The effect of marine algae (*Gracilaria verrucosa*) formulated feed on the growth rate, survival rate and chemical composition of abalone (*Haliotis squamata)* reared in marine submersible cages

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Abstract. The use of formulated feed in the marine abalone rearing is one effort to anticipate the dependency on the common practice of using fresh feed/natural feed. The study aimed to formulate an alternative feed by utilizing marine algae (*Gracillaria verrucosa*), fish meal, and bran. The study was performed in Lae-Lae Island waters, Makassar, from May to October 2016. This study employed one-month-old abalone juveniles with an average of 5 g initial weight, devided in two stages, i. e. experimental culture condition and experimental diet and feeding. The parameters observed in in this study are the chemical composition of the tissue of abalone and the relative growth. The results indicated that the formulated feed did not significantly affect the growth rate, survival rate, and chemical composition of tropical abalone reared in the marine submersible cages (P>0.05). However, the growth and survival rate of the abalone had a high percentage. Different percentages in marine algae supplementation contributed to similar reponse regarding growth rate, survival rate and chemical composition of the soft tissue of tropical abalone. In addition, after supplying formulated feed, protein composition also indicated an increase.
Key Words: *Gracillaria verrucosa*, natural feed, pellet.

Introduction. Abalone (*Haliotis squamata*) is a species of the gastropod genus *Haliotis* (Bangoura et al 2014) and is commonly known as a sea snail. In Indonesia, particularly in South Sulawesi, abalone is commonly known as a 'seven-perforation sea snail' (Hadijah et al 2013). Abalone is generally found in the marine areas, living in the rocky substrate around the coastal waters and reefs (Leighton 2009). Due to its high nutritional content, compared to other molluscs, abalone is considered to be a premium food commodity (Huang et al 2018; Qi et al 2010). HDL cholesterol content found in abalone meat is low. A recent study performed by Liu et al (2020) confirmed that abalone viscera, although it is considered waste, could help increase high-density lipoprotein content. This substance has a very positive effect on preventing atherosclerosis, a major cause for coronary vascular disease (CVD). Abalone is an export commodity because it is a special food in various parts of the world, such as East Asia, the United States, and some European countries (Australian Bureau of Agricultural and Resource Economics and Sciences 2017; Cook 2014). Abalone is cultivated in China, Japan, New Zealand and the Philippines (Gallardo & Buen 2003; Kijima et al 2002). Clarke (2004) reported that South Africa and Australia are two other major supplier countries for 80% of the high-quality abalone products in the Hong Kong market. Meanwhile, countries like Indonesia and the Philippines are incapable of supplying abalone products, since the product quality cannot meet the market requirement.

The development of abalone farming has encouraged various fields of study such as the physiology of abalone digestion, the application of forage science for abalone, abalone feeding behavior, natural or formulated feed optimization and others (Robertson-Andersson et al 2008; Troell et al 2006). Some of the most frequently researched topics are improving the production in aquaculture, the breeding, and juvenile rearing of abalone (Effendy et al 2018). Studies concerning the biological reproduction and ecology were also conducted on tropical abalone (Budi et al 2015; Hadijah 2017; Hadijah et al 2008, 2013).

Abalone is generally found foraging on a variety of natural seaweeds or marine algae. Studies concerning the formulated and natural diets in abalone farming have been performed in the last 4 decades and new challenges always emerge in improving the optimal production and growth of abalone (Kirkendale et al 2010; Mulvaney et al 2013). Today, many abalone farmers are using feed combinations, formulated feeds and alternative local feeds, while other farmers rely only on commercial feed. Commercial feed generally contains 20-50% crude protein, 30-60% carbohydrates, 1.5-5.3% crude fiber, 6% ash, and 4.126 cal g^{-1} gross energy (Stone et al 2014). A study performed by Budi et al 2015 suggested that natural feed such as *Gracillaria* sp. contributed to a better growth and survival rate of the tropical abalone compared to other types of marine algae. This positive response resulted from essential acids such as arginine, histidine, lysine, methionine, valine, phenylalanine and non-essential amino acids such as alanine, aspartic acid, cystine, glutamic acid, glycine and serine (Bautista-Teruel 2003; Dawczynski et al 2007). However, the use of natural feeds in tropical abalone farming is considered inefficient due to the high feed conversion ratio. In addition, natural feed can only be available in a particular season.

Research concerning the use of formulated feed in the abalone juvenile rearing was performed by Hadijah (2017), which investigate the effect of a 25% protein content supplementation. However, previous studies have never reported the abalone rearing technology in the submersible cage utilizing formulated feed. Feed formulation is important to analyze because abalone's growth may be influenced by feed availability and other biological and physical environmental factors, such as population density, stressful conditions, seasons, and temperature (Huchette et al 2003; Saunders et al 2008). Therefore, in this study, the focus is on the effect of marine algae *Gracillaria verrucosa* as feed to the abalone growth reared in submersible cages.

Material and Method

Experimental culture conditions. This study was performed in Lae-Lae Island waters, Makassar, from May to October 2016. The coordinates of the submersible cages location was -5.135165, 119.390667, Makassar Strait, on the northern beach of Lae-Lae Island. Some environmental parameters were determined. Water salinity was measured by using a refractometer, the surface temperature with a thermometer. The ocean current velocity was measured with a current meter.

This study employed one-month-old abalone juveniles with an average of 5 g initial weight. The submersible cages for the abalone were made of a plastic rectangular container with small holes to enable water flow. The plastic containers were designed to be attached to one another. To ensure the lid remains closed, the two attached containers were locked using T clips. The containers were stored in an iron cage with a size of L×l×h = 120 cm × 30 cm × 15 cm. Each iron cage could store up to 4 plastic containers. There were 3 units of cages and, overall, there were 12 units of plastic containers. Each container stocked 30 abalone juveniles. 360 abalone juveniles were stocked in the submersible cages in total.

Water salinity, temperature and ocean current velocity were measured daily, by using a refractometer, a thermometer and with drifting floats, respectively.

Experimental diet and feeding. The 360 abalone juveniles reared in cages were supplied with feed equal to 20% of their body weight, daily. The formulated feed supplied to the abalone varied in the concentration of *Gracillaria verrucosa* paste: 0% (T4), 25% (T3), 30% (T2), and 35% (T1). The marine algae were processed by traditional sun-drying. After the marine algae dried, 250 g of the algae were mixed with 100 mL of water and heated until it turned into paste. To produce a smooth texture, the algae paste was mixed with other feedstuffs to produce pellets. Feedstuffs of formulated feed contained fishmeal, corn starch, bran, marine algae flour, wheat flour, starch, binders, vitamin and mineral mix (Table 1).

The paste processing was one of the methods to improve adhesiveness in the produced pellet. Marine algae paste was dried to reduce moisture content to 10%. The process of feed production was performed in the Integrated Agricultural Laboratory, Bosowa University, Indonesia. Chemical analysis of feed and abalone was conducted in the Chemical Feed Laboratory, Faculty of Animal Science, Hasanuddin University, Indonesia by proximate analysis.

Table 1

The composition of formulated feed for abalone juveniles (*Haliotis squamata*)

Feedstuff composition (%)	*Formulated Feed*			
	T1	T2	T3	T4
Local fish meal	33	33	33	33
Bran flour	17	17	17	17
Corn starch	10	10	10	10
Marine algae paste	35	30	25	0
Wheat flour	-	-	-	25
Starch	1	6	11	11
Fish oil	2	2	2	2
Vitamin mix	2	2	2	2
Total	100	100	100	100

Notre: T1 - formulated feed with 35% algae paste; T2 - formulated feed with 30% algae paste; T3 - formulated feed with 25% algae paste; T4 - control diet with 0% seaweed paste; CP - crude protein; CF - crude fat; CFib - crude fiber; NFE - nitrogen free extract.

Experimental feed was provided twice per day, for 8 weeks in the morning and evening. The formulated feed was randomly supplied to the juvenile rearing containers. Each treatment had 3 replications.

Experimental parameters. The chemical composition of the experimental diet including the crude protein, nitrogen-free extract (NFE) and energy were analyzed using the proximate analysis at the initial and final period of the experiment based on AOAC (2005) guidelines.

The relative growth of experimental abalone was determined by using a 0.1 g precision electronic scale at the start, middle and end of the experiment. 10 samples were collected randomly. Relative Growth Rate (RGR) was measured after the following formula (Watanabe et al 1983):

$$RGR=[(W_t - W_o)/W_o]x100$$

Where: RGR - relative growth rate (%); W_o - initial average weight (g); W_t - the final average weight (g).

Biomass growth was measured by weighing the experimental abalone at the initial, middle, and final period of the experiment. 10 samples were collected randomly. Biomass growth was measured by using the following formula (Huisman et al 1986):

$$BG=Wt–Wo$$

Where: BG - biomass growth (g); W_o - initial average biomass (g); W_t - final average biomass (g).

The survival rate of abalone was observed at the initial and the final period of the study and was measured by using the following formula (Effendie 2002):

$$SR=(N_t/N_0)x100$$

Where: SR - survival rate (%); N_t - total survived post-juvenile abalone; N_0 - total abalone juveniles stocked.

The chemical composition of the soft body of abalone was determined by proximate analysis, including crude protein, nitrogen-free extract (NFE), and energy at the initial and final stage of the experiment based on the guidelines by Watanabe et al (1983).

Data analysis. All data were converted to mean values and were analyzed by one-way analysis of variance (ANOVA). The SPSS Windows v.20 software was employed to identify the differences among treatments. Any significant effect observed in the experimental parameters was further analyzed by conducting the least significant difference at a significance level of 5%.

Results and Discussion

The chemical composition of experimental feed. The result of the formulated feed chemical analysis indicated that the highest crude protein, crude fat, and crude fiber were identified in T1 (28.6%), T2 (8.95%), and T2 (1.02%), respectively. In contrast, the lowest crude protein, crude fat, and crude fiber were identified in T4 (23.94%), T3 (7.19%), and T4 (0.78%), respectively. The average results of the chemical analysis of formulated feed in this study are presented in Table 2.

Table 2

Chemical composition of formulated feed in each treatment

Formulated feed	*Composition (%)*						*Energy (Kcal kg^{-1})*
	Water	*CP*	*CF*	*CFib*	*NFE*	*Ash*	
T1	7.91	28.60	8.62	0.88	34.35	19.63	4.810
T2	7.63	28.10	8.95	1.02	36.19	18.12	4.937
T3	8.24	26.69	7.19	0.80	40.13	16.95	4.877
T4	8.09	23.94	7.43	0.78	47.89	11.87	5.234

Note: T1 - formulated feed with 35% algae paste; T2 - formulated feed with 30% algae paste; T3 - formulated feed with 25% algae paste; T4 - control diet with 0% seaweed paste; CP - crude protein; CF - crude fat; CFib - crude fiber; NFE - nitrogen free extract.

Growth and survival rate of abalone. The results of the ANOVA test indicated that supplying formulated feed had no significant effect (p>0.05) on the absolute growth, relative growth and survival rate of abalone reared in marine submersible cages. The highest absolute and relative growth was observed in T1 (0.63 g; 25.77%), and the lowest was observed in T2 (0.73 g; 16.03%). The highest survival rate was observed in T3 (99%), and the lowest in the T1 (81%). The average absolute growth, relative growth and survival rate of abalone during the experiment are presented in Table 3.

Table 3

Average absolute growth, relative growth and survival rate of abalone (*Haliotis squamata*)

Formulated feed	*Growth*		*Survival rate (%)*
	Absolute (g)	*Relative (%)*	
T1	0.63	25.77	81
T2	0.37	16.03	96
T3	0.45	18.15	99
T4	0.38	16.69	93

Note: T1 - formulated feed with 35% algae paste; T2 - formulated feed with 30% algae paste; T3 - formulated feed with 25% algae paste; T4 - control diet with 0% algae paste.

The chemical composition of the soft tissue of abalone. The results of ANOVA indicated that supplying the formulated feed to the abalone had no significant effect (p>0.05) on the soft tissue chemical composition of abalone juveniles. However, T4 generated the highest crude protein content (26.37%), and T2 generated the lowest crude protein content (24.4%).

The highest crude fat content was observed in T2 (1.08%), while the lowest crude fat content was observed in T4 (1.05%). The highest crude fiber content was observed in T1 (0.56%), while the lowest crude fiber content was observed in T4 (0.24%). The data of the chemical analysis of the soft tissue in the beginning and end of the experiment are presented in Table 4.

Table 4
Chemical composition of abalone (*Haliotis squamata*) soft tissue in the beginning and end of the experiment

	Treatment	*Composition (%)*					
		Water	*CP*	*CF*	*CFib*	*NFE*	*Ash*
Start of experiment	-	62.94	22.11	0.99	0.10	10.57	3.29
End of experiment	T1	61.09	25.60	1.06	0.56	8.18	3.51
	T2	59.77	24.40	1.08	0.40	11.09	3.26
	T3	56.79	24.66	1.07	0.34	13.47	3.67
	T4	63.11	26.37	1.05	0.24	5.61	3.62

Note: T1 - formulated feed with 35% algae paste; T2 - formulated feed with 30% algae paste; T3 - formulated feed with 25% algae paste; T4 - control diet with 0% seaweed paste; CP - crude protein; CF - crude fat; CFib - crude fiber; NFE - nitrogen free extract.

The protein content is important for the growth of abalone. Protein and lipids are the main energy sources for marine animals, including abalone, during early growth (Litaay 2001). Improvement through the formulated feed is expected to generate an increase in production growth and cost reduction (Bansemer et al 2016). Table 2 showed that a crude protein content above 20% of the formulated feed is capable of supplying the need of the abalone. This is in accordance with Spencer (2002), who states that the required crude protein in feed for abalone ranges from 20 to 53%. Coote et al (2000) found that a 27% protein content contributes to the optimal growth of juvenile abalone (*H. levigata*). In contrast, a study experimenting a type of feed with 20-28% crude protein content did not significantly affect abalone growth. As an epiphyte grazer, it is suitable for abalone to consume feed with a low protein content, since it can adapt its feeding behavior. According to Patadjai et al (2009), the necessary amount of protein required by the abalone ranges from 17-27%. The potential use of marine algae as a carbohydrate substitute in this study showed a positive effect descriptively. The use of high carbohydrate feed may result in a more ecofriendly effect. This feed is characterized by a high digestibility rate, encouraging a low metabolism waste of nitrogen and phosphorus that contaminate the water. As the fish resources from capture fisheries decline, the availability of fish meal as an important protein source in feeds similarly declines (Garcia-Esquivel & Felbeck 2006). Therefore, the optimal use of a carbohydrate source could be a good alternative to overcome this issue.

According to Fleming et al (1996), the formulated feed for abalone should have a high crude protein (20-50%) and carbohydrate (30-60%) content, and a low lipid (1.5-5.3%) and fiber (2-6%) content. The energy from the experimental feed, ranging from 4810 to 5234 kcal kg^{-1}, could be sufficient for abalone reared in submersible cages. The fat content of the experimental feed ranged from 7.19 to 8.95%. Fat content in feed plays an important role in the marine animal's growth, serving as energy and essential fatty acid source. It also helps maintain the important membrane and cell structures of an organ, and helps liposoluble vitamin absorption (NRC 1993). The carbohydrate content of the experimental feed ranged from 34.35 to 47.89% and was considered the optimal percentage for herbivore demersal abalone (Thongrod et al 2003). The carbohydrate content is important because it will function as a non-protein energy source and will reduce the protein consumption for growth. Carbohydrate sources that can be used in artificial feeds include starch, corn starch, rice flour and wheat flour. In this study, the carbohydrate source in the formulated feed was corn starch and algae meal. Currently, various commercial feeds can be found on the market with 20-50% crude protein content, 30-60% carbohydrates, 1.5-5.3% crude fat content and 0-3% crude fibers (Fleming et al 1996).

Abalone supplied with formulated feed descriptively showed a positive response in terms of growth. This is related to the role of formulated feed composed of good-quality feedstuff, such as crude protein with balanced amino acid composition, crude fat, carbohydrates, fibers, vitamins and minerals (Patadjai et al 2009). High growth rate of abalone can be achieved with a proper composition of feed available throughout the year (Fitzgerald 2008). However, it is necessary to understand that the utilization of feed by an organism does not only rely on feed composition, but also on other factors, including endogenous and exogenous enzymes (Garcia-Esquivel & Felbeck 2006). Bautista-Teruel (2003) reported that *H. asinina* supplied with formulated feed showed a positive response in terms of growth compared to the abalone supplied with natural feed. In this study, the formulated feed treatments did not significantly affect the absolute and relative growth, or the survival rate. This was possibly due to the processing of the *G. verrucosa* paste. The process of drying multiple times the marine algae pellets could affect negatively the nutrient content of the formulated feed.

The inaccurate, unequal and long period of drying process, together with sudden changes in temperature may result in the alteration of the chemical composition of marine algae (Farida 2002). According to Troell et al (2006), dried marine algae processed into pellets may result in the insignificant growth rate of abalone compared to natural/fresh marine algae. Moreover, the drying process contributed to reducing antioxidant content in the feedstuff. The intensive drying will reduce phytochemicals, including natural antioxidants significantly (Gupta et al 2011; Lim & Murtijaya 2007). Abalone growth was also affected by the feed consumption rate. The easier the absorption process and the consumption are, the better is the abalone growth. Feed consumption rate is also affected by anti-nutritional compounds and feed texture. Furthermore, abalone prefers macroalgae with a soft texture (Viera et al 2005). The formulated feed in this study was processed into pellets with a rougher texture. Although the palatability was relatively high, it was assumed that these are the contributing factors to the low effect. In addition, the rearing condition of abalone could also affect the growth. One of those factors is the quality of seawater (Karim et al 2017). In the current study, the average salinity concentration was 32.5‰, and the water temperature had an average of 27.5°C. The ocean current velocity ranged from 0.04-0.07 m s^{-1}. The range of abalone survival rate in the final period of the experiment was relatively high (81-99%). A few studies mentioned that even though abalone prefers marine algae, a frequent supply of marine algae in the rearing period could result in slow and heterogeneous growth (Priyambodo et al 2005; Stickney 2000; Susanto et al 2010; Vivanco-Aranda et al 2011). The experiment indicated that the growth of abalone supplied with formulated feed did not show any significant effect. However, a 35% proportion of *Ulva* sp. meal may contribute to a better growth rate. Other studies mentioned that the protein content of abalone meat supplied with formulated feed is significantly higher compared to the control group (Adiasmara et al 2016).

Based on the proximate analysis of abalone soft body, it was descriptively identified that the crude protein, crude fat, fiber and ash content increased after supplied with formulated feed. In spite of this, from the overall perspective, the chemical composition of the soft tissue of abalone did not significantly differ (p>0.05) from the start to the end of the experiment. This was caused by nutrient input of the experimental feed, which had a similar contribution to the chemical composition of the soft tissue. This is in line with the findings of Patadjai (2011), who reported that abalone may consume low protein feed and present equal soft body protein composition as abalone fed with high protein feeds such as fish meal, shrimp meal, or sea urchin meal. However, some other studies showed that abalone supplied with a high protein content feed will produce a high protein content in the soft tissue (Effendy et al 2018).

Conclusions. Based on the results, it can be concluded that the implementation of formulated feed in the tropical abalone reared in the submersible cages did not significantly affect the growth, survival rate, and chemical composition of the soft tissue. However, descriptively, it showed a positive response. The protein level and the inclusion of algae meal at different concentrations in formulated feed showed an equal response in growth, survival rate, and chemical composition of tropical abalone.

The utilization of various marine algae may substitute other carbohydrate sources due to massive availability in the natural habitat. However, it is necessary to mention that marine algae could be formulated with softer texture and better processing.

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