**Incorporation of Fermented Cassava Leaf Flour into Commercial Feed to Enhance the Growth of Tilapia Fry (*Oreochromis niloticus*)**

**Abstract**

The success of cultivation is influenced by several factors, one of which is feed. This research was conducted to enhance the quality of feed and evaluate the benefits of incorporating fermented cassava leaf flour into feed on the growth and survival of Nile tilapia (*Oreochromis niloticus*) fry. The research design used a Completely Randomized Design (CRD). Data analysis involved analysis of variance (ANOVA) and Duncan's test. Three treatment levels and a control were applied in the research. A: Incorporating 15% fermented cassava leaf flour, B: Incorporating 25% fermented cassava leaf flour, C: Incorporating 35% fermented cassava leaf flour, D: Using 100% commercial feed. The study indicated that the inclusion of fermented cassava leaf flour in the feed significantly affected absolute weight growth (p<0.05). The optimal treatment was observed with the incorporation of 15% fermented cassava leaf flour in treatment A, where the average weight of tilapia seeds reached 3.49 g. The highest absolute length growth was recorded at the optimal treatment dose in treatment A, with a value of 2.05 cm in comparison to the other treatments. The survival rate results demonstrated that the highest value of 83% was achieved with the best treatment of 15% fermented cassava leaf flour. The water quality in the rearing media remains within the appropriate range for tilapia cultivation.

**Key words:** Nile Tilapia; growth; fermented cassava leaf flour; feed.

Introduction

Nile tilapia (*Oreochromis niloticus*) is a valuable fishery commodity with significant economic importance. It is highly favored among the Indonesian population for its excellent flavor, firm texture, and rich nutritional content, making it suitable for various processed products (Li et al, 2023). As one of the top export commodities, tilapia is experiencing a growing demand (Lusiana et al., 2021). This increased demand is reflected in the rising production of tilapia each year. The Directorate General of Aquaculture (2020) reported average tilapia production of 1,474,742 tons in 2019 and 1,235,514 tons in 2020. This data indicates that tilapia is a fishery commodity with bright and profitable prospects.

Feed is a crucial factor that influences the growth and survival of cultured fish. Forage, presented as wet feed or flour, serves as a cost-effective source of protein. Local feed ingredients, which fish farmers may not yet be familiar with, can also be utilized to prepare fish feed, including rubber seed cake, cassava leaves, bananas, and kale. Several studies have examined sources of vegetable raw materials, such as cassava leaf flour (Mohidin et al., 2023).

Fish growth occurs when the feed consumed contains appropriate protein content and maintains a proper protein-energy balance. The availability and balance of energy sourced from protein enable it to serve as a building block for growth, while non-protein energy from fats and carbohydrates provides the necessary energy source (Carbon & Pasiakos., 2019). However, as a primary source of nutrients and energy, feed represents the largest component in utilizing natural resources, which must be available in sufficient quantities and balanced to meet growth needs and ensure easy digestion. Cassava leaves act as an alternative feed ingredient that can be incorporated into fish feed formulations. Additionally, cassava leaves offer valuable nutritional content (Amare et al., 2024). This study aimed to determine the effect of adding cassava leaf flour on absolute length, absolute weight, and survival rate, and to identify the appropriate dose of cassava leaf flour to be added to commercial feed that can support the growth and survival of tilapia fry based on the parameters of absolute length, absolute weight, and survival rate.

# **Materials and Methods**

## Experimental Set-Up

## This research was conducted at the Tatelu Freshwater Aquaculture Center (BPBAT) in the Dimembe sub-district of North Minahasa, North Sulawesi, from August to September 2023. The method used in this study was a completely randomized design (CRD). The study included four treatments and three replications, resulting in twelve experimental units, with each experimental unit containing 20 tilapia fry measuring 3 to 5 cm. The treatments were as follows: (A) Feeding 85% commercial feed + 15% fermented cassava leaf meal, (B) 75% commercial feed + 25% fermented cassava leaf meal, (C) 65% commercial feed + 35% fermented cassava leaf meal, (D) 100% commercial feed.

**Preparation of fermented cassava leaf flour**

Cassava leaves were washed, chopped, and steamed for 15 minutes. Afterward, the steamed cassava leaves were dried until they were no longer hot. Next, evenly add 0.5% yeast per kilogram to the cassava leaves. They were placed in a jar and sealed tightly with duct tape for 4 days. Then, the cassava leaves should be sun-dried for 2 days. Finally, the cassava leaves were ground using a flour grinder.

**Mixing cassava leaf meal with commercial feed**

Commercial feed is weighed according to the desired dose, along with cassava leaf flour. Water was sprayed onto the feed, and cassava leaf meal was added and mixed thoroughly. The feed was aerated for half a day. After the feed dried, it was placed in a labeled jar and it was stored at room temperature.

### Preparation of rearing media

### The rearing media used in this study consisted of 12 aquariums, each measuring 60 cm x 40 cm x 40 cm. Before using the aquariums, they were thoroughly cleaned and dried. Once dry, each aquarium was labeled according to the randomized treatment assigned and filled with 40 liters of water, with aeration provided in each aquarium.

### Stocking of Test Fish

### Nile tilapia fry that have been acclimated in temporary holding tanks are then placed into each 40-liter maintenance container at a density of 1 fish per 2 liters. The rearing duration for tilapia fry is 40 days.

**Feeding**

The test fish used in this study were tilapia fry during the rearing period, fed according to the treatment. Feeding is conducted at a rate of 5% of the biomass weight of the tilapia fry. The feed was provided in the morning and evening.

**Water Quality Monitoring**

The water parameters observed during the study included measurements of temperature, pH, and dissolved oxygen (DO). Water quality assessments were conducted every two days at 08:00 and 16:00 using a portable multiparameter device to measure temperature, pH, and DO throughout the study period. Absolute length, absolute weight, and survival rates were also recorded.

The body length of tilapia fry was measured every 10 days. Length measurements were taken by placing the fry on a flat surface, such as a cutting board, and measuring them with a ruler. The measurement results were recorded, and the average body length per individual in each treatment was calculated. To determine the absolute length, the formula used was from Abdourhamane et al. (2021). Absolute weight measurements were taken every 10 days using digital scales. Before weighing, the container filled with water was weighed and then balanced. The fry were weighed individually, and the average body weight per individual was calculated. Absolute growth was determined using the Abdourhamane et al. formula (2021), while the Survival Rate Observation represents the percentage of aquatic biota that survive during the rearing period. Survival calculations were performed using the Abdourhamane et al. formula (2021).

**Data Analysis**

The obtained data, specifically Absolute Weight, Absolute Length, and Survival Rate (SR), were analyzed using analysis of variance (ANOVA) to determine the differences between each treatment.

**Results & Discussion**

**Absolute Weight Gain**

Figure 1. Absolute Weight Growth of Tilapia Fry (Oreochromis niloticus) Following the Addition of Fermented Cassava Leaf Flour to Feed Over a 40-Day Rearing Period.

The results of the statistical analysis showed that the type of feed provided influenced weight gain (P <0.05). Based on the picture above, the best treatment was achieved with the addition of 85% commercial feed and 15% fermented cassava leaf flour (treatment A), where the average weight of tilapia fry reached 3.49 grams. In contrast, the lowest results were seen in treatment D, which involved the addition of 100% commercial feed, where the average weight of tilapia fry increased to only 1.85 grams.

The growth observed in tilapia during the study was attributed to the feed consumed, which included fermented cassava leaf flour. The differences in growth among the various treatments resulted from variations in the feed's content (Niode, et al. 2017). Cassava leaves are one of the raw materials for protein sources, and they enhance digestibility due to their high levels of plant material and flavonoids, beneficial for increasing fish appetite (Samsugiartini, 2006 in Nugraha, et al. 2022). High-quality feed is defined as feed that meets all nutritional requirements of fish. The quality of feed can be assessed based on its nutritional content, including protein, fat, carbohydrates, minerals, and vitamins. If the feed lacks protein, fish growth will be hindered, as the protein in body tissues will be used to maintain essential bodily functions. Conversely, if there is an excess of protein in the feed, the surplus will be expelled as nitrogen in the form of ammonia (Mulyasari 2011).

The addition of fermented cassava leaves leads to variations in the weight and length of fish. Enzymes from controlled fermentation microorganisms can break down fibrous materials that are difficult to digest, transforming them into feed that fish can easily digest and absorb. Enzymes produced during fermentation boost the nutritional value of feed ingredients by reducing crude fiber, increasing protein, and enhancing other feed nutrients (Rachmawati et al., 2024).

**Absolute Length Growth**

The results of length growth obtained in the study conducted for 40 days show the results that can be seen in the Figure below:

Figure 2: Absolute Length Growth (cm) of Tilapia Fry (Oreochromis niloticus) After the Incorporation of Fermented Cassava Leaf Flour into Feed for a 40-Day Rearing Period.

The results of the statistical analysis indicated that the feed treatment influenced the absolute length growth (P<0.05). As shown in the picture above, the average length of tilapia fish varied. Treatment A, which involved the addition of 15% fermented cassava leaf flour, produced a length of 2.05 cm. In treatment B, with the addition of 25% fermented cassava leaf flour, the length measured at 1.54 cm. Treatment C, which contained 35% fermented cassava leaf flour, achieved a length of 1.38 cm, while treatment D, consisting solely of 100% commercial feed, reached a length of 1.07 cm.

In this study, treatment A, which included 15% fermented cassava leaf flour, achieved the best outcomes, resulting in an average growth of tilapia fish fry reaching 2.05 cm. In contrast, treatment D, which involved 100% commercial feed, exhibited the lowest growth in length, with an average of only 1.07 cm for tilapia fish fry. The protein content in feed influences both high and low growth rates in fish (Elangovan et al., 2017). Protein is a critical factor affecting fish growth as it aids in forming new tissue essential for developing and maintaining the body. When the protein content in feed is excessively high, only a portion will be absorbed and utilized for repairing damaged body cells, while the remainder is converted into energy (Revi et al. 2013).

**Survival Rate**

Figure 3. Survival rate (%) of Nile Tilapia Fry (*Oreochromis niloticus*) after incorporating Fermented Cassava Leaf Flour for a 40-day rearing period.

The results of the statistical analysis indicated that the feed treatment affected survival (P<0.05). According to the picture above, the survival rates of tilapia fish varied: treatment A, which included 15% fermented cassava leaf flour, produced a survival rate (SR) of 83%. This was followed by treatment B, which contained 25% fermented cassava leaf flour and an SR of 78%, and treatment C, which had 35% fermented cassava leaf flour, resulting in an SR of 73%. Finally, treatment D, which did not include fermented cassava leaf flour, had an SR of 68%. Both internal and external factors influence survival. Internal factors arise from the individual fish, while external factors relate to the quality of the feed and water. This aligns with Vardian et al. (2013), who claimed that survival can be affected by biotic and abiotic factors. Biotic factors encompass the age of the fish and their ability to adapt to the environment, whereas abiotic factors involve the availability of food and the quality of the water in their living medium (Mahavadiya et al., 2018).

The high survival rate can be attributed to the effective use of the provided feed by tilapia fry, which creates favorable environmental conditions in the maintenance media that support fish survival and reduce stressors that could lead to mortality during the rearing period (Nurfitasar et al., 2020). The survival rate of tilapia showed no significant differences across treatments and remained high (68-83%), likely due to the fish's positive response to the feed.

**Water Quality**

The water quality parameters observed include temperature, pH, and DO. The results of water quality measurements obtained during the study can be seen in the following table:

Table 1. Water quality parameters; pH, DO and Temperature

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatments** | **pH** | **DO** | **Temperature** |
| **A** | 8 | 6,9 | 26 - 27ºC |
| **B** | 8 | 6,7 | 26 - 27ºC |
| **C** | 8 | 6,6 | 26 - 27ºC |
| **D** | 7,9 | 6,4 | 26 - 27ºC |
| **SNI** | 6,5-8,5 | >5 ppm | 25-30oC |

According to Table 1, the average water quality data collected during the study remained within a reasonable range. The results of the water quality observations indicate that variations in research findings can be attributed to different treatments, with no impact from the quality of the water media.

Conclusion

Based on the discussion above, the following conclusions can be drawn: the addition of fermented cassava leaf flour significantly affects (P<0.05) the absolute weight growth, absolute length growth, and survival of tilapia (*O. niloticus*). A dosage of 15% fermented cassava leaf flour in commercial feed is identified as the optimal amount that promotes absolute weight growth, absolute length growth, and survival of tilapia (*O. niloticus*). During the 40-day rearing period, water quality parameters remained within a suitable range to support the growth and survival of tilapia. In light of these conclusions, further research is necessary to explore the effects of adding cassava leaf flour at a lower dosage in commercial feed for the optimal growth of tilapia (O. niloticus) fry.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

# **Reference**

1. 1. Abdourhamane T., Comlan G., Tovignon Z., Sima Obiang C., Tuncha Ng., Mba D., Beyeme A.M., Beyeme, Mb., Ondo J.P., Kimsé M., Irié Bi., Zoro A., Boukila B., Obame E.L.C. 2021. Study of the zootechnical performance of tilapia cabrae and Oreochromis schwebischi in a control environment: the case of the mbolet fish farm in Lambaréné, Gabon. 2. 1-7.
2. 2. Amare T.A., Storebakken T., Mørkøre T. 2024. Potency of cassava leaf as protein source for Nile tilapia (Oreochromis niloticus). Aquacult Int 32, 10197–10214 (2024). https://doi.org/10.1007/s10499-024-01657-3
3. 3. Carbone J.W., Pasiakos S.M. 2019. Dietary Protein and Muscle Mass: Translating Science to Application and Health Benefits. Nutrients. 2019 May 22;11(5):1136. doi: 10.3390/nu11051136.
4. 4. Elangovan P., Felix Samson., Nathan F., Ahilan, B. 2017. An overview on the significance of fish nutrition in the aquaculture industry. 349-355.
5. 5. Li Q., ​​Huang Y., Zhang Front. Nutr. 10:1153323. doi: 10.3389/fnut.2023.1153323
6. 6. Lusiana E.D., Musa M., Ramadhan S. 2021. Determinants of Nile tilapia's (Oreochromis niloticus) growth in aquaculture ponds in Batu, Indonesia. Biodiversity 22: 999-1005.
7. 7. Mahavadiya D., Sapra D., Rathod V., Sarman V. 2018. Effect of biotic and abiotic factors in feeding activity in teleost fish: A review. 6. 387-390.
8. 8. Megawati R.A., Arief M., Alamsyah M.A. 2012. Addition of feed with different crude fiber content on feed digestibility in stomached fish and non-stomached fish. Scientific Journal of Fisheries and Marine Sciences 4: 187– 189.
9. 9. Mohidin S.R.N.S.P., Moshawih S., Hermansyah A., Asmuni M.I., Shafqat N., Ming L.C. 2023. Cassava (Manihot esculenta Crantz): A Systematic Review for the Pharmacological Activities, Traditional Uses, Nutritional Values, and Phytochemistry. J Evid Based Integr Med 28:2515690X231206227. Doi: 10.1177/2515690X231206227
10. 10. Mulyasari. 2011. The Potential of Cassava Leaves as One Source of Raw Material for Fish Feed. Proceedings of the Aquaculture Technology Innovation Forum. Bogor: 901-904.
11. 11. Ng, W. K. and Wee, K. L. 2003. The Nutritive Value of Cassava Leaf Meal in Pelleted Feed for Nile Tilapia. Aquaculture, 83(1-2): 45-58
12. 12. Niode, Abdul Rahman, Nasriani Nasriani, and Ad Mahmudy Irdja. 2017. Growth and Survival of Tilapia Seeds (Oreochromis niloticus) on Different Artificial Feeds. Akademika 6.2.
13. 13. Nugraha, Nugie, and Indra Suharman. "Utilization of Cassava Leaf Flour (Manihot Utilisima Pohl) Fermented by Trichoderma in Feed on the Growth of Freshwater Pomfret (Colossoma macropomum) Seeds." SEBATIN Aquaculture Journal 3.2: 68-82.
14. 14. Rachmawati D., Elfitasari T., Chilmawati D., Yuniarti T. 2024. The effect of phytase enzyme in feed on growth performance, protein digestibility, feed utilization efficiency, mineral content, and body nutrient composition in Nile tilapia (Oreochromis niloticus) fingerlings. AACL Bioflux 17(5): 20096-2108. http://www.bioflux.com.ro/aacl
15. 15. Revi Nardi, Yunaedi Basri, Elfrida. 2013. Evaluation of the Use of Local Raw Material-Based Feed on Nutrient Value in Tilapia (O. niloticus). Department of Aquaculture. Faculty of Fisheries and Marine Sciences. Bung Hatta University, Journal of Fisheries and Marine Sciences, 2(1): 1-9.
16. 16. Vardian, A, K., Subandiyono., Pinandoyo. 2013. The Effect of Differences in Tilapia F5 Strains (Larasati, Red, Black) Given Feed with an E/P Value of 10.96 Kcal/G Protein on Growth and Survival. Journal of Aquaculture Management and Technology, 2(4): 108-114. https://ejournal3.undip.ac.id/index.php/jamt/article/view/4811.