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Digestibility of fermented copra meal for fish as a plant protein source in the Saline tilapia (*Oreochromis niloticus*) Seeds

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Abstract. Copra meal is an alternative protein source as a fish meal in fish feed because the nutritional value is almost the same. However, copra meal has a relatively high crude fiber content, so efforts are needed to reduce crude fiber content through the fermentation process using the microbe *Rhizopus* sp. which produces cellulase enzymes. This study aims to determine the digestibility level of fermented copra meal in feed as a source of vegetable protein in the maintenance of saline tilapia (*Oreochromis niloticus*) seeds. The research was conducted with the addition of fermented, dried, and powdered copra meal to the feed with a concentration of (A) 0%; (B) 15%; (C) 30%, and (D) 45%. The fish were given test feed daily with a feeding frequency of once per day. The results showed that giving 15% of the fermented copra meal as a plant protein source in the feed gave a better total feed digestibility compared to the addition of unfermented copra meal.

1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the most popular commodities in farmed fish and becomes an animal protein supply for humans. The flesh is white, moist, and mild-flavored and, as such, versatile for cooking in a variety of menus. Tilapia is also a potential fish for aquaculture because it can adapt to environmental conditions with a wide range of salinity [1]. Such as saline tilapia developed by BPPT in 2008. Saline tilapia can live in waters with high salinity to salt levels > 20 ppt, even in marine waters with salinity up to 32 ppt [2]. However, apart from the wide adaptability of the environment, another factor influencing the success of fish farming is feed. Feed is one of the key factors in aquaculture activities. Its contribution can reach 70% of the total production costs in intensive aquaculture activities, especially for the protein feed component [3].

The availability of feed is often an obstacle in intensive aquaculture because the carrying capacity of natural food from the cultivation area is minimal. For fish to grow normally, supplementary feed is needed. The supplemental feed can be in the form of fresh feed and artificial feed. The use of artificial animal protein supply for humans. The flesh is white, moist, and mild-flavored and, as such, versatile for cooking in a variety of menus. Tilapia is also a potential fish for aquaculture because it can adapt to environmental conditions with a wide range of salinity [1]. Such as saline tilapia developed by BPPT in 2008. Saline tilapia can live in waters with high salinity to salt levels > 20 ppt, even in marine waters with salinity up to 32 ppt [2]. However, apart from the wide adaptability of the environment, another factor influencing the success of fish farming is feed. Feed is one of the key factors in aquaculture activities. Its contribution can reach 70% of the total production costs in intensive aquaculture activities, especially for the protein feed component [3].

The availability of feed is often an obstacle in intensive aquaculture because the carrying capacity of natural food from the cultivation area is minimal. For fish to grow normally, supplementary feed is needed. The supplemental feed can be in the form of fresh feed and artificial feed. The use of artificial

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feed has several advantages. The quality of feed can be controlled and produced in large quantities as needed. The handling technique is easier, its availability can be arranged, and the transportation system is easier.

The main problem in aquaculture is the high price of commercial feed. Feed as a source of energy for growth is a production cost component with the largest amount, namely 40-89% [4]. In addition, the commercial feed has a protein content of around 26-30%, and if the management of the feed is not good, it can accumulate organic matter so that high ammonia levels can accelerate the decline in water quality [5]. This is one of the factors for the increase of disease organisms in fish.

Currently, the component of artificial feed for fish is dominated by the use of fish meal as the primary protein source. On the other hand, fish meal production tends to be unstable due to stagnant fishing yields. Therefore, it is necessary to have an alternative source of feed protein that has a nutritional value performance that is relatively equivalent to a fish meal or can meet the needs of cultivated fish to grow optimally.

One of the vegetable ingredients and sources of terrestrial protein, which is agricultural and industrial waste, can be used in herbivorous red tilapia feed is copra meal. Copra meal flour is a waste from copra processing to produce coconut oil.

Copra meal flour has a relatively high protein content of around 18-24%, although it also has a relatively high crude fiber content of 13-16% [6]. To reduce the crude fiber content of copra meal, a fermentation process can be done using microbes that produce cellulase enzymes.

The copra meal fermentation using *Rhizopus* sp. gave the best results in improving the quality of copra meal by increasing the protein content and increasing the digestibility coefficient of protein and fat for milkfish when compared to *A.niger, S. cerevisiae,* and *B.* subtilis [7]. This study aims to determine the digestibility of fermented copra meal at different doses of saline tilapia. The success in improving the quality of copra meal through the fermentation process of the *Rhizopus* sp. The fungus has made this feed a choice to replace feed with animal protein sources, namely fish meal.

2. Materials and Methods

2.1. Fermented Copra Meal

Copra meal fermentation was carried out based on the method developed by [7] using the type of microbe *Rhizopus* sp. The copra meal fermentation method stages were carried out by sterilizing the copra meal using an autoclave, then cooling it, and then adding the fungal microorganism *Rhizopus* sp. left to stand (Fermentation). The fermentation results were dried in an oven (at a temperature of 50 °C) then grinded, followed by a proximate test.

2.2. Proximate Analysis

The fermented copra meal which has been made into flour, is then analyzed proximate to see its feasibility as an ingredient for milkfish feed. Proximate analysis was carried out based on the Kjeldahl method [8].

2.3. Digestibility Analysis

Stool collection was carried out after feeding, water was removed to dispose of the remaining feed by opening the lower faucet. Before fecal collection, fish were adapted to the test feed for 1 week, and then the fecal collection was carried out every three hours to prevent nutrient leaching in the feces. The collected feces were immediately stored in the freezer until sufficient for analysis and then freeze-dried. Chromium levels in feed and fecal samples were analyzed according to the Takeuchi [9] procedure. The digestibility coefficient of dry matter, protein, fat, and Nitrogen Free Extract (NFE) of the test feed was calculated by the formula [9]:

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$$ADC\,(\%) = 100 * \left\{1 - \left(\frac{M_D * A_F}{M_F * A_D}\right)\right\} \quad (1)$$

ADC = Apparent digestibility coefficient of dry matter, crude protein, fat, and NFE; MD and MF are chromium levels in feed and feces. AD and AF are nutrient levels in feed and feces, respectively.

5 **Table 1**. Feed composition for digestibility trials of copra meal (%)

Feed ingredients	Treatment with additions			
	A	B	C	D
Fish meal	20	20	20	20
Copra cake (%)	0	15	30	45
Tofu waste	18	18	13	6
noodle waste	21	16	16	10
Rice bran	25	15	10	10
Tapioca flour	15	15	10	8
6 Vitamin mix	0.5	0.5	0.5	0.5
Mineral mix	0.5	0.5	0.5	0.5
Chromeoxide	0.7	0.7	0.7	0.7

Information:
Treatment A = feed without the addition of fermented copra meal
Treatment B = 15% of the fermented copra meal
Treatment C = 35% of the fermented copra meal
Treatment D = 45% of the fermented copra meal

2.4. Protein analysis

The sample of 0.5 grams was weighed and put into a Kjedhal flask. Added a mixture of ± 2 grams of selenium and a few grains of boiling stone, then added 10 ml of concentrated sulfuric acid (H_2SO4). Make a blank (selenium + boiling stone + 10 mL concentrated sulfuric acid). Heat it over the digester starting from a low temperature of 1.5 and 3.0 on the digester tool until the reaction is complete. If the solution is in a 100 ml volumetric flask, carefully impart it with distilled water. Let it cool, then distill (distillate). The pipette 10 ml of the squeezed solution is put in a ready distillation tool, add 5 ml of saturated NaOH. It has collected in a 4% ml boric solution and the BCG indicator (Bromogresol green) 2 - 3 drops. Titrate with $HClSO_4$ until it is orange. Record the final volume, calculated by the formula [9]:

Formula:

$$\%\ Nitrogen = \frac{mL\ HCl\ x\ NHCl\ x\ 14\ x\ 10}{\text{Weight of sample in mg}} \quad (2)$$

Note:

(%) N	= Nitrogen
HCl	= chloride acid (mL)
NHCl	= ammonium chloride
14	= molecule weight of nitrogen
Weight of sample in mg	= total weight of example (mg)

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$$\% \, Protein = \% \, N \times 6.25 \quad (3)$$

Note:
% Protein = Protein Percentage
% N = Nitrogen
6.25 = Factor of number (factor of conversion protein content)

2.5. Data analysis
Digestibility data obtained from this study were analyzed for variance (ANOVA). If the variance test results show a significantly different effect, then the LSD test was carried out using the IBM SPSS Statistics 21 Analysis Program.

3. Results and Discussion

3.1. Feed Digestibility
The observations of the digestibility test for feed added with fermented copra meal can be seen in Table 2.

Table 2. Feed composition for digestibility trials of copra meal (%)

Repetition	Treatment			
	A (0%)	B (15%)	C (30%)	D (35%)
1	50.01	48.9	47.99	45.56
2	51.09	49.76	47.01	46.9
3	49.89	47.76	46.78	46.98
Average	50.33± 0.66^{a}	48.80± 1.00^{b}	47.26± 0.53^{b}	46.48±0.80^{b}

Note: The same letter on the same line shows no significant difference (P> 0.0%)

Based on the table above, it can be seen that the highest feed digestibility was obtained from the control feed without the addition of copra meal flour. However, the analysis of variance did not show any difference (P> 0.05) with treatment B (15%) with the addition of copra meal flour. However, the two treatments, A and B, statistically showed a difference (P <0.05) with treatment C and D, although between treatments C and D did not statistically show any difference (P> 0.05) the same. This indicates that the addition of copra meal resulting from the fermentation of above 15% can affect the digestibility of the feed in tilapia seeds. Fish that are the size of the digestive organ are still in a simple form. Even though they are perfect, they still need help from digestive enzymes to digest food (exogenous enzymes). Therefore, the tilapia seeds have not been able to fully digest the food that has been added to the fermented copra meal flour until the addition is above 15-45% because as the addition of copra meal flour increases, the content of feed fiber increases.

The digestibility of feed protein during the study can be seen in Table 3 as follows:

Table 3. Digestibility of feed protein obtained during the study

Repetition	Treatment			
	A (0%)	B (15%)	C (30%)	D (35%)
1	40.89	43.09	40.09	40.98
2	43.97	42.09	40.98	41.3
3	44.98	41.98	41.02	40.09
Average	43.28±2.13a	42.386±0.61a	40.69±0.53a	40.79±0.63a

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Based on Table 3, it can be seen that the highest feed protein digestibility was obtained from treatment A (control), which ranged from 43.28%, and the lowest was obtained from treatment C, which was around 40.69%, although statistically, all treatments were the same there was no difference (P> 0, 05). This is because the protein content in all feeds is almost the same, so that that fish can use the same feed protein. In addition, tilapia is still the size of a seed, so it uses more feed protein as its energy source compared to the fats and carbohydrates in the feed. Furthermore, it is said that the digestibility value of this protein is related to the value of the amino acid content obtained in copra meal because feed ingredients containing more amino acids, especially essential amino acids, will be easily absorbed by fish. As a result, increasing the protein digestibility coefficient. Proximate test results data for each treatment are presented in Table 4

From the proximate test data above, it can be seen that the protein level in treatment B (15%) has a protein content of 36.65%, which is the highest value which means that it has met the optimum protein content for fish, namely 25-50%. According to Watanabe and Cho [10], protein has an important role in normal tissue function, defense, and growth. Protein in its function is the main constituent of the fish body and plays an important role as enzymes and hormones that support metabolism.

Table 4. Results of Feed Proximate Analysis in Each Treatment

Treatment	Parameter (%)				
	Ash	Water	Fat	Protein	Crude Fiber
A (control)	6.79	8.97	3.50	27.50	3.67
B (15%)	10.72	8.18	3.53	36.65	5.14
C (30%)	10.73	9.16	4.43	34.72	4.43
D (45%)	9.78	9.32	4.67	29.70	5.37

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Fat is an organic compound that is insoluble in water but dissolves in organic solvents as the most important energy source for the growth and survival of fish [11]. A good feed generally contains 4 - 18% fat. While the fat content of feed B is 3.53%, then the fat content in the feed can be said to be in a suitable category.

Ash is a residue produced during the combustion process of organic matter in inorganic compounds in oxides, salts, and minerals. The total ash contained in a product is limited in quantity. The ash content in the feed represents the mineral content of the feed, and the appropriate level is 3-7% [12]. The ash content in feed B is 10.72%. This indicates a very high ash content, not following the needs of the fish but has a water content of 8.18%.

Crude fiber is an indigestible part of carbohydrates and is not an essential nutrient in fish. Most of the vegetable fiber is difficult to digest by fish [13]. Crude fiber will cause impurities in the culture container, but it is still needed to facilitate the excretion of feces by forming clumps of dirt [14]. According to [15], the optimal crude fiber content supporting fish growth is 4-8% in tilapia. The proximate test result of crude fiber in treatment B was 5.14%. Compared with the literature, the test feed has crude fiber content following the needs of fish. The excellent fish feed can meet balanced nutrition and according to the needs of fish for optimum growth.

4. Conclusion

The addition of fermented copra meal to the feed resulted in a higher digestibility level compared to unfermented copra meal.

Acknowledgment
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