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RESEARCH PAPER

# Effects of Substitution of Fish Meal (FM) and Macroalgae (MA) with Soybean Meal and Rice Bran in a Commercial Juvenile Abalone (*Haliotis duscus hannai*) Diet on Growth Performance

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**Abstract**

The effects of fish meal (FM) and macroalgae (MA) substitution with fermented soybean meal and rice bran were tested on a commercial diet in juvenile abalone. Abalone (21600) individuals were distributed equally to four diet treatments. Four experimental diets were prepared: a Std diet, FM50, FM50+MA50 and FM50+MA100. The standard diet (Std) consists of FM (140 g/kg), fermented soybean meal (250 g/kg), corn gluten meal (34 g/kg), shrimp meal (30 g/kg), wheat flour (200 g/kg), dextrin (50 g/kg) and MA (250 g/kg). *Undaria* and *Laminaria* were also prepared. The feeding trial lasted for 16 weeks. Survival of abalone fed the formulated diets was higher than that of abalone fed the *Undaria* and *Laminaria*. Weight gain and specific growth rate of abalone fed the Std diet were higher than all other diets. Substitutability of the fermented soybean meal for FM seemed to be rather limited in the commercial diet for abalone farms. However, another 500 g/kg MA in the commercial diet could be substituted with rice bran as long as 500 g/kg MA were substituted with rice bran. The FM50+MA50 diet is the most recommendable for abalone farm in considering feed cost.

*Keywords*: Abalone (*Haliotis discus hannai*), fish meal (FM), macroalgae (MA), fermented soybean meal, rice bran.

## Introduction

Annual abalone aquaculture production in Korea was about 20 metric tons in 2000 and reached 8,982 metric tons in 2014 (FishStatJ, 2015). This trend is likely to continue due to its continuing high consumer demand and continued farm expansions to meet the demand.

Fish meal (FM), which has been commonly used as a primary protein source in aquafeeds, prices have increased 285% since 2001 (FAO, 2014) and are projected to continue increasing due to a shortage of catchable FM sources in wild and continuing expansion of global fish meal fed aquaculture. Macroalgae (MA), which has been used as a replacement or feed additive, prices are also becoming more costly due to expansion of the biofuel industry to develop MA for ethanol extraction.

In Korea, abalone farmers are likely to continue to feed abalone on MA such as *Undaria pinnatifida* Harvey or *Laminaria japonica* Areschoug during the winter season on their farms as these MA are grown naturally and harvested in the wild. Although its nutrient content, such as protein (amino acid) and lipid (fatty acid) do not satisfy the complete dietary requirement for abalone, *Haliotis discus hannai* Ino (Uki, Kemuyama, & Watanabe, 1986a; Mai, Mercer, & Donlon, 1995a, b), farmers prefer feeding abalone on MA for the convenience of farm management. This may result in poorer growth rates and increase the production cost of abalone over commercial feeds. Generally during summer, the dry or salted MA is commonly fed to abalone. However, the nutrients in the dry or salted MA are easily destroyed in the drying or salting process and this can also result in poorer growth rates.

One of the largest production costs in aquaculture operations is feed and a compromise needs to be made between a nutritionally balanced and cost effective feed (Neori & Nobre, 2012). As FM and MA are the largest components of commercial abalone feeds and prices continue to increase, alternatively sources need to be identified and their performances evaluated against existing commercial feeds. Globally research has shown that a well formulated feed will produce better growth rates that a single algal species diet (Lee, 1998; Cho, Park, Kim, Yoo, & Lee, 2006; Kim, Lee, Han, Kim, & Park, 1998; Cho, Park, Kim, & Yoo, 2008; Naidoo, Maneveldt, Ruck, & Bolton, 2006; Gracia-Esquivel &

Felbeck, 2009; Dang, Li, Speck, & Benkendorff, 2011; Kim *et al*., 2015; Myung *et al*., 2016).

Studies by Uki, Kemuyama, & Watanabe (1985a, b, 1986a) have shown that plant protein (soybean and cottonseed meal) are good alternative sources for casein, and can produce the best weight gain of abalone, *H. discus hannai*, compared to other plant proteins tested (Lee, Yun, & Hur, 1998). Cho *et al*. (2008) also reported that growth of abalone fed a combined fish and soybean meal diet or a combined fish, soybean and crustacean meal diet was comparable to that of abalone fed a casein-basal diet. In addition, FM at 350 g/kg could be completely replaced with soybean meal at 580 g/kg with an additional 5 g/kg methionine supplementation, as it is lacking in the plant protein source (Cho, 2010). Therefore, soybean meal seems to be a promising alternative protein source for FM in abalone feed.

Fermentation of soybean meal increases protein content, eliminates trypsin inhibitors, and reduces peptide size (Hong, Lee, & Kim, 2004; Kook, Cho, Hong, & Park, 2014). Dietary inclusion of fermented plant protein sources, such as soybean and cottonseed meals also produced a promising effect on performance of Nile tilapia, *Oreochromis niloticus* Linne and black sea bream, *Acanthopagrus schlegeli* Bleeker (Lim & Lee, 2011; Zhou *et al*., 2011). A study by Kim *et al*. (2015) on abalone, *H. discus* Reeve revealed that 1000 g/kg substitution of *L. japonica* with rice bran, [an agriculture byproduct and rich in the nutrients such as crude protein and vitamins (Gao, Kaneko, Hirata, Toorisaka, & Hano, 2008)], at 200 g/kg in the diet was successful in terms of abalone weight gain. However, the work needed to be trialed under commercial farm conditions.

The aim of this paper is to investigate the substitution effect of FM and MA with fermented soybean meal and rice bran in commercial abalone diet on growth performance of juvenile abalone at commercial farm.

## Materials and Methods

### Preparation of Abalone and Rearing Conditions

Juvenile abalone, *H. discus hannai*, were purchased from a private hatchery and transferred to Joeun abalone farm (Wando, Jeollanamdo, Korea). Abalone were acclimated for two weeks and fed with the dry *L. japonica* once a day at the ratio of 2-3% of total biomass. A 6 ton concrete flow-through raceway tank (120 cm×690 cm×50 cm; water volume: 4.14 ton) at a flow rate of 42.3 L/min with sand filtered seawater (temperature range: 17.9 to 22.8°C (mean±SD: 20.2±0.01°C) was divided into 3 sections (120×230×50 cm; water volume: 1.38 ton) by a plastic-framed net (mesh size of 5 mm), and 4 concrete tanks were used for this experiment. Juvenile abalone (n = 21600) individual averaging 3.6 g were randomly distributed into each section (n=1800 per section). Aeration was supplied into each raceway and the photoperiod followed natural conditions. The experimental diets were fed to abalone once a day (17:00) at a satiation level with a little leftover (about 2-3% of biomass). Dead abalone were removed daily and the tanks were cleaned twice a week. The feeding trial lasted for 16 weeks. At the end of the feeding trial, 200 abalone were randomly sampled from each treatment and collectively weighed.

### Preparation of the Experimental Diets

Four experimental diets and the dry *Undaria* and *L. japonica* were prepared (Table 1). Four diets are referred to as follows: a Std, FM50, FM50+MA50 and FM50+MA100 diets. The standard diet (Std) is a commercial juvenile abalone diet and consists of FM and shrimp meal, fermented soybean, what flour and MA (250 g/kg, a mixture of *Undaria* and *Hizikia fusiforme* Harvey at a ratio of 1:1) as the protein and carbohydrate sources and was formulated to satisfy dietary nutrient requirements for (Uki *et al*., 1986a; Mai *et al*., 1995a, b). The 500 g/kg FM (70 g/kg of diet), and combined 500 g/kg FM and 500 g/kg MA (125 g/kg of diet), and combined 500 g/kg FM and 1000 g/kg MA (250 g/kg of diet) were substituted with the same amount of fermented soybean meal, and combined fermented soybean meal and rice bran, respectively, in the Std diet, referred to as FM50, FM50+MA50 and FM50+MA100 diets. Four diets were pelletized by an extruded pelleter (Jyoda, Japan) in Ewha Oil and Fat Industry Co. Ltd. (Busan, Korea). Pellets were round-shape, and their sizes were 5 mm in diameter (1.5 mm in thickness). *Undaria* and *L. japonica* were prepared to compare effect of the experimental diets with that of MA on the growth performance of abalone.

### Analytical Procedures of the Diets and Carcass

Abalone from each treatment were sampled and frozen for chemical analysis (100 at the beginning and 50 at the trial termination). Prior to examination, all samples were slightly thawed, followed by separation of the shell and soft-body tissue. Shell length and width were measured to 1.0 mm using 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 a condition index for abalone. Specific growth rate (SGR, %/body weight gain day) was calculated using the formula:

$$SGR = [(\ln(Wf) - \ln(Wi))/\text{days of feeding}]\times 100,$$

where In(Wf) = natural log of the final mean weight of abalone and In(Wi) = natural log of the initial mean weight of abalone Britz (1996).

The pooled separated soft body tissue from the sampled abalone from each diet was then

**Table 1.** Feed formulation of the experimental diets (g/kg, DM basis)

| | Experimental diets | | | | | |
|---|---|---|---|---|---|---|
| | Std | FM50 | FM50+MA50 | FM50+MA100 | *U. pinnatifida* | *L. japonica* |
| Fish meal (FM)[1] | 140 | 70 | 70 | 70 | | |
| Fermented soybean meal[2] | 250 | 320 | 320 | 320 | | |
| Corn gluten meal | 34 | 34 | 34 | 34 | | |
| Shrimp meal | 30 | 30 | 30 | 30 | | |
| Wheat flour | 200 | 200 | 200 | 200 | | |
| Dextrin | 50 | 50 | 50 | 50 | | |
| Macroalgae (MA)[3] | 250 | 250 | 125 | | | |
| Rice bran | | | 125 | 250 | | |
| *Spirulina* | 5 | 5 | 5 | 5 | | |
| Yeast | 10 | 10 | 10 | 10 | | |
| Sea aroma | 1 | 1 | 1 | 1 | | |
| Choline chloride(500g/kg) | 5 | 5 | 5 | 5 | | |
| Vitamin premix[4] | 5 | 5 | 5 | 5 | | |
| Mineral premix[5] | 20 | 20 | 20 | 20 | | |
| *Nutrients* (g/kg) | | | | | | |
| Dry matter | 987 | 988 | 978 | 981 | 860 | 887 |
| Crude protein | 363 | 361 | 380 | 383 | 207 | 90 |
| Crude lipid | 14 | 14 | 37 | 68 | 8 | 4 |
| Carbohydrate[6] | 424 | 435 | 464 | 467 | 424 | 581 |
| Ash | 199 | 190 | 119 | 82 | 361 | 325 |

[1]Fish meal (FM): Alaska pollack meal.
[2]Fermented soybean meal was purchased from CJ CheilJedang Corp. (Seoul, Korea).
[3]Macroalgae (MA) were the mixture of *Undaria* and *Hizikia fusiforme* at a ratio of 1:1.
[4]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.
[5]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.
[6]Carbohydrate was calculated as the difference between 1000 and the sum of crude protein, crude lipid and ash contents.

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 h and ash was determined using a muffle furnace at 550°C for 4 h. All methods were according to AOAC (1990) practices. Amino acid composition of the experimental diets were 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 h at 110°C.

### 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). Percentage data was arcsine-transformed prior to statistical analysis.

## Results

Amino acid profiles of the experimental diets are given in Table 2. The essential amino acids, such as arginine, isoleucine, lysine, methionine, threonine and valine were relatively low in the FM50 diet, but arginine, histidine, isoleucine, leucine, phenylalanine and valine were relatively high in the FM50+MA50 and FM50+MA 100 diets compared to the Std diet.

Fatty acid profiles of the experimental diets are presented in Table 3. A sum of n-3 highly unsaturated fatty acid (HUFA) content were relatively high in the Std diet compared to that of the FM50, FM50+MA50 and FM50+MA100 diets, especially linolenic acid (18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexanoic acid (DHA, 22:6n-3) decreased in proportion to an amount of substitution of FM and MA with the fermented soybean meal and rice bran, respectively, but linoleic acid (18:2n-6) content increased. No EPA and DHA was also found in MA (*Undaria* and *L. japonica*) diets.

Survival of abalone fed the all formulated diets was significantly higher ($P<0.0001$) than that of abalone fed the *Undaria* and *L. japonica* (Table 4). Survival of abalone fed the *Undaria* was significantly higher ($P<0.05$) than that of abalone fed the *L. japonica*. Weight gain (g/abalone) and SGR (%/day) of abalone fed the Std diet were significantly higher ($P<0.0001$) than those of abalone fed the all other diets, and followed by the FM50, FM50+MA50 and FM50+MA100 diets, *Undaria* and *L. japonica* in that in order.

The longest shell length was obtained in abalone fed the Std diet, followed by the FM50, FM50+MA50 and FM50+MA100 diets, *L. japonica*, and *Undaria* (Table 5). The widest shell width was observed in

**Table 2.** Amino acid profiles of the experimental diets (g/kg, DM basis)

| | Experimental diets | | | | | |
|---|---|---|---|---|---|---|
| | Std | FM50 | FM50+MA50 | FM50+MA100 | *U. pinnatifida* | *L. japonica* |
| Alanine | 19.0 | 18.1 | 19.0 | 18.7 | 19.3 | 4.7 |
| Arginine | 21.3 | 20.5 | 22.2 | 22.9 | 7.3 | 2.6 |
| Aspartic acid | 34.0 | 34.4 | 36.4 | 35.8 | 14.0 | 15.6 |
| Cystine | 4.5 | 4.6 | 5.0 | 5.1 | 1.4 | 1.0 |
| Glutamic acid | 59.5 | 60.2 | 64.3 | 64.1 | 19.8 | 25.6 |
| Glycine | 19.5 | 17.3 | 18.3 | 18.0 | 8.2 | 3.4 |
| Histidine | 7.4 | 7.5 | 8.3 | 8.5 | 2.2 | 1.0 |
| Isoleucine | 14.4 | 14.2 | 15.7 | 15.5 | 6.4 | 2.4 |
| Leucine | 26.9 | 26.9 | 28.9 | 28.5 | 11.1 | 4.1 |
| Lysine | 18.1 | 16.9 | 18.2 | 18.0 | 7.2 | 2.8 |
| Methionine | 5.7 | 5.3 | 5.6 | 6.1 | 2.8 | 1.1 |
| Phenylalanine | 16.1 | 16.3 | 17.5 | 17.2 | 3.7 | 2.9 |
| Proline | 18.4 | 18.1 | 19.2 | 19.6 | 6.5 | 3.1 |
| Serine | 16.8 | 16.9 | 17.0 | 16.8 | 6.0 | 2.8 |
| Threonine | 14.2 | 14.0 | 14.3 | 14.0 | 6.4 | 3.0 |
| Tyrosine | 10.6 | 10.5 | 11.3 | 11.7 | 4.2 | 1.8 |
| Valine | 16.2 | 15.9 | 17.6 | 17.5 | 7.8 | 3.3 |

**Table 3.** Fatty acid compositions of the experimental diets (g/kg of total fatty acid)

| | Experimental diets | | | | | |
|---|---|---|---|---|---|---|
| | Std | FM50 | FM50+MA50 | FM50+MA100 | *U. pinnatifida* | *L. japonica* |
| 14:0 | 36.1 | 32.1 | 16.2 | 10.0 | 55.9 | 88.4 |
| 15:0 | n.d | n.d | n.d | n.d | 9.6 | n.d |
| 16:0 | 192.0 | 194.8 | 174.2 | 172.9 | 273.6 | 155.0 |
| 18:0 | 25.1 | 25.4 | 19.8 | 18.6 | 27.0 | 13.2 |
| 20:0 | n.d | n.d | 4.1 | 4.6 | 6.3 | n.d |
| 21:0 | n.d | n.d | n.d | n.d | 5.8 | n.d |
| 22:0 | n.d | n.d | 2.8 | 2.9 | n.d | n.d |
| 24:0 | n.d | n.d | 4.5 | 4.9 | n.d | n.d |
| ∑Saturates | 253.2 | 252.3 | 221.6 | 213.9 | 378.2 | 256.6 |
| 14:1n-9 | 2.9 | 3.2 | n.d. | n.d. | 7.1 | 5.7 |
| 16:1n-9 | 35.6 | 27.4 | 13.3 | n.d. | 8.1 | 27.5 |
| 16:2n-6 | n.d | n.d | n.d | 8.2 | n.d | n.d |
| 17:1n-9 | n.d | n.d | n.d | n.d | 5.7 | 6.0 |
| 18:1n-9 | 176.3 | 170.1 | 293.7 | 335.9 | 115.1 | 210.6 |
| 18:1n-11 | 42.6 | 38.8 | 20.0 | 14.8 | n.d. | n.d. |
| 20:1n-9 | 29.8 | 19.0 | 12.1 | 9.5 | n.d. | n.d. |
| 22:1n-9 | 18.7 | 10.2 | 4.8 | 2.8 | n.d. | 6.2 |
| 24:1n-9 | 2.9 | n.d. | n.d. | n.d. | n.d. | n.d. |
| ∑Monoenes | 308.8 | 268.7 | 343.9 | 371.2 | 136.0 | 256.0 |
| C18:2n-6 | 260.2 | 312.4 | 344.0 | 363.1 | 61.1 | 85.1 |
| C22:2n-6 | n.d. | n.d. | n.d. | n.d. | 5.7 | n.d. |
| C18:3n-3 | 30.7 | 39.4 | 26.5 | 21.8 | 61.9 | 64.4 |
| C18:3n-6 | n.d. | n.d. | n.d. | n.d. | 8.9 | 31.4 |
| C18:4n-3 | 13.9 | 15.5 | 9.4 | 3.6 | 102.7 | 70.3 |
| C20:3n-3 | 5.3 | 5.1 | 4.2 | n.d. | 106.8 | 145.8 |
| C20:3n-6 | n.d. | n.d. | n.d. | n.d. | 5.2 | 6.0 |
| C20:4n-3 | 2.9 | n.d. | n.d. | n.d. | 4.9 | n.d. |
| C20:5n-3 | 60.9 | 52.4 | 26.8 | 14.5 | n.d. | n.d. |
| C22:5n-3 | 7.3 | 5.0 | n.d. | n.d. | 57.6 | 62.8 |
| C22:6n-3 | 50.0 | 43.4 | 20.8 | 11.9 | n.d. | n.d. |
| ∑n-3 HUFA | 126.4 | 105.9 | 51.8 | 26.4 | 174.5 | 214.6 |
| Unknown | 6.9 | 5.6 | 2.7 | n.d. | 70.9 | 21.5 |

n.d. indicates not detected.

abalone fed the Std diet, and followed by the FM50, FM50+MA50 and FM50+MA100 diets, *Undaria*, and *L. japonica*. Shell height of abalone fed the formulated (Std, FM50, FM50+MA50 and FM50+MA100) diets were not significantly different among treatments, but significantly taller ($P<0.0009$) than that of abalone fed the *Undaria* and *L. japonica*. The soft body weight of abalone fed the Std diet was

**Table 4.** Survival (%), weight gain (g/abalone) and specific growth rate (SGR) of juvenile abalone fed experimental diets for 16 week

| Experimental diets | Initial weight (g/abalone) | Final weight (g/abalone) | Survival (%) | Weight gain (g/abalone) | SGR[1] (%/day) |
|---|---|---|---|---|---|
| Std | 3.6±0.00 | 7.5±0.01$^{a}$ | 85.1±0.48$^{a}$ | 3.87±0.011$^{a}$ | 0.63±0.001$^{a}$ |
| FM50 | 3.6±0.00 | 6.5±0.01$^{b}$ | 84.8±0.75$^{a}$ | 2.94±0.005$^{b}$ | 0.52±0.000$^{b}$ |
| FM50+MA50 | 3.6±0.00 | 6.1±0.01$^{c}$ | 84.0±0.48$^{a}$ | 2.49±0.014$^{c}$ | 0.46±0.002$^{c}$ |
| FM50+MA100 | 3.6±0.00 | 6.0±0.02$^{c}$ | 84.9±0.11$^{a}$ | 2.40±0.021$^{c}$ | 0.44±0.003$^{c}$ |
| *U. pinnatifida* | 3.6±0.00 | 5.6±0.04$^{d}$ | 80.4±0.18$^{b}$ | 2.00±0.038$^{d}$ | 0.38±0.006$^{d}$ |
| *L. japonica* | 3.6±0.00 | 4.6±0.06$^{e}$ | 72.4±0.27$^{c}$ | 1.02±0.063$^{e}$ | 0.22±0.012$^{e}$ |

Values (means of duplicate±SE) in the same column sharing the same superscript letter are not significantly different (P>0.05).
[1]Specific growth rate (SGR, %/day) = [(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 5.** Shell length (mm), shell width (mm), shell height (mm), soft body weight (g) and the ratio of soft body weight to total weight of abalone fed the experimental diets for 16 week

| Experimental diets | Shell length (mm) | Shell width (mm) | Shell height (mm) | Soft body weight (g) | Soft body weight/total weight |
|---|---|---|---|---|---|
| Std | 41.7±0.14$^{a}$ | 28.5±0.01$^{a}$ | 8.6±0.14$^{a}$ | 4.3±0.05$^{a}$ | 0.61±0.004$^{a}$ |
| FM50 | 40.6±0.05$^{b}$ | 28.0±0.33$^{ab}$ | 8.6±0.01$^{a}$ | 4.0±0.02$^{ab}$ | 0.60±0.003$^{a}$ |
| FM50+MA50 | 40.0±0.10$^{c}$ | 27.2±0.41$^{b}$ | 8.6±0.20$^{a}$ | 3.8±0.21$^{b}$ | 0.60±0.013$^{a}$ |
| FM50+MA100 | 39.7±0.00$^{d}$ | 27.1±0.09$^{b}$ | 8.3±0.12$^{a}$ | 3.7±0.04$^{b}$ | 0.61±0.004$^{a}$ |
| *U. pinnatifida* | 38.1±0.08$^{e}$ | 25.9±0.02$^{c}$ | 7.2±0.05$^{b}$ | 3.1±0.08$^{c}$ | 0.61±0.002$^{a}$ |
| *L. japonica* | 38.2±0.00$^{e}$ | 25.9±0.05$^{c}$ | 7.2±0.13$^{b}$ | 3.1±0.05$^{c}$ | 0.60±0.006$^{a}$ |

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

significantly heavier (P<0.002) than all other diets, except for the FM50 diet. However, the ratio of the soft body weight to total weight of abalone was not significantly (P>0.4) different among the diets.

Moisture and ash content of the soft body of abalone was not significantly (P>0.6) different among the diets (Table 6). However, 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 FM50+MA50 diet was also significantly higher (P<0.0003) than that of abalone fed the *Undaria* and *L. japonica*, but not significantly different from that of abalone fed the Std and FM50 diets. Crude lipid content of the soft body of abalone fed the FM50+MA50 and FM50+MA100 diets was significantly higher (P<0.0002) that of abalone fed the all other diets. Crude lipid content of the soft body of abalone fed the Std and FM 50 diets was also significantly higher that of abalone fed the *Undaria* and *L. japonica*. Crude protein and lipid content of the soft body of abalone was directly reflected from dietary protein and lipid contents.

## Discussion

The several essential amino acids, such as arginine, isoleucine, lysine, methionine, threonine and valine were low in the FM50 diet, but arginine, histidine, isoleucine, leucine, phenylalanine and valine were high in the FM50+MA50 and FM50+MA 100 diets compared to the Std diet (Table 2). All essential amino acids were found, but relatively low in the *Undaria* and *L. japonica* in this study, agreeing with Daweczynski, Schubert, and Jahreis (2007)'s study. Mai, Mercer, and Donlon (1994) also reported that the several essential amino acids, such as arginine, methionine, threonine and histidine were the limiting factor in 6 species of MA, *Ulva lactuca* Linne, *Chondrus crispus* Stackh, *Palmaria palmate* Linnaeus, *Alaria esculenta* Linne, *Laminaria digitata* Hudson and *Laminaria saccharina* Linne, for abalones, *H. tuberculata* Linnaeus and *H. discus hannai*. Histidine was also reported to be a limiting factor when fish meal was substituted with various sources of animal and/or plant protein sources (Cho, 2010).

A sum of n-3 HUFA content were high in the Std diet compared to that of the FM50, FM50+MA50 and FM50+MA100 diets, especially linolenic acid, EPA and DHA decreased in proportion to an amount of substitution of FM and MA with the fermented soybean meal and rice bran, respectively, but linoleic acid content increased. Similar EPA and DHA contents in soybean and rice bran oils were reported by Krishna, Hemakumar, and Khaton (2006) and Grisdale-Helland *et al*. (2002). No EPA and DHA was also found in MA, *Undaria* and *L. japonica*, diets in this study, partially agreeing with Dawezynski *et al*. (2007)'s study showing that 132 and 162 g/kg of EPA were detected in *Undaria pinnatifia* and *Laminaria* sp., but no DHA was detected in all seaweeds investigated. The requirement of n-3 HUFA was reported to be 10 g/kg in the diet containing 50 g/kg lipid, being equivalent to 0.5 g/kg in the diet for abalone, *H. discus hannai* (Uki, Kemuyama, &

**Table 6.** Chemical composition (g/kg) of the soft body of abalone at the end of the 16 week feeding trial

| Experimental diets | Moisture | Crude protein | Crude lipid | Ash |
|---|---|---|---|---|
| Std | 773±2.0$^{a}$ | 168±1.6$^{bc}$ | 9±0.4$^{b}$ | 28±1.2$^{a}$ |
| FM50 | 778±1.2$^{a}$ | 167±0.8$^{bc}$ | 8±0.8$^{b}$ | 27±0.4$^{a}$ |
| FM50+MA50 | 775±6.9$^{a}$ | 172±0.8$^{b}$ | 16±0.8$^{a}$ | 25±0.0$^{a}$ |
| FM50+MA100 | 769±0.8$^{a}$ | 184±1.2$^{a}$ | 17±0.8$^{a}$ | 25±0.4$^{a}$ |
| *U. pinnatifida* | 770±0.4$^{a}$ | 163±1.6$^{c}$ | 6±0.4$^{c}$ | 29±0.4$^{a}$ |
| *L. japonica* | 771±0.0$^{a}$ | 158±1.2$^{d}$ | 6±0.4$^{c}$ | 27±0.4$^{a}$ |

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

Watanabe, 1986b). However, the requirement of n-3 HUFA was all satisfied in the all diets, and ranged from 0.9 g/kg (*L. japonica*) to 1.9 g/kg (FM50+MA50 diet). Mai, Mercer, & Donlon (1996) reported that EPA played a prominent role for 2 species of abalone, *H. tuberculata* and *H. discus hannai*, and n-3 and n-6 polyunsaturated fatty acid (PUFA) seemed to be essential for abalone, *H. discus hannai*.

Poor performance in survival, weight gain and SGR of abalone fed the *Undaria* and *L. japonica* could be explained by the poor nutritional quantity and quality of essential amino and fatty acids in these MA (Tables 2 and 3). Similarly, MA commonly used as feed in wild did not produce the optimal performance of abalone (Lee, 1998; Cho *et al*., 2006; Kim *et al*., 1998; Naidoo *et al*., 2006; Cho *et al*., 2008; Gracia-Esquivel & Felbeck, 2009; Dang *et al*., 2011; Myung *et al*., 2016).

Poorer weight gain of abalone fed the FM50, FM50+MA50 and FM50+MA100 diets was observed compared to that of abalone fed the Std diet in this study. However, unlike this study, Cho (2010) showed that FM at 350 g/kg could be completely replaced with soybean meal at 580 g/kg with 5 g/kg methionine supplementation in the diet for juvenile abalone. The differences in performance of abalone between this study and Cho (2010)'s study could have resulted from the lack of methionine supplementation in this study. However, Lee *et al*. (1998) reported that the plant protein sources (soybean meal or cotton seed meal) without supplementation of amino acids and are likely to be deficient in plant protein sources could replace casein or FM in the diet for abalone, *H. discus hannai*. Fish meal could also be successfully replaced with a combined animal and plant protein sources in abalone diets (Guzman & Viana, 1998; Bautista-Teruel, Fermin, & Koshio, 2003; Cho, 2010).

Another reason for the poorer weight gain of abalone fed the FM50, FM50+MA50 and FM50+MA100 diets compared to that of abalone fed the Std diet could be due to the fermentation of soybean meal in this study. Abalone may not utilize the fermented soybean meal well. This is why all 500 g/kg fish meal substituted diets with the fermented soybean meal regardless of substitution of MA with rice bran produced poorer weight gain in this study. Similarly, juvenile abalone (*H. discus*) fed the FM-basal diet with 250 g/kg fermented soybean meal achieved poorer weight gain than that fed the FM-basal diet with 250 g/kg soybean meal in the 16-week feeding trial (Cho, 2016; unpublished data). Nutritional values of fermented soybean meal varied depending on physical conditions of fermentation (Kook *et al*., 2014). More studies to elucidate the reason why abalone fed the diet containing fermented soybean meal showed poorer growth performance than that fed diet containing soybean meal is needed. Unlike this study, however, dietary inclusion of the fermented plant protein sources, such as soybean meal and cottonseed meal produced promising effect on performance of fish (Lim & Lee, 2011; Zhou *et al*., 2011).

Unlike Kim *et al*. (2015)'s study, 50% and 100% substitution of MA with rice bran in the FM50+MA50 and FM50+MA100 diets, respectively, produced poorer weight gain of abalone compared to the Std and FM50 diets in this study. The differences could have resulted from those in MA used in both studies. MA used in this study are the mixture of *Undaria* and *Hizikia fusiforme* at a ratio of 1:1, but a single *L. japonica* was used in the former. Abalone fed the diets (Std and FM50 diets) containing the mixture of *Undaria* and *Hizikia fusiforme* in this study outgrew over that fed the control diet containing *L. japonica* in Kim *et al*. (2015)'s study. The mean SGR was 0.58%/day in this study, but 0.46%/day for the latter. Therefore, substitution effect of MA with rice bran in this study was probably masked. The combined MA produced slightly, but not significantly, improved weight gain of abalone, *H. discus hannai* and *H. laevigata* Donovan, over a single MA (Qi *et al*., 2010; Dang *et al*., 2011).

No difference in weight gain and SGR of abalone fed the FM50+MA50 and FM50+MA100 diets (Table 4) in this study could indicate that another 50% MA in the commercial diet could be substituted with rice bran as long as 500 g/kg MA were substituted with rice bran. Abalone are known to be herbivorous and feed mostly on MA, which is usually low in protein and lipid, but high in carbohydrate, 400-500 g/kg in the wild (Thongrod, Tamtin, Chairat, & Boonyaratpalin, 2003). Unlike this study, however, Kim *et al*. (2015) reported that 400 g/kg substitution of *L. japonica* with rice bran at 200 g/kg in the diet achieved the best specific growth rate of abalone, *H. discus* and concluded that 100% substitution of *L. japonica* with rice bran at 200 g/kg in the diet was successfully made without retardation of weight gain when juvenile abalone were fed on the experimental diets trailed over *L. japonica*. However, a 16 week trail may not be a sufficiently long enough time give that abalone remain on farms for considerably longer periods. Similarly, the leaf meal, *Moringa oliefera* Lamarck, and freshwater aquatic fern, *Azolla pinnata* R. Brown, are promising alternative feed ingredients for *H. asinina* (Linnaeus) culture (Reyes & Fermin, 2003).

Biological criteria of abalone measured in this study (shell length, shell width, shell height and soft body weight), except for the ratio of the soft body weight to total weight seemed to be closely related to growth rate of abalone. Similarly, biological criteria of abalone agreed with growth rate of abalone (Bautista-Teruel *et al*., 2003; Cho, 2010; Myung *et al*., 2016).

Crude protein and lipid content of the soft tissue directly related to dietary protein and lipid contents in this study, and agreed with other studies (Mai *et al*., 1995a, b; Thongrod *et al*., 2003; Cho *et al*., 2008; Garcia-Esquivel & Felbeck, 2009; Cho, 2010; Kim *et al*., 2015; Myung *et al*., 2016).

In conclusion, the substitutability of fermented soybean meal for fish meal seems to be rather limited in commercial diets for abalone farm. However, another 500 g/kg MA in the commercial diet could be substituted with rice bran as long as 500 g/kg MA were substituted with rice bran. In considering feed cost, the FM50+MA50 diet is the most recommendable for abalone farm.

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