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The University of Western Australia

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Enhancing the Role of Maritime Education and Training for Supporting The World Connectivity through Safe, Secure and Efficient Shipping on Clean Oceans.

PURPOSE:

The aim of the conference is to provide a platform to the researchers, experts and practitioners from academia, governments, NGOs, research institutes, and industries to meet and share cutting-edge progress in the fields of maritime Education. It provides an opportunity to enhance understanding of the relationships between knowledge and research related to Maritime Education.

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LIST OF PAPERS

This paper is presented in The First International Conference on Maritime Education and Training November 17th, 2016, Semarang, Indonesia

REMARKABLE AFFECT OF DIETS ON ABALONE (*HALIOTIS ASININA*) GROWTH AND SHELL PIGMENTATION

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ABSTRACT Absolute growth and shell color of juvenile *H. asinina* was monitored over a period of 2-months in an experiment consisting of 8 treatments with 3 replicates (n = 10 individuals per replicate). The treatments were: fresh red seaweed (*Gracillaria verrucosa*), and green algae (*Ulva lactuca*) and 6 formulated feeds using different seaweed meals. This study is an attempt made on the effects of partially replacing fish meal with algae (*U. lactuca*) of the formulated feeds on the growth This preliminary study investigated the effect of seaweed and formulated feed on the growth, the shell color of this abalone and survival of abalone. The results showed that the absolute growth of abalone fed by macroalgae *G.verrucosa* higher than abalone fed with other feed and significantly different from the makroalgae *U. lactuca* and others feeds formulated. Results showed that abalones fed the seaweed *G. verrucosa* and formulated feed with *G. verrucosa* meal had dark - brown shells. However, abalones fed with makroalgae *U. lactuca* and either of the formulated feeds (with spirulina) and formulated feeds (with *U. lactuca* meal) remarkably had the same good growth performance and green shell appearance. Investigations showed that seaweeds meal could be the better replacement for fish meal in pelleted feeds of *H. asinina*.

Keywords – abalone, growth, formulated feed, seaweed, seaweed meal

I. INTRODUCTION

The feed commonly used in abalone cultivation is macroalgae such as *Gracilaria* sp, which contains nutrients proven to increase abalone growth rate (Effendy, 2007; Male *et al*, 2010). But the use of macroalgae feed is still limited, because its availability is still dependent on nature, Because it is required an alternative feed by utilizing seaweed as one of the feed ingredients in formulation feed. Some countries in the world have conducted experiments or research to use formulation feed on abalone cultivation activities (Ferreira, 2015).

In the manufacture of formulation feed, many factors can affect the success rate. Among them is the source of raw materials used, the source of raw materials and types of binders used. The content of the raw material and the accuracy of the binder type are important in formulation feed, as it will affect the nutrient content and stability of the formulation feed. Several types of seaweed in recent years began to be used as additives in formulation feed (O'Mahoney *et al.*, 2014) on fish culture, using *Gracilaria* macroalgae (Al-Asgah *et al.*, 2016) and ulva (Ergun *et al* ., 2008). Seaweed is used in formulation feed because seaweed can be a source of nutrients and can also serve as a binder because it contains hydrocolloid compounds (Anggadiredja *et al.*, 2006). *Gracilaria* is known to have a high agar content, and the extract agar is usually used as a binder in formulation feed (Bautista Teruel and Milamenna, 1999). Ulva sp species, commonly used for protein, pigment, vitamin, and mineral content.

Artificial feed is a mixture of various types of food ingredients, whether vegetable or animal that is processed so it is easy to consume and is also a source of nutrition for the organism. The artificial feed to be provided by the aquaculture animal should have a complete formulation, containing ingredients that can

be synthezed dan also can promote growth and. Similarly, trials on addition of ulva and *Gracilaria* meals as a source of nutrition in *H. asinina* are still very poorly found, although in *H. laevigata* had been studied (Bansemer, 2015). Therefore, in the experiment, there were trials of several types of formulation feed and natural feed to see the effect on growth and shell pigmentation of abalone cultured.

II. METODOLOGY

A. Time and Place

This research was conducted in May to July 2016. Feed production activity and feeding of animal test was conducted at Hatchery abalone at Tapulaga Village, Soropia District, Konawe Regency, Southeast Sulawesi.

B. Experimental animal

Seven to nine - month-old *Haliotis asinina* with initial shell length of 25 – 40 mm. This research conducted in Randomized Block Design. There were 8 (eight) levels of treatment with using fresh seaweed dan formulation feed using different binder. The study was divided into 3 groups based on abalone shell length, where group 1 was abalone with size 25-30 cm, Group 2 with size 31-35 mm and group 3 with size 36-40 mm, produced from abalone hatchery at laboratory of Abalone hatchery in Southeast Sulawesi Indonesia. The abalone were acclimated for two weeks and stocked in a 100-ton concrete land-based tanks filled with aerated sea water.

C. Experimental feed preparation

Two seaweeds *G. verrucosa* and *Ulva fasciata* (Diet B) collected from wild were used as natural feed to compared to the formulaied diets. All seaweeds were transported to hatchery, cleaned from mud then put in aerated seawater fiber tank until used. Six formulated diets based on different feed ingredient were used see Tabel 1. The major dietary protein source used were fish meal, shrimp meal, spyruline and soybean meal while wheat flour was used as carbohydrate source. Mineral and vitamin mix were added to diet during diet preparation. All the experimental diets were formulated to have crude protein content approximately 32-35%.

All food ingredients were finely ground in 60 µm then mix with others pre-weighed ingredients including vitamins and minerals mix. 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 die. The wet pellet were then dried under solar drying to reach the moisture content decreased about to10%. The dried uncut pellet were put in plastic container and refrigerated until used. Proximate composition of all experimental feed were analyzed in duplicated (Table 1 and 2).

D. Feeding experiment

160 of juvenile abalone were stocked in 18 of rearing basket and placed in a 100-ton concrete land-based tanks filled with aerated sea water. Where each basket containing 10 juvenile abalone per randomly allocated experimental feed (three replicated per treatment). This research was conducted for 2 months. Provision of macroalgae feed is done every 2 days as much as 10-20% of body weight and formulation feed is given 2x a day at 06.30 Wita and 18.30 Wita as much as 0.5-2% of abalone body weight.

A recirculation system was maintained at approximately 50 per minute. Water temperature was ranged from 28 to 29°C, salinity varied from 33 – 35‰. Nitrate, nitrite, ammonia and pH in the rearing tank were 0.0019-0.008, 0.1 - 0.3ppm, 0.002 - 0.005, and 8.5, respectively. Abalones were fed *ad libitum* twice a day, early morning and about to night. The feed remaining that settled on the

Table 1. Feed Formulation and Proximate composition of formulated Feed

Feed ingredient	Feed Formulation					
	Agar extract (Formulated 1)	Carragenan extract (Formulated 2)	Sago meal (Formulated 3)	Flour (Formulaied 4)	*G. verrucosa meal* (Formulated 5)	*U. fasciata meal* (Formulated 6)
	%					
Fish meal	15	15	15	16	13	13
Shrimp meal	15	15	15	15	11	11
Soybean meal	13	13	13	15	12	12
spyruline	10	10	10	7	10	10
Flour	30	30	30	40	35	35
Agar Extrcact	10	-	-	-	-	-
Carragenan extract	-	10	-	-	-	-
G. verrucosa meal	10	-	-	-	12	-
U. fasciata meal	-	10	-	-	-	12
Sago meal	-	-	-	-	-	-
Vit+Min Mix	7	7	7	7	7	7
Proximate composition (%)						
Energy and lipid (Kkal/100g)	48.96	44.28	46.98	56.52	46.08	48.96
Total Energy (Kcal/100g)	327.96	323.56	331.94	337.32	338.28	330.72
Water	12.07	9.4	9.68	10.48	8.91	9.44
Ash	12.74	15.86	13.86	13.04	12.92	14.68
Total Lipid	5.44	4.92	5.22	6.28	5.12	5.44
Crude Protein	31.86	32.61	33.34	35.77	34.58	34.54
Total Carbohidrate	37.89	37.21	37.9	34.43	38.47	35.9

Vitamine and mineral Content of each 1 kg of vitamine dan mineral mix.

Calcium (Ca) : 32,5%
Phospor (P) : 1,0%
Iron (Fe) : 6 g
Mangan (Mn) : 4 g
Iodine (I) : 0,075 g
Copper : 0,3 g
Zinc : 3,75 g
Vit B_{12} : 0,5 mg
Vit D_3 : 50.000 IU

Table 2. Proximate Composition of natural / fresh seaweed

No	Nama Sampel	Hasil Analisis				
		Kadar Air (%)	Kadar Abu (%)	Kadar Protein (%)	Kadar Lemak (%)	Karbohidrat (%)
1	*G. verrucosa (Segar)*	89.22	5.6	1.68	<0.02	3.78
2	*U. fasciata (Segar)*	84.76	5.32	2.2	0.83	6.53

bottom tank was siphoned out also twice a day before feeds were given, and further dried then weigh to get the feed consumption. The feeding experiment lasted for 60 days.

E. Parameters observed

Absolute Growth

Growth calculation is measured in two ways: growth calculation based on shell length change and growth calculation based on body weight change using formula:

Absolute growth based on shell length change according to Effendie (1997) that is:

$Li = Lt - Lo$

Where:
Li = absolute growth of average interval length
Lt = average length at time t
Lo = average length at the beginning of the study
Absolute growth based on weight changes according to Effendie (1997), namely:

$Wi = Wt - Wo$

Where:
Wi = absolute growth of average body weight interval
Wt = average weight at time-t
Wo = average body weight at the beginning of the study
Statistical analysis

The different in absolute growth among various dietary treatments were analyzed using Analysis of Variance with Tukey Post-hocTest and considered significant at $P<0.05$, while the effects of amino acid content in feed on abalone meat were analyzed using Correlation and regression test (Gomez and Gomez, 1984). Data gathered were analyzed using SPSS versi 19 (Wijaya, 2011).

III. RESULTS AND DISCUSSION

A. Results

On the observation and measurement during 60 days of research, obtained absolute growth data length and weight and appearance of abalone shell color as follows:

Table 3. Absolute growth of weight and shell length of *H. asinina*

Treatments	Absolute Growth (Mean ±Std. Deviation)	
	Length (mm)	Weight (g)
A = G. verrucosa (Segar)	7.09±1.08[a]	7.37±2.60[a]
B = U. fasciata (Segar)	1.11±.16[c]	.75±.26[b]
C = Formulated feed (1)	2.85±.88[b]	.78±.46[b]
D = Formulated feed (2)	2.54±.73[bc]	1.17±.74[b]
E = Formulated feed (3)	2.61±.45[bc]	1.43±.63[b]
F = Formulated feed (4)	2.93±.80[b]	1.6±.703[b]
G = Formulated feed (5)	2.3±.21[bc]	1.801±.215[b]
H = Formulated feed (6)	3.67±.67[b]	2.80±1.03[b]

* Data with the different letter indicate treatments that significantly different from each other within columns (ANOVA, Tukey post-hoc tests at $p < 0.05$)

Based on Table 3 above, the higest absolute growth average of shell length was on treatment A (macroalga G. fresh verrucosa) with value 7.09 ± 1.08 mm, then on H treatment (feed formulation with the addition of U. lactuca meal) with a value of 3.67 ± .67mm and F Treatment (Feed formulation with Flour) with a value of 2,933 ± .801. Subsequently following was, the treatment C (feed formulation with agar extract) with a value of 2.85 ± .88 mm, Treatment E (Formulation Feed with Sago) with a value of 2.61 ± .45 mm, Treatment D (Formulated Feed with Carragenan) with a value of 2.54 ± .73 mm, G treatment (feed formulation with the addition of flour of *G. verrucosa*) with a value of 2,317 ± .206 mm and the lowest absolute growth of shell length was at treatment B (fresh macroalgae U. lactuca) with a value of 1.11 ± .168 mm.

Based on the results of the analysis of variance (ANOVA) showed that there was significantly different between treatments (P <0.05) and Tukey's test further showed that between different feed treatments showed different effects on the absolute growth of the shell. Anova results showed that A treatment (fresh macroalga *G. verrucosa*) with a value of 7,085 ± 1,078 mm, showed the highest growth value compared to those growths using 7 different types of feed. Furthermore, between treatments C, D, E, F, G and H showed no significant effect between one treatment with other treatment on the growth of juvenile shell. Furthermore, Tukey's test showed that the feeding treatment of C, F and H showed an effect of absolute growth of higher and significantly different shell length gorwth (P <0.05) compare to treatment B, while treatment D, E and G showed that absolute growth of shell lengths that were not significantly different (P> 0.05) with treatment B, although it was known that the lowest absolute growth of shell length was at treatment B (fresh macroalgae U. lactuca) with a value of 1.11 ± .168 mm.

Based on 60-day research results, the absolute growth rate based on body weight in Table 3 above shows that the highest absolute growth of body weight that was in treatment A (macroalga G. fresh verrucosa) with a value of 7,373 ± 2597 g. Then in the H treatment (feed formulation with the addition of U. lactuca meal) with a value of 2.80 ± 1.03 g. Subsequently, the treatment was G (feed formulation with the addition of *G. verrucosa* flour) with a value of 1.801 ± .215 g, and F Treatmet (Formulated with Wheat Feed) with a value of 1.6 ± .703 g, Treatment E (Formulation Feed with Sago) With the value of 1.43 ± 63g,

Treatment D (feed formulation with Carragenan) with a value of 1.17 ± .74 g, Treatment C (feed formulation with agar extract) with a value of 0.78 ± .46 g, and the absolute growth of the lowest body weight that is in treatment B (Fresh macroalgae U. lactuca) with a value of .75 ± .26 g.

The results of the analysis of the variability of the different types of feed on the growth of juvenile abalone body weight showed that there was influence between treatments (P <0.05) and Tukey's test further showed that between different feed treatments showed different effect on absolute growth Body weight. From Tukey test results found that treatment A (macroalga *G. verrucosa* fresh) with a value of 7,373 ± 2597 g showed the highest growth value compared to the growth of body weight juvernil by using 7 types of other feed and very different significantly on the growth of juvenile abalone body weight. While the effect of A feed on the growth of juvenile abalone body weight showed a very real difference with the use of feed B, C, D, E, F, G and H. Furthermore Tukey test on the use of feed B, C, D, E, F, G And H on the growth of juvenile abalone body weight showed the same effect or unreal differences between one type of feed with other types of feed.

The descriptive color test of abalone shell growth

Results of rearing of juvenile abalone fed different fresh seaweed and different formulated feed showed different shell growth colors, namely: treatment A, shell color of abalon consuming macroalga *G. verrucosa* had dark brown shell; treatment B. Abalon which consumes macroalga *U. fasciata* shell greenish color; Treatment C (formulation feed using binder from agar extract), the visible color of the shell is dark brown, with white patches and pale greenishness on the abalone shell. Treatment D (formulated feed using binder from carrageenan extract) The visible color of the shell is brown in color, appears to fade from the previous color to light brown and also looks green line of growth. Treatment E (formulation feed using binder of sago flour) Seen in the expansion of the shell is dark brown, slightly young green spots. Treatment F (formulation feed using wheat flour) The visible color of the shell is dark brown, white patches and young greenish on abalone shells . Treatment G. Abalon that consumed formulation feed *G. verrucosa* shell greenish color with white spots; and treatment H. (Abalon that consumed formulation feed using *U. fasciata meal) had* Color greenish.

B. DISCUSSION

Growth Absolute growth represents the difference between change in size (length and weight) over a given period during the study (Affandi and Tang, 2002). From the results of the research conducted during 60 days of maintenance, the absolute growth rate of total length and weight in each treatment showed varying results with increasing age and abalone size (*H. asinina*). The average absolute growth of abalone shell and body weight (*H. asinina*) fed artificial feed with different binder.

Absolute growth represents the difference between change in size (length and weight) over a given period during the study (Affandi and Tang, 2002). Results of the research conducted during 60 days of rearing, the absolute growth rate of total length and weight in each treatment showed varying results with increasing age and abalone size (*H. asinina*). The average absolute growth of abalone shell and body weight (*H. asinina*) fed natural / fresh seaweed and formulated feeds with different binders was shown in Table 3. Proteins content of macroalgae are generally low, the results of research conducted by Nufajrie *et al.*, (2014) on *G. verrucosa* is 8.06% and the research conducted Patadjai, (2011) at *U. fasciata* at 5.84%. Although the protein content of macroalgae *G. verrucosa* is relatively low, but it is proven to stimulate the growth of the abalone, it is presumably because the metabolism of abalone have the ability to synthesize or convert polysaccharides derived from seaweed to be new muscle tissue (wight) and shell growth (length). Makroalga *G. verrucosa* showed the absolute growth rate based on the highest shell length and body weight and was significantly different from the other seven treatments that were consistent with the daily feed intake level. The highest daily feed intake was also shown in *G. verrucosa* macroalgae feed at 1.55 g / day / day and significantly different from daily consumption of the other three treatments. There are several factors that can be expected to affect this among others that is because the treatment A which is a *Gracilaria* type macroalga allegedly has a high power atraktability than other treatments. It can be seen from the high feed intake of abalone to feed macroalgae species *G. verrucosa* compared to other treatments, this is in line with research that has been done Effendy (2007) and Nurfajrie *et al.*, (2014) that

the feed *G. verrucosa* showing the level of feed consumption and growth was higher than on other macroalgae feeding. Another type of *Gracilaria* is also *G. edulis* according to Patadjai research, (2011) also shows the highest feed consumption compared to other macroalgae. The high power and palatabilty atraktability feed on macroalgae species *G. verrucosa* allegedly caused by the presence of metabolites or compounds in seaweed which can be a source of pleasure for abalone attractants that make abalone interested in approaching the feed and tend to consuming more feed of macroalgae species *G. verrucosa*. This is similar to the research that Viana *et al.*, 1994 has conducted in the attractiveness and palatability test, where macroalgae provide the highest response than formulation feeds indicating that fresh seaweed can be a source of attractant to abalone. In addition to the factors atractability and palatability, another suspected case could affect the high growth and daily feed intake in feed macroalgae *G. verrucosa* ie morphology *G.verrucosa* feed that has a shape and texture of food such as stems are small and softer so it supports the activities of grazing and the capabilities of abalone in changing polysaccharides derived from seaweed because it has enzymes that can digest cell walls of seaweed tissue such as the enzyme cellulase, agarase, karagenase, alginase and pectinase enzyme (Kuncoro *et al.*, 2013).

The feed components in formulation feed use binder extracts to have nutrients that can increase growth so that the energy intake obtained from the consumed feed is fully used for the growth of shell length. Nutrition shows the basic nutrients (biochemical components) needed to support all metabolic systems to perform their respective functions (Litaay, 2005). In addition, abalone has the ability to hydrolyze carbohydrates in the form of agar, which not only serves as a binder, but also plays a role in supplying carbohydrate needs in the abalone. This is supported by Knauer, (1994) who states that only four algae carbohydrates can be Hydrolyzed by abalone such as alginic, agar, and carrageenan acids. The raw materials used in formulation feed are fish meal and shrimp flour. In addition to functioning as a source of protein, fish meal and shrimp flour also serves as attractants that can lure abalone to approach the feed. This is consistent with the statement by Sinaga *et al.* (2015) that shrimp flour contains a glycine amino acid which is a lure, which can stimulate the abalone attraction in the feed. Rahmawati, (2013) added that fish meal is the primary choice of protein source in fish feed formulation because fish meal has good digestibility and pallatability. In addition, the binder used is a source of carbohydrates that can be used abalon to meet their needs. This is supported by Viera, (2014) who stated that abalons can consume feed with carbohydrate content ranging from 40-50%. The high content of carbohydrates in the diet can increase abalone growth because it has an enzyme that can meghidrolisis carbohydrates and can synthesize non-essential fatty acids from within the body.

Proximate analysis showed that protein content in all formulation feeds in this study ranged between 32-35% and the protein content was still within the optimal range for abalone growth, Ai statement Angell *et al.*, (2012) that the optimal protein content in artificial feed for abalone is ranged between 27% -47%, fat content in feed formulation is ranged between 4.9-5.2% and carbohydrate content ranged from 37.21-37.90% , Fat and carbohydrate content is still within the optimal range for abalone growth. The results showed that feed formulation with *G. verrucosa meal, U. fasciata meal, sago, flour, extract agar and extract carragenan* could be the source of binder / adhesive in formulation feed because seaweed contains hydrocoloid compound and also fikokoloid compound. According to Anggadiredja (2006), hydrocolloid compounds are indispensable in a product because they function as a gelling, stabilizer, emulsifier, and suspending agent so they can act as binders. The feed formulations in this study contain sufficient nutrients for the growth of juvenile abalone *H. asinina* but show a lower growth rate compared to G. verrucosa macroalgae feed. The lower growth rate of abalone fed on formulation feed is due to the low feed intake presumed to be caused by formulation feed having less power of atraktability and palatibility resulting in lower feed intake. Patadjai (2009), said that the success of a feed formulation to be used as an alternative natural feed is in addition to nutritional quality, is also determined by the acceptability and preference of animals to the artificial feed. This depends on whether the attractiveness of an artificial feed even more the palatability of the feed. Atractability and palatability of artificial feed is determined by the composition of the feedstock used in the feed formulation. In this study, the less attractive macroalgae that have been introduced in the formulation feed is suspected because the macroalga used has become flour and it is assumed the given percentage of 13 grams can cause the crude fiber content of the cellulose to be high in formulation feed. In contrast to the results of the Bautista-Teruel and Millamena study (1999) using formulation feed from extracts for seaweed to show higher growth rates than macroalgae feed. And also there are several stages or process of seaweed processed into flour among the drying process. In the

drying process it is suspected that there is an altered extract compound which eventually causes less attractive formulation feed. The absolute growth based on the the lowest length and body weight is shown in fresh *U. fasciata* macroalgae, but during observation this fresh macroalgae feed has the highest daily feed intake rate after *G.verrucosa* macroalgae and on the other hand also shows the highest FCR value and is significantly different from the other three treatments. The high value of FCR on *U. fasciata* macroalgae feed is due to the low abalone ability in converting the consumed feed into body weight. This may indicate that *U. fasciata* macroalgae feed has a low biological value or low feed grade where most of the consumed feed is thought to be used only for survival and activity so that little is used for growth, and it is thought that the feed consumed is used as energy for Metabolic processes. Based on the research of Santi *et al.*, (2012) and Brownlee *et al.*, (2012) say that abalone has limited ability to digest the macroalgae of *Ulva sp.* Which contain several types of polysaccharides such as xylan, ulvan, and cellulose. Both types of polysaccharides (xylan and ulvan) are more easily digested by bacteria found in the gut whereas cellulose is a type of polysaccharide that is very difficult to digest by aquatic organisms, especially abalons.

IV. CONCLUSION

In terms of shell length, abalons fed with fresh *G. verrucosa* showed significantly higher growth than abalone given fresh *U. fasciata* and both feed formulations with macroalgae flour binders. However, the feeding of formulations with *U. fasciata* binder shows significantly different growth with fresh *U. fasciata*. Whereas between fresh *U. fasciata* and feed formulations with binder *G. verrucosa* and between the two feed formulations with different binders, each did not differ significantly. In terms of body weight, the udsed of *G. verrucosa as feed* showed significantly higher growth than fresh *U. fasciata*, and all formulation feeds. But between fresh *U. fasciata* and all feed formulations tested did not Show a significant difference. Abalone which is given fresh macroalgae and feed formulations containing macroalgae flour as a binder shows insignificantly different insights. The results showed that the use of formulation feed with different binder did not give effect to abalone growth and abalone (*H. asinina*) maintained on IMTA system.

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