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Attractability and palatability of formulated diets incorporated with fermented aquatic weeds meal (FAWM) for Asian catfish *Clarias batrachus* fingerling

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Abstract: The inclusion of protein sources from aquatic weeds is limited in the commercial aquafeed industry due to the variability of their nutritional profile, palatability issue, and levels of anti-nutritional factors (ANFs). This research set out to observe the physical, biochemical, and bacteriological properties, as well as the attractability and palatability of various types of fermented aquatic weeds meal (FAWM) treated diets for Asian catfish production. Four experimental diets (30% crude protein) were prepared by replacing 50% protein of control diet with different FAWM and labeled as D_0 (control group), D_1 (fermented *Azolla*), D_2 (fermented *Pistia*), and D_3 (fermented *Eichhornia*). The physical properties of FAWM diets in terms of pellet durability index, bulk density, water stability, floatability, and swelling test showed significant ($p<0.05$) alterations among the diet groups in this research. The color and flavor of FAWM diet groups were characterized by a greenish-black and brown, along with a strong fermented flavor, while control diet showed typical color and odor. Both total bacteria (TB) and lactic acid bacteria (LAB) were considerably ($p<0.05$) greater in FAWM groups when those compared to the control diet. The diet containing fermented *Azolla* (D_1) had remarkably ($p<0.05$) higher attractability (53.33%) and palatability (14.22 mg/g) than other tested diets. Additionally, the outcomes of taste preference assessment revealed that the fish exhibited a significant ($p<0.05$) preference for pellets from the D1 diet. Though the significantly higher growth performance and feed utilization were occurred in the Despite the control diet displaying significantly better growth performance and feed utilization, the fish fed with fermented *Azolla* diet experienced an increased final weight (g), weight gain (%), Specific growth rate (%/day), feed conversion ratio, and protein efficiency ratio amongst the FAWM diet groups. Based on the above findings, it can be concluded that incorporating FAWM, specially fermented *Azolla* meal could assist in generating low-cost, more palatable, attractable, and high quality aquafeed for Asian catfish production in captivity.

INTRODUCTION

One of the main reasons for the sharp rise in demand for fish and seafood is the enormous rise in global population. Aquaculture has a great potential to meet the protein need for global population and has a significant contribution to the raising global fish production, reaching about 82 million tons in 2018 (FAO, 2020). Fish feed plays a fundamental role in sustaining the aquaculture production in captivity. However, fish nutrition is critical as feed is the most expensive part in the aquaculture system, being approximately 60% of the entire production cost (Craig *et al*., 2017; Prabu *et al*., 2017; Daniel, 2018). Protein and lipid are the vital components in the diets that are highly required by fish for their optimum growth, survival, and reproduction. Moreover, fish meal and fish oil are considered the best animal derived protein and lipid source in fish diet (Hodar *et al*., 2020). According to Shepherd and Jackson (2013) around 4.4 to 4.6 kg whole fish is often needed to generate 1 kg fish meal (FM), while Liland *et al*. (2012) reported that about 12.2 kg of fish is required to produce 1 kg fish oil. Marine pelagic fish such as mackerel, anchovies, herring, and sardines are some of the most significant sources of FM in aqua-diet (Merino *et al*., 2012). It is projected that the global aquaculture will not be able to meet its protein needs by 2050 because of FM production over reliance on marine fisheries resources (Jiang *et al*., 2018; Kari *et al*., 2022), which poses a serious problem for the entire world. Meanwhile, their limited supply, high price, and unavailability are the major constrains that drive up the feed cost. Therefore, the exploring and developing of alternative bio-active and functional nutrient ingredients from indigenous sources have recently caught substantial interest in the aqua-feed industry (Suma *et al*., 2023). The utilization of aquatic weeds may be the great option to fill the existing problem.

Aquatic weeds are the promising fish and animal feed ingredients that are widely distributed in the waterbodies of Bangladesh. Aquatic weeds' superior nutritional composition has recently allowed FM to be partially or completely replaced (Debnath *et al*., 2018; Ghosh *et al*., 2021; Nandi *et al*., 2023). Naseem *et al*. (2021) documented that aquatic weed meal comprises of about 11 to 32% crude protein, 2.9 to 16.81% crude lipid, 8 to 31% crude ash, and very high amino acids, minerals and vitamins content depending on the choice of ingredients used. According to another study, the use of these plants in the aquaculture feed has dual benefits of eco-friendly management of aquatic weeds and the potential to replace FM in fish feed formulation (Ali and Kaviraj, 2018).

It is crucial to note that the inclusion of plant protein in diets dramatically decreased the feed cost due to their local availability, low price, and abundance. However, a number of scientists have reported that the aquaculture feed industry should require an alternative protein supplement to replace high valued FM, which is highly appreciated but has a limited supply and a great demand (Bairagi *et al*., 2002; Yılmaz *et al*., 2004; Sadique *et al*., 2018). Therefore, several studies have been conducted to explore potential alternatives for FM by using novel protein source from aquatic weeds (Table 1). The key factor affecting the aquaculture industry's sustainability is access to high-quality and reasonable priced ingredients (Ghosh and Roy, 2017; Goswami *et al*., 2020). However, our current understanding on the utilization of aquatic weeds meal in animal diets, including their working mechanisms, animal health and environmental impacts, is limited, indicating significant gaps in knowledge in this area. In depth research is required to overcome the existing knowledge deficit and produce inexpensive, nutritionally sound and environmental friendly fish feed. This short review highlights the recent advances, challenges, opportunities, product development, and sustainability aspects associated with the use of aquatic weeds as a viable alternative to traditional feed ingredients in the aquaculture industry in order to examine the efficiency of aquatic weed meal-based diets in fish production and cost-effective management in future.

MATERIALS AND METHODS

Experimental fish collecting and holding

A group of 1,000 fingerlings of *C. batrachus* (mean weight: 3.00±0.05g) were bought from a commercial hatchery, transported to the laboratory with proper aeration and acclimatized in a large aquarium (90cm x 30cm x 20cm, length x width x depth) for two weeks. During acclimatization, commercial diet (ACI catfish feed limited, Bangladesh), with 32% crude protein, 6% crude lipid was given to fish twice a day to satiety. Before the commencement of the experiment, 360 fish were individually weighed and distributed into twelve HDPE tanks (90L) with a total of 30 fish in each tank. The experimental design consisted of four treatments, each replicated three times.

Preparation of Fermented Aquatic Weeds Meal (FAWM)

Three raw freshwater aquatic weeds viz., water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and duckweed (*Azolla pinnata*) were collected from several haor region at Habiganj, Sylhet, Bangladesh. After that, they were subjected to sorting, cleaning and sun-drying until the moisture content was below 10% and then ground into fine powder. Subsequently, a modified semi-solidify fermentation (SSF) process was used to make FAWM in line with (Zulhisyam et al., 2020; Kari et al., 2022; Nandi et al., 2023). Briefly, about 0.001% of *Lactobacillus* spp. was uniformly mixed with fine powder in three separate bowls and then assembled with 10% molasses. Then, with little addition of water, all ingredients were mixed and kept in a plastic container for 3 weeks to finish the process.

Diet formulation and feeding trial

Four experimental diets (30% crude protein) were formulated with 50% protein substitution of control diet total protein and designed as D_0 (control) with 0% FAWM, D_1 (fermented *Azolla*), D_2 (fermented *Pistia*), and D_3 (fermented *Eichornia*). The diet formulation ingredients such as fishmeal, wheat bran, rice bran, soybean oil, fish oil, vitamin and mineral premix, and carboxymethyl cellulose were brought from the commercial market and then subjected to grinding and mixing with FAWM powder at respective levels (Table 1). Afterward, the mixture was pelleted (2mm) screened, sun-dried (moisture content <10%), and then placed in zip-lock bags in three separate airtight containers. Fish were fed 2 times a day with experimental diets at station level for 90 days.

Table 1. Diet formulation and proximate composition

Ingredients (%)	**Diets(%FAWM)**			
	D_0	**D_1**	**D_2**	**D_3**
Fish meal[1]	38	15	16	17
Fermented *Azolla* meal[2]	0	43	0	0
Fermented *Pistia* meal[3]	0	0	45	0
Fermented *Eichhornia* meal[4]	0	0	0	47
Soybean meal	12	10	10	10
Flour	20	16	13	10
Corn starch	19	5	5	5
Soybean oil	3	3	3	3
Fish oil	2	2	2	2
Vitamin and mineral premix[5]	3	3	3	3
CMC[6]	3	3	3	3
Total	100	100	100	100
Proximate composition (%)				
Moisture	9.45	9.60	9.41	9.91
Crude protein	30.55	30.49	30.28	30.01
Crude lipid	7.45	7.29	7.30	7.12
Crude fibre	8.01	8.12	8.31	8.95
Crude ash	9.33	9.34	9.24	9.30
NFE[7]	35.21	35.16	34.46	34.71

[1]FM: Fish meal, crude protein, 62 %; crude lipid, 8.61%; ash, 17.95%; moisture, 11.98%.
[2]Fermented Mosquito fern: Crude protein, 34.78%; crude lipid 9.2%; ash,13%; crude fibre,19.6%; moisture,13.6%
[3]Fermented Water lettuce: Crude protein, 32.95%; crude lipid 8.3%; ash,11%; crude fibre,21.3%; moisture,14.4%
[4]Fermented Water hyacinth: Crude protein, 30.12%; crude lipid 5.3%; ash, 16.3%; crude fibre, 26.1%; moisture, 13.92%

[5]Vitamin and mineral premix (g/kg premix): Vitamin A, D, E, K, C, B_1, B_2, B_6, B_{12}, KCL,90; KI,0.04; $CaHPO_4.2H_2O$,500; Nacl,40; $CuSO_4.7H_2O$,4; CoO_4,0.02; $FeSO_4.7H_2O$,20; $MnSO_4.H_2O$,3; $CaCo_3$,215; MgOH,124; Na_2SeO_3,0.03; Na_2SeO_3,0.03; NaF1
[6]CMC: Carboxymethyl cellulose
[7]NFE: Nitrogen-free extract

Proximate composition analysis of experimental diets

Each experimental diet was undergone to proximate analysis according to the method proposed by AOAC (2000) with three replicates of each diet. Moisture content was determined using a hot air oven (105℃ for 24 hours), crude protein content was estimated using the Kjeldahl technique, lipid content was measured using the Soxhlet device, crude fiber content was quantified using a hot extraction device and ash content was evaluated by muffle furnace at 550°C for 6 hours.

Bacterial load determination in experimental diets

Standard methods according to Nandi et al. (2023) with some modifications were used to determine the total bacteria (TB) and total viable lactic acid bacteria (LAB) in diets. In short, about 1g sample was weighed and mixed with the help of a vortex mixer. After that the sample was serially diluted to 10^{-10} and about 10 µl of the diluted sample was spread homogenously onto nutrient agar (HiMedia, India) and MRS agar (HiMedia, India) media in triplicate plates. Plates were dried with laminar air flow and incubated for 48 hours for TB and LAB counts at 37℃. Colonies ranged from 30-300 were taken into consideration of bacterial quantification analyses.

Measurement of physical properties of experimental diets

The physical parameters of formulated pellets were measured as outlined by Zulhisyam et al. (2020) and Nandi et al. (2023).

Expansion ratio (ER): A total number of 50 pellets were randomly chosen and a Vernier caliper was employed to measure their diameter. The experiment was repeated at least 3 times and the average feed diameter was taken. The expansion ratio of test diets as a percent was estimated by using the following formula:

ER (%) = 100 X (average diameter of feed pellets- average diameter of original commercial feed)/ Average diameter of original commercial feed

The bulk density: About 1 kg of each test diet in triplicate was gently filled into a previously tarred measuring cylinder (1000 ml) and then weighed on a measuring scale. The mean value was recorded and noted. The bulk density of feed was estimated by the following formula:

Bulk density (kgm^{-3}) = Weight of feed particles in a cylinder (kg)/ Total volume occupy (m^3)

Pellet durability index (PDI): A tumbling box tester (Seedburo, Chicago IL, USA) was used to measure the PDI of each experimental diet. In short, about 25g of feed particles of triplicate diets were taken in a mechanical instrument for measuring the initial total weight (Wi). Then the pellets were tumbled for 10 minutes in a tumbling tester box at a rate of 50 revolutions per minute. The pellets were then sieved on a 1 mm mesh size sieve to separate the produced dust and re-weighed (Wr) by using an electronic balance. The Pellet durability index (PDI) is calculated as:

PDI (%) = (Wr/Wi) X100

Water stability: Water stability of each test diet was measured for a period of 20 minutes. This was done by taking of 25g of each replicate diet into a 0.5 mm wire mesh screen, inserted into 1000 ml beaker containing water. After the end of the duration, the remaining portion of the feed was dried in a hot-air oven at 105°C for 24 hours. As a result of the dissolution, the final weight represents the dry weight of the sample. Water stability was then calculated as follows:

Water stability (%) = 100 X (Weight of retained whole pellets after immersion / Initial total weight of feed pellets)

Floatability: A 1000 ml beaker was used for the determination of floatability of diets. In brief, 50 randomly chosen pellets of each diet in triplicate were dropped on the surface of the beaker containing water at room temperature. The pellets were observed for 20 minutes and then the sum number of floating feeds was counted. Floatability is calculated using the following equation:

Floatability (%) = 100 X (Average number of floating pellets after 20 minutes/ Average total number of initial feed)

Swelling test: The feed pellets underwent a swelling test using water in a petri dish with 20 ml of water at room temperature. The diameter of 10 randomly selected pellets of each type was measured and dropped on the surface of the water. After sank, they were left submerged for 2, 5, and 10 minutes. At the end of immersion for a definite time, excess water was drained and gently regains the pellets on an absorbent paper. Thereafter, the diameter of feed particles was re-measured and recorded. The swelling was calculated as a percentage by using the following expression:

Swelling (%) = (Total diameter of swollen pellet/ total diameter of dry pellet) X 100

Organoleptic properties of experimental diets: The organoleptic characteristics of formulated feed are examined through sensory observation in the laboratory. The color, odor, and texture of the feed were recorded after the formulation of experimental diets. The water-color in treatment tanks was observed every morning and afternoon during the feeding trial.

Assessment of Attractability and Palatability

Assessment of attractability

Attractability of each test diet was performed according to Al-Souti et al. (2019) and Suresh et al. (2011) method with some modifications. Four rectangular glass tanks (76 cm x 45cm x 45cm, length x width x height) were used and a black polyethylene sheet was covered around them for acclimatization to human presence. Four glass tanks with dimensions of 76 cm in length, 45 cm in width, and 45 cm in height were employed and enveloped with a black polyethylene sheet to prevent the penetration of light. After that, ten randomly selected catfish (mean weight 5.00±0.26 g) from each treatment group were released into separate acclimatization chamber. Subsequently, following a one-minute interval, a quantity of 2 grams of feed (distributed across pellets labeled D_0- 117, D_1-106, D_2-154, and D_3-149) was introduced into the feeding chamber. The wooden frame was then lifted upwards, permitting the fish to access the feeding chamber. The number of fish was counted which were approaching to capture the feed within 5 mins. The countdown timer commenced as soon as the fish entered the feeding zone to approach the pellets. The timer was started as soon as the fish entered the feeding zone to approach the pellets. The calculation of feed attractability involved determining the percentage of catfish present in the feeding chamber based on the collected data.

Attractability (%) = (Number of fish in feeding zone/ number of stocked fish) X 100

Assessment of palatability

The palatability of fish was tested in line with the standard methodology by Al-Souti et al. (2019) with a few alterations. Four rectangular glass tanks (76 cm x 45 cm x 45 cm, length x width x height) were filled with water and ten randomly chosen (avg. weight 5.00±0.29 g) fish from every test fish group were introduced into the aquaria. After 1 hour of acclimatization, 2g of the test diet was introduced into the tank and fish were given 10 minutes to consume the feed. After that, the fish were carefully placed into a bowl, mass-weighed, and immediately released into the aquarium. The unconsumed feed was carefully gathered from the water's surface using a mesh net and subsequently dried in a hot air oven set at 60℃ for 8 hours. Following this, the dried feed was weighed, and the corresponding values were documented. Feed consumption was calculated as milligram of feed per gram of fish biomass. Each diet was tested 7 times at the same time of the day commencing at 9 am. The palatability of each diet was calculated as follows:

Feed consumption (palatability) = milligram of feed/ gram of fish biomass

Test preferences of Asian catfish

For this purpose, a 9-point hedonic scale (Pimentel et al., 2015; Mohan et al., 2018) was used with some modifications. Five randomly selected fish (Avg. wt. 7.00±0.05g) were placed in the aquarium and then 10 randomly selected pellets from each diet were given at one time. After that, the number of pellets consumed within 3 minutes was carefully observed and recorded. The hedonic scale was formed based on the preference for pellets that were being consumed (1 dislike extremely up to 9 for like extremely). Each diet was tested four times at 3-hour intervals.

Measurement of experimental fish growth parameters

After a 90-day feeding trial, at least 10 randomly selected fish were collected from each tank and were also subjected to anesthesia with MS_{222} in the laboratory. The final weight and length of each fish were determined. The survival in every tank was checked at the end of the trial. The following parameters were calculated for the growth performance of fish and feed utilization (Zulhisyam et al., 2020, Kabir et al., 2023) as follows:

i. Weight gain, WG (%) =100 X (Final weight- Initial weight)/ Initial weight
ii. SGR (%/day) = 100 X In (final weight)-In (initial weight)/ Initial weight
iii. Feed conversion ratio (FCR) = Total feed intake / Live weight gain
iv. Protein efficiency ratio (PER) = Live weight gain / Crude protein fed
v. Survival rate (%) = 100 X (Number of surviving fish/ Total number of fish at the start of the experiment)
vi. Condition factor (CF) = 100 X (Weight of body, g)/ (Total length of fish, cm)^3

Measurement of Water quality of treatment tanks

The temperature, pressure, pH, ammonia, nitrite, nitrate, and dissolved oxygen of each experimental tank water were recorded once a week by using a multiparameter probe (HI 9828, YSI Incorporation, yellow Spring, OH, USA).

Statistical analysis

The mean value of the collected dataset was subjected to one-way analysis of variance (ANOVA) to test the significant difference between the control and treatment groups. Before analysis, normality, and homogeneity of data were done through Shapiro-Wilk and Levene's test. Followed by, Duncan’s test was performed to evaluate the homogeneity of variances. Finally, the results were presented as mean ± standard error (SE) at a significant level of $p<0.05$. IBM SPSS Statistics version (26.0) was used to carry out all statistical analyses.

RESULT AND DISCUSSION

Physical properties of experimental diets

The physical properties of experimental diets are presented in Table 2. Aquafeed pellets with improved physical properties substantially lower dust production and feed waste. This study showed significant differences ($p<0.05$) in the bulk density, PDI, floatability, and water stability of various experimental diets, except expansion ratio. The significantly lowest ($p<0.05$) bulk density and PDI were found in fermented *Azolla* diet (D_1) although no obvious variations were observed in the PDI value of fermented *Pistia* and fermented *Eichhornia* based diets. The findings of this current study were consistent with previous reports (Kiki Haetami et al., 2017, Wan et al., 2023). Additionally, Fahrenholz (2012) and Mammeri (2020) indicated that better pellet quality was obtained from higher PDI. Nevertheless, the fermented *Azolla* treated diet exhibited a notably low bulk density, accompanied by a statistically significant increase ($p<0.05$) in the percentage of floatability. These outcomes can likely be attributed to the effects of solid-state fermentation, which led to the expansion of the pellet's surface area, a reduction in density and an increase in floatability. This finding was also supported by Chukeatiroteet et al. (2017) and Maulana et al. (2020) who reported that the production of volatile compounds during fermentation including esters, alcohols, aldehydes, ketones, furans, and aromatic compounds increased floatation performance. Moreover, the size of feed pellet is determined by the size of the grown species, which have lower densities and can float on the water's surface for extended periods (Regupathi et al., 2019).

Apart from this, diet D_2 (fermented *Pistia*) had the significantly highest ($p<0.05$) water stability among the treatment groups. This might be due to the better binder capacity of corn starch and other ingredients. Kannadhason et al. (2008) stated that improved water stability can result from high starch gelatinization of feed ingredients, which can also improve feed digestibility and feed expansion during production. Meanwhile, Dawood and Koshio (2020) reported that fermented diets often have increased water stability, which can aid fish in efficiently ingesting feed. The swelling data displayed a notable distinction ($p<0.05$) among the various diet types. The observed particles exhibited a maximum expansion of 167.28% of their diameter in the D2 diet (fermented *Pistia*) after being immersed in water for 10 minutes. The variation in FAWM possibly accounts for the observed differences. An essential determinant of quality in aqua feed production is the extent of feed swelling. The greatest pellet swelling signifies the structural robustness of the pellet, accompanied by minimal disintegration and nutrient leaching (Fagbenro & Jauncey, 1995; Zulhisyam et al., 2020).

Table 2. Physical properties of experimental diets. Data expressed as Mean ± SE

Parameters	Diets			
	D_0	D_1	D_2	D_3
Feed diameter (mm)	2.02±0.01	2.01±0.01	2.02±0.02	2.04±0.01
ER (%)	2.02±0.01	2.01±0.01	2.02±0.02	2.04±0.01
Bulk density (kgm^{-3})	482.00±15.09^{a}	328.67±1.15^{d}	374.67±6.11^{b}	356±6.00^{c}
PDI (%)	98.40±0.11^{a}	98.03±0.21^{b}	95.52±0.58^{b}	94.97±0.75^{b}
Floatability (%)	52.67±3.05^{d}	100.00±0.00^{a}	94.00±2.00^{b}	88.00±2.01^{c}
Water stability (%)	85.27±0.32^{b}	88.05±0.78^{ab}	90.04±0.82^{a}	85.85±1.30^{b}

ER: Expansion ratio; PDI: Pellet durability index. Different alphabets as superscripts in each row presented significant difference ($p<0.05$).

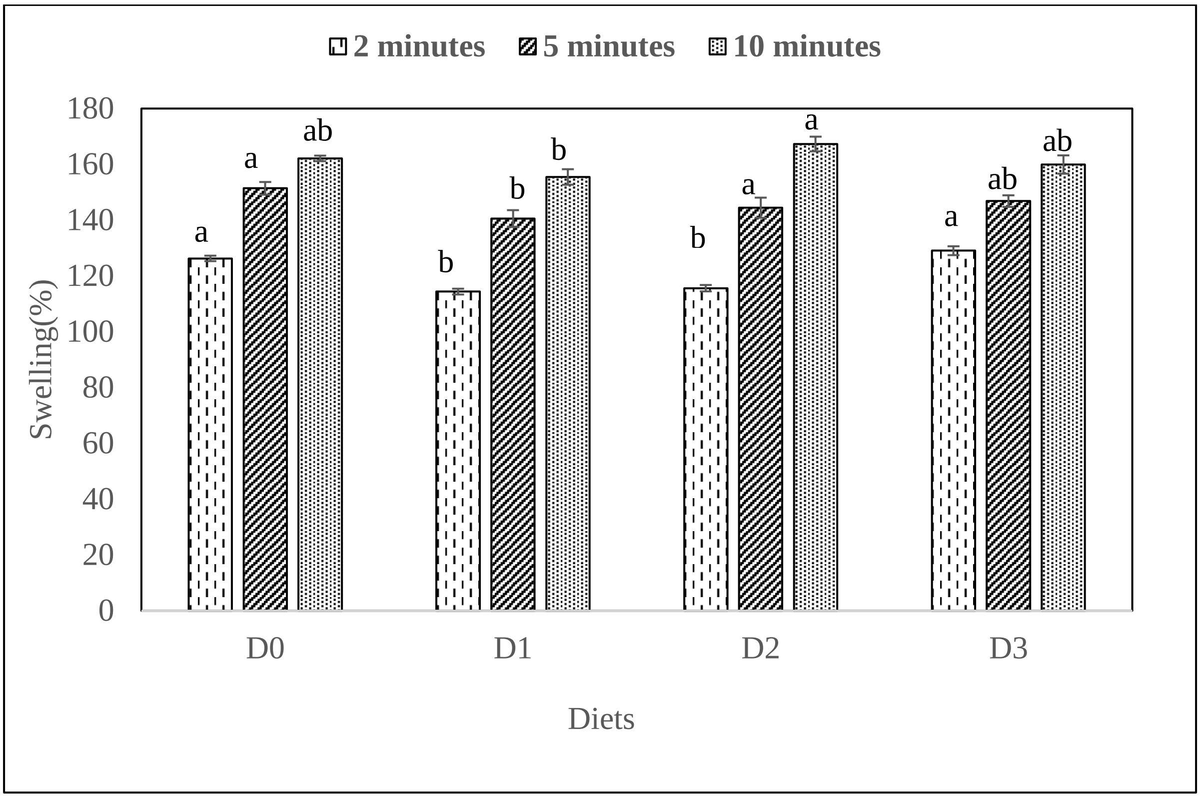

Figure 1: Swelling trends over 2, 5, 10 minutes of experimental diets D_0, D_1, D_2, and D_3. Data expressed as Mean ± SE.

Organoleptic properties of experimental diets

The organoleptic properties of experimental diets and water color during trial are shown in Table 3. The control diet showed a deep yellowish-brown color while FAWM groups showed deep greenish-black color in the diet containing fermented *Azolla,* but fermented *Pistia*, and *Echhornia* diet showed greenish-brown color. Different pigments in aquatic macrophytes and other chemical compounds might be the reason for this color of FAWM groups after pelleting. Again, diet contained *Azolla* only felt smooth surface texture. In this study, molasses and dry particles of aquatic weeds created a fermented flavor that immediately distinguished it from traditional odor. The result was steady with Zulhisyam et al. (2020). The color of the water in the experimental tanks turned turbid after seven days. This might be for the use of different FAWM diets in tanks which is line with the statement of Shamsuddin et al. (2017).

Table 3. Organoleptic properties of experimental diets

Parameters	Diets (FAWM)			
	D_0	D_1	D_2	D_3
Feed color	Deep yellowish brown	Deep greenish black	Lighter-greenish brown	Light-greenish brown
Feed texture	Rough surface	Smooth surface	Rough surface	Rough surface
Feed odor	Fishy odor	Strong fermented flavor	Minor fermented flavor	Stronger fermented flavor
Water-color	Yellowish turbid	Greenish turbid	Brownish turbid	Brownish turbid

Assessment of attractability and palatability of experimental diets

The consumption of substantial quantities of plant proteins has prompted considerable interest in the utilization of stimulants or attractants to enhance the palatability of fish feed. The outcomes of the attractability assessment, as depicted in Figure 2, revealed a noteworthy increase ($p<0.05$) in attractability of fish towards the D_1 diet (fermented *Azolla*), with a percentage of 53.33%, followed by the D_2 diet (fermented *Pistia*), the control diet (D_0), and the D_3 diet (fermented *Eichhornia*). This outcome could potentially be attributed to the fermentation process, which enhanced the D_1 diet by providing it with a smooth surface texture and an appealing flavor, thus contributing to its heightened attractiveness. This is corroborated by the research of Herawati et al. (2020), who documented that the amino acid glycine acted as a natural attractant in fermented *Lemna minor* meal when tested on *Oreochromis niloticus*.

On the other hand, the palatability of experimental diets is presented in Figure 3. Palatability exhibited an ascending trend in D_1 diet, followed by D_0, and D_3. However, in D_2 diet containing fermented *Pistia*, palatability experienced a significant decline. This result might be because of presence of ANFs, high crude fibre, and other indigestible chemicals in FAWM. According to Yamamoto et al. (2010), ANFs, high crude fiber, and other hazardous substances significantly affected the palatability qualities of diets. Meanwhile, Zulhisyam et al. (2020) observed that turbidity due to nutrient leaching results in less palatability of diets.

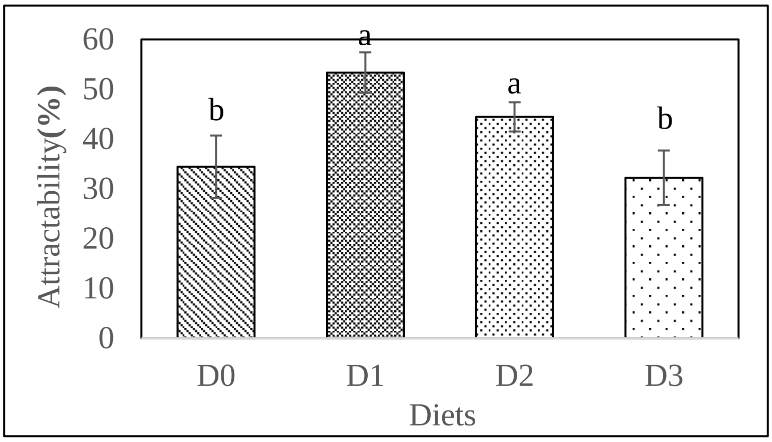

Figure 2. Attractability of different experimental diets at 5 min. values expressed as Mean ±SE. Different superscripts was presented as significant differences ($p<0.05$).

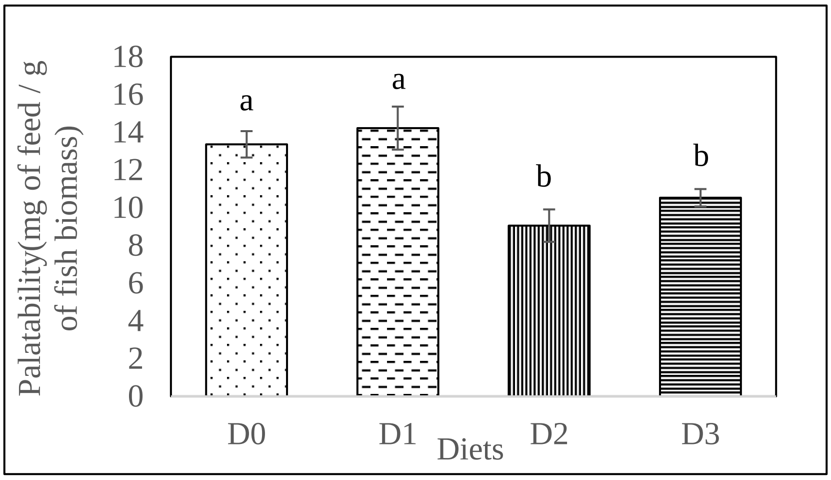

Figure 3. Palatability (mg of feed/g of fish biomass) of experimental diets within 10 minutes. Data expressed as mean ± SE. Different superscripts were present as significant differences ($p<0.05$).

Test preference of fish

Animals use their senses of smell, taste, and sight to differentiate between foods that evoke pleasurable or unpleasant eating-related sensations. It plays a crucial role in determining the palatability of potential food. The results of the sensory assessment of Asian catfish to experimental diets are presented in Figure 4. Between the FAWM and the control diet, there were significant ($p<0.05$) differences in the test preference of diets by experimental fish. Fish prefer D_1 (fermented *Azolla*) diet over the two other diets and barely like D_0 (without FAWM). This could be attributed to the presence of stimulants in the test diet. This discovery, as affirmed by Kasumyan and Doving (2003), indicated that the gustatory system (including preferences based on both oral and external taste) played a role in the behavioral reactions to food brought in with the fish. Additionally, factors like water temperature and low pH were found to be capable of altering taste preferences (Kasumyan, 2019). Meanwhile, numerous studies have shown plant macromolecules are used to modify the physicochemical, bacteriological, and sensory properties of food in addition to their nutritional value as a source of energy and amino acids (Hasan et al., 2018; Huang and Nitin, 2019; Zulhisyam et al., 2020).

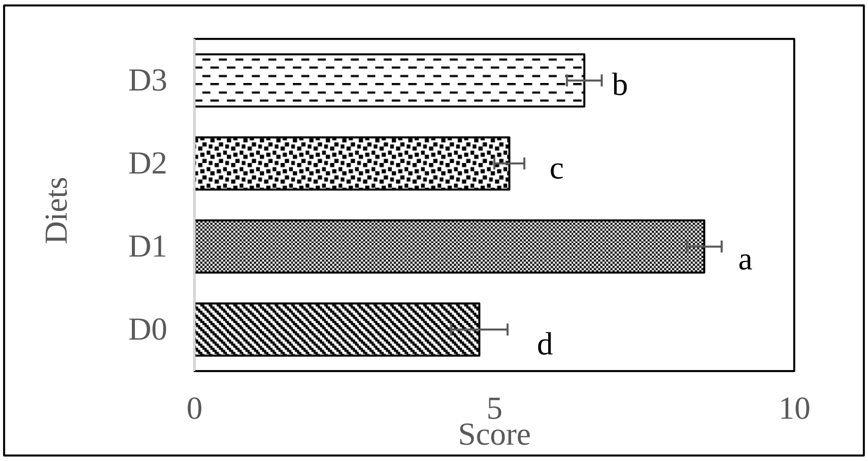

Figure 4. Taste preference of fish to experimental diets. Data were expressed as mean ± standard error (SE). Different superscripts represent significant differences ($p< 0.05$).

Total bacteria (TB) and lactic acid bacteria (LAB) in experimental diets

Table 4 presents the TB and LAB of experimental diets. The results revealed that TB and LAB are significantly ($p<0.05$) varied between the control and FAWM diets, although no obvious difference was observed among the bacterial loads between FAWM diet groups. Semi-solidify fermentation might be the reason for increased lactic acid bacteria in FAWM group diets. Van Vo et al. (2015) revealed that *Lactobacillus* fermentation modified the nutrition quality, higher inclusion level of alternative ingredients and different polysaccharides might have potential prebiotics in the fish intestine (Ringø et al., 2010). Additionally, Zulhisyam et al. (2020) and Nandi et al. (2023) documented corresponding results. Additionally, Aliyu -A et al. (2019) stated that probiotic bacteria's fermentation of dietary components effectively reduces the disease-causing bacteria in the gut.

Table 4. Total bacteria and lactic acid bacteria in experimental diets. Data expressed as Mean ± SE.

Parameters	**D_0**	**D_1**	**D_2**	**D_3**
TB (C.F.U/g) X 10^7	$7.97±0.03^b$	$13.60±.78^a$	$15.13±0.47^a$	$15.03±1.95^a$
LAB (C.F.U/g) X 10^4	$1.50±0.15^b$	$7.40±0.55^a$	$9.00±1.27^a$	$8.567±0.31^a$

Growth performance of Asian catfish

The growth performance of Asian catfish fed with different test diets is presented in Table 5. The majority of the growth parameters including final weight, weight gain, SGR, and CF were significantly ($p<0.05$) varied among the experimental diets. The D_0 (control) diet showed the notably highest weight gain (708.11%) and final body weight (28.32g) which corresponds to the result of Sogbesan et al., (2015) but among the FAWM diet groups, D_1 (fermented *Azolla)* diet fed fish showed significantly ($p<0.05$) higher FW, WG, and SGR. These outcomes might be because semi-solidify fermentation increased the digestion and absorption of feed nutrients, reduced the hardness of the fiber and ANFs. Rafeay et al., (2023) noted that 20% replacement of fresh green *Azolla* gave the highest values of growth parameters with better feed conversion and protein efficiency ratio. Moreover, Magouz et al. (2020) also reported that the 10-20% inclusion of *Azolla* increased the growth of Nile tilapia. However, in the case of FAWM, this inclusion level can be increased to 40-50% in catfish diets with better health and growth (Iskandar et al., 2019; Irabor et al., 2022a; Nandi et al., 2023).

Again, the feed utilization parameters in terms of FCR and PER also varied among the experimental diets. The D_0 diet treated fish showed significantly ($p<0.05$) better FCR but the highest mean value (1.81) of FCR observed in D_3 contained fermented *Eichhornia*. The presence of ANFs of other during processing may be the result of this poor FCR. Zhang et al. (2019) and Su and Chen (2020) reported that these factors could alter the nutrient balance of the diet, reduce palatability, and disturb the digestive process, and growth which ultimately decreases feed efficiency. However, the survival rate showed no significant difference ($p> 0.05$) not among the treatment groups. Similar results were found by Emshaw et al. (2023) while evaluating the effect of fermented water hyacinth meal on juvenile Nile Tilapia.

Table 5. Growth performance of Asian catfish fed for 90 days with experimental diets. Data expressed as Mean ± SE.

Parameter	Diets (% FAWM)			
	D_0	D_1	D_2	D_3
IW (g)	3.50±0.00	3.50±0.00	3.50±0.00	3.50±0.00
FW (g)	28.32±0.25^a	25.51±0.08^b	23.52±0.05^c	20.90±0.09^d
WG (%)	708.11±7.21^a	627.75±2.33^b	571.88±1.44^c	496.96±2.38^d
SGR (%/day)	1.88±0.01^a	1.79±0.00^b	1.72±0.00^c	1.61±0.01^d
FCR	1.30±0.01^d	1.51±0.01^c	1.72±0.00^b	1.81±0.01^a
PER	2.58±0.02^a	2.21±0.01^b	1.94±0.00^c	1.84±0.00^d
SR (%)	95.00±2.89	98.33±1.67	96.67±1.67	96.67±1.67
CF	0.58±0.01^c	0.75±0.00^b	0.85±0.00^a	0.84±0.01^a

IW: Initial weight; FW: Final weight; WG: Weight gain; SGR: Specific growth rate; FCR: Feed conversion ratio; PER: Protein efficiency ratio; SR: Survival rate; CF: Condition factor. Different superscripts in each row indicate significant differences ($p<0.05$).

Water quality of treatment tank

According to Suma et al. (2023), aquaculture production success heavily depends on water's hydrological characteristics. The results of hydro-ecological variables are presented in Table 6. This findings showed that the majority of the hydrological parameters in terms of pH, DO, pressure, and nitrate were significantly ($p<0.05$) different among the treatment group except for temperature, ammonia, and nitrite ($p>0.05$). The possible reason for these may be due to the same water source and different FAWM may lead to variations in other parameters. All the parameters across the treatments were within the acceptable range which was previously reported by authors Nandi et al. (2023); Irabor et al. (2022a) for catfish culture in captivity.

Table 6. Water quality of treatment tank. Data expressed as Mean ± SE.

Parameters	Diets			
	D_0	D_1	D_2	D_3
Temperature (°C)	29.39±0.32	29.3±0.35	28.99±0.33	29.27±0.01
pH	7.5±0.06^{a}	7.04±0.01^{b}	7.12±0.01^{b}	7.10±0.00^{b}
DO (ppm)	5.35±0.01^{c}	5.28±0.02^{d}	5.42±0.01^{a}	5.40±0.01^{ab}
Pressure(mm)	752.66±1.45^{a}	751.26±0.69^{ab}	749.33±0.33^{b}	751.26±0.59^{ab}
Ammonia(ppm)	0.13±0.01	0.11±0.01	0.13±0.01	0.14±0.01
Nitrite(ppm)	0.24±0.13	0.06±.01	0.07±0.01	0.12±0.01
Nitrate(ppm)	0.68±0.02^{c}	0.57±0.01^{d}	0.75±0.01^{a}	0.72±0.01^{ab}

DO: Dissolved oxygen; different superscripts in each row denote significant differences ($p<0.05$).

CONCLUSION

Based on the experimental diets' physical and bacteriological properties, attractability, palatability, taste preference, and growth of Asian catfish, it is concluded that the incorporation of FAWM, especially *Azolla* could assist in generating more palatable and attractive aquafeed for Asian catfish production in captive conditions.

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