FM50, FM50 + MA50 and FM50 + MA100 diets, then *L. japonica* and *U. pinnatifida* (Table 3). The widest shell width was observed in abalone fed the Standard diet, and followed by the FM50 + MA50, FM50 and FM50 + MA100 diets, then *L. japonica* and *U. pinnatifida*. The soft body weight of abalone fed the all formulated diets was significantly ($P < 0.05$) greater than that of abalone fed the MA.

3.3. Proximate Composition of the Soft Body of Abalone

Moisture content of the soft body of abalone fed the FM50 diet was significantly ($P < 0.05$) higher than that of abalone fed the FM50 + MA50 diet, *U. pinnatifida* and *L. japonica*, but not significantly ($P > 0.05$) different from that of abalone fed the Standard, FM50 + MA100 diets (Table 4). Crude protein content of the soft body of abalone fed the FM50 + MA100 diet was significantly higher ($P < 0.05$) than that of abalone fed the all other diets. Crude protein content of the soft body of abalone fed the Standard and FM50 + MA50 diets was also significantly higher ($P < 0.05$) than that of abalone fed the FM50 diet, *L. japonica* and *U. pinnatifida*. Crude lipid content of the soft body of abalone fed the FM50 + MA100 diet was significantly higher ($P < 0.05$) that of abalone fed the all other diets. Crude lipid content of the soft body of abalone fed the FM50 + MA50 diet was also

Table 2. Survival (%), weight gain (g/abalone) and specific growth rate (SGR) of juvenile abalone fed the experimental diets for 16 weeks at abalone farm

Experimental diets	Initial weight (g/abalone) n = 200	Final weight (g/abalone) n = 100	Survival (%) n = 2 tanks	Weight gain (g/fish) n = 2 tanks	SGR[1] (%/day)
Standard	4.3 ± 0.01	11.8 ± 0.04[a]	96.4 ± 0.06[a]	7.54 ± 0.048[a]	0.88 ± 0.005[a]
FM50	4.3 ± 0.01	10.1 ± 0.06[b]	96.4 ± 0.06[a]	5.77 ± 0.066[b]	0.73 ± 0.007[b]
FM50 + MA50	4.3 ± 0.01	8.6 ± 0.03[c]	96.4 ± 0.06[a]	4.33 ± 0.028[c]	0.61 ± 0.002[c]
FM50 + MA100	4.3 ± 0.01	8.7 ± 0.07[c]	96.4 ± 0.06[a]	4.36 ± 0.068[c]	0.61 ± 0.007[c]
U. pinnatifida	4.3 ± 0.01	7.4 ± 0.02[e]	93.3 ± 0.01[b]	3.15 ± 0.009[e]	0.48 ± 0.000[e]
L. japonica	4.3 ± 0.01	8.0 ± 0.01[d]	93.4 ± 0.05[b]	3.76 ± 0.020[d]	0.55 ± 0.003[d]

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

[1]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. Shell length (mm), shell width (mm), shell height (mm), soft body weight (g/individual) and the ratio of soft body weight to total weight of abalone fed the experimental diets for 16 weeks at abalone farm

Experimental diets	Shell length (mm)	Shell width (mm)	Shell height (mm)	Soft body weight (g)	Soft body weight/total weight
Standard	41.9 ± 0.20^{a}	28.9 ± 0.30^{a}	8.9 ± 0.33^{a}	4.7 ± 0.01^{a}	0.63 ± 0.007^{a}
FM50	40.3 ± 0.11^{b}	27.3 ± 0.10^{bc}	8.3 ± 0.12^{abc}	4.0 ± 0.05^{b}	0.62 ± 0.005^{ab}
FM50 + MA50	39.5 ± 0.10^{c}	27.6 ± 0.35^{b}	7.8 ± 0.02^{bc}	3.9 ± 0.07^{bc}	0.64 ± 0.006^{a}
FM50 + MA100	39.2 ± 0.03^{c}	27.0 ± 0.13 ^{bc}	8.4 ± 0.18^{ab}	3.7 ± 0.01^{c}	0.61 ± 0.001^{ab}
U. pinnatifida	37.5 ± 0.20^{e}	25.3 ± 0.20^{d}	7.6 ± 0.01^{c}	3.0 ± 0.02^{d}	0.59 ± 0.005^{b}
L. japonica	38.5 ± 0.10^{d}	26.6 ± 0.01^{c}	8.0 ± 0.12^{bc}	3.1 ± 0.06^{d}	0.57 ± 0.006^{c}

Values (means of pooled from 50 abalone in duplicate tanks ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

Table 4. Chemical composition (%) of the soft body of abalone at the end of the 16-week feeding trial at abalone farm

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
Standard	76.1 ± 0.16^{ab}	16.8 ± 0.08^{b}	1.1 ± 0.04^{c}	2.0 ± 0.04^{c}
FM50	76.3 ± 0.16^{a}	16.5 ± 0.12^{c}	1.1 ± 0.04^{c}	1.9 ± 0.04^{cd}
FM50 + MA50	75.1 ± 0.61^{b}	16.9 ± 0.08^{b}	1.8 ± 0.04^{b}	1.8 ± 0.00^{cd}
FM50 + MA100	76.2 ± 0.12^{ab}	17.3 ± 0.04^{a}	2.0 ± 0.04^{a}	1.7 ± 0.00^{d}
U. pinnatifida	72.6 ± 0.08^{c}	15.5 ± 0.08^{d}	0.9 ± 0.00^{d}	2.5 ± 0.08^{b}
L. japonica	72.3 ± 0.00^{c}	16.3 ± 0.04^{c}	1.1 ± 0.04^{c}	2.8 ± 0.04^{a}

Values (means from duplicate tanks ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

significantly higher ($P < 0.05$) that of abalone fed the Standard and FM50 diets, *L. japonica* and *U. pinnatifida*. The highest ash content was obtained in abalone fed the *L. japonica*, and followed by *U. pinnatifida*, the Standard, FM50, FM50 + MA50 and FM50 + MA100 diets.

3.4. Cumulative Mortality of Abalone Subjected to Various Stress Conditions

No mortality of abalone was observed during 30 h air exposure, except for 1 dead abalone from each container receiving the *U. pinnatifida* and *L. japonica* at 22 h after air exposure (Fig. 5). The cumulative mortality of abalone fed the MA (*U. pinnatifida* and *L. japonica*) and Standard and FM50 diets was significantly ($P < 0.05$) higher than that of abalone fed the FM50 + MA100 diet, but not significantly ($P > 0.05$) from that of abalone fed the FM50 + MA50 diet at 100 h after air exposure.

In the salinity stress experiment, the cumulative mortality of abalone fed the *U. pinnatifida* and *L. japonica* was significantly ($P < 0.05$) higher than that of abalone fed the all formulated diets, except for Standard diet, at day 7 (Fig. 6), but no significant difference in the cumulative mortality was found among the formulated diets. The lowest cumulative mortality was observed in abalone fed the FM50 + MA100 diet, followed by the FM50 + MA50, FM50 and Standard diets, then *L. japonica* and *U. pinnatifida*.

In the temperature stress experiment, the cumulative mortality of abalone fed the *L. japonica*, and *U. pinnatifida* was significantly ($P < 0.05$) higher than that of abalone fed the all formulated diets at 80 h after temperature change (Fig. 7). The lowest cumulative mortality was obtained in abalone fed the FM50 + MA50 diet, followed by the FM50, FM50 + MA100, FM50 and Standard diets, then *L. japonica* and *U. pinnatifida*.

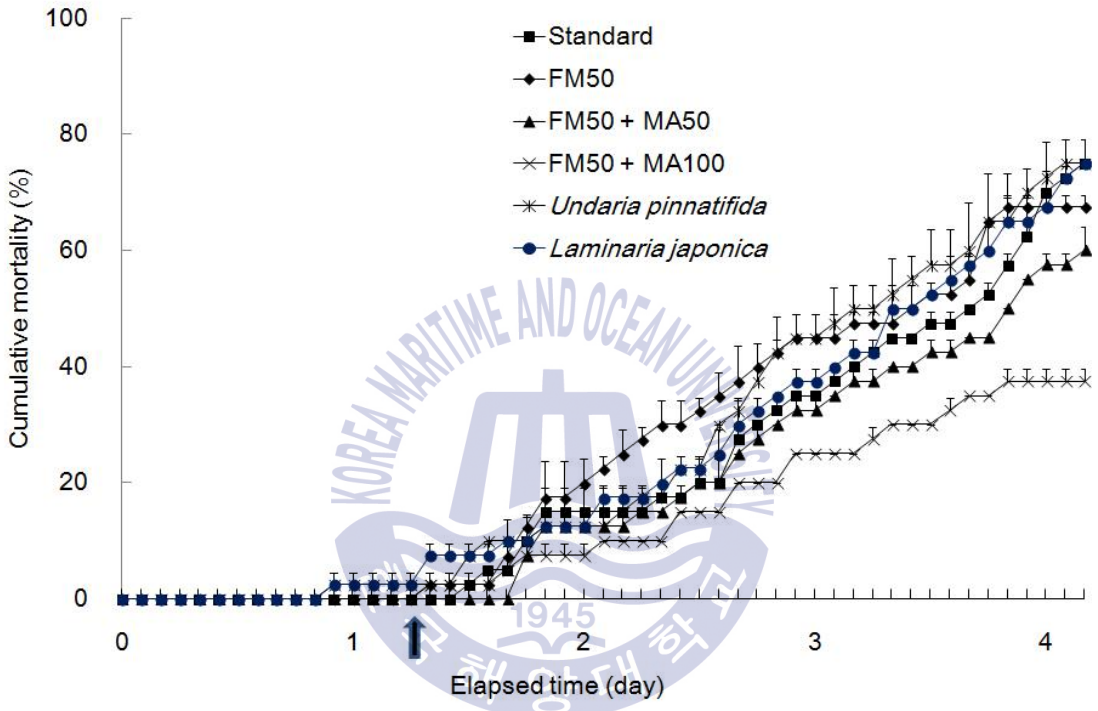

Figure 5. Cumulative mortality (%) of abalone fed the experimental diets for 16 weeks, and then subjected to air exposure (means from duplicate tanks ± SE). Arrow (⬆)indicates the time abalone were re-immersed in water.

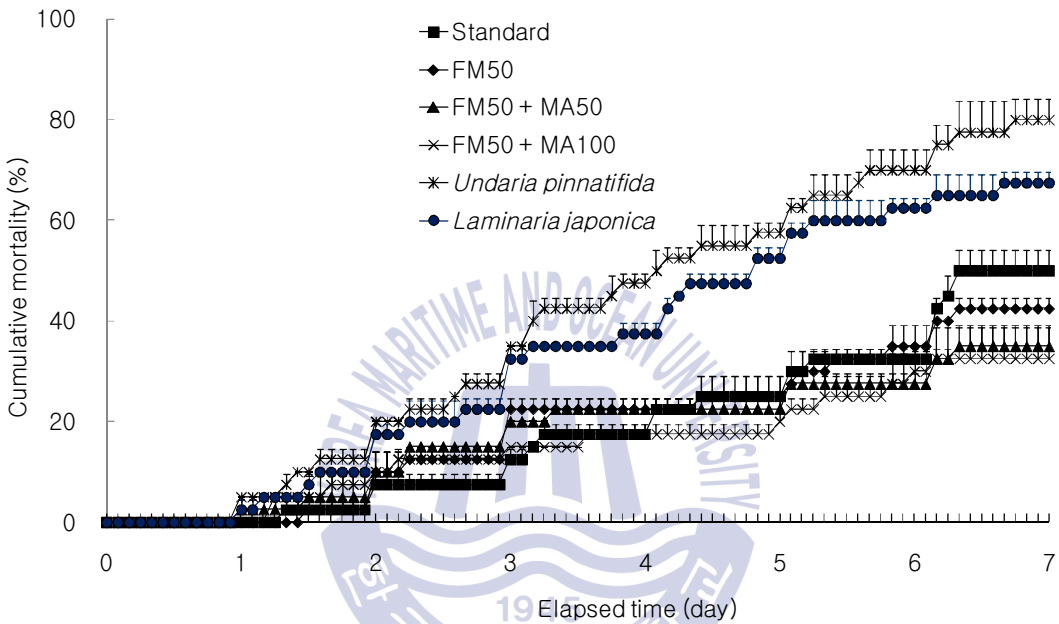

Figure 6. Cumulative mortality (%) of abalone fed the experimental diets for 16 weeks, and then subjected to sudden salinity change (means from duplicate tanks ± SE).

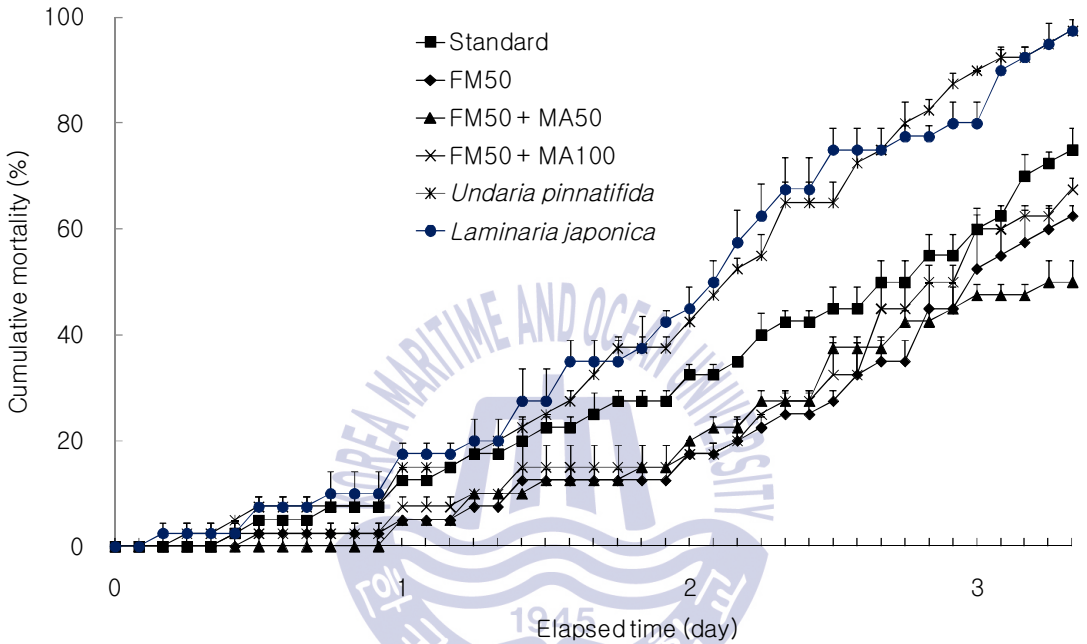

Figure 7. Cumulative mortality (%) of abalone fed the experimental diets for 16 weeks, and then subjected to sudden temperature change (means from duplicate tanks ± SE).

4. Discussion

Since abalone are slow eaters, water stability of the diet can be one of the most important factors determining growth of abalone and water pollution from farms. Dry matter and crude protein content in the all formulated diets and *U. pinnatifida* sharply decreased to less than 50 % of the initial contents within 12 h after seawater immersion, and then continued to slowly decrease after 24, 48 and 72 h. This indicates that dry matter and crude protein of the formulated diets were leaching out primarily within 12 h after seawater immersion. Higher retention of the initial dry matter, crude protein, crude lipid and ash content in all the formulated diets compared to those in the MA (*U. pinnatifida* and *L. japonica*) after 48 h after seawater immersion, indicated that water stability of the all formulated diets was higher than that in the MA. Bautista-Teruelet et al. (2003) reported that water stability of the formulated diets was estimated to be 64% at 24 h when animal and plant protein sources was tested for abalone (*H. asinina*). Gómez-Montes et al. (2003) showed that ash content decreased by 66% to 74% of the known levels of diets after 12 h immersion in seawater when dietary optimum protein and energy ratio was determined for abalone (*H. fulgens*). Mai et al. (1995a) estimated average water stability of the experimental diets containing different lipid levels ranging from 0.6-11.58% to be about 90 and 84% at 8 and 24 h in water, respectively. The relatively high value of water stability of the experimental diets resulted from the use of feed binder (18% Na alginate) compared to values [(22.5% (*L. japonica*) - 73% (FM50 + MA50 diet)] in this study. Sales & Britz (2003) reported that the lowest dry matter leaching (1.1 and 1.6%) was obtained in corn gluten and fish meals, but highest (12 and 12.8%) for legumes (lupins and

faba beans) when dry matter leaching of experimental diets containing different protein sources (FM, corn gluten meal, soya bean meal, cottonseed meal, sunflower meal, canola meal, peanut meal, lupins, fafa beans) was measured after 16 h in seawater immersion. Overall, the well- formulated diet is highly recommendable due to advantages of high water stability compared to the dry MA. Abalone farmers are likely to bottom-clean their farm with flow-through system twice a week when the well-formulated diet was daily supplied at proper feeding ratio in Jeju Special Self-Governing Province in Korea (personal communication).

Weight gain and SGR of abalone fed the all formulated diets were higher than those of abalone fed the MA in this study, agreeing with other studies (Lee et al. 1998, Kim et al. 1998, Cho et al. 2006, Cho et al. 2008a & b, Garcia-Esquivel & Felbeck 2009, Dang et al. 2011, Myung et al. 2016, Kim et al. 2016a) showing that the formulated diets produced better growth performance in several abalones than the MA. Poor weight gain and SGR was found in abalone fed the diets substituting 50% FM with the fermented soybean meal regardless of substitution of MA (FM50, FM50 + MA50 and FM50 + MA100), compared to those of abalone fed the Standard diet. This indicated that 50% substitution of FM with the fermented soybean meal suppressed the growth performance of juvenile abalone. Unlike this study, however, Lee et al. (1998) reported that 31% casein could be completely replaced by 40% white FM, 39% soybean meal or 55% cotton seed meal in the experimental diets without retardation of weight gain of juvenile abalone (*H. discus hannai*) in a pilot-scale 18 week feeding trial. Lee (1998) also showed that a single protein source of 33% casein or 41% white FM in the extruded diet could be replaced by the combination of 40% soybean meal and 15% cottonseed meal without deterioration of weight gain in juvenile

abalone (*H. discus hannai*) in a pilot-scale 4 month feeding trial. Therefore, suitability and/or feasibility of alternative plant protein source for FM in the extruded diet must be tested prior to a practical application.

The mean SGR of juvenile abalone averaging 4.3 g reared at mean temperature of 19.5°C was relatively high, 0.61%/day in this study compared to 0.58%/day for abalone averaging 3.6 g at mean temperature of 20.2°C in Kim et al. (2016b, unpublished data). The higher SGR in larger abalone at lower temperature in this study indicates that the growth rate of abalone (*H. discus*) is faster than that of abalone (*H. discus hannai*). A proper study to determine the growth rate of two species of abalone at different temperature conditions is needed for direct comparison.

Abalones are known to be herbivorous and feed mostly on MA, which is usually low in protein and lipid, but high in carbohydrate, e.g. 40-50% in the wild (Thongrod et al. 2003). Unlike Kim et al. (2016a)'s study showing that 100% substitution of *L. japonica* with rice bran at 20% in the diet could be made without retardation of growth of juvenile abalone (*H. discus*) in a pilot-scale of the 4-month feeding trial, weight gain and SGR of abalone fed the FM50 + MA50 diet were reduced compared to those of abalone fed the FM50 diet, but comparable to those of abalone fed the FM50 + MA100 diet in this study. This indicates that the rest 50% of MA can be replaced with rice bran after 50% substitution of MA with rice bran in the formulated diet for abalone is made.

Higher protein and lower ash content of the soft body of abalone fed the formulated diets compared those of abalone fed the MA was well reflected from nutrient contents in the experimental diets in this study, agreeing with other studies (Mai et al. 1995a & b, Thongrod et al. 2003, Cho et al.

2008a, Garcia-Esquivel et al. 2009, Cho 2010, Kim et al. 2016a & b, Myung et al. 2016).

The cumulative mortality of abalone fed the MA, Standard and FM50 diets was higher than that of abalone fed the FM50 + MA100 diet at 100 h after air exposure in this study. Exposing abalone to air can affect the animal's physiological state, which in turn may result in a reduction in growth of abalone (*H. laevigata* and *H. rubra*) (Edwards et al. 2000), health of abalone (*H. rubra*) (Song et al. 2007) or survival of abalone (*H. tuberculate*) (Malham et al. 2003). Hooper et al. (2011, 2014) demonstrated effects of movement with short term air exposure on physiological and histological changes of farmed Australian hybrid abalone (*H. laevigata* × *H. rubra*). Wells & Baldwin (1995) reported that lactate and tauropine increased, while ATP and energy charge decreased in abalone (*H. iris* and *H. australis*) after 24 h air exposure and demonstrated that stress responses might affect meat quality and survival during transport.

The cumulative mortality of abalone fed the MA was higher than that of abalone fed the all other diets at 96 h after sudden salinity change, but no difference in the cumulative mortality was found among the formulated diets. Cheng et al. (2004a) showed that Taiwan abalone (*H. diversicolor supertexta*) transferred from salinity 30 to salinity 20, 25 and 35 had reduced immune ability and decreased resistance against *V. parahaemolyticus* infection. When abalone (*H. discus discus*) was subjected to physical stresses such as thermal, low-salinity and hypoxic stress, they utilize antioxidant and immune defense mechanisms to overcome stress (Zoysa et al. 2009).

The cumulative mortality of abalone fed the *L. japonica* and *U. pinnatifida* was higher than of abalone fed the all other diets after 60 h after sudden

temperature change. Cho & Kim (2012) showed that survival of abalone (*H. discus hannai*) was affected by feed type, but not by water temperature, but weight gain was by both feed type and water temperature when juvenile abalone was fed with either the formulated diet or *Lamninaria japonica* at different temperature conditions (20, 23 and 26°C) for 12 weeks. David et al. (2014) demonstrated that a pattern of mortality in response to high water temperatures that differed for age classes and dietary intervention reduced mortality in larger (3-year old) abalone at high temperature (26°C). For instance, survival of 2-year old greenlip abalone (*H. laevigate*) fed the commercial or *Ulva lactuca* at 26°C was all over 90%, but survival (65%) of 3-year old abalone fed the commercial diet was lower than that (90.2%) of abalone fed the *U. lactuca* at 26°C or either diet (over 97.5%) at 18 and 22°C when abalone were fed with either *U. lactuca* or commercial diet for 36 days at different temperature conditions (18, 22 and 26°C). High summer temperature is one of the most stressful environmental problems confronted by the Australian abalone aquaculture industry and immunosuppression and organ damage of abalone are likely to be involved in the increased incidence of disease outbreak (Hooper et al. 2007, Travers et al. 2008, Dang et al. 2012, Hooper et al. 2014). Cheng et al. (2004b) reported that Taiwan abalone (*H. diversicolor supertexta*) subjected to high (32°C) temperature had reduced innate immunity and resistance against *V. parahaemolyticus* when it was held in salinity 30 seawater at 28°C, injected with *V. parahaemolyticus*, and then transferred to 20, 24, 28 and 32°C. When the temperate abalone (*H. midae*) were subjected to two constant temperatures (14 and 19°C) for one month, long-term 19°C exposure was more stressful to abalone than a 14°C exposure of the same duration (Vosloo et al. 2013). Exposure to higher temperature generally led to higher activities of the antioxidant

enzymes measured, but lowered total antioxidant capacities of the abalone. The relatively higher cumulative mortality of abalone fed the MA compared to the all formulated diets before being subject to various stress conditions in this study indicated that abalone fed the formulated diets were more resistant to various stress conditions commonly occur during year-round abalone culture than the MA in farm. This may result from the fact that those fed the formulated diets have better nutritional status and are likely to have better physiological health than the MA.

II. Conclusion

In conclusion, water stability of the all formulated diets was relatively higher compared to that of the MA (*U. pinnatifida* and *L. japonica*) at 24, 48 and 72 h after seawater immersion. The well-formulated diets produced better growth performance of abalone over the MA. Abalone subjected to the various stress conditions (salinity and temperature changes) after being fed with the all formulated diets was more resistant than those fed the MA.

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