Artificial feed composition for growth and protein and fat retention of humpback grouper, *Cromileptes altivelis*

Diana Y. Syahailatua, Jane L. Dangeubun, Abdul M. Serang

State Fisheries Polytechnique Tual, Jln Langgur Sathean Km 6, Souheast Maluku, Indonesia. Corresponding author: A. M. Serang, serang@yahoo.co.id

Abstract. This study aims to find growth rate and protein content of humpback grouper, *Cromileptes altivelis*, through the utilization of hardmilk wood plant, *Alstonia acuminata*, and other local materials as artificial feed composition. This experiment used 12-13 cm long-humpback grouper, *C. altivelis*. They were reared in the aquarium filled with 100 liters of seawater at a density of 6 individuals per aquarium. There were 4 experimental feed types with addition of different concentration of *A. acuminata* plant, 0.1% (A), 0.2% (B), 0.3% (C), 0.4% (D), and control treatment (without *A. acuminate* addition). Protein content of each treatment wa the same, 45%. The study applied a Complete Randomized Design with 3 replications. Results revealed that treatment B gave the highest mean growth rate with mean daily weight increment of 4 g and daily length increment of 0.011 cm, and mean absolute weight increment of 73 g and absolute length increment of 4.594 cm. As conclusion, feeding *C. altivelis* with addition of 0.2% *A. acuminata* can raise the growth rate with high survival rate.
Key Words: local material, *Alstonia acuminata*, aquarium culture, experiment.

Introduction. Good seed and feed preparations, in quality or quantity, are required in fish culture for production development (Mutiasari et al 2017). Feed is one of the important components in culture activities to support growth and survival of the cultivated fish. Culture activities generally use commercial feed covering about 60-70% of the total production cost (Arief et al 2014), and therefore, knowledge on nutrition and practical feeding of fish are important for a successful fish culture (Zakaria et al 2012). This part makes feed be important to study in order to improve the nutrition at the right dose to stimulate the growth of the cultivated fish. One of the attempts to reduce the feed cost is the use of good quality, easily available, and affordable local feed ingredients, and their need as artificial feed is not competitive with other human necessity (Arief et al 2008).

Fish nutritional needs are fulfilled due to the availability of food items. Complete feed nutritional content is always related with materials used to create feed formulation (Marzuqi & Anjuzari 2013), and the utilization of nutrients, such as protein and fat, is highly correlated with digestion. Fish digesting ability is highly influenced by feed nutrient content. Carnivorous fish like Atlantic salmon cannot well digest particle nutrients from algae due to negative interaction between some components of the algal products and proteolytic enzymes (Norambuena et al 2015). According to Mudjiman (2004), all energies needed by fish naturally come from protein. Thus, protein is employed for growth and body maintenance. Besides, the energy used for body maintenance can come from fat and carbohydrate. These energy sources can be utilized to replace the role of protein as energy source for this purpose.

According to Khan & Abidi (2012), fish can grow from food consumption, and growth can only occur if the energy need for body maintenance and other functions is fulfilled. Protein utilization depends upon its availability, source, energy, growth-influencing non-protein in the food, food conrversion, nutrient retention efficiency, and body composition. Protein is a major source of energy for fish. The availability of good quality protein in the diet will yield good growth performance (Bhat et al 2014).

Insufficience of feed protein will cause drastical decline of termination of growth or loss of body weight, since the fish can take protein from several tissues to maintain the function of more vital tissues (Iskandar & Fitriadi 2017). Protein as nutrient component in fish feed is very expensive, particularly for carnivorous fish, such as humpback grouper, *Cromileptes altivelis*. Therefore, feed protein content must be in optimum amount with balanced amino acid composition that is able to maximally support its utilization for fish growth (Usman et al 2010). They also found that difference in optimum need for protein is affected by many factors, such as species, fish size, and food material composition. Protein and fat are important components of fish feed, the former as energy source for survival and growth beside carbohydrate and the latter as the largest energy source for fish body. Grouper is a carnivorous fish that tends to necessitate high protein food content, 45-55% (Laining et al 2003; Kabangga et al 2004). Feeding appropriate nutritional food content can yield healthy and high quality fish products (Febrianti et al 2016). Food type, number, and quality will certainly determine the fish survival and production (Sutarmat et al 2004).

Feed quality is not only restricted to its nutritional value, but also physical characteristics, such as solubility, digestibility, color, odor, and taste. Food quality is also affected by the use of raw materials. Good feed selection can be based on nutritional value (Meyer & Fracalossi 2004). Food rich in nutrients has given better fish growth (Bhat el 2014). Good quality feed administration will promote the final target of optimal production, so that study on feed nutrition, composition, and physical characteristics needs to be pursued. The use of hardmilk wood *Alstonia acuminata* as fish feed is intended to increase the fish growth, the feed digestibility efficiency, and the resistance to diseases. This study was carried out to know the effect of *A. acuminata* on fish growth, survivorship, protein, and fat, feed quality combination (through proximate analysis of feed material and fish meat) and to know the most effective dose treatment for growth development and protein retention in *C. altivelis*.

Material and Method. This study was conducted for 28 days from March 13th to April 9th, 2017 in Mariculture Laboratory of State Fisheries Polytechnique, Tual. Test animals were 12-13 cm long *C. altivelis* juveniles, with weight range of 31-35 gr. The fish were taken from hatchery in Mariculture Center, Ambon. The juveniles were reared using 15 units of 100 L-aquaria, each of which was filled with 80 L of sterile seawater at the density of 8 ind aquarium^{-1}. Water of each aquarium was siphoned and replaced daily as much as 75% of the water volume. Feed composition is presented in Table 1.

Table 1

Composition of experimental feed (g/100 g feed) with different protein and protein energy (kkal DE/g protein = C/P)

No	*Feed composition (gram)*	*Treatment*				
	Materials	*A*	*B*	*C*	*D*	*Control*
1	*A. acuminata* meal	0.1	0.2	0.3	0.4	0
2	Fish meal	39.5	39.5	39.5	39.5	39.5
3	Shrimp meal	11.7	11.7	11.7	11.7	11.7
4	Cow's blood meal	10	10	10	10	10
5	Moringa leaf flour	3.4	3.4	3.4	3.4	3
6	Bran flour	9.7	9.6	9.5	9.4	10.2
7	Fish oil	9	9	9	9	9
8	Mineral Mix[1]	8.3	8.3	8.3	8.3	8.3
9	Vitamin Mix[2]	8.3	8.3	8.3	8.3	8.3
	Total	100	100	100	100	100

Notes: 1. Mineral mix composition (per Kg feed): 138.58 mg of Fe citrate, 219.9 mg of $ZnSO_47H_2O$, 123.79 mg of $MGSO_4$, 11.79 mg of $CUSO_45H_2O$, 2.39 mg of $COSO_47H_2O$, 5.06 mg of KIO_3, 1.28 mg of Cr^{3+}, and 7.00 mg of Selenium regen (Watanabe 1988); 2. Vitamin mix (per Kg feed) composition: Vitamin A 4000 IU, Vitamin D3 2000 IU, 200 mg of Vitamin E, 8 mg of Vitamin K, 32 mg of Vitamin B1, 40 mg of Vitamin B2, 32 mg of Vitamin B6, 0.04 mg of Vitamin B12, 120 mg of Pantotenic acid, 160 mg of Nicotinic acid, 8 mg of Biotin, 300 mg of Inositol (Watanabe 1988).

The feed used as experiment consisted of 4 types added with different concentration of *A. acuminata,* A (0.1%), B (0.2%), C (0.3%), and D (0.4%), and control treatment without addition of *A. acuminata*. The study utilized Complete Randomized Design with 3 replications. Factor of protein content was 45% and energy-protein ratios of the feed before proximate analysis were 8 and 9.5 kcal DE/g protein, belonging to optimal ratios for various fish species (Gatlin III 2010). Feeding was given ad libitum twice a day, morning and afternoon.

Growth and survivorship of C. altivelis. Increase in absolute weight and relative growth and the survival rate were calculated using Effendie (1997). Weight and length growth of *C. altivelis* were recorded weekly using 0.1 g-balance and callipper, respectively.

Daily relative growth rate (Gr) was calculated as follows:

$$Gr = \frac{Wt - Wo}{Wo \times t} \times 100 \quad \text{or} \quad Gr = \frac{Lt - Lo}{Lo \times t} \times 100 \qquad (1)$$

Where Gr = daily growth rate (%), Wt = body weight at the end of experiment, Wo = initial body weight, Lt = body length at the end of experiment, Lo = initial body length and t = time.

Absolute growth (AG) was calculated as follows:

$$AG = Wt - Wo \quad \text{or} \quad AG = Wt - Wo \qquad (2)$$

Survival rate was obtained as $S = \frac{Nt}{No} \times 100$ (3)

Where S = survival rate (%), Nt = number of individuals at the end of culture, and No = number of individuals at the beginning of culture.

Feed proximate analysis*.* Feed proximate analysis included protein, fat, coarse fiber, ash, and water content. The proximate analysis was done on feed materials, feed, and meat of *C. altivelis* at the end of the study.

Protein Retention (PR) and Fat Retention (FR) were calculated following Watanabe (1988) as follows:

$$PR = \frac{\text{End body protein weight – initial body protein weight (g)}}{\text{Total protein weight consumed (g)}} \times 100\ \% \qquad (4)$$

$$LR = \frac{\text{End body fat weight – initial body fat weight (g)}}{\text{Total fat weight consumed (g)}} \times 100\ \% \qquad (5)$$

The effect of experimental feed on the measured variables was statistically estimated using ANOVA. If there is a significant effect detected among the treatments, further test will be done using honest significant difference (HSD) test (Steel & Torrie 1993).

Results and Discussion. Most fish farmers use complete diets, those containing all the required protein (18-50%), lipid (10-25%), carbohydrate (15-20%), ash (< 8.5%), phosphorus (< 1.5%), water (< 10%), and trace amounts of vitamins, and minerals (Craig & Helfrich 2009). Several previous studies on protein need determination of economic cultured fish found that feed protein varied between 30-55% (Giri et al 1999).

Proximate content of the artificial feed and fish protein content is presented in Table 2. Water content is an important component in feed materials, and food materials hold a different amount of water, either from animal or plant source. It directly influences the food stability and quality (Sundari et al 2015). Hence, determination of water content is the most important and extensive to do in food processing and testing. Proximate analysis found that water content of control treatment approached to treatment A, the lowest among the treatments. The water content of all feed in this study was relatively the same due to similar drying process in the oven, at 50^{o}C for 24 hours. According to Darsudi et al (2008), water content difference can be affected by the water content of the materials mixed with excessive water, holding technique and holding room condition. High water content of the feed could promote fungi growth and damage the feed.

Table 2

Proximate analysis of the artificial feed of *C. altivelis*

Sample code	*Water content (%)*	*Ash (%)*	*Protein (%)*	*Fat (%)*	*Carbohydrate (%)*	
					Coarse fiber	*NNEM*
Control	4.09	28.95	38.67	14.65	4.17	9.47
A	4.03	24.94	42.61	12.06	3.52	12.84
B	5.17	26.75	42.14	13.27	4.36	8.31
C	4.41	24.54	43.19	12.87	3.54	11.45
D	4.44	27.98	41.37	14.04	3.73	8.44

Note: NNEM = non-nitrogen extract material.

In fish feed, ash is retained in follow-up materials. Good feed ash content should be less than 12%, since ash content affects the fish digestibility and growth (Setyono 2012). Our study found that the highest ash content of the feed was recorded in Control treatment, 28.95%, followed by treatment D, B, A, and the lowest in treatment C, 24.54%, respectively. The highest mean protein content of the fish feed was recorded in treatment A, 42.61%, and the lowest in Control treatment, 38.67%. The highest carbohydrate content of the coarse fiber occurred in treatment B, 4.36%, followed by control treatment, D, C, and then treatment A, respectively, while the highest NNEM value was recorded in treatment A followed by C, control treatment, D, and the lowest in treatment B (Table 2). This result indicates that the feed composition has met the standard nutritional requirement to support good growth and health of *C. altivelis*.

Growth of C. altivelis*.* Daily growth rate is estimated to present percent daily weight increment of the fish. Results showed that each treatment reflected increased weight and length increment. Treatment B had significantly different effect on daily weight growth from treatment A, C, and control, but no significant difference from treatment D. Also, there was no significantly different effect between treatment A, C, and control and between treatment A, D and control. Treatment B and D did not have significantly different effect on daily weight increment as well.

The highest mean daily weight growth rate of *C. altivelis* until day-28 was found in treatment B, 4.00%, followed by D, A, Control, and the lowest in treament C, 1.53% (Table 3), while the highest mean daily length growth rate occurred in treatment B, 0.011%, followed by A, control, and the lowest in treatments C and D, 0.004.

ANOVA showed that there was significant difference between absolute length increment in treatment B and other treatments, but no significant difference among treatment A, C, D, and control. The highest mean absolute weight growth at day-28 was recorded in treatment B, 73 g, followed by treatment D, A, control, and the lowest in treatment C, 24.16 g, while the highest mean absolute length increment was recorded in treatment B, 4.594 cm followed by treatment A, control, D, and the lowest in treatment C, 1.2 cm (Table 3). It means that treatment B yields more than twice the lowest mean absolute weight increment of treatment C and control treatment and almost 2 to 3 times mean absolute length increment of other treatments. Mean daily weight increment measurement was intended to know daily percent weight growth, while the measurement of mean absolute weight is to record the mean weight increment at the end of the study. Both data sources used the same initial and final weight. The former employed the value of daily measurement, while the latter used the difference between initial weight and the weight at the end of the study. Nevertheless, those are real data recorded during the study.

Table 3
Growth parameter and protein content of C. *altivelis* until day-28, and comparison of treatment effect on fish growth and survivorship

Treament	*Initial weight (gr)*	*Final Weight (gr)*	*Mean (daily weight increment (%)*	*Mean daily length increment (cm)*	*Mean absolute weight increment (gr)*	*Mean absolute length increment (cm)*	*Survival rate (%)*	*Mean protein content after treatment (%)*
Control	33.33	63.16	2.22[ab]	0.005	29.833[a]	2.078[a]	100	17.37
A	32.67	75.67	2.52[ab]	0.007	43.000[a]	2.828[a]	100	17.94
B	31.67	104.67	4.00[c]	0.011	73.000[b]	4.594[b]	100	18.13
C	33.00	57.17	1.53[a]	0.004	24.167[a]	1.200[a]	100	17.56
D	33.33	84.33	3.50[bc]	0.004	51.00[ab]	1.656[a]	100	17.76

Note: values with different letter at the same column indicate significant difference in HSD test, while values with similar alphabet at the same column indicate non-significant difference.

Protein is a food ingredient which is highly needed for fish growth (Agustono 2014). Its utilization for fish growth is influenced by fish size, age, feed protein quality, feed energy, water temperature, and feeding frequency. If protein energy exceeds the requirement, the consumption level will decline, and intake of other nutrients including protein will reduce as well. Increase in protein content in the body reflects that the fish are capable of utilizing the feed protein for living needs (Subagiyo & Djunaedi (2011), such as metabolisms, damaged cell repair, and growth. When food carbohydrate is not sufficiently available, protein will be used to fulfill the energy necessity (Haetami 2012).

Based on protein analysis of fish meat, it was found that the highest protein content was recorded in treatment B, 18.13%, and the lowest was found in control treatment, 17.37% (Table 3). It could result from that *C. altivelis* is carnivorous. According to Gatlin III (2010), this group is very efficient at using dietary protein and lipid for energy but less efficient at using dietary carbohydrates.

Therefore, this study assumes that *C. altivelis* digested more protein than fat and carbohydrate to gain energy for growth. Addition of *A. acuminata* will raise protein level of the feed and affect protein retention in the fish body so that the fish grow bigger, and reversed condition could occur if the feed contains low protein. In this study, the feed formulation is supposed to have fulfilled the nutrient absorption in the fish body, so that *C. altivelis* could well use the food energy under protein, fat, and carbohydrate equilibrium.

Protein retention. Protein retention reflects feed protein proportion stored as protein in fish body tissue during rearing process (Maulidin et al 2016). The present finding showed that protein retention of *C. altivelis* rose, indicating that the fish could optimally utilize the feed so that the body weight increases due to optimum digestion, absorption, and being able to convert it to be flesh. This finding is supported by Maulidin et al (2016) who studied the effect of papain enzyme addition on meat quality of snakehead that protein retention increases with protein content of the feed.

Statistical test revealed that there was no significantly different effect among treatments on protein retention ($p > 0.050$). In spite of that, the highest protein retention was recorded in treatment of feed B, 36.29%, followed by A, 27.28%, D, 22.53%, C, 20.83%, and control 17.13%, respectively (Table 4). Protein as major energy source is beneficial for fish growth. Insufficient feed protein content will reduce the fish growth rate (Widaksi et al 2014).

Fat retention. Difference in fat content could result from variations in feed quality depending upon sorts of fish meat used and feed processing. Fat is one of the major energy sources needed by fish and plays important role in feed storage. According to Darsudi et al (2008), good fish feed holds protein, fat, carbohydrate, vitamin and mineral content that supports the nutrient need of the cultured fish. The nutrient need should be complete and balanced in order to support the physiological processes and metabolism in the fish body. They also found that the fish feed administered at the appropriate formula and time will be able to raise the digestibility and the absorptibility of the feed nutrient in the digestive tract. It will impact on growth development and fish survivorship.

The highest fat retention occurred in treatment B, 19.71%, followed by treatment A, control, D, and the lowest in treatment C, 13.46% (Table 4). It could result from increased fat consumption as a result of high fat content of the feed, while the unused fat is stored as body fat. This is supported by Serang et al (2007) that high fat content of portunus crab is caused by increased fat consumption of the crab. Low fat retention in treatment C reflects that *C. altivelis* cannot utilize the fat as protein sparing effect in tissue formation. It is in line with Mukti at al (2014) who find low fat retention in eel (*Anguilla* sp.) indicating that eel cannot utilize fat as protein sparing effect in tissue formation.

Table 4

Protein and fat retention

Treatment	*Protein retention*	*Fat retention*
A	27.28	18.66
B	36.29	19.71
C	20.83	13.46
D	22.53	17.67
Control	17.13	17.79

Survival rate. Survival rate is percent number of fish living in certain period of time, and it is usually influenced by food and environmental conditions. Bad food and environmental conditions will negatively affect the fish health and reduce survivorship. Fish of high survivorship is needed to obtain good growth (Effendie 1979). During 28 days of *C. altivelis*, feeding artificial feed with addition of *A. acuminata*, the survivorship of all test fish reached 100%. Addition of *A. acuminata* is capable of increasing endurance system in the body and stimulating fish appetite, so that the fish look healthy and strong. It could be proved that during the study there was no electricity power for 3 days causing all groupers in the culture tank died, but the test fish were alive, even though groupers were known very susceptible to changes in water temperature and salinity. Previous finding (Dangeubun et al 2013) found that administration of coarse extract of *A. acuminata* skin at 200 ppm yielded the highest mean response to increase in total leukocytes, monocytes, lymphocytes, and neutrophil, so that it could raise the survivorship of *C. altivelis* up to 94.44%.

Several previous studies have confirmed that addition of natural materials in feed formulation could contribute to aquaculture development. Maulidin et al (2016) shows that addition of papain enzyme gives significant effect on all parameters, but snakehead (*Chana stritata*) fries with the best growth recorded at treatment 3.05% kg^{-1} feed. Addition of turmeric (*Curcuma longa*) flour to Pangasius catfish (*Pangasianodon hypophthalmus*) feed at the dose of 480 mg turmerict/100 g feed can increase the daily growth rate of the fish (Dewi 2015). Turmeric flour addition to the fish feed could raise the fish appetite indicated with increased food consumption, followed with increase in final body weight and daily growth rate in carp. According to Putri et al (2016), addition of natural material in small amount to the feed formulation functions to maintain the characteristic of the feed nutrition, as preservative, binding material, appetite stimulator. Thus, addition of *A. acuminata* to increasing fish appetite and maintaining the characteristic of feed nutrition has highly contributed to fish feed production development from natural materials.

Conclusions. Treatment B with addition of *A. acuminata* (0.2%) could increase the growth rate of *C. altivelis* compared with other treatments. In 28 days of culture, the highest mean weight growth was found in treatment B, 73 g, followed by treatment D, A, and control, and the lowest in treatment C, 24.167 g. The highest mean absolute length increment was also recorded in treatment B, 4.594 cm, followed by treatment A, control, and D, and the lowest in treatment C, 1.2 cm. The highest protein and fat retentions were also recorded in treatment, 36.29% and 19.17%, respectively. This study found that the present feed formulation has given positive impact on protein retention of the fish, *C. altivelis* in particular, resulting in good growth, both absolute and daily growth, and excellent survivorship (100%) during the culture experiment.

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Authors:
Diana Y. Syahailatua, State Fisheries Polytechnique, Tual, Jln. Langgur Sathean Km 6, Southeast Maluku, Indonesia, e-mail: yulanda@yahoo.id
Jane L.Dangeubun, State Fisheries Polytechnique, Tual, Jln. Langgur Sathean Km 6, Southeast Maluku, Indonesia, e-mail: janelindakorompis@yahoo.com
Abdul Malik Serang, State Fisheries Polytechnique, Tual, Jln. Langgur Sathean Km 6, Southeast Maluku, Indonesia, e-mail: serang@yahoo.co.id

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