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The effect of adding coconut pulp resulting from *Aspergillus oryzae* fermentation in commercial feed on the growth of Nile tilapia (*Oreochromis niloticus*)

PURI ELYANA, ARTINI PANGASTUTI♥, ESTU RETNANINGTYAS NUGRAHENI
Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A Surakarta 57 126, Central Java, Indonesia. Tel./fax.: +62-271-663375, ♥email: pangastuti_tutut@yahoo.co.id

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Abstract. *Elyana P, Pangastuti A, Nugraheni ER. 2018. The effect of adding coconut pulp resulting from* Aspergillus oryzae *fermentation in commercial feed on the growth of Nile tilapia (*Oreochromis niloticus*). Cell Biol Dev 2: 33-42.* Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) is one type of fish with the potential as an animal protein source. Quality feed, particularly feed containing basic protein nutrients, is required to increase fishery product production. Coconut pulp is one type of household waste with a relatively high nutritional content, particularly protein, and the potential to be processed into fish feed ingredients. Coconut pulp is initially processed through a fermentation process with *Aspergillus oryzae* (Ahlb.) Cohn, which is expected to increase protein digestibility. This study aims to determine the effect of adding fermented coconut pulp as a mixture in feed on the protein content and growth of Nile tilapia. This study used an experimental method called Completely Randomized Design (CRD). This study lasted 60 days and included four treatments containing fermented coconut pulp and commercial pellets. The composition of each treatment I, II, III, and IV was 75%:25%; 50%:50%; 25%:75%; and 0%:100%. Each treatment was replicated three times. Data analysis revealed that adding 75% fermented coconut pulp to the feed increased the water, fat, and crude fiber content by 25.72%, 20.36%, and 10.56%, respectively. In addition, the growth of Nile tilapia increased after they were fed fermented coconut pulp. The concentration of adding coconut pulp to the optimal feed for Nile tilapia growth and protein content was 25%. However, it is necessary to improve the nutritional composition of the feed to increase growth and meat protein.

Keywords: Coconut pulp, feed, fermentation, *Oreochromis niloticus*

INTRODUCTION

Indonesia is a country rich in biodiversity. One of these riches is the diversity of germplasm species and fishery resources in freshwater, coastal, and marine waters. That is an excellent natural potential for developing the Indonesian fisheries industry. Fish is an outstanding source of animal protein for meeting the Indonesian people's nutritional needs. Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) is a freshwater fish that has the potential to be a source of animal protein (Azwan et al. 2011). Nile tilapia meat contains 17.5% protein, 4.1% fat, and 74.8% water (Suyanto 2002). Nile tilapia has several advantages, including quick growth, high environmental tolerance, a large body size, good taste, a high survival rate, and ease of maintenance. However, to meet the nutritional needs of humans as consumers, the nutritional quality of the fish must also be considered.

Feed is essential for aquaculture species to grow properly and effectively. Quality feed ingredients can help increase fishery product production. Unfortunately, cost constraints are still a frequent impediment. Feed is the most expensive component of aquaculture production (can reach 60-70%). High feed prices in Indonesia are caused by imported raw materials such as soybean meal, fish meal, and even corn, even though the country has been self-sufficient (Amri 2007). Indonesia is vulnerable to market fluctuations due to its reliance on imported raw materials for feed. As a result, it is critical to investigate more sustainable approaches and strategies, such as developing the domestic feed raw material industry, while also ensuring that the origin of these ingredients can be easily traced. Plant protein sources can be used as animal protein sources.

Coconut pulp as a potential vegetable source for animal feed should be investigated as a fish feed additive. Aside from being readily available, using coconut pulp as a vegetable component in fish feed is expected to boost the nutritional value of the feed. Coconut pulp contains 13.35% water, 17.09% protein, 9.44% fat, 23.77% carbohydrates, 5.92% ash, and 30.4% crude fiber (Mujiman 1985). According to Derrick (2005), the crude protein content of coconut pulp reaches 23%, and its easily digestible fiber content makes coconut pulp suitable for use as feed ingredients. Using a biotechnology approach through fermentation is one way to increase the usability of protein and the value of the benefits of coconut pulp. According to Miskiyah et al. (2006), fermentation coconut pulp with *Aspergillus niger* Tiegh. mold increased protein content by 130%, from 11.35% to 26.09%.

Aside from *A. niger*, the mold of *Aspergillus oryzae* (Ahlb.) Cohn can be used to boost the nutritional value of feed ingredients, particularly protein content. The *A. oryzae* is the mold that produces the most enzymes, including a-amylase, a-galactosidase, glutaminase, protease, and β-

glucosidase. The most important of these enzymes are protease and amylase enzymes, which work to break down protein and starch from substrates. For example, the a-amylase enzyme produces glucose by breaking the a-1,4 bond, whereas the β-glucosidase enzyme breaks the β-1,6 bond in the branched-chain and converts dextrins to glucose (Purwoko 2007). The *A. oryzae's* protease enzyme converts long polymer chains from proteins into amino acids, increasing amino acid nitrogen and total acid levels (Gandjar 1977).

The objectives of this study were as follows: (i) to determine the nutritional content of the feed, which included protein, fat, carbohydrate, ash, and water content after the addition of coconut pulp fermented by *A. oryzae*; and (ii) in determining the nutritional content of the feed after the addition of coconut pulp fermented by A. oryzae. (ii) To assess the growth of Nile tilapia after feeding with coconut pulp fermented *A. oryzae* at various concentrations. (iii) To identify the optimal concentration of coconut pulp fermented *A. oryzae* to feed to boost Nile tilapia growth.

MATERIALS AND METHODS

Research site and time

This study was carried out at the Biology Sub-Laboratory of the Faculty of Mathematics and Natural Sciences Central Laboratory, the Soil Science Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, and the PBIAT Janti Satker, Klaten, Central Java, Indonesia.

Materials

The main materials used were a pure culture of *A. oryzae* from the Inter-University Center, Universitas Gajah Mada, Yogyakarta, Indonesia. Other materials were fresh coconut pulp from household waste and male Nile tilapia.

Experimental design

This experiment used a completely randomized design consisting of four treatments, each with three replications. The treatment is shown in Table 1.

Creating work culture

PDA (3 g) and distilled water (77 mL) were put in a beaker, then heated to boiling on a hot plate and stirred with a magnetic stirrer until homogeneous. Next, the PDA solution was placed in 4 ml test tubes, tightly closed with cotton and aluminum foil, tied with a rubber band, and autoclaved at 121°C for 15 minutes. The tube was then tilted so that the PDA inside was tilted and no moisture was present before inverting its position and waiting for it to cool. Next, *A. oryzae* spore suspension was inoculated into slanted agar media and incubated for 3-5 days at room temperature. Finally, the culture was ready for use as a working culture, while the remainder was stored as stock culture in an incubator at 4ºC.

Coconut pulp fermentation

Ten (10) kg of coconut pulp were sun-dried. After the coconut pulp had dried, it was mashed, and 800 ml of water was added. The mixture of water and coconut pulp was steamed for 30 minutes, then cooled on a Formica plastic. After cooling, minerals containing 360 g $(NH_4)2SO_4$, 200 g Urea, 75 g NaH_2PO_4, 25 g $MgSO_4$, and 7.5 g KCl were added, followed by 10 oses of *A. oryzae* spores. It was then mixed and stirred until homogeneous. The mixture was placed on a plastic tray with a thickness of 1 cm and then fermented aerobically at room temperature for 2 days. Afterward, the mixture was wrapped in plastic, compacted without air (enzymatic process occurred), and incubated at room temperature for 2 days. Then, the mixture was dried, ground into pellets, and stored (Purwadaria et al. 1995a,b).

The pond with a size of 15m x 3m x 0.5m was divided into 12 plots with a size of 1m x 1m each. Each plot was filled with water up to 30 cm from the bottom of the pool. Then 180 male Nile tilapia fish that were 2 months old were put in plots, each plot consisting of 15 fish. Each plot was also given an aerator to increase water aeration. Before treatment, the Nile tilapia were acclimatized for 10 days. After acclimation, 5 fish were taken in each plot to collect data on the initial protein content of the study.

Supporting data to determine pond water quality is the water's physical and chemical conditions, which is the fish's environment. The data measured include DO and temperature measured by DO meter, pH measured by pH meter, and ammonia content measured by distillation.

The weight of the fish as measured by the O'Hauss scale and the standard length of the fish as measured with a ruler were the variables used to determine the growth of the fish. Fish were fed various rations that included varying concentrations of fermented coconut pulp. Feeding occurs thrice daily, at 08.00, 12.00, and 16.00. According to Afrianto and Liviawaty (2005), the feeding interval is 4 hours because the fish require food every 3-4 hours after eating. When the temperature rises, Nile tilapia activity rises, and the fish become hungry quickly. Feed concentration was up to 5% of the Nile tilapia's body weight, with 3 replications in each treatment. Every day, before feeding in the morning, the rest of the feed and feces were cleaned, and as much water as was issued was added (Rukmana 1997).

Water quality data was collected once every 10 days, followed by measurements of total weight and standard length of fish, and data collection was done in the morning before feeding. The value of protein retention was determined by observing the fish protein content test at the beginning and end of the study. In addition, the muscle tissue (meat) of the dorsal part of Nile tilapia from each treatment was collected.

Table 1. Comparison of fermented coconut pulp concentration and commercial pellets

Commercial treatment (%)	Fermented coconut pulp (%)	Pellets (%)
P_I	75	25
P_{II}	50	50
P_{III}	25	75
P_{IV}	0	100

Analysis of feed nutrition before and after fermentation

Measurement of protein content using the Kjeldahl Method

Samples of 1-2 grams were taken and placed in a Kjeldahl flask with 3 grams of the digestion mixture (1 part $CuSO_4$ and 9 parts K_2SO_4), and 20 ml concentrated H_2SO_4. The Kjeldahl flask was heated on a heating stove until the solution's original black color changed to a clear color during heating. After the digestion, the Kjeldahl flask was cooled, the inner surface of the flask was rinsed with distilled water, and the solution was mixed until homogeneous. The destruction sample solution was placed in a steam distillation device, and three drops of phenolphthalein indicator were added. The collecting solution was placed in a beaker (containing 50 mL of a 2% boric acid solution and 5 drops of Tashiro's indicator) under the cooler tip, which was immersed in the collecting solution. The concentrated NaOH solution was then gradually poured until the sample solution became alkaline. The distillation was complete when the distillate dripping reacted neutrally to red litmus, and the reservoir solution turned green. Next, the reservoir solution was titrated with 0.1 N HCl solution until the color returned to pink.

Protein content was calculated by the following formula (Sudarmadji 1997):

$$\text{Protein content} = \frac{\text{mL HCl x N HCl titrate x 14 x 6.25}}{\text{g sample x 1000}} \text{ X 100\%}$$

Measurement of fat content

The filter flask (extraction) containing boiling stone grains was dried in a dryer at 105°-110°C for 1 hour, then cooled in a desiccator and weighed (a). The sample was weighed to 1 g (X), placed in a filter sleeve, and wrapped in cotton. The filter sleeve was inserted into the Soxhlet, and then chloroform filtered until clear. The filter flask was dried in a dryer at 105°-110°C for 1 hour, then cooled in a desiccator until a constant weight was obtained. (b).

The following formula calculated fat content:

Fat content = b-a/x.100%

Where:
b : Constant (final) weight of the pumpkin
a : Pumpkin initial weight
X : Sample weight (Anggorodi 1979)

Measurement of the crude fiber content

The filter flask (extraction) containing boiling stone grains was dried in a dryer at 105°-110°C for 1 hour, then cooled in a desiccator and weighed (a). The sample was weighed to 1 g (X), placed in a filter sleeve, and wrapped in cotton. The filter sleeve was inserted into the Soxhlet, and then chloroform filtered until clear. The filter flask was dried in a dryer at 105°-110°C for 1 hour, then cooled in a desiccator until a constant weight was obtained:

Crude fiber content = (Y-Z-A)/X.100%

Where:
Y : Weight of filter paper after final drying
Z : weight of filter paper after initial drying
A : Weight of filter paper after curing
X : Sample weight (Anggorodi 1979)

Water level measurement

The bottles and caps were weighed and dried at a temperature of 105°-110°C for 10-12 hours before being cooled in a desiccator for 30 minutes and weighed. Next, a 1 g sample was placed in a dried bottle. Finally, the bottle and its contents were weighed, and the contents were dried at 105°-110°C until a constant weight was obtained (B). The following formula calculated the water level:

Water content = (A-B)/A.100%

Where:
A: Initial bottle weight
B: Constant bottle weight (Tillman et al. 1989)

Measurement of ash content

Porcelain was dried in a dryer at a temperature of 105-110°C, then cooled in a desiccator and weighed (X). Next, a sample of 1 gram was put into the porcelain (Y), then burned on a bunsen until no smoke came out. Next, the porcelain dish and the burned sample were put in an oven at 400°C until the sample turned white, then cooled and weighed (Z). The following formula calculated ash content:

Ash content = Z-Y/X.100%

Where:
Z : Final weight of porcelain cup and sample
X : Porcelain cup weight
Y : Sample weight (Anggorodi 1979)

Measurement of carbohydrate levels

The measurement of carbohydrate content is carried out using the "Carbohydrate by Difference" method (Nugroho 1999):

% carbohydrate = 100% - (protein+lipid+ash+water)%

Nile tilapia growth analysis

Measurement of the growth of Nile tilapia: (i) The Weight of the Nile tilapia was weighed using an O'Hauss scale. (ii) The standard length of the Nile tilapia was measured from the tip of the front of the head to the crease of the base of the caudal fin using a ruler and millimeter paper.

The following formula calculated the degree of Survival (Effendi in Fuad 1996):

S = Nt/No x 100%

Where:
S : Survival rate
Nt : Number of fish at the end of the study
No : Number of fish in the initial study

The following formula calculated the daily Growth Rate (Effendi in Fuad 1996):

$$SGR = \frac{\text{In Wt} - \text{In Wo}}{t2 - t1} \times 100\%$$

Where:
Wt : Final Weight of fish
Wo : Initial Weight of fish
t_1 : Start time (days)
t_2 : Finish time (days)
SGR : Daily growth rate (%)

Protein retention (PR), according to Buwono (2004), was calculated by the following formula:

$$PR \quad \frac{\text{JPS end (g)} - \text{JPS start (g)}}{\text{JPB (g)}} \quad X\ 100\%$$

Where:
JPS *end*: The amount of protein stored in the fish body at the end of the study (g)
JPS *start*: The amount of protein stored in the fish body at the start of the study (g)
JPB: The amount of protein given (g)

Feed Efficiency (FE), according to Huisman in Ing Mokoginta et al. (1995) calculated by the following formula:

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

Where:
Wt : Final weight of Nile tilapia
Wo : Initial Weight of Nile tilapia
D : The Weight of dead Nile tilapia
F : weight of feed given

Sampling technique

This study used random sampling as its sampling technique. Three replications were performed to test the nutritional content of the feed, fish protein content, and growth of each treatment group.

Data collecting technique

For 60 days, data on Nile tilapia growth was collected every 10 days. The growth of Nile tilapia was observed three times by weighing and measuring the standard length of Nile tilapia as a growth parameter. The research data includes observational data. In addition, the protein content of the fish was measured at the beginning and end of the study.

Data analysis technique

The results of the observations were analyzed using analysis of variance to determine whether or not the effect on the parameters measured in this study is real (ANOVA). If the treatment has a significant effect or is significantly different, the DMRT (Duncan's Multiple Ranges Test) was used with a test level of 5% to pinpoint the location of the difference in influence between treatments.

RESULTS AND DISCUSSION

Coconut pulp fermentation

One of the methods used to convert coconut pulp into feed using *A. oryzae* is fermentation. The fermentation process consists of two stages: aerobic fermentation and anaerobic fermentation. Similar studies on coconut cake have been conducted in the past (Purwadaria et al. 1995a,b). Mycelium was present during the growth of *A. oryzae* during the fermentation process. The appearance of fine thread-like fibers and the pulp compaction indicates the mycelium's growth. As a result, after fermentation, unfermented coconut pulp has a different structure, color, odor, and chemical composition (Table 2).

Table 2 shows that the water content increased by 12.84% after fermentation. The high increase in water content was caused by *A.* oryzae's respiration process, which increased the water content in the pulp. Meanwhile, after fermentation, the ash content of coconut pulp decreased from 5.92% to 3.15%. This decrease was caused by the fact that *A. oryzae* required mineral salts as cofactors for enzymes involved in metabolism during its growth (Advisory Committee on Technology Innovation 1979).

After fermentation, the fat content of coconut pulp increased from 9.44% to 20.35%. This increase in fat content is possible due to the *A. oryzae* mold's low lipase activity, which prevents it from optimally degrading fat into fatty acids, and its high amylase activity, which allows it to remodel carbohydrates optimally.

Furthermore, fat is not used as an energy source during fermentation; carbohydrates are the primary energy source. This metabolism allows for the conversion of various carbohydrates into fat (Kasmidjo 1990). Acetyl Co-A is the key compound that connects carbohydrate metabolism to fatty acid synthesis. If the cells in the body have more glucose than they require for energy, they will convert some of the acetyl Co-A produced by glucose catabolism into fatty acids (Wilbraham and Matta 1992).

Table 2. Proximate analysis of coconut pulp before and after fermentation using *Aspergillus oryzae*

Analysis	Before fermentation (%)	After fermentation (%)
Water	13.35	26.19
Ash	5.92	3.15
Fat	9.44	20.35
Protein	13.09	13.63
Crude fiber	30.40	10.15
Carbohydrate	23.77	26.53

Coconut pulp's protein content increased from 13.09% to 13.63%. Compared to previous studies, the increase in protein levels was very small. According to Miskiyah et al. (2006), the protein content of coconut pulp increased from 11.35% to 26.07% or 130% after fermentation with *A. niger*. This minor increase in protein content was most likely caused by *A. oryzae*'s activity of low protein consumption compared to amino acid synthesis. Fungi can synthesize amino acids, including phosphoenolpyruvate and a-ketoglutarate. Molds produce proteins and amino acids by utilizing the carbon and nitrogen skeletons found in the substrate (Cochrane 1958 in Gusmanizar and Rahman 2000).

The amount of energy in the feed can be estimated using crude fiber. The three components of crude fiber are cellulose, hemicellulose, and lignin. After fermentation, the crude fiber content of coconut pulp decreased from 30.40% to 10.15%. The high activity of the cellulase enzyme in *A. oryzae* mold allowed it to degrade cellulose and hemicellulose in coconut pulp, decreasing fiber content. A fish feed with a low crude fiber content will be easily digested because fish do not have cellulase enzymes in their digestive tract that can degrade cellulose and hemicellulose (Gusmanizar and Rahman 2002).

Fish feed

According to Sahwan (2002), one of the most important factors in maximizing fishery productivity is the availability of feed in sufficient quantities, on time, and with good nutritional value. That is because the natural feed content available at the location is insufficient to meet the needs of the fish, necessitating the use of additional feed. However, feed quality must also be considered because it significantly impacts the fish growth rate.

There were four types of feed treatments in this study, each with a different composition: treatment I was 75% fermented coconut pulp and 25% commercial pellets, treatment II was 50% fermented coconut pulp and 50% commercial pellets, treatment III was 25% fermented coconut pulp and 75% commercial pellets, and treatment IV (control) was 100% commercial pellets. A feed quality test was performed to determine the nutritional value of feed in each treatment, which included protein, fat, carbohydrate, ash, crude fiber, and water content levels. According to statistical analysis (appendices 1-6), each test in each treatment produces a significant difference (Table 3).

The water content test determines the amount of water in the feed for each treatment. According to Sahwan (2002), the water content in the feed should not exceed 10% so that fungus does not overgrow the feed. Statistical analysis showed a significant difference between treatments III and IV, indicating that treatments III and IV had different water content. Treatments I and II were also noticeably different from treatments III and IV. The higher the percentage of fermented coconut pulp, the higher the water content in each treatment, as shown in Table 3. This high-water content is caused by *A. oryzae* respiration in the pulp during the fermentation process, which increases the water content in the pulp, causing an increase in the water content of the feed.

The ash content of the feed indicates the amount of mineral content in the feed (Jangkaru 1974). Fish require minerals during the growth process, but only in trace amounts. Calcium (Ca) and phosphorus (P) is required for bone formation and to keep body tissues functioning normally. Iron (Fe) is required for the formation of red blood cells, and manganese (Mn) is required for reproduction (Sahwan 2002). Statistical analysis shows a significant difference between treatments, indicating that each has a different ash content. According to Table 3, the higher the percentage of fermented coconut pulp added to the feed, the lower the ash content of the feed. The highest ash content was 9.35% in treatment IV (control), and the lowest ash content was 3.40% in treatment I. The decrease in ash content was caused by the fact that *A. oryzae* required mineral salts as cofactors for enzymes that were important in metabolism during its growth, resulting in a decrease in ash content in the feed in each treatment with varying levels of fermented coconut pulp.

The presence of fat in the feed affects its taste and texture. Mudjiman (1985) states that the ideal fat content for fish feed ranges from 4 to 18%. According to statistical analysis, there was a significant difference between treatments, indicating that the fat content of each treatment differed. According to Table 3, the percentage of high-fat content in the feed was followed by a high percentage of fermented coconut pulp addition. Treatment IV (control) had the lowest fat content of 5.73%, and treatment I had the highest fat content of 20.36%. The increase in fat content was possible because the lipase enzyme activity in *A. oryzae* was low, making degrading the fat in coconut pulp into fatty acids less efficient. Furthermore, fat is not used as an energy source during fermentation; carbohydrates are the primary energy source. This metabolism enables the conversion of various carbohydrates to increase the fat content of coconut pulp, thereby influencing feed treatment.

Table 3. Nutritional data for feed after the addition of fermented coconut pulp

Treatment	The nutritional content of the feed					
	Water level (%)	Ash (%)	Fat (%)	Protein (%)	Crude fiber (%)	Carbohydrate (%)
P_I	25.72^{c}	3.40^{a}	20.36^{d}	13.40^{a}	10.56^{b}	37.13^{a}
P_{II}	24.11^{c}	5.03^{b}	14.79^{c}	18.17^{b}	8.22^{ab}	37.91^{a}
P_{III}	19.35^{b}	6.68^{c}	12.23^{b}	23.46^{c}	6.03^{a}	38.29^{a}
P_{IV}	10.61^{a}	9.35^{d}	5.73^{a}	29.34^{d}	5.66^{a}	44.98^{b}

Notes: Numbers followed by different letters indicate a significant difference in the DMRT test at the 5% test level in the vertical direction. PI: 75% fermented coconut pulp and 25% commercial pellets, PII: 50% fermented coconut pulp and 50% commercial pellets, PIII: 25% fermented coconut pulp and 75% commercial pellets, PIV: 100% commercial pellets (Control)

Carbohydrates are the last element in the fish growth process because the fish's ability to digest carbohydrates is very low. Therefore, fish carbohydrate needs are relatively small and tend to be used as a source of carbon skeletons for protein synthesis (Tacon 1987). The highest carbohydrate content in treatment IV (control) was 44.98%, and the lowest in treatment I was 37.13%. The analysis showed treatments I, II, and III significantly differed from treatment IV. From Table 3, it can be seen that the low carbohydrates in treatments I, II, and III were probably because carbohydrates were used as the main energy source during the coconut pulp fermentation process by *A. oryzae*, causing low carbohydrates in the feed.

Protein is a chemical compound needed by the fish body as a source of energy and for growth. The research results show that the relationship between growth and protein content is directly proportional; the more protein content in the feed, the higher the growth. Table 3 above shows that the highest protein content in treatment IV was 29.34%, and the lowest protein content in treatment I was 13.40%. The higher the addition of fermented coconut pulp in the feed, the lower the protein content of the feed. That was due to the low activity of protein consumption compared to the synthesis of amino acids by *A. oryzae* during the fermentation process. Molds carry out the biosynthesis of proteins and amino acids by utilizing the carbon and nitrogen skeletons available in the substrate.

Table 3 shows that the crude fiber content in the feed is increasing along with the large percentage of fermented coconut pulp, even though the crude fiber is difficult to digest by fish. From Table 3, it can be seen that in treatment I, with the addition of 75% of fermented coconut pulp, the highest crude fiber content was 10.57%. The high crude fiber content in fish feed will affect the digestibility and absorption of food substances in the fish's digestive tract. The crude fiber content of less than 8% will improve the structure of the feed, but if the crude fiber exceeds 8% will reduce feed quality (Djajasewaka 1995).

Feed efficiency (FE)

The growth of fish with indicators of fish weight and the amount of feed given during the study can show feed efficiency (Figure 1). Feed efficiency calculates the amount of feed that enters the fish's digestive system for the body's ongoing metabolic processes, one of which is growth. Protein is fish's primary energy source, followed by fat and carbohydrates. Therefore, protein is a nutrient that is required for growth. Protein utilization for growth is influenced by several factors, including fish size/age, protein content, feed energy content, water temperature, and feeding level (Mokoginta et al. 1995).

Food is digested in the intestine and then transported to the liver via blood vessels via passive diffusion, active transport, and pinocytosis. Protein is absorbed in the form of amino acids, which are then transported to the liver to be converted back into protein, which has been tailored to the needs of the fish body (Wedemeyer 1996). The qualitative and quantitative composition of the amino acid mixture determines the pattern of amino acid absorption in the intestine. Amino acids are taken up by blood capillaries from the mucosa after active absorption by intestinal mucosal cells and transported in plasma and body tissues for metabolism (Noor 1990).

The higher the feed efficiency value, the faster the growth rate. According to the above study's findings, treatment IV had the highest feed efficiency of 0.28 grams, while treatment I had the lowest. The feed efficiency value (FE) was 0.28, meaning that for every gram of feed, 0.28 grams was digested into the fish's digestive system. This high FE value is followed by a good nutritional content of the feed (Table 3), one of which is the low crude fiber content of 5.66%, making the feed easy to digest for fish. Furthermore, protein and carbohydrate levels are high, allowing for rapid growth. Meanwhile, the low FE value was followed by the feed's low nutritional content, with a high crude fiber content of 10.56%. Whereas crude fiber in feed should not exceed 8% because it interferes with digestion and absorption of food substances, lowering the quality of fish feed (Mudjiman 2004).

Nile tilapia growth

Growth is an increase in length and weight over a specific period. Growth is influenced by nutrients (food), which include protein, fat, carbohydrates, vitamins, and minerals, as well as water and oxygen, in addition to genetic factors and hormones. Protein is the most important feed substance for fish weight gain, according to Djajasewaka and Suhenda (1992). Feeds with a high protein content will be more effective and efficient for growth because protein, like fat and carbohydrates, is an important substance. Growth hormone has the greatest influence on protein utilization for growth. Growth hormone increases the transport of amino acids across the membrane or speeds up the chemical process of protein synthesis, increasing tissue protein.

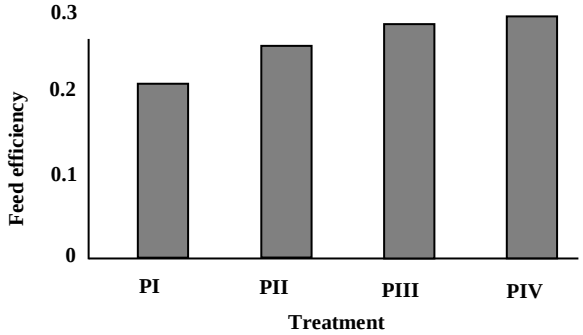

Figure 1. Feed efficiency during research. Notes: PI: 75% fermented coconut pulp and 25% commercial pellets, PII: 50% fermented coconut pulp and 50% commercial pellets, PIII: 25% fermented coconut pulp and 75% commercial pellets, PIV: 100% commercial pellets (control)

Nile tilapia fish weight

The results of the research and statistical analysis of Nile tilapia growth with weight indicators revealed a significant difference ($p<0.05$) from each treatment. Treatment IV (control), which contained 100% commercial pellets without the addition of fermented coconut pulp, produced the greatest weight gain. While treatment I, which contained 75% fermented coconut pulp and 25% commercial pellets, produced the lowest weight gain (Table 4).

This Nile tilapia weight difference demonstrates a proportional relationship between protein quantity and fish weight growth. The more feed protein there is, the more effective it is at increasing fish weight. According to Utojo (1995), the amount and type of essential amino acids in the feed determines the amount and type of protein in the body, among other things.

Compared to the results obtained with commercial pellet feed alone, the value of weight gain in Nile tilapia with a combination of fermented coconut pulp and commercial pellet feed was still relatively small. That does not, however, preclude the use of feed with this fermented coconut pulp mixture. According to the DMRT test, there was no significant difference between treatment III, which still contained 25% fermented coconut pulp, and treatment IV, which contained 100% commercial pellets, so feed that only consisted of commercial pellets could be replaced with feed that also contained fermented coconut pulp, a 25% content in addition to commercial pellets as one of the raw materials. That is due to the nutritional content of the feed being sufficient to support fish growth.

Nile tilapia fish length

The results of the research and statistical analysis of Nile tilapia growth with weight indicators revealed a significant difference ($p<0.05$) from each treatment. Treatment IV (control), which contained 100% commercial pellets without the addition of fermented coconut pulp, produced the greatest weight gain. While treatment I, which contained 75% fermented coconut pulp and 25% commercial pellets, produced the lowest weight gain (Table 4).

This Nile tilapia weight difference demonstrates a proportional relationship between protein quantity and fish weight growth. The more feed protein there is, the more effective it is at increasing fish weight. According to Utojo (1995), the amount and type of essential amino acids in the feed determines the amount and type of protein in the body, among other things.

Compared to the results obtained with commercial pellet feed alone, the value of weight gain in Nile tilapia with a combination of fermented coconut pulp and commercial pellet feed was still relatively small. That does not, however, preclude the use of feed with this fermented coconut pulp mixture. According to the DMRT test, there was no significant difference between treatment III, which still contained 25% fermented coconut pulp, and treatment IV, which contained 100% commercial pellets, so feed that only consisted of commercial pellets could be replaced with feed that also contained fermented coconut pulp, a 25% content in addition to commercial pellets as one of the raw materials. That is due to the nutritional content of the feed being sufficient to support fish growth.

Nile tilapia fish protein content

The fish's body converts the protein in the feed into protein based on its requirements (Table 5). There are two basic chemical processes for protein synthesis: amino acid synthesis and the conjugation of suitable amino acids to form each type of protein in each cell. That is the most fundamental growth process because growth is impossible without large-scale protein production (Fujaya 2004).

Protease and peptidase enzymes secreted by the intestines catalyze the breakdown of proteins into amino acids. Amino acids are required for protein synthesis, which plays a role in replacing damaged cells and forming body tissues, increasing tissue protein. This increase in tissue protein is reflected in the fish's increased body weight and length (Murray et al. 1996).

Protein analysis and analysis of variance ($p<0.05$) revealed no significant difference ($p>0.05$) between treatments. According to Djuanda (1981), some food consumed is converted into energy used for living activities, and some food is expelled from the body. As a result, not all of the protein in the fish's diet is converted into meat. Furthermore, the physiological ability of fish influences the formation of meat protein.

Table 4. Nile tilapia fish weight and length

Treatment	Fermented coconut pulp level (%)	Fish weight (gram)	Fish length (cm)
PI	75	26.66^{a}	11.38^{a}
PII	50	27.78^{a}	11.84^{ab}
PIII	25	32.09^{ab}	12.83^{bc}
PIV	0	35.97^{b}	13.76^{c}

Note: Numbers followed by letters indicate a significant difference in the DMRT test at the 5% test level. PI: 75% fermented coconut pulp and 25% commercial pellets, PII: 50% fermented coconut pulp and 50% commercial pellets, PIII: 25% fermented coconut pulp and 75% commercial pellets, PIV: 100% commercial pellets (Control)

Table 5. Nile tilapia fish protein content

Treat-ment	Fermented coconut pulp level (%)	Feed protein content (%)	Fish protein content (%)
PI	75	13.40	13.40
PII	50	18.17	18.17
PIII	25	23.50	23.50
PIV	0	29.34	29.34

Notes: Numbers followed by letters indicate a significant difference in the DMRT test at the 5% test level. PI: 75% fermented coconut pulp and 25% commercial pellets, PII: 50% fermented coconut pulp and 50% commercial pellets, PIII: 25% fermented coconut pulp and 75% commercial pellets, PIV: 100% commercial pellets (Control)

Protein retention

Protein retention depicts the amount of protein provided that can be absorbed and used to build or repair damaged cells and by the fish body for daily metabolism (Figure 2). The amount of protein that can be absorbed and used by the body as a building block determines whether or not fish grow quickly. For fish to grow normally, the ration or feed must contain enough energy to meet daily metabolic energy needs and enough protein to meet the development needs of new body cells.

The protein retention value represents the protein deposition index in body tissues (used for growth). Protein retention refers to the amount of protein stored in the body of a fish. The use of feed protein is expected to increase body protein or cause growth (Suhenda et al. 2003).

According to Figure 2, treatment I had the lowest protein retention of 0.056%, while treatment IV (control) had the highest protein retention of 0.089%. The low protein retention is most likely caused by the feed's low protein content, which cannot meet the energy requirements to build or repair damaged body cells and fish metabolism daily. Protein is a very important nutrient that is required for body maintenance, tissue formation, replacement of damaged body tissues, and increasing body protein in growth, according to Cowey and Sargent (1979).

The energy content of the feed also influences protein utilization to form tissue. The higher the energy content of the feed, the better the utilization of protein by the fish body, resulting in increased body tissue formation.

Daily growth rate

The growth rate of fish will accelerate as the protein content of the feed increases. Fish use protein feed for body maintenance, tissue growth, protein addition to the body, and tissue replacement (Cowey and Sargent 1979). Figure 3 depicts the daily growth rate for each treatment.

Figure 3 shows that treatment IV had the highest daily growth rate (control). The treatment with the lowest daily growth rate was treatment I, which contained 75% fermented coconut pulp with a protein content of 13.40%. In addition to the low protein content, the crude fiber content of the treatment I fed was still high, causing Nile tilapia growth to be lower than in other treatments. Fiber has several physiological effects, including reducing nutrient availability. Additionally, fish digestive organs are less able to digest fiber perfectly, resulting in decreased nutritional intake and low growth.

The low daily growth rate in each feed treatment with the addition of fermented coconut pulp indicates that Nile tilapia is less responsive to the feed given and cannot consume feed optimally, resulting in a low growth rate because the body's energy supply is reduced.

The amount and balance of feed nutrients affect the growth rate, which means that the nutritional composition of feed raw materials can complement each other's nutrient needs, increasing the growth rate and nutritional content of fish. For example, Table 3 shows that the feed containing fermented coconut pulp generally has a less balanced nutritional composition than the control feed, with high water, fat, and crude fiber content.

Survival rate

The survival rate in this study was relatively high. Suyanto (2002) believes that a 30-50% mortality rate is still considered normal. Nile tilapia generally died after sampling, specifically during length measurement and weighing. The most common causes of fish mortality are bacterial, fungal, vitamin C deficiency, and nutritional imbalances in feed (Sutarmat et al. 2003).

There was no significant difference based on the analysis of variance, implying that the survival percentage in each treatment is nearly the same (Table 6). That demonstrates that the inclusion of fermented coconut pulp in the feed does not affect Nile tilapia survival.

The observations during the study's 60-day period revealed that dead Nile tilapia did not indicate disease attacks on Nile tilapia. That is demonstrated by the lack of morphological organ damage in the fish body due to bacterial or fungal attacks. After 24 hours, dead fish usually float to the water's surface.

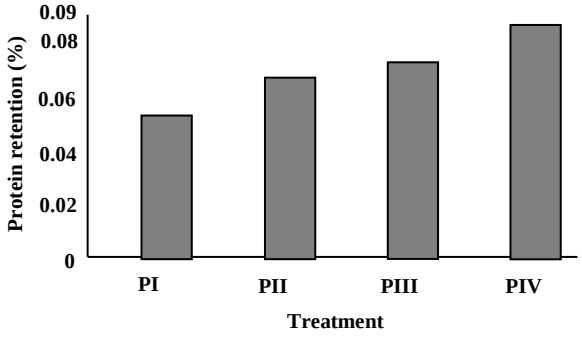

Figure 2. Protein retention. Notes: PI: 75% fermented coconut pulp and 25% commercial pellets, PII: 50% fermented coconut pulp and 50% commercial pellets, PIII: 25% fermented coconut pulp and 75% commercial pellets, PIV: 100% commercial pellets (control)

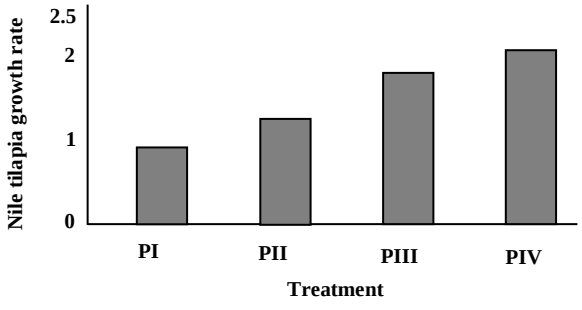

Figure 3. Nile tilapia growth rate during the research. Notes: PI: 75% fermented coconut pulp and 25% commercial pellets, PII: 50% fermented coconut pulp and 50% commercial pellets, PIII: 25% fermented coconut pulp and 75% commercial pellets, PIV: 100% commercial pellets (control)

Table 6. The survival rate of Nile tilapia

Treatment	Survival rate (%)
PI	98.89[a]
PII	96.67[a]
PIII	96.67[a]
PIV	97.78[a]

Notes: PI: 75% fermented coconut pulp and 25% commercial pellets, PII: 50% fermented coconut pulp and 50% commercial pellets, PIII: 25% fermented coconut pulp and 75% commercial pellets, PIV: 100% commercial pellets (Control)

Table 7. Water quality data during the study

Day -	Water quality parameters		
	Temperature (ºC)	pH	DO (ppm)
0	26.9	7.47	2.10
10	27.1	7.07	2.82
20	28.7	7.47	2.55
30	27.3	7.90	2.21
40	30.0	8.01	3.75
50	29.5	7.93	3.48
60	29.6	7.74	3.80

Water quality

Water is the most important medium or habitat for fish life. Therefore, an adequate water supply will solve various problems in intensive fish farming. In addition, good water quality is one of the keys to success in fish farming. The water parameters observed in this study include temperature, pH, and DO (oxygen content). Water quality parameter data can be seen in Table 7.

Table 7 shows that the water temperature ranges from 26.9 to 30.0ºC. The temperature range is still ideal for Nile tilapia growth. Suyanto (2002) states that the ideal temperature for Nile tilapia growth is between 25 and 30ºC. Water temperature influences fish appetite and metabolic processes. Food digestion in fish occurs slowly at low temperatures, whereas digestion occurs more quickly at high temperatures.

In this study, the degree of acidity (pH) ranged from 7.07-8.01. This pH range is favorable for Nile tilapia habitat and growth. According to Sherif and Feky (2009), the pH range for optimal growth is 7-8, while the pH range for Nile tilapia habitat is 6-8.5. One of the most important environmental factors for fish life is oxygen content. If the dissolved oxygen concentration is low, the organism being reared appetite decreases, affecting growth.

In this study, the dissolved oxygen (DO) content ranged from 2.10 to 3.80 ppm. This range falls below fish life's 4 ppm minimum oxygen concentration limit. Therefore, the low DO levels in the waters in this study were caused by the water coming from the earth's bowels, which had a low oxygen content. Nile tilapia, on the other hand, can tolerate DO levels as high as 1 ppm, but its growth is not optimal (Kordi 2000).

According to the study, the following conclusion is drawn: (i) adding 75% fermented coconut pulp to commercial pellets increases the water, fat, and crude fiber content by 25.72%, 20.36%, and 10.56%, respectively. On the other hand, the control feed had the highest ash content, protein content, and carbohydrates, which were 9.35%, 29.34%, and 44.98%, respectively. (ii) The growth of Nile tilapia was increased after feeding with fermented coconut pulp. (iii) The concentration of addition of fermented coconut pulp in the optimal feed for the growth of tilapia is 25%.

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