Protein content and free amino acid composition of abalone (*Haliotis asinina*) broodstock fed by different fresh macroalgae and formulated diet

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Abstract. This study aimed to determine the protein content and free amino acid composition of abalone (*Haliotis asinina*) broodstock fed by fresh macroalgae and formulated diet. Hatchery produced abalones (16 month old) were used in this study. They were fed with 3 species of macroalgae: *Gracilaria verrucosa* (A), *G. edulis* (B) and *Ulva lactuca* (C) and 2 formulated feeds (D and E) for 60 days. The result showed that the protein content of abalone fed by macroalgae and formulated diets from the highest were 18.79±1.19% (B), 17.70±1.05% (A), 17.52±0.26% (C), 16.67±0.31% (D) and 14.59 ±0.56% (E), respectively. All of the fifteen amino acids (L-Isoleucine, L-Glutamic acid, L-Phenylalanine, L-Serine, L-Histidine, L-Threonine, L-Proline, L-Tyrosine, L-Leucine, L-Aspartic acid, L-Lysine HCL, Glycine, L-Arginine, L-Alanine, L-Valine) of broodstock abalone fed formulated feed (D) were higher than those of *G. verrucosa*, formulated feed (E; with binder agar), *G. edulis* and *U. lactuca*. Four free amino acids (Glycine, L-Serine, L-Proline and L-Alanine) content of abalone broodstocks hepatopancreas fed by macroalgae showed no significant different than those fed by formulated feeds. Abalone broodstocks fed by formulated feed (D) and *G. verrucosa* contained higher glutamic acid, glicyne, aspartic acid and arginine and faster maturation and spawning time compare to those of broodstock fed with formulated feed (e) and natural feed (*G. edulis* and *U. lactuca*).

Key Words: natural and formulated feed, aquaculture,, broodstocks, abalone.

Introduction. Abalone (Haliotidae, *Haliotis*) are widely cultured species, and considered as premium seafood (Wang et al 2014). Therefore, abalone became one of the most highly valued seafood products in the world (Minh et al 2010; Qi et al 2010; Cook 2014). One of the species, Donkey ear tropical abalone, *Haliotis asinina* is known as the fastest growing among other abalone species. In the wild, adult abalone are predominately sedentary, scraping off attached or catching drift macroalgae (Allen et al 2006), but also including benthic microalgae when macroalgae is limited, for example within tropical coral reef environments (Sawatpeera et al 1998).

Macroalgae are grown or harvested to feed farmed abalone and their diets reflect species-specific dietary preferences, macroalgae biogeography, and seasonal availability (Mulvaney 2016). In contrast, some regions do not have the capacity to harvest wild macroalgae and therefore rely on formulated pellet feeds. Feed selection is an important consideration in developing an abalone culture system. In the hatchery, the quality of feed is very important, as the provision of nutritionally well-balanced diets with suitable protein, carbohydrate, and lipid profiles can significantly increase the survival, growth rates, health and reproduction of abalone broodstock (Mulvaney 2016).

Macroalgae or formulated feeds were used for maturation of abalone broodstock in the hatchery (Bautista-Teruel et al 2001). Seasonally changes in naturally abundance of macroalgae will influence the macroalgae availability for abalone, while formulated diet was used during lack of natural feed. Formulated feeds for abalone are being used intensively in some countries such as Japan, China, Australia, New Zealand, Mexico and South Africa with different species (Britz 1996; Britz et al 1997; Bansemer et al 2014). In

Australia, the use of mixed algal diets, including the use of nutrient enhanced macroalgae, increased the growth rates of the juvenile hybrid abalone (*H. rubrax, H. laevigata*) when compared to formulated feeds (Mulvaney 2016), while in another experiment shows reduced growth rates in juvenile green lip abalone *H. laevigata* (Bansemer et al 2016). The differences of these feeding probably due to differences in abalone species, feed formulations, macroalgae species, and experimental design.

The diverse nutritional value of mixed algal diets provides a good balance of nutrients for abalone. Individually, most species of marine algae have a low protein composition (generally less than 20% of dry weight), which is assumed to be inadequate to fulfil the protein requirements of faster growing, commercially produced abalone (Fleming et al 1996; Johnston et al 2005). However, Viera et al (2005) found that the growth rates of *H. tuberculata* were more likely affected by the specific amino acid (AA) composition within the macroalgae rather than the total protein content. It also has been found that the amino acids level including free amino acid influence the taste abalone which vary according to the seasonal change (Hwang et al 1997).

Establishment of optimal dietary requirements of AA and characterization of alternative protein/AA sources have been a major focus of fish nutrition research. Amino acids are potentially important ingredients for development functional and environmentally oriented aquafeeds to enhance the efficiency and profitability of global aquaculture production (Li et al 2009). Amino acids are also important energy sources of clams and fish larvae (Rønnestad et al 1999; Ambariyanto & Hoegh-Guldberg 1999; Ambariyanto 2000; Ambariyanto et al 2013), which also can be done through fish feed such as *Artemia* (Jusadi et al 2015).

Abalone is capable to convert or synthesize amino acids to maintain the amino acid balance in case of lack from the diet (Tung 2010). Also, amino acids have an important role in metabolism, especially as regulators in metabolic pathway needed for maintenance, food intake, nutrition utilization, reproduction and response on environmental stress (Santiago & Lovell 1988; Li et al 2009; Wu 2010). Li & Gatlin (2006) reported that glutamic acid, glicyne, and aspartic acid has important role in fish reproductive activity (Wu 2010). Those reports indicated that amino acid can be reflected quantitatively in meat and qualitatively through the biological activity.

Formulated feed has been intensively developed for growth acceleration and for flavor improvement but not for maturation and reproduction of abalone broodstocks. It is important to understand the feed that contains protein and free amino acids will affect the reproduction (maturation and spawning) of abalone. Formulated improvement feed need to be developed in order to produce high quality of abalone *Haliotis asinina* broodstock. This study investigates the influence of the given feed (natural feed and formulated feed) on protein and free amino acids composition of abalone broodstock (*H. asinina*).

Material and Method. This study was conducted in 2016 at Abalone Research and Development, Southeast Sulawesi, Indonesia. Sixteen-month-old *H. asinina* (55-60 mm shell length) produced from abalone hatchery by natural spawning at Abalone Research and Development, Southeast Sulawesi, Indonesia were used in this experiment. These abalones were acclimated for two weeks and stocked in a 126-tons concrete land-based tanks filled with aerated sea water. Proximate and free amino acid content of these abalones were analyzed.

Three seaweeds species i.e. *Gracilaria verrucosa* (diet A), *G. edulis* (diet B) and *Ulva lactuca* (diet C) collected from wild were used as natural feed. All seaweeds were transported to hatchery, cleaned from mud then put in aerated seawater fiber tank until used. Two formulated diets based on different feed ingredient were used namely wheat flour (diet D) and agar (diet E) (Table 1). Major dietary protein source used were fish meal, spirulina and soybean meal while wheat flour was used as carbohydrate source. Mineral and vitamin mix were added to diet during diet preparation. Measurement of two formulated feeds resulted in two different crude protein content i.e. 35.77% (D) and 31.8% (E).

All food ingredients were finely ground in 60 µm then mixed with other pre-weighed ingredients, including vitamins and minerals mix, to produce pellets. Cooked wheat flour and agar were added to the diet while dried mixture were being mixed. The mixing was stopped if the mixture turn to dough with the consistency lead to dough to pass through a pelletizer with a 2 mm diameter. The wet pellet were then dried under solar drying to reach the moisture content decreased about 10%. The dried uncut pellet were put in plastic container and refrigerated until used. The protein content and free amino acid profile of abalone meat were analyzed. Samples were transported using styrofoam box and to keep the temperature stable jelly ice was used. Crude protein content was analyzed by Kjeldhal method as recommended by the AOAC (1990). Free amino acid content was measured by using HPLC (High Performance Liquid Chromatography) with the chromatographic principles recommended by Metusalach (1995).

The statistical analysis used in this study was the Randomized Block Design followed by Tukey Test to determine the differences between the tests (Zar 1984).

Table 1

Composition of formulated diets used in the experiment (%)

Ingredients	*Formulated diet (D)*	*Formulated diet (E)*
Fish meal	15	16
Shrimp meal	15	15
Soybean meal	13	15
Spirulina	10	7
Wheat flour	30	40
Extract Agar	10	0
Vit & Min mix	7	7

Results and Discussion

Protein content. Abalone fed with different folmulated diets have relatively higher protein content in the meat than those fed different natural fresh macroalgae. This is thought to be influenced by nutrition, enviromental condition and metabolic ability of abalone. The result showed that the crude protein content of abalone fed by formulated feed (D), *G. verrucosa*, formulated feed (E), *G. edulis* and *U. lactuca* were 18.79±1.19%, 17.70±1.05%, 17.52±.26%, 16.67±.31% and 14.59±.56%, respectively.

Results of proximate analysis of all fresh macroalgae used as feed showed a protein content of 1.8 to 2.2%. The protein content values in the macroalgae are very small compare with the value of the protein content in abalone meat (Figure 1). This suggests that abalone has a special metabolic ability, where the abalone have the enzymes involved in the metabolic process that is very effective in the conversion of feed into protein macromolecules in the body.

The results of several studies have shown that abalone fed with high protein content have higher meat protein content than those fed with low protein. Utilization of feed by an organism not only depends on the composition of the foodstuff but is also influenced by various factors including endogenous and exogenous enzymes (Garcia-Esquivel & Felbeck 2006). This is especially true for organisms such as abalone that are herbivorous species that in their habitat consume seaweed rich in polysaccharides, especially cellulose which has low digestibility. However, with the presence of cellulase enzymes in the abalone gastrointestinal organs, the nutrients in seaweed can be digested and absorbed by abalone and metabolized to meet the needs of abalone. Optimalization of the digestive process is also inseparable from the potential of digestive system owned by abalone as pointed out by Monje & Viana (1998), that with certain mechanics cellulose can stimulate the production of cellulase enzymes.

Abalone is a species of herbivore that utilizes seaweed as a source of nutrients for the necessities of life. The digestive process of herbivore animals, both land animals and marine animals, involves digestion of chemicals and microbial digestion. Similarly, for the abalone as Monje & Viana (1998) have pointed out that the presence of cellulose enzymes in the gastrointestinal organs other than the result of endogenous secretions is also produced by the microflora commonly found in the digestive organs of molluscs.

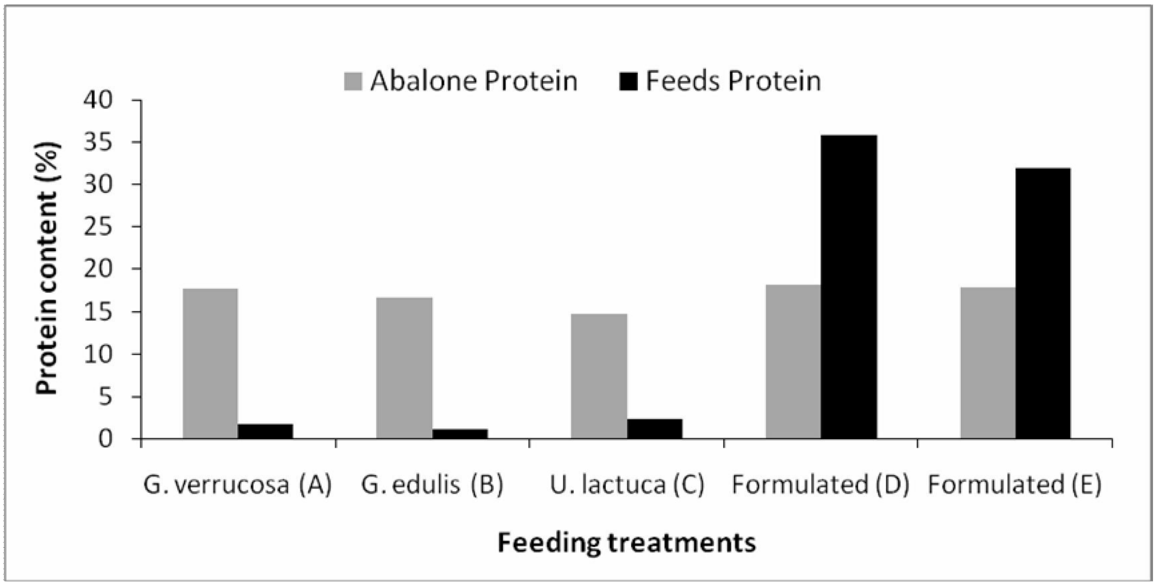

Figure 1. Crude protein content of feeds (macroalgae i.e. *G. verrucosa*, *G. edulis* and *U. lactuca* and formulated feeds) and abalone (*Haliotis asinina*).

In this study, the extracts to be used as binders as well as energy sources in artificial feed formulations were tested. The use of agar as a binder is done with the consideration that *Gracilaria* is a seaweed that has high agar content and is the best feed to support the growth and smoothness of abalone reproduction cycle especially *H. asinina* species (Effendy & Sarita 2004). The biological effects shown by *Gracilaria* suggest that abalone digestive organs produce enough enzyme agarose to optimize the digestion process of feed.

In addition to the presence of enzymes that play a role in the process of digestion, the proximate content of feed consumed also affect the abalone condition, especially in terms of achieving the growth rate. This has been proved by Viana et al (1996) and Lee (2004) that juvenile abalone fed with higher proteins feed had higher growth rates. Improvements to abalone diet formulation, concentrating on optimizing dietary protein level for environmental change and life stages, will ultimately lead to increased growth production and reduced expenses (Bansemer et al 2014). Further, they add that the growth rates of abalone fed by formulated diets are typically higher than those fed live macroalgae, as feed producers are able to formulate diets to contain optimal dietary protein and energy, and provide a suitable amino acid profile for growth. Thus it suggests that abalone has digestive mechanisms that can adapt to the feed consumed.

Total amino acid. The results showed that higher amino acid levels in two formulated feeds have higher protein content and amino acids than from given natural diets (Table 2). The amino acid content of feed in treatment D (feed formulation #1) was expressed quantitatively in abalone meat. Meanwhile, it was also known that almost all of the amino acid content of feed in treatment E (feed formulation #2) was not quantitatively expressed in abalone meat, although all types of amino acids were lower in natural feeds than in formulated feeds but it did not mean that the amino acid content of abalone meat fed natural feeds were lower than those fed formulated feed. This concept relies on the basis that the requirement of dietary amino acid composition is similar to the soft tissue of the animal (Bansemer et al 2014).

All of the fifteen amino acids (L-Isoleucine, L-Glutamic acid, L-Phenylalanine, L-Serine, L-Histidine, L-Threonine, L-Proline, L-Tyrosine, L-Leucine, L-Aspartic acid, L-Lysine HCL, Glycine, L-Arginine, L-Alanine, L-Valine) of broodstock abalone fed formulated feed D were higher than those of *G. verucosa*, formulated feed E (with binder agar), *G. edulis* and *U. lactuca*. Several free amino acids (Glycine, L-Serine, L-Proline and L-Alanine) content of abalone hepatopancreas fed natural and formulated feeds showed no significant difference with those of broodstocks abalone.

Some of free amino acids (L-Glutamic acid, L-Arginine, L-Isoleucine, L-Threonine, L- L-Valine, L- Lysine, L-Leucine, L-Histidine, L-Tyrosine and L-Phenylalanine) content of abalone fed formulated feed #1 was not significantly differrent or higher than that of natural feed (*G. verrucosa*) but it was significantly different to those meat of abalone fed by formulated feed #2 and natural feeds (*G. edulis* and *U. lactuca*). While, free amino acids (L-Glutamic acid, L-Arginine, L-Isoleucine, L-Threonine, L- L-Valine, L-Lysine, L-Leucine) content of abalone broodstocks fed by natural feed (*G. verrucosa*) was not significantly different to those of abalone broodstocks fed by formulated feed #2 and natural feeds (*G. edulis* and *U. lactuca*). Other amino acids (L-Histidine, L-Tyrosine and L-Phenylalanine) of abalone broodstocks fed by *G. verrucosa* was not significantly higher than that of fed formulated feed #2 and *U. lactuca* but it was significantly different to those of fed by *G. edulis*.

The results showed that during the study abalone which was given formulated feed showed different reproduction activities with those given the natural feed. Nutrient content in the diet including amino acids is quite effective to give a positive effect on the process of reproduction. It is suspected that the amino acids involved in reproductive activity are glutamine, glycine and aspartic acid (Li & Gatlin 2006). That indicates that although the quantity of amino acids is not reflected in abalone but qualitatively it seems that the amino acid content has a positive effect on abalone biological activity.

In addition to feeding factors, there are other factors contributing to the reflection of total amino acid content in abalone meat such as the synthesis or conversion of amino acids. This is consistent with Tung's (2010) assertion that abalone has the ability to convert or synthesize amino acids to maintain the equilibrium of amino acids in its body if it can not be obtained from feed. Another factor contributing to the total amino acid content in abalone meat is the role of every amino acid in the organism, where each amino acid has a particular role in the metabolic process. Recent studies have found that some of the amino acids and their metabolic outcomes show a very important role as regulators in the metabolic pathway needed for growth, rearing, food intake, nutrient utilization, defense, reproduction and response to environmental stress (Li et al 2009).

The analysis of total amino acid content showed that almost all amino acids in formulated feeds showed a higher content than those found in natural food. In contrast to the abalone flesh, which suggests that not all total amino acids in formulated fed abalone meat have a higher content than those given natural feed. This can be seen in some amino acid found in abalone meat given *G. verrucosa* and formulated feed containing flour binder even higher than those fed with formulated feed containing agar binder. This is supported by Viera et al (2005) who found that the growth rates of *H. tuberculata* were more likely affected by the specific amino acid composition within the macroalgae rather than the total protein content.

Another thing which also one factor determining the amino acid content in the body is the requirement of the organism itself. If the amount of amino acid absorbed exceeds requirement of the organism, then the excess of amino acids will be deaminated then the carbon bone will be synthesized to become a stored product (storage product) and the amine group is excreted (Lehninger 1976; Berg et al 2001). From the biochemical point of view, an amino acid can be synthesized not only from other amino acids but also from non amino acid components such as intermediate in the Krebs cycle. In addition, amino acids have an important role in metabolism, especially regulators in the metabolic pathways required for rearing, food intake, nutrient utilization, care, reproduction and response to environmental stress (Li et al 2009). Spawning is more often shown by those fed formula feeding during conducting attribute research on the effect of amino acid in reproductive activity. This is similar to the results reported by Li & Gatlin (2006) in which glutamic acid, glycine, and aspartic acid have an important role in fish reproduction activities, including arginine (Li et al 2009). Arginine has been shown to improve spermatogenesis, embryonic survival, immune function, cardiovascular function, and reproductive function (Wu 2010).

Table 2

Free amino acid composition of abalone meat (*H. asinina*) fed with different natural and formulated feed

No.	Free amino acid	*Treatment A (G. verrucosa)*	*Treatment B (G. edulis)*	*Treatment C (U. lactuca)*	*Treatment D (Formulated 1)*	*Treatment E (Formulated 2)*
1	L-Isoleucine	3982.5±322[ab]	2790.2±810[a]	2694.8±208[a]	5228.3±629[b]	3183.8±645[a]
2	Glutamate	17977±2757[ab]	14108.7±5256[a]	12421.1±559[a]	24073.9±3580[b]	14933.9±2484[a]
3	Phenylalanine	3895.5±452[bc]	2367.4±363.6 [a]	2784.8±294[ab]	4930.2±692.6 [c]	2781.7±694.8[ab]
4	L-Serine	6056.8±900[a]	4846.7±1428[a]	5771.1±1682	7781.6±1300[a]	5642.7±2308
5	L-Histidine	17977±2757[ab]	14108.7±5256[a]	12421.1±559[a]	24073.9±3580[b]	14933.9±2484[a]
6	L-Threonine	6343.5±766[ba]	4398.1±1409[b]	4659.8±399[b]	8095.5±1103[a]	4963.2±1274[b]
7	L-Proline	5389.2±1336	5369.1±1463	4526.2±581	6631.2±2103	5184.0±1344
8	L-Tyrosine	3555.2±366[ba]	2092.8±408.8[c]	25131± 305[cb]	4500.7±740[a]	2430.7±638[cb]
9	L-Leucine	7845.7±1055[ab]	5476.0±1502[a]	5296.3±334[a]	10520.6±1418[b]	5827.1±1037[s]
10	Aspartate	10670±1619[ab]	8357.6±3251[a]	7410.2±510[a]	13830.0±2133[b]	9236.5±1587[ab]
11	L-Lycin HCL	7324.0±395[ab]	5594.1±2308[a]	4877.4±232[a]	10400.6±1281[b]	6428.1±1068[a]
12	Glycine	11958.4±3416	11269.3±2667	10538±1026	14681.6±4995	11266.3±4005
13	L-Arginine	11579±1490[ab]	8046.9±1709[a]	8355±1068[a]	15386.9±3253[b]	8548.9±2787[a]
14	L-Alanine	7087.9±1069	6174.4±2070	5427.3±375	9363.2±1781	6419.5±1491
15	L-Valine	4330.1±300[ab]	3133.8±912[a]	3045.58±274[a]	5688.8±638[b]	3621.7±830[a]

Note: Different letter notations show significant differences between treatments.

Conclusions. Fifteen amino acids content of abalone broodstock fed formulated feed #1 were higher than those of fed by *G. verrucosa*, formulated feed #2 (with binder agar), *G. edulis* and *U. lactuca*. There is no effect of natural and formulated feeds on four free amino acids (Glycine, L-Serine, L-Proline and L-Alanine) content of hepatopancreas abalone broodstocks. Abalone broodstock fed formulated feed #1 and *G. verrucosa*, which were both contents higher glutamic acid, glycine, aspartic acid and arginine compare to those of broodstock fed with formulated feed #2 and natural feed (*G. edulis* and *U. lactuca*).

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