Journal of Materials and Environmental Science
ISSN: 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2022, Volume 13, Issue 03, Page 236-251

http://www.jmaterenvironsci.com

Azolla: A organic feed for fish farming – Review

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Received 29 March 2021,
Revised 28 March 2022,
Accepted 29 March 2022

Keywords
- ✓ Azolla
- ✓ Protein source
- ✓ Nile tilapia
- ✓ Growth rate
- ✓ Rohu
- ✓ Fish meal

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Abstract

Azolla is an aquatic fern belongs to the family *salviniaceae.* Azolla is rich in proteins contains essential amino acids and minerals. Use of azolla in the feed industry has been in practice for various advantages such as availability, cost effectiveness, sustainability etc. Azolla is a good replacer of protein from costly sources such as fish oil and fish meal dependent on feeding behaviours of the fish species. They can be used in the form of raw and fresh, powdered, dried, fermented, cooked, concentrates etc. It can be used as direct feed, partial or supplementary replacement to fish meal in preparation of fish feed. The different types of fishes like nile tilapia, fringed- lipped peninsula carp, tilapia zilli, thai silver barb, rohu, prawn, mrigal carp, orange fin labeo, black tiger shrimp, patin fish, gift tilapia, shabbout were used for the testing of azolla feed. This paper is an attempt to make extensive review on potential use of azolla in fish feed industry.

1. Introduction

1.1 Origin

Azolla is a aquatic fern and small leafed floating plant, The term Azolla is derived of two Greek terms Azo (to burn) and Allyo (to kill), refering the inability of plants to survive in dry conditions [1]. It is from the family Azollaceae and the genus Azolla. The azolla are divided into two subgenera: Euazolla and Rhizosperma [2]. Euazolla has 5 species, namely A. caroliniana Willd., A. mexicana Presl., A. microphylla Kaulf., A. rubra., and A. filiculoides and Rhizosperma has only 2 species called A. pinnata and A. nilotica [3].

1.2 Distribution

Azolla is native to the subtropics, tropics, and warm temperate places of Asia, America, and the Africa [4],[5]. It normally grows in aquatic ecosystems such as ponds, stagnant waters, canals, paddy fields or

ditches. Azolla caroliniana is found in the Caribbean and Eastern North America, *A. mexicana* in the Americas from Northern South America to Western North America, *A. filiculoides* in the Americas from to Western North America to Alaska, *A. nilotica* in East Africa from Sudan to Mozambique, *A. microphylla* in subtropical and tropical America, and *A. pinnata* is found in most of Asia and the coast of tropical Africa [6]. Three Azolla sp. i.e. *A. microphylla,* and *A. pinnata* and are commonly found all over the Indian subcontinent. Kannaiyan and Kumar (2006) [7] reported that Azolla species distribution links to fresh water ecosystems of tropical and temperate regions across the world.

1.3 Morphology

Azolla in water look like a red or green carpet [8]. The roots of Azolla plants always immersed in water. Azolla can be found in triangular shape with 1.5-3 cm length and 1-3 cm breadth. The Azolla macrophyte, called a frond, ranges from 1 cm to 2.5 cm in length in species such as *A. pinnata* and the largest species like *A. nilotica* [9]. Azolla consist a main rhizome which divided into secondary rhizomes, it has small leaves consecutively arranged. The roots absorb all nutrients from water and in shallow water they may touch the soil, absorb nutrients from it. Leaf consists of two lobes, a ventral lobe and a chlorophyllous aerial dorsal lobe that is partly merged in water. In every dorsal lobe there is a leaf canal, which holds the interdependent Anabaena Azolla.

2. Growth and cultivation of azolla:

Azolla can harvest nine tons of protein per hectare of pond per year. In laboratory condition, Azolla has been testified to be able to double the biomass in less than 2 days and in beneficial field conditions within 3-5 days and 5- 10 days in normal field situations.

a) Small ponds of 320 meter size should be made in typical Azolla pond.
b) 10-15 cm standing water should be there in the ponds.
c) Culture of green Azolla 50-200 g/sqm along with single super phosphate (20 kg/ha) as a phosphorus source should be mixed and release into the pond.
d) Rapid growth of Azolla plants forms a green color mat just like a carpet in the ponds within 14-21 days.
e) In summer season Azolla can be produced at regular interval of 21 days.
g) In winter season growth rate of Azolla plant is reduced due to low temperature and moisture stress. Therefore, Azolla should be produced after 30 days of interval during this season.

These information's are summarised from (Rai, 2010) [10] (Katole *et al.,* 2017) [11]

3. Chemical Composition

Azolla contains 20-37 % protein [12],[13]. However, the digestible protein of the Azolla is 56.6 % [14] which limits the inclusion of higher level of Azolla in poultry diets. The chemical score guide showed the potential of Azolla meal as a good cause of protein. Azolla contains 0.47-0.53 % leucine and lysine, 0.11-0.17 % methionine, 0.53-0.55 % threonine, 0.14-0.15 % tryptophan. The nutrient composition of azolla is shown in the table – 1. Apart from this valine and arginine are the predominant important amino acids while tryptophan and the sulphur containing amino acids were insufficient [15][16].

3.1 Nutrients composition of different azolla (Table 1)

Total Ash in this study were similar with values of Bolka (2011) [22] and Parashuramulu *et al.,* (2013) [23] whose values were in the range from 16.21% was reported by Prasanna *et al.*, (2011) [20]. Whereas

Subudhi & Singh (1978) [24] reported 10.50%-15.82% of total ash in dried azolla. The higher value (24.26) of total ash was reported by Cheeryl *et al.,* (2014) [25] while this value was 28.7% were also reported by Lukiwati *et al.,* (2008) [26] .

Nutrients	Percentage	ppm	Reference
Crude protein	17.59		(Bhatt et al., 2020) [1]
Crude fiber	16.54		
Total ash	25.28		Balaji et *al.,*(2009) [17]
Calcium	1.67		
Phosphors	0.46		(Kathirvelan et al., 2015) [13]
Iron	0.231		
Manganese	0.205		Chattereji *et al.,*(2013) [18]
Sodium	0.777		
Potassium	2.19		Kavya (2014) [19]
Copper		15.90	Prasanna *et al.,*(2011)[20]
Zinc		46.77	
Magnesium		0.155	Ayyappan (2000) [21]
Moisture		5	

4. As nutritional supplement for livestock

Azolla is used as food supplement for variety of animals like chickens, ducks, cattle, goat, pigs, fish and rabbits. Seultrope, (1967) [27] conducted an experiment and reported that Azolla can be used as food for pigs and cattle. Various authors have conducted studies on the usage of azolla as livestock feed and their influence on growth is explained in the table 2. Das *et al.,* (1994) [28] observed that digested Azolla slurry residual after biogas production was suitable as fish pond fertilizer. Murthy, et al., (2013) [29] fed 2 kg fresh Azolla per day to the milking cows replacing 50% of concentrate for 3 months and observed that Azolla maintained good dairy performance while decreasing feed labour costs by 16.5% and milk production costs by 18.5%. Parthasarathy, *et al.,* (2002) [30] observed that 5 % replacement broiler ration with dried Azolla was quite profitable and safe for broiler production. Ali *et al.,* (1995) [31] led a trial with feeding broiler chicken with maize and soybean meal 10% substituted by dried *A. pinnata* and observed that feed cost significantly decreased without affecting the meat production resulting higher net return. Rai *et al.,* (2012) [32] conducted a trial and observed that layer birds fed with fresh Azolla had a developed body weight at 8 weeks or started egg production at 40 and 72 days.

5. The below table gives status of azolla as sustainable feed for fish (Table 2)

Sl. No	Fish Name		Habitat	Methods	Time	Remarks	Author/s
	Common	Scientific					
1.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Four isonitrogenous and isolipidic diets were formulated to include 0%, 10%, 20%, and 30% Azolla powder. 375 fish with similar body weight were distributed across 12 Habas, which were fixed in an earthen pond in a random manner, with 25 fish per Haba. The feeding rate was 3% of body weight and the fish were visually fed twice daily.	Feeding trial lasted for 90 days.	• Positive effects on the digestive enzymes, intestinal morphometry, immune functions, and growth rate. • The ideal inclusion from 10 to 20% of the diet.	Magouz *et al.,* 2020[33]
2.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Three isonitrogenous and isocaloric diets containing three levels of azolla 0, 10, and 20, respectively, as a partial substitution of fish meal, were fed to three triplicate ponds of male O. niloticus.	Experimental trial lasted for 90 days.	• Diet with 20% azolla observed similar growth compared with fish fed a diet containing fish meal.	Abou *et al.*, (2008)[34]
3.	Fringed-lipped peninsula carp	*Labeo fimbriatus*	Fresh water	Azolla was used along with spirogyra powder at 4:1 ratio Partial substitute of fish meal at the rate of 0, 25, 50, 75 and 100% were prepared.	The feeding trail was conducted for 60 days	• Can be used for the replacement of fish meal by 25% in the diets.	Sheeno and Sahu, (2006)[35]
4.	Nile tilapia	*Oreochromis niloticus*	Fresh water	For the experimental study three different isonitrogenous diets were formulated by incorporating azolla, lemna, and water hyacinth respectively.	Three groups of juveniles of Oreochromis niloticus	• The inverse relationship was observed between growth and azolla meal levels as per the statistical analysis.	Bag and Mahapatra, (2011)[36]

					consisting 50 number of fishes per group were fed for 90 days		
5.	Tilapia zilli	*Coptodon zilli*	Fresh water	Sun-dried Azolla meal was used in partial substitution at 0, 25, 50, 75, or 100% of the control diet on an equal weight basis.	Experimental feeding trial which lasted for 91 days	• Fish fed fresh Azolla lost weight irrespective of the initial fish size. • Weight loss increased significantly with increasing fish size expressed as percentages of initial weight.	Mohsen, (2008) [37]
6.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Azolla was dried and grinded into fine powder and used as principal protein source for fish feed formulation. (Bhosale *et al.,* 2010).	The feeding trail was conducted for 30 days	• Azolla meal showed better weight gain in O. niloticus along with increased body protein content and decreased body lipid content.	Nancy and Amalarani, (2016) [38]
7.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Dried Azolla powder cultured in the secondary effluent was included at levels of 20.7, 34.4, and 48.2% of the total weight of the diets. The percentage of weight gain of the fish was recorded.	Experimental feeding trial which lasted for 21 days	• Azolla can replace about 20% of Tilapia feed.	Nobuyuki & Shunji, 2000 [39]
8.	Fringed-lipped peninsula carp	*Labeo fimbriatus*	Fresh water	Four test diets were prepared by replacing the groundnut oilcake and rice bran from control feed at 10 (10% A), 20 (20% A), 30 (30% A) and 40% (40% A) with dried and powdered azolla. Fish in randomly selected triplicate tanks were fed one of the four experimental diets	Experiment lasted for 75 days	• It indicates the incorporation of azolla up to 40% in the diet of L. fimbriatus. • Fry-to-fingerling rearing does not affect fish growth and survival.	Gangadhar *et al.,* 2015[40]

				and the control diet once daily (Biswas, Jena, & Singh, 2006) at 10% of body weight during the first month, followed by 7% during the second month and 5% during the last 15 days (Jena et al., 2005)			
9.	Thai Silver Barb	*Barbonymus gonionotus*	Fresh water	Five treatments (T1 to T5) were designed to vary in CFF (commercial fish feed) substitution rate of 0%, 25%, 50%, 75%, and 100% with A. pinnata. Twenty fish with an approximate initial size of 3.90 g were transferred into the cages in five separate treatments with three replications. The fish were fed with floating commercial pellet feed.	Experiment lasted for 56 days	• The study suggests that a one-fourth proportion of CFF (commercial fish feed) replacement with fresh *A. pinnata* could be a bearable another option to save the cost of Thai silver barb production and offer high-profit margins.	Mousumi *et al.,* 2018[41]
10.	Rohu	*Labeo rohita*	Fresh water	A basal diet was prepared using groundnut oilcake, rice bran, Soya-bean meal, wheat flour and mineral mixture. For the preparation of experimental diet, Azolla was mixed in basal diet in different quantities.	Experiment lasted for 60 days	• Study indicates a better growth rate, • Specific growth rate, • Food conversion efficiency • High gross conversion efficiency of fingerling fed	Ramesh *et al.,* 2017[42]
11.	Rohu	*Labeo rohita*	Fresh water	The dried Azolla powder was used as a feed ingredient in the diet of Labeo rohita.	The growth of fish was assessed on test diets over a period of 150 days.	• Azolla is a good source of protein and it can be combined upto 25% level in the diet of Labeo rohita safely. • Azolla in fish diets reduces the fat content in muscle of fish.	Suriya Narayana Datta, (2011)[43]

12.	Prawn	*Macrobrachium Rosenbergii*	Fresh water	The experimental groups were fed with the respective concentrations of fishmeal replaced with S. platensis, C. vulgaris and A. pinnata inclusion levels of 25%, 50%, 75% and 100% incorporated diets. The feeding was scheduled two times a day (6:00 am and 6:00 pm).	Experiment lasted for 60 days	• The non-enzymatic antioxidants such vitamin C and E were significantly elevated in experimental fed groups. • The elevation of vitamin C and E were recorded higher in 50% of the C. vulgaris incorporated feed PL group followed by the 50% of S. platensis and A. pinnata incorporated feed	Radhakrishan *et al.,* 2010 [44]
13.	Mrigal carp	*Cirrhinus mrigala*	Fresh water	Four test diets were prepared by replacing groundnut oil cake and rice bran from control feed (C) at 10 (T1), 20 (T2), 30 (T3) and 40% (T4) with dried and powdered azolla. Fish in randomly selected triplicate tanks were fed with one of the four diets once daily at 10% of body weight during the first month, followed by 7% during the second month and 5% during the last 15 days.	Experiment lasted for 75 Days	• As seen in the present study, a decreasing trend in the performance of mrigal, in terms of growth parameters and FCR, becoming significant at 40% level of azolla incorporation, is an indication that dietary incorporation of azolla beyond 30% is not desirable in fry to fingerling rearing.	Gangadhar *et al.,*2014 [45]
14.	Orangefin labeo	*Labeo calbasu*	Fresh water	Four experimental diets were formulated for each ingredient replacing groundnut oil cake and rice bran of the basal diet at 10, 20, 30 and 40% levels.	Experimental feeding trial which lasted for 45 days	• The study reveals the usefulness of azolla for inclusion in calbasu diets. Azolla can be used up to 30% without affecting the digestibility	Gangadhar *et al.,* 2017 [46]

15.	Black tiger shrimp	*Penaeus monodon*	Fresh water	Five isonitrogenous experimental diets containing approximately 40% crude protein were formulated by replacing 0, 25, 50, 75, and 100% of the protein from soybean meal with protein from azolla meal.	Experimental feeding trial which lasted for 56 days	• The azolla meal as a plant protein source without any adverse effects on growth and survival, feed utilization efficiency, and palatability.	Sudaryono, 2006 [47]
16.	Tilapia zilli	*Coptodon zilli*	Fresh water	Base diet was replaced with different levels of Azolla meal. Thus, five tested diets were formulated by mixing either 0.0, 25.0, 50.0, 75.0 or 100.0% Azolla meal with preformulated fish feed.	Experiment lasted for 90 Days	• An optimum inclusion level of Azolla meal should be incorporated at a level not more than 25% for the feeding of T. zillii. • Good weight gain, specific growth rate and higher mortality rate.	Abdel *et al.,* 1998[48]
17.	Prawn	*Macrobrachium rosenbergii*	Fresh water	Five experimental diets were formulated to be isonitrogenous. The control diet was not supplemented either with A. pinnata or with Digestin TM and classified as control (T 1). In (T 2) diet, all WB content was replaced by A. pinnata without Digestin TM supplementation. Diets T 3, T 4 and T 5 were formulated as T 2 diet and supplemented with increasing levels of Digestin TM (1%, 2%, and 3%, respectively).	Experimental feeding trial which lasted for 84 days	• Based on the cost diff erential between the two ingredients, there are economic advantages to complete replacement of wheat bran with *A. pinnata* for dietary of *M. rosenbergii.* • Therefore, from an economic perspective, the diet containing *A. pinnata*, supplemented with Digestin ™ at the level of either 2% (T 4) or 3% (T 5) can be considered more cost eff ective for prawn, M.	Goda *et al.*, 2017[49]

						rosenbergii PLs compared to other experimental diets	
18.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Whole plants of *Azolla pinnata* were harvested and 30% crude protein, were formulated, Diets No.1, 2, 3 and 4 contained 10% DUM (Dried azolla meal) treated with different concentrations of NaOH, and diets No.5,6,7 and 8 contained 20% DAM treated with different concentrations of NaOH. Each ingredient was ground and thoroughly mixed with the other dietary ingredients, vitamins and minerals mixtures.	Experimental feeding trial which lasted for 84 days	• Nile tilapia can utilize of dried Ulva (*Ulva lactuca*) meal at a level of 10 % or dried azolla (*Azolla pinnata*) meal at a level of 20 % with sodium hydroxide (NaOH) treatment at a level 3.0 % in the diet instead of plant protein (soybean meal) without any adverse effect on fish health.	Eman *et al.,* 2010[50]
19.	Patin Fish	*Pangasius djambal*	Fresh water	Three pools, each sized 3×5×1 m3, with a stocking density of 500 fish/15 m3 were treated by different feeding: pellets; pellets coated with probiotic; pellets plus supplement of A. pinnata (3:1). Feeding was given for twice a day.	The fish were cultured for 7 months.	• Patin fish added with A. pinnata shows a better profile of fatty acids compare to control and fish fed with pellet plus probiotic.	Ika *et al.,* 2015[51]
20.	GIFT tilapia	*Oreochromis niloticus*	Fresh water	Four isonitrogenous (32%) and isocaloric (17 KJ kg-1 DM) experimental diets were formulated and used to replace fish meal with Azolla meal at 0, 150, 300 and 450 g kg−1. 120 healthy GIFT tilapia fingerlings (body weight 3.3 ± 0.32g) were randomly stocked at a rate of 10 fishes per container in 12 plastic containers (70L capacity). Experimental animals were	60 days	• The present study revealed the effective incorporation of Azolla meal as a fish feed ingredient in the GIFT tilapia diet for the first time. The venture for using this Azolla meal have brought the positive impact in the growth of fishes at 15% inclusion level with enhanced blood serum	Sebastian *et al.*, 2020[52]

				fed with formulated diets at 5% of their body weight in two rations for all the treatments throughout the trial.		biochemistry performance thereby reducing the cost of fish feed in the tilapia culture.	
21.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Experimental diets were designed to contain 30% crude protein, 300 Kcal (calorie) digestible energy/100g and protein/energy ratio about 70mg protein/Kcal (calorie). presents the constituents and composition of tested diets. About 50% of soybean meal protein (equal about 30% of total protein in the control diet) was substituted by azolla meal. Azolla meal is incorporated into the tested diets at levels, 10.6, 21.2, 31.8 and 42.2% of the diet.	The experiment ran for 90 days	• Azolla meal at a maximum level of 31.8% was suitable as a dietary protein supplement for tilapia when combined 50% replacement for soybean protein, without any adverse effect on growth performance, survival rate, feed use and economical parameters.	Ebrahim *et al.,* 2007[53]
22.	Nile tilapia	*Oreochromis niloticus*	Fresh water	The graded levels of the palm kernel cake at 20, 40, 60, 80 and 100% were replaced with aquatic fern to make up a total of 6 treatments. The treatments were randomly assigned to six concrete tanks in duplicates, each randomly stocked with 10 fingerlings of Oreochromis niloticus.	The experiment ran for 84 days	• The treatments containing azolla pinnata had subjectively better results than the control, particularly in treatment of 20% palm kernel cake replaced with the fern, where the best results were obtained.	Abioye *et al.,* 1993[54]
23.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Fish were fed with six isonitrogenous (29.2% CP) and isoenergetic (16.9 kJ.g-1) diets formulated to contain 0% (A0), 10% (A10), 20% (A20), 30% (A30), 40% (A40) and 50% (A50) of AM using	The experiment ran for 90 days	• In fish fed diets containing AM in gradual replacement of FM, significantly higher deposition of ARA and lower (n-3) fatty acids in fish was resulted.	Abou *et al.,* 2010[55]

				locally available ingredients and the freshwater fern A. filiculoides.			
24.	Nile tilapia	*Oreochromis niloticus*	Fresh water	The experimental diets were formulated to contain three levels of Azolla (0, 10, and 20%), in partial substitution of fish meal.	Fish were fed for 90 days	• There were no significant differences between treatments, suggesting that similar yearly productions were obtained between fish fed with the diet containing 20% of Azolla and those fed with the control diet (0% Azolla).	Abou *et al.,* 2012[56]
25.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Fresh azolla was sun dried, finely ground and its proximate analysis performed. Dry azolla was incorporated into 5 practical diets containing approximately 30% crude protein 390kcal Ge/100g to replace 25, 50, 75, and 100% of the fish meal protein, carbohydrate and lipid respectively.	The experiment was conducted 70 days	• The use of A. pinnata as a food source for tilapia and adults is limited. Less than 25% of the dietary fish meal protein may be replaced by azolla.	Sayed, (1992)[57]
26.	shabbout	*Tor grypus*	Fresh water	An aquaponic system integrating fish culture and plant production was used. The plants, embedded in a gravel filter, extract organic wastes from the water and the purified water was recycled back to the fish tanks at a daily exchange rate of 5% of the tank volume. Nitrosomonas and nitrobacter bacteria were added to the gravel beds to enhance the decomposition of nitrogenous	The experiment was conducted for 84 days	• The final average weight, mean weight, gain of fish fed with D1 were significantly higher than those of fish fed with D2, D3 and D4.	Gökçınar and Bekcan, 2015[58]

				compounds. Three replicates of 10 fish per tank were established f or each treatment.			
27.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Cultured Azolla in pond and examined its potential as a fish feed. In a feeding experiment with Tilapia nilotica, a diet containing 20.7, 34.4 and 48.2% of the total weight of dried azolla were given for 3 weeks.	Experimental feeding trial which lasted for 21 days	• It was observed that azolla can replace about 20% of tilapia feed, which indicates the beneficial effect of the use of aquatic plant.	Shiomi and Kitoh, (2001) [59]
28.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Using dry azolla meal as a sole protein source for feeding tilapia Oreochromis niloticus. The inclusion levels of azolla meal were 0, 15, 20, 30, 40 and 45% on dry weight basis in diet.	Experimental feeding trial which lasted for 30 days	• Considering cost of feed, the study recommended to use 45% azolla incorporated diet for tilapia in a fertilized pond.	Fiogbe, (2004) [60]
29.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Eleven diets were prepared containing 30% crude protein while fish meal was substituted at a rate 5, 10, 20, 30 and 100% by azolla powder.	Experimental trial lasted for 56 days	• Improved growth and protein utilization efficiency has been reported for diets with 10% - 15%**.**	Fasakin (2008) [61]
30.	Nile tilapia	*Oreochromis niloticus*	Fresh water	Commercial feed was replaced by fermented azolla leaves with different supplementation levels, i.e. 0%, 30%, 60%, and 90% and fed to fishes.	Performed for two, six, eight, and ten days	• Azolla meal fermented for two days has given the best results.	Utomo *et al.,* (2011) [62]

Conclusion

Comparing all the results we came to the conclusion that the fishes fed with Azolla meal showed comparatively more weight gain and showed greater increase in length. The body protein content was found to be highest in fishes that were fed with Azolla. Such locally offered cheap and quality ingredient can expand living and food security of the poor by contributing to developments in aquaculture production and by guaranteeing the supply of a healthy animal protein to the consumer

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