International Journal of Agriculture and Biosciences 2024 13(3): 318-324.
https://doi.org/10.47278/journal.ijab/2024.124

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Nutritional Value of Alligator Weed (*Alternanthera philoxeroides*) and Its Application for Herbivorous Aquaculture Feed

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ABSTRACT

This study investigates Alligator weed's nutritional value and potential application (*Alternanthera philoxeroides*) as a feed ingredient in herbivorous aquaculture. The nutrient composition of Alligator weed was analyzed, revealing a high content of crude protein (18.5g/100g), crude fiber (21.0g/100g), and essential minerals such as calcium (1,200mg/100g) and potassium (2,350mg/100g). Phytochemical analysis identified significant amounts of flavonoids (2.35g/100g) and phenolic compounds (3.80g/100g), indicating potential antioxidant properties. Antioxidant activity was assessed using the DPPH assay, with methanol extracts showing the highest activity (79.4% inhibition at 10mg/mL). The application of Alligator weed in aquaculture feed was evaluated in several herbivorous fish species, including tilapia, grass carp, silver carp, common carp, and freshwater prawns. Optimal inclusion levels were determined to be 20%, resulting in improved growth performance, feed conversion ratios (FCR), and survival rates across all species. The findings suggest that Alligator weed is a promising, sustainable feed ingredient that can enhance the health and productivity of herbivorous aquaculture species. Further research on long-term effects and commercial viability is recommended to fully harness its benefits.

Keywords: Alligator weed, Antioxidant, Aquaculture feed, Herbivorous fish, Nutritional value.

Article History
Article # 24-675
Received: 20-Jun-24
Revised: 19-Jul-24
Accepted: 24-Jul-24
Online First: 27-Jul-24

INTRODUCTION

Alligator weed (*Alternanthera philoxeroides*) is an invasive aquatic plant found in various parts of the world, particularly in tropical and subtropical regions (Tanveer et al., 2018). Originating from South America, this species has rapidly spread due to its high adaptability and aggressive growth patterns, often leading to significant ecological and economic impacts (Clements & Jones, 2021). Traditionally considered a nuisance, recent studies have begun to explore its potential as a resource, particularly in the context of sustainable aquaculture (Wu et al., 2023; Suraiya et al., 2024).

Herbivorous aquaculture, including the farming of species such as tilapia, grass carp, and certain freshwater prawns, requires a steady supply of plant-based feed. Given the increasing costs and environmental concerns associated with conventional fish feed ingredients like soybean meal and fishmeal, there is a pressing need to identify alternative, sustainable feed sources (Mitra, 2020; Albrektsen et al., 2022). Alligator weed, with its rapid growth and high biomass yield, presents a promising candidate for such purposes.

Cite this Article as: Serdiati N, Islamy RA, Mamat NB, Hasan V and Valen FS, 2024. Nutritional value of alligator weed (*Alternanthera philoxeroides*) and its application for herbivorous aquaculture feed. International Journal of Agriculture and Biosciences 13(3): 318-324. https://doi.org/10.47278/journal.ijab/2024.124

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Preliminary analyses of alligator weed indicate that it possesses a favorable nutritional profile for herbivorous fish. It is rich in proteins, essential amino acids, and fibers, while also providing vitamins and minerals necessary for the growth and health of aquatic species (Li et al., 2020). Additionally, its high palatability and digestibility make it an attractive feed component. Utilizing alligator weed as an aquaculture feed could potentially reduce feed costs, mitigate the environmental impact of feed production, and contribute to the management of this invasive species.

This paper aims to provide a comprehensive evaluation of the nutritional value of alligator weed and assess its applicability as a feed ingredient in herbivorous aquaculture. By examining its nutrient composition, growth performance of fish fed with alligator weed-based diets, and potential environmental benefits, this study seeks to establish a foundation for integrating this invasive plant into sustainable aquaculture practices.

MATERIALS & METHODS

Plant Material Collection and Preparation

Alligator weed (*Alternanthera philoxeroides*) was collected from a natural habitat located in Malang, East Java, Indonesia. The leaves of the plants were harvested and thoroughly washed with distilled water to remove any adhering dirt and contaminants. Following the cleaning process, the leaves were air-dried in a shaded area for 48 hours. Subsequently, the leaves were oven-dried at 60°C until a constant weight was achieved. The dried leaves were then ground into a fine powder using a laboratory mill. Finally, the powdered material was stored in air-tight containers at room temperature (±25°C) until further use.

Nutritional Composition Analysis

The proximate composition of Alligator weed powder was analyzed using standard AOAC (Association of Official Analytical Chemists) methods (Aletan & Kwazo, 2020). The crude protein content was determined using the Kjeldahl method, while crude fat was measured by Soxhlet extraction. The crude fiber content was analyzed using the Van Soest method. Ash content was determined by incinerating the samples at 550°C in a muffle furnace. Carbohydrate content was calculated by difference.

The mineral content of Alligator weed was analyzed using atomic absorption spectrophotometry. Calcium (Ca), Magnesium (Mg), Iron (Fe), and Zinc (Zn) contents were measured following wet digestion of the samples. Phosphorus (P) content was determined calorimetrically. Potassium (K) and Sodium (Na) contents were measured using a flame photometer.

Vitamin A, Vitamin C, and Vitamin E contents in Alligator weed were determined using high-performance liquid chromatography (HPLC). The samples were prepared and extracted following standard protocols before being subjected to HPLC analysis.

Amino acid composition was analyzed using high-performance liquid chromatography (HPLC) following acid hydrolysis of the Alligator weed samples. The contents of lysine, methionine, threonine, and valine were quantified and reported.

The fatty acid profile was determined using gas chromatography (GC). The samples were subjected to lipid extraction followed by methylation. Linoleic acid (Omega-6) and α-linolenic acid (Omega-3) contents were then measured using gas chromatography (GC).

Phytochemical Screening

Qualitative phytochemical analysis was conducted to detect the presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, terpenoids, glycosides, steroids, coumarins, and anthocyanins in the Alligator weed powder. Standard procedures as described by published methods (Akbar et al., 2021).

Antioxidant Activity using DPPH Assay

The antioxidant activity of the Alligator weed extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay (Khandker et al., 2022). Different concentrations (1, 5, and 10mg/mL) of the extracts were prepared in water, methanol, and ethanol. The antioxidant activity was measured by mixing 1mL of the extract with 2mL of 0.1mM DPPH solution in methanol. The mixture was incubated in the dark for 30min, and the absorbance was measured at 517nm using a spectrophotometer. Vitamin C was used as the reference standard. The percentage inhibition of DPPH radical was calculated using the formula:

$$\text{DPPH Inhibition } (\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

Fish Feed Preparation

Experimental diets were formulated by incorporating Alligator weed powder at different inclusion levels (10, 20, and 30%) into a basal diet. The ingredients of the basal diet included fishmeal, soybean meal, cornmeal, wheat flour, fish oil, vitamins, and minerals. The ingredients were thoroughly mixed, and the diets were pelletized using a laboratory pellet mill. The pellets were dried at 40°C and stored in sealed plastic bags until feeding trials.

Fish Feeding Trials

Herbivorous fish species (Tilapia, Grass Carp, Silver Carp, Common Carp, and Freshwater Prawns) were obtained from a local aquaculture farm. The fish were acclimatized for two weeks in 1000-liter tanks with continuous aeration. During the acclimatization period, the fish were fed a commercial diet.

For the feeding trials, fish were randomly distributed into 12 tanks (3 replicates per diet). Each tank contained 30 fish of similar size and weight. The fish were fed the experimental diets twice daily at 3% of their body weight for eight weeks. Growth performance parameters, including weight gain, specific growth rate (SGR), and feed conversion ratio (FCR), were recorded. The survival rate was monitored throughout the trial period.

Data Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test to determine significant differences between the means of different treatment groups. Statistical significance was set at $P<0.05$. All

statistical analyses were performed using SPSS software.

RESULTS & DISCUSSION

Nutritional Compositions

The analysis of Alligator weed (*Alternanthera philoxeroides*) indicated a rich nutritional profile, which is promising for its application as an alternative feed ingredient in herbivorous aquaculture. The nutritional composition of Alligator weed (*Alternanthera philoxeroides*) per 100g of dry weight was determined and is summarized in the following table:

Table 1: Nutritional Compositions of Alligator Weed (*Alternanthera philoxeroides*)

Nutrient	Content per 100g (Dry Weight)
Proximate Composition	
Crude Protein	18.5g
Crude Fat	3.2g
Crude Fiber	21.0g
Ash	14.7g
Carbohydrates	42.6g
Minerals	
Calcium (Ca)	1,200mg
Phosphorus (P)	310mg
Potassium (K)	2,350mg
Magnesium (Mg)	320mg
Sodium (Na)	45mg
Iron (Fe)	35mg
Zinc (Zn)	4.8mg
Vitamins	
Vitamin A	1,450IU
Vitamin C	35mg
Vitamin E	3.1mg
Amino Acids	
Lysine	1.2g
Methionine	0.4g
Threonine	0.9g
Valine	1.0g
Fatty Acids	
Linoleic Acid (Omega-6)	1.1g
α-Linolenic Acid (Omega-3)	0.3g

The crude protein content of 18.5g/100g dry weight suggests that Alligator weed can serve as a substantial protein source. Protein is essential for the growth and maintenance of body tissues in fish. The crude fat content, measured at 3.2g, although moderate, provides essential fatty acids required for various physiological functions. The high crude fiber content (21.0g) is beneficial for digestive health, although it may necessitate careful consideration in diet formulation to prevent excessive fiber that could impair nutrient digestibility (Annamalai et al., 2021).

The ash content of 14.7g indicates a significant presence of inorganic minerals, which are critical for various metabolic processes in fish (Lall & Kaushik, 2021). Carbohydrates, accounting for 42.6g, provide an energy source, though their utilization efficiency varies among different fish species (Maas et al., 2020; Phan et al., 2021).

The mineral analysis revealed that Alligator weed is particularly rich in calcium (1,200mg), potassium (2,350mg), and magnesium (320mg). Calcium and magnesium are vital for skeletal development and metabolic functions, while potassium plays a crucial role in osmoregulation and cellular functions. The presence of iron (35mg) and zinc (4.8mg) is also noteworthy, as these minerals are essential for oxygen transport and enzyme functions, respectively. The relatively low sodium content (45mg) is beneficial, considering the need to manage sodium levels in aquaculture systems.

Vitamins A, C, and E were found in significant amounts. Vitamin A (1,450IU) is essential for vision, immune function, and growth. Vitamin C (35mg) acts as an antioxidant and is necessary for collagen synthesis and immune function. Vitamin E (3.1mg) is another potent antioxidant that protects cellular membranes from oxidative damage. The presence of these vitamins supports the use of Alligator weed to enhance the overall health and stress resistance of fish.

The amino acid profile includes lysine (1.2g), methionine (0.4g), threonine (0.9g), and valine (1.0g). These essential amino acids are required for protein synthesis and overall growth (Zhao et al., 2019; Ahmad et al., 2021). Lysine and methionine are often limited in plant-based diets, making their presence in Alligator weed particularly beneficial for balanced nutrition.

The fatty acids analysis showed linoleic acid (1.1g) and α-linolenic acid (0.3g), which are omega-6 and omega-3 fatty acids, respectively. These fatty acids are important for maintaining cell membrane integrity, reproductive performance, and anti-inflammatory responses (Kumar et al., 2022; Shi et al., 2022). The presence of these fatty acids in Alligator weed can contribute to the essential fatty acid requirements of fish.

The nutrient-rich profile of Alligator weed suggests its potential as a sustainable feed ingredient in herbivorous aquaculture. The high protein content, coupled with essential minerals and vitamins, can support growth, health, and productivity in fish. Studies have demonstrated that plant-based feeds can effectively replace conventional fishmeal and other animal-based feed ingredients without compromising growth performance and health (Barrows et al., 2023).

Phytochemical Screening

The phytochemical screening of Alligator weed (*Alternanthera philoxeroides*) revealed the presence of a variety of bioactive compounds, which are known for their potential health benefits, and therapeutic properties. The phytochemical composition of Alligator weed (*Alternanthera philoxeroides*) per 100g of dry weight was determined and summarized in Table 2.

Table 2: The results of phytochemical composition of Alligator weed (*Alternanthera philoxeroides*)

Phytochemical	Content (g/100g dry weight)
Alkaloids	0.65
Flavonoids	2.35
Tannins	1.20
Saponins	1.75
Phenolic Compounds	3.80
Terpenoids	0.95
Glycosides	0.50
Steroids	0.45
Coumarins	0.30
Anthocyanins	0.20

The alkaloid content was found to be 0.65g per 100g dry weight. Alkaloids are known for their diverse pharmacological activities, including anti-inflammatory,

analgesic, and antimicrobial effects. The presence of alkaloids in Alligator weed suggests potential applications in enhancing the disease resistance and overall health of fish, as supported by studies indicating the benefits of alkaloids in aquaculture feeds (Bussabong et al., 2021).

Flavonoids were measured at 2.35g/100g dry weight. Flavonoids are well-recognized for their antioxidant properties, which can help in reducing oxidative stress and enhancing the immune response in fish. The inclusion of flavonoid-rich ingredients in fish diets has been shown to improve growth performance and health (Xu et al., 2021; Ghafarifarsani et al., 2022). The significant flavonoid content in Alligator weed thus supports its use in aquaculture to promote fish health and resilience.

The tannin content was found to be 1.20g/100g dry weight. Tannins possess astringent properties and have been reported to enhance gut-health by modulating gut microbiota and reducing pathogen load (Sallam et al., 2021). However, high levels of tannins can have antinutritional effects, potentially inhibiting protein and mineral absorption (Choi & Kim, 2020). Therefore, the tannin content in Alligator weed needs to be balanced carefully in aquaculture feed formulations to harness their benefits without adverse effects.

Saponins were present at 1.75g/100g dry weight. Saponins have been reported to exhibit antimicrobial, antiparasitic, and immunostimulatory activities. They can enhance nutrient absorption by increasing the permeability of intestinal cells (Wang et al., 2022; Zhang et al., 2023). The presence of saponins in Alligator weed suggests potential benefits for improving fish health and growth when included in diets at appropriate levels.

The highest phytochemical content was observed for phenolic compounds, with 3.80g/100g dry weight. Phenolic compounds are known for their strong antioxidant properties, which can protect fish against oxidative damage and support overall health. The role of phenolic compounds in enhancing stress resistance and immune function in fish has been highlighted in various studies (Ahmadifar et al., 2020; Ahmadi et al., 2022). Therefore, the high phenolic content in Alligator weed underscores its potential as a beneficial feed ingredient in aquaculture.

Terpenoids were found at 0.95g/100g dry weight. Terpenoids possess anti-inflammatory and antimicrobial properties, which can help in reducing disease incidence and promoting health in aquaculture species. Their presence in Alligator weed adds to its therapeutic potential.

The glycoside content was determined to be 0.50g/100g dry weight. Glycosides can contribute to various biological activities, including antioxidant and anti-inflammatory effects. Their role in improving fish health through dietary inclusion is supported by research indicating positive impacts on growth and disease resistance.

Steroids were measured at 0.45g/100g dry weight. Steroids are involved in regulating various physiological processes, including growth and reproduction. Their presence in Alligator weed can contribute to enhancing growth performance and reproductive health in aquaculture species.

Coumarins and anthocyanins were present at 0.30 and 0.20g/100g dry weight, respectively. Both compounds are known for their antioxidant properties, with coumarins also exhibiting antimicrobial and anti-inflammatory activities. Anthocyanins are particularly effective in scavenging free radicals and protecting cells from oxidative stress (Tena et al., 2020). The presence of these compounds in Alligator weed further supports its use in promoting fish health and stress resilience.

The phytochemical composition of Alligator weed indicates a rich presence of bioactive compounds with potential benefits for aquaculture. The inclusion of Alligator weed in fish diets could enhance growth performance, health, and disease resistance, owing to its diverse phytochemical profile. However, further research is recommended to optimize its inclusion levels and evaluate long-term effects on different aquaculture species.

Antioxidant Activity

The antioxidant activity of Alligator weed (*Alternanthera philoxeroides*) extract was assessed at various concentrations (1mg/mL, 5mg/mL, and 10mg/mL) using different solvents (water, methanol, and ethanol). The antioxidant activity of Alligator weed extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay comparing with Vitamin C serving as the reference standard. The results, expressed as the percentage of DPPH radical inhibition, are summarized in the following Table 3.

Table 3: The antioxidant activity of Alligator weed extract comparing with Vitamin C.

Sample	Dose (mg/mL)	Solvent	Antioxidant Activity (DPPH % inhibition)
Alligator Weed Extract	1	Water	30.5
	5	Water	55.2
	10	Water	68.7
	1	Methanol	40.3
	5	Methanol	63.8
	10	Methanol	79.4
	1	Ethanol	35.1
	5	Ethanol	58.4
	10	Ethanol	72.5
Vitamin C (Standard)	1	Water	85.6
	5	Water	92.3
	10	Water	95.1

The antioxidant activity of Alligator weed extract in water exhibited a dose-dependent increase. At 1mg/mL, the inhibition of DPPH radicals was 30.5%, which increased to 55.2% at 5mg/mL and 68.7% at 10mg/mL. Although the activity was significant, it remained lower than that of Vitamin C, which showed 85.6, 92.3, and 95.1% inhibition at corresponding concentrations. The results indicate that Alligator weed possesses moderate antioxidant properties when extracted with water.

Methanol as a solvent resulted in higher antioxidant activity compared to water. At 1mg/mL, the DPPH radical inhibition was 40.3%, which increased to 63.8% at 5mg/mL and 79.4% at 10mg/mL. The methanolic extract exhibited stronger antioxidant activity than the aqueous extract at all tested concentrations. This suggests that methanol is more effective in extracting antioxidant compounds from

Alligator weed. The efficiency of methanol in extracting phenolic compounds with high antioxidant activity.

Ethanol extraction also showed a dose-dependent increase in antioxidant activity. The DPPH radical inhibition was 35.1% at 1mg/mL, 58.4% at 5mg/mL, and 72.5% at 10mg/mL. The antioxidant activity of the ethanolic extract was higher than that of the aqueous extract but slightly lower than that of the methanolic extract. Ethanol is known to effectively extract both polar and non-polar compounds, which may account for the moderate antioxidant activity observed.

Vitamin C, used as the reference standard, exhibited the highest antioxidant activity across all concentrations, with DPPH radical inhibition percentages of 85.6, 92.3, and 95.1% at 1, 5, and 10mg/mL, respectively. The superior antioxidant activity of Vitamin C highlights its potent free radical scavenging ability, as extensively documented in literature.

The study demonstrates that Alligator weed extracts possess significant antioxidant activity, with methanol being the most effective solvent for extracting antioxidant compounds. The presence of phenolic compounds, flavonoids, and other phytochemicals in Alligator weed likely contributes to its antioxidant properties. These findings are consistent with previous studies indicating the antioxidant potential of plant-based extracts rich in phenolic and flavonoid compounds.

The antioxidant properties of Alligator weed can have several applications in aquaculture. Antioxidants are known to improve the health and stress resistance of fish by neutralizing free radicals and reducing oxidative stress. Incorporating Alligator weed into aquaculture feeds could enhance the overall health and resilience of fish, thereby improving growth performance and survival rates. Moreover, the use of natural antioxidants from plant sources is an environmentally sustainable alternative to synthetic antioxidants.

Application of Alligator Weed in Herbivorous Aquaculture Feed

The growth performance, feed conversion ratio (FCR), and survival rate of various herbivorous fish species were evaluated at different inclusion levels of Alligator weed (*Alternanthera philoxeroides*). The results are summarized in the following Table 4.

The results indicate that the inclusion of Alligator weed in the diet of various herbivorous aquaculture species positively influences growth performance, feed conversion ratio (FCR), and survival rate, with the optimal inclusion level identified as 20%.

Tilapia

Tilapia exhibited improved growth performance with an inclusion level of 10% Alligator weed, achieving an FCR of 1.5 and a survival rate of 95%. At the 20% inclusion level, growth performance was optimal, with an FCR of 1.3 and a survival rate of 92%. However, at the 30% inclusion level, growth performance and survival rate decreased slightly, indicating that excessive inclusion might not be beneficial. Moderate inclusion of plant-based feeds improves fish growth and health.

Grass Carp

Grass carp showed improved growth performance and an FCR of 1.4 at the 10% inclusion level, with a survival rate of 93%. The optimal inclusion level was 20%, resulting in the best growth performance, an FCR of 1.2, and a survival rate of 91%. At the 30% inclusion level, growth performance was moderate, with an FCR of 1.5 and a survival rate of 88%. The balanced inclusion of alternative plant-based feeds enhances nutrient utilization and growth in grass carp.

Silver Carp

Silver carp showed improved growth performance at the 10% inclusion level, with an FCR of 1.6 and a survival rate of 94%. The optimal inclusion level was 20%, with an FCR of 1.4 and a survival rate of 91%. At the 30% inclusion level, the FCR increased to 1.7, and the survival rate decreased to 89%, indicating a decline in growth performance with higher inclusion levels. Moderate levels of plant-based feed ingredients improve growth efficiency in silver carp.

Common Carp

Common carp exhibited improved growth performance with a 10% inclusion level, achieving an FCR of 1.5 and a survival rate of 92%. The 20% inclusion level resulted in optimal growth performance, an FCR of 1.3, and a survival rate of 90%. At the 30% inclusion level, growth

Table 4: Application of Alligator Weed in Herbivorous Aquaculture Feed with growth performance, feed conversion ratio (FCR), and survival rate as parameters.

Fish Species	Inclusion Level of Alligator Weed (%)	Growth Performance	Feed Conversion Ratio (FCR)	Survival Rate (%)
Tilapia	10	Improved	1.5	95
	20	Optimal	1.3	92
	30	Moderate	1.6	90
Grass Carp	10	Improved	1.4	93
	20	Optimal	1.2	91
	30	Moderate	1.5	88
Silver Carp	10	Improved	1.6	94
	20	Optimal	1.4	91
	30	Moderate	1.7	89
Common Carp	10	Improved	1.5	92
	20	Optimal	1.3	90
	30	Moderate	1.6	87
Freshwater Prawns	10	Improved	1.7	90
	20	Optimal	1.5	88
	30	Moderate	1.8	85

performance was moderate, with an FCR of 1.6 and a survival rate of 87%. These findings highlighted the benefits of incorporating plant-based ingredients into the diet of common carp to enhance growth and health.

Freshwater Prawns

Freshwater prawns showed improved growth performance with a 10% inclusion level, an FCR of 1.7, and a survival rate of 90%. The optimal inclusion level was 20%, with an FCR of 1.5 and a survival rate of 88%. At the 30% inclusion level, the FCR increased to 1.8, and the survival rate decreased to 85%, indicating that higher inclusion levels might not be as beneficial. The results demonstrated that moderate inclusion of plant-based feed components can improve the growth and survival of freshwater prawns.

Alligator weed in the diet of herbivorous aquaculture species at 20% appears to be optimal for enhancing growth performance, feed conversion ratio, and survival rate. The presence of beneficial phytochemicals and nutrients in Alligator weed likely contributes to these positive outcomes. However, higher inclusion levels may lead to diminished returns, possibly due to the presence of antinutritional factors or imbalances in nutrient composition.

Conclusion

The study demonstrates that Alligator weed (*Alternanthera philoxeroides*) is a valuable ingredient in the diets of herbivorous aquaculture species, with an optimal inclusion level of 20% significantly enhancing growth performance, feed conversion ratio, and survival rates. The study's key findings underscore the substantial impact of advanced aquaculture management and technology on sustainable water resource utilization, with evidence suggesting that innovative techniques enhance productivity and environmental conservation. The integration of efficient water management practices is shown to be crucial for improving the overall sustainability of aquaculture operations. Nevertheless, limitations include the study's focus on a specific geographic region, which may affect the generalizability of the results, and its short-term duration, which does not address long-term impacts or potential unforeseen consequences. Future research should aim to conduct longitudinal studies across diverse regions to validate and broaden these findings, and explore the socio-economic effects of advanced aquaculture technologies on local communities. Continued investigation into scalable, innovative solutions is vital for the sustainable advancement of the aquaculture industry.

Acknowledgment

The authors would like to thank the Integrated Research Laboratory (IRL) Brawijaya University, and the SATU JRS 647/UN3/3023 funding for all support and facilities.

Conflict of Interest: There is no conflict of interest.

Author Contributions

N.S. was responsible for the initial research design and data collection. R.A.I. conducted the statistical analysis and interpretation of the data. N.B.M. contributed to the literature review and drafting of the manuscript. V.H. provided critical revisions and contributed to the final editing of the manuscript. F.S.V. assisted in the experimental procedures and provided additional data analysis. All authors reviewed and approved the final version of the manuscript.

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