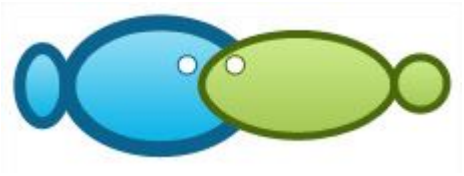

Effect of artificial feed enriched with *Alstonia acuminata* plant on humpback grouper *Cromileptes altivelis* parents

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Abstract. This study aims to examine artificial feed formula application with addition of *Alstonia acuminata* plant on humpback grouper *Cromileptes altivelis* parents. It was carried out in Kelanit waters, Southeast Maluccas. The experimental fish were *C. altivelis* parents with body weight of 1027-1455 g. They were kept in 12 floating fish cages of 1 x 1 x 2 m at a density of 10 ind plot^{-1} with 3 replications. The artificial feed was enriched with *A. acuminata* of 0.1 g (treatment A), 0.2 g (treatment B), 0.3 g (treatment C), and 0.4 g (treatment D) in 100 g feed. Feeding was done twice a day, between 06.00 and 07.00 a.m. and between 17.00 and 18.00 p.m. as much as 5% of fish biomass. Protein content of the feed was 45%. The study employed group randomized design for a 30 day-observation. Results showed significantly different effect of *A. acuminata* plant addition to daily growth rate, and feed consumption. The highest specific growth rate, weight increment, daily feed consumption, and feed efficiency and the lowest feed conversion ratio were recorded in treatment D, addition of 0.4 g *A. acuminata*.
Key Words: feed, growth, feed consumption, feed conversion ratio.

Introduction. The humpback grouper, *Cromileptes altivelis* (Valenciennes, 1828), is one of the most valuable reef fishes, especially in Southeast Asia (Na-Nakorn et al 2010). A wide market sharing and expensive prices make the cultivation of groupers produce high profits. However, in grouper aquaculture, they are faced with various obstacles ranging from seeds, diseases, and quality artificial feed (pelleted feed) (Ottolenghi et al 2004).

Development of pelleted feed for cultured fish is an important aspect in aquaculture industry (Shapawi et al 2011). In intensive aquaculture, artificial feed plays a very important role to meet the fish need for their growth. According to Laheng et al (2016), the use of plant material in fish feed begins to get more attention in order to increase the health status of the organisms and the feed utilization efficiency. One of the plants with potential to increase feed utilization efficiency is *Alstonia acuminata*.

The use of *A. acuminata* as a feed is intended to increase growth and feed digestion efficiency, in addition to increasing resistance to disease. Dangeubun et al (2013) found that application of coarse extract of *A. acuminata* skin at a dose of 200 ppm gave the highest mean response to the development of total leukocytes, monocytes, lymphocytes, and neutrophils that makes the survival of tiger grouper *Mycteroperca tigris* increase up to 94.44%. It is also supported by Dangeubun & Syahailatua (2015) that methanol extract of the stem skin of *A. acuminata* contains alkaloid, phenol, flavonoid, steroid, and tannin that are potential to be an immunostimulant in grouper. The use of *A. acuminata* as feed material is intended to raise the fish growth and the feed digestive efficiency, beside resistance to diseases (Syahailatua et al 2017; Serang et al 2018).

Those findings reveal that *A. acuminata* plant is potential as immunostimulant and could be used as feed material for grouper cultivation. Several *A. acuminata* utilization-related studies have been done as immunostimulant (Dangeubun & Syahailatua 2015; Dangeubun & Metungun 2017), while feeding experiments with addition of *A. acuminata* plant to the feed were performed by Syahailatua et al (2017) and Serang et al (2018) in humpback grouper *C. altivelis,* and Dangeubun et al (2019) in fish seeds of several

different species and found that addition of *A. acuminata* plant as much as 0.2 g $^{-100\ g\ of\ feed}$ could raise the absolute growth, daily growth rate, feed consumption, feed efficiency, survivorship of *C. altivelis* and *Lates calcalifer*.

Application of *A. acuminata* plant in the feed to the humpback grouper *C. altivelis* spawners is the next step of the research development of *C. altivelis* seeds. This study was aimed at addressing the application of the artificial feed formula enriched with different concentration of *A. acuminata* plant addition to *C. altivelis* spawners.

Material and Method. This study was carried out for 30 days, from March 19th to April 17th, 2019, in the seawater of Kelanit village, southeast Mallucas (Figure 1). The test fish used was the parent fish *C. altivelis* of 1027-1455 g. The test fishes were reared in 12 floating net cages of 1 x 1 x 2 m at a density of 10 ind plot^{-1} with 3 replications. The experiment used artificial feed with addition of 0.1 (treatment A), 0.2 (treatment B), 0.3 (treatment C) and 0.4 (treatment D) g of *A. acuminata* to 100 g feed. Fish meal, cow's blood meal, moringa leaf flour, bran flour, fish oil, mineral mix, and vitamin mix were used as well (Table 1). Protein content of the feed was 45%. Feeding was given twice a day, in intervals 06.00-07.00 am and 17.00-18.00 pm as much as 5% of fish biomass. It employed Randomized Block Design with 3 replications for a 30-day observation period. The parent fish were taken from Marine Fish Hatchery and fishermen in Tual.

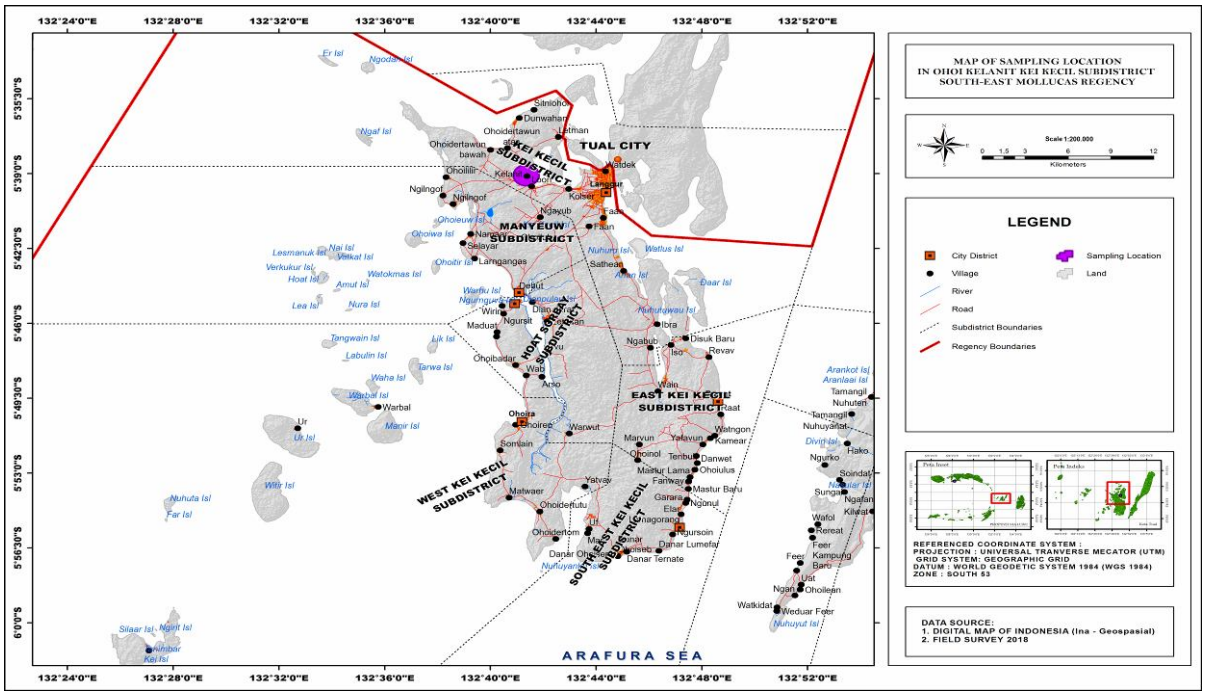

Figure 1. Map of the study site.

Table 1

Feed composition

No.	*Feed materials*	*Composition (g)*			
1	*A. acuminata* meal	A (0.1)	B (0.2)	C (0.3)	D (0.4)
2	Fish meal	39.5	39.5	39.5	39.5
3	Shrimp meal	11.7	11.7	11.7	11.7
4	Cow's blood meal	10	10	10	10
5	Moringa leaf flour	3.4	3.4	3.4	3.4
6	Bran flour	9.7	9.6	9.5	9.4
7	Fish oil	9	9	9	9
8	Mineral mix	8.3	8.3	8.3	8.3
9	Vitamin mix	8.3	8.3	8.3	8.3
	Total	100	100	100	100

Parameters measured were specific growth rate, weight gain, absolute weight growth, feed efficiency, daily feed consumption, and feed consumption ratio. To obtain growth data, the fish weight was recorded once a week. The body weight measurement used a 0.1 g-table balance.

Specific growth rate (SGR) and weight gain (WG) of fish were calculated following Samad et al (2014):

$$SGR = \frac{\ln W2 - \ln W1}{T} \times 100$$

where: SGR = specific growth rate (% day^{-1});
W1 = initial weight (g);
W2 = final weight (g);
T = the number of days in the feeding periods (day).

$$WG\ (\%) = \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{initial weight (g)}} \times 100$$

Feed efficiency was calculated as follows (Watanabe 1988):

$$FE = \frac{(B_t + Bd) - B_0}{F} \times 100$$

where: FE = feed efficiency (%);
Bt = fish weight at the end of study (g);
Bd = dead fish weight during the study (g);
Bo = fish weight at the beginning of study (g);
F = amount of feed consumed during the study (g).

Parameters statistically tested were specific growth rate, weight gain, absolute growth rate, and feed efficiency. ANOVA was applied to see the feed effect on the measured variables. If there is a significant difference ($p < 0.05$) between treatments, Honest Significant Difference (HSD) test was applied (Steel & Torrie 1993).

Results and Discussion

Growth performance of fish parents. The highest specific growth rate of the fish spawners during the study was recorded in treatment D with addition of 0.4 g *A. acuminata,* 0.74±0.13% d^{-1} and the lowest in treatment A, addition of 0.1 g *A. acuminata*, 0.37±0.16% d^{-1} (Figure 2). The present study showed much better specific growth rate than that reported by Fauzi et al (2008) who reared *C. altivelis* at a density of 25 ind. m^{-3} and fed with chopped fish for 2 month-culture period, 0.2% d^{-1}. Hassaan et al (2018) reported that the use of yeast extract of 15 $g^{-kg\ feed}$ significantly gave better specific growth and protein efficiency ratio of Nile tilapia *Oreochromis niloticus*.

Maulidin et al (2016) revealed that addition of papain enzyme in the artificial feed gave good effect on growth of stripped snakehead *Channa striata* with the best dose of 3% papain $^{-kg\ feed}$, but did not influence the survivorship. Huang et al (2011) found that addition of thiamin could increase growth and enzymatic activity of juvenile Jian carp's intestine. Thiamin need of juvenile Jian carp (8.0-60.2 g) based on percent weight gain (PWG) is 1.02 mg kg^{-1}, while the growth performance of rainbow trout tends to rise in addition of soybean meal feed, narrow-leaf or yellow lupin meals (Glencross et al 2011).

Serang et al (2018) reported that addition of *A. acuminata* of 0.2 $g^{-100\ g\ feed}$ gave the highest mean daily weight growth rate of humpback grouper *C. altivelis* as much as 4.00%, and daily length growth as much as 0.011% in 28 days. Similar finding is also reported by Dangeubun et al (2019) for white snapper *L. calcalifer* in 60 days, 4.11% for mean daily weight growth rate and 0.23% for mean daily length growth rate. According to Setiawati et al (2017), daily growth rate of *Pangasius* sp. is 1.13% after fed with

addition of 0.1% or 0.1 $g^{-100\ g\ feed}$ of cinnamon leaf extract. Therefore, feed formulation with addition of 0.2 g *A. acuminata* has given future expectation for marine fish culture development.

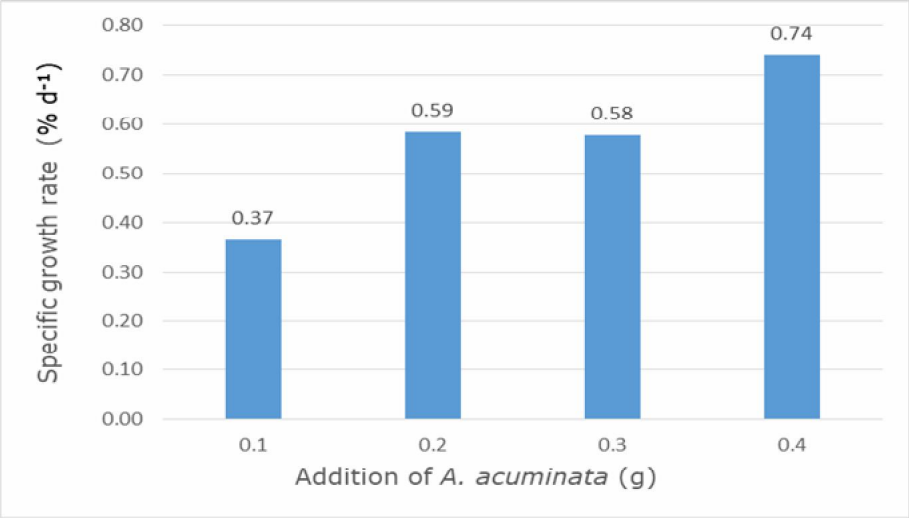

Figure 2. Specific growth rate of *C. altivelis*.

The highest weight growth occurred in treatment D, addition of 0.4 g *A. acuminata,* 24.99±4.73%, and the lowest in treatment A, addition of 0.1 g *A. acuminata,* 11.68±5.41% (Figure 3). Statistically, treatments gave significant different effect on weight growth of the fish parents ($p < 0.05$). Growth effect also occurred in pikeperch juveniles fed with feed enriched with plant oil (Kowalska et al 2011) and *Paralichthys olivaceus* fed with feed enriched with *Scutellaria baicalensis* (Cho et al 2013) yields weight increment between 545 to 637%, but application of *S. baicalensis* extract does not significantly influence the weight increment of *P. olivaceus* ($p > 0.05$).

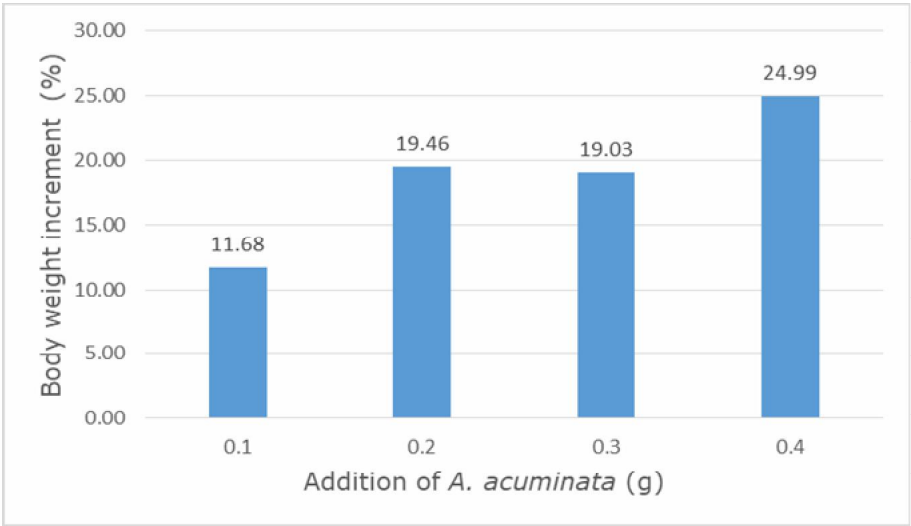

Figure 3. Body weight increment of *C. altivelis*.

Figures 2 and 3 demonstrate that the highest specific growth and body weight increment was recorded at the addition of 0.4 g *A. acuminata*, followed by addition of 0.2 g, then 0.3 g and the lowest occurred at the addition of 0.1 g *A. acuminata*. This finding is similar to that reported by Opiyo et al (2019) who adds 4 g kg^{-1} *Saccharomyces cerevisiae* to the feed that yields high growth performance. Syahailatua et al (2017) found that during 28 day-culture period, addition of *A. acuminata* could increase the body defense system and stimulate the appetite of humpback grouper *C. altivelis*, so that the fish look healthy and strong.

Furthermore, finding the artificial feed formulation enriched with *A. acuminata* plant has given positive impact on fish protein retention, especially *C. altivelis* seeds, yielded good absolute and daily growth, and 100% survivorship. High growth performance resulted from addition of 0.4 g *A. acuminata* to 100 g feed is optimum concentration needed by mature *C. altivelis* to stimulate the appetite, increase the body defense of the fish parent, and to make the feed be able to be well digested. *A. acuminata* contains phenolic compounds, coumaric acid. The administration of external skin extract of *A. acuminata* helps the body defense system fight the bacterial infection (Dangeubun 2012). Coumaric compound in the external skin extract of *A. acuminata* can ruin the cell membrane of the bacteria and can bind the bacteria DNA that the DNA does not express, then kill the bacteria (Lou et al 2012). In addition, the feed protein content of 45% could provide sufficient protein as extra energy source. This extra energy is utilized for growth since growth can only occur if the energy needs for living processes and other functions are met.

Feed efficiency, daily feed consumption, and feed conversion ratio. Statistical test showed that feeding with addition of different dose of *A. acuminata* plant gave significantly different effect on the absolute growth, mean specific growth rate, weight growth, and daily feed consumption ($p < 0.05$), while there was no different effect on feed efficiency and feed conversion ratio ($p > 0.05$) (Table 2).

Table 2

Mean specific growth rate (SGR), body weight increment (WG), feed efficiency (FE), daily feed consumption (DFC), and feed conversion ratio (FCR)

Parameter	*Addition of A. acuminata (g)*			
	A (0.1)	*B (0.2)*	*C (0.3)*	*D (0.4)*
SGR (% d^{-1})	0.37 ± 0.16^{b}	0.59 ± 0.27^{ab}	0.58 ± 0.13^{ab}	0.74 ± 0.13^{a}
WG (%)	11.68 ± 5.41^{b}	19.46 ± 9.74^{ab}	19.03 ± 4.84^{ab}	24.99 ± 4.73^{a}
FE (%)	62.65 ± 20.25^{a}	65.93 ± 13.24^{a}	63.44 ± 13.66^{a}	73.94 ± 15.71^{a}
KPH (g)	67.10 ± 9.07^{c}	99.69 ± 34.13^{bc}	131.13 ± 5.13^{ab}	148.78 ± 0.16^{a}
FCR	1.70 ± 0.47^{a}	1.56 ± 0.35^{a}	1.63 ± 0.34^{a}	1.40 ± 0.34^{a}

Note: different letters on the same row indicates significant difference ($p < 0.05$).

Stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) fed with commercial feed enriched with 0.5% and 1% of cinnamon powder yields FCR of 3.18±0.63 and 3.66±1.59, respectively. On 90th day FCR did not differ significantly ($p > 0.05$) (Begum et al 2018). For fish fed well prepared diets, FCR values < 1.0 have been reported, although it generally ranges between 1.2 and 1.5. Ahmad et al (2011) used 0% (control), 0.5, 1 and 1.5% of cinnamon powder to rear Nile tilapia and the FCR values obtained from each treatment of cinnamon powder addition are 2.21±0.022, 2.05±0.015, 1.95±0.01, 1.94±0.01, respectively.

High feed efficiency indicates good feed consumption for growth (Iskandar & Elrifadah 2015). The feed efficiency (Table 2) for all treatments of *A. acuminata* addition is higher than that reported by Setiawati et al (2017) in *Pangasius* catfish, 41.89%, fed with feed enriched with 0.1% cinnamon leaf extract. Hassaan et al (2018) found that body weight of Nile tilapia linearly rose, while FCR linearly declined with increased addition of yeast extract to the feed. Dangeubun et al (2019) found 77.42±7.411% feed efficiency of white snapper *L. calcalifer* with addition of 0.2 g *A. acuminata* to 100 g feed.

Dada (2015) reported feed additives are edible substances that are added to animal feeds in small quantity to enhance the feed quality so that it enhances growth performance, feed utilization and reduces mortality in fish. Several studies revealed that spices and medicinal plants can be used as feed additive in aquaculture for better growth and survival and microbial disease treatment as well (Immanuel et al 2004).

Setiawati et al (2016) evaluated the growth performance of Asian catfish (*Pangasianodon hypophthalmus*) fed with diets containing *Cinnamomum burmannii* leaf powder and extract. They recorded highest SGR (1.18±0.05%) in fish fed with diet

containing 1% cinnamon leaf powder for 60 days. Ahmad et al (2011) found that the fish fed the diet containing 1% cinnamon showed the highest average body weight, weight gain percentage, and SGR in comparison with other diets.

Conclusions. Feeding with addition of 0.4 g *A. acuminata* gave the best growth of humpback grouper *C. altivelis* parents. The highest feed efficiency and daily feed consumption and the lowest feed conversion ratio were found in addition of 0.4 g *A. acuminata* to 100 g feed. There was also significantly different effect of *A. acuminata* plant dose on daily growth rate, weight growth, and daily feed consumption. Addition of 0.4 g *A. acuminata* to the feed of humpback grouper parents could be one of the efforts to increase the fish parent's growth in the future.

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