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Utilization of Fermented Coconut Epidermis Using Oncom Mushroom (*Neurospora sitophila*) on the Growth Performance of Red Tilapia (*Oreochromis niloticus*) Fingerlings

Fauziyah Nur Afisha [a*] and Kiki Haetami [a]

[a] *Fisheries Department, Faculty of Fisheries and Marine Science, Padjadjaran University, JL. Raya Bandung-Sumedang, Hegarmanah Jatinangor, Sumedang 45363, West Java, Indonesia.*

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study aims to analyze the effect of feed based on coconut husk waste fermented *Neurospora sitophila* on the growth of red tilapia, and to determine the optimal dose of coconut husk waste. This research was carried out in May – June 2021, at the Aquaculture Laboratory Building IV, Faculty of Fisheries and Marine Sciences, Padjadjaran University Bandung, West Java45363 Indonesia. This study used a completely randomized design with four treatments and four replications. Treatment A (Control), Treatment B (feed containing 25% coconut husk filler), treatment C (feed containing 50% coconut husk filler), and treatment D (feed containing 75% coconut husk filler). Parameters observed were Specific Growth Rate, Feed Conversion Ratio, Survival Rate, Feed characteristics and water quality (Temperature, pH, DO, Ammonia). The results showed that treatment C(feed containing 50% of coconut husk filler) gave significantly

**Corresponding author: Email: fauziyah18001@mail.unpad.ac.id, fauziahnurafisha@gmail.com;*

different results in the parameters of SGR and FCR, but not significantly different in survival rates. Water quality parameter values are in good condition for the growth and survival of red tilapia. The highest SGR value in treatment C was 1.26%. The lowest FCR value was in treatment C of 1.90. The most optimal dose of fermented coconut husk for the growth of red tilapia is treatment C.

Keywords: Red Tilapia (Oreochromis niloticus); coconut husk; growth; survival.

1. INTRODUCTION

Tilapia fish (*Oreochromis niloticus*) in Indonesia is one of the freshwater fish that has high economic value and is an important commodity in the world's freshwater fish business [1]. The advantages of tilapia compared to other consumption fish are that tilapia can grow fast only with low-protein feed, spawn all year round, are omnivorous, have thick flesh, and the taste of meat similar to red snapper [2]. Red tilapia is widely cultivated because it is easy to maintain and breed and has a high tolerance of changes in water quality. Cultivating tilapia, especially in enlargement activities, has the most important factor, namely the availability of feed in sufficient quantities.Fish feed is one of the most important factors in a fish farming business. Feed must contain all the necessary nutrients such as carbohydrates, fats, proteins, minerals and vitamins and essential amino acids in sufficient and balanced quantities. About 50% or even more of production costs is determined by feed [3].

The weakness in making fish feed so far is not optimizing the potential of local feed [4]. The increasing demand for feed and the price of refined rice bran causes counterfeiting of rice bran by mixing it with husks, so the quality will decrease due to the presence of lignin which is anti-nutritional [5]. Other antinutrients present in bran apart from lignin content are phytates which are part of crude fiber.

Utilization of alternative materials as raw feed materials is one solution to this problem. Raw feed materials must have good nutritional value, be easy to obtain, easy to process, not contain poisons, relatively cheap prices, and not constitute human staple food [6]. Coconut husk flour can be used as an alternative raw material for feed up to 15% [7]. Coconut husk flour also contains organic acids needed by freshwater fish such as linoleic acid to help growth and certain hormone precursors [8].

Coconut husk is the inner skin of coconuts whose waste is found in many traditional markets.Coconut husk can be used as a complementary ingredient for feed raw materials because it can be a source of energy. The content of substances found in coconut husk consists of 7.75% protein, 26.48% fat, 15.19% crude fiber, 2.79% ash, 0.03% Ca, and 0.21% P based on results of analysis at the Animal Feed and Nutrition Laboratory, Faculty of Animal Husbandry Unpad 2006. The presence of coconut shell waste in the feed formulation is predicted to have a good response by red tilapia because it contains a metabolic energy of 3328 kcal/kg. The high content of fatty acids in the feed causes the feed to be difficult to digest [9], High crude fiber content will reduce the metabolic energy of feed ingredients, due to a decrease in the digestibility of the material so that nutrient absorption is notoptimal [10]. One effort to overcome the high content of crude fiber and fat is by fermentation.

Fermentation is a process of chemical change in an organic substrate through the activity of enzymes produced by microorganisms [11]. Fermentation aims to increase the crude protein content and reduce the crude fiber of the substrate. The optimum process of fermentation depends on the type of organism [12]. The fermentation process requires a certain percentage of inoculum and a certain fermentation time, the more doses of inoculum used, the more material will be broken down.Natural fermentation technology from coconut husk waste material with oncom mushrooms is one of the processing technique efforts. Utilization of palawija and coconut commodities (especially coconut husk waste) can still be increased to the maximum extent possible through fermentation technology, and can be used as an alternative feed ingredient and supplement in the supply of feed. The results of the Proximate Test for the Food Substance Composition of Coconut Epidermis are fermented based on the analysisLaboratory of Ruminant Animal Nutrition and Animal Feed Chemistry, Faculty of Animal Husbandry Unpad.

Fermented fiber materials are usually fermented using microorganisms in the form of mold, because this type of fungus is capable of breaking down crude fiber. One of the molds that has high cellulolytic activity and is often used in the fermentation of fiber materials isNeurospora sithopila or better known as oncom mushrooms [13]. This mold also has high lipolytic activity which hydrolyzes triglycerides into free fatty acidsand the ability to decompose protein into amino acids [14].

*Neurospora sitophila*is a mold that belongs to the subdivision Eumycophyta, class Ascomycetes, order Sphriales and family Sordoriaceae.Molds of the Genus Neurospora have long been known and have been studied since 1843. Species *Neurospora Sitophila* has been widely used in laboratory research since 1941. This mold can grow well with growth temperatures between 20–30ºC under aerobic conditions [15]. This mold grows on media containing cellulose and produces the enzyme β-glucosidase. This mushroom also has high lipolytic activity which hydrolyzes triglycerides into free fatty acids. Fermentation use *Neurospora sitophila* provides another advantage, namely the increased content of carotene produced from conidia which are orange in color. Increasing the nutritional value of the substrate allows fish to digest fermented products better. The advantage of fermentation by the carotenegenic mold Neurospora when compared to sources from plants to produce β-carotene is that it is more efficient in terms of time, place and cost because it does not require time to grow plants, large and heavy equipment and large areas. This fungal fermentation process makes food reddish yellow [12].

Based on this discussion, in this research it is necessary to examine the effect of adding fermented coconut husk by *Neurospora sitophila* asfiller on red tilapia feed. The right dose in the addition of fermented coconut husk by *Neurospora sitophila*is expected to promote good growth in red tilapia.

2. MATERIALS AND METHODS

Research on the utilization of fermented coconut husk using oncom mushrooms (*Neurospora sitophila*) on the growth performance of red tilapia (*Oreochromis niloticus*) with parameters of growth in length, weight, SR (Survival Rate), SGR (Spesific Growth Rate), FCR (Feed Convertion Ratio), and water quality was carried out at the Aquaculture Laboratory Building IV, Faculty of Fisheries and Marine Sciences, Padjadjaran University, Bandung, West Java, 45363Indonesia in May to June 2021.

The tools and materials used in this research were aquariums with a size of 30 x 40 x 40 cm^3 as many as 16 pieces, 1 unit fiber tub with a size of 70 x 70 x 70 cm^3, aeratorpump atman hp 12000, hose, aeration hose, aeration stone, scoop, digital scale with an accuracy of 0.1gr, tray, plastic zip lock,pH meter, Dissolved Oxygen meter Lutron DO-5510, Ammonia test kit merk prodac, Red Tilapia (*Oreochromis niloticus*) measuring 7-12 cmcmas 160 tails, Oncom Mushroom (*Neurospora sitophila*), coconut husk.

The method used in this research is a laboratory experimental method, using a completely randomized design (CRD) consisting of four treatments and four replications. The treatment given was basal feed containing fermented coconut husk filler*Neurosporasitophila*, with the following combination or composition: (A) Basal feed with 100% bran filler, (B) Feed containing 25% coconut husk filler, (C) Feed containing 50% coconut husk filler, (D) Feed containing 75% coconut husk filler.

Parameters observed in this research include absolute body weight growth, survival and water quality. Observation of weight growth and water quality was observed once every 7 day for 42 day. This research did not use recirculation, so the water in the aquarium was cleaned by a third every day. The parameters observed were the increase in the weight of red tilapia as the main data. The water quality measured was temperature, degree of acidity (pH), dissolved oxygen content (DO), and ammonia content $(NH)._3$).

2.1 Procedure

2.1.1 Preparation of rearing containers

Preparation of the container begins with cleaning the aquariumwith a size of 30 x 40 x 40 cm^3 from dirt or scale that sticks to the walls and bottom using soap. After the aquarium is clean, check for leaks and then dry it in the sun. Furthermore, the tanks and aquariums were disinfected using potassium permanganate solution for about 24 hours, then filled with water with a volume of 45 liters and given sufficient aeration. The aquarium can be used after one day of water filling.

Table 1. Food substance composition of coconut epidermis

No.	Content	Coconut Epidermis (2006)	Fermented Coconut Epidermis (2019)
1.	Protein	7,7	11,32
2.	Fat	26,48	18,47
3.	Coarse Fiber	15,19	11,79
4.	Ash Rate	2,79	2,16

2.1.2 Preparation of test feed

The feed formulation is prepared in advance. Then do the fermentation on the coconut husk by*Neurosporasitophila.* After that, the fermented coconut husk is ground into flour, put it into the ration formulation that was previously designed. Each feed ingredient is weighed according to the formulation, then mixed and pelletized by the machine*pelleting*, the last is drying using the oven.

2.1.3 Maintenance of test fish

The test fish to be used in this research were red tilapia with a length of 7 - 12 cm and weighing between 9-16 g as many as 10 in an aquarium containing 45 liters of water volume, with a density of 1 fish / 4,5 L. After acclimation, the tilapia were stocked in an aquarium. Furthermore, tilapia are reared by feeding as much as 3% of the fish's body weight with a frequency of 2 times, namely at 08.00 am and 03.00 pm

2.1.4 Research implementation

The research was carried out for 42 days. Observations on fish growth, survival and water quality (pH, temperature, ammonia and DO) were carried out every seven days. Aquarium siphoning was carried out daily and the water was changed periodically during the observation period. The amount of feed given is adjusted to the total weight of fish in each maintenance container. The resulting red tilapia seed weight data will be used to measure the feed weight for each period.

2.2 Observation

2.2.1 Survival rate

The pass percentage is the ratio between the number of test fish that lived at the end of the study and the initial fish in one period in one population [16]. Survival orsurvival rate (SR) is calculated to determine the mortality rate of the test fish during the study, survival can be calculated based on formula [17]:

$$SR = \left(\frac{Nt}{No}\right) \times 100\%$$

Explanation:

SR = Survival rate
N_t = Number of fish at the end of the study
N_0 = Number of fish at the beginning of the study

Table 2. Preparation of test feed

Material	Treatment feed composition (g)			
	A	B	C	D
Fish Flour	13,58	13,58	13,58	13,58
Soybean Flour	13,58	13,58	13,58	13,58
Bran	69,82	52,36	34,91	17,45
Fermented Coconut Epidermis *)	0	17,45	34,91	52,79
Tapioca	1	1	1	1
Premix	2	2	2	2
Total	100	100	100	100
Protein (%)	25,77	25,77	25,77	25,77
Fat (%)	9,72	10,85	11,98	13,11
Fiber (%)	6,53	7,19	7,86	8,52
DE (kcal/kg)**)	2667,69	3063,46	3459,22	3854,99
DEP/P	103,50	118,86	134,21	149,57

*Is:*Proximate Analysis and Calculation Results **) DE (Digestible Energy) = 70% x GE (Gross Energy)*

2.2.2 Feed convertion ratio

Feed conversion is calculated using theformula [18]:

$$FCR = \frac{F}{(Wt+D)-Wo}$$

Explanation:

FCR = *Feed Convertion Ratio*
W_o = Weight of test fish at the beginning of the research(g)
Wt = Weight of test fish at the end of research(g)
D = Weight of dead fish during rearing (g)
F = Amount of feed given(g)

2.2.3 Spesific growth rate

Observation of daily growth rate was carried out once every 7 days during the study. The formula used in calculating the Daily Growth Rate (LPH) uses the formula [19]:

$$\alpha = \frac{(\ln Wt - \ln W0)}{t_1 - t_0} \times 100\%$$

Explanation:

α = Daily Growth Rate of weights
wt = average weight at the end of treatment (day t)
wo = initial average weight of treatment (day 0)

2.2.4 Water quality

Good water quality plays an important role in efforts to improve the quality of fish growth. One of the criteria for good water quality is according to the needs of each type of fish. Fish will live healthy and show good growth performance in an environment with appropriate water quality [20]. Observation of water quality consists of temperature, pH, Dissolved Oxygen, and ammonia. Measurement of water quality in this study was carried out every observation period.

Temperature, pH and DO testing procedures:

1. The pH meter and DO meter are calibrated first
2. Then it is turned on and immersed in the test water, wait until the temperature, pH and DO values appear
3. Then neutralized again

Ammonia testing procedure:

Put 10 ml of water from the maintenance aquarium into the tube, add reagent 1, shake for 5 seconds, add reagent 2, shake for 5 seconds then add reagent 3, wait for 5 minutes

2.2.5 Data analysis

Data on water quality and feed characteristics were analyzed descriptively. SR data (*Survival Rate*), SGR (*Spesific Growth Rate*), and FCR (*Feed Convertion Ratio*) obtained then analyzed the F-test variance at 5% level. If in the analysis of variance there is a significant difference (P <0.05), then the test is continued with Duncan's test to determine differences between treatments. The following is the F-test formula:

$$F_{hit} = \frac{KTP}{KTG}$$

H_0 is homogeneous data, while it is non-homogeneous data. If there is a significant difference in the analysis of variance, then the test is continued with the Duncan test with the following formula:

$$D = d_{a.p.v}\, x \frac{\sqrt{KTG}}{n}$$

Explanation:

P = the distance between the two levels of treatment p
v = error free degrees
a = real level
KTP = the middle square of the treatment
KTG = middle square error

Table 3. Measurement of water quality

No	Parameter	Unit	Measuring Device
1.	Suhu	ºC	Termometer
2.	*Dissolved Oxygen*	Mg L^{-1}	DO Meter
3.	pH	-	pH Meter
4.	Ammonia	Mg L^{-1}	Ammonia *test kit*

3. RESULTS AND DISCUSSION

3.1 Feed Convertion Ratio

Feed conversion value is a value that describes the efficiency of a feed for growth [19]. One of the factors that affect the feed conversion ratio is the digestibility of the feed. Feed digestibility can also be affected by crude fiber content.Crude fiber has very low nutrition, but in certain amounts it is recommended to use it to accelerate peristalsis in the intestines and help feces clot [21] in Febriyanti (2017).Red tilapia is a tilapia fish that cannot digest feed ingredients that have high fiber content. If the fiber content in the feed ingredients is too high, it will accelerate the feed through the intestines, so that the nutrients absorbed are reduced and cause low protein absorption by the tilapia body [22].

The need for crude fiber for tilapia fingerlings is 6-8% [23]. From this range it is known that treatment A, treatment B, treatment C are still in the range of red tilapia crude fiber requirement because it has a range of 6.4 – 7.8%.Crude fiber content of more than 8% in the feed can reduce the quality of the feed structure [24]. This shows that the feed D treatment exceeds the crude fiber limit for tilapia because it has 8.2% fiber, thereby reducing the quality of the feed structure which causes decreased absorption.

The results of the statistical analysis showed that feeding with different compositions of coconut husk produced a significant difference in the FCR value. FCR values were then analyzed using Duncan's multiple range test at the 95% level of confidence. The best FCR value results are in treatment C because it has the lowest FCR value and is still within the limits of fat and fiber according to the needs of tilapia (Fig. 1).

The results showed that Treatment D (feed containing 75% of coconut husk filler) was the treatment that had the worst FCR among the other treatments. Besides being seen from the fiber content, another component that supports that treatment D is the most difficult treatment for fish to absorb due to the fat content of the feed. Fat is an important component needed by fish after protein. Fish need fat as energy which is used to synthesize fatty acids which cannot be synthesized by the fish themselves. Coconut husk contains organic acids needed by tilapia.Coconut husk flour contains organic acids in the form of linoleic acid to help growth and certain hormone presecutors [8]. A fat content in feed of 12% will result in maximum development [25]. Whereas treatment D (based on 75% fermented coconut husk) had a fat content above 12%.

3.2 Growth

Observation of the growth rate of red tilapia is a supporting parameter observed to determine the effect of adding oncom mushroom fermented coconut husk on the growth of red tilapia. The results of measuring the weight growth of Red Tilapia in this research showed that different feeding treatments resulted in different average values. There was an increase in the average growth from the first observation to the sixth observation in each treatment, indicating that the feed given was able to be eaten and utilized by tilapia (Fig. 2).

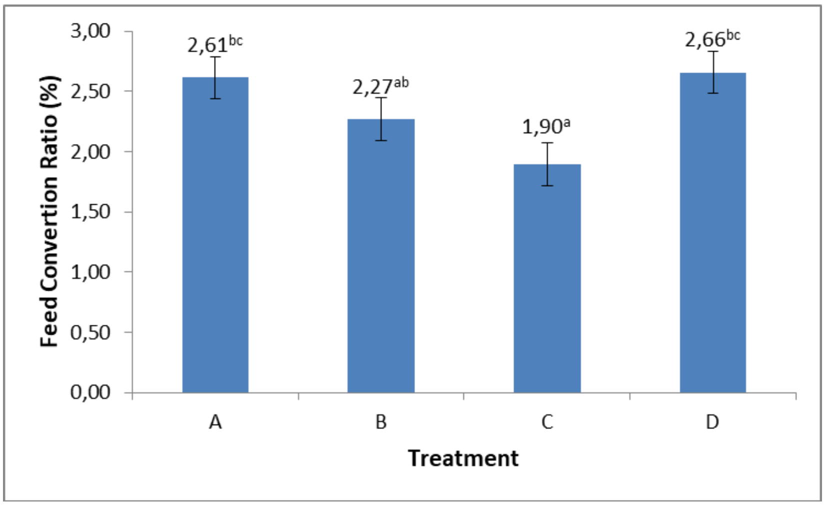

Fig. 1. FCR value for each treatment

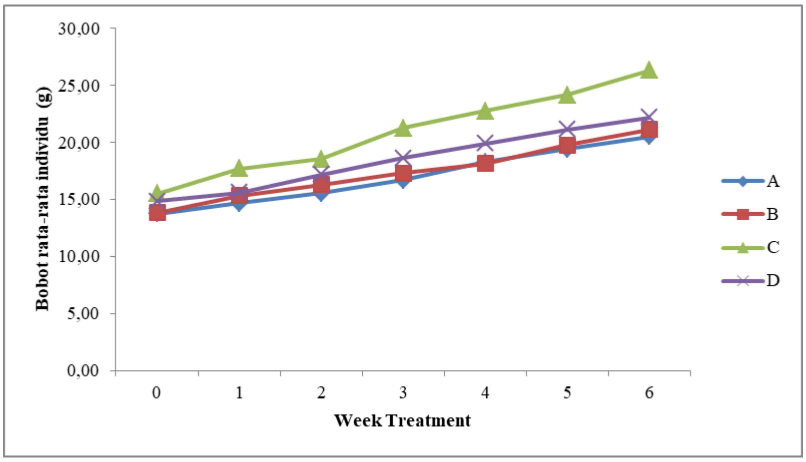

Fig. 2. Increase in average tilapia weight

The average yield of tilapia growth during the study was then followed by the calculation of SGR (Spesific Growth Rate). The results of statistical analysis, obtained the average absolute weight of fish in the control treatment (A) which was significantly different from the average absolute weight in the treatment of feed containing coconut husk filler (Fig. 3).

The results of measuring the absolute weight growth of tilapia produce various values. This difference is caused by differences in nutritional quality in the feed treatment. Some of the roles of nutrition in aquaculture are being able to influence production costs, fish growth, fish health, waste production [26].

The SGR value was then analyzed using a Table of variance and showed a significantly different effect on each treatment (so that it was continued with Duncan's multiple range test.Based on the results of Duncan's multiple range test at 95% confidence level, treatment C produced the highest SGR value because the fat content in the feed was sufficient for tilapia. Coconut husk flour has a high fat content of around 20-33% [27]. Fish generally obtain fat from feed [28]. Fat is a component needed by fish after protein. Fish need fat as an energy source, to synthesize fatty acids that the fish cannot synthesize themselves. Fat content in feed of 12% will result in maximum development [25]. Feed treatment D had the lowest yield due to the high fat content exceeding the fat content in the feed, namely 12.6. The high content of saturated fatty acids in the feed causes the feed to be difficult to digest so that the growth of fish on feed D gives the lowest yield [9].

3.3 Survival Rate

Survival is a comparison between the number of organisms that live at the end of the study and the initial number of organisms at the time of stocking expressed as a percentage, the greater the percentage value indicates the more organisms that live during rearing [19]. In fish farming death is a determinant of business success. The supporting factors that resulted in a high survival value in this study were supported by the feed given, stocking density settings and environmental conditions in the rearing medium. Giving coconut coir fermented oncom mushrooms to the test fish feed can meet nutritional needs and maintain their survival.

The survival rate of red tilapia for 42 days of rearing in each treatment has a range of 93% - 100% (Table 4), this value is still high because it is above the standard value for survival quality of production tilapia, namely 75% [29]. There are three categories to describe the range of survival rates, among others are ; SR > 50% is included in the good category, 30% > SR > 50% is included in the medium category, and SR < 30% is categorized as unfavorable [30], in Mulyaniet al. (2014),. Based on the survival rate during the study, it was found that the survival rate of red tilapia fed fermented coconut husk was in a good category.

3.4 Water Quality

Water quality parameters are one of the factors that affect the survival of red tilapia. The role of water quality affects the metabolism and stress level of fish so that it plays an important role in increasing the production of fish farming. The water quality parameters observed in the research were pH, DO, temperature and ammonia. Water quality data collection in the research was carried out every 7 days. The results of observations of water quality parameters are presented in Table 5.

The pH of water is a determinant of chemical processes in water. pH levels that are too high or too low cause fish to become stressed.tilapia growth will be optimal at pH 7-8 [31]. The results of pH analysis during the study showed that the average for all treatments ranged from 7-7.7, still within the tolerance limits for tilapia fish or in good condition. pHwhich is not optimal can cause stress fish, susceptible to disease, as well as low productivity and growth [32]. In addition, pH plays an important role in the field of aquaculture because it relates to the ability to grow and reproduce.

Dissolved oxygen is one of the limiting factors in waters. The minimum value of dissolved oxygen levels for fish farming is 3 ppm [33]. Dissolved oxygen content affects the process of oxidation and reduction of organic and inorganic materials [34]. based on the results of these measurements, it is known that the dissolved oxygen content in tilapia rearing media is at the optimum value. This is reinforced by the statement that the optimal dissolved oxygen content for fish growth is around 5-8 ppm [35].

Temperature is an abiotic factor that affects the metabolism and growth of fish.Temperature is an abiotic factor that affects the metabolism and growth of fish.the temperature of the pond or waters that can be tolerated by tilapia is 15 - 37°C (Princess 2018). Based on the measurement results, the temperature range in the maintenance aquarium is between 23.6 - 24.7°C. Aquatic organisms such as fish and shrimp can live well in the temperature range of 20°C – 30°C. Changes in temperature below 20°C or above 30°C cause fish to experience stress which is usually followed by a decrease in digestibility [36].

Table 4. Red tilapia survival for 42 days

Treatment	Survival rate (%)
A. (Control, Basal feed with filler bran as much as 100%)	100
B. (Artificial feed containing filler epidermis coconut as much as 25%)	93
C. (Artificial feed containing filler epidermis coconut as much as 50%)	96
D. (Artificial feed containing filler epidermis coconut as much as 75%)	100

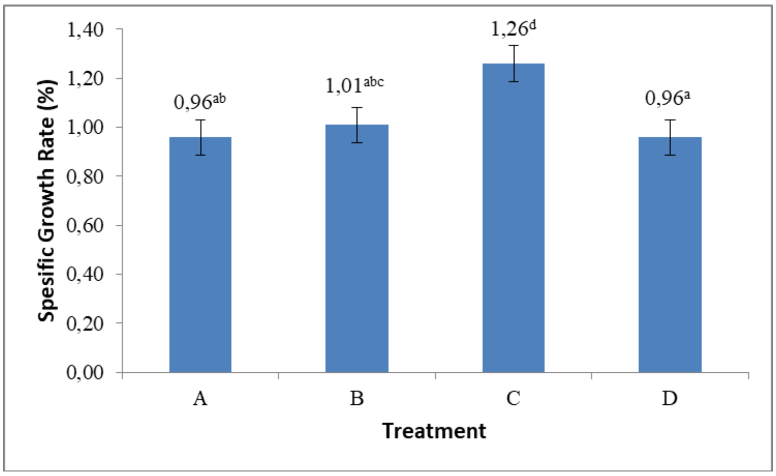

Fig. 3. SGR value of each treatment

Table 5. Observation results of red tilapia water quality

Parameter	Result	Reference (BSNI.2009)
pH	7-7,8	6,5-8,5
DO (ppm)	4,8-5,3	≥3ppm
Temperature (°C)	25,3-26,1	25-30
Ammonia ($mg.L^{-1}$)	0.0014 - 0.0040	

Ammonia (NH3) is a water quality parameter which is a big problem for fish and in fish farming activities.Ammonia levels should be < 0.1 mgL^{-1} [37]. The concentration of ammonia in the rearing medium for tilapia is still within the threshold when compared to the questions above, so it does not threaten the survival of red tilapia.

4. CONCLUSION

The conclusion that can be drawn from this research is the optimal dose of fermented coconut husk utilizationNeurosporasittophila 50% yielded the highest daily growth rate (SGR) of 1.26%, the highest feed conversion ratio (FCR) of 1.9%, the highest survival rate of 96%.Application for the addition of fermented coconut huskNeurosporasittophila in filler material in the best fish feed is as much as 50%. Seeing the benefits of using coconut husk, further research is needed to find out whether coconut husk can be used as the main ingredient in preparing feed for fish.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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