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The study increasing doses of plants *Alstonia acuminata* as artificial feed in accelerating the growth and survival of groupers fish (*Chromileptes altivelis*)

Abdul Malik Serang*, Jane Lulinda Dangeubun, Diana Y. Syahailatua

Polytechnic State Fisheries Tual, Jl. Kingdom Langgur-Sathean Km 6 Langgur Southeast Maluku District, Indonesia

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Abstract

This research aims to determine the growth rate and the stability of ducker grouper (*Chromileptes altivelis*) by utilizing *Alstonia acuminata* plant as artificial feed. Ducker grouper used in this experiment is seed stadia. Ducker grouper is kept in aquarium filled with water as much as 50 liters with dense stocking of 6 tails per aquarium. The feed used as experimental feed consisted of 4 types with the addition of different *A. acuminata* plants ie A (0,1), B (0,2), C (0,3), and D (0,4) and control. Protein content of each treatment is the same ie 45%. The experimental design used was a complete randomized design with 3 replications. The results showed that feed B gave an increase to the addition of the average length of 17.62cm and an average weight of 108.33 grams higher than with other feed treatment and control feed. Feed B also provides absolute growth, and the highest daily growth rate. While F test showed significant at 5% level (P <0,05).

* **Corresponding Author:** Abdul Malik Serang ✉ amserang@gmail.com

Introduction

Nutritional requirements of fish as a determinant of growth depends on the feed. In intensive cultivation systems, artificial feed plays an important role than natural food because of its adequate availability. Artificial feed with good nutritional value obtained from the feed. Artificial feed is known to increase the carrying capacity of the cultivation system (Devaraj *et al.*, 1976). The use of *A. acuminata* as a feed is intended to improve the growth and efficiency of feed digestion, but also to increase resistance to disease.

Based on the results of the study (Dangeubun *et al.*, 2013), rough extract of *A. acuminata* skin with 200 ppm treatment resulted in the highest average response on the total increase of leucocytes, monocytes, lymphocytes and neutrophils so as to increase tiger grouper 94.44%. This study found the number of lymphocytes before infected at a dose of 200 ppm 75.5% and increased up to 82.35%.

This indicates that humoral immune response of fish in good condition, so that the process of resistance to foreign objects and the formation of antibodies. Based on the results of the study (Dangeubun *et al.*, 2013) it was found that pure isolate from *A. acuminata* leaf acetate extract is known to contain the structure of methyl-hydroxy-2-methoxy-3 (2-oxohexyl) benzoate compound while based on the results of LC-MS crude extract *A. acuminata* aqueous methanol is known to contain the dominant compound of coumaric acid indicated by a spectrum with a molecular mass of 339,0468. This compound is thought to be p-coumaroyl quinic acid ($C_{16}H_{18}O_8$) which belongs to the class of phenolic compounds (Dangeubun, 2012). The results of this study are strongly suspected that *A.acuminata* plants have potential as immunostimulants.

Research on *A. acuminata* plant is still limited to the study of *A. acuminata* as antibacterial and *A. acuminata* as immunostimulant by immersion method. While for *A. acuminata* as feed for the improvement of life graduation and growth improvement has not been done. It is expected that the combination of *A. acuminata* feed with local ingredients serves to increase growth associated with the production process while producing superior fish that are resistant to disease. Therefore, this study was conducted to examine the effect of artificial feeding with the addition of *Alstonia acuminata* with other local ingredients that functioned to accelerate the growth and graduation rate of ducker grouper (*Chromileptes altivelis*).

Materials and Methods

The study was conducted for 28 days starting from March 13, 2017 until April 09, 2017 at the Mariculture Polytechnic Laboratory of Tual State Fisheries. Test animals used in this study were ducker grouper (*Chromileptes altivelis*) with a length of 12 - 13cm ± 0.2 and weight ranging from 31 to 35 grams. The seeds were obtained from hatching and maintenance at the Ambon Sea Cultivation Park Hall. The seeds are kept by using a 100 liter aquarium volume of 15 pieces. Each aquarium is filled with 50 liters of sterile sea water with a density of 6 tails/aquarium. Every day, 75% of water axes and turnovers are made in each aquarium. The feed used as experimental feed consisted of 4 types with the addition of different *A. acuminata* plants ie A (0,1), B (0,2), C (0,3), and D (0,4) and control. Protein content of each treatment is the same ie 45%. The experimental design used was a complete randomized design with 3 replications. Feeding done 2 times a day ie in the morning and evening. The feed is given until the fish is full. Feed composition is shown in Table 1.

Table 1. Test feed composition.

No	Materials	Feed Composition (gr)				
	Treatment	A	B	C	D	K
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

No	Materials	Feed Composition (gr)				
	Treatment	A	B	C	D	K
6	Brain 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

Note:

1. Mineral mix composition (per Kg feed) : Fe citrate 138,58 mg, $ZnSO_47H_2O$ 219,9 mg, $MGSO_4$ 123.79 mg, $CUSO_45H_2O$ 11,79 mg, $COSO_47H_2O$ 2,39 mg, KIO_3 5,06 mg, Cr^{3+} 1,28 mg, Selenium region 7,00 mg (Watanabe, 1988).
2. Vitamin mix composition (per Kg feed): Vitamin A 4000 IU, Vitamin D3 2000 IU, Vitamin E 200 mg, Vitamin K 8 mg, Vitamin B1 32 mg, Vitamin B2 40mg, Vitamin B6 32 mg, Vitamin B12 0.04 mg, Pantotenat acid 120 mg, Nicotinec acid 160 mg, Biotin 8 mg, Inositol 300 mg (Watanabe, 1988).

Table 2. Proximate composition of the experimental feed.

Proximatecomposition (% dry materials)	Treatment Feed				
	A	B	C	D	Control
Water content	4,03	5,17	4,41	4,44	4,09
Protein	45,44	45,49	45,18	45,39	45,32
Fat	12,57	13,99	13,46	14,69	15,27
Ash	24,94	26,75	24,54	27,98	28,95
Coarse fiber	3,52	4,36	3,54	3,73	4,17
NNEM	12,84	8,31	11,45	8,44	9,47

Note: NNEM = Non-nitrogen extract material.

Measurable parameters include absolute growth rate, daily growth rate and live graduation rate. To know the growth of ducker grouper, the measurement of body weight gain and body length of ducker grouper. Measurements are done every week. Measurement of body weight using the scales sit with 0.1 grams of accuracy, body length by using the sliding term.

Daily growth rate of repeatedly was calculated based on the equation use by Bambang, Rusdi, Ismidan, dan Rahmawati (2011).

$$G = \frac{Ln\ wt - Ln\ wo}{t} \text{ x } 100\%$$

Where,

G : Daily growth rate

Lt : The growth of the test organisms at the end of the research (gr)

Lo : The growth of the test organisms at the beginning of the research (gr)

T : Maintenance time (day)

Absolute growth was calculated with reference formula from Effendie (1997):

$$Lm = Lt - Lo$$

Where:

Lm: Absolute growth (cm)

Lt: The average length of the end of the research (cm)

Lo: Pertumbuhan panjang rata-rata pada awal penelitian (cm).

Survival rate measured based on Effendie (1997) equation:

$$S = \frac{Nt}{No} \text{ x } 100$$

Where.

S: Sorvival rate (%)

Nt: Number of individual in the end of experiment (tail)

No: Number of individual in the beginning of the experiment (tail)

Parameters tested statistically are: daily growth rate and absolute growth. To know the effect of test feed on the measured variable is used variance analysis (F test). If there is any difference between the treatments then proceed with BNJ Test (Steel and Torrie, 1993).

Result and discussion

Growth

The results of the measurements showed that in each treatment and control there was an increase in the growth of the weight and length of the ducker grouper's seeds. The average daily growth rate of the highest grouper ducker seeds on the 28th day was achieved in treatment B of 4.00% followed by D, A, Control, and the lowest in treatment C of 1.93% (Fig. 1). While the average growth rate of the highest daily length was achieved at treatment B that is 0,011% then followed by A, control, and the lowest at treatment of C and D that is equal to 0,004% (Fig. 2).

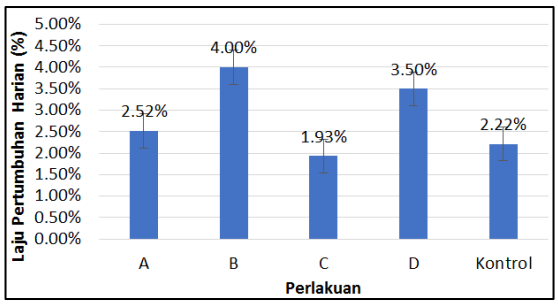

Fig. 1. Average growth rate daily weight of ducker grouper seeds during the maintenance period.

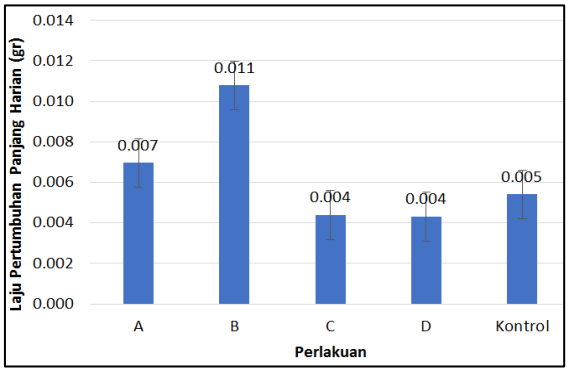

Fig. 2. Average growth rate daily length of ducker grouper seeds during the maintenance period.

In the treatment of B feed with the addition of *A. acuminata* as much as 0.2gr/100gr feed resulted in the highest growth rate of weight and growth rate of the highest length. The rate of specific growth rates obtained in this study is much better than that reported by Fauzi *et al.* (2008) which stated that duck grouper fish kept by floating net cage system with stocking density of 200 tail/8 m^3 (25 tail/m^3) and fed trash fish experienced an average growth of 0.2%/day for 2 months maintenance period.

This is in accordance with the opinion of Suyanto (2009) that both the bad growth of fish is determined by the quality of water, feed, stocking density, and the size of the keramba used. Growth can only occur if the energy requirements for the maintenance of living processes and other functions are met. Growth in grouper seeds is affected by body size variation, smaller body size leading to slower growth (Sutarmat *et al.*, 2006). Mudiarti and Zainuddin (2016) reported that the interaction results of biomass and salinity treatments showed that the artemia biomass treatment of 200% at 31 ppt salinity had the highest relative weight growth value. While the results of the study Hamzah *et al* (2012) seen that fish fed with the addition of selenometionin dose 4 and 16 mg Se / kg of feed has a daily growth rate and feed efficiency is higher than the fish group without the addition of Se. The average absolute weight of the highest grouper ducker seeds on the 28th day was achieved in the B treatment of 73,000 gr then followed by D, A, Control, and the lowest at the C treatment of 24.167 gr (Fig. 3). While the highest average length of absolute growth was achieved at treatment B that is equal to 4,594 cm then followed by A, Control, C and lowest at treatment D that is equal to 1,656 cmr (Fig. 4).

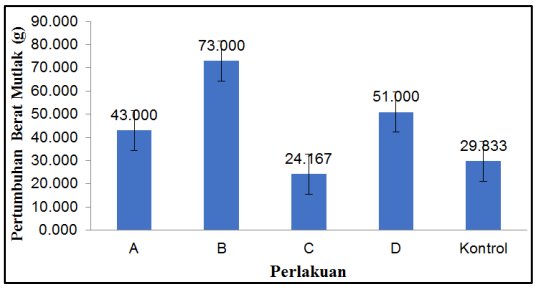

Fig. 3. Average absolute growth in weight of ducker grouper seeds during the maintenance period.

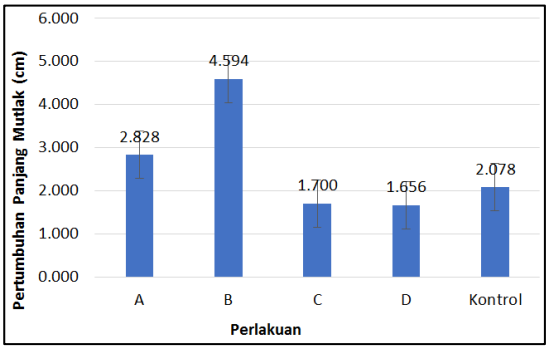

Fig. 4. Average absolute growth in length of ducker grouper seeds during the maintenance period.

Growth is a change in the length or weight of an organism over a period of time. In Fig. 3 and 4, it is seen that the absolute weight growth is higher than the growth of absolute length. Feed B produces absolute growth and absolute growth of high length. This may be due to the addition of *A. acuminata* of 0.2g/100g of feed is the optimum *A. acuminata* content for the ducker grouper so that the feed is digested faster and produces better growth. The results of Maulidin *et al* (2016) showed that the addition of papain enzyme in the artificial feed had a significant effect on all parametric treatment except the survival rate of canna fish seeds (*Channa striata*) where the best growth was obtained in a 3.0% papain/kg feed treatment. The result of statistical test in Table 3 shows that feed treatment with the addition of *A. acuminata* gives significant effect to absolute growth, daily growth rate in length and weight at all treatments (P<0.05). The highest absolute growth in length at treatment B was 4.593 cm followed by treatment A, control, C and the lowest treatment D which was 1.657cm. While the highest absolute growth in weight at treatment B that is equal to 73.000gr followed by treatment D, A, control and the lowest treatment C that is equal to 24,167gr. The highest growth rate was achieved in treatment B of 4,007% followed by treatment of D, A, control and the lowest in treatment C amounted to 1,913%.

Table 3. Notation results for absolute growth and daily growth rate.

Treatment	Growth					
	Absolute Length (cm)		Absolute Weight (gr)		Daily Growth Rate (%)	
A	2.830	a	43.000	a	2.531	ab
B	4.593	b	73.000	b	4.007	c
C	1.697	a	24.167	a	1.913	a
D	1.657	a	51.000	ab	3.493	bc
Control	2.077	a	29.833	a	2.205	ab

Note: The numbers followed by different letters in the same column show significantly different Duncan test, whereas the number followed by the same letter in the same column shows no significant difference.

Survival Rate

During the maintenance period of grouper seed stability in all treatments and 100% control (Table 4).

Table 4 shows the percentage of survival rate of (*C. altivelis*) during the uniform maintenance period (28 days) ie 100% in all feed treatment. This suggests that the addition of *A. acuminata* may result in maximal survival rate for ducker grouper seeds (*C. altivelis*). The results obtained by Hermawan *et al* (2015) the average survival rate of the tiger grouper is the highest in feeding in the form of yolk and tembang of 100% ± 0.00 and the lowest in feeding in the form of pellets and pepetek of 98.89% ± 1,92.

Table 4. Survival rate of grouper fish during the study.

Week	Survival Rate Based on Treatments (%)				
	A	B	C	D	Control
0	100	100	100	100	100
I	100	100	100	100	100
II	100	100	100	100	100
III	100	100	100	100	100
1V	100	100	100	100	100

A survival rate of 100% in all treatments and not affected by the addition of *A. acuminata* up to a dose of 0.4 g/100g of feed indicates that this dose has not caused toxicity and is still tolerable by ducker grouper seeds. The same results were also obtained by Hamzah *et al.* (2012) with the addition of selenometionin 0 to 16 mg Se / kg doses of juvenile groupers of duck that is 100%. Research studies conducted by (Dangeubun, 2012) on the utilization of *A. acuminata* and *A. scholaris* showed that *A. acuminata* contain active compounds that are antibacterial. The results of this study are strongly suspected that *A.acuminata* plants have potential as immunostimulants.

Water Quality

The results of water quality parameter measurements during the study period are shown in Table 5 below.

Table 5. Water quality parameters during maintenance period.

No	Parameters	Range
1	Suhu (°C)	26 - 29
2	Salinitas (ppt)	32 - 33
3	pH	7,44 - 8,93
4	Dissolved oxygen (ppm)	5,13 - 5,38

The treated temperature is considered to be good for the growth of grouper fish in accordance with Tiskiantoro (2006) who reported that the optimal temperature for the cultivation of grouper duck *C. altivelis* is 27-32°C. While water temperatures were recorded during the study reported by Dody and Dinawanti (2016), which ranged from 27 to 30°C. The results of Hermawan *et al.* (2015) showed that water temperature during the maintenance of tiger grouper (*Epinephelus fuscoguttatus*) ranged from 28 - 33°C. The ideal temperature for tiger grouper fish maintenance ranged from 27-28,5°C (Sari *et al.* 2009) and 25 - 32°C (DKP 2007).

The range of salinity value of waters during maintenance period is considered good enough for duck grouper growth according to Kordi & Ghufran (2004) opinion that optimal salinity for ducker grouper growth in floating net cages ranges from 33-35 ppt. Mudiarti and Zainuddin (2016) reported that the optimal value of salinity to length is salinity of 31 ppt, then began to decrease at salinity 35 ppt. The best result at 31 ppt salinity is the isoosmotic media salinity for the duck grouper, where the greatest energy is used to support its growth.

Similarly, the pH of water can affect the level of water fertility because it affects the life of microorganisms, including phytoplankton and zooplankton. This water pH range corresponds to the pH required by ducker grouper for its growth (Kordi & Ghufran, 2004) because fish cultivation will work well in water with a pH of 6.5-9.0, while the highest fish appetite is obtained at pH 7.5-8.5.

Dwiyanti (2006) also reported that the duck grouper that was kept sensitive to pH changes and liked a pH value of about 7.0 to 8.5. Dody and Dinawanti (2016) obtained a pH value of 7.04 - 7.27.

Dissolved Oxygen (DO) is a limiting factor for aquatic biota. Water biota including ducker grouper will live normally if the availability of oxygen in the water is sufficient. The content of oxygen dissolved in water is affected by the temperature at which dissolved oxygen is inversely proportional to temperature. Dissolved oxygen (DO) in the study obtained by Hermawan *et al.* (2015) ranged from 4.9 to 6.23mg/L. The optimum dissolved oxygen content that supports the maintenance of grouper fish ranges from 4-8 mg/L (DKP 2007). This means that dissolved oxygen during maintenance (5.13 - 5.38 ppm) is optimal to support the survival and growth of ducker grouper.

Conclusions

From this research it can be concluded that feeding with the addition of *A. acuminata* 0.2gr can increase the growth of best grouper of duck. Therefore, in making a feed formulation for ducker grouper (*C. altive*lis) seedlings are expected to add 0.2 grams of *A. acuminata.*

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