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Utilization of Fermented Mangrove Propagules (*Rhizophora mucronata*) as Feeding Material for Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The research was carried out at the Laboratory of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran for culture and observation of fish and at the Poultry Nutrition Laboratory, Faculty of Animal Husbandry, Padjadjaran University on the fermentation process and the manufacture of test feeding. The purpose of this study was to determine the optimal percentage of *Aspergillus niger* fermented mangrove propagules meal in artificial feeding on the growth rate of nile tilapia. Tilapia fry used were 5-7 cm in size with an average weight of 4.97 ± 0.2 g. The study used a Completely Random Design (CRD) with five treatments and three repetition. Feeding is formulated on the percentage of fermented mangrove propagules meal use, that is 0; 2,5; 5; 7.5; and 10%. The main parameters observed were nutritional quality of fermented products, daily growth rate, feeding efficiency and survival. The data obtained were analyzed using F Test and continued with Duncan's Test if there were differences between treatments. The results showed that the addition of mangrove propagules fermented about 10% level. It gives results that there is no difference between the daily growth rate, feeding efficiency and survival whic is the same as those in tilapia. This is shows that mangrove propagulees can be used in fish feeding without causing negative effects on the growth of nile tilapia fry.

***Keywords*:** Mangrove Propagulee, Fermentation, Growth, Feeding Efficiency, Tilapia, *Rhizophora mucronata, Oreochromis niloticus*

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1. INTRODUCTION

Tilapia is a fish whose habitat is in fresh water but can also live in a wide range of salinity or euryhaline and able to live in deep and wide waters such as brackish water ponds to sea cages (floating net cages) up to 30-35 ppt salinity. Tilapia is one of the main commodities that contribute to increased aquaculture production. The production of Tilapia in 2014 amounted to 999,695 tons, an increase in 2017 of 1.15 million tons or an increase of 3.6 percent from 2016 which reached 1.14 million tons [1].

Indonesia's coastal area has a high biodiversity. About 202 species of mangrove species have been identified and thrived, one of them is *Rhizophora mucronata*. Mangrove fruit that has undergone germination is called propaguleee. Mangrove fruit is easily found along the coast. Cultivation activities are inseparable from the provision of quality food. Feeding production should be based on consideration of fish nutritional needs, quality of raw materials and economic value. Currently many alternative feeding ingredients have been developed to reduce imported materials. These ingredients must be abundant in availability and the price is relatively cheap but on the other hand alternative ingredients usually have a deficiency in their nutritional content.

Mangrove propagule *R. mucronata* contains protein of 2.93%, crude fat of 0.21%, ash of 2.09%, moisture content of 15.25% and crude fiber of 25.98%. One way to reduce crude fiber is by biological treatment through a fermentation process using microorganisms that are able to degradating the components of crude fibers such as mold *Aspergillus niger*. [2] states that *A.niger* can produce extracellular enzymes such as amylase, cellulase and hemicellulase which can decompose crude fiber into simpler compounds (monosaccharides). The result also showed a marine-derived fungus NIOCC #3 (*Aspergillus niger*) isolated from such substrates produced a thermostable (at 55 °C) xylanase active at pH 8.5 and cellulase-free, so can be use to gradual breakdown of wood structure. The use of mangrove propagule as an alternative feeding that has improved nutritional value through fermentation techniques is expected to meet the needs of non-protein energy sources and can reduce the use of imported materials. Optimizing the use of local feeding ingredients is expected to overcome the problem of feeding which is part of the cultivation series.This study aims to determine the addition level of mangrove propagules in artificial feeding which can produce the highest growth, feeding efficiency and survival in nile tilapia fry.

2. METHODS

The study was conducted at the Laboratory of Non Ruminant Nutrition and Animal Food Industry, Faculty of Animal Husbandry for fermentation processes, feeding manufacturing, and Aquaculture Laboratory, Faculty of Fisheries and Marine Sciences for fish farming activities

2. 1. Materials and Tools of Research

The materials used in this study were 5-7 cm nirvana strain tilapia with an average weight of 4.97 2 0.2 g as many as 300 tails, mangrove propagules, *A.niger* isolates, and test feeding formulated with fish meal, soybean meal, corn meal, fine bran, tapioca meal, fish oil and premix. The tools used are aquariums with a size of 20 × 30 × 40 cm^3, aeration blowers, thermometers, DO meters, pH meters, fiber tanks, heaters, gauze shavings, and brand AND EK-120G of digital scales.

2. 2. Research Methods

The research was carried out by using a Completely Random Design method (CRD) with 5 treatments and 3 repetitions. The treatment given was the difference in the proportion of fermented mangrove propagule meal in feeding, it is (0%, 2.5%, 5%, 7.5%, 10%).

2. 3. Research Procedurs

2. 3. 1. Preparation Stage

Mangrove propagule fermentation preparation began with the stages of making the media to selectively extract Touge Agar (ETA), making *A. niger* solid inoculums and Total Plate Count (TPC). After preparation, 4.9 kg of propagules are cut to size ±3 cm, then washed thoroughly and steamed using autoclave for 60 minutes at 120 °C. After cold, *A. niger* inoculum is mixed with mangrove propagule pieces at a dose of 2 grams/kg. and incubated for 6 days. Furthermore, fermented propagules were dried using sunlight, and their nutritional content was analyzed and used using proximate analysis.

Preparation of test feeding in accordance with iso protein feeding formulations that have been determined using percent square method (Table 1).

Table 1. Feed Formulations Used During the Research

Materials	Treatment (%)				
	A	B	C	D	E
Fish meal	33,00	33,42	33,81	34,20	34,58
Soybean meal	33,00	33,42	33,81	34,20	34,58
Corn meal	12,00	10,33	8,69	7,05	5,42
Fine bran	12,00	10,33	8,69	7,05	5,42
Fermented propagule meal (FPM)	0	2,5	5	7,5	10
Tapioca meal	7	7	7	7	7
Fish oil	2	2	2	2	2
Top mix	1	1	1	1	1
Total	100	100	100	100	100

2. 3. 2. Research Implementation

The fish was acclimatized for 7 days and given a test feeding to be able to adjust to the feeding during the study. The first day of sampling is to find out the initial weight of the fish and determine the dose of feeding given. The fish was cultured in aquarium as many as 20 fish / aquarium. Feeding is carried out three times a day, with a dose of 5% of fish biomass. The number of dead fish is recorded every day. Weighing weights and measurements are carried out every 7 days, while water quality (DO, pH and temperature) were measured every 10 days.

2. 3. 3. Observation Parameters

Parameters observed in this study are as follows:

1) The nutritional value of propagules from fermentation is carried out using proximate analysis. Parameters observed included crude protein, crude fiber, fat, ash, water content and extract material without nitrogen (BETN).
2) Daily growth rate (DGR) of fish can be calculated using the formula [3], as follows:

$$\text{DGR (\%)} = \left[\sqrt[n]{\frac{Wt}{Wo}} - 1\right] \text{x } 100\%$$

Explanation
DGR = Daily growth rate (%)
Wt = Final average weight (g)
Wo = Initial average weight (g)
t = Maintenance period (days)

3) Survival Rate

The survival rate is calculated by the equation [4], as follows:

$$SR = \frac{Nt}{No} \text{x } 100\%$$

Note :
SR = survival rate (%)
Nt = Fish number at the end of the research (individu)
No = Fish number at the beginning of the research (individu)

4) Feeding efficiency

Feeding eficiency can be counted with the equation as follows [5]:

$$EP = \frac{(Wt + D) - Wo}{JKP} x\ 100\%$$

Note :
EP = Feeding efficiency (%)
Wt = Fish biomass at the end of maintenance period (g)
Wo = Fish biomass at the beginning of maintenance period (g)
D = Fish weight that died during the study (g)
JKP = The amount of food consumed during the research (g)

5) Water Quality

Water quality parameters measured included temperature, Dissolved Oxygen (DO), and acidity (pH) which was carried out at the beginning of the study and every 10 days in each aquarium.

2. 3. 4. Data Analysis

Fermented products were analyzed descriptively while the effect of treatment given on daily growth rate, feeding efficiency and survival in test fish were analyzed statistically using analysis of variance with F test at 5% test interval.

3. RESULTS AND DISCUSSION

3. 1. Nutritional Value of Fermented Products

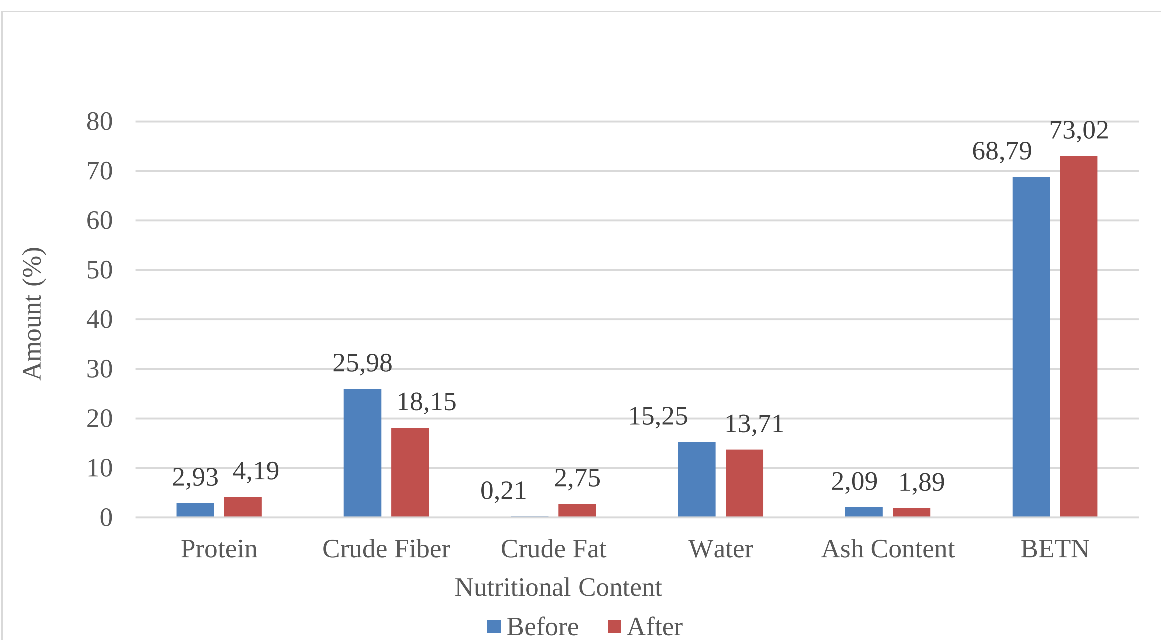

Figure 1. Mangrove Propagules Nutrition Content, Before and After Fermentation

Fermentation is a process that occurs through the work of microorganisms or enzymes to convert complex organic materials such as proteins, carbohydrates and fats into simpler molecules. The enzyme produced by *A. niger* can help digestion such as cellulase, lipase, amylase, protease, phytase, β-gluconase, and xylanase [6]. Microbial enzymes are capable of degrading toxic chemical compounds of industrial and domestic wastes either via degradation or conversion.

The results of fermentation can cause an improvement in the nutritional value of the product and the properties of the basic ingredients such as increasing digestibility, causing the taste and aroma are preferred. Fermentation carried out for 7 days showed a change in the nutritional value of propagule fermentation when fermented with *A. niger*.

Decrease in crude fiber from 25.98% to 18.15% can occur because *A. niger* is a type of cellulotic yeast, which can secrete cellulase enzymes to degrade cellulose into simpler components, such as cellobiose, and glucose. Decrease in value indicates that there has been an overhaul of crude fiber into glucose. Increased protein levels occur due to the work of microbes and the addition of protein contained in the microbial cells themselves. The increase of protein in the fermentation process could be caused by an increase in the amount of yeast mass. Fermentation to produce single cell protein have application in animal nutrition, including fish foodstuff area as aroma and vitamin carrier, and to improve the nutritive value of feed [7].

The decrease occurred at the fermented ash content of mangrove propagules. The ash content comes from the residual combustion of feeding ingredients, which contain no organic matter. The decrease in ash content is influenced by the use of minerals to maintain the life of microorganisms even in small amounts. Fermentation can also reduce water content by 10.09%. Time and temperature can affect the value of water content (moisture) of fermented product [8]. During fermentation process, the moisture content of substrate will decreased, due to the activity of mold metabolism and releasing heat that made the fermentation temperature increased. Increasing temperature was followed by evaporation of the water content in substate.

The similar result was found in fermentation using silage. The association of *L. fermentum* with L. *buchneri* increased the aerobic stability of silage with high humidity [9]. Crude fat has increased from 0.21% to 2.75% after fermentation. The increase could be as a result of extensive break down of large fat molecule to simpler fatty acid units due to the high activity ofthe lipolytic enzymes which could have resulted in fat increase [10].

The BETN content of a feeding ingredient is highly dependent on other components, such as ash, crude protein, crude fiber and crude fat. Calculation of BETN content includes a 100% reduction in the total amount of crude protein content, crude fat, crude fiber and ash.

The nutritional improvement by using microbes are well documented. Several species of Aspergillus genus have been used to ferment the feedstuff like *A. oryzae* [11,12], *A. usamii* [13], *A. awamori* [14], *A. niger* [15]. Fermentation with Aspergilli almost completely eliminates phytate, resulting in a protein source for feed with highly available phosphorus as well as zinc [16].

3. 2. Daily Growth Rate

Fish growth is defined as changes in fish weight, size and volume as time increases. The growth of tilapia for 42 days of maintenance can be seen by the average weight gain per week in each treatment (Figure 2).

Increased body weight of fish indicates that fish are experiencing growth. This shows that the feeding provided can meet the nutritional needs of tilapia fry during maintenance period. Growth will occur if there is residual energy in the feeding after metabolic needs and body maintenance are met. The growth rate of tilapia getting results that were not significantly different in each of these treatments showed that all percentages of the use of mangrove propagules meal could still be received by fish. The daily growth rate is in the range of 1.12% -1.34%. The acceptance of fish to feeding can be due to the level of palatability that is good in terms of appearance, flavour and texture which is quite favored by fish. The fermentation process can cause changes in flavour and taste and reduce antinutrients in raw materials so that fish can receive feeding added by propagules. In addition, feeding containing propagules has the same physical properties as commercial feeding which has increasingly dark colors with the addition of propagules, has a hard texture and is quite difficult to break so it is not easily breakdown and has a high resistance in water. Artificial feeding can be produced which is preferred by fish, nutritional qualified, not easily broke down in water, and have no toxic ingredient for fish [17].

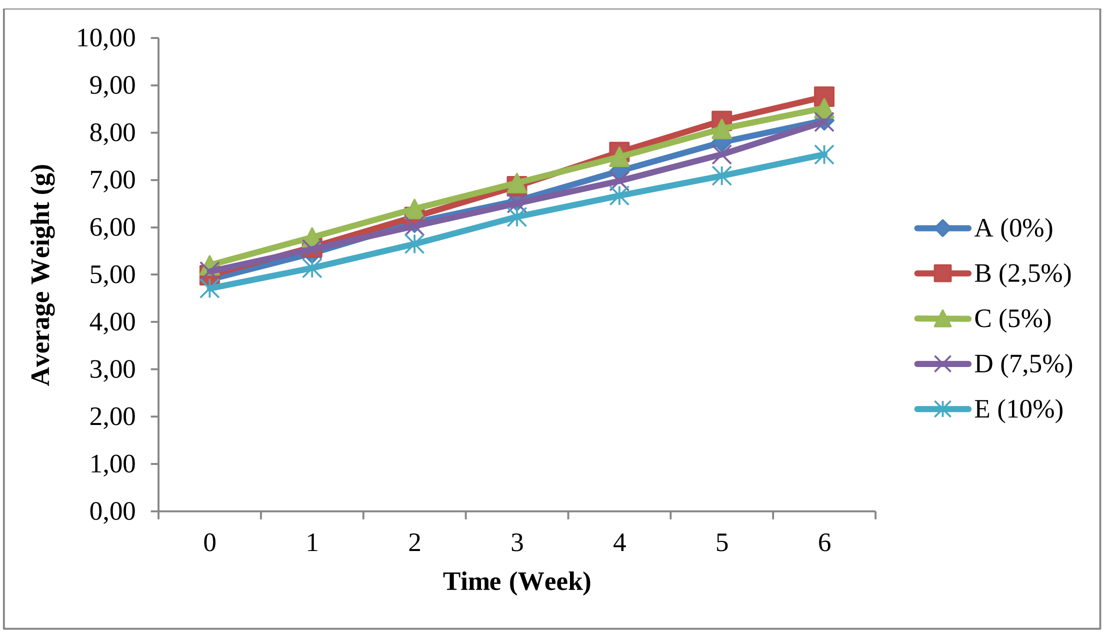

Figure 2. Average Weight of Tilapia Fry During the Study

The positive response of feed can be seen by increasing the amount of feeding consumption every week. The range of feeding consumption value is 297.90-335.06 g during the maintenance period. If the amount of feeding is consumed and digested optimally well by fish, it will produce a high rate of fish growth. The higher the growth rate shows the better the feeding is used for growth. This value is influenced by the content of artificial feeding, especially in protein and crude fiber. The crude protein content contained in all treatment feedings is in the range needed for the growth of tilapia seeds, it is 31.47-29.61%. Protein content in artificial feeding for black tilapia fry is in the range of 20-25% [18].

Crude fiber in feeding A (FPM 0%) and B (FPM 2.5%) respectively 5.96% and 6.44% while feeding C, D and E have crude fiber content of more than 8%, which is 8, 12%, 8.70% and 9.76%. The content of crude fiber more than 8% will reduce the quality of fish feeding, and decrease the consumption [19]. This can cause a decrease in the rate of fish growth in the treatment of C, D and E.

In general, in each treatment has a nutrient value that is not much different and relatively the same, resulting in a daily growth rate that is not significantly different. This result is consistent with that obtained by [20] that the addition of fermented husks up to 10% does not affect the daily growth rate but can reduce the digestibility value in fish due to high crude fiber and suspected antinutrient factor. The results of [21] show that the use of fermented mango (*Mangifera indica*) were similar (P>0.05) in fish feed diet, whereas the administration *Pistia stratiotes* results in a growth rate daily between 0.072-0.104% [22]. This growth rate is quite good considering the value growth rate of at least 1% [23].

3. 3. Feeding Efficiency

Feeding efficiency is an illustration of the feeding given utilization so as to improve fish growth. The greater the value of feeding efficiency, the more efficient the fish utilizes the food consumed for its growth. Addition of mangrove propagule meal of 2.5% has an average value of feeding efficiency of 22.44%, treatment E of 20.87%, treatment A and D respectively 20.58% and 19.53% and treatment C is 19.20% (Figure 3). The results of the analysis of variance in the test interval (5%) showed that the use of FPM up to 10% had no significant effect on feeding efficiency.

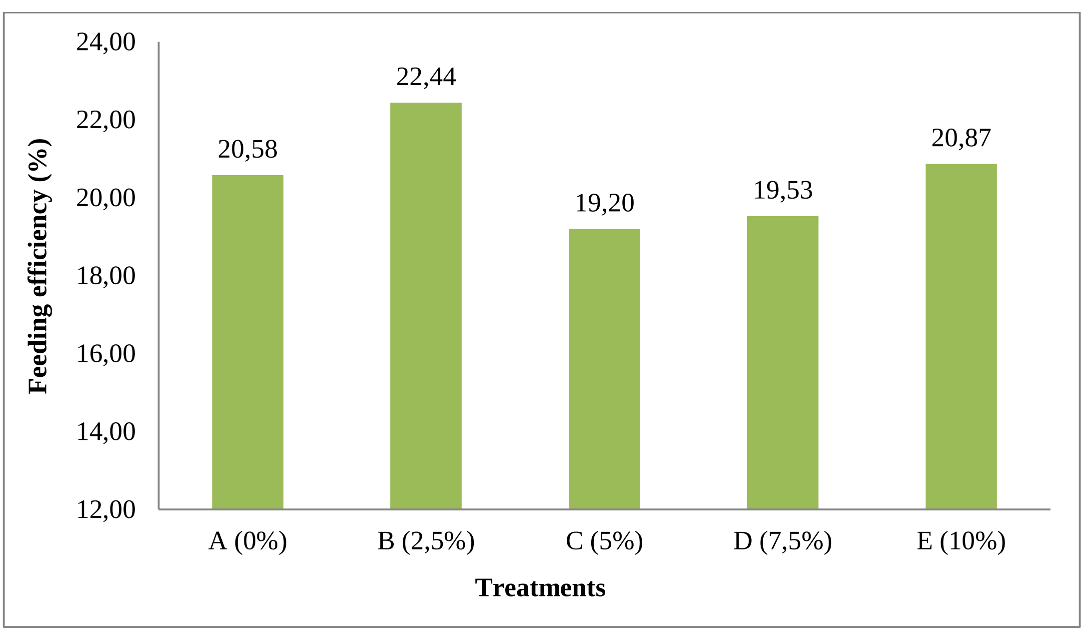

Figure 3. Feeding Efficiency of Tilapia Fry

This shows that all treatment feeding given by fermented propagule is still acceptable and favored by fish. Fermentation results are easily digested, have an pleasant taste and aroma to be preferred [24]. Feeding which was given additional mangrove propagule meal showed no difference with the control feeding so that it could be used as an alternative feeding material to the level of 10%.

Value of efficiency affects the rate of growth produced, the higher the value of feeding efficiency will be directly proportional to the rate of growth. This is evident, because treatment B (FPM 2.5%) has the highest daily growth rate compared to other treatments. Different things happened in treatment E where even though feeding efficiency was high compared to other treatments but had the lowest daily growth rate. It is suspected that the food consumed by fish is used more for metabolic processes and body maintenance, but not for growth. Feeding can be said to be good if the value of feed efficiency is more than 50% or even close to 100%. The results of [25] showed that the efficiency of tilapia feeding by using more than 49.74%. In general, the average value of feeding efficiency in this study was low, in the range of 19.20-22.44%. *A. niger* was increase the nutritional quality of rapeseed meal as a diet for nile tilapia [26]. A high protein energy ratio can affect low feeding efficiency. The high protein energy ratio shows that non-protein energy in feeding is very high in energy. If the energy in feeding is higher than the fish's needs, the fish will quickly fill up before it can utilize protein and other components in feeding. Protein value of energy ratio for optimal growth of fish ranges from 8-9 kcal / g [27], whereas the value of the protein ratio of the treatment energy is more than 9 kcal / g or in the range of 12.85-13.30 kcal.

The use of fermented mangrove propagule in feeding even though it produces low feeding efficiency values (19.20–22.44%) but has a good impact on production costs. In terms of price, the feeding added by propagule has a relatively cheaper price compared to control feeding (Table 2). This further supports the use of mangrove propagule as an alternative feeding because it is in accordance with [26] regarding the requirements that need to be considered in utilizing alternative feeding ingredients, including nutrient content, palatability, price and availability of feeding ingredients

Table 2. Price of Tilapia Feeding

Ingredient	Price per Kg (Rp)	Price of Feeding Treatment (Kg)				
		A	B	C	D	E
Fish meal	10.200	3366	3408,84	3448,62	3488,4	3527,16
Soybean meal	8.800	2904	2940,96	2975,28	3009,6	3043,04
Corn meal	5.000	600	516,5	434,5	352,5	271
Fine Bran	3.500	420	361,55	304,15	246,75	189,7
Fermented Propagules Meal	0	0	0	0	0	0
Tapioka Meal	8.400	558	558	558	558	558
Top Mix	18.800	376	376	376	376	376
Fish Oil	12.500	125	125	125	125	125
		8379	8286,85	8221,55	8156,25	8089,9

Sources: Ingredient Feeding Store CV. Missouri 2016

3. 4. Survival

The degree of survival can be known by dividing the number of fish that lived at the end of the study with the number of fish at the beginning of the study. The results showed that each treatment had a high survival value (Figure 4). The results of analysis of variance in the 5% test interval showed that the addition of mangrove propagules was not significantly different from the survival of nile tilapia fry.

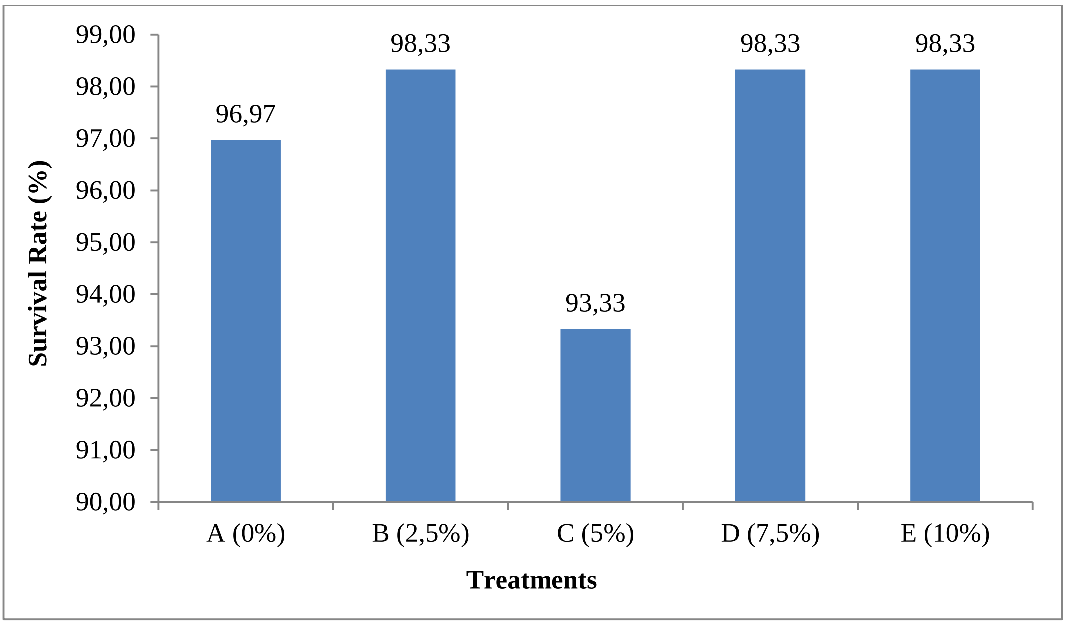

Figure 4. Survival of Tilapia Fry

Table 3. Range of Water Quality Values During the Research

Treament	Water Quality Parameter		
	Temperature (°C)	pH	Dissolved Oxygen (ppm)
A	25-26	7,53-7,87	4,24-5,40
B	25-28	7,49-7,80	3,50-7,80
C	25-29	7,41-8,00	3,40-7,80
D	25-29	7,41-7,89	3,28-5,50
E	25-28	7,6-7,92	3,58-7,00
Standard SNI (2009)	25-32	6,5-8,5	≥3

Based on these results, the fish can adapt to the feeding that is added with mangrove propagules well, also with the quality of water given by mangrove propagule feeding. Feeding containing mangrove propagules up to 10% does not have a negative effect on the survival of tilapia. The high survival of tilapia fry shows that feeding can be used well to grow and survive. If the quality and quantity of feeding given is sufficient for the nutritional needs of the fish, the fish body's endurance system will be stronger to dispel foreign objects or bacteria that harm the body. Water quality is one of the factors that influence survival. In general, the quality of water in the culture medium in this study is in the recommended range for nile tilapia [28-31] (Table 3).

4. CONCLUSIONS

1) The addition of 2.5% fermented propagule meal gave the highest daily growth rate of 1.34%, feed efficiency 22.44% and survival of 98.33%
2) Addition of mangrove propagule meal from *A. niger* fermentation in artificial feeding of nile tilapia fry can be used and does not give a negative influence on the growth rate, feeding efficiency and survival of tilapia fry to the level of 10%.

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