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Effect of Using Fermented Coconut Testa by *Rhizopus oryzae* Went & H.C. Prinsen Geerligs, (1895) on the Growth of Red Tilapia Seeds (*Oreochromis niloticus* Linnaeus, 1758)

Rida Oktapiani*, Kiki Haetami, Evi Liviawaty, Walim Lili

Fish Department, Faculty of Fishery and Marine Science, Padjadjaran University,
Jl. Raya Bandung – Sumedang Km 21, Jatinangor 45363, Indonesia

*E-mail address: rida17001@mail.unpad.ac.id

ABSTRACT

This study aims to determine the effect of the level of use of fermented coconut testa from Rhizopus oryzae for four days on optimal artificial feed on the growth of red tilapia fry. The method used in this research is an experimental method using a completely randomized design (CRD), which consists of five treatments and three replications. The treatments used were differences in the level of use of fermented coconut testa in feed (A) 0%, (B) 5%, (C) 10%, (D) 15%, and (E) 20%. Maintenance was carried out for 42 days. Parameters observed were nutritional value of fermented products, daily growth rate, feed conversion ratio, and water quality (temperature, pH and dissolved oxygen). Daily growth rate, feed conversion ratio, and water quality were observed every 7 days. Daily growth rate and feed conversion ratio data were analyzed using the Analysis of Variance (ANOVA) with a 95% confidence level, followed by Duncan's multiple spacing test, while the nutritional content of fermented products and water quality were analyzed descriptively. The results showed that the use of coconut testa fermented by *R. oryzae* for four days as much as 15% in the feed formulation gave the highest daily growth rate of red tilapia fish, namely 1.29% and feed conversion ratio of 2.36.

***Keywords*:** Red tilapia, coconut testa, fermentation, *Rhizopus oryzae*, growth, *Oreochromis niloticus*

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1. INTRODUCTION

Red tilapia (*Oreochromis niloticus*) entered Indonesia in 1981 from the Philippines and in 1989 from Thailand [1] Based on data from the Directorate General of Aquaculture, Ministry of Fisheries and Marine Affairs (KKP) in 2016-2017 there was an increase in national tilapia production by 3.6 percent. Tilapia is included in one of the fishery commodities that deserves to be the mainstay of aquaculture products because it is in demand from the domestic market to foreign markets [2].

Feed is an important part of cultivation activities and is one of the determinants of the success of cultivation. Feed consumes 60-80% of the total cost of cultivation production [3]. Meanwhile, the price of feed is still relatively high due to the availability of feed ingredients that depend on the quality of imports. Therefore, to reduce the cost of feed, it is necessary to have alternative feed ingredients from local materials available in the surrounding environment. Alternative feed ingredients must be easy to obtain, relatively cheap in price, and have a fairly high nutritional content [4]. One of the raw materials that can be used as an alternative feed ingredient is coconut testa.

Coconut testa is the inner skin of the coconut shell which is commonly found in traditional markets as waste and animal feed. Coconut testa contains 7.75% protein, 26.48% fat, 15.19% crude fiber. The high fat and crude fiber content causes the coconut testa content to be a limiting factor in its use. So it is necessary to process alternative feed ingredients, namely through a fermentation process to improve nutritional quality, break down complex compounds into simple ones so that they are easily digested, and reduce crude fiber [5].

Mold is a microorganism capable of using cellulose as a carbon source for its growth [6]. *R. oryzae* produces cellulase, xylanase, pectinase, and amylase enzymes that can reduce fiber and carbohydrate content [7]. In addition, *R. oryzae* also has proteolytic and lipolytic abilities for increasing protein, breaking down fats into fatty acids and glycerol [8]. The total colony of mold in coconut testa fermented at a dose of 3% for 96 hours resulted in the highest colony average of 7.72 x 106 CFU. In the log (exponential) phase, the highest mycelium production occurred at the incubation time of 48 hours to 96 hours characterized by fungal growth which was marked by the appearance of white colonies [9].

R. oryzae fermented coconut testa is expected to increase the nutritional value and facilitate the absorption of nutrients that can be used as feed ingredients that can reduce feed production costs and increase the growth of red tilapia.

2. MATERIALS AND METHODS

This research was conducted at the Aquaculture Laboratory, Building IV, Faculty of Fisheries and Marine Sciences, Padjadjaran University for the manufacture of feed, maintenance period, and observation of test fish. The tools used in research activities are an aquarium measuring 40 x 20 x 30 cm3, steamer, tray, fiber tub, blower and, aeration installation, DO meter, heater, pellet printing machine, millimeter block, pH meter, filter, drain, hose, and a thermometer.

The materials used in this research activity were Rhizopus oryzae mold inoculum, 70% alcohol, red tilapia with a length of 7-9 cm and an average weight of 7-8 grams as many as 250 fish from the Cibiru Fish Seed Center, heat-resistant plastic, chlorine, coconut testa waste as much as 3 kg, raw materials for artificial feed consisting of fish meal, soybean meal, wheat pollard, corn flour, fine bran, tapioca flour, premix, and warm water. The method used is an experimental method with a completely randomized design (CRD) consisting of 5 treatments and 3 replications. The treatments given were differences in the level of use of fermented coconut testa in feed formulations, A (0%), B(5%), C (10%), D (15%), and E (20%).

2. 1. Research Preparation

Coconut Testa Fermentation

The coconut testa obtained from the market are cleaned using running water and then sterilized by steaming at a temperature of 70-90 °C for 40 minutes. The coconut testa was weighed and then put into a heat-resistant plastic and added with 3%/kg Rhizopus oryzae solution, homogenized and the plastic was given a hole. The epidermis was fermented for four days in a fermentation cabinet under aerobic conditions at a temperature of 25-30 °C. After fermentation, the coconut testa is dried using an oven, and mashed with a blender so that it can be used as feed ingredients.

Feed Making

The manufacture of feed is done by making feed formulations from several ingredients using the Pearson Square method. The process of making fish feed begins with the reduction of the size of the raw materials, mixing, printing, drying, packaging and storage.

The feed formulation can be seen in Table 1.

Table 1. Feed Formulation.

Raw material	Treatment Feed Composition (%)				
	A	B	C	D	E
Soybean Meal	16.54	16.65	16.76	16.87	16.98
Fish flour	16.54	16.65	16.76	16.87	16.98
Cornstarch	21.31	19.57	17.83	16.09	14.35
Polar	21.31	19.57	17.83	16.09	14.35
Fine bran	21.31	19.57	17.83	16.09	14.35
Tapioca/Binder	2	2	2	2	2
Premix	1	1	1	1	1
Fermented Coconut Testa	0	5	10	15	20
Total	100	100	100	100	100
Proteins (%)	28	28	28	28	28

Raw material	Treatment Feed Composition (%)				
	A	B	C	D	E
Fat (%)	5.88	6.57	7.26	7.95	8.65
Crude Fiber (%)	5.42	5.79	6.16	6.53	6.90
DE (kcal/kg)*	2323.29	2391.58	2459.87	2528.16	2596.45
DE/P	8.30	8.54	8.78	9.01	9.25
Note: *) DE (Digestible Energy) = 75% x GE (Gross Energy)					

Acclimatization

Red tilapia were acclimatized in fiber tanks which were given aeration and commercial feed with a frequency of twice a day in the morning and evening for ± 7 days before being put into the aquarium.

Maintenance Media Preparation

The aquarium was washed and sterilized using a chlorine solution of 1-2 ppm which was aerated for 24 hours, then rinsed until it was clean and odorless and then dried. The aquarium is filled with 20 liters of water and aerated for 24 hours.

2. 2. Main Research

Red tilapia were reared for 42 days in an aquarium of 10 in 20 liters. Feeding as much as 3% of the weight of the fish with the frequency of feeding twice at 08.00 and 16.00. Observations on daily growth rate, feed conversion ratio, and water quality (temperature, dissolved oxygen, and pH) every 7 days for 42 days of rearing.

2. 3. Observed Parameters

Nutritional Value of Fermented Products

The samples were analyzed proximately in dry weight, the samples observed were samples of unfermented coconut testa and coconut testa that had been fermented for four days. This research was conducted by comparing the nutritional content of unfermented coconut testa and fermented coconut testa. The nutritional content observed included protein, crude fat, crude fiber, nitrogen-free extract (BETN), ash and water.

Daily Growth Rate

The daily growth rate is calculated using the formula:

$$DGR = \frac{Ln(Wt) - Ln(Wo)}{T} \times 100\%$$

Note :
DGR = Daily growth rate (%)
Wt = Weight of red tilapia at the end of the study (grams)
Wo = Weight of red tilapia at the beginning of the study (grams)
T = Length of maintenance time (days)

Feed Conversion Ratio

The feed conversion ratio is calculated using the formula:

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

Note :
FCR = Feed Conversion Ratio.
Wo = Weight of red tilapia at the beginning of the study (grams)
Wt = Weight of red tilapia at the end of the study (grams)
F = Amount of feed given (grams)
D = Weight of red tilapia that died during the study

Water quality

The observed water quality parameters include temperature, degree of acidity (pH), and dissolved oxygen (DO). Water quality parameters were measured every 7 days for 42 days.

2. 4. Data Analysis

Data on daily growth rate and feed conversion ratio were statistically analyzed using analysis of variance (ANOVA) at the 95% confidence level to see the effect. If the analysis of variance obtained a significant difference (P < 0.05%), then continued with Duncan's multiple distance test to determine the difference between treatments. Meanwhile, data on changes in nutrition of fermented coconut testa and water quality were analyzed descriptively.

3. RESULT

3. 1. Nutritional Value of Fermented Products

Fermentation is a process of chemical change in an organic substrate through the activity of enzymes produced by microorganisms. In addition, physically there is little change in the substrate. On the 3rd day of fermentation, there is a little mycelium on the surface of the substrate and there is water vapor. The appearance of mycelium to the substrate surface was due to the number of mold colonies in the log phase on day 3 or 72 hours. The fungus *R. oryzae* reached the log (exponential) phase at 48-96 hours of fermentation and stationary phase at 96-144 hours [9]. Physical changes were quite significant on day 4 or 96 hours, coconut testa had a solid shape because the substrate was held together by white *R. oryzae* mycelium like fermented tempeh products (Figure 1). *R. oryzae* has a characteristic white mycelia, when mature the white mycelia will be covered by sporangium which is brownish gray [8]. In addition, coconut testa has a distinctive fermented smell. The characteristic odor of acidic fermentation is produced by volatile odor substances from organic acids excreted by microorganisms [10].

Figure 1. Fermented Coconut Testa on Day 0 (left) and Day 4 (right)

While the chemical changes include the levels of protein, fat, ash, carbohydrates, and water on the substrate can be known through proximate analysis. The results of the proximate analysis of unfermented coconut testa and fermented coconut testa for four days can be seen in Table 1.

Table 1. Nutrient Content of Coconut Testa Before and After Fermentation

No.	Content	Coconut Testa (%)*	Fermented Coconut Testa (%) *	Change (%)
1.	Protein	5,75	11,61	+101,91
2.	Fat	17,19	20,55	+19,55
3.	Carb Fiber	26,48	14,23	-46,26
4.	Ash	2,75	2,73	-0,73
5.	BETN	52,62	50,88	-3,31
6.	Water	6,28	3,04	-51,59

Note: * Results of Proximate Analysis of Ruminant Animal Nutrition and Animal Feed Chemistry Laboratory (2020)

After four days of fermentation of coconut testa, there was an increase in protein content of 101.91%. The increase in protein content was caused by the activity of molds capable of producing protease enzymes from secondary metabolites. *R. oryzae* is a mold that can produce protease enzymes [11]. In addition, increasing the number of *R. oryzae* colonies could increase the crude protein content of the substrate because the mold is a source of single cell protein and its mycelia contain 31-50% protein [12]. In addition to an increase in protein content, there is also an increase in fat content in the fermented substrate. The increase in fat in the fermented coconut testa was due to the lipolytic activity produced by the mold. The increase in lipolytic enzymes in the fermentation substrate will cause the hydrolysis of fats into fatty acids and glycerol [13]. In addition, the increase in fat was accompanied by an increase in the biomass of *R. oryzae*, which components of the cell wall and plasma membrane are fat components in the form of phospholipids and lipoproteins [7]. Ash comes from the rest of the feed burning material, the decrease in ash content in the fermentation results is influenced by the use of minerals by microorganisms to maintain life [14]. Then the decrease in crude fiber content in the material was caused by the activity of *R. oryzae* mold. This mold produces enzymes that are used to break down polysaccharides such as cellulase, xylanase, pectinase, and amylase into oligosaccharides and glucose which causes a decrease in carbohydrate and fiber content [7]. The calculation of the BETN content included a 100% reduction in the total content of crude protein, crude fat, crude fiber and ash [14]. Fermentation also reduces the water content of coconut testa from 6.28% to 3.04%. This is due to a decrease in the ability of the material to retain water due to starch degradation by microorganisms so that a lot of bound water is liberated, the texture of the material becomes soft and porous. This causes evaporation of water during the drying process in the same drying period [15].

3. 2. Daily Growth Rate

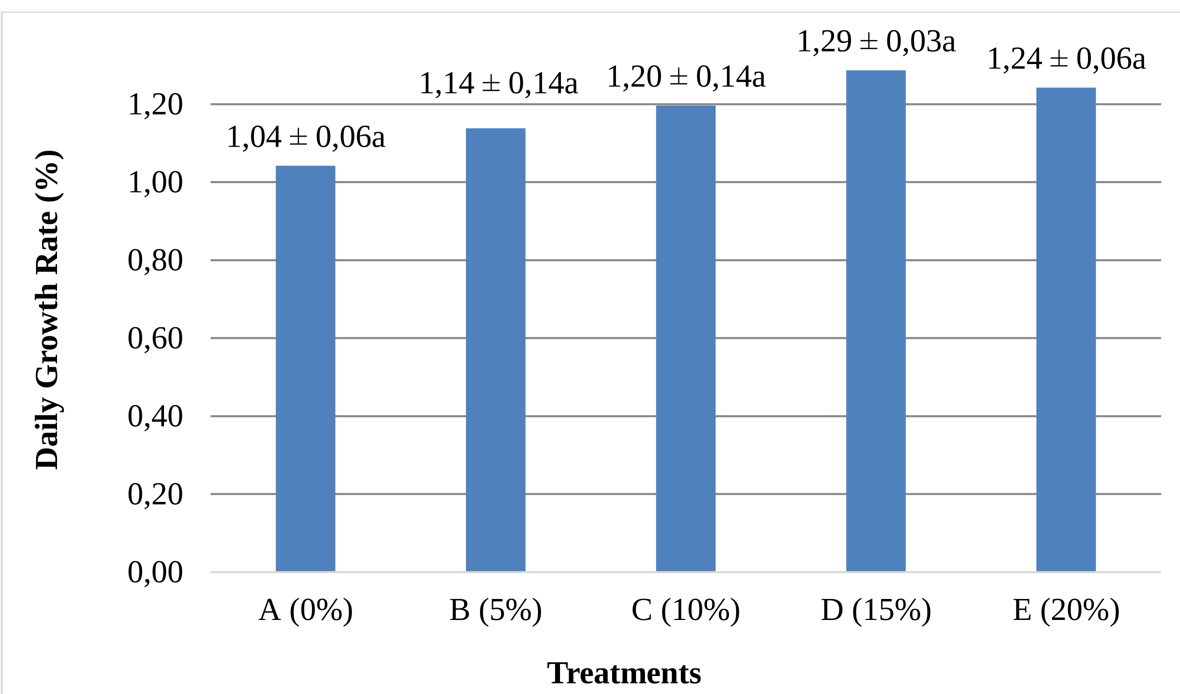

Figure 2. Red Tilapia Daily Growth Rate

Growth is an increase in length and weight in a certain period the daily growth rate of red tilapia can be seen in Figure 2.

The growth rate of red tilapia fry ranged from 1.04%-1.29%. Based on the results of analysis of variance in the use of fermented coconut testa in feed formulations, it did not have a significantly different effect on the growth rate of red tilapia (P> 0.05). The highest daily growth rate was produced by treatment D (15%), while the lowest growth rate was produced by treatment A (control), which was 1.04%. There was no significant effect on the level of use of fermented coconut testa, because in the preparation of the feed formulation, it was composed of isoprotein and isoenergetic. So all treatments get the same opportunity to support an increase in growth rate. Isoprotein feeding resulted in the same growth rate because the protein content obtained by fish was the same between treatments, as well as the energy content in the feed which did not differ much causing the growth rate to be not significantly different [16]. The nutritional content of feed with the use of coconut testa is shown in Table 2.

Table 2. Nutritional Content of Feed

Content	Treatments (%)					Nutritional Requirements (%)
	A*	B*	C*	D*	E*	
Protein	28	28	28	28	28	25-30 [28]
Fat	5.88	6.57	7.26	7.95	8.65	6-8 [22]
Coarse Fiber	5.42	5.79	6.16	6.53	6.90	7 [22]
E/P	8.30	8.54	8.78	9.01	9.25	8-9 [21]

Note : *) Conversion of the results of the analysis of feed ingredients according to Hartadi, et al. 1990 and Pearson Square.

Nutritional requirements treatment D (15%) had the most optimum nutritional composition that could support the growth of red tilapia. The fat content contained is still within the range of fish nutritional needs that can support fish growth. The presence of fat in the feed can balance the use of protein for metabolic activities and body maintenance which is commonly called the protein sparing effect, so that the protein contained in the feed can be used for growth [18].

Treatment A (0%) produced the lowest growth rate compared to other treatments. The fat content in feed A (0%) was 5.88%, which means the fat content required by red tilapia was within the optimum limit for fat adequacy in the feed. Feed must contain balanced nutrition according to the nutritional needs of fish, so that there is a complementary effect between feed ingredients. The treatment E (20%) had a fat content of 8.65% which exceeded the optimum limit for red tilapia fat requirements. Fat levels that exceed the optimum limit for fat requirements can cause fat accumulation which causes fish to feel full quickly and a decrease in feed consumption which results in decreased fish growth [18]. Crude fiber contained in treatments A (0%) and B (5%) was not sufficient to meet the fiber needs of tilapia.

While the use of 10-20% coconut testa has a higher fiber content, this is supported by the presence of microbial enzymes produced by *R. oryzae*. However, crude fiber content that is too high will decrease growth because intestinal emptying takes longer and feed digestibility decreases [19]. The presence of *R. oryzae* in the coconut testa fermentation process does not cause adverse effects on the substrate because this mold has the ability to reduce anti-nutrients, increase nutritional value, produce antibacterial, antioxidant, antimicrobial, antiaflatoxin compounds in feed [20]. So that the use of fermented coconut testa up to 20% does not have a bad effect on growth.

The nutritional content that is balanced and in accordance with what fish needs will be used by fish as a source of energy used for growth or metabolism. Treatment A (0%) had the lowest growth rate with a protein energy of 8.30 kcal/kg and treatment E (20%) had the highest energy compared to other treatments, which was 9.3 kcal/kg. The protein energy value of all treatments was the optimal DE/P value for fish growth, which ranged from 8-9. However, if DE/P exceeds the optimal limit, it will reduce feed consumption [21].

3. 3. Feed Convertion Ratio

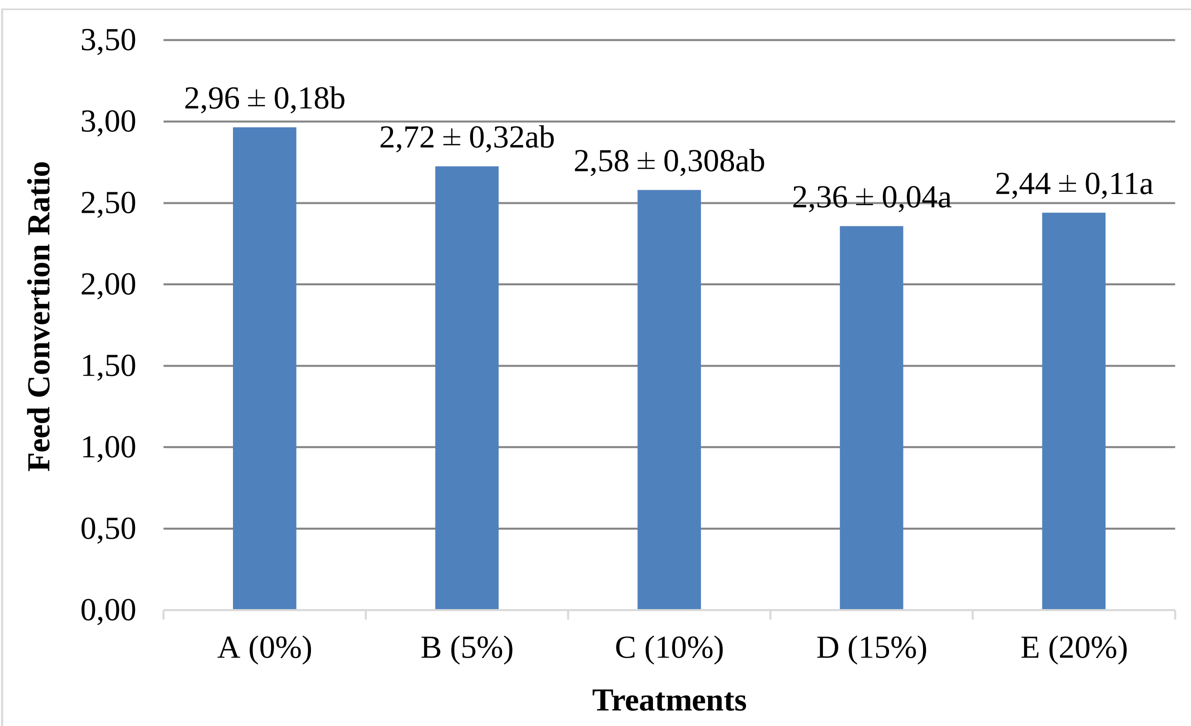

Figure 3. Feed Conversion Ratio

Feed conversion ratio (FCR) is a comparison between the amount of feed given to the total weight of fish produced at the end of the research. The smaller the feed conversion value indicates that the feed provided is more efficient and able to be utilized properly by fish, so that the incoming energy can be utilized for the growth process [2]. The resulting feed conversion ratio is quite varied, ranging from 2.36 to 2.96. The histogram of the feed conversion ratio can be seen in Figure 3. Treatment D (15%) resulted in the lowest feed conversion value compared to other treatments, which was 2.36, meaning that feeding 2.36 grams would produce 1 gram of fish meat. The results of analysis of variance showed that the level of use of fermented coconut testa resulted in a significantly different feed conversion ratio ($p<0.05$). Treatment D (15%) was significantly different from treatment A (0%), this was because the nutrient content in the feed was different between treatments which gave different feed conversion values. According to [23], the factors that affect feed conversion in fish are influenced by density, weight, age, temperature, and feed (quality, quantity and frequency of feeding).

The feed conversion value produced by treatment D (15%) was lower than treatment A (0%). Coconut testa which is used as a feed ingredient is a fermented product which means that the compounds in it are simple because *R. oryzae* produces protease, lipase, amylase, and pectinase enzymes that make it easier for fish to digest feed containing fermented coconut testa [8]. Feed without the use of coconut testa will affect the digestibility of feed, because the compounds contained in the feed ingredients are complex compounds compared to fermented products.

The final product of fermentation contains compounds that are simpler and easier to digest than the original ingredients [29]. Digestibility is related to the rate of gastric emptying and the response to feed. The longer the gastric emptying, the longer the feed will be submerged in water which will result in a decrease in feed quality. According to [21], feed that is slower to respond to fish will experience nutrient leaching in the water before entering the fish's stomach. In addition, Treatment A (0%) had the highest feed conversion value because it had the lowest protein energy compared to other treatments. Protein energy in feed affects feed conversion [24].

Treatment D with the use of 15% fermented coconut testa in the feed formulation had a low feed conversion, but not much different from treatment E (20%). Based on research [25], the use of coconut testa as much as 5% on red tilapia fry resulted in the highest feed efficiency value, but could be utilized up to 15%. Meanwhile, fermented coconut testa can be used as an alternative feed ingredient for up to 20% of the feed components and does not have a negative effect on growth. This indicates that the mold *R. oryzae* can utilize cellulose as a carbon source for its growth substrate in coconut testa and increase fish digestibility of feed by using coconut testa [6].

The use of fermented coconut shells for four days in the feed formulation resulted in a fairly high feed conversion value (2.42-2.96), because the FCR value of fish generally ranged from 1.5 to 2.5 [26]. However, the use of coconut testa has a good impact on production costs and growth rates. Feed formulation using fermented coconut testa as an alternative feed ingredient can reduce the cost of red tilapia feed production.

3. 4. Water quality

Water quality parameters measured during the study included temperature, Dissolve Oxygen (DO), and acidity (pH). The results of water quality measurements during maintenance can be seen in Table 3.

Based on table 3, it can be seen that the water quality observed in all treatments including temperature, dissolved oxygen, and pH is still in the range of water quality standards based on [27], this is because water quality is maintained by changing water, providing aeration, and siphoning during fish rearing [30-35].

Table 3. Water Quality.

Treatments	Observed parameters		
	Temperature (^oC)	Dissolve Oxygen (mg/L)	pH
A (0%)	26-29	5,1-6,3	7,18-7,80
B (5%)	26-29	5,2-6,3	7,36-7,75
C (10%)	26-29	5,2-6,3	7,33-7,76
D (15%)	26-29	5,3-6,5	7,34-7,75
E (20%)	26-29	5,2-6,6	7,37-7,78
Quality standards (SNI 7550:2009)	25 - 32	$\geq$3	6,5 - 8,5

4. CONCLUSIONS

The results of the research showed that the use of coconut testa fermented by *R. oryzae* for four days in the feed formulation did not significantly affect the growth rate of red tilapia seeds, ranging from 1.04% - 1.29% per day. The use of coconut testa as much as 15% in the feed formulation resulted in the best feed conversion of 2.36. However, the use of coconut testa fermented by *R. oryzae* for four days can be used up to 20% as an alternative feed ingredient.

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