



Thesis for the Degree of Master of Science

# Growth and body composition effects of tuna byproduct meal (TBM) substituted for fish meal in the diet of juvenile abalone, *Haliotis discus*



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### 국문요약

# 전복, Haliotis discus 치패용 배합사료내 어분의 참치가공부산물 대체 시 성장 및 체구성에 미치는 영향

#### 정원관

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## 요약

본 연구는 국내 전복 주요 양식 대상 종 중 하나인 까막전복, Haliotis discus에서 배합사료내 어분의 참치가공부산물(Tuna byproduct meal: TBM) 대체 시 성장 및 체조성에 미치는 영향을 조사 하였다. 전복 치패(평균 무게 1.29 g) 1,260미를 임의로 선별하여 70 L 플라스 틱 수용기 18개에 각각 70미씩 수용하였다. 사육실험은 16 주간 진행 하였으며, 사료는 매일 1회 만복 시 까지 손으로 공급하였다. 사료 6 종류(TBM0, TBM25, TBM50, TBM75, TBM100 및 ST)를 실험에 이용 하였으며, 3반복 수행하였다. TBM0는 단백질원으로 어분 28%와 대두 박 13%를 함유하였고, 어분의 25, 50, 75 및 100%를 참치가공부산물로 대체한 TBM25, TBM50, TBM75 및 TBM100를 사료실험군으로 설정 하였으며, 자연산 먹이인 다시마(ST)를 공급하는 실험군을 설정 하였 다. 배합사료내 어분을 참치가공부산물로 대체함에 따라 사료내 필수 아미노산인 Isoleucine, Lysine 및 Valine의 함량은 감소하였다. 증체량 과 일일성장률(SGR)은 TBM25의 실험군에서 다른 실험군보다 유의하 게 높게 나타났다(P < 0.05). 가식부의 조단백질 함량은 어분의 참치가



공부산물 대체량 증가에 비례적으로 감소하였다. 결론적으로, 전복 치 패용 배합사료내 어분 함량이 28%일 때, 어분의 75%를 참치가공부산 물 대체 시 증체량 및 일일성장률의 손실이 없는 것으로 판단된다.

Key words: 전복, Haliotis discus, 어분, 참치가공부산물, TBM





# Growth and body composition effects of tuna byproduct meal (TBM) substituted for fish meal in the diet of juvenile abalone, *Haliotis discus*

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#### Abstrat

The effects on growth and body composition that result from tuna byproduct meal (TBM) substituted for fish meal in the diet of juvenile abalone, *Haliotis discus*, were determined. One thousand two hundred and sixty juvenile abalone were randomly distributed into 18 70-L plastic rectangular containers. Six experimental diets were prepared in triplicate. The TBM0 diet included 28% fish meal and 13% soybean meal as the protein source. Twenty-five, 50, 75, and 100% of the fish meal were substituted with TBM, referred to as TBM25, TBM50, TBM75, and TBM100 diets, respectively. Finally, salted sea tangle (ST) was prepared. The essential amino acids, such as isoleucine, lysine and valine, tended to decrease with the dietary substitution of TBM for fish meal in the experimental diets. The weight gain and specific growth rate (SGR) of abalone that were fed the TBM25 diet were significantly higher than those of abalone that were fed the other diets (P < 0.05). The crude protein



content of the soft body of the abalone linearly decreased with dietary substitution of TBM for fish meal. In conclusion, as much as 75% of the fish meal in the diet of abalone can be replaced with TBM without a retardation in weight gain and SGR of the abalone when 28% fish meal was included.

Key words: Abalone, Haliotis discus, Fish meal, TBM, Tuna byproduct meal





#### 1. Introduction

Fish meal has been commonly used as a protein source in the commercial aquafeed including abalone feed. Recently, however, the international market price of fish meal has continued to increase sharply as a result of the expansion of aquaculture, a high demand for fish meal as a protein source in aquafeed, and a reduction in fish meal resources, such as sardine or mackerel, in the wild. Therefore, the development of a new feed ingredient to replace fish meal in aquafeed is urgently needed.

In the past, sources of animal (meat meal, meat and bone meal, blood meal, and feather meal) and plant (soybean and corn gluten meals) proteins have been commonly used in aquafeed as an alternative protein source to fish meal. However, the former cannot be safely used as an alternative protein source for fish meal anymore because of the high risk of the transfer of from land animals to humans, including diseases diseases such as foot-and-mouth disease, mad cow disease and bird-flu. The latter also cannot be economically used because of the expansion of the biofuel industry to develop soybean and corn as a seed stock for ethanol production around the world. In addition, the use of plant proteins in aquafeed is limited as a result of some nutrient inhibitors (Olli et al. 1994; Suh et al. 2003). Therefore, the use of another feed ingredient as a substitute for fish meal in aquafeed that is both safe in terms of disease transference and economic viability is highly needed.

A commercially available tuna byproduct meal (TBM) has been recently developed by HIF Co., Ltd. in Changwon-si, Gyeongsangnam-do, Korea. TBM is potentially a very important alternative animal protein source for fish meal in aquafeed because of its high nutrient content, with crude protein and lipid levels greater than 60 and 15%, respectively. TBM is a mixture of tuna



(skipjack tuna, *Katsuwonus pelamis* and yellowtail tuna, *Thunnus albacares*) byproducts, which primarily consist of tuna head, bone, fin, blood and skin that remain after the tuna-canning process, mixed with soybean meal at a ratio of 4:1. TBM is fermented by *Bacillus* sp. for 48 hours and then dried. Our previous studies have revealed that up to 30 and 40% of fish meal could be replaced with TBM without a retardation in the growth of juvenile olive flounder, *Paralichthys olivaceus*, and rockfish, *Sebastes schlegeli*, respectively (Jeon et al. 2014; Kim et al. 2014). Similarly, tuna muscle byproduct powder could replace 50% of fish meal protein without a reduction in growth performance of the olive flounder (Uyan et al. 2006). A combination of other fishery byproducts, such as scallop and squid meal, with fermented soybean meal effectively replaced fish meal in the diet of red sea bream, *Pagrus major*, and olive flounder without a reduction in growth (Kader et al. 2011, 2012).

Cho et al. (2008) showed that abalone, *Haliotis discus hannai*, that were fed with combined fish meal and soybean meal diet or a combined fish meal, soybean meal and crustacean meal diet grew well compared to the abalone that were fed a casein-basal diet, which was reported to be a good protein source for abalone (Uki et al. 1985, 1986). In addition, the growth rate of abalone, *H. fulgens*, that were fed a combined abalone viscera silage and soybean meal diet was comparable to that of abalone that were fed a fish meal-based diet, but both produced a better growth rate in abalone than a commercial diet (Guzman and Viana 1998). Bautista-Teruel et al. (2003) also reported that the combined animal and plant protein sources, such as fish meal, shrimp meal defatted soybean meal and/or *Spirulina*, achieved a better growth rate in abalone, *H. asinina*, than was achieved with a combined plant source (defatted soybean meal and *Spirulina*) alone. Thus, TBM is a promising alternative to fish meal as a protein source in abalone feed.



In this study, the effects on growth and body composition that result from TBM substituted for fish meal in the diet of juvenile abalone, *H. discus*, were determined.





#### 2. Materials and Methods

#### 2. 1. Preparation of Abalone and Rearing Conditions

Juvenile abalone were purchased from a private hatchery and transferred to an abalone farm (Ocean and Fisheries Research Institute, Jeju Special Self-Governing Province, Jeju, Korea). Before the initiation of the feeding trial, the abalone were acclimated to the experimental conditions for 4 weeks and fed with dry sea tangle once a day at a ratio of 2-3% of total biomass. One thousand two hundred and sixty juvenile abalone averaging 1.29 g (1.5 cm in shell length) were randomly distributed into 18 70-L plastic rectangular containers (120 cm  $\times$  36 cm). Nine containers were placed into each of two 9-ton concrete flow-through raceway systems (water volume: 3 tons) operating at a flow rate of 48.3 L/min. Sand-filtered seawater at a temperature ranging from 16.7 to 21.8°C (mean  $\pm$  SD: 18.4  $\pm$  0.99°C) was supplied throughout the feeding trial. Aeration was supplied to each raceway, and the photoperiod followed natural conditions. Experimental diets were fed to the abalone once a day at a satiation level with a little leftover food (2-3.5% biomass). Dead abalone were removed daily, and the bottoms of the containers were siphon-cleaned everyday. The feeding trial lasted for 16 weeks. At the end of the feeding trial, the abalone from each container were collectively weighed.

#### 2. 2. Preparation of the Experimental Diets

Six experimental diets were prepared in triplicate (Table 1). The TBM0 diet included 28% fish meal and 13% soybean meal as the protein sources. The TBM diet used 12% wheat flour and 4% dextrin and 3% squid liver oil and 2% soybean oil as the carbohydrate and lipid sources, respectively. Twenty-five, 50, 75 and 100% of the fish meal were substituted with TBM



TABLE 1. Ingredients c	LE 1. Ingredients of the experimental diets (%, DM basis)						
			Experim	ental diets			
	TBM0	TBM25	TBM50	TBM75	TBM100	ST	
Ingredient (%)							
Fish meal	28	21	14	7	0		
(CP:72.7%, CL:11.3%)	20	- 1	11	,	Ŭ		
Soybean meal	13	13	13	13	13		
Tuna byproduct meal (TBM) <sup>1</sup>	0	7	14	21	28		
(CP:61.0%, CL:15.8%)	Ū	,	11	- 1	20		
Wheat flour	12	12	12	12	12		
Dextrin	4	4	4	4	4		
Sea tangle	10	10	10	10	10		
Squid liver oil	3	3	-3	3	3		
Soybean oil	2	2	2	2	2		
Sodium alginate	22	22	22	22	22		
Vitamin premix <sup>2</sup>	2	2	2	2	2		
Mineral premix <sup>3</sup>	4	4	4	4	4		
Nutrients (%, DM basis)	101	10.4		3			
Dry matter	87.8	90.7	90.9	92.1	89.6	35.6	
Crude protein	31.3	29.9	28.9	28.0	26.9	7.6	
Crude lipid	9.2	9.4	9.5	9.6	10.1	1.2	
Ash	15.6	15.7	16.2	16.2	16.9	65.5	

TABLE 1. Ingredients of the experimental diets (%, DM basis)

ST = salted sea tangle.

<sup>1</sup>Tuna byproduct meal (TBM) was purchased from HIF Co., Ltd.

<sup>2</sup>Vitamin premix contained the following amounts, which were diluted in cellulose (g/kg mix): L-ascorbic acid, 200;  $\alpha$ -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<sub>3</sub>, 4; A, 1.5; D<sub>3</sub>, 0.003; choline chloride, 200; cyanocobalamin, 0.003.

<sup>3</sup>Mineral premix contained the following ingredients (g/kg mix): NaCl, 10; MgSO<sub>4</sub>·7H<sub>2</sub>O, 150; NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 250; KH<sub>2</sub>PO<sub>4</sub>, 320; CaH<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O, 200; Ferric citrate, 25; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 4; Calcium lactate, 38.5; CuCl, 0.3; AlCl<sub>3</sub>·6H<sub>2</sub>O, 0.15; KIO<sub>3</sub>, 0.03; Na<sub>2</sub>Se<sub>2</sub>O<sub>3</sub>, 0.01; MnSO<sub>4</sub>·H<sub>2</sub>O, 2; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.1.



(HIF Co., Ltd), referred to as the TBM25, TBM50, TBM75 and TBM100 diets, respectively. The experimental diets satisfied the dietary nutrient requirements of abalone (Mai et al. 1995a, 1995b). Finally, salted sea tangle (ST) was prepared to compare the effect of the experimental diets on the performance of the abalone.

Next, 22% sodium alginate was added to all experimental diets. Thereafter, all the ingredients were mechanically mixed, and water was added at a ratio of 1:1. A paste was made from each of the diets using an electronic mixer and shaped into 0.15cm thick sheets that were then cut by hand into 1 cm<sup>2</sup> flakes. The flakes were then dipped into an aqueous solution of 5% CaCl<sub>2</sub> for 1 minute, and the excess solution was drained naturally. The flakes were then dried naturally for 2 days and stored at -20°C until use.

#### 2. 3. Analytical Procedures of the Diets and Carcass

Twenty abalone at the start and ten abalone at the termination of the feeding trial were sampled from each container and frozen for chemical analysis. 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 millimeters with a digital caliper (Mitutoyo Corporation, Kawasaki, Japan), and the ratio of soft body weight to body weight (the soft body weight + the weight of the excised shell) was calculated to determine a condition index for abalone. The specific growth rate (SGR, % body weight gain/day) 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 pooled and separated soft body tissue of all the abalone from each container were then homogenized and used for proximate 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 hours; and ash was determined using a muffle furnace at 550°C for 4 hours. All the methods were performed according to AOAC (1990) practices. The amino acid composition of the experimental diets was determined by using a high-speed amino acid analyzer (Hitachi L-8800, Tokyo, Japan) after which the samples were hydrolyzed in 6 N HCl for 24 hours at 110°C.

#### 2. 4. Statistical Analysis

One-way ANOVA (Analysis of variance) and Duncan's multiple range test (Duncan 1955) were used to determine the significance of the differences among the means of the different treatments using SAS version 9.3 (SAS Institute, Cary, NC). Percentage data were arcsine transformed prior to statistical analysis.

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#### 3. Results

The amino acid profiles of the experimental diets are given in Table 2. The essential amino acids, such as isoleucine, lysine and valine tended to decrease with the dietary substitution of TBM for fish meal in the experimental diets. All essential and non-essential amino acid contents in the ST diet were relatively low.

The survival of abalone fed with the experimental diets substituting TBM for fish meal was not significantly different (P > 0.05) among treatments, but it was significantly higher (P < 0.05) than that of abalone fed with the ST diet (Table 3). The weight gain and SGR of ablaone that were fed the TBM25 diet were significantly higher (P < 0.05) than those of abalone that were fed the the TBM0, TBM50 and TBM75 diets were significantly higher (P < 0.05) than those of abalone that were fed the TBM0, TBM50 and TBM75 diets were significantly higher (P < 0.05) than those of abalone that were fed the TBM100 and ST diets. The weight gain and SGR of abalone that were significantly higher (P < 0.05) than those of abalone that were fed the TBM100 diet were significantly higher (P < 0.05) than those of abalone that were fed the TBM100 diet were significantly higher (P < 0.05) than those of abalone that were fed the TBM100 diet were significantly higher (P < 0.05) than those of abalone that were fed the TBM100 diet were significantly higher (P < 0.05) than those of abalone that were fed the TBM100 diet were significantly higher (P < 0.05) than those of abalone that were fed the ST diet.

The shell length and width of abalone that were fed the TBM25 and TBM50 diets were significantly longer and wider (P < 0.05) than those of abalone that were fed the TBM100 and ST diets, but they were not significantly (P > 0.05) different from those of abalone that were fed the TBM0 and TBM75 diets (Table 4). The shortest shell length and narrowest shell width were obtained in abalone fed with the ST diet. The shell height of abalone that were fed the TBM0 diet was significantly (P < 0.05) greater than that of abalone that were fed the TBM100 and ST diets, but it was not significantly different (P > 0.05) from that of abalone that were fed the TBM25, TBM50 and TBM75 diets. The soft body weight of abalone that were fed the TBM25, TBM50 and TBM75 diets was significantly heavier



			Experim	ental diets		
	TBM0	TBM25	TBM50	TBM75	TBM100	ST
Alanine	1.49	1.53	1.47	1.50	1.37	0.07
Arginine	1.44	1.53	1.55	1.47	1.35	0.04
Aspartic acid	2.54	2.62	2.53	2.54	2.34	0.13
Cystine	0.27	0.28	0.30	0.31	0.30	0.02
Glutamic acid	4.07	4.17	4.01	4.01	3.69	0.17
Glycine	1.45	1.54	1.54	1.61	1.55	0.06
Histidine	0.58	0.59	0.57	0.57	0.54	0.02
Isoleucine	1.13	1.11	1.07	1.09	1.03	0.05
Leucine	1.96	2.01	1.91	1.93	1.78	0.08
Lysine	1.79	1.80	191.66	1.64	1.46	0.05
Methionine	0.55	0.57	0.56	0.53	0.50	0.01
Phenylalanine	1.14	1.15	1.10	1.14	1.03	0.05
Proline	1.22	1.20	1.24	1.27	1.24	0.04
Serine	1.05	1.16	1.14	1.15	1.04	0.05
Threonine	1.06	1.13	1.09	1.09	0.99	0.06
Tyrosine	0.69	0.76	0.79	0.76	0.68	0.04
Valine	1.32	1.29	1.24	1.25	1.17	0.06

TABLE 2. Amino acid profiles of the experimental diets (DM % in the diet)

Experimental diets	Initial weight (g/abalone)	Final weight (g/abalone)	Survival <sup>1</sup> (%)	Weight gain (g/abalone)	SGR <sup>2</sup> (%/day)
TBM0	$1.29 \pm 0.001$	2.97 ± 0.012	$85.7 \pm 5.15^{a}$	$1.68 \pm 0.013^{b}$	$0.33 \pm 0.002^{b}$
TBM25	1.29 ± 0.001	3.06 ± 0.007	$86.7 \pm 2.90^{a}$	$1.77 \pm 0.005^{a}$	$0.34 \pm 0.000^{a}$
TBM50	1.29 ± 0.002	2.98 ± 0.017	$87.6 \pm 2.08^{a}$	$1.69 \pm 0.015^{\rm b}$	$0.33 \pm 0.002^{b}$
TBM75	1.29 ± 0.003	2.96 ± 0.013	$84.8 \pm 0.95^{a}$	$1.67 \pm 0.012^{b}$	$0.32 \pm 0.002^{b}$
TBM100	$1.29 \pm 0.004$	2.68 ± 0.008	$86.7 \pm 0.95^{a}$	$1.39 \pm 0.005^{\rm c}$	$0.28 \pm 0.001^{\circ}$
ST	$1.29 \pm 0.002$	2.08 ± 0.006	$69.5 \pm 0.48^{b}$	$0.79 \pm 0.007^{d}$	$0.19 \pm 0.001^{d}$

**TABLE 3.** Survival (%), weight gain (g/abalone), and specific growth rate (SGR) of juvenile abalone, *Haliotis discus*, fed with the experimental diets substituting tuna byproduct meal (TBM) for fish meal with for 16 weeks

<sup>1</sup>Values in the same column sharing a common superscript are not significantly different (P > 0.05).

 ${}^{2}SGR = [(\ln(W_{f}) - \ln(W_{i}))/days of feeding] \times 100$ , where  $\ln(W_{f}) = natural log of the final mean weight of abalone and <math>\ln(W_{i}) = natural log of the initial mean weight of abalone.$ 



Experimental diets	Shell length <sup>1</sup> (mm)	Shell width (mm)	Shell height (mm)	Soft body weight (g)	Soft body weight/total weight
TBM0	$34.0 \pm 0.23^{ab}$	$23.2 \pm 0.04^{ab}$	$7.2 \pm 0.19^{a}$	$2.3 \pm 0.03^{ab}$	$0.62 \pm 0.004^{a}$
TBM25	$35.4 \pm 0.64^{a}$	$23.9 \pm 0.39^{a}$	$7.2 \pm 0.11^{ab}$	$2.6 \pm 0.08^{a}$	$0.61 \pm 0.008^{a}$
TBM50	$35.3 \pm 0.52^{a}$	$23.7 \pm 0.36^{a}$	$7.1 \pm 0.07^{ab}$	$2.6 \pm 0.10^{a}$	$0.61 \pm 0.003^{a}$
TBM75	$34.7 \pm 0.27^{ab}$	$23.3 \pm 0.18^{ab}$	$7.0 \pm 0.19^{abc}$	$2.5 \pm 0.24^{a}$	$0.60 \pm 0.003^{a}$
TBM100	$33.2 \pm 0.12^{bc}$	$22.5 \pm 0.08^{b}$	$6.8 \pm 0.06^{bc}$	$2.1 \pm 0.06^{b}$	$0.58 \pm 0.006^{b}$
ST	$31.8 \pm 0.27^{\circ}$	$21.1 \pm 0.19^{\circ}$	$6.7 \pm 0.06^{\rm c}$	$1.7 \pm 0.04^{\rm c}$	$0.56 \pm 0.003^{\rm c}$

TABLE 4. Shell length (mm), shell width (mm), shell height (mm) and the ratio of soft body weight to total weight of abalone, *Haliotis discus*, at the end of the 16 weeks feeding trial

<sup>1</sup>Values in the same column sharing a common superscript are not significantly different (P > 0.05).



(P < 0.05) than that of abalone that were fed the TBM100 and ST diets, but it was not significantly different (P > 0.05) from that of abalone that were fed the TBM0 diet. The lowest soft body weight was observed in abalone that were fed the ST diet. The ratio of soft body weight to total weight of abalone that were fed the TBM0, TBM25, TBM50 and TBM75 diets was significantly higher (P < 0.05) than that of abalone that were fed the TBM100 and ST diets. The lowest ratio of soft body weight to total weight was observed in abalone that were fed the ST diet.

The moisture content of the soft body of abalone fed with the TBM25 diet was significantly higher (P < 0.05) than that of abalone that were fed the other diets (Table 5). The moisture content of the soft body of abalone that were fed the TBM100 diet was significantly higher (P < 0.05) than that of abalone that were fed the TBM75 diet but was not significantly different (P> 0.05) from that of abalone that were fed the TBM0, TBM50 and ST diets. The crude protein content of the soft body of the abalone linearly decreased with dietary substitution TBM for fish meal. The lowest crude protein content of the soft body was obtained in abalone that were fed the ST diet. The crude lipid content of the soft body of abalone fed with the TBM0, TBM25, TBM50, TBM75 and TBM100 diets was significantly higher (P < 0.05) than that of abalone fed with the ST diet. However, the ash content of the soft body of abalone that were fed the TBM0, TBM25, TBM50, TBM75 and TBM100 diets was significantly lower (P < 0.05) than that of abalone that were fed the ST diet.



**TABLE 5.** Proximate composition (%, wet weight basis) of the soft body of abalone, *Haliotis discus*, fed with the experimental diets substituting tuna byproduct meal (TBM) for fish meal for 16 weeks

Diets	Moisture <sup>1</sup>	Crude protein	Crude lipid	Ash
TBM0	$77.2 \pm 0.01^{bc}$	$24.0 \pm 0.04^{a}$	$2.3 \pm 0.03^{a}$	$3.2 \pm 0.07^{b}$
TBM25	$77.7 \pm 0.04^{a}$	$23.5 \pm 0.09^{b}$	$2.4 \pm 0.04^{a}$	$3.1~\pm~0.03^{b}$
TBM50	$77.2 \pm 0.08^{bc}$	$22.9 \pm 0.10^{\circ}$	$2.4 \pm 0.05^{a}$	$3.2~\pm~0.07^{\rm b}$
TBM75	$77.2 \pm 0.09^{\circ}$	$22.2 \pm 0.19^{d}$	$2.4 \pm 0.03^{a}$	$3.2~\pm~0.04^{\rm b}$
TBM100	$77.4 \pm 0.07^{b}$	$21.6 \pm 0.29^{e}$	$2.3 \pm 0.03^{a}$	$3.3~\pm~0.04^{b}$
ST	$77.2 \pm 0.04^{bc}$	$19.4 \pm 0.10^{\rm f}$	$1.6 \pm 0.09^{\rm b}$	$3.6 \pm 0.08^{a}$

<sup>1</sup>Values in the same column sharing a common superscript are not significantly different (P > 0.05).



#### 4. Discussion

Because higher survival, weight gain and SGR were observed in abalone fed with the experimental diets substituting TBM for fish meal compared with abalone that were fed the ST diet, a common feed at commercial abalone farms in Korea, abalone that were fed the experimental diet seemed to grow well in this study. This was further supported by the low essential and nonessential amino acid contents in the ST diet compared with all other experimental diets (Table 2). Similarly, the well-formulated or commercial diet achieved a better weight gain in abalone than did macroalgae, which was probably because of the rich nutrient content in the former (Viana et al. 1993; Daume et al. 2007; Cho et al. 2008; Garcia-Esquivel and Felbeck 2009; Dang et al. 2011). In addition, Mai et al. (1994) reported that two species of abalone, H. tuberculata and H. discus hannai had similar amino acid requirements and that some essential amino acid, such as arginine, methionine, threonine or histidine, which are likely to be deficient in macroalgae, might be limiting factors for the growth of both abalones when fed with six species of macroalgae. Naidoo et al. (2006) also reported that the fresh kelp, fresh kelp combined with epiphyte, Carpoblepharis flaccida, commercial diet (Abfeed<sup>®</sup>) or a combined diet produced better growth of abalone, H. midae with regard to weight gain compared with a diet of dried kelp (blades, stipe and pellets).

A significant improvement in weight gain and SGR of abalone that were fed the TBM25 diet compared with abalone fed the TBM0 diet was observed in this study, and this was probably because of the higher content of some essential amino acids, such as arginine, histidine, leucine, lysine, methionine, phenylalanine and threonine in the former.

No difference in weight gain and SGR was found in abalone that were fed



the TBM0 diet compared with abalone that were fed the TBM50 and TBM75 diets; however, the significantly higher weight gain and SGR compared with TBM100 diet in this study indicated that up to 75% of fish meal could be replaced with TBM without a retardation in weight gain and SGR in abalone. Another reason for the poor weight gain and SGR of abalone that were fed the TBM100 diet could be the low dietary protein content (26.9%) compared with that of the other TBM diets (above 28%). In addition, most of the essential amino acid content, such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine and valine sharply decreased when 100% of the fish meal was substituted with TBM. In determining the suitability of a protein source as an alternative to fish meal in the diet of abalone, essential amino acids, such as lysine and methionine, are the primary consideration (Shipton and Britz 2001; Bautista-Teruel et al. 2003; Cho et al. 2008). In an earlier study (Cho 2010), histidine was shown to be another amino acid that determines the suitability of an alternative protein source to replace fish meal in the diet of abalone, H. discus hannai.

In contrast to the results of this study however, only 30 and 40% of fish meal could be replaced with TBM without a retardation in the growth of juvenile olive flounder (Kim et al. 2014) and rockfish (Jeon et al. 2014), respectively. This difference could be explained by the fact that abalone is herbivorous (Thongrod et al. 2003) and more effectively utilizes the soybean meal in TBM than do carnivorous fishes.

Biological parameters (shell length, shell width, shell height, soft body weight and the ratio of soft body weight to total weight of the abalone) were relatively well reflected from the growth of abalone in this study. All biological parameters of the abalone fed with the ST diet were poorer than those of the abalone fed with the experimental diets except for the TBM100 diet.



The linear increase in the crude protein content of the soft body of abalone fed with diets substituting TBM for fish meal reflects the dietary crude protein content of the experimental diets in this study. In addition, the lower crude protein and higher ash content of the soft body were obtained in abalone fed with the ST diet compared with abalone fed with the experimental diets substituting TBM for fish meal. Similarly, the dietary substitution of fish meal with the animal and/or plant protein sources affected the proximate composition of the soft body of the abalone (Mai et al. 1995a, b; Thongrod et al. 2003; Cho et al. 2008; Garcia-Esquivel and Felbeck 2009; Cho 2010; Myung et al. In press). The effects of substituting TBM substituted for fish meal in the commercial feed for farmed abalone are worth studying in the future.





### ${\rm I\hspace{-1.5mm}I}$ . Conclusion

As much as 75% of the fish meal in the diet of abalone, *Haliotis discus* can be replaced with tuna byproduct meal (TBM) without a retardation in weight gain and specific growth rate (SGR) of the abalone when 28% fish meal was included. Significant improvements in weight gain and SGR were obtained in abalone fed with the TBM25 diet substituting 25% of the fish meal with TBM compared with abalone fed with the TBM0 diet.





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