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# The use of artificial feed in *Haliotis squamata* farming in submerged cage culture system at Lae-Lae island, Makassar

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## ABSTRACT

This study investigated the use of a combined formula of artificial feed and fresh marine algae *Gracilaria* sp in a submerged cage culture system and its effect on *Haliotis squamata* relative growth and survival rates. The experiment was performed in Lae-Lae Island, Makassar from May to October 2016. The sample Abalone juvenile larvae were 1.5 cm in size and stocked at a density of 30 pcs/cage. The cage size was 30 cm x 15 cm x 7 cm and equipped with a 2-inch Paralone pipe. This experiment employed a completely random design (CRD) with 3 treatments and 3 replicates. The applied treatment included the combination of artificial feed and fresh marine algae *Gracilaria* sp. Each treatment included: A. 25% artificial feed + 75% fresh marine algae, B. 50% artificial feed + 50% fresh marine algae, C. 75% artificial feed + 25% fresh marine algae, and was given at the proportion of 20% of the abalone's weight. The results of the study indicated that the use of a combined artificial feed with fresh marine algae (*Gracilaria* sp.) had no significant effect on relative growth and survival rates of *Haliotis squamata.* Although feed treatment did not significantly affect the relative growth and survival rate, descriptively, treatment A contributed to the most optimal relative growth and the survival rate compared to treatments B and C.

**Keywords:** abalone, *Gracilaria* sp, survival rate.

# O emprego de ração artificial na criação de *Haliotis squamata* cultivado em sistema de cultura em gaiola submersa na ilha de Lae-Lae, Makassar

## RESUMO

Este estudo tentou investigar o envolvimento de uma fórmula combinada de ração artificial e algas marinhas frescas *Gracilaria sp* em um sistema de cultivo em gaiola submersa e seu efeito no crescimento relativo de *Haliotis squamata* e nas taxas de sobrevivência. O experimento foi realizado na Ilha Lae-Lae, Makassar a partir de 2016. As larvas juvenis de Abalone da amostra tinham 1,5 cm de tamanho e foram estocadas a uma densidade de 30 unidades / gaiola. O tamanho da gaiola era de 30 cm x 15 cm x 7 cm e equipado com um tubo

Paralone de 2 polegadas. Este experimento empregou um delineamento inteiramente ao acaso (CRD) com 3 tratamentos e 3 repetições. O tratamento aplicado incluiu a combinação de ração artificial e algas marinhas frescas *Glacilaria sp*. Cada tratamento incluiu: A. 25% de ração artificial + 75% de algas marinhas frescas, B. 50% de ração artificial + 50% de algas marinhas frescas, C. 75% de ração artificial + 25% de algas marinhas frescas. Cada tratamento foi administrado na proporção de 20% do peso do abalone. Os resultados do estudo indicaram que o uso de um alimento artificial combinado com algas marinhas frescas (*Gracilaria sp*.) não teve efeito significativo no crescimento relativo e nas taxas de sobrevivência de *Haliotis squamata*. Embora o tratamento com ração não tenha afetado significativamente o crescimento relativo e a taxa de sobrevivência, significativamente, o tratamento A contribuiu para o crescimento relativo ideal e a taxa de sobrevivência em comparação com os tratamentos B e C.

**Palavras-chave:** abalone, *Gracilaria* sp, taxa de sobrevivência.

## 1. INTRODUCTION

The abalone *Haliotis squamata* is a species of sea snail or gastropod with a significant economic value (Ardi *et al.*, 2020; Yun *et al.*, 2020). These abalones are in great demand in countries such as Japan, the US, Europe, Columbia, and Canada (Cook, 2014; ABARES, 2017). This high demand is caused by abalone's low cholesterol content, rich nutrition, and delicacy (Grandiosa, 2020). In Japan, New Zealand, and Philippines, abalones are cultured (Grandiosa, 2020; Gallardo and Buen, 2003). In Indonesia, however, abalone fishing is heavily relied upon by local fishermen. Continuous, unregulated abalone fishing may eventually lead to population decline. Unfortunately, in Indonesia, this natural resource is not yet optimally utilized (Wuryandari, 2014). The abalone population decline was marked by the small size (Omar *et al*., 2006) and lower volume of catches (Hadijah and Zainuddin, 2007; 2008). Similar problems also occurred in other places, including California. In the last decade, such conditions contributed to the updated status of abalone population to an endangered species or critically endangered (Gruenthal, 2007). Although the status of the Indonesian abalone population is categorized as harvestable resources, continuous exploitation from fishing activities will eventually cause a population decrease. Therefore, one initiative to encourage abalone population conservation is abalone breeding. However, the effort of abalone conservation through breeding is still limited due to a number of factors, including feed availability and insufficiency of abalone larvae. To achieve abalone culture and conservation success, it is necessary to be attentive to the aspects of rearing, feeding, and sufficient larvae (Giri *et al*., 2015; Palumbi, 2003). To improve abalone production, breeding can be performed in submersible cages (Hadijah, 2017; Hadijah and Zainuddin *et al*. 2015; Li and Ong, 2017). Another challenge for marine abalone culture is the lack of information about good feed quality for abalone growth and survival. Generally, feeding macroalgae as a natural feed for abalone (Hadijah and Zainuddin, 2014; Hwang *et al*., 2014) would result in inefficient nutrition fulfillment (Kemp *et al*., 2015). However, some researchers have studied the use of macroalgae as a feed for abalones. According to (Bansemer *et al*., 2016; Chojnacka, 2012), feeding macroalgae to abalones could improve health, product quality, and feeding behavior of abalones compared to the formulated feed (Packer *et al*., 2016). On the other hand, live macroalgae feeding for abalone may cause another problem, considering its high moisture content capable of causing diseases and infection (Bautista-Teruel *et al.*, 2003; Vandepeer, 2006).

Another research by (Hadijah, 2017; Hadijah, 2015) suggested that marine algae *Gracilaria* Sp contributed to the most optimal growth and survivability of tropical abalone compared to other types of feed. Unfortunately, the use of marine algae as natural feed in tropical abalone farming is considered to be inefficient due to the high feed ratio and resource

reliance on seasonal algae harvesting. Based on those facts, this research focused on the implementation of artificial feed combined with fresh marine algae in marine abalone farming as an alternative effort to reduce artificial diet use.

## 2. RESEARCH METHODS

### 2.1. Experimental and Culture Conditions

Water quality parameters including water turbidity and depth were measured at the initial stage of the experiment. Water temperature measurement and feeding were performed simultaneously once every three days. The mean water temperature throughout the trial was sea-surface temperature (27-28$^{o}$C). Sea water salinity was measured by using a refractometer resulting in 32-33 ppm. The measurement of ocean currents was estimated using drifting floats. Phosphate and nitrate concentration in the water was collected both at the beginning and at the end of the experiment. Salinity and pH measurement was performed at different times. Dissolved oxygen was measured regularly in the morning and observed once every two weeks along with the in-situ abalone sampling. The experiment was conducted in Lae-Lae Island waters, Makassar, South Sulawesi, Indonesia, from May to October 2016. This study employed one-month-old abalone larvae (*Haliotis squamata*) with a 5-gram initial weight. The seeds were originally from the Bali Gondol Sea seed center and were transported to the site by airplane (a 10-hour trip). After arrival the seeds were acclimatized at the Takalar Brackish Water Seed Center for 24 hours. During the acclimatization, they were given fresh feed in the form of *Gracilaria* seaweed. The submersible cages for the abalone were made of a plastic rectangular vessel with small holes to enable the water flow. Easily obtained media to serve as abalone submersible cages were plastic containers designed to attach one another. To ensure the container's lid did not open, the two attached containers were locked using a T clip. These containers were stored in an iron cage with a size of 120 cm L x 30 cm W x 15 cm H. Each iron cage stored 4 plastic containers. There were 3 iron cages, and therefore 12 units of the plastic containers (Figure 1).

**Figure 1.** Abalone Rearing Containers.

A container with Abalone Juvenile larvae stocking density of 30 pcs/container (Figure 2).

Once all the containers were stocked with abalone larvae, the containers were attached to an iron rack and submerged (Figure 3).

**Figure 2.** Abalone larvae were stocked in the container.

**Figure 3.** Positioning the containers with abalone larvae.

## 2.2. Experimental diet and feeding

The abalones were fed with a combination of artificial feed and fresh marine algae at a proportion of 20% abalone body weight. The experimental diet based on the treatment was randomly applied to the larvae rearing container (Bautista-Teruel and Millamena, 1999). Feedstuffs of artificial feed were composed of fish flour, corn starch, bran, wheat flour, starch as binder, vitamin mix and mineral mix (Table 1).

**Table 1.** The Composition of Formulated Feed for Abalone Larvae.

| N° | Name Feedstuff | Percentage |
|---|---|---|
| 1 | Local fish meal | 33 |
| 2 | Bran flour | 17 |
| 3 | Corn Starch | 10 |
| 4 | Wheat Flour | 25 |
| 5 | Starch | 11 |
| 6 | Fish Oil | 2 |
| 7 | Vit-Mix | 2 |
| | Total | 100 |

Feed formulation was pelletized and dried until moisture content remained 10% and dry cracks appeared on the pellets. Proximate analysis was performed on the dried pelleted feed to identify the nutritional content in the formula. The results of the proximate analysis are presented in Table 2.

**Table 2.** Chemical composition of formulated feed in each treatment.

| N° | Content | Percentage |
|---|---|---|
| 1 | Water | 8.36 |
| 2 | Crude Protein (%) | 23.16 |
| 3 | Crude fat (%) | 6.27 |
| 4 | Crude fiber (%) | 0.47 |
| 5 | NFE (%) | 45.69 |
| 6 | Ash (%) | 16.05 |
| 7 | Energy (Kcal/kg) | 4907 |

Description: Analysis Results of Livestock Feed Chemistry Laboratory Faculty of Animal Science, Hasanuddin University (2016).

### 2.3. Treatments and Experimental Design

The research design adopted a completely randomized design (CRD) with 3 treatments and 3 replicates. Each of the treatments applied the combination of artificial feed and fresh marine algae *Gracilaria* sp. Each treatment encompassed: A. 25% artificial feed + 75% fresh marine algae, B. 50% artificial feed + 50% fresh marine algae, C. 75% artificial feed + 25% fresh marine algae.

### 2.4. Observed Parameters

Relative growth was calculated with the following formula (Watanabe *et al*., 1983) (Equation 1):

$$RG = \frac{Wt-W0}{W0}\ x\ 100 \quad (1)$$

Where:

RG = Relative Growth (%)

$W_o$ = initial average weight (g)

$W_t$ = final average weight (g)

The survival rate of abalone was observed at the initial and the final period of the experiment and was measured using the following formula (Effendie, 2002) (Equation 2):

$$S = \frac{Nt}{N0} \text{ x } 100\% \quad (2)$$

Where:

S = survival rate (%)

$N_t$= total survived post-larvae abalone (pcs)

$N_0$= total Abalone Juvenile larvae stocked (pcs)

### 2.5. Data Analysis

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

## 3. RESULT AND DISCUSSION

The data of average relative growth in each treatment is presented in Table 3.

**Table 3.** Relative growth resulted from each treatment.

| Treatment | Relative Growth (%) |
|---|---|
| A | 2.48±0.10[a] |
| B | 2.35±0.07[a] |
| C | 2.36±0.12[a] |

Description: A. 25% artificial feed + 75% fresh marine algae, B. 50% artificial feed + 50% fresh marine algae, C. 75% artificial feed + 25% fresh marine algae; Description: Superscript letters within the same row indicated no significant effect from the treatment (P>0,05).

Analysis of Variance indicated that the combination of artificial feed and fresh marine algae *Gracilaria* sp had no significant effect on the relative growth of Abalone Juvenile larvae (P>0.05)>. This indicated that the combination of artificial diet and fresh marine algae contributed to an equal relative growth of abalone larvae. The relative growth of Abalone Juvenile larvae in each treatment did not indicate any significant effect. The equal results of relative growth were most probably caused by a number of positive factors including good water quality and good nutrient absorption from the combination of artificial feed and fresh marine algae *Gracilaria sp*. According to Allen *et al*. (2006), the supplementation of *Gracilaria sp* in abalone feed may serve as a stimulant for a more effective feed compared to the commercial feed. Feeding the abalone with a combination of 75% *Gracilaria sp* and 75% artificial feed seemed to be absorbed effectively. According to Capinpin and Corre (1996), there are three factors affecting the abalone preference to the algae. These included the metabolite compounds (Paul *et al*., 2006; Winter and Estes, 1992), the morphology and the texture of algae, as well as the necessary nutrients for abalone growth. The supplementation of *Gracilaria sp.* as feed could encourage the appropriate growth for abalone *H. squamata* farming (Hadijah, 2017; Hadijah *et al*., 2020). In addition, the rearing circumstance significantly affects abalone growth. One of the parameters is water salinity (Boamah *et al.*, 2020; Kong *et al.*, 2017).

The observation result of abalone's survival rate is presented in Figure 4.

The results of ANOVA indicated that the combination of artificial feed and marine algae *Gracilaria sp* did not affect the survival rate of Abalone juvenile larvae significantly (P>0.05). The absence of a significant difference among the treatments demonstrated the complementary roles between fresh natural diet and artificial feed despite the percentage difference. Although no significant difference in absolute and relative growth, the ranges of abalone survival rate by the end of the experiment were descriptively optimal, accounting for 97-99%.

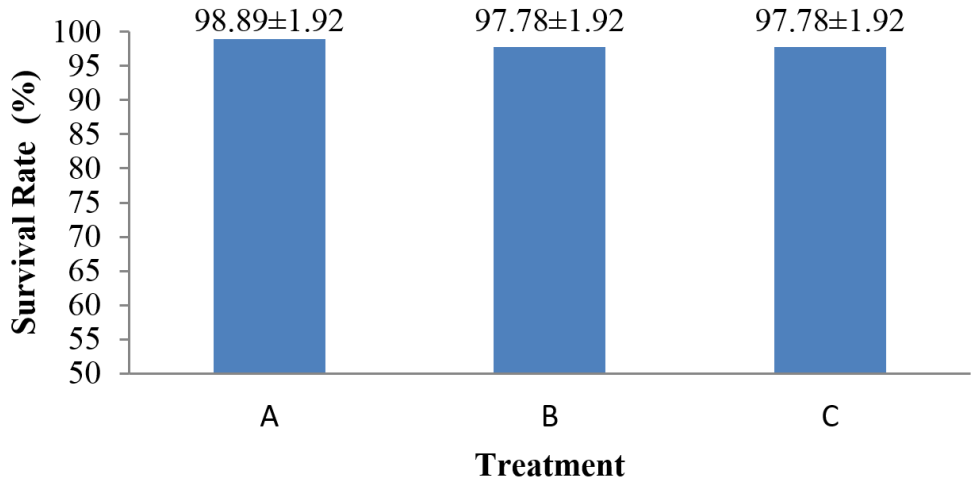


**Figure 4.** Abalone's Survival Rate in Each Treatment.

By understanding the abalone's natural feeding behavior as an herbivore epiphyte grazer adhering to the substrates, it is acceptable for the abalone to consume a low-protein feed. According to Patadjai *et al*. (2009), the protein required by abalone ranged from 17-27%. Fleming *et al*. (1996) confirmed that the properly formulated feed for abalone should contain high protein content (20-50%) and carbohydrate (30-60%), low lipid (1.5-5.3%), and fiber (2-6%). The nutritional content of the experimental feed in this study (Table 2) was considered appropriate to support the abalone survival rate. Coote *et al*. (2000) confirmed that the crude protein required by abalone ranges from 27-40% The use of dried macroalgae flour as feedstuff in a formulated feed for abalone could become a practical option (Viera *et al*., 2012; O'Mahoney *et al*., 2014). Results from different studies indicated that Abalone Juvenile larvae could consume pelletized formulated feed and have optimal growth and survival rates when supplied with fresh feed (Bautista-Teruel *et al*, 2003; Patadjai *et al*., 2009). The substitute feed from macroalgae such *as U. pertusa* flour could substitute soy and fish meal as a protein source in formulated feed for juvenile Halitos asinine (Santizo-Taan *et al*., 2020).

Abalones fed with formulated feed responded positively with an optimal survival rate. This is connected with the feedstuff composition of formulated feed that sustains growth, including protein, balanced amino acid, fat, carbohydrate, fiber, vitamin, mineral, *binder*, and *attractant* (Daniel, 2018). An artificial feed could be produced from alternative local feedstuff and is capable of improving the potential of marine animal growth, such as white leg juvenile shrimp (Zainuddin *et al*., 2020) or abalone. According to Hadijah *et al*. (2020), different percentages of marine algae supplementation contributed to a similar response to growth, survival rate, and tropical abalone soft tissue's chemical composition. In addition, protein composition also indicated an increase after feeding on artificial feed. A number of carbohydrate sources that can be used in artificial feed included starch, corn starch, rice flour and wheat flour (Freeman, 2001). Currently, there are various commercial feeds available in the market containing 20-50% protein, 30-60% carbohydrates, 1.5-5.3% fat, and 0-3% crude fiber (Fleming *et al*., 1996). Bautista-Teruel *et al.* (2003) reported that *H. asinina* supplied with formulated feed showed a positive response of growth compared to abalone supplied with natural feed.

Coote *et al*. (2000) reported that increasing feed protein content to 27% could optimize the growth of abalone *H leveigata*. In this experiment, the supplemented animal protein was a fish meal, shrimp cephalothorax flour, and crab gonad meal, while the plant protein source was obtained from soybean meal.

The absence of significant difference in the Abalone juvenile larvae survival rate indicated that the combination of artificial feed and fresh feed contributed equal output of survival rates.

This was because of the feed nutritional content that could sustain the Abalone juvenile larvae survival. High survival growth of Abalone juvenile larvae was stimulated by the available and sufficient nutrients such as protein, carbohydrates, fat in the supplied feed for abalone larvae. Some research also suggests that although abalones prefer to forage marine algae, frequent marine algae supplementation during the rearing period may result in lower and heterogenous growth (Stickney, 2000; Priyambodo *et al*., 2005).

## 4. CONCLUSION

The results of the study indicated that the use of a combined artificial feed with fresh marine algae (*Gracilaria* sp.) had no significant effect on relative growth and survival rates of *Haliotis squamata.* Although feed treatment did not significantly affect the relative growth and survival rate, descriptively, Treatment A (25% artificial feed + 75% fresh marine algae) contributed to the most optimal relative growth and the survival rate compared to Treatments B (50% artificial feed + 50% fresh marine algae) and C (75% artificial feed + 25% fresh marine algae).

## 5. ACKNOWLEDGEMENT

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