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Changes in Proximate Content of Macroalgae *Ulva Sp* during Co-culture with Abalone *Haliotis squamata* in Coastal Waters of West Timor-Indonesia

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ABSTRACT

Abalone is luxurious sea food that fetches a very high price. To fulfil increasing market demand, aquaculture is a necessity. Feed is prerequisite for developing aqua culture for this species. In the absence of artificial diets, abalone growers have to depend entirely on macro algae such as *Ulva sp* harvested from natural population. Abalone are known to consume large amount of algal diet, thus dependence on wild algae could lead to a heavy exploitation of algae population. One of strategies to maintain the supply of *Ulva sp* is by culturing together with *H. squamata* in the same area. However, there is no information whether the cultured algae would match the nutritional quality of algae collected from the wild. This research evaluated the proximate composition of *Ulva sp* growing in two culture systems i.e., cage culture in open sea and recirculating aqua culture system (RAS) in land. The proximate of the cultured Ulva were compared with those freshly collected from the wild. Results showed that protein content differed significantly among the two cultured Ulva and the wild ones ($P<0.05$). Ulva cultured in RAS contained the highest level of protein (16.89±0.87 % dw), followed by those cultured in cage (11.29±0.31% dw), and the lowest occurred in the wild Ulva. Cultured and wild Ulva had high carbohydrate content ranged from 46.56±5.03 to 49.29±3.5% dw, but ANOVA showed no significant differences among the source of algae ($P>0.05$). Lipid was a minor component in Ulva with levels ranged from 0.25±0.05 to 0.9±0.31 % dw. There were significant differences in the lipid content among source of algae ($P<0.05$) with the highest level observed on Ulva grown in RAS, followed in decreasing order by Ulva grown in the cage and the wild ones. This study recommends that abalone growers should culture Ulva because it improves the nutritional quality of the algae and reduces dependence on the wild Ulva that could threaten the natural algal population.

Keywords: Ulva, abalone, co-culture,RAS, cage culture,WestTimor

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1. Introduction

Abalone (Haliotis squamata) is one of the highest valued sea-foods in Indonesia fetching a price of Rp 300k ($30)/kg. The high market values encourage overexploitation leading to possible extinction of wild stock of abalone species in West Timor and surrounding islands (Gimin and Sunadji, 2010). Though their exploitation is now banned, abalone collectors are still harvesting the abalone in remote habitats. To protect natural abalone population from collapsing and, at the same time, fulfil the market's demand, development of aqua culture is a necessity.

A number of factors determine the success of the local abalone aquaculture. Among these is the availability of suitable feed. In the absence of formulated diets, abalone growers depend on algal feed, particularly Ulva and Gracilaria, which are harvested from natural algal beds. Abalones are herbivorous animals that consume high amount of algae. Developing of their aqua culture could lead to a heavy exploitation of the natural algal beds. Thus, ideally abalone

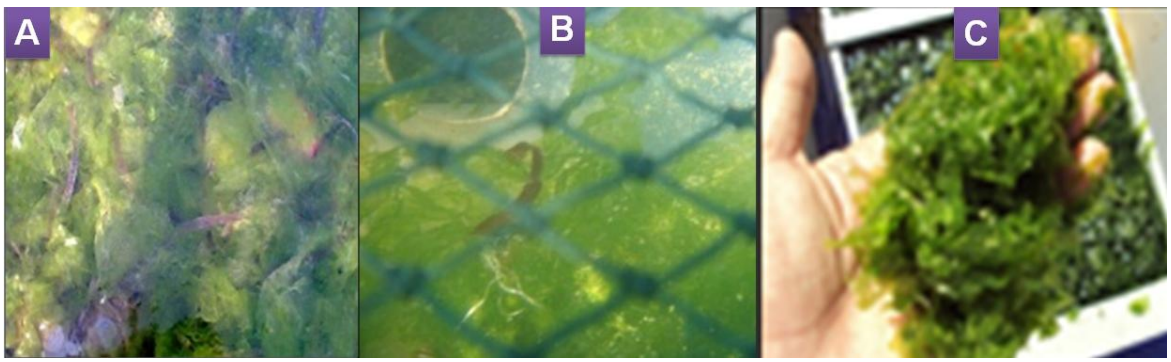

Fig. 1: A. Wild Ulva sp; B. Ulva sp in half-drum cage; C. Ulva sp in recirculation aquaculture system

aqua culture should run simultaneously with algal culture. However, culturing algae for feeding abalone is a new idea and could be rejected by abalone growers due to extra labour. Also, there is some doubt whether the cultured algae could match the quantity and the quality of Ulva harvested from natural environment.

This research was carried out to clarify the effects of co-culturing Ulva with abalone on proximate composition of the algae in order to make recommendation for the abalone growers and some modification of the present practice of abalone aqua culture in West Timor.

2. Materials and Methods

2.1. Collection and culture of Ulva sp

The algae Ulva sp (Fig. 1A) was collected from the intertidal region in Tablolong ($10^o15'801"$ S; 123^o $28'664"$ E), West Timor-Indonesia between June to November 2015. The alga was stocked in perforated half-drum cages (Fig. 1B) at the rate of 750 g/L and let to grow. The algal cages were set side by side with abalone (*Haliotis squamata*) culture cages so that meta-bolic wastes from abalone could pass through the algal cages. The whole cages were set in coastal water about 300 m from the low water mark where strong current prevails. The Ulva sp was also stocked in a land-based recirculating aqua culture system (RAS) (Fig. 1C). Each unit of RAS consisted of components such as abalone tank (100 L), algal tank (300 L), submersible pump (60 L/h), mechanical filter and protein skimmer (60 L/min). The system applied no water exchange except for the supplement of evaporated water with fresh seawater. The Ulva was used as bio-filter and nutrient absorber.

The samples of both cultured and wild Ulva sp were washed in running water, rinsed in 2% ammonium formate, and blotted dried. The samples were put in an oven at 60 ^oC for 48 hours until dry. The dried samples were then pulverized to powder with mortar and pestle, then passed through 500 µm metal sieves. The dried powder was kept in sealed plastic sample bags then stored at -20 ^oC freezer prior to chemical analysis.

2.2. Preparation of samples for proximate analyses

The samples of both cultured and wild Ulva sp were washed in running water, rinsed in 2% ammonium formate, and blotted dried. The samples were put in an oven at 60 ^oC for 48 hours until dry. The dried samples were then pulverized to powder with mortar and pestle, then passed through 500 µm metal sieves. The dried powder was kept in sealed plastic sample bags then stored at -20 ^oC freezer prior to chemical analysis.

2.3. Analytical method

For proximate analysis, total Kjeldahl nitrogen was determined by standard AOAC (1990) methods. Total protein content was calculated by multiplying Kjeldahl nitrogen by 6.25. Carbohydrate was determined by colorimetric method of Dubois et al. (1956) using glucose as standards. The dried algal powder were extracted with 0.5 M H_2SO_4 and centrifuged at 2000 rpm for 10 minutes. Into 1 mL aliquot of supernatant, 1 mL 5% phenol reagent was added and vortex mixed, then 5 mL concentrated H_2SO_4 was added. Absorbance of extracts and glucose standards were measured after 30 minutes at 485 nm using a Hitachi U-1100 UV-Vis spectrophotometer. Total lipids were determined using methanol-chloroform extraction method according to Bligh and Dyer (1959). Into a 100 mg dried sample, 5 mL methanol, 10 mL chloroform and 4 mL HP-water were

added then followed by ultra sonification. After filtering with Whatman 40, the filtrate was transferred to 50 mL beaker and evaporated to dryness using hot plate.

The lipid fraction was then rinsed out of dried residue with 5mL chloroform into a 25 mL tarred beaker and allowed to evaporate. The lipid was weighed to calculate total lipid content (%) of sample mass.

2.4. Statistical analysis

The differences in proximate content among the wild and two cultured Ulvasp were evaluated using a one-way ANOVA test, and the significant differences among the means were tested using an LSD test. All statistical analyses were performed using the software IBM SPSS Version 22.

3. Results and Discussion

3.1. Protein Content

The range of protein content in wild Ulva sp and Ulva from the two cultured systems (cage culture and RAS) ranged from 7.38 to 16.89%. From ANOVA, all samples showed significant differences ($P<0.05$) in protein content. The lowest protein content was observed on Ulva collected from the wild. Between the cultured Ulva, the alga grown in RAS contained significantly ($P<0.05$) more protein than those grown in cage culture (Fig. 2A).

The higher levels of protein content observed in Ulva growing on co-culture systems with abalone, either in the cage culture or in the land-based recirculating aqua culture system (RAS), would be related to its production under high nitrogen culture condition than those collected from nature where seawater contain low nutrient concentrations. Ulva has a good capability for removing nutrients, particularly nitrogen and phosphate from animal effluents (Robertson-Andersson et al., 2008; Macchiavello and Bulboa, 2014). Previous studies reported that Ulva can assimilate as much as 100% for NH_4^+ and at least 80% for NO_3^- generated by abalone aquaculture (Macchiavello and Bulboa, 2014; Robertson and Andersson et al., 2008) and these nitrogen compounds increase protein and pigment content of the algae (Viera et al., 2005; Figueroa et al., 2009). Also, Ulva under culture, either in cage or in recirculating aqua culture system, that receives effluent from cultured animals (fish, crustacean or molluscs), benefit not only from a rich source of ammonia but also from an important and free source of dissolved inorganic C coming from animals respiration that becomes available for algal photosynthesis (Figueroa et al., 2009).

Protein content of Ulva from both of the culture systems was significantly higher than those of the wild algae. Each of the cultured Ulva had a protein of at least 11%, while those harvested from the wild contained only 7.38%. This confirms results reported by several authors who have shown that culture of Ulva in nutrient-rich waters increases its protein content from 11% to over 32% in dry weight (Shpigel et al., 1999; Boarder and Shpigel, 2001). Genus Ulva are known to be able to remove up to 90% of dissolved nitrogen from aqua culture effluents (Boarder and Sphigel, 2001) and use it to form protein. Culturing Ulva in aqua culture systems, i.e., in cage or in RAS, like the present study exposed the algae to higher concentration of dissolved nitrogen than those algae living in natural environment. As a result, protein content in the cultured Ulva increased. Previous studies reported that the culture of Ulva in nutrient-rich water increases their protein content roughly 3 to 10 fold (Sphigel et al., 1999; Boarder and Sphigel, 2001). Increases in the protein content of the cultured Ulva in the present study ranged from 1.5 to 2.3 fold compared with the natural algae.

Between the cultured Ulva, the algae grown on RAS contained significantly higher protein content than those grown in cage. Several studies shown that in recirculating systems, with minimum water exchange, nitrogenous compounds in the forms nitrate (NO_3^-) and ammonium (NH_4^+), might build up, accumulate and reaches high concentration (Losordo et al., 1998; Metaxa et al., 2006; Cahill et al., 2010). Macroalgae are known to be able to take up NO_3^- and NH_4^+ simultaneously and at the same rate (Harrison et al., 1986). Those algae with a high surface to volume ratio, such as Ulva, have a high nutrient uptake rate (more membrane surface for uptake) (Harrison and Hurd, 2001). Therefore, when Ulva is exposed to high concentration of NO_3^-, the algae reduces this compound to NO_2^- and, later to NH_4^+. The latter is

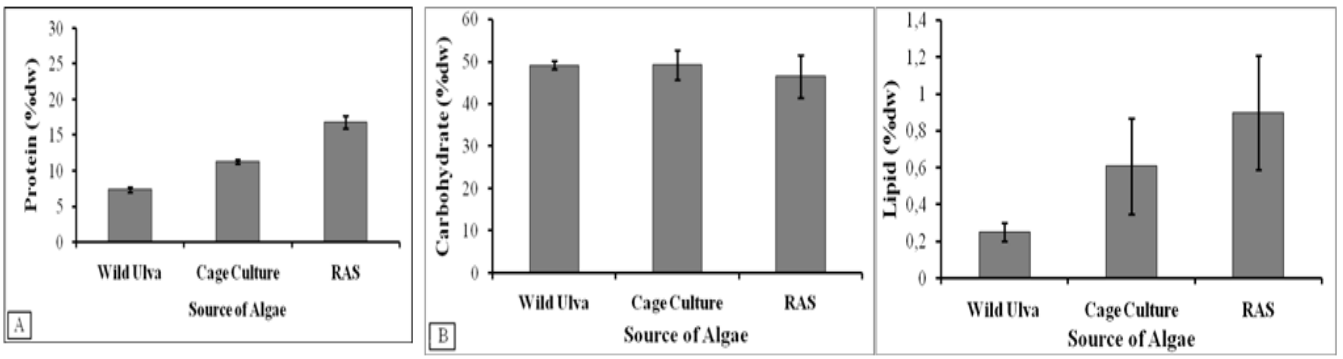

Fig. 2.Protein, Carbohydrate and Lipid content in Ulva sp harvested from the wild and from aqua culture systems (values are means ± SD; N=3)

then converted to form amino acids (Harrison and Hurd, 2001). However, when the algae are exposed to low N or N starved, amino acids also decrease. This could be the reason of why those Ulva growing in RAS had higher protein content than those in cage culture in open sea where effluents from abalone cages were flushed away quickly and little retained by Ulva in neighbouring cages.

Several studies have shown that there is positive correlation between protein content in algal diets and growth rate of abalone under culture (Britz, 1996; Sphigel et al., 1999; Naidoo et al., 2006; Qi et al., 2010). Results of the present study showed that Ulva produced under aqua culture systems will be benefiting for abalone *H. squamata*, not only from higher protein content than wild Ulva, but also by maintaining good water quality within abalone culture facilities through nutrients uptake recorded in the present study means that Ulva could be a good diet for *H. squamata*. Indeed high levels of dietary carbohydrate are required by abalone to enhance growth (Thongrod et al., (2003).

3.2. Total Lipid

The total lipid content was a minor proximate component Ulva sp and its percentage ranged from 0.25 to 0.90% (Fig. 2C). Nevertheless, ANOVA showed that there are significant differences ($P<0.05$) in the previously (Ratana-arporn and Chirapart, 2006; Rameshkumar et al., 2012). Though there were significant differences between the wild and the cultured Ulva, this would not be prime consideration high carbohydrates (Mai et al., 1995; Durazo-Beltran et al., 2004). Limited utilization of dietary lipids could also be due to their low metabolic rate (Viera et al., 2005). Therefore, lipids are unlikely to play a significant role as an energy source in abalone feeds.

Some authors reported that for optimal growth of abalone like *H. tuberculata*, an algal diet should have a for selecting diet for abalone. Abalone species have low lipid requirement which is an adaptive response among herbivorous aquatic animals living in habitats where natural algal diets usually have low lipids and lipid content. The highest lipid content was observed on Ulva grown in RAS, followed by those produced from cage culture, and the lowest recorded on Ulva collected from the wild. In comparison to protein and carbohydrate, total lipid content in both wild and cultured Ulva was very low (<1%) but this result fell within the ranges reported

3.3. Carbohydrate Content

Carbohydrate content was a major component in Ulva sp collected from the wild or aquaculture systems. All the dried Ulva contained 46.56 to 49.16 % of carbohydrate (Fig. 2B). Ulva sp grown on RAS had the lowest carbohydrate content. However, the ANOVA showed no significant differences ($P>0.05$) in carbohydrate content between the wild algae and the cultured ones.

Carbohydrate was the main proximate component of Ulva harvested from the natural environment and from aqua culture. Abalones use the carbohydrate as the main energy source (Pérez-Estrada et al., 2014) and most Haliotis species require dietary carbohydrate ranges from 43 to 48% (Sales and Janssens, 2004). The high requirement for carbohydrate among abalone species related to the presence of various enzymes to hydrolyse complex carbohydrates (Fleming et al., 1996) and the utilization

of to synthesize non-essential lipids (Viera et al., 2005). High levels of carbohydrate of at least 46% balanced levels of protein (>15%; optimum 28-35%), lipid (3-5%), carbohydrate (20-30%), with no toxic substances or phenolic compounds (Mercer et al., 1993; Mai et al., 1995; Viera et al.,2005; Qi et al., 2010).

The protein and lipid contents in the present study were below those levels. However, like other herbivores, abalones are able to compensate for the lower nutritional value by increasing their feed intake (Qi et al., 2010). Studies on suitable algal diets for tropical abalone *H. squamata* are scarce and no information on levels of balanced nutrients. Common practice in *H. squamata* hatcheries and grow-out farms in Indonesia is by provisioning a mixture of algae Gracilaria and Ulva, both contain low protein and lipid, yet this practice can produce high growth rate (>1.5% g/d) (Gimin et al., 2014). It is likely that feed compensation takes place or the tropical abalone might have low requirement for protein and lipid.

4. Conclusion

In conclusion, culturing the algae Ulva instead of harvesting directly from the nature is promising because not only the nutritional quality of the algae increases, but also from ecological point of view as this practice could avoid over exploitation of wild macro algae for feeding abalone. In addition, the co-culture Ulva and abalone provides other benefits such as, water quality improvement around the abalone farming site as well as removal significant amount of dissolved nutrients coming from abalone aqua culture.

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