E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2025; 13(3): 224-229

www.fisheriesjournal.com
Received: 03-03-2025
Accepted: 05-04-2025

Isagani P Angeles Jr
1Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela 3320, Philippines
2Institute of Fisheries, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines
3Freshwater Fisheries Center, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines

Melrose M Guerrero
1Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela 3320, Philippines
2Institute of Fisheries, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines
3Freshwater Fisheries Center, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines

Mary Joy L Asprec
1Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela 3320, Philippines
2Institute of Fisheries, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines
3Freshwater Fisheries Center, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines

Marites R Castro
1Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela 3320, Philippines
2Institute of Fisheries, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines
3Freshwater Fisheries Center, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines

Laila M Gallego
Institute of Fisheries, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines

Adelaida T Calpe
Department of Science and Technology, Philippine Council for Agriculture, Aquatic, and Natural Resources Research and Development, Los Baños, Laguna 4030 Philippines

Corresponding Author:
1Provincial Institute of Fisheries, Isabela State University, Matusalem, Roxas, Isabela 3320, Philippines
2Institute of Fisheries, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines
3Freshwater Fisheries Center, Isabela State University, San Fabian, Echague, Isabela 3309, Philippines

Field testing of azolla *Azolla filiculoides* and *Amaranthus spinosus* Kolitis leaf meal for improved tilapia in pond culture

Isagani P Angeles Jr, Melrose M Guerrero, Mary Joy L Asprec, Marites R Castro, Laila M Gallego and Adelaida T Calpe

DOI: https://www.doi.org/10.22271/fish.2025.v13.i3c.3093

Abstract
This study evaluated *Azolla filiculoides* and *Amaranthus spinosus* leaf meal as an alternative diet for Nile tilapia. Fish were fed commercial feeds (Control), alongside formulated 20% Azolla (A20) and 50% ASLM (ASLM50) protein sources on alternate days.
Results showed that growth parameters, final weight, weight gain, specific growth rate, feed conversion ratio, and percentage survival were comparable among all treatments.
Cost and return analysis showed that both A20 and ASLM50 had higher net income, return on investment, and shorter payback periods than that of Control. ASLM had the highest ROI (59.63%) and shortest payback period (1.68 years), closely followed by A20, while Control had the lowest.
Overall, the incorporation of A20 or ASLM50 in Nile tilapia diets enhanced growth performance and increased profitability, supporting their use as sustainable and cost-effective alternatives to conventional commercial feeds.

Keywords: Nile tilapia, *Azolla filiculoides*, *Amaranthus spinosus*, growth performance, cost effectiveness

1. Introduction
The nutrient quality of feed ingredients is one of the major prerequisites apart from availability (which sometimes is a function of cost and season) for the production of good quality feeds. Tilapia feed generally constitutes 60-70% of the operational cost in intensive and semi-intensive aquaculture systems [1]. The feed must be well-balanced with all the necessary nutritional contents. A careful selection of feed ingredients for use in aquafeed will play a vital role in the nutritional and economic success of farmed fish. The ever-increasing cost of fish feed has engendered research interests in ways of replacing it totally or partially with other less-expensive protein diets of comparable feed ingredients [2, 3]. There is a need for a research effort to evaluate the nutritive value of different non-conventional feed resources, including terrestrial and aquatic macrophytes [4]. Therefore, the major prerequisite for profitable fish farming is the availability of suitable feeds, formulated from locally available cheap ingredients that will supply adequate nutritional requirements for cultured fish [5, 6].
Azolla plants gained their importance in aquaculture due to their high crude protein content (13% to 30%) and essential amino acid composition (rich in lysine) compared with most green forage crops and other aquatic macrophytes [7]. Anitha *et al.* [8] stated that sundried Azolla contains 22.48% crude protein, 4.5% oil, 14.7% crude fiber, 17.34% ash, and 40.98% nitrogen-free extract. Azolla can fix atmospheric nitrogen due to the presence of a symbiotic cyanobacterium, *Anabaena azollae*, and because of this property, it has been widely exploited as a biofertilizer for rice plants. In addition, it has several other uses such as food, feed, biogas production, and hyperaccumulator of heavy metals [9].
Amaranth is similarly advantageous; it is a versatile plant since it can grow under a broad range of climates, soil, and cultivar systems, and is resistant to heat and drought with no major diseases [10, 11]. However, the most valuable characteristic of *Amaranth spp*. is that they contain a high proportion of high-quality protein [12]. In view of this, the use of amaranth as a feed

ingredient has become inevitable for the carrying capacity of culture systems and can enhance fish production by many folds [13].

In the completed project of ISU funded by DOST-PCAARRD titled "*Amaranthus spinosus* Leaf Meal as Potential Protein Source for Nile Tilapia", Azolla and Amaranthus *spinosus* "Kolitis" Leaf Meal (ASLM) enhanced growth performance, improved resistance of Nile tilapia against *Aeromonas hydrophila* infection, and provided a better cost-benefit ratio. In addition, Azolla stabilizes both the antioxidant capacity and the metabolic response of Nile tilapia, while ASLM has potential antimicrobial and quorum-sensing properties against *A. hydrophila* and *Chromobacterium violaceum* [14].

Considering the increasing prices of feed ingredients, it will be helpful to the tilapia aquaculture industry to search for potential feed supplements that could not only enhance feed efficiency but also strengthen the health status of tilapia. The research initiatives on the utilization of plant-based as aquafeed could be one of the challenges in addressing the problem of the higher cost of feed to sustain the aquaculture industry in the country. Moreover, the utilization of plant-based feed with high nutritive value as supplemental feed has taken a new dimension in meeting the fish's nutritional requirements at the very least cost.

2. Materials and Methods

2.1 Selection of the Farm Cooperators

Tilapia farm cooperators from Ramon, San Mateo, and Echague, Isabela, along with Central Luzon State University and the Maligaya Science City of Muñoz, were identified and coordinated through the Municipal Agriculture Office, City Agricultural Office, State Universities and Colleges, and the Bureau of Fisheries and Aquatic Resources for field testing. Cooperators were briefed on the study's objectives and scope to ensure they had the necessary knowledge and skills for proper implementation. They were also assisted in carrying out the field testing. The study was conducted in ponds measuring approximately 500m² or smaller, with a stocking density of 5 fish per square meter.

2.2 Preparation and Collection of Azolla and ASLM

Fresh Azolla was sourced from the culture tanks of the Freshwater Fisheries Center, while fresh Amaranthus was collected from Barangay Annafunan, Echague, Isabela. The leaves were thoroughly cleaned and left to air-dry for several days. Once dried, they were initially ground into a coarse powder using a high-capacity grinding machine for use in formulation. The plant materials were then stored in a drying room for two weeks to complete the air-drying process. Afterward, they were finely ground using a mechanical grinder.

2.3 Feed Formulation with Azolla or ASLM

The optimal concentration levels of Azolla and ASLM, determined from previous studies, were applied in this field testing. Ingredients were carefully weighed and combined in the appropriate proportions based on the formulation composition outlined in Tables 2 and 3. The mixture was then processed into pellets using a pelletizer. The resulting pellets were air-dried for 24 hours and stored in a refrigerator at 4°C until they were ready for feeding.

2.4 Field testing on the use of Azolla or ASLM as feed for tilapia production

The study assessed the use of formulated feeds containing Azolla or ASLM in selected farm cooperators in Isabela and Nueva Ecija. A feeding trial was conducted to evaluate their effects on the rearing performance of tilapia in a pond-based culture system, lasting for at least 16 weeks. Optimum concentration levels of Azolla or ASLM, based on the results of our previous studies was used in this field testing. Three types of feeds were tested during the feeding trials: (1) commercial feed (practice in the farm), (2) formulated feed with Azolla, and (3) formulated feed with ASLM (Table 1).

Table 1: Experimental diet

Treatments	Description
Control	Commercial feed (practice in the farm)
A20	Formulated feed with Azolla
ASLM50	Formulated feed with ASLM

2.5 Fish rearing, feeding and sampling

Nile tilapia fingerlings were sourced from a private hatchery in San Mateo, Isabela, and the Freshwater Aquaculture Center in the Science City of Muñoz. They were transported in well-oxygenated plastic bags and stocked in grow-out farms operated by cooperators in Isabela and Nueva Ecija. The field testing was closely monitored throughout the study. The fingerlings were randomly weighed and stocked in fishponds at a density of 5 fish per square meter. Feeding was conducted twice daily, at 0900 h and 1500 h, at a rate of 10% of their body weight. Formulated feeds were provided on alternate days. Fish weights were recorded every two weeks during sampling, and the daily feed ration was adjusted accordingly. Sampling was conducted using a cast net to catch the fish. Throughout the study, water quality parameters—including temperature, pH, and dissolved oxygen (D.O.)—were consistently monitored.

Table 2: Formulation (g kg−1 dry weight) of the experimental diet with Azolla *Azolla filiculoides*

Ingredients	Experimental diet (g)	
	Control (C)	A20
Fish meal		300
Soy Bean Meal (SBM)		150
Corn bran		100
Corn starch		180
Azolla (20%)		200
Soybean oil		50
Vitamins		10
Minerals		10
Proximate composition		
Crude protein %		30.70
Crude Fibre %		2.62
Total ash %		10.40
Crude lipid %		10.67
Dry matter %		89.87

Table 3: Formulation (g kg−1 dry weight) of the experimental diet with *Amaranthus spinosus* "Kolitis" Leaf Meal

Ingredients	Experimental diet (g)	
	Control (C)	ASLM50
Fish meal		220
ASLM (47%)		470
Wheat flour		65
Rice bran		40
Corn oil		50
Soybean meal		65
Cassava meal		20
Vitamin premixa		20
Mineral premixb		20
Corn starch		10
Salt (NaCl)		20
Proximate composition		
Crude protein %		29.55
Crude Fat %		<0.10
Moisture content %		10.66
Total ash %		16.79
Total carbohydrates %		43
Total calories %		290.20

[a] Commercial formula (mg premix kg-1 diet). Vitamins (mg): retinol, 1000; thiamine; 1200; riboflavin; 2000; pyridoxine; 1000; cyanocobalamin; 200; ascorbic acid (Stay C), 5000, cholecalciferol, 2400; a tocopherol, 1000; pantothenic acid, 400; choline chloride, 1600; folic acid, 2500; nicotinic acid, 1800; biotin, 1200; inositol, 3000; paraminobenzoic acid, 3200.
[b] Minerals (mg): cobalt, 400; copper, 2100; iron, 2000; iodine, 1600; manganese, 4000; zinc 2000; selenium, 400.

2.6 Analysis of growth performance and survival

Weight sampling of Nile tilapia was conducted every 2 weeks. Nile tilapia were collected from the experimental ponds using net and placed on a bucket with water of similar temperature. Weight was obtained using a digital scale. The experimental fish was weighed individually. The quantity of feed given was adjusted after weight sampling.

The calculations were performed using the following formula:

Daily Feeding Ration (DFR) (g) = average weight of fish x feeding rate x quantity stocked

Weight Gain (WG) = final weight - initial weight/initial weight X 100

Specific Growth Rate (SGR) (%) = 100 x ln W2 - ln W1/ T (number of days in the feeding period)

Feed Conversion Ratio (FCR) = feed intake/weight gain

Percentage Survival (%SUR) = final count/ initial count x 100

2.7 Monitoring and evaluation

The field testing at each farm cooperator was closely monitored, covering all stages from feed formulation to actual grow-out production, including conditioning, tilapia stocking, sampling, and feeding. Additionally, supervision was conducted during data collection and record-keeping.

2.8 Data Analysis

One-way analysis of variance (ANOVA) was performed to determine the effect of formulated diets on WG (%), SGR (%), FCR, and survival. Tukey's test was carried out to determine the differences between treatments. The significant level applied to all analyses was set at 5%. SAS software version 9.0 (SAS Institute, Inc., Cary, NC) was used for statistical analysis.

3. Results

3.1 Growth and survival of Nile tilapia *Oreochromis niloticus*

After 16 weeks of rearing, results showed that growth parameters—final weight, weight gain, specific growth rate, feed conversion ratio, and percentage survival—were comparable among all treatments, as presented in Table 4 and Figure 1.

3.2 Water quality parameters

The water quality parameters of the Nile tilapia in pond pond-based culture system, as shown in Table 5, demonstrate that pH levels, temperature, and dissolved oxygen are within the ideal range for optimal fish growth and health.

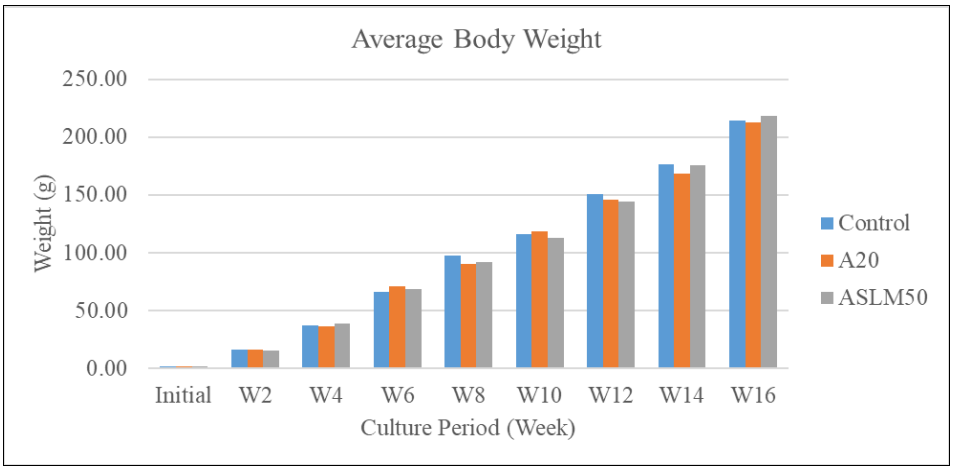
3.3 Cost and return analysis

Cost and return analysis showed that both A20 and ASLM50 had higher net income, return on investment, and shorter payback periods than the Control. ASLM had the highest ROI (59.63%) and shortest payback period (1.68 years), closely followed by A20, while Control had the lowest (Table 6).

Table 4: Mean growth performance and percentage survival of of Nile tilapia *Oreochromis niloticus* fed with Azolla *Azolla filiculoides* and *Amaranthus spinosus* Leaf Meal (ASLM) after 16 weeks of rearing.

Trt[1]	Growth Parameters					
	Wi[2] (g)	Wf[3] (g)	WG[4](%)	SGR[5](%)	FCR[6]	%SUR[7]
Control	2.04 ±0.31	214.06 ±25.70	12610.50±1600.28	4.76±0.14	1.96±0.09	59.47±6.41
A20	1.93±0.20	212.97±25.85	12671.90±1394.47	4.76±0.14	1.97±0.11	62.74±10.65
ASLM50	1.92±0.20	218.49±18.91	12953.00±1444.5	4.28±0.47	1.96±0.12	59.97±7.58

[1]Treatments: Control= Commercial diet; A20= Practical diet with 20% Azolla; ASLM= Practical diet with 47% ASLM.
[2]Wi: Initial weight
[3]Wf: Final weight
[4]WG (%): Percentage of weight gain
[5]SGR: Specific growth rate
[6]FCR: Feed conversion ratio
[7]%SUR: Percentage survival



*__Note:__ Treatments: Control= Commercial diet; A20= Practical diet with 20% Azolla; ASLM50= Practical diet with 50% ASLM

Fig 1: Growth performance of Nile tilapia *Oreochromis niloticus* after sixteen weeks of rearing experiment

Table 5: Mean values of water quality parameters of experimental ponds under three treatments.

Parameters	Control	A20	ASLM50	Suitable Range
pH	7.71±0.16	7.60±0.08	7.72±0.11	6.5 - 9
Dissolved Oxygen (mg/L)	5.24±0.87	5.50±0.82	5.31±0.94	> 3 mg/L
Temperature (°C)	26.22±2.02	26.58±1.89	27.01±1.79	25-30°C

*__Note:__ Treatments: Control= Commercial diet); A20= Practical diet with 20% Azolla; ASLM50= Practical diet with 50% ASLM

Table 6: Summary of cost and return analysis based from extrapolated data of Nile tilapia *Oreochromis niloticus* grow-out culture.

Treatments[1]	Gross sale[2]	Total culture cost[3]	Net income after tax[4]	Return on investment[5]	Payback period[6]
C	1,421,529.65	1,021,958.00	485,835.90	47.54%	2.10
A20	1,603,408.54	1,050,871.98	615,856.08	58.60%	1.71
ASLM50	1,572,341.44	1,025,170.54	611,295.26	59.63%	1.68

*__Note:__ [1] Treatments: C= Control (Commercial diet); Azolla= Practical diet with 20% Azolla; ASLM= Practical diet with 47% ASLM.
[2]Gross sale= total weight of fish X average selling price
[3]Total culture cost = Capital investment + Operating capital + Depreciation Cost / year
[4]Net income after tax = Net income before tax - 15% provision tax
[5]Return on Investment = (Net Income after tax / Operating Capital + Depreciation Cost) X 100
[6]Payback Period = Total culture cost / Net income after tax

4. Discussion

This study aimed to assess the feasibility of using Azolla and ASLM as cost-effective dietary alternatives for tilapia aquaculture. As omnivorous fish, tilapia possess a certain degree of metabolic efficiency in digesting plant-based food sources due to structural adaptations in their digestive system. However, fishmeal remains a primary ingredient in commercial fish diets worldwide due to its high digestibility and rich essential amino acid content, particularly lysine. Despite this, ongoing research seeks to identify alternative ingredients that can reduce feed costs while maintaining optimal fish growth [15, 16]. Several studies have proposed alternative protein sources to enhance fish growth cost-effectively [17, 3, 18]. Feed quality significantly influences tilapia growth, with high-quality ingredients like fishmeal generally promoting superior growth performance, whereas low-quality plant protein sources may yield lower growth rates [19, 20].

The current field-testing study with Nile tilapia fed diet with A20 and ASLM50 was based on prior research by the Freshwater Fisheries Center. Previous findings showed that replacing up to 20% of fishmeal with Azolla supported optimal tilapia growth, while 50% ASLM substitution further enhanced growth, improved resistance to *Aeromonas hydrophila* infection, and offered a better cost-benefit ratio. These results provided the rationale for conducting field trials in several municipalities of Isabela and Nueva Ecija.

In this field-testing study, fish were fed commercial feeds (Control) alongside formulated A20 and ASLM50 protein sources on alternate days. Results showed that growth parameters—final weight, weight gain, specific growth rate, feed conversion ratio, and percentage survival—were comparable among all treatments, indicating that both Azolla and ASLM can be viable alternatives to traditional fishmeal-based feeds. These findings align with research by Zhou & Yue [21] and Mahboob [22], who reported successful fishmeal replacement in *O. niloticus* diets without negatively impacting growth. Similarly, a study by Kumar *et al.* [23] reported no significant differences in growth performance and feed utilization parameters (FCR, PER, ALC, and ER) when comparing heated *Jatropha platyphylla* kernel meal (H-JPKM) with fishmeal. The results from this study indicate excellent nutrient utilization.

The absence of significant differences in SGR among the experimental fish further supports these conclusions and is consistent with findings by Oso *et al.* [24], who observed that substituting fishmeal with compounded Bambara nut diets (up to 75%) had no significant effect on weight gain, percentage weight gain, or SGR in tilapia fingerlings. Similarly, Makori *et al.* [25] reported an SGR of 3.7%/day in *O. niloticus* reared in earthen ponds, with the current study's values falling within a moderate range, reinforcing the suitability of plant-based protein sources for tilapia.

Feeding *Oreochromis niloticus* fingerlings with diets

containing up to 30% *Azolla filiculoides* and agricultural by-products has been shown to support acceptable growth rates in tropical wetland earthen ponds, as demonstrated by Kanangire [26] and Pouomogne and Ombredane [27]. In the present study, feed conversion ratios (FCR) remained comparable across all treatments, with the Control and ASLM50 diets yielding the lowest FCR (1.96), closely followed by A20 (1.97), indicating efficient nutrient utilization. These results are consistent with findings by Aliu & Okolie [28] and Zamal *et al.* [29], who observed similar efficiency with alternative plant-based feeds. Percentage survival was also assessed, with A20 exhibiting the highest survival rate (62.74%), followed by ASLM50 (59.87%) and the Control (59.47%), corroborating earlier research by Mosha *et al.* [30] that reported improved survival with Azolla inclusion.

The integration of Azolla and *Amaranthus spinosus* as alternative protein sources in combination with commercial feeds presents a promising strategy for sustainable tilapia farming. This approach has the potential to reduce feeding costs, promote environmentally friendly aquaculture practices, and sustain optimal fish growth. From an economic perspective, incorporating Azolla into tilapia diets could significantly lower feed costs, which are a major variable expense in fish production. This finding aligns with previous studies by Abu *et al.* [31] and Jimoh *et al.* [32], which concluded that using cheaper feed ingredients increases profitability.

Additionally, Magouz *et al.* [33] confirmed that 20-25% Azolla inclusion in fish diets does not negatively affect growth, feed efficiency, immune response, or overall fish health. The beneficial properties of Azolla are attributed to its antioxidant and immunostimulant compounds, including phenols, carotenoids, flavonoids, and tannins [34, 35, 36]. Numerous studies have investigated the partial or complete substitution of plant protein ingredients with Azolla meal, reporting no adverse effects on fish performance [33, 37, 30].

Research on amaranth-based feeds in different farming systems has also demonstrated that amaranth meal enhances fish immune function, growth performance, and gut microbiota enzymatic activity [38, 39, 40]. Similarly, Angeles *et al.* [14] reported that fish fed with 50% ASLM (*Amaranthus spinosus* Leaf Meal) exhibited improved growth performance, likely due to the balanced essential nutrients in the diet. The present study further supports the potential of amaranth meal to improve fish feed quality and microbiological safety.

In grow-out culture, the net income after tax for A20 and ASLM diets increased by 13% and 8%, respectively, compared to the Control. Similarly, return on investment (ROI) for Azolla and ASLM was also increased by 14% and 8%, respectively, compared to the Control. This enhanced economic performance is attributed to the low production costs of Azolla and Amaranthus, both of which are easily cultivated and require minimal input. Studies analyzing the economic framework of aquaculture are essential to determine the financial viability of such investments [41]. Several studies have examined the economics of tilapia rearing by evaluating system profitability based on different management factors (e.g., harvest, rationing, size heterogeneity) and investment aspects [42, 43, 44, 45].

5. Conclusion

Overall, the incorporation of A20 (20% *Azolla filiculoides*) or ASLM50 (50% *Amaranthus spinosus* leaf meal) improved growth performance and profitability in Nile tilapia aquaculture, demonstrating that both can serve as viable alternative diets to traditional commercial feeds. This approach offers a promising strategy for sustainable and cost-effective tilapia farming.

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