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Bioconversion of Coconut Testa by *Rhizopus Oryzae* on Chemical Changes and Performance of Red Tilapia (*Oreochromis Niloticus*)

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ABSTRACT: The nutritional content that is balanced and in accordance with the needs of fish will be utilized by fish as a source of energy used for growth. The used of feed alternative from coconut testa bioconversion has been investigated for its effect on the growth performance of red tilapia stadium fingerling. The chemical composition of coconut testa, survival rate and water quality (temperature, pH and dissolved oxygen) were analysed descriptively. The experiment was carried out experimentally using a Completely Randomized Design (CRD), which consisted of four levels of using coconut testa bioconversion by *Rhizopus oryzae* in feed (0%, 5%, 10%, 15%, and 20%). The performance parameters measured consisted of Average daily gain, feed efficiency, and protein efficiency ratio. Data were analyzed using the Analysis of Variance (ANOVA) with a 95% confidence level, and Duncan's multiple distance test. The chemichal change, survival rate. The study showed that the bioconversion of R. oryzae coconut testa up to 20% in the feed formulation could provide the performance of red tilapia fry, with the highest value obtained at the use of 15% with an average feed efficiency of 42.43 ± 0.71.

KEYWORDS: Red tilapia, coconut testa, bioconversion, *Rhizopus oryzae*, performance.

INTRODUCTION

The provision of sustainable artificial feed both in quality and quantity is one way to increase the value of aquaculture production. The feed given must be in accordance with the nutritional needs of fish for the survival, growth, and development of fish. Inefficient feed will increase production costs while excessive and inedible feed will cause toxins that are harmful to fish survival(Siregar & Adelina, 2012). Tilapia is quite efficient in energy utilization, because it is omnivorous with various types of feed, both vegetable and animal (M, 2018); (Nugroho, B.H., Basuki, F. dan Wisnu R, 2013). The requirement that feed raw materials be suitable for use in the manufacture of feed is to have high nutritional value, abundant and continuous availability, also has a fairly cheap price (Septinova et al., 2019). Coconut testa, the outermost part of the coconut flesh, is often obtained as coconut grated waste or the rest of the processed nata de coco food industry. Coconut testa processing through bioconversion is one of the efforts made to improve the quality of feed ingredients. Based on the results of the proximate analysis, the composition of the coconut testa consists of 8.10% crude protein, 48.72% crude fat, 31.3% crude fiber, 2.38% ash (Salza Biila Nuha et al., 2019). The crude fiber content in fish feed is a limiting factor because it can interfere with the digestibility of feed in the digestive system (Józefiak et al., 2018). Mold is a microorganism capable of using cellulose as a carbon source for its growth(Endrawati & Kusumaningtyas, 2018).

Rhizopus oryzae produces cellulase, xylanase, pectinase, and amylase enzymes that can reduce fiber and carbohydrate content (Hsiao et al., 2014). These filamentous microorganisms have high resistance levels and can live in unfavorable conditions (Mustabi et al., 2018). Meanwhile, the high crude fat content will cause the feed to be easily oxidized, rancidity and result in the accumulation of fat in the fish intestines, liver or kidneys so that the fish's appetite is reduced. The fungction of mold of *R oryzae* was digestive process with protease, lipase, and amylase metabolite enzymes and enhance the nutrients can be easily absorbed by the fish body (López-Otín & Bond, 2008). Bioconversion of sago peels using R. oryzae causes changes in crude fiber content from 28.16% to 10.82% (Hamdat et al., 2010).

R. oryzae is safe for consumption because it does not produce toxins and can increase the digestibility of plant-based feed ingredients (Cantabrana et al., 2015)

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The aim of the study was to determine the yield level of coconut pering waste bioconversion by *R. oryzae* mold in the formulation on changes in nutrition and feed efficiency in tilapia. The expected benefit of this research is to provide information about the use of waste coconut husks from the bioconversion of R. oryzae to improve feed conversion of tilapia. The hypothesis of this research is that the addition of coconut husk bioconversion by *R. oryzae* can increase feed efficiency and protein efficiency of red tilapia seed feed.

MATERIALS DAN METHOD

The materials used in the feeding experiment were coconut pering waste, fish meal, soybean meal, refined rice bran, and vitamin mix. *Rhizopus oryzae* mold inoculum powder was prepared, with instrumentation steamer, fermentation cabinet, plastic and tray. The bioconversion process uses *R. oryzae* inoculum in powder form (powder), based on the method developed by (Cantabrana et al., 2015). Fermentation is carried out aerobically with a dose of 2% inoculum and an incubation time of 4 days at a temperature of 25°C. Steaming is carried out at a temperature of 70-90°C for 40 minutes to function as a sterilization of the coconut testa material. After fermentiation, the coconut husk is dried in a drying cabinet for 2 days, then mashed with a blender so that it can be used as feed ingredients

Feeding trials used red tilapia as much 250 ind. fish of 5-7 cm fingerling stage with an initial weight of 6±0.5 grams. The stocking of each aquarium is 10 fish with a stocking density of 1 fish / 2 liters. The frequency of feed that will be given is twice a day at 08.00 and 16.00 WIB with feeding as much as 3%. Maintenance is carried out for 42 days and siphoning every day to remove residual feed and feces as well as adding water according to the volume of wasted water

The results of the bioconversion were analyzed proximately(Length, 2013; AOAC 2000) and the changes in dry weight, protein and fat content were calculated. crude fiber, and nitrogen-free extract (BETN). The feed formulation was prepared in isoprotein (28 percent), with the treatment of using coconut testa bioconversion results as 0%, 5%, 10%, and 15%. The composition of feed ingredients and formulations are presented in Table 1.

Table 1. Nutrient Composition and Feed Formulation

Ingredient	Treatments (%)				
	A	B	C	D	E
Fish meal	16.54	16.65	16.76	16.87	16.98
Soy bean meal	16.54	16.65	16.76	16.87	16.98
Corn meal	21.31	19.57	17.83	16.09	14.35
Wheat bran	21.31	19.57	17.83	16.09	14.35
Rice bran	21.31	19.57	17.83	16.09	14.35
Coconut Testa Bioconversion	0.00	5.00	10.00	15.00	20.00
Binder	2.00	2.00	2.00	2.00	2.00
Vitamin-mineral mix	1.00	1.00	1.00	1.00	1.00
Total	100	100	100	100	100
Crude Protein (%)	28.00	28.00	28.00	28.00	28.00
Crude 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) (He & Wurtsbaugh, 1993)

The experimental method using a completely randomized design (CRD), consists of on artificial feed which five levels of treatment of coconut testa were A 0%, B 5%, C 10%, D 15%, and E 20%.

Variable Observed:

1. Average Daily Gain

Average daily growth (ADG) was calculated using the formula from (Nurhadi et al., 2019):

$$\mathbf{ADG} = \frac{Wt - Wo}{t}$$

Information:

ADG = Daily average growth (g/day)
Wt = Average weight of tilapia at the end of the study (g/head)
Wo = Average weight of tilapia at the beginning of the study (g/head)
t = Length of maintenance

2. **Feed Efficiency ;(He & Wurtsbaugh, 1993; Effendie,1997):**

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

Information :
EP = Feed efficiency (%)
Wo = Weight of tilapia at the beginning of the study (grams)
Wt = Weight of tilapia at the end of the study (grams)
F = Amount of feed given (grams)
D = Weight of tilapia that died during the study

3. **Survival Rate (Effendie,1997):**

$$SR = \frac{Nt}{No} \times 100\%$$

Information:
SR = Survival rate or survival (%)
No = Number of fish at the initial of the study (biomass)
Nt = Number of fish at the end of the study (biomass)

4. **Water Quality**

Water quality parameters were observed every 7 days including temperature, degree of acidity (pH), and dissolved oxygen (DO).

Data analysis.

The data obtained consisted of data from chemical test results, identification of fat content of fatty acids and amino acids of the product descriptively. Data on growth and meat quality of juvenile fish were analyzed using analysis of variance (ANOVA) and differences between treatments were analyzed by Duncan's multiple-distance test with 95% confidence level.

RESULTS AND DISCUSSION

Bioconversion Product Description

Bioconversion is a process of overhauling organic waste through a fermentation process by microorganisms to become an energy source (Hsiao et al., 2014). Physical changes in the substrate along with the growth of the mold, which was shown by the change of the uniform white color and the dense texture of the mycelium produced by the mold *R. oryzae*, on the fourth day (Figure 1).

Figure 1. Coconut testa and Coconut testa bioconversion

Figure 1 shows that there is a change in the color and texture of the coconut testa on day 4, which indicates that mold growth is at the end of the log phase to the beginning of the stationary phase. According to (Endrawati & Kusumaningtyas, 2018), a mold of *R. oryzae* is one of the filamentous fungi that can be used as a source of probiotics in the bioconversion process because it is able to convert proteins into bioactive peptides as antimicrobials and antibiotics. In the bioconversion process, chemical changes occur that change the substrate which included the nutritional content of proximate results, amino acids and fatty acids as a medium for growing microorganisms into useful organic products (Table 2).

Table 2. Nutritional content of proximate results, several amino acids and fatty acids from Testa and Coconut Testa Bioconversion (CTB) by *R oryzae*

No	Ingredient	Coconut testa (%)*	CTB by *R oryzae* (%)**
1.	**Proximate Analysis**		
	Crude Protein (%)	5.75-7.75	11..61
	Crude Fat (%)	17.19-26.48	20.55
	Crude Fiber (%)	26.48	14.23
	NFE	52.62	50.88
	Carbohydrate	79.10	65.00
	Ash	2.75	2.73
2	NEAA	0.5	0.6
	L- Glutamat	ND	0.44
	L-Alanin	0.7	1.11
	Glisin	0.35	0.4
	Asam aspartat	ND	0.49
	Tirosin	ND	0.24
3	**Fatty acid composition**		
	Hexanoic/caproate	ND	0.87
	Octanoic	3.9	11.52
	Decanoic	3.8	7.24
	Dodekanoac	40.9	53,47
	Tetradekanoat	20.9	18,65
	Heksadecanoat/palmitate	ND	8,26
	9,12 oktadekadienoic	ND	ND
	9 oktadekanoic	5.3	ND

Note : * Laboratory of Ruminant Animal Nutrition and Animal Feed Chemistry (2020)
** Hasil Analisis SIG (2000)

The results of the proximate analysis of coconut testa experienced an increase in protein, and a decrease in fat and crude fiber (Table 2). *R. oryzae* activity can increase protein levels due to the presence of single cell proteins, also synthesize various kinds of products such as organic acids (lactic and fumaric acids), volatile compounds and enzymes (cellulases, proteases, tannases, xylanases, pyruvate decarboxylases, and lipases)(Length, 2013;López-Fernández et al., 2020). The presence of lipase enzymes can facilitate the breakdown of fats into fatty acids. Protease enzymes are enzymes to catalyze the hydrolysis of peptide bonds into short oligopeptides and free amino acids that are easily absorbed by the body (López-Fernández et al., 2020). One of the protease enzymes produced is aspartate protease. Aspartate protease is widely used in commercial food processing to improve taste and nutritional content (Hsiao et al., 2014). From Table 2, it appears that there was an increase in aspartate and glutamate as substances that can increase feed palatability. Volatile compounds are one of the secondary metabolites of organic acids resulting from the excretion of microorganisms that cause a distinctive odor (López-Fernández et al., 2020).

The decrease in crude fiber content as a result of bioconversion by *R. oryzae* is an indication of the activity of cellulolytic molds. *R. oryzae* can produce lignolytic and phytase enzymes which are useful for breaking down crude fiber and breaking covalent bonds between proteins and phytic acid. *R. oryzae* has amylase, cellulase, and xylanase enzymes which are enzymes that degrade complex carbohydrates into soluble carbohydrate products (NFE) which can be used as a component of energy sources for fish. The activity of *R. oryzae* causes degradation of starch (amylum) and hydrolysis so that added value products are obtained which physically make the texture of the material soft and porous (Dewi & Purwoko, 2005).

The fat content of the coconut testa substrate varied from 17.19 to 26.48% (Table 2), and after bioconversion the total fat was 20.55%. According to Sukma et al.(2018), the components that make up the cell wall and plasma membrane of *R. oryzae* are fat components in the form of phospholipids and lipoproteins. Table 2 shows that there was an increase in the fatty acid composition of the bioconverted coconut testa. However, the content of omega 3 and omega 6 fatty acids was not detected in the coconut testa bioconversion by *R oryzae*. The results of this study also inform that the results of coconut testa bioconversion can improve the quality of fat in order to make it easier for fish to absorb nutrients and support growth rates.

Performance and Feed Efficiency

The performance of red tilapia fed with the use of coconut testa is shown in Table 3.

Table 3. Fish biomass weight and performance of experimental feeding results

	A (0%)	B (5%)	C (10%)	D (15%)	E (20%)
Initial weight	80,63± 0,59	80,43± 0,51	81,13± 0,76	80,87± 0,25	80,83± 0,65
Final weight	124,93±2,51	129,88±7,00	134,23±6,74	138,86±1,38	136,27±3,90
AWG	44,30±3,08	49,44±7,35	53,10±7,45	57,99±1,63	55,43±3,65
ADG	1,04 ± 0,06a	1,14 ± 0,14a	1,20 ± 0,14a	1,29 ± 0,03 a	1,24 ± 0,06 a
FCR	2,96 ± 0,18b	2,72 ± 0,32ab	2,58 ± 0,30ab	2,36 ± 0,04 a	2,44 ± 0,11 a
Feed Eff.	33,82 ± 2,07a	37,07± 4,63ab	39,12± 4,40ab	42,43± 0,71b	41,05± 1,84b
PER	1,21± 0,07a	1,32± 0,17ab	1,40 ± 0,16 ab	1,52± 0,03 b	1,47± 0,07 b
Survival rate	93,3± 0,06a	96,7± 0,06a	96,7± 0,06a	96,7± 0,06a	96,7± 0,06a

Table 3 shows that the growth rate (ADG) showed results that were not significantly different (P>0.05), this was because all treatment feeds were formulated with iso-protein and showed that the feed given was able to be utilized as energy for the growth process. The Feed Efficiency and Protein Efficiency Ratio (PER) showed significantly different results between treatments (P < 0.05). The histogram of feed efficiency and PER values is presented in Figure 2.

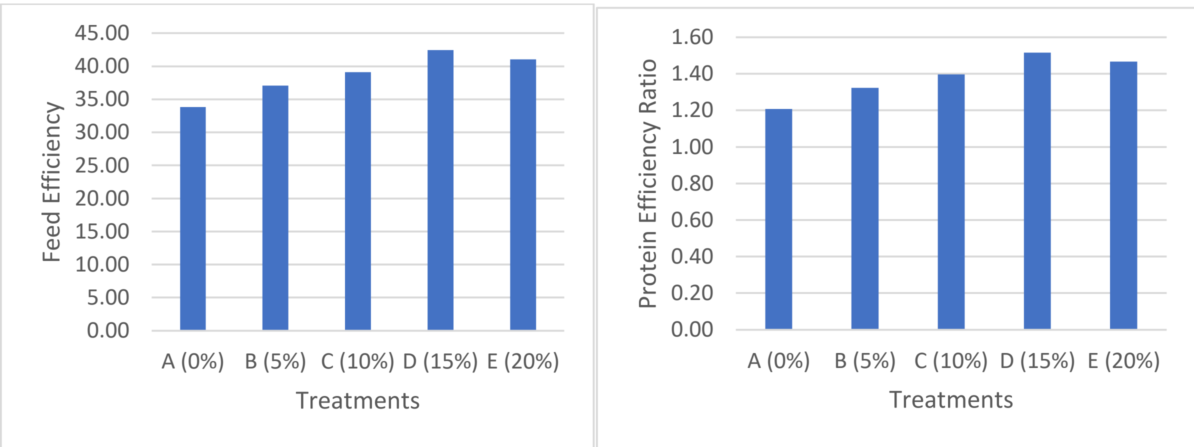

Figure 2. Feed Efficiency dan Protein Efficiency Ratio

The results of analysis of variance showed that the level of use of bioconversion results resulted in significantly different feed conversion ratios, feed efficiency and protein efficiency ratios (p<0.05). From the results of research by (N. Rendika et al., 2019), the addition of R. *oryzae* inoculum additives in feed pretreatment can increase protein digestibility as indicated by the addition of weight of the esophagus, crop, small intestine, cecum and large intestine in monogastric animals. The lipolytic activity produced by the mold causes the hydrolysis of fats into fatty acids and glycerol (Ratledge & Wynn, 2020), as well as the improvement of fatty acids (Table 2). Mold has nucleotide and membrane components in the form of phospholipids and lipoproteins that can help absorb food substances (Purwadaria et al., 2004).

The level of use of coconut testa products by 15% and 20% had a real significance (P<0.05), higher than the coconut testa treatment without the bioconversion process (0%). Coconut testa which is used as a feed ingredient in the treatment formulation is a bioconversion product which means that the compounds in it are simple because *R. oryzae* produces protease, lipase, amylase, phytase and pectinase enzymes that make it easier for fish to digest feed (Selle & Ravindran, 2007).

The protein energy value of all treatments was the optimal DE/P value for fish growth according to Pickard et al., (2020) which ranged from 8-9 (Table 1). DE/P values and digestibility were related to gastric emptying rate and feed response. If the energy of the feed is excessive, the feed tends to be slower to respond to by the fish and will experience nutrient leaching in the water before entering the fish's stomach (Haetami et al., 2017).

From the results of this study, increasing fat and energy content from increasing levels of coconut testa use can improve protein energy balance (DE/P) and affect performance. If the energy level is low, monogastric animals can efficiently utilize energy from protein or from glucogenic amino acids through the gluconeogenesis process, but this can result in reduced protein for growth (Abun et al., 2021). This can be seen from the lower performance results at the level of treatment using coconut testa with bioconversion results from 0-10% (Table 3).

Treatment D with the use of coconut testa bioconverted as much as 15% in the feed formulation had the highest feed efficiency, then treatment E (20%). Based on research (Salza Biila Nuha et al., 2019) the use of coconut testa as much as 5% on red tilapia fish resulted in the highest feed efficiency value, but could be utilized up to 15%. Meanwhile, fermented coconut husk 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 shows that the fungi *R. oryzae* can utilize cellulose as a carbon source for its growth substrate and increase the digestibility of fish to feed (Mustabi et al., 2018).

Feed formulation using fermented coconut testa as an alternative feed ingredient can reduce the cost of red tilapia feed production. *R. oryzae* is a mold that is safe for consumption because it does not produce toxins that have been used as detoxification agents against food toxins, such as Ochratoxine and also to increase the digestibility of plant feed ingredients (Cantabrana et al., 2015). In addition, *R. oryzae* was able to produce phytase enzymes during incubation on the fourth day at 25°C and produced optimum amylase at 30°C (Kanti, 2017). Phytase enzymes can increase nutrient absorption and regulate nutrient excretion (such as phosphorus, nitrogen, and minerals) and can hydrolyze phytic acid (reserved phosphate elements) which can maximize the absorption of feed nutrients so as to increase fish growth (Selle & Ravindran, 2007). Survival is the ratio between the number of living fish in a certain period of time compared to the total number of fish at the beginning of maintenance. Fish with high survival have high endurance and tolerance levels for the environment. Siregar & Adelina (2012) stated that survival or life span can be influenced by biotic and abiotic factors. Biotic factors consist of age and ability of fish to meet feed needs. Meanwhile, abiotic factors include food availability and the quality of living media. Based on the further analysis using Duncan's multiple range test at the 95% confidence level, the survival rate of red tilapia was not significantly different.

Table 4. Water Quality from Feeding Trial

Range	Treatments					Refference
	A	B	C	D	E	
Temp,(℃)	26,1 – 31,2	25,8 – 31,4	26,1 – 30,9	25,8 – 31,4	26 -29	20- 33
pH	7,18-7,80	7,36-7,75	7,33-7,76	7,34-7,75	7,37-7,78	6-8,6
DO (mg/l)	5.1 – 6.3	5.1 – 6.3	5.1 – 6.3	5.1 – 6.5	5.1 – 6.6	>5

Note: a) Moniruzzaman et al., (2015).

Based on the results of observations that have been carried out every week, data on water quality and survival rate give results that are not significantly different from artificial feed without using coconut testa waste. the results of bioconversion, so that it can be used as an alternative feed ingredient for making artificial red tilapia feed up to 20 percent. This is understandable because red tilapia is an adaptable fish, resistant to disease, and can utilize various types of feed. According to (Wardoyo, 2007), tilapia has the potential to be cultivated because it occupies a strategic position in the export market, is easy to breed, grows fast, is resistant to disease, and eats everything (omnivores).

The results showed that the range of survival values in red tilapia ranged from 93.33% to 100%, which means that the use of coconut testa bioconverted up to 20% did not cause fish death so that the dose was included in the safe limit to be mixed with feed. According to (Moniruzzaman et al., 2015), the survival value of fish in the experiment is influenced by water quality, especially pH and ammonia. The survival values indicated that the water quality parameters throughout the experimental period were within the required range for tilapia. Survival rate is the ratio between the number of fish that live at the end of maintenance and the number of fish that live at the beginning of maintenance. Life pass can be used as a benchmark to determine the tolerance and ability of fish to live. Things that affect the value of survival is good feeding so that it can support fish growth through energy obtained from feed. This is also confirmed by Hoque & Tasrin (2018), factors that affect the level of survival are abiotic and biotic, including competitors, density, population, age and the ability of organisms to adapt to their environment.

CONCLUSION

Based on the results of research and analysis, it can be concluded that the use of coconut testa through bioconversion by *R. oryzae* has a significant effect on feed efficiency and protein efficiency ratio of red tilapia seeds. The use of coconut testa as much as 15% resulted in the highest feed efficiency of 42.43% and the highest protein efficiency ratio of 1.52%. However, the use of coconut testa was not significantly different to the survival rate of red tilapia fry and did not produce a negative effect on the water quality of the rearing media. The use of as much as 20% does not have a negative impact on feed efficiency and protein efficiency ratio so as to maximize the use of alternative feed ingredients.

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