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Physical and Chemical Analysis of Fish Feed Based on Fermentation of Kelakai Leaf (*Stenochlaena palustris* (Burm.F.) Bedd)

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***Abstract*— *Kelakai (Stenochlaena palustris) can be used as an ingredient in fish feed because it has a high protein content. The problem with the use of kelakai for fish feed is that the crude fiber content is quite high, and the improvement of this nutritional value can be done through the fermentation process. The purpose of this study was to analyze the physical and chemical properties of fish feed based on fermented kelakai leaves. The research was conducted at the Nutrition Laboratory, Faculty of Fisheries and Marine Affairs, Lambung Mangkurat University Banjarbaru, South Kalimantan Province for ± 1.5 months. This study used a completely randomized design (CRD) with 3 treatments, namely Treatment (A) non-fermented kelakai leaf-based feed, treatment (B) fermented kelakai leaf-based feed with a dose of probiotics 5%, and Treatment (C) Fermented feed based on a 10% dose of probiotics, with test parameters namely physical test and chemical test of feed. The results showed that the longest durability of feed was destroyed after 1 hour 02 minutes in the non-fermented treatment, while the best feed buoyancy was in the treatment of kelakai leaf fermentation-based feed at a dose of 5% for 43.75 seconds. Kelakai leaf fermented feed with doses of 0%, 5%, 10% showed the value of protein content (25.78%-26.28%), crude fiber (7.67%-7.96%), fat (3.32%- 6.88%), and contains 8 essential amino acids (L-Phenylalanine, L-Leucine, L-Valine, L-Arginine, L-Lysine, L-Leucine, L-Threonine, L-Histidine) and 7 non-amino acids. essential oils (L-Serine, L-Glutamic acid, L-Alanine, glycine, L-Aspartic, L-Tyrosine, L-Proline) where the highest value was in the fermented feed based on the 5% dose of tapioca flour.*

***Keywords*— *Physical analysis, proximate, amino acids, fermentation, kelakai, fish feed*.**

I. INTRODUCTION

Feed is one of the important components in aquaculture activities, but the costs incurred for feed can reach 60% of production costs. An alternative that can be done is to make feed independently by utilizing local ingredients that are abundantly available and contain the right nutrients. Kelakai (Stenochlaena palustris) can be used as an ingredient in fish feed because it has a high protein content.

The results of the research by Fatmawati and Fauzana (2016), stated that the young shoots of the young kelakai leaf contained 27.31% protein compared to the old leaves of 26.79%. The constraint on the use of kalakai for fish feed is the high crude fiber content of 10.45% for young leaves and 15.62% for old leaves (Fatmawati and Fauzana, 2016), while fish needs for crude fiber are not more than 8% (Fatmawati and Fauzana, 2016). Mujiman, 2000). It also contains vitamins and minerals which include ferrum (4153 mg/100 g), vitamin C (41 mg/100 g), protein (2.36%), beta-carotene (6.69 mg/100 g) and folic acid (1.13 mg/100 g) (Petricka, Makiyah & Mawarti, 2018).

Improvement of nutritional value can be done through the fermentation process of feed ingredients or feed using microbes or probiotics (Arief, Fitriana and Subekti, 2014). Fermentation products are generally easily biodegradable and have a higher nutritional value than the original material (Winarno, Fardiaz and Fardiaz, 1980). This is not only caused by the catabolic nature of microbes or breaks down complex components into simpler ones so that they are easier to digest, but also can synthesize several complex vitamins. Another benefit of fermentation is that food materials are more resistant to storage and can reduce the toxic compounds they contain, so that the economic value of the basic ingredients is much better (Pamungkas, 2011).

Fish feed is said to be of good quality if after being processed and made, has the appropriate shape and texture for buoyancy and good density for durability (Mujiman, 2000), and contains the nutritional and nutritional values needed by fish. Quality feed after being made must contain 20-70% protein, 15% carbohydrates, 10% fat, and 5% vitamins, water and minerals (Murtidjo, 2001).

The use of kelakai leaves as raw material for fish feed has not been widely used, so information on the use of kelakai leaves in fish feed is still limited. A study of the nutritional content of fermented kelakai leaf-based feed is very necessary to see its potential as a physically appropriate feed ingredient and has nutritional content that is suitable for use for fish.

II. RESEARCH METHODS

This research was carried out within 1.5 months starting from March to May 2022. The research was carried out at the Nutrition Laboratory, Faculty of Fisheries and Marine ULM for the manufacture of feed and physical testing of the feed. Proximate analysis was carried out at the Chemistry and Animal Feed Laboratory, Faculty of Agriculture ULM and amino acid analysis at the SIG Laboratory Bogor.

The tools and materials needed during this research are listed in Table 2.1 and Table 2.2.

Table 2.1 Tools Used

Tools	Description
Sttionery	Recording data
Oven	Drying ingredients
Flour tool	Refining ingredients
Feed mold	Print feed
Digital scale	Weighing materials
Scissors	Scissirs materials
Sieve	Sieve material
Plastic	Storing fermented feed
Label paper	Give information
Measuring cup	Observing feed buoyancy
Erlenmeyer	Observing the durability of feed
Pots and stove	Steaming ingredients
Small basin	Making feed
Jar	Storing pellet feed

Table 2.1 Materials Used

Material	Use
Kelakai Flour	Pellet feed ingredients
Bran	Pellet feed ingredients
Tapioca flour	Pellet feed ingredients
Vitamin mix	Pellet feed ingredients
Fish oil	Pellet feed ingredients
Probiotik EM4	Fermented ingredients
Molasses	Fermented material
Aquades	Mixing molasses probiotics

Research procedure

1. Preparation of the kelakai leaf flour:

-Young kelakai leaves are separated between leaves and stems.

-The kelakai leaves are then dried by drying directly in the sun or put in an oven with a maximum temperature of 60°C until the kelakai leaves are dry.

-The dried kelakai leaves are mashed using a flour, then filtered to produce fine flour.

2. Fermentation of kelakai flour:

-The kelakai flour is weighed and then poured into a tray to be stirred homogeneously with distilled water until it is in a moist condition.

kelakai flour is put in a plastic bag and steamed for 15 minutes.

EM4 probiotics are activated first by adding molasses which has been diluted with distilled water. Molasses used as much as 3% of the dose of kelakai flour, following the use of molasses in the ration in general is 3% (Widayati and Widalestari, 1996).

-Probiotic EM4 which was homogenized with molasses was added to the cooled kelakai leaf flour according to the treatment doses (0.5% and 10%).

-The homogenous kelakai leaf flour with probiotics is put in a plastic bag, tightly closed and tied and allowed to stand for 7 days at room temperature.

3. Manufacture of fermented feed based on kelakai leaves:

-The ingredients for the feed are fish meal, kelakai leaf flour, bran, tapioca vitamin mix flour and fish oil, with the following compositions Fatmawati and Fauzana (2016), namely 30% kelakai flour, 45% fish meal, 13% bran flour, flour tapioca 10%, vitamin mineral mix 1% and fish oil 1%.

-All feed ingredients that have been mixed evenly are then molded into pellets and dried under direct sunlight or in an oven at a maximum temperature of 40°C.

Experiment Design and Treatment

This research was carried out using an experimental method using a Completely Randomized Design (CRD) with 3 treatments as follows:

A : Non-fermented kelakai leaf-based feed (0% probiotic)

B : Feed based on fermented kelakai leaves with a 5% dose of probiotics

C : Fermented feed based on kelakai flour with a dose of 10% probiotics

Observation Parameter

1. Physical test (feed resistance in water and feed buoyancy). The durability of the feed in water is calculated based on the length of time the feed is destroyed after being put into the water, while the buoyancy of the feed is done by dropping the pellet into the water in a container (20cm high) followed by counting the time for the feed to touch the water until it sinks (Handajani and Widodo, 2004). 2010)
2. Chemical test, namely nutrient content based on proximate analysis which includes crude protein, crude fat, crude fiber, water content and ash content.

3. Amino Acid Content

Data analysis

Data analysis was carried out on the resistance of the feed in the water and the buoyancy of the feed starting from the normality test, diversity test, ANOVA and the mean difference test (Hanafiah, 2014). The data on the nutritional content and amino acids of the feed were analyzed in tabulated form and compared with the relevant literature.

III. RESULTS AND DISCUSSION

3.1. Feed Resistance in Water

The data from the observation of the endurance of the feed in the water can be seen in Table 3.1 Figure 3.1

Table 3.1 Average Endurance Test of Feed in Water (Hours)

Repetition	Treatment		
	A	B	C
1	1,01	0,20	0,30
2	1,01	0,25	0,29
3	1,02	0,15	0,31
4	1,05	0,30	0,32
5	1,03	0,36	0,33
Total	5,12	1,26	1,55
Average	1,02± 0,02	0,25± 0,08	0,30± 0,02

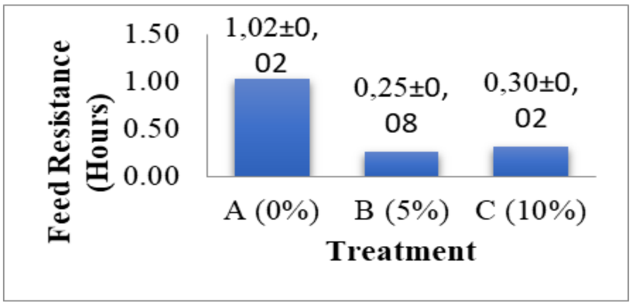

Fig.3.1 Graph of Feed Resistance Average in Water

Table and Figure 3.1. showed that the highest average feed resistance was found in treatment A (1.02 hours or 60 minutes) then treatment C (0.30 hours or 18 minutes), and the last one was treatment B (0.25 hours or 15 minutes).

The results of the Lilifors normality test and the Barlett homogeneity test on the resistance of feed in water showed Lmax (0.34) < Ltab 5% (3.49) where the data spread normally, and X2 count (0.01943) <X2 table 5% (

15,507) and X2table 1% (20,090) shows homogeneous data.

The results of the analysis of diversity (ANOVA) of feed resistance showed that Fcount > from Ftable 5% and less than Ftable 1%, so it was decided to accept H1 and reject H0 or fermented feed based on tapioca flour was significantly different to the resistance of feed in water, where the feed treatment without fermenting the leaves of anchovies, their resistance to water was better than other treatments.

Pellets made of adhesive take ± 1.05 hours to disintegrate (Krisnan and Ginting, 2009). The quality of feed is seen from the durability, at least for 10 minutes in the water (Mujimam, 2000). The fermented fermented fermented feed using EM4 can be used as an alternative feed for aquaculture activities, because of its dry, compact and not easily destroyed physical characteristics when put into water. Good quality feed can last more than 15 minutes for catfish feed, 5 minutes for carp feed, and tilapia feed for 90 hours (Utomo, 2015).

3.2. Feed Buoyancy

The data from the observation of feed buoyancy can be seen in Table 3.2 and Figure 3.2.

Table 3.2 Average Feed Buoyancy Test (Second)

Repetition	Treatment		
	A	B	C
1	12,68	41,25	32,45
2	11,19	43,35	31,12
3	10,52	45,37	34,53
4	10,10	43,52	32,68
5	11,08	45,25	38,25
Average	11,11± 0,98	43,75 ±1,68	33,81± 2,77

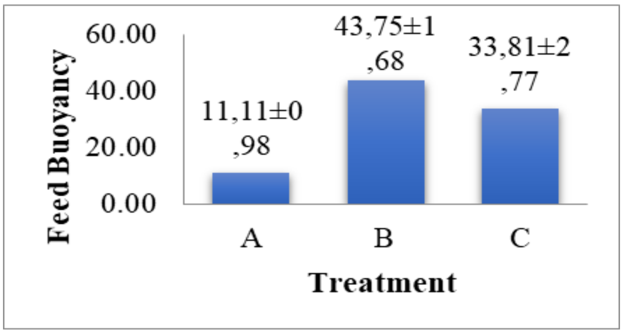

Fig.3.3 Feed Buoyancy

Table and Figure 3.2 show the highest average feed buoyancy in treatment B (43.75 seconds) followed by treatment C (33.81 seconds) and finally treatment A (11.11 seconds).

The results of the Lilifors normality test and the Barlett variance homogeneity test on feed buoyancy showed Lmax (0.35) < Ltab 5% (3.49) where the data spread normally, and X2 count (2.084) < X2table 5% (15.507) and X2table 1% (20,090) shows homogeneous data.

The results of the analysis of diversity (ANOVA) of feed buoyancy showed that Fcount (366,762) > Ftable 5% (3.89) and Ftable 1% (6.93), so it was decided to reject H0 and accept H1 or fermented feed based on tapioca flour. feed float in water. Fermented feed based on malaya leaves with 5% probiotics is the best treatment for feed buoyancy.

Feed made from fermented kelakai flour using probiotic EM4 needs additional treatment because in the process of making feed it does not use a feed making machine that is capable of floating (extruder) so that the feed sinks quickly and this feed can be given to catfish and shrimp (Afrianto and Liviawaty). , 2005). The pellet category is physically good, that is, it has a high quantity of water stability and density, is impact resistant, but has a medium water absorption capacity and a low expansion ratio (Krisnan and Ginting, 2009). In certain circumstances, pelleted feed only needs to float a few minutes before being eaten by fish (Handajani and Widodo, 2010).

3.3. Feed Nutrient Content

3.3.1. Proximate Analysis Results

The data from the proximate analysis of pellet feed based on the fermentation of macaque leaves can be seen in Table 3.3. following :

Table 3.3. Yield Nutritional Content of Feed (%)

Parameters	Treatment		
	A	B	C
Water	4,39	12,66	13,28
Ash	10,25	11,75	12,12
Crude Protein	25,78	25,78	26,28
Coarse Fiber	7,96	7,96	7,67
Crude Fat	6,88	3,32	4,98

1. Moisture Content

According to Winarno (2015) the water content in the feed indicates the freshness and durability of the feed. The value of the water content of the feed in this study was between 4.39%-13.28% which was still within the tolerance limit for fish growth, where 70%-90% of the wet weight was the water content in fish food (Mujiman, 2000).

2. Ash content

According to Irawati (2008) to determine whether the feed is good or not, selecting synthetic and native feeds, a calculation of the ash content of the feed is carried out which is a parameter of a material. The results of the proximate analysis of feed ash content in this study ranged from 10.25%-12.12%, this range was still within the tolerance limit for fish growth.

Ash content cannot describe the appearance and amount of minerals contained in the feed. The higher the ash content, the lower the quality of the feed (Suparjo, 2010). The maximum ash content in fish feed is 15% (Mujiman, 2000).

3. Crude Protein

The results of the crude protein value of pellet feed based on fermented macaque leaves in this study were between 25.78%-26.28% According to Mujiman (2000), the amount of protein ranged between 20%-60% needed by fish in a feed, and 30-36 % is the optimum protein requirement. Fish will not improve if the protein quality is less than 6% wet weight which is controlled by its amino acid content.

4. Coarse Fiber

According to Suparjo (2010) crude fiber is partly derived from plant cell walls which contain plant components. Crude fiber is a variety of fibers that are not easily crushed. The results of crude fiber feed in this study ranged from 7.67%-7.96%, this range was sufficient for fish growth. The function of fiber is to facilitate the digestive process and does not have growth substances (Nonok and Fitazar, 2011).

5. Fat

Sources of stamina, essential fatty acids, phospholipids, sterols and an introduction to the process of absorbing vitamins mixed in are the benefits of fat (Murtidjo, 2001).

The results of the crude fat content of the feed in this study ranged from 4.98 - 6.88, this range was still within the tolerance limit for fish growth. According to Mujiman (2000), the need for fat in fish feed ranges from 4-18%.

3.3.2. Amino Acid Content

Table 3.4 shows that the plant based diet contains 8 essential amino acids, namely phenylalanine, leucine, valine, arginine, lysine, leucine, L-Threonine, histidine and 7 non-essential amino acids, namely serine, glutamic acid, L-Alanine, glycine, aspartate. , tyrosine, proline.

Table 3.4. Feed Amino Acid Content

Amino Acid(%)	Treatment		
	A	B	C
L-Serine	0,61	0,90	0,69
L-Glutamic Acid	1,54	2,40	1,65
L-Phenylalanine	0,53	0,76	0,59
L-Isoleucine	0,49	0,83	0,55
L-Valine	0,71	1,07	0,76
L-Alanine	0,79	1,19	0,85
L-Arginine	0,62	0,87	0,64
Glycine	0,68	1,09	0,80
L-Lysine	0,67	1,19	0,70
L-Aspartic Acid	1,14	1,69	1,18
L-Leucine	0,93	1,46	0,98
L-Tyrosine	0,25	0,36	0,28
L-Proline	0,55	0,79	0,60
L-Threonine	0,59	0,88	0,65
L-Histidine	0,22	0,37	0,27

Source : SIG Labotratory Bogor 2022

The highest amino acid value in treatment B was followed by treatment C, then the lowest was treatment A. The results of the analysis showed that the fermented feed of kelakai flour contained amino acids that met the needs of fish, where the content of L-Glutamic acid, one of 15 amino acids, had the highest value in treatment B. of (2.40%).

The amino acid compositions are shown in Table 3.4 and the overall results for the compositions of treatments A, B, and C show varying values. The top amino acids from the feed test results include L-Glutamic acid, L-Aspartic, L-Leucine, lysine and valine,

Protein quality is related to the amino acid profile it contains. Amino acids are classified according to the body's ability to synthesize and their metabolic needs. This classification is known as essential and non-essential amino acids. Most animals including fish require 10 amino acids, namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenyl alanine, threonine, tryptophan and valine (NRC, 1993). The need for essential amino acids in ornamental fish such as goldfish ranges from 3.4% to 11.8% (Fiogbe & Kestemont 1995) higher than consumption fish such as Japanese eel (Anguilla japonica), carp (Cyprinus carpio), catfish (Ictalurus punctatus) and salmon (Oncorhynchus tshawytscha) which only ranged from 0.5% to 6.0% (NRC 1993).

Lysine is one of the ten essential amino acids that can be used as a reference amino acid, there are several reasons to choose lysine as a reference amino acid. First, a major function of lysine in the animal body is the deposition of protein tissues, since its requirements are not affected by other metabolic roles. Second, depending on the fish species and the type of feedstock, lysine usually has a major role in limiting amino acids because it is known that the need for lysine is much greater than for other amino acids (Miles & Chapman 2007).

The need for the essential amino acid lysine for the body of fish is between 4-6% of the ration protein (Agustono, Widodo and Paramita, 2010). According to FAO (2014) the lysine requirement of omnivore fish is 2.07%.

The optimal lysine amino acid content in feed for the growth of sunu grouper fry is 2.84% (Giri et al, 2009). The feed in this study contained the highest lysine at 1.19% which was still equivalent to the lysine content in general commercial feed. Commercial feed currently available has a lysine content of 1.41% (Khalida, Agustono and Paramita, 2017).

Cowey & Tacon (1983) suggested that the amino acid requirement for fish is essential and should be linked or even regulated by the pattern of amino acid presence in muscle tissue. Imbalance of the amino acid profile in the diet can reduce food intake and reduce the efficiency of utilization of essential amino acids.

IV. CONCLUSION

Physical properties of fermented kelakai flour feed with a dose of 0%, 5%, 10% seen from the durability of the feed in water, the highest dose of 0% of the feed was destroyed after 1 hour 02 minutes, while the best feed buoyancy was in the treatment of fermented kelakai-based feed with a dose of 5 % with a value of 43.75 seconds.

Chemical properties of fermented kelakai flour feed with a dose of 0%, 5%, 10% showed the value of protein content (25.78%-26.28%), crude fiber (7.67%-7.96%), fat (3.32 %-6,88%), and contains 8 essential amino acids (L-Phenylalanine, L-Leucine, L-Valine, L-Arginine, L-Lysine, L-Leucine, L-Threonine, L-Histidine) and 7 fatty acids non-essential amino acids (L-Serine, L-Glutamic acid, L-Alanine, glycine, L-Aspartic, L-Tyrosine, L-Proline) where the highest value was in fermented feed based on the dose of 5% tapioca flour.

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V. GRATITUDE

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