



USE OF AZOLLA IN FISH FEED AS FISHMEAL SUBSTITUTE

Hundare S. K*, Ranadive A. B and Lende S. R

Center of Excellence in Aquaculture, Kamdhenu University, Gandhinagar, Gujarat, India

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ABSTRACT

The development of sustainable aquaculture depends on the establishment of alternative feedstuffs to fishmeal. Due to the rising cost of commercial fish feeds, the farmers are looking for alternative feeds in order to make aquaculture a viable and attractive venture. Several species of aquatic plants could be potential sources of fish meal replacers in aquaculture. Azolla is an effective substitute for fishmeal. This article has reviewed the importance and uses of different forms of azolla in fish feed as fishmeal substitute.

Key words:

Azolla, Fishmeal substitute

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INTRODUCTION

Global agriculture sector is facing impacts of rapid climate change, economic and financial uncertainty and growing competition for natural resources. The decrease in the agriculture production needs to be balanced by aquaculture sector. Thus, the set aims for the contribution and conduct of fisheries and aquaculture towards food security and nutrition are the use of natural resources so as to ensure sustainable development in economic, social and environmental terms. In 2016, global aquaculture production was 110 million tonnes consisting 54 million tonnes of finfish, 17 million tonnes of molluscs, 8 million tonnes crustaceans, 30 million tonnes aquatic plants and 1 million tonnes of other aquatic animals (FAO, 2018).

In global aquaculture production, Asia contributed 101.5 million tonnes and ranked first among remaining producers. Among this, major groups were finfish (47.7 million tonnes), aquatic plant (30 million tonnes), molluscs (16 million tonnes) and crustaceans (7 million tonnes) (FAO, 2018). Being a second largest producer, India contributed 5.2 million tonnes in Asia's production in terms of 4.7 million tonnes of finfish and 0.5 million tonnes of crustaceans (FAO, 2018). In broad terms, aquaculture production systems used for producing these aquatic animals and plants can be divided into feed-dependent systems or fed aquaculture (e.g. finfish and crustaceans) and non-fed aquaculture systems, where culture is predominately dependent on the natural environment for food, e.g. aquatic plants and molluscs (FAO, 2016).

In Asia, fed aquaculture accounted for 54 percent of the region's total aquaculture production, indicating that almost half of Asia's aquaculture production comes from non-fed aquaculture. In contrast to the Asian situation, finfish and crustacean aquaculture production in Europe is 100 per cent dependent on aquafeeds. Asia is the largest global consumer of aquafeed ingredients (FAO, 2016). Aquaculture production, mainly of crustaceans and finfish, relies on farm-made or complete industrial diets. About two third of protein in aquatic diets comes from fish meal and its feeding value has caused its extensive use in aquaculture diets. Thus, with expansion of aquaculture industry, demand for fish feed has been increased with a huge pressure on fish meal industry. The reduction in catches of pelagic fishes, the sole resources of quality fish meal has resulted in rapid hike in prices which ultimately affected feed cost, thereby increase in operational cost of farming (FAO, 2016).

Fish nutritionists have made several attempts to partially or totally replace fish meal with less expensive, locally available protein sources. Due to the current high cost of fish meal, there is an intense pressure to re-evaluate common alternative protein sources to determine how best to use them in preparing low fish meal aquafeeds (Hardy and Tacon, 2002). There are several protein sources that have the potential of replacing fishmeal in aquaculture feeds without affecting the growth performance of fish (Tacon and Metian, 2009). In recent years, utilization of aquatic plants and weeds having high food values as feed ingredients has taken a new dimensions in producing the much required animal protein at low cost. The cost of production of aquafeed can be reduced using this ecofriendly and sustainable resources (Gangadhar, 2015).

***Corresponding author: Hundare S. K**

Center of Excellence in Aquaculture, Kamdhenu University, Gandhinagar, Gujarat, India

Azolla has a higher crude protein content (ranging from 19 to 30 per cent) than most green forage crops and aquatic macrophytes and rather favorable essential amino acid (EAA) composition for animal nutrition (rich in lysine), it has also attracted the attention of livestock, poultry and fish farmers (Cagauan and Pullin, 1991).

Expansion of Tilapia culture is attributed to high resistance to diseases, ability to survive at low oxygen tensions and ability to feed on wide range of foods. Even though, slight variations exist among tilapia species, nutrient requirements are primarily affected by the size of the fish (El-Sayed and Teshima, 1992). The global tilapia production in 2015 is 5.6 million tonnes and Asia contributes 4.1 million tonnes in which Nile tilapia contributed 2.8 million tonnes (FAO, 2017). The importance of Nile tilapia as an inexpensive protein source in rural communities, its geographical distribution, economic potential ability to reproduce in captivity, availability and in some countries inexpensive market price (Landau, 1992) has made Nile tilapia a preferred fish species for culture in many parts of the world (Fasakin *et al.*, 2008).

A nutrient requirement for optimum growth for tilapia is dependent upon the quality of source, fish size or age and energy contents of the diets. The average protein requirement varies between 45 - 50 as stated by Stickney (1997). The minimum requirement of dietary lipid in tilapia diets is 5% but improved growth and protein utilization efficiency has been reported for diets with 10% - 15% (Ng and Chong, 2004).

Research studies have shown that the protein of azolla plant is 23% - 30% which includes 55% of the amino acid (Gokcinar and Bekcan, 2015).

Azolla is an aquatic fern consisting of short branched floating stem, bearing roots which hang down in water. The most remarkable characteristics of azolla is its symbiotic relation with nitrogen fixing blue green algae. Therefore, for centuries, it has been used as a biofertilizer as well as green manure in rice crop of southern China and northern Vietnam. Azolla has higher protein content (19 - 30%) than most green forage crops and aquatic macrophytes and essential amino acids favorable for animal nutrition. Azolla can be a valuable protein supplement of many species including ruminants, poultry, pig and fish (Hasan, 2009).

There are seven extant *Azolla* species are recognized (some taxonomists recognized 6 species), which are distributed widely from temperate to tropical regions. Few research studies have indicated that the azolla plant has 23% - 30% crude protein content including 55% of the amino acid in present (Gokcinar and Bekcan, 2015).

Dry azolla powder has been used as a feed ingredient for different species such as, tilapia (Antoine *et al.*, 1986, 1987; Pantastico *et al.*, 1986; Micha *et al.*, 1988; Santiago *et al.*, 1988; El-Sayed 1992; Fioqbe *et al.*, 2004; Abou *et al.*, 2007; Ebrahim *et al.*, 2007; Tawwab, 2008; Fasakin, 2008; Abou *et al.*, 2011; Bag and Mahapatra, 2011; Agbede *et al.*, 2001), African catfish (Fasakin and Balogun, 1998), common carp (Ahmad, 2003) and rohu (Gangadhar *et al.*, 2015). In other forms viz. fermented azolla (Utomo *et al.*, 2001), azolla protein concentrate (Sundarano, 2006) were also used as a feed ingredient in fish diets.

In some experiments, other aquatic plants such as, lemna meal, water hyacinth meal were used in combination with of azolla

meal in diet of Mozambique tilapia (*Oreochromis mossambicus*) by Bag *et al.* (2012).

Thus, various research studies have indicated that apart from fresh azolla, various forms such as azolla meal, dry azolla powder, fermented azolla and azolla protein concentrate (APC) were used for partial substitution of fish meal, which has reflected in reduction of feed cost.

Azolla meal

Jayatissa (1996) has used *Azolla* spp. (*Filicophyta: Azollaceae*) as the major source of feed during pond culture of macrophytophagous fish species *Ctenopharygodon idella*, *Oreochromis niloticus*, *Trichogaster pectoralis*, *O. mossambicus*, *Etropolis suratensis*, *Labeo rohita* and *Cirrihinus mrigala*. Feed acceptance trial was conducted for these seven species using *Azolla imbricata* and *Azolla pinnata* species. Except *Ctenopharygodon idella*, remaining six species have recorded higher growth rates fed with azolla than the other aquatic weeds such as *Elodea Canadensis*, *Nitella flagellifera*, and *spirodella polyriza*. The growth rates of fish species tested, except that of *Ctenopharygodon idella*, were significantly (at 5% level) higher than that on dry formulated feed. Thus, study concluded that azolla can be used as a cheap source of feed for the grass carp and tilapia species.

Shiomi and Kitoh (2001) have cultured *Azolla filiculoides* 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. As per the results, diet B containing 20.7% azolla, displayed the same effect as the control for 2 weeks, and then induced a 5.2% decrease in Tilapia weight after 3 weeks. Diets C and D exerted the same effect on the growth of Tilapia after 3 weeks of feeding with a 17% decrease of growth compared to the control. Based on the results, it was observed that azolla can replace about 20% of tilapia feed, which indicates the beneficial effect of the use of aquatic plant.

There are six isonitrogenous diets (CP 27.5%) were formulated by Fioqbe (2004) 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. Comparing growth results, juveniles fed with 15% of azolla meal have exhibited best growth followed by azolla free diet. Considering cost of feed, the study recommended to use 45% azolla incorporated diet for tilapia in a fertilized pond.

Similarly, Sudaryono (2006) have studied the effect of different inclusion of azolla meal (*Azolla pinnata*) as a substitution of defatted soybean meal in juveniles of black tiger shrimp (*Penaeus monodon*). The levels of incorporation were 0, 25, 50, 75 and 100% of total soybean meal. All diets were isonitrogenous containing 40% crude protein. At the end of the feeding trial, the statistical analysis of data revealed that, there is no significant difference in the growth parameters. Also no significant difference was noticed in feeding preference test on the diets. Thus, use of azolla meal as an alternative plant protein source to soybean meal for *Penaeus monodon* juveniles was recommended. Thus, cost of feed can be reduced.

Abou *et al.* (2007) have studied effect of stocking density on growth, yield and profitability of farming Nile tilapia, *Oreochromis niloticus*, fed with azolla diet in earthen ponds.

In first experiment, Nile tilapia (*Oreochromis niloticus*) fingerlings were fed with three isonitrogenous and isocaloric diets viz. A30, A35 and A40 containing 30%, 35% and 40% azolla, respectively, for 90 days. At the end of experiment, no significant difference was found in growth parameters and production ($P > 0.05$) values. In second experiment, three different stocking densities, 1, 3 and 5 m⁻², were assigned to three treatments T₁, T₂ and T₃ respectively. Fish were fed twice daily with diet A40. On the basis of growth values and economic return, it was concluded that Nile tilapia could be raised at a density of 3 fish m⁻² using 40% azolla incorporated diet to improve production.

Growth performance of Nile Tilapia (*Oreochromis niloticus*) fingerlings was studied by Ebrahim *et al.* (2007) using diets containing azolla Meal as a source of protein. *Azolla nilotica* was incorporated into experimental diets at various levels (10.6, 21.2, 31.8 and 42.2% of the diets) to replace about 50% of soybean meal protein in the control diet (30% crude protein). Based on results, it was observed that substitution of azolla meal at a maximum percentage of 31.8% can replace about 50% of soybean meal protein in fingerlings tilapia diet without any adverse effect on growth performance, feed efficiency and survival rate.

Preference of the omnivorous- macrophagous fish, *Tilapia zillii* (Gervais), to consume a natural free-floating fern, *Azolla pinnata* was studied by Tawwab (2008). In the experiment, fish with different initial body weights were fed with five diets containing dry azolla meal as replacements of the control diet (20% crude protein) at levels of 0 (control), 25, 50, 75, or 100% for 13 week. As per the results of experiment, fish growth was reduced when the level of dry azolla meal in the diet exceeds 25%. The mortality rate was increased significantly with increasing azolla level in the diet. Carcass composition has shown increases in moisture and ash contents and decreases in dry matter, crude protein, and total lipids in accordance with the increase of azolla level in the diets. Thus, it was could be concluded that the optimum inclusion level of dry azolla meal should be no more than 25% in *T. zillii* diet.

Abou *et al.* (2011) have studied the effect of replacement of fish meal by azolla meal in diets of Nile tilapia (*Oreochromis niloticus*) on growth and fatty acid profile. Six isonitrogenous diets (29.2% crude protein) were formulated with inclusion levels at 0, 10, 20, 30, 40 and 50% of azolla meal. It was observed that the weight gain and SGR were decreasing as the levels of azolla inclusion were increasing from 10%. The FCR was significantly increased when azolla meal levels has exceeded 20%. Fatty acid profile has indicated that replacement of FM by AM, produced fish with lower (n-3) highly unsaturated fatty acids mainly docosahexaenoic acid, and higher arachidonic acid ($P < 0.05$). The n-3/n-6 fatty acid ratio ranged from 0.08 to 0.17 and was lower in fish fed A40 and A50 ($P < 0.05$). As per results, high azolla meal in diets affected growth of Nile tilapia and thus could be incorporated in diets up to 30% without any adverse effect on fish fatty acid composition.

A comparative study was conducted by Abou. (2013) to evaluate the efficiency of azolla incorporated diet solely and in combination with natural food organisms, in terms of growth and fatty acid composition. Rearing of Nile tilapia fingerlings was carried out in tanks and earthen ponds for 90 days. Six test diets with 0, 10, 20, 30, 40, and 50% azolla meal were

prepared for feeding. In both experiments, inverse relationship was observed between growth and azolla meal levels as per the statistical analysis.

Dry Azolla powder

Evaluation of sun-dried water fern, *Azolla africana* and Duckweed, *Spirodella polyrrhiza* as feed ingredient in practical diets for Nile Tilapia, *Oreochromis niloticus* juvenile was done by Fasakin (2008). Eleven Diets were prepared containing 30% crude protein while fish meal was substituted at a rate 5, 10, 20, 30 and 100% by azolla and duckweed powder. Feeding was carried out at 5% of body weight/day in two equal portions for 8 weeks. Water fern at 5% level diet has shown good results of survival rate (%), weight gain (%), specific growth rate, feed conversion ratio while Duckweed showed higher results in 10% inclusion level. Comparative study of results indicated that the inclusion of sun dried duckweed and water fern meal at 10 and 5% dietary inclusion, respectively was satisfactory in terms of biological performance. Thus, it was concluded that replacement for fish meal by water fern and duck weed in practical diets appears feasible, particularly in development of low-cost diet for Nile tilapia.

Abou *et al.* (2008) have conducted a study on preliminary assessment of growth and production of Nile tilapia, *Oreochromis niloticus* L., fed with azolla based diets in earthen ponds. Three isonitrogenous (27% CP) and isocaloric (4019.14 kcal/g) diets A0, A10, and A20 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* for 90 days. The azolla-free diet (0% azolla) served as a control. As analysis of result, it can be concluded that a diet with 20% azolla provided similar growth to Nile tilapia compared with fish fed a diet containing fish meal. However, more research is needed with diets containing more than 20% of azolla.

Gangadhar *et al.* (2015) have evaluated the effect of azolla incorporation on the growth and survival of *Labeo fimbriatus* during juvenile rearing. The azolla incorporation levels were 10, 20, 30 and 40% for partial replacement of GOC and rice bran in pelleted diets. The fishes were fed with 10% of body weight during first month, followed by 7% during second month and 5% during last 15 days. The results indicated that there was no effect of azolla incorporation on growth and survival of *Labeo fimbriatus*. The study indicated the possibility of incorporating azolla in diets of *L. fimbriatus* up to 40% during fry-to-fingerling rearing, resulting in savings in feed cost.

Fermented Azolla

To improve the nutritional value of azolla for fish and subsequently to increase their incorporation level into fish diets, it is necessary to ferment the ingredient to improve rate of digestibility (Velasquez *et al.*, 2011).

Fermented *Azolla spp.* leaves were utilized as a feed ingredient of tilapia *Oreochromis spp.* by Utomo *et al.* (2011). During the study, fermentation of azolla meal was performed for two, six, eight, and ten days (AF2, AF6, AF8, AF10) using *Trichoderma harzianum* as the fermentor. Commercial feed was replaced by fermented azolla leaves with different supplementation levels, i.e. 0%, 30%, 60%, and 90% and fed to fishes. Azolla meal fermented for two days (AF2) has

shown the best results. The results concluded that, fermentation can increase the nutritional quality of azolla meal and most optimal supplementation level in the diet of tilapia is 30%.

Azolla protein concentrate (APC)

Sheeno and Sahu. (2006) have studied the effect of azolla protein concentrate (APC) on growth of *Labeo rohita* fry. During the experiment with it was used along with spirogyra powder (SP) at 4:1 ratio was used as partial substitute of fish meal. Five isonitrogenous and isocaloric diets with APC-SP substitution at the rate of 0, 25, 50, 75 and 100% were prepared. The feeding trail was conducted for 60 days. The results showed that, the growth and other biological parameters were unaffected by the dietary substitution of APC-SP in diets of *Labeo rohita* fry. Thus, it was concluded that APC is a good source of protein and can be used for the substitution of fish meal by 10% in the diets of *Labeo rohita* fry.

Azolla meal with other plant protein

Agbede *et al.* (2001) have conducted a study to evaluate the preferential feeding of fishes among 3 aquatic weeds such as *Azolla filiculoides* (water fern), *Elodea sp.* and *Pistia stratiotes* (water lettuce). Sixty juvenile *Oreochromis niloticus* (Nile Tilapia) were fed with (three species) of aquatic weed, namely *Azolla filiculoides* (water fern), *Elodea sp.* and *Pistia stratiotes* (water lettuce) reared to determine which of the weeds will be selectively consumed, and preferred of all. A control group of twenty fishes were fed with compounded feed. The selectivity of the weeds was observed based on their utilization as food source, and *Azolla filiculoides* was found to be highly utilized than *Elodea sp.* and the roots of *Pistia stratiotes*. Highest growth parameters were observed in fishes fed with compounded feed followed by *Azolla filiculoides* and *Elodea sp.* On the other hand, fishes exhibited negative growth trend when fed with *Pistia stratiotes*. Thus the results of the study proposed that *Azolla filiculoides* can be effectively used as feed ingredient for *Oreochromis niloticus*.

Effect of spirulina (*Spirulina platensis*) inclusion in diets formulated for ornamental fish guppy was studied by Derneasi *et al.* (2010). Different levels of spirulina (*Spirulina platensis*) were incorporated in feeding diets to evaluate its effect on growth and feeding conversion for a period of 90 days. The 15 circular plastic aquariums with a volume of 10 L were used for rearing 20 juveniles in each treatment. Commercial feed was used as a control while it was replaced at 10, 20, 30 and 40 % spirulina incorporated diet. As per the results, highest growth rate, weight gain and feed conversion ratio was observed in 40 % spirulina diet but no significance difference was found between specific growth rate.

A comparative study of fatty acid profile was conducted by Bag and Mohapatra. (2011) to study the effect of diets incorporated with different aquatic weeds viz. *Azolla* (*Azolla pinnata*), lemna (*Lemna minor*) and water hyacinth (*Eichhornia crassipes crassipes*). For the experimental study three different isonitrogenous diets (30% CP) were formulated by incorporating azolla (48%), lemna (46%), and water hyacinth (47%) respectively. Three groups of juveniles of *Oreochromis niloticus* consisting 50 number of fishes per group were fed for 90 days after completion of the research. All treatments were significantly higher than control in

respective value of weight gain, protein efficiency ratio and gonadosomatic index. It was predicted that lemna possibly contains better quality of protein which in turn influence the somatic as well as reproductive growth in fishes fed with lemna diet. After analysis of fatty acid profile no significant difference were observed in accumulation of PUFA, especially n-3PUFA. Negligible variation were noticed n-3/n-6 ratio.

Effect of fermented *Cladophora* containing diet on growth performance and feed efficiency of tilapia *Oreochromis niloticus* species was analysed by Dewi *et al.* (2014). Five different isoprotein diets (30% CP) were formulated by substituting plant based protein by fermented *Cladophora* in the range of 0 to 40%. Fishes were fed at the rate of 3% of body weight and rearing was continue for 42 days. According to result the tilapia fed with 10% fermented *Cladophora* recorded highest growth rate, feed efficiency, protein digestibility and essential amino acid index. The growth rate of 10% fermented *Cladophora* treatment was 30% higher than the control diet of fishes may affect in cost reduction and gain in production biomass..

Toyosi. (2015) has used cassava leaf meal to partially replace fish meal in the diet of *Clarias gariepinus*. Four dry diets were formulated at 40% crude level added in the feed 0, 10, 20 and 30% inclusion level. During the days period fishes were fed at the rate of 5% of their body weight.

Partial replacement of fishmeal with cassava leaf (*Manihot esculenta*) meal was carried out by Toyosi. (2015) to evaluate its effect on the growth performance of *Clarias gariepinus* juveniles. Four dry diets were formulated at 40% crude protein were fed to juveniles at 0%, 10%, 20% and 30% inclusion level of cassava leaf meal where the 0% served as the control. The experiment was conducted for 91 days. The fish were fed at 5% body weight twice daily morning and evening. The best results were obtained in the treatment 20% inclusion of cassava leaf meal but there was no significant difference in the growth parameters between 20% inclusion level and the control diets.

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