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UTILIZATION OF FERMENTED AZOLLA MICROPHYLLA FLOUR MIXTURE AS FRESHWATER BAWAL FISH (*Colossoma macropomum*) FEED TO ACCELERATE FISH GROWTH PROCESS

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Abstract

Bawal fish is popular in many Asian cuisines and is often served grilled, fried, or steamed. It is known for being a good source of protein and essential nutrients, making it a healthy and nutritious option for seafood lovers. Meanwhile, fermented Azolla flour contains high levels of nutrients, such as protein, vitamins, and minerals, which can help improve fish growth. The nutrients in the fermented Azolla flour can be utilized by the fish to build body tissues and accelerate body mass growth. The aim of this study is to evaluate the impact of using fermented Azolla microphylla flour in artificial feed for the growth of bawal fish fry and the best percentage of fermented Azolla microphylla flour in the feed to enhance fish growth. The study used a completely randomized design (CRD) with 1 factor, 5 treatments, and 2 replications. The treatments in this study were PA (30% FTA), PB (40% FTA), PC (50% FTA), PD (100% PK), PE (0% FTA) in the feed. The test feed was given at 5% of the biomass weight, twice a day at 07.00 and 17.00 WIB. The bawal fish fry used had an average size of 5.19 ± 0.71 cm and an average weight of 2.57 ± 0.08 g, with a stocking density of 15 fish/m3. The results of the study showed that the administration of fermented Azolla powder increased the growth of bawal fish fry. The use of 40% fermented Azolla microphylla flour (PB) produced the best results in terms of fish length growth at 4.85%, weight growth at 6.5%, and the highest protein content at 72.56% with the use of 30% Azolla microphylla fermentation.

Keywords: *Bawal Fish, Fermented Azolla Flour, Fish Growth, Protein*

1. INTRODUCTION

The freshwater bawal fish has shown great potential for aquaculture, but to ensure optimal growth, providing the right feed is crucial. Growth, measured as an increase in mass over time, is closely linked to the nutritional needs of the fish. Just like any other living organisms, freshwater bawal fish require a balanced diet containing proteins, carbohydrates, fats, vitamins, and minerals for their metabolic, physiological, and biochemical processes throughout their life cycle. The role of feed in determining fish growth is significant, and growth estimation can be achieved through the analysis of frequency data on length or weight.

Micro-scale nutrients from the feed are metabolized within the fish's body, where they are transformed into essential chemicals used for growth and providing energy for various activities, including reproduction. Therefore, it is essential that fish feed meets the daily energy requirements of the fish and promotes optimal growth at each stage of their life. The key components of fish feed that support growth include proteins, carbohydrates, lipids, minerals, and vitamins, while factors like water and oxygen are also critical for their growth (Nugraha et al., 2020).

One potential source of rich protein in fish feed is the Azolla plant, specifically Azolla *microphylla*, which is abundant in Indonesian rice fields. This plant can serve as an additional feed option due to its relatively high protein content, ranging from 25% to 30%, making it beneficial for fish growth. Additionally, the fermentation process can reduce the crude fiber in Azolla, making it easier for fish to digest. Through fermentation, indigestible materials such as cellulose and hemicellulose are broken down into simple sugars, facilitated by microorganisms.

Table 1. Components in Azolla Flour per 100 grams

Component	Percentage
Crude Protein	24-30%
Crude Fiber	12,38%
Nitrogen	43,35%
Ash	19,33%
Calcium	1,64%
Phosphorus	0,34%

With these considerations in mind, the study aims to determine the ideal composition of feed for freshwater bawal fish. Test feed formulations consisting of wheat flour, shrimp head, soybean flour, bran, and vegetable oil are prepared and processed according to feed processing reference procedures, with different treatments to be assessed. The finished feed will then undergo tests on freshwater bawal fish to evaluate its suitability, and the feed's nutrient content will be analyzed in the laboratory to ensure it meets the nutritional needs of the fish. This research seeks to enhance the understanding of how the right feed composition can contribute to the optimal growth of freshwater bawal fish in aquaculture settings.

2. RESEARCH METHODS

The method of this study is a pure experimental method with a Completely Randomized Design (CRD) and repeated observations through 5 treatments (A, B, C, D) and one control (K), each replicated twice. The treatments in this study were as follows:

a. PA = Feed mixture with 30% fermented azolla dosage
b. B = Feed mixture with 40% fermented azolla dosage
c. PC = Feed mixture with 50% fermented azolla dosage
d. PD = Feed mixture with 0% fermented azolla dosage
e. Pk = 100% Commercial Feed

The research was divided into two parts: feed formulation and feed testing. Feed formulation was carried out at the Laboratory of Natural Sciences, Faculty of Teacher Training and Education, Lambung Mangkurat University, located at Jalan Brigjen H. Hasan Basry, Campus I, FKIP ULM, for 60 days. Feed testing was conducted at the Freshwater Aquaculture Production, located at Jalan Mentaos Timur RT 01/RW 03, Kelurahan Mentaos, Kec. Banjarbaru Utara, Kota Banjar Baru, Prov. South Kalimantan, for 14 days. The study used freshwater bawal fish fry with a size of 3-4 cm. The feed

given to the freshwater bawal fish in this study was in pellet form. The containers used had a size of 40 cm x 30 cm x 40 cm with a water depth of 40 cm. The equipment used included scoops, plastic basins, digital scales, millimeter blocks, cameras, writing tools, thermometers, and pH meters.

The study conducted laboratory tests, including protein content and proximate analysis of the fish feed used during the observation. The parameters used in this research were the weight growth rate of the fish, fish length, and supporting data such as water quality and fish survival rate. The data obtained from the feed testing on the freshwater bawal fish will be analyzed using SPSS application with One Way ANOVA analysis. The processed feed will also be tested for its nutritional content at the Laboratory of Medicine, Lambung Mangkurat University.

3. RESULTS AND DISCUSSION

3.1.Result

3.1.1. Data on Protein Content Analysis Results on Feed

To find out the protein content of the processed feed made before giving it to the fish, the processed feed is tested first, the results of laboratory tests on processed feed are shown in table 2.

Table 2. Result of nutrient content on feed used during research

Treatment Code	Sample Code	Protein (%)	Fats (%)	Carbo hydrate (%)	Moisture Content (%)	Ash Content (%)	Fiber Content (%)
C	P1	72,56	49,41	46,12	2,01	56,04	64,12
B	P2	64,08	41,67	40,83	1,91	49,13	53,09
A	P3	51,23	30,17	39,97	1,45	40,24	50,11
D	P5	43,15	29,66	36,84	1,39	40,19	44,03
K	P4	65,35	32,91	40,08	1,90	29,02	33,02

According to the results of the protein content test in table 2, the composition of sample P4 shows a protein content of 65.35%. This treatment is the control because it uses 100% commercial feed. The composition of the sample code (P1) has a high level of protein because the mixture of fermented azolla flour is the most at 50%. The composition of the sample code (P5) so the amount of protein content is the lowest compared to the amount of protein content of other treatments. The reason for the low protein content in this feed is because the protein source only comes from shrimp head flour and soybean flour. The composition of the sample code (P3) gives a protein content of 51.23%, the feed with the sample code P3 is the second lowest amount of protein after the P5 treatment with protein sources derived from 30% fermented azolla flour, shrimp head flour and soybean flour. The composition of the feed sample code P2 shows a protein content of 64.08% as the lowest protein content after P5 and P3. While the composition of sample code P1 shows protein levels of 72.56% as the most protein content because it has the amount of fermented azolla flour by 50%.

3.1.2. Results of Hypothesis Testing Analysis of Freshwater Bawal Fish Growth

This table analyzes the impact of azolla flour fermentation mixture in processed fish feed on the growth of freshwater pomfret.

Table 3. Fish Growth

Treatments	Repeat 1 (cm)	Repeat 2 (cm)	Total	Average
A	0,28	0,23	0,51	0,255
B	0,51	0,46	0,97	0,485
C	0,31	0,26	0,57	0,285
D	0,33	0,5	0,83	0,415
K	0,33	0,23	0,56	0,28

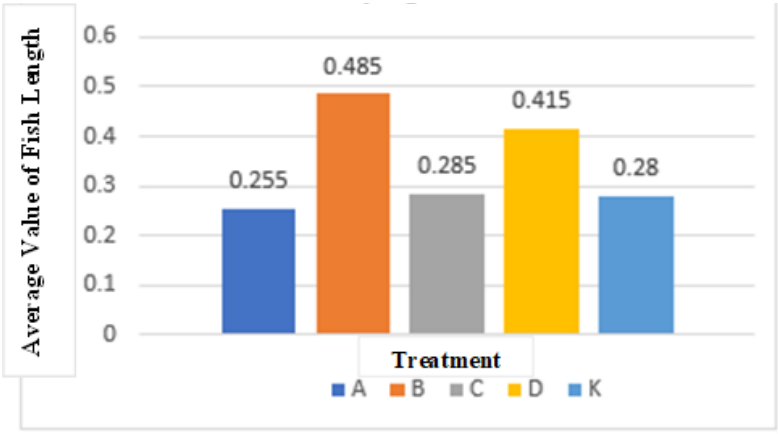

Figure 1. Graphic of Absolute Length Growth of Fish

Description:

a. Treatment A: 30% mixture of fermented Azolla *microphylla* flour
b. Treatment B: 40% mixture of fermented Azolla *microphylla* flour
c. Treatment C: 50% mixture of fermented Azolla *microphylla* flour
d. Treatment D: 0% mixture of fermented Azolla *microphylla* flour
e. Treatment K: Commercial feed (MS Prima Feed).

Absolute length growth refers to the daily increase in the body length of fish larvae. Based on the table above, it is observed that Treatment B, which consists of a 40% mixture of fermented Azolla flour, has the highest average growth rate of 0.485 cm. Following that, Treatment D, which has a 0% mixture of fermented Azolla flour, shows a growth rate of 0.415 cm. Treatment K, using commercial feed (MS Prima), and Treatment C, a 50% mixture of fermented Azolla flour, have slightly lower growth rates of 0.28 cm and 0.285 cm, respectively. Lastly, the smallest growth rate is observed in Treatment A, which uses a 30% mixture of fermented Azolla flour, with a growth rate of 0.285 cm. Statistical analysis using SPSS revealed that the data on weight growth is not normally distributed, but it is homogenous. The *Kruskal-Wallis* test showed no significant difference in the average values among the treatments. Further analysis using Duncan's test was conducted to examine specific differences between each treatment.

Table 4. Results of Duncan's Test for Absolute Length Growth

Treatments	N	1	2
A	86	5,2535	
C	87	5,3471	5,3471
D	83	5,3482	5,3482
K	88		5,3773
B	75		5,3893
Sig.		0,116	0,506

The Duncan test table shows that treatment B has a value that is not significantly different. Treatment B is a feed with a mixture of fermented azolla flour as much as 40%. This shows the effect compared to other feed treatments.

3.1.3. Analysis of Fish Weight Growth

Absolute weight growth is the actual average size change, namely the average weight at the beginning and end of the study (Effendie, 2002).

Table 5. Absolute Weight Growth

Treatments	Repeat 1 (cm)	Repeat 2 (cm)	Total	Average
A	0,53	0,62	1,15	0,575
B	0,44	0,86	1,30	0,65
C	0,40	0,24	0,64	0,32
D	0,58	0,71	1,24	0,62
K	0,61	0,58	1,19	0,28

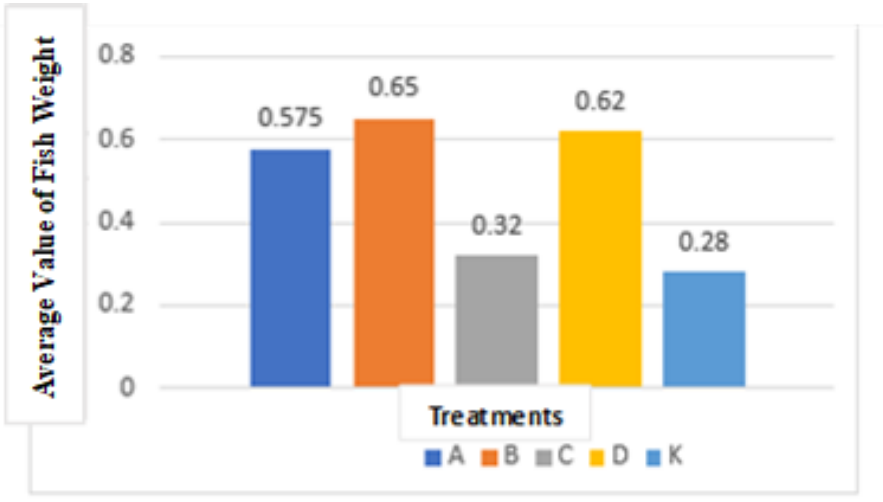

Figure 2. Graphic of Absolute Weight Growth of Fish

Description:

a. Treatment A: 30% mixture of fermented Azolla *microphylla* flour
b. Treatment B: 40% mixture of fermented Azolla *microphylla* flour
c. Treatment C: 50% mixture of fermented Azolla *microphylla* flour

d. Treatment D: 0% mixture of fermented Azolla *microphylla* flour
e. Treatment K: Commercial feed (MS Prima Feed).

Absolute weight growth is the growth of the fish body every day. Based on the table above, it is known that treatment B, namely 40% azolla flour fermentation mixture, has the highest growth rate of 0.65 grams. After that, treatment D is 0% mixed fermentation of azolla flour. Then treatment A is 30% mixed fermentation of azolla flour and treatment C is 50% fermentation of azolla flour, and the last has the smallest absolute weight growth value is treatment K with 100% commercial feed (MS Prima Feed).

In addition, statistical tests were carried out using SPSS, the results for the normality test of weight growth data in each treatment had P-count <0.05, so the data were not normally distributed. Then a homogeneity test was held where the P-count sig value. (0.336) > α (0.05) then the result is H0 accepted, meaning this data is homogeneous. After that, the *Krusskal Wallis* test results in a P-count value (0.082) < α (0.05) so there is a difference in the average value, then the further test will be continued with the Duncan test to see the value of the real difference in each treatment.

Table 6. Duncan Test Results of Absolute Length Growth

Treatment	N	1	2
C	87	2,8068	
A	86	2,8899	2,8899
D	83	2,9870	2,9870
B	88		3,0415
K	75		3,0652
Sig.		0,074	0,094

Based on the results in the table above, the information obtained for treatment C is not significantly different from treatment A and D, while treatment C is significantly different from treatment B and K. In treatment A can be stated as not significantly different from treatment E, B and K. The Duncan test table shows that treatment B has a different value compared to treatments A, C, D and K. Treatment B is a feed with a mixture of fermented azolla flour as much as 40%. This shows the effect compared to other feed treatments.

3.1.4. Supporting Data Results

Table 7. Survival of Freshwater Bawal Fish during Maintenance

Treatment	Survival Rate		Total	Survival (%)
	Beginning	Final		
A	30	27	3	90%
B	30	29	1	96,6%
C	30	28	2	93,3%
D	30	22	8	86,6%
K	30	26	4	73,3%

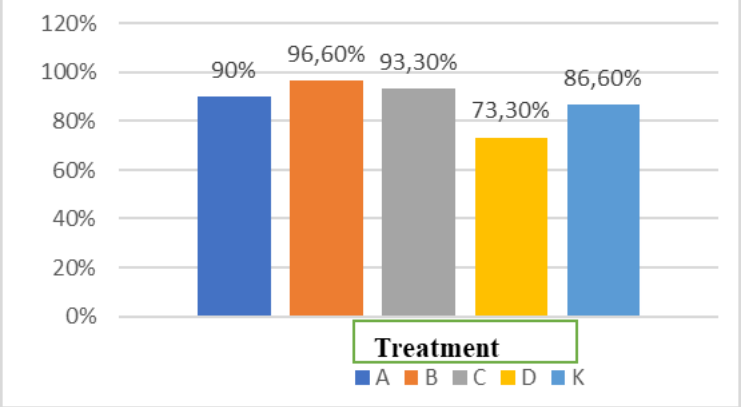

Figure 3. Graphics of Fish Survival

(Perdana et al., 2016) said, "survival rate is not directly affected by feed, but is affected by biotic factors (age and ability of fish to adjust to the living environment) and abiotic factors (food availability and quality of living media).

3.2. Discussion

3.2.1. The Influence of Fermented Azolla Microphylla Flour Variations on the Length Growth of Freshwater Bawal Fish

In this study, five types of feed were used to observe the length growth of freshwater bawal fish larvae. The feeds included processed feed with 0% fermented Azolla *microphylla* flour, 30% mixture, 40% mixture, 50% mixture, and 100% commercial feed. During the 14-day observation period, significant differences were found in the length growth of fish larvae among the treatments. Treatment PB with an average length growth of 0.485 cm showed the highest growth rate, followed by Treatment PD with an average length growth of 0.415 cm. The study suggests that the administration of fermented Azolla *microphylla* flour in Treatment PD significantly impacts the length growth of freshwater bawal fish ($P<0.05$). The proper protein intake is essential for body cell maintenance, tissue formation, and tissue replacement in fish, influencing both length and weight growth.

The study indicates that the dosage of fermented Azolla flour is proportionate to the daily protein needs of freshwater bawal fish larvae. Based on the test results in Table 2, the highest protein content was found in Treatment PC at 72.56%, followed by Treatment PK at 65.35%, Treatment PB at 64.08%, Treatment PA at 51.23%, and the lowest protein content in Treatment PD at 43.15%. Treatment PB, which received the highest protein intake, showed the highest average length growth, indicating that the length growth of freshwater bawal fish is proportional to the appropriate protein content in their feed. Protein is a vital nutrient needed for cell maintenance, tissue formation, tissue replacement, and serves as a significant energy source and growth influencer for fish, affecting both length and weight growth.

Based on figure 1, it can be seen that there is no direct relationship between the increasing dosage of Azolla given and the increase in length growth. However, the dosage of fermented Azolla feed is directly related to the daily protein needs of freshwater bawal fish larvae. The most suitable dosage for 14-day-old freshwater bawal fish larvae to

receive a protein intake of 64.08% per daily feed provision. In the study by Rahayu et al. (2020) on Baung fish, the protein content in fermented Azolla *microphylla* flour varied with P0V0 at 23.85%, P0V1 at 29.33%, P0V2 at 35.41%, and P0V3 at 39.97%. The best result was obtained in Treatment P0V3 with a protein content of 39.97%, resulting in a length growth of 10 cm in one month of Baung fish rearing.

ANOVA analysis using Duncan's test shows that the administration of fermented Azolla *microphylla* flour significantly affects the length growth of freshwater bawal fish ($P<0.05$). Treatment PB shows the most favorable results. Proper protein intake optimizes body cell maintenance, tissue formation, and tissue replacement, providing suitable energy sources and preventing wastage through fish respiration. The availability of protein in feed significantly affects fish growth (length and weight). (Sukadi, 2016) strengthens this finding by stating that the nutritional content determines the quality of feed. Protein is one of the essential nutritional needs for fish, and insufficient protein in feed can hinder fish growth.

3.2.2. The Influence of Fermented Azolla microphylla Flour Variations on the Weight Growth of Freshwater Bawal Fish

Growth refers to the increase in length over a period of time and the increase in size and weight. It is a complex biological process influenced by various factors (Effendie, 2002). There are two types of length growth: total length and standard length. Total length is measured from the head to the end of the tail, while standard length is measured from the head to the midpoint of the tail fin. Growth is always influenced by external and internal factors (Wiadnya et al., 2017).

During the rearing process, there were changes in the weight growth of freshwater bawal fish. Based on the table above, Treatment B, which had 40% fermented Azolla flour mixture, showed the highest growth rate at 0.65 grams. Treatment D, which had 0% fermented Azolla flour, had the lowest growth rate. Treatment A, with 30% fermented Azolla flour mixture, and Treatment C, with 50% fermented Azolla flour, showed intermediate weight growth. The last treatment, K, with 100% commercial feed (MS Prima Feed), had the lowest absolute weight growth.

Furthermore, statistical analysis using SPSS showed that the weight growth data for each treatment did not follow a normal distribution with P-count < 0.05. However, the data were homogenous according to the homogeneity test (P-count sig. = 0.336 > α = 0.05). The *Kruskal-Wallis* test yielded a P-count value of 0.082 < α = 0.05, indicating a significant difference in the average weight. Consequently, a further Duncan test was conducted to observe the real differences in each treatment.

According to the results in Table 7, Treatment C was not significantly different from Treatments A and D, while Treatment C showed significant differences compared to Treatments B and K. Treatment A was not significantly different from Treatments E, B, and K. The Duncan test table shows that Treatment B had differences in weight compared to Treatments A, C, D, and K. Treatment B, with a 40% mixture of fermented Azolla flour, demonstrated a significant influence compared to the other feed treatments.

Based on figure 2, it can be seen that there is no direct relationship between the increasing dosage of Azolla given and the increase in weight growth. However, the dosage of fermented Azolla feed is directly related to the daily protein needs of freshwater bawal fish larvae. The most suitable dosage for 14-day-old freshwater bawal fish larvae to receive a protein intake of 64.08% per daily feed provision.

ANOVA analysis using Duncan's test shows that the administration of fermented Azolla microphylla flour significantly affects the weight growth of freshwater bawal fish ($P<0.05$). Treatment B (PB) shows the most favorable results. Proper protein intake optimizes body cell maintenance, tissue formation, and tissue replacement, providing suitable energy sources and preventing wastage through fish respiration. The availability of protein in feed significantly affects fish growth, whether in length or weight. This is supported by (Sukadi, 2016), who stated that the nutritional content, such as protein, determines the quality of feed and inadequate protein in feed can hinder growth. (Cherryl et al., 2014) and (Kathirvelan et al., 2015) further strengthen this point, indicating that dried Azolla *microphylla* leaves can be a potential natural protein source for non-conventional feed with low lignin content (Cherryl et al., 2014; Kathirvelan et al., 2015).

3.2.3. Survival Rate of Freshwater Bawal Fish

The Survival Rate is a parameter used to determine the percentage of fish survival during the rearing period (Fitriani et al., 2022). The survival rate of freshwater bawal fish observed during this study ranged from 73.3% to 96.6%. The survival rate varied between treatments. Fish mortality during the study was caused by pathogens that caused wounds on certain parts of the body, such as fins and tails. These wounds worsened the fish's condition, leading to their death. Not all fish could survive during the second week of the study. Furthermore, fish survival is influenced by their ability to adapt to different environments. This variation in adaptability results in varying survival rates for each treatment. According to Lakshmana in (Armiah, 2010), "the factors that influence the high or low survival rate are biotic factors, including competitors, population density, age, and the organism's ability to adapt to the environment." In aquaculture, mortality is a determinant of the success of rearing efforts.

4. CONCLUSION

Based on the findings of this study, it can be concluded that the provision of various Azolla *microphylla* flour variations have a significant impact on the length and weight growth of freshwater bawal fish. The analysis results indicate that the addition of different doses of Azolla flour variations influences both the length and weight growth of the fish. Treatment B, with a 40% Azolla dose combined with other materials such as shrimp head flour, wheat flour, soybean skin flour, and bran, showed the most favorable results. This suggests that careful consideration of Azolla dosage and its combination with other ingredients can be crucial for optimizing the growth of freshwater bawal fish in aquaculture practices.

Therefore, incorporating fermented Azolla *microphylla* flour in fish feed formulations can be a promising approach to enhance the growth and overall health of freshwater bawal fish. By observing the significant effects of Azolla flour variations on both length and weight growth, fish farmers and researchers can explore its potential application in commercial aquaculture to improve fish growth rates and production efficiency. Additionally, the study highlights the importance of protein intake in fish feed, as it plays a critical role in maintaining body cells, tissue formation, and tissue replacement. Adequate protein provision in the diet can positively impact fish growth,

and the use of Azolla *microphylla* flour provides a natural and potentially sustainable source of protein for freshwater bawal fish.

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