

RESEARCH ARTICLE

Utilization of Fermented *Moringa* and *Trichanthera Gigantea* Leaves as Protein Sources in Fish Meal Production

A. Ajit*, N. I. I. Johari, A. Z. Sulaiman, N. A. M. Azman

Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebu Persiaran Tun Khalil Yaakob, 26300, Gambang, Kuantan, Pahang, Malaysia

ABSTRACT - The increasing demand for sustainable protein sources in aquaculture drives innovation in fish meal production. This study investigates the use of fermented leaves, specifically *Moringa* leaves and *Trichanthera gigantea* leaves, as alternative protein sources. The objective is to identify the optimal ratio of these fermented leaves in fish meal to enhance protein concentration. Fish feed formulations with varying ratios undergo fermentation and drying, followed by analysis of their protein, lipid, moisture, and ash content. The protein content is standardized using a Bovine Serum Albumin (BSA) calibration curve and assessed pre-fermentation via the Lowry assay. Additionally, the floatability of the fish pellets is evaluated to determine their suitability in aquatic environments. Results indicate that a formulation ratio containing 45% *Moringa* leaves, 45% rice bran, and 10% starch is the most promising, with a protein content of 28.94%, lipid content of 10.37%, moisture content of 10.08%, and ash content of 7%. The conclusion emphasizes that *Moringa* leaves serve as a superior protein source compared to *Trichanthera gigantea* leaves for fish meal production, thereby supporting sustainable aquaculture practices.

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1.0 INTRODUCTION

Aquaculture production is predicted to expand by 37% from 2016 to 109 million tonnes by 2030. This expansion is necessary to satisfy the estimated 20% increase in world fish consumption [1]. As aquaculture continues to grow, the demand for quality fish feed is also rising. Farmers are required to supply high-quality fish feed to ensure optimum growth of fish, which consequently increases production costs in the industry. Fish meal is a highly valuable protein source that is extensively used in animal diets and is a critical component of fish feed formulations. The primary reason for the rising price of fish meal is the source of protein used in the production of fish feed. The manufacturing process, which involves the drying and grinding of fish into a powder, not only contains protein but also essential fatty acids, amino acids, and a mineral that is essential for the overall health of aquatic species [[2]]. The metabolism of fish is significantly improved by protein, which serves as an essential component for a variety of metabolic processes. Similarly, amino acids are essential for the growth and development of fish. The recommended components of a freshwater fish diet are protein (25-40%), fatty acids (5-12%), vitamins (0.2-3%), and minerals (7-9%) [3]

The traditional protein sources from animal waste, such as fish offal, meat and bone are harder to find and more expensive due to high demand in other industries. As the demand for aquaculture products increases worldwide, there is a growing need to find alternative protein sources that are both sustainable and affordable. Therefore, researchers are now exploring options like plant-based proteins and insect meals, which offer good nutritional value and are less harmful to the environment [[4]]. The innovative use of fermented leaves in the production of fish meal has garnered attention as the demand for sustainable and alternative protein sources intensifies [5]. The nutritional quality of leaves is improved through fermentation, which increases the protein content and improves digestibility. This research is essential for the development of a sustainable strategy to address the increasing protein demands in aquaculture. It involves the experimentation with the ratios of fermented leaves in fish meal to determine the most effective combination for feed utilization.

Plants can be a valuable addition to fish meals in aquaculture systems. Furthermore, leaves can be easily grown and harvested, making them a sustainable and environmentally friendly alternative to fish meals. It is also a drought-resistant crop, making it ideal for areas with limited water resources. *Moringa* leaves are high in protein, containing up to 23-30% protein by dry weight, which is comparable to many fish meals. *Moringa* leaves also contain significant nutrients, a range of essential amino acids, vitamins, and minerals that are beneficial for fish health and growth [6]. *Trichanthera gigantea* leaves are also one of the alternatives that is suitable to replace fish meal. It is a fast-growing perennial tree species native to Central and South America. The tree features big leaves and tiny white tubular blooms and can reach a height of 20 meters. It contained 88.44 % of dry matter, 18.21-23.9 % of crude protein, 12.5-23.8 % of crude fiber, 2.66 % of crude fat, 21.80 % of ash, 11.56% of moisture, 5 % of calcium, 0.41 % of phosphorus, and 2,983 kcal/kg of gross energy [7],

[8] Rice bran, a widely available co-product of rice milling in Asia, is commonly incorporated into freshwater aquafeeds as an energy ingredient supplying digestible carbohydrates and residual oil, as well as B-vitamins, minerals, tocopherols, γ -oryzanol, and dietary fiber [9], [10]. It can moderate feed cost by partially replacing more expensive proteins and may provide functional components that support gut health.

Starch from maize, cassava/tapioca, wheat, broken rice, or other local sources provides nutritional functions in aquafeeds [10]. Starch enhances pellet cohesiveness and water stability when it gelatinizes during conditioning or extrusion. It also functions as a carrier matrix that promotes uniform nutrient distribution and provides energy that can preserve dietary protein for growth in freshwater fish that use carbohydrates, and it affects feed intake behavior, sinking properties, and pellet texture [11], [12].

Integrating plant-based ingredients into aquaculture diets presents a more sustainable and eco-conscious alternative to conventional fish meal. The ease of cultivation and harvest, coupled with the drought-resistant nature of leafy plants, makes them suitable for regions with limited water resources. Among these, *Moringa* leaves have high protein content, emerges as a promising substitute [13], [14]. Similarly, *Trichanthera gigantea* leaves, with substantial nutrient content, proves valuable for replacing fish meal [15].

Therefore, this study addresses an important knowledge gap in sustainable aquaculture by examining the effects of different inclusion ratios of *Moringa oleifera* and *Trichanthera gigantea* leaves as protein sources in fish pellets. While previous research has documented the individual nutritional properties of these plants, limited attention has been given to their combined application in achieving a balanced nutritional profile for fish diets. This research aims to identify the optimal ratio of these plants as alternative protein sources in feed formulation, thereby contributing to the sustainability and nutritional adequacy of aquaculture practices. The outcomes of this study are expected to provide scientifically grounded recommendations for developing sustainable, nutritionally balanced, and cost-effective fish feeds, ultimately supporting the long-term resilience of the aquaculture sector.

2.0 MATERIALS AND METHOD

2.1 Materials

Moringa leaves, *Trichanthera gigantea* leaves, rice bran, starch, cornstarch, yeast (*Saccharomyces cerevisiae*), vitamin, *Lactobacillus* (EM4), brown sugar.

2.2 Preparation of *Moringa* and *Trichanthera gigantea* leaves

Approximately 150 g of *Moringa* and *Trichanthera gigantea* leaves were collected for each type. The leaves were oven-dried at 60°C for up to 8 hours. After drying, the leaves were ground into powder using a blender and sieved to obtain a uniform particle size.

2.3 Fish feed formulation

The ratios of the fish meal ingredients were determined based on a total weight of 30 g. The formulations included *Moringa* leaves or *Trichanthera gigantea* leaves, starch, and rice bran. The specific ratios for these ingredients are outlined in Table 1.0 and Table 2.0, which show four different sample formulations with varying percentages of the main components. Starch was kept at a consistent 10% of the sample weight. Following these ratios, the ingredients were thoroughly mixed. An additional 0.6 g of vitamin premix and 2.4 g of corn starch were subsequently added to the formulation [16].

Table 1. The ratio of the selected ingredients for *Moringa* leaves

| Ingredient | Sample-1 (%) | Sample -2 (%) | Sample-3 (%) | Sample-4 (%) |
|--------------------------|--------------|---------------|--------------|--------------|
| Rice bran | 0 | 30 | 45 | 60 |
| <i>Moringa</i> leaves | 90 | 60 | 45 | 30 |
| Starch | 10 | 10 | 10 | 10 |

Table 2. The ratio of the selected ingredients for *Trichanthera gigantea* leaves

| Ingredient | Sample-1 (%) | Sample-2 (%) | Sample-3 (%) | Sample-4 (%) |
|---|--------------|--------------|--------------|--------------|
| Rice bran | 0 | 30 | 45 | 60 |
| <i>Trichanthera</i> <i>gigantea</i> leaves | 90 | 60 | 45 | 30 |
| Starch | 10 | 10 | 10 | 10 |

2.4 Fermentation of fish feed formulation

A fermentation reagent was prepared by adding 0.33 g of yeast (*Saccharomyces cerevisiae*), 0.66 mL of *Lactobacillus* (EM4), and 0.66 mL of brown sugar to 33 mL of water. This reagent was mixed with the fish feed ingredients as prescribe

in Table 1.0 and Table 2.0, and 10% starch was added to improve binding. The mixture was then transferred to a closed container and fermented for 4 days. After fermentation, the mixture was dried at 135°C for 2 hours and shaped into pellets. The pellets were stored in airtight plastic jars at room temperature.

2.5 Analysis for protein, lipid, moisture, and ash content

2.5.1 Standardisation of calibration curve for Bovine Serum Albumin (BSA)

A stock solution of BSA with a concentration of 1 mg/mL. Then, the stock solution is diluted to create a series of standard solutions with concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL. The protein was determined by using the Lowry method. In this assay, three reactions solution were prepared as Lowry solution which was solution A, solution B, and solution C with a ratio of 100:1:1 volume basis, respectively [17]. For solution A, 2.86 g of sodium hydroxide (NaOH) and 14.31 g of sodium carbonate (Na₂CO₃) were dissolved in 500 mL of deionized water. Solution B was prepared by dissolving 1.42 g of copper (II) sulfate pentahydrate (CuSO₄.5(H₂O)) in 100 mL deionized water. For solution C, it was prepared by dissolving 2.86 g of sodium tartrate dihydrate (Na₂Tartrate.2(H₂O)) in 100 mL deionized water. Folin Reagent was freshly prepared for every assay by mixing 5 mL of 2N Folin and Ciocalteu's Phenol Reagent with 6 mL of deionized water. Then, the assay was initiated by mixing 0.5 mL sample with 0.7 mL of Lowry solution. The mixture sample was incubated for 20 min at room temperature under dark conditions. After 20 min, the mixture sample was immediately added to 0.1 mL of Folin Reagent and mixed it. Then, the mixture was incubated for 30 min at room temperature under dark condition. After 30 min incubation, the mixture was analysed in UV-visible spectrometers and the absorbance was measured shortly. The protein content was analysed by measuring the absorbance at 750 nm using UV-visible spectrometers. The protein standard curve was established by BSA protein standard.

2.5.2 Protein determination using Lowry assay

The protein content determination process, all the samples were extracted by mixing 1g of samples with 50 mM hydrochloride acid (HCl)-Tris buffer at a 1:5 ratio for 45 min at room temperature. After centrifugation at 10,000 rpm for 10 min, the supernatant was collected and filtered. Then, 0.5 mL of the sample was used in the Lowry solution method for the calibration curve standardization using BSA protein standards [17]. For the Lowry solution, three solutions (A, B, and C) were prepared at a ratio of 100:1:1, respectively. Solution A included 2.86 g of sodium hydroxide (NaOH) and 14.31 g of sodium carbonate (Na₂CO₃) dissolved in 500 mL of deionized water. Solution B consisted of 1.42 g of copper (II) sulfate pentahydrate (CuSO₄.5(H₂O)) in 100 mL deionized water. Solution C was prepared by dissolving 2.86 g of sodium tartrate dihydrate (Na₂Tartrate.2(H₂O)) in 100 mL deionized water. Folin Reagent was freshly prepared for each assay by combining 5 mL of 2N Folin and Ciocalteu's Phenol Reagent with 6 mL of deionized water. The assay involved mixing 0.5 mL of the sample with 0.7 mL of the Lowry solution, followed by a 20 min incubation at room temperature in the dark. Afterward, 0.1 mL of Folin Reagent was added, and the mixture was incubated for an additional 30 min. The absorbance was measured at 750 nm using UV-visible spectrometers. The protein content was analysed based on the absorbance values, with a standard curve established using BSA protein standards.

2.5.3 Lipid determination using Folch method

The Folch method was employed for lipid extraction [18]. A 0.15 g sample, which contained 0.15 mL of water, was homogenized with 3 mL of chloroform: methanol (2:1, v/v). The resulting homogenate was then filtered, and the filtrate volume was adjusted to 20 mL using chloroform and methanol (2:1, v/v). The filtrate was mixed with 4 mL of water, creating an upper phase containing water and methanol with hydrophilic compounds and a lower phase containing chloroform with lipids. The upper phase, containing hydrophilic compounds, was completely removed using a droplet. The aluminium tray was weighed, and the lower phase was poured into the tray, with the weight recorded. The tray with the lower phase was then heated on a hot plate at 40°C until lipids were formed. The lipids, along with the aluminium tray, were weighed to determine the extracted lipid weight by using Equation 1 and Equation 2.

$$\text{Weight of lipids} = (\text{weight of container} + \text{extracted lipids}) - (\text{weight of container}) \quad (1)$$

The weight of the lipids in the samples was determined by weight difference:

$$\text{Lipid content (\%)} = \frac{\text{weight of lipids (g)}}{\text{weight of samples (g)}} \times 100 \quad (2)$$

2.5.4 Moisture content

The sample was weighed to determine its initial weight and then dried in an oven at 135°C for 2 hours. After the drying process, the sample was removed from the oven and placed at room temperature. Subsequently, the sample was weighed again after cooling down to obtain its final weight. The difference in weight between the initial and final weights represented the amount of moisture in the sample. Finally, the percentage of moisture in the sample was calculated by using Equation 3 by dividing the weight of the moisture by the initial weight of the sample and multiplying by 100%.

$$\text{Moisture content (\%)} = \frac{(\text{Initial weight of sample (g)} - \text{Final weight of sample (g)})}{\text{Initial weight of sample (g)}} \times 100 \quad (3)$$

2.5.5 Ash content

Initially, the sample and a porcelain crucible were weighed. Subsequently, a 2 g sample was placed into the porcelain crucible. The crucible that contained the sample, was then subjected to a furnace at a temperature of 550°C. After the heating process, it was allowed to cool in at room temperature and then re-weighed. The percentage of ash was calculated using the provided formula in Equation 4:

$$\text{Ash (\%)} = \frac{\text{Mass of dry samples (g)}}{\text{Mass of ash (g)}} \times 100 \quad (2)$$

2.5.6 Floatability

The floatability assessment method employed a systematic procedure. Initially, the optimal ratio was determined, selecting Sample 3 was selected with composed of 45% *Moringa leaves*, 45% rice bran, 10% starch, 8% cornstarch, and 2% vitamins. Subsequently, 20 pellets from this ratio were collected and placed in a 500 mL water medium. The experiment commenced with the initiation of the timer. Readings of the floating fish pellets were recorded at 2-minute intervals for a duration of 30 minutes. The data collected during the experiment were then subjected to calculations using a predetermined formula (Equation 5) to evaluate the floatability characteristics of the fish pellets. This method allowed for a comprehensive analysis of the pellets' buoyancy over the specified timeframe, providing valuable insights into their performance in aquatic environments.

$$\text{Pellet afloat (\%)} = \frac{\text{Final number of feed pellets afloat}}{\text{Initial number of feed pellets}} \times 100 \quad (5)$$

3.0 RESULTS AND DISCUSSION

The protein, lipid, moisture, and ash contents of fish feed formulations containing fermented *Moringa oleifera* and *Trichanthera gigantea* leaves were evaluated and are discussed in this section. The analysis aims to compare the nutritional composition of both plant-based ingredients to assess their potential in fish feed applications.

3.1 Protein content

Protein content analysis conducted prior to fermentation revealed notable differences among the sample components, as illustrated in Figure 1. *Moringa leaves* exhibited the highest protein content at 16.04% while *Trichanthera gigantea* leaves displayed a slightly lower protein content of 13.67%. These findings underscore the distinctive nutritional profiles of the individual components, with *Moringa leaves* standing out as a particularly rich source of protein. The variation in protein content among the samples provides valuable insights into the potential nutritional contributions each component can make to the fish meal. The selection and combination of these raw materials, along with the subsequent fermentation process, will play an important role in determining the final protein composition of the fish meal and, consequently, its suitability for supporting the dietary needs of the target fish species.

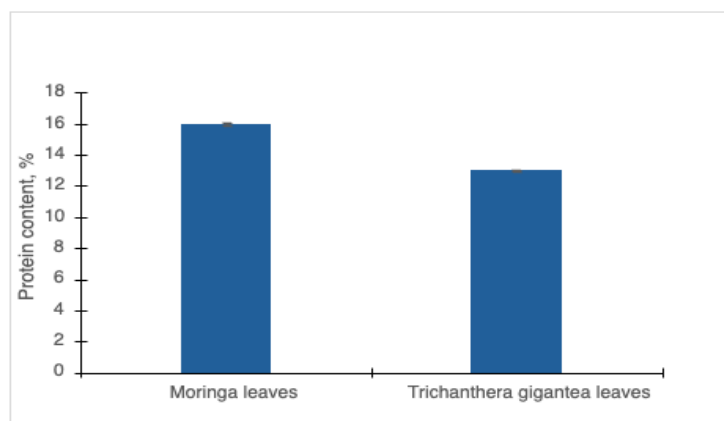


Figure 1. The protein content in *Moringa* and *Trichanthera gigantea* leaves before fermentation.

Figure 2 presents the protein levels in *Moringa oleifera* and *Trichanthera gigantea* leaves following the fermentation process. Protein is a vital nutrient in aquaculture feed formulation, playing a crucial role in muscle development, tissue regeneration, and metabolic energy fish. The analysis of protein content after fermentation (Figure 2) shows that the *Moringa* leaf samples had a significantly higher protein content compared to before-fermentation level of 16.04%. For example, Sample 3 with *Moringa* leaves showed a protein content of 28.94%, an increase of approximately 12.9% from before-fermentation level of the leaves alone. Similarly, the *Trichanthera gigantea* samples also showed an increase from the initial 13.67%. This demonstrates the effectiveness of fermentation and the inclusion of these leaves in boosting the protein content of the feed. The analytical results clearly shows that *Moringa* leaves have a significantly higher protein content compared to *Trichanthera gigantea* leaves. This observation indicate the *Moringa* may be a superior nutritional potential as a plant-based protein source and suitability for partial substitution of conventional protein ingredient in aquafeed development.

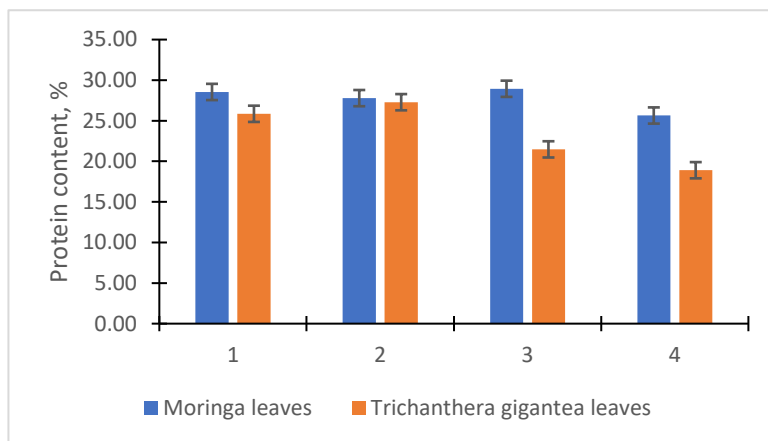


Figure 2. The protein content in *Moringa* and *Trichanthera gigantea* leaves after fermentation.

The preference for *Moringa* is further supported by Raju et al., 2024 its protein content, which ranges from 23% to 30%, compared to the slightly lower range of 18% to 23% for *Trichanthera gigantea* [19]. This shows that while fermentation increases the protein levels in both, *Moringa* remains the superior choice for fish meal due to its consistently higher protein content. Fermentation is often employed to enhance the nutritional value of feed by improving digestibility and breaking down anti-nutritional factors. This suggests that while fermentation may improve other nutritional aspects or digestibility, its effect on protein levels in *Trichanthera* leaves might be minimal, requiring alternative methods to boost protein content for fish feed production. The substantial difference in protein levels highlights *Moringa*'s potential as a sustainable and enriching alternative for fish feed formulations. This higher protein content in *Moringa* leaves can be attributed to its biochemical composition, which includes a greater concentration of essential amino acids and other proteins, enhancing its nutritional value.

3.2 Lipid content

Lipids serve as an additional energy source and play a crucial role in the absorption of vitamins and the formation of cell membranes. The previous studies have reported lipid contents of 4–10% for *Moringa oleifera* leaves and 3–7% for *Trichanthera gigantea* leaves [4], [5], [7], representing their natural composition before fermentation. In this study, the lipid content of the formulated fish meals supplemented with *Moringa oleifera* and *Trichanthera gigantea* leaves increased to 7.00–12.13% after fermentation, which is higher than before fermentation values of 4–9%. These findings are in line with the results of Alawiyah et al. (2022), who also reported higher lipid contents following fermentation, with *Moringa* ranging between 5–9% and *Trichanthera gigantea* between 2.5–6.8%. Overall, the results confirm *Moringa oleifera* consistently demonstrates a superior lipid profile compared to *Trichanthera gigantea*, both before and after fermentation.

The lipid levels in Samples 2, 3, and 4 were significantly higher than in Sample 1 because of the increased use of rice bran, which contains 15–25% lipids [20]. Adding rice bran raised the overall lipid concentration in the formulated feeds and improved their nutritional composition. The higher lipid content increased the energy density of the diet and provided a readily available energy source that is important for fish growth, metabolic processes, and feed efficiency.

3.3 Moisture content

Figure 4 presents the moisture content in *Moringa* and *Trichanthera gigantea* leaves. Moisture content is a critical factor affecting the nutritional composition, shelf life, and overall quality of fish feed. Maintaining optimal moisture levels is essential to prevent mold growth and minimize nutrient degradation. The data in Figure 4 show that *Moringa* leaves have a lower moisture content compared to *Trichanthera gigantea* leaves, indicating that *Moringa* holds less water, making it a more suitable ingredient for long-term storage.

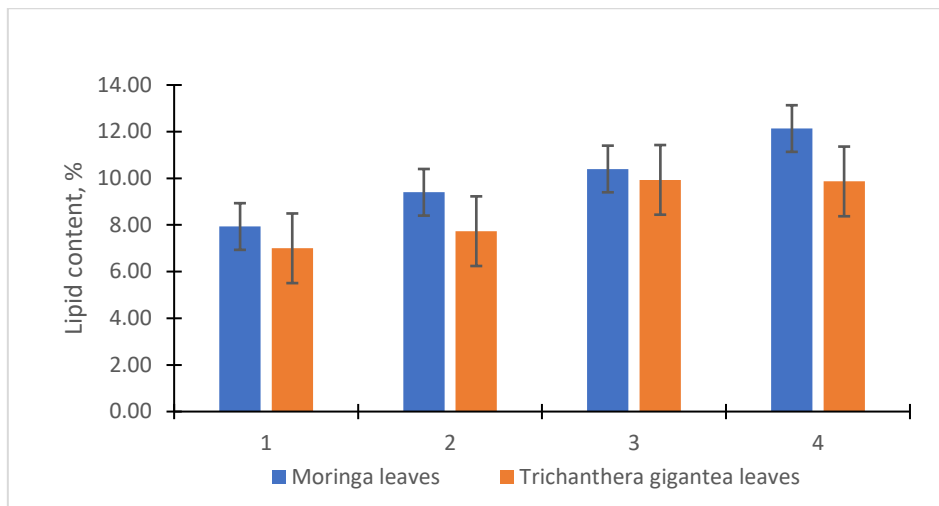


Figure 3. The lipid content in *Moringa* and *Trichanthera gigantea* leaves

This observation aligns with the findings of Alawiyah et al. (2022), who noted that *Trichanthera gigantea* leaves tend to absorb and retain more moisture, which can increase the risk of spoilage in fish feed. In contrast, the lower moisture content of *Moringa* leaves reduces this risk, contributing to its potential as a more stable ingredient in fish meal production [8], [21], [22], [23].

The moisture content in Sample 1, which consists solely of leaves is lower compared to Samples 2, 3, and 4, which include rice bran. The higher water absorption capability of rice bran likely contributes to the increased moisture content in these samples [9]. This elevated moisture level can be attributed to rice bran's ability to absorb and retain more water during processing, which results in higher moisture levels compared to Sample 1. The inclusion of rice bran introduces a variable that significantly impacts the overall moisture content of the formulations, as seen in previous studies examining the role of rice bran in aquatic feed.

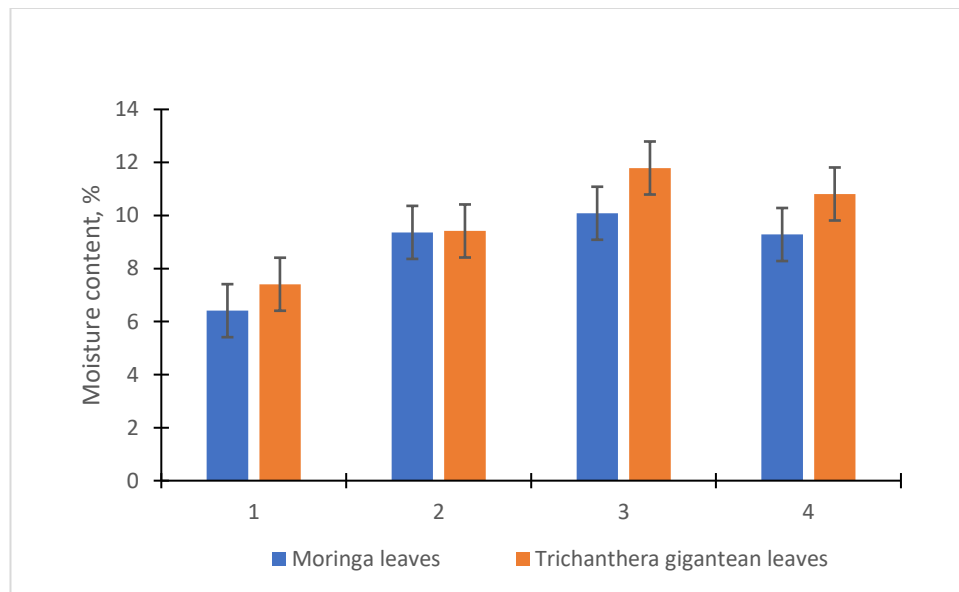


Figure 4. The moisture content in *Moringa* and *Trichanthera gigantea* leaves

3.4 Ash content

Ash content in plant materials represents the total mineral content and consists of inorganic minerals remaining after the combustion of organic matter. These minerals, such as phosphorus and calcium, are essential for bone development and other physiological functions in fish. Based on Figure 5, *Trichanthera gigantea* leaves contain a higher ash content than *Moringa* leaves, with *Trichanthera gigantea* exhibiting 16% to 20% ash content compared to *Moringa*'s 8% to 12%. In Samples 3 and 4, the ash content is observed to be lower than in Samples 1 and 2. This difference can be attributed to the higher moisture retention which could be due to the increased moisture retention caused by the inclusion of rice bran.

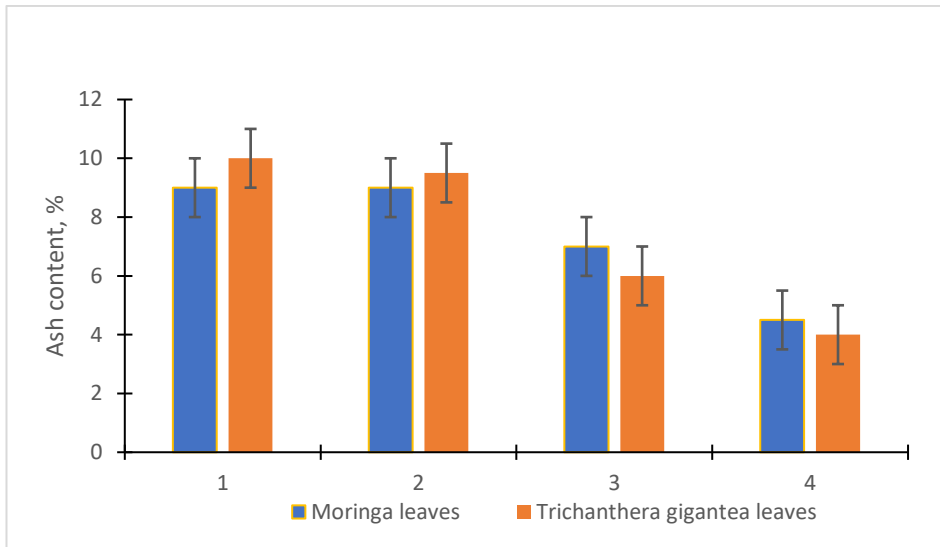


Figure 5. The ash content in *Moringa* and *Trichanthera gigantea* leaves

This increased moisture content may cause a dilution effect, reducing the overall percentage of ash in Samples 3 and 4. This relationship between moisture retention and ash content emphasizes the impact of rice bran on the nutritional profile of the samples, consistent with the findings of Rajput et al. (2022), who noted similar interactions between moisture and mineral content in rice bran-based feed formulations. The relationship between moisture retention and ash content highlights the impact of rice bran composition on the overall nutritional characteristics of the samples [10]

3.5 Floatability of sample

Sample 3 of Moringa leaves was likely chosen due to its higher protein content, along with its well-balanced nutritional profile and favorable physical properties. These qualities make it an excellent candidate for optimization, particularly in terms of floatability. Testing this sample could lead to advancements in fish feed formulations that not only meet nutritional requirements but also perform effectively in aquatic environments.

According to Figure 6, the floatability of Sample 3 shows a gradual decline over a 30 min period, highlighting the potential challenge of relying on a formulation comprising 45% *Moringa leaves*, 45% rice bran, and 10% starch for fish pellet production. Despite an initial strong performance with 20 pellets afloat at 2 minutes, the count consistently drops to 12 by the end, indicating increased water absorption and subsequent sinking. This decline suggests that the formulation's water retention properties could be improved to enhance floatability. During the fermentation process, an additional 10% starch was incorporated to enhance the binding properties of the pellets, bringing the total starch content to 20%. Starch plays a critical role in both expansion and binding in the final product, which is essential for ensuring the buoyancy of aquatic feed.

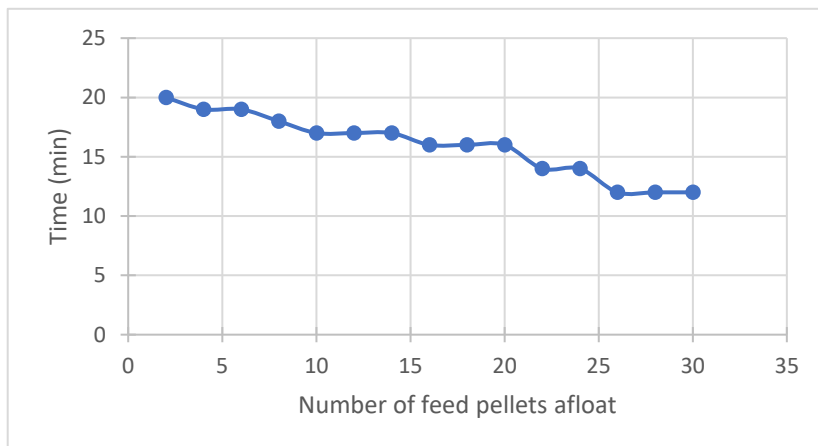


Figure 6. Number of feed pellets afloat for Sample 3 of *Moringa* leaves vs time

4.0 CONCLUSION

In conclusion, the comparative analysis highlights that *Moringa leaves* boast a higher protein content than *Trichanthera gigantea leaves*, positioning them as a more suitable and preferable option for incorporation into fish meal production. This finding underscores the significance of considering *Moringa leaves* as an alternative protein source in nutritionally enriched fish meal formulations. Upon an overall assessment, Sample 3 that contains 45% *Moringa leaves*, 45% rice bran and 10% starch is chosen due to its favourable nutritional composition. It yields a protein content of 28.94%, falling within the best range of 25% to 40% required for fish nutrition. Additionally, the lipid content at 10.37% aligns with the recommended range of 5% to 12%, meeting the lipid requirements for fish. The moisture content at 10.08% is within the appropriate range of 7% to 11%, fulfilling the moisture needs of fish. Furthermore, the ash content is at 7%, falling within the range of 7% to 9%, meeting the ash requirements for fish nutrition. These factors collectively establish Sample 3 as a promising choice for fish meal production, offering a well-balanced nutritional profile that aligns with the dietary needs of fish.

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6.0 CONFLICT OF INTEREST

The authors declare no conflicts of interest.

7.0 AUTHORS CONTRIBUTION

N.I.I Johari: Investigation, Writing-original draft, Visualisation.

N.A.M Azman: Methodology, Data curation, Resources.

A. Ajit : Conceptualisation, Project administration.

A.Z Sulaiman: Writing-review & editing,

11.0 REFERENCES

- [1] S. Fathi, A. N. Harun, S. Rambat, and N. A. Tukiran, "Current Issues in Aquaculture: Lessons from Malaysia," *Adv Sci Lett*, vol. 24, no. 1, pp. 503–505, Mar. 2018, doi: 10.1166/asl.2018.12051.
- [2] FAO, "The production of fish meal and oil," 1986.
- [3] R. D. Miles and F. A. Chapman, "Benefits of Fish Meal in Aquaculture Diets," *EDIS*, vol. 2006, no. 12, 2006, doi: 10.32473/edis-fa122-2006.
- [4] S. Helmiati, Rustadi, A. Isnansetyo, and Zuprizal, "The replacement of fish meal with fermented *Moringa leaves* meal and its effect on the immune response of red tilapia (*Oreochromis sp.*)," in *IOP Conference Series: Earth and Environmental Science*, 2021. doi: 10.1088/1755-1315/919/1/012057.
- [5] S. Helmiati, Rustadi, A. Isnansetyo, and Zuprizal, "Replacement of fish meal with fermented *moringa leaves* meal and its effect on the growth performance of red tilapia (*Oreochromis sp.*)," *AACL Bioflux*, vol. 13, no. 3, 2020.
- [6] J. Cao *et al.*, "*Moringa oleifera* leaf protein: Extraction, characteristics and applications," 2023. doi: 10.1016/j.jfca.2023.105234.
- [7] A. N. Alawiyah, Rd. H. Supratman, and - Mansyur, "Kualitas Fisik Pelet Ransum Kelinci yang Mengandung Berbagai Tingkat *Trichanthera gigantea*," *Pastura*, vol. 12, no. 1, 2022, doi: 10.24843/pastura.2022.v12.i01.p01.
- [8] M. Rosales, "*Trichanthera gigantea* (Humboldt & Bonpland.) nees: A review," 1997.
- [9] X. Yu, H. Li, X. Wu, and W. Wu, "Effect of dephenolization degree of rice bran on the structure of rice bran protein," *LWT*, vol. 184, 2023, doi: 10.1016/j.lwt.2023.115104.
- [10] S. Isah and J. Okosun, "Nutritional and Anti-nutritional Compositions of Rice Bran as a Potential Animal Feed," *Int Res J Pure Appl Chem*, vol. 24, no. 6, 2023, doi: 10.9734/irjpac/2023/v24i6835.
- [11] R. Strathman and G. Yan, "Optimising Starch Transformation in Aquafeed Extrusion for Enhanced Water Stability and Performance," *Aquafeed Journal*, vol. 2, no. 1, 2023, doi: 10.61985/aj2401.
- [12] H. Cheng, H. Wang, S. Ma, M. Xue, J. Li, and J. Yang, "Development of a water solubility model of extruded feeds by utilizing a starch gelatinization model," *Int J Food Prop*, vol. 25, no. 1, 2022, doi: 10.1080/10942912.2022.2046055.

- [13] A. M. Jiménez-Monreal, F. A. Guardiola, M. Ángeles Esteban, M. Antonia Murcia Tomás, and M. Martínez-Tomé, "Antioxidant activity in gilthead seabream (*Sparus aurata* L.) fed with diet supplemented with moringa," *Antioxidants*, vol. 10, no. 9, 2021, doi: 10.3390/antiox10091423.
- [14] Fatmawati *et al.*, "Intervention of Giving Moringa Biscuits (*Moringa Oliefera*) Mix Sori Fish Flour to Increased Blood Hemoglobin Levels in Young Girls, Kendari, INDONESIA," *Pharmacognosy Journal*, vol. 15, no. 2, 2023, doi: 10.5530/pj.2023.15.64.
- [15] F. O. Libatique, "Growth Performance, Blood Dynamics and Sensory Characteristics of Broilers Fed with Madre de Agua (*Trichanthera gigantea*) Leaf Meal," *Linker: The Journal of Emerging Research in Agriculture, Fisheries and Forestry*, vol. 2, no. 1, 2021.
- [16] S. V. Bhosale, M. P. Bhilave, and S. B. Nadaf, "Formulation of fish feed using ingredients from plant sources: Reasearch notes," *Research Journal of Agricultural Sciences*, vol. 1, no. 3, 2010.
- [17] J. H. Waterborg and H. R. Matthews, "The Lowry method for protein quantitation.," 1994. doi: 10.1385/0-89603-268-x:1.
- [18] K. W. WASHBURN, "A Modification of the Folch Method of Lipid Extraction for Poultry," *Poult Sci*, vol. 68, no. 11, 1989, doi: 10.3382/ps.0681425.
- [19] D. N. N. N. K. N. R. B. S. G. R. S. S. Raju, "Effect of Moringa, Subabul and Guava Leaves Either Sole or in Combination on Nutrient Utilization, Nitrogen Balance of Ram Lambs," - *Indian Journal of Animal Research (Agricultural Research Communication Centre)*, 2024.
- [20] B. L. Tan, M. E. Norhaizan, and L. C. Chan, "Rice Bran: From Waste to Nutritious Food Ingredients," 2023. doi: 10.3390/nu15112503.
- [21] H. A. Jack, L. M. Cranston, J. L. Burke, M. Knights, and P. C. H. Morel, "The effects of increasing concentrations of *Trichanthera gigantea* leaves in pellets on the nutritive value and short-term intake of diets of grass plus pellets offered to lambs reared under tropical conditions in the Caribbean," *Tropical Grasslands-Forrajes Tropicales*, vol. 9, no. 3, 2021, doi: 10.17138/TGFT(9)383-390.
- [22] A. N. Alawiyah, Rd. H. Supratman, and - Mansyur, "Kualitas Fisik Pelet Ransum Kelinci yang Mengandung Berbagai Tingkat *Trichanthera gigantea*," *Pastura*, vol. 12, no. 1, 2022, doi: 10.24843/pastura.2022.v12.i01.p01.
- [23] M. Rosales, "*Trichanthera gigantea* (Humboldt & Bonpland.) nees: A review," 1997.