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A Review on Significance of *Azolla* Meal as a Protein Plant Source in Finfish Culture

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Abstract

The increase in costs and demand of protein from conventional resource necessitates fish farmers and hatcheries manager to incorporate cheap and locally available ingredients in fish diets. Among protein plant sources, *Azolla* seems to be good replacer of protein from expensive sources such as fish meal and fish oil depending on feeding habits of the fish species. It contains high crude protein content (13% to 30%) and essential amino acid (EAA) composition (rich in lysine) than most green forage crops and other aquatic macrophytes. A review was conducted on significance of *Azolla* meal as a protein plant source in finfish culture, mostly focus was on Tilapia species and family *Cyprinidae*. About 30 published online journal papers, from Research gate and Google scholar in aquaculture nutrition were reviewed. Among reviewed papers revealed that, the dietary *Azolla* supplementation at certain level have a positive effect on feed utilization and protein conversion ratio, mobilization and utilization of glycogenic amino acids, and growth performance. Therefore, this review suggests that, 10-45% *Azolla* inclusion level can be incorporated in the diet for Tilapia species, except for *T. zillii* which requires more than 40% protein contents. While in fish belong to the family *Cyprinidae*, the inclusion level should be 10-50% for Rohu, and 10-25% for the rest of family members, except *Labeo fimbriatus* which didn't shows any effect up to 40% *Azolla* inclusion level in a diet.

Keywords: Fish growth performance; Protein plant source; *Azolla* meal; Tilapia species; Family *Cyprinidae*

Introduction

Aquaculture is the fastest growing food producing sector and is perceived to have the greatest potential to meet the growing demand for aquatic food [1]. World aquaculture production is likely to grow continuously, but at slow rate [2]. In Tanzania, aquaculture is largely a subsistence activity practiced in the coastal and inland areas [3,4]. The sector is mainly dominated by tilapia species, *Orechromis niloticus* and African catfish, *Clarius gariepinus* which accounting around 90 per cent of the total inland production. In the coastal areas particularly Zanzibar, it is dominated by sea weed, *Eucheuma denticulatum* farming [5].

The increase in costs and demand of protein from conventional resources necessitate fish farmers in developing countries [6] including Tanzania to incorporate cheap and locally available ingredients in fish diets. Recent literatures reported that, the utilization of high food value aquatic plants are used to supplement fish diets.

A floating freshwater, *Azolla pinnata* is one of the aquatic plants with high biomass and protein production which can be used as a direct fish feed or diet ingredient of an alternative protein source [7]. *Azolla* has gained its importance in aquaculture due to higher crude protein content (13% to 30%) and essential amino acid (EAA) composition (rich in lysine) than most green forage crops and other aquatic macrophytes [6]. In spite of its attractive nutritional qualities and relative ease to produce in ponds, reports on use of *Azolla* in aquaculture are extremely limited. However, it is well documented in some shellfish such as black tiger shrimp *Penaeus monodan* [8] and finfish such as carps [9] and Nile tilapia [10].

These fish species have been reported to convert raw protein from *Azolla* into the best edible protein, thus reduces the cost of production of feeds [11]. Also, it is reported to have important components which enhance performance of fish. Cohen et al. [12] reported the presence of the 3-Deoxyanthocyanins which are the only known flavonoids of *Azolla*. In addition, Mithraja et al. [13] reported various antioxidants like phyto-constituents such as tannins, phenolic contents and flavonoids from *Azolla* crude extract. Therefore, this review aimed to explore the significance of *Azolla* in fish diets from the recent studies conducted by several researchers in the field of aquaculture nutrition.

Literature Review

Origin, classification and distribution of *Azolla* species

Azolla is a genus of aquatic ferns and small leafed floating plants, native to the tropics, subtropics, and warm temperate regions of Africa, Asia, and America [14]. It is very sensitive to lack of water in aquatic ecosystems such as stagnant waters, ponds, ditches, canals or paddy fields. These areas may be seasonally covered by a mat of *Azolla* associated with other free-floating plants species such as Duckweed (*Lemna minor* L.), Water lettuce (*Pistia stratiotes* L.), Water caltrop (*Trapa natans* L.), Water meal (*Wolffia* Horkel ex Schleid) and mud-rooting species such as Hornwort (*Ceratophyllum demersum* L.), Water purslane (*Ludwigia palustris* L.) and Knot weed, *Polygonum arenastrum* [15].

Literature shows that, *Azolla* domestication dates back to the 11th century in Vietnam [16], and the genus was botanically established by Lamarck in 1783 [15]. The *Azolla* are categorized either into subgenera or taxonomic "section" level. In subgenera classification, *Azolla* are divided into two genera based on the sporocarp characters: *Euazolla* and *Rhizosperma* [16]. *Euazolla* is further classified into the taxonomic "section" level [17] which have 5 species, namely Willd (*A. caroliniana*), Lam (*A. filiculoides*), Presl (*A. Mexicana*), Kaulf (*A. microphylla*) and *A.*

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rubra [18]. While, the former genera (*Rhizosperma*) has only 2 species called Decne. ex Mett. (NI), *A. nilotica* and R. Br, *A. pinnata* [19].

According to Kannaiyan and Kumar [15], *Azolla* species are distributed all over the world in fresh water ecosystems of temperate and tropical regions. Some literature has indicated that species of genera *Euazolla* have originated from North and South America while *Rhizosperma* originated from Africa, Asia and Australia (Table 1) [15,20].

Impacts of *Azolla* species in nature

Azolla is the one of the world's fastest growing aquatic macrophytes which can be doubling in only 2-5 days [21,22]. Though, it has various benefits, are also considered as annoying weeds in nature, particularly *A. pinnata* and *A. filiculoides* [23]. Many studies have mentioned *Azolla* as a weed [24-26]. For instance, the North American native *A. filiculoides* has invaded many places in Iran [25,27], Europe and South Africa [26], where it is now considered as an important exotic weed. *A. pinnata* is another example of an obnoxious weed [16,28]. This fern became naturalized in North Carolina (US) in 1999, where it continues to be present [24], and also in New Zealand where it displaced the native *A. rubra* in most parts of the country. In fact, such invasive aquatic ferns are major concern for biologists and ecologists dealing with conservation and management of wetland ecosystems due to the threats they may pose to the rich original biological diversity.

Azolla may have important harmful and irreversible impacts on wetlands as they may change the local fauna and flora [29,30]. Also, they may reduce the ecological quality through changes in biological, chemical and physical properties of aquatic ecosystems [31]. According to Olenin et al. [32], some of the biological changes consist of eradication of susceptible or rare species, alteration of native communities and algal blooms. While physical-chemical changes involve the modification of substrate conditions and the shore zones, alterations of oxygen and nutrient contents, pH and transparency of the water and accumulation of pollutants. In addition, these invaders can survive and reproduce in a wide range of environmental conditions [33,34].

Importance of *Azolla* in fish pond

Azolla can be used either directly or indirectly in the fish pond, due to higher percentage in nutrients composition on dry weight basis (Table 2) and other constituents such as minerals, chlorophyll, carotinoids, amino acids, and vitamins [35]. It can be used as fish food in *Azolla*-fish pond culture and contributes directly to weight gain of macrophytophagous fish [36]. Also, it has been reported that, *Azolla* tends to increase production of fish faeces which directly consumed by bottom dwellers which in turn used as an organic (nitrogenous) fertilizer to increase overall pond productivity. In addition, the high rates of decomposition of *Azolla* make it a suitable substrate for enriching the detrital food chain or for microbial processing such as composting, prior to application in ponds [36]. However, the contribution of *Azolla* to aquaculture sector is promising, it may not ensure high productivity when used alone. Hence, it can be a useful supplement to natural feed in low-input aquaculture and can reduce high dependency on fish meal and fish oil from the nature [37].

Significance of *Azolla* meal

Among published papers reviewed, Tilapia species (*Oreochromis niloticus, Tilapia mossambica*, *Tilapia zillii*) and Family *Cyprinidae* (*Labeo rohita, Catla catla*, *labeo calbasu, Labeo fimbriatus*, *Ctenopharyngodon idella, Barbonymus gonionotus*) were mostly reported to utilize *Azolla* when incorporated into the diets.

Tilapia Species

Nile tilapia, *Oreochromis niloticus*

The aquatic fern *Azolla* has been successfully used in tilapia culture as feed ingredients [38-42]. Some authors have been studied on growth performance and survivability of tilapia fingerlings by providing *Azolla* partially or fully as a component in the fish feed [43]. Therefore, most of the literatures reviewed reported the improvement on growth performance, feed utilization and survival rate on Nile tilapia fry at the increased dietary inclusion of *Azolla* up to a certain level. For instance, Santiago et al. [44,45] reported that, Nile tilapia fry fed rations containing up to 42% of *A. pinnata* outperformed fish fed a fishmeal-based control diet. Also, Micha et al. [46] reported highest performance in *Tilapia rendalli* fingerlings when fed feeds incorporated with *Azolla*. In contrast, Abou Youssouf [10] reported that, the final mean weight of Nile tilapia decreased as *Azolla* inclusion level increased from 0% to 50% in the experimental diets (Figure 1). Similar results have been

Genera	Species	Origin and Distribution
Euazolla	*A. filiculoides*	Southern South America, and Western North America to Alaska
	A. caroliniana	Eastern North America, Central America, North South America, the Caribbean, Mexico and West Indies
	A. mexicana	Northern South America to British Columbia, Western North America and Eastward to Illinois
	A. microphylla	Western and Northern South America to Southern North America and the West Indies
Rhizosperma	*A. pinnata*	Tropical Africa and Southern Africa, South East Asia, Japan and Australia
	A. nilotica	Central Africa, upper Nile Sudan, Uganda, Tanzania, Congo and Namibia

Source: Carrapiço et al. [20] and Kannaiyan and Kumar [15].

Table 1: Worldwide distribution of *Azolla* species.

Constituents	*Azolla* (% Content)
Crude protein	13-30
Crude fat	4.4-6.3
Cellulose	5.6-15.2
Hemicellulose	9.8-17.9
Lignin	9.3-34.8
Ash	9.7-23.8

Source: Ayyappan [37].

Table 2: Nutrient composition (%) of *Azolla* on dry weight basis.

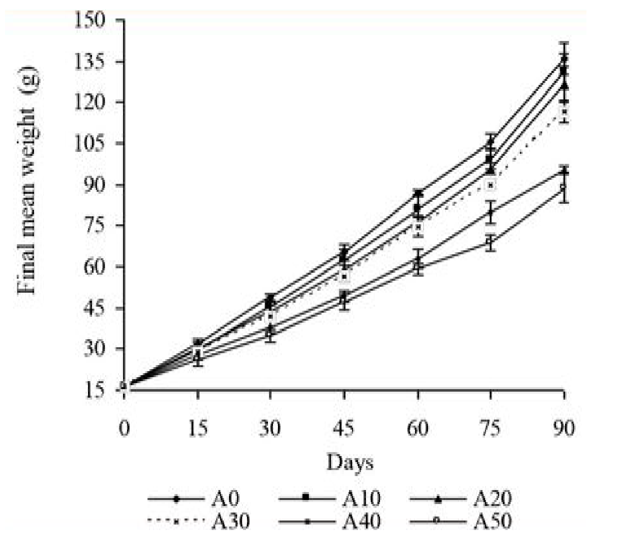

Figure 1: Changes in fortnight mean weight (mean ± S.D.) of Nile tilapia fed in earthen ponds for 90 days.

reported by Abou et al. [47] when he fed fish with a diet containing 20% of *Azolla* at 30% *Azolla* cover.

Tilapia mossambica

Earlier studies have been reported the improvement in feed utilization and increased growth in *Tilapia mossambica*. According to Sithara and Kamalaveni, [48] the biochemical studies on this fish species reported that, the protein, carbohydrate and lipid contents in liver and muscles were increased when fish fed a diets containing wheat bran and rice bran (control feed), wheat bran, rice bran and *Azolla* in the ratio of 25:25:50 (experimental feed) for the duration of 90 days. Similar results reported by Micha et al. [46].

Tilapia zillii

Several researches have been conducted in *Tilapia zillii* based on the knowledge of being microphagous omnivore fish. However, Abdel-Halim et al. [49], reported poor growth performance of *T. zillii* fry when fed a diet replaced with either 0, 25, 50, 75 or 100% *Azolla pinnata* meal (Table 3). Similarly, Micha *et al.* [46] reported a decreased growth of *T. rendalli* when *Azolla* was incorporated in their diets.

Family *Cyprinidae*

Rohu, *Labeo rohita*

Among fish cultured in family *Cyprinidae*, Rohu is the most commercial fish with maximum market demand and acceptability as food by the consumers due to its test and flesh quality [1]. Various kind of supplementary feeds have been tried to accelerate growth and production of fish per unit area [50], including *Azolla*. Several studies have been focused on growth and survival of herbivorous fishes including Rohu fingerlings by providing *Azolla* species partially or fully as a component in the fish feed [11,43]. According to Panigrahi et al. [6], the highest percentage weight gain and growth parameters of Rohu fingerlings were found in T2 group fed with 40% *Azolla* followed by T3 fed with 50% *Azolla* (Tables 4 and 5). Similarly, Das et al. [51] reported significantly increased growth up to 40% level of *Azolla* inclusion and then significantly decreased growth when the level of *Azolla* increased to 56.8% and 63.6% in the diets. Also, Kumari et al. [1] reported better growth performance of Rohu fingerlings when fed 200g/kg feed *Azolla* supplemented diet. In contrast, Mohanty and Dash [52] reported higher weight gain and good utilization in Rohu fry fed with *A. caroliniana* at 60% inclusion level, comparing diets with 30, 40 and 50% *Azolla* incorporation.

Catla (*Catla catla*)

Several studies conducted in other carp species reported the efficiently utilization of *Azolla* inclusion diets. For instance, Catla which is an economically important South Asian freshwater fish, reported a higher growth rate and compatibility with other major carps, surface feeding habit, and consumer preferences. In a study conducted by Umalatha et al. [35] reported that, incorporation of *Azolla* up to 20% did not have any adverse effect on dry matter and protein digestibility, both decreasing ($p<0.05$) at higher inclusion levels (Table 6). Similarly, Asadujjaman and Hosain [53] reported poor growth of Catla fed *Azolla* as compared to those fed control diet consisting of rice bran, wheat bran and mustard cake (30:30:40). However, in other carp species the different results have been reported. For instance, Ahmad [54] reported high growth performance in common carp, *Cyprinus carpio* L. fingerlings when fed *Azolla* incorporation diet.

Orange fin labeo, *Labeo calbasu*

Orange fin labeo, *Labeo calbasu* is an herbivorous fish belonging to family *Cyprinidae*, found commonly in rivers and freshwater lakes around South Asia and South-East Asia [55]. It is a bottom dweller and can tolerate high turbid water during dry season [56]. It is considered as herbivorous fish feeding mainly on vegetable matter, followed by crustaceans and other insect larvae. It feeds on algae (10%), higher plants (48%), protozoa (12%), crustaceans (10%), molluscs (5%),

Diet	Initial weight (g/fish)	Final weight (g/fish)	Weight gain (g /fish)	SGR (%g/day)	Mortality (%)
1 (0%)	2.30 ± 0.01	4.72 ± 0.2[a*]	2.42 ± 0.21[a]	0.79[a]	6.67 ± 0.0[b]
2 (25%)	2.22 ± 0.01	4.65 ± 0.2[a]	2.43 ± 0.22[a]	0.82[a]	10.00 ± 3.3[b]
3 (50%)	2.32 ± 0.09	3.14 ± 0.1[c]	0.82 ± 0.08[c]	0.33[c]	3.00 ± 3.3[b]
4 (75%)	2.35 ± 0.01	2.72 ± 0.01[cd]	0.37 ± 0.03[cd]	0.16[cd]	16.67 ± 3.3[ab]
5 (100%)	2.27 ± 0.03	2.49 ± 0.01[d]	0.22 ± 0.01[d]	0.10[d]	23.34 ± 3.3[a]

*Figures in the same column not having the same letters are significantly different ($p<0.05$). Source: Abdel-Halim et al. [49].

Table 3: Growth performance of *Tilapia zillii* fry fed on diets containing different levels of *Azolla* meal.

Experimental diets	Ingredients		
	***Azolla* sp. powder**	**Rice bran**	**Groundnut oil cake**
T_0 feed	NIL	50	50
T_1 feed	20	40	40
T_2 feed	40	30	30
T_3 feed	50	25	25

Source: Panigrahi et al. [6]

Table 4: Percentage composition of different ingredients in experimental diets.

Growth parameters	Treatments			
	T_0	T1	T2	T_3
Percentage weight gain	175.62 ± 2.31	197.17 ± 6.19	281.57 ± 7.21	239.33 ± 5.24
SGR	0.55 ± 0.01	0.60 ± 0.01	0.73 ± 0.01	0.67 ± 0.01
FCR	4.21 ± 0.09	3.79 ± 0.61	2.93 ± 0.18	3.49 ± 0.11
Survival (%)	76	80	100	100

Source: Panigrahi et al. [6]

Table 5: The growth parameters and experimental diets.

mud and sand (15%) [57]. Gangadhar et al. [55] reported the highest (p<0.05) digestibility values of this species at 10% *Azolla* inclusion level. However, some researcher reported that, *Azolla* can be incorporation up to 30% without any adverse results in Orange fin labeo and other species, which indicates the superiority of Azolla as a feed ingredient over other plant protein sources.

Labeo fimbriatus

Freshwater herbivorous fishes like *Labeo fmbriatus* feed mainly on unicellular algae, filamentous algae and parts of higher aquatic plants [58]. Among published papers reviewed, only one publication has been found reported insignificant differences in the growth parameters of *L. fmbriatus* fry receiving *Azolla*-incorporated diets (up to 40% inclusion level) [58].

Grass carp, *Ctenopharyngodon idella*

The Grass carp, *Ctenopharyngodon idella* is a rapid growing, phytophagous, cyprinid fish indigenous to the large rivers of China and Siberia [59]. Several studies have been conducted in this species and the results have been shown almost the same trend as in Catla, Orange fin labeo and Thai Silver barb. According to Nekoubin and Sudagar, [60], the highest food conversion ratio (FCR) was observed in *Azolla* (*A. fliculoids*) (62.18 ± 4.29) which had significant difference (P<0.05) from other treatments. Similarly, Ayyappan, [61] reported that, grass carp and common carp recorded a weight gain of 174 and 35.8g/fish respectively and utilized *Azolla* to the extent of 30% inclusion level.

Thai Silver barb, *Barbonymus gonionotus*

Thai silver barb, *Barbonymus gonionotus* is an omnivorous species in origin [62], an exotic fish of Bangladesh belonging to the family *Cyprinidae*. It has good palatability, high yield potential, and very large market demand [63,64]. Das et al. [65] reported that, the highest average weight gain (AWG) and specific growth rate (SGR) were observed in fish fed at T1 (0% *Azolla*) compared to other treatments. However, the general growth and production performance of fish was higher in T2 (25% *Azolla*) and was gradually decreased with the increase in the levels of supplementary *A. pinnata*. (Tables 7 and 8).

Similar results have been reported in several studies with the same purpose of replacing fish meal by the plant protein in Nile tilapia [66,67].

Feeds	Total DMD	Protein Digestibility	Fat Digestibility	NFE Digestibility
		Azolla		
Control	60.20 ± 2.20[cd]	68.26 ± 1.91[c]	82.79 ± 1.47[a]	79.31 ± 1.90[a]
10%	62.48 ± 0.18[d]	75.45 ± 4.03[cd]	87.08 ± 0.29[b]	86.66 ± 0.72[c]
20%	55.61 ± 0.64[c]	65.44 ± 0.65[c]	89.80 ± 0.08[c]	83.74 ± 1.25[bc]
30%	49.34 ± 2.04[b]	55.64 ± 2.58[b]	95.29 ± 0.57[d]	82.14 ± 0.48[ab]
40%	36.21 ± 3.33[a]	44.91 ± 1.92[a]	96.83 ± 0.70[d]	81.14 ± 1.16[ab]
		Soy bean		
Control	60.61 ± 2.29[a]	69.21 ± 2.65[a]	80.93 ± 0.52[a]	76.36 ± 1.88[a]
10%	60.25 ± 1.76[a]	68.76 ± 1.74[a]	81.16 ± 0.17[a]	76.68 ± 1.15[a]
20%	60.08 ± 1.10[a]	71.37 ± 2.59[a]	82.70 ± 1.84[a]	77.10 ± 0.87[a]
30%	63.25 ± 0.98[a]	72.25 ± 0.64[a]	86.43 ± 0.80[b]	80.68 ± 0.81[b]
40%	68.71 ± 0.14[b]	84.96 ± 0.15[b]	88.32 ± 0.08[b]	84.83 ± 0.19[c]
		Silkworm pupa		
Control	61.28 ± 3.26[b]	68.57 ± 3.41[a]	85.20 ± 1.35[a]	78.61 ± 0.19[a]
10%	58.61 ± 0.40[ab]	69.04 ± 0.33[a]	84.18 ± 0.22[a]	77.37 ± 0.06[a]
20%	58.43 ± 0.52[ab]	70.87 ± 0.45[a]	88.00 ± 0.39[b]	79.56 ± 0.47[a]
30%	59.00 ± 1.77[ab]	69.64 ± 1.29[a]	94.66 ± 0.87[c]	85.73 ± 0.54[b]
40%	55.31 ± 2.63[a]	67.17 ± 1.41[a]	93.72 ± 0.30[c]	85.40 ± 0.63[b]
Source: Umalatha et al. [35].				

Table 6: Digestibility (%, mean ± SD) of dry matter, protein and fat by *Catla* fed experimental feeds.

Treatments	Protein (%) (Dry Matter)		Protein (g/day)		Feed Quantity (g in Wet Weight/day)	
	CFF	*A. pinnata*	CFF	*A. pinnata*	CFF	*A. pinnata*
T_1 (Control)	100	0	5.46	0	17.63	0
T_2	75	25	4.10	1.36	13.23	15.27
T_3	50	50	2.73	2.73	8.81	30.64
T_4	25	75	1.36	4.10	4.40	46.03
T_5	0	100	0	5.46	0	61.27
Source: Das et al. [64]						

Table 7: Experimental design and feeding dose (at initial level) at different treatments.

Treatments	T_1	T_2	T_3	T_4	T_5
IAW (g)	3.90 ± 0.13	3.90 ± 0.11	3.90 ± 0.29	3.90 ± 0.09	3.90 ± 0.08
FAW (g)	30.93 ± 0.4 [a]	30.68 ± 0.4 [a]	24.55 ± 0.45 [b]	19.81 ± 0.25[c]	15.20 ± 0.39 [d]
AWG (g)	27.03 ± 0.16 [a]	26.78 ± 0.10 [a]	20.65 ± 0.19 [b]	15.91 ± 0.46[c]	11.30 ± 0.34 [d]

SGR (% day^{-1})	3.70 ± 0.14^{a}	3.68 ± 0.16^{a}	3.28 ± 0.11^{b}	2.90 ± 0.08^{c}	2.43 ± 0.18^{d}
SR (%)	99.33 ± 1.15^{a}	98.67 ± 1^{a}	99.33 ± 0.58^{a}	98 ± 1^{a}	99.33 ± 0.58^{a}
CF	2.35 ± 0.035^{a}	2.29 ± 0.032^{a}	2.12 ± 0.13^{a}	1.90 ± 0.40^{a}	1.59 ± 0.095^{b}
HSI	1.41 ± 0.03^{a}	1.32 ± 0.04^{a}	1.74 ± 0.06^{b}	2.03 ± 0.04^{c}	2.44 ± 0.09^{d}
FCR	0.88 ± 0.09^{a}	0.93 ± 0.17^{a}	1.15 ± 0.12^{b}	1.66 ± 0.15^{c}	2.64 ± 0.06^{d}
PER	2.98 ± 0.03^{a}	2.94 ± 0.02^{a}	2.26 ± 0.08^{b}	1.75 ± 0.07^{c}	1.24 ± 0.02^{d}

IAW: Initial Average Weight, FAW: Final Average Weight, AWG: Average Weight Gain, SGR: Specific Growth Rate, SR: Survival Rate, NPR: Net Production Rate, CF: Condition Factor, HSI: Hepatosomatic Index, FCR: Feed Conversion Ratio, PER: Protein Efficiency Ratio.
Means with different superscripts (a, b, c and d) are significantly different (p<0.05). (Source: Das et al. [64]).

Table 8: Growth performance of Thai silver barb *Barbonymus gonionotus* fed varying compositions of *A. pinnata* and commercial fish feed after 56 days.

Discussion

Basing on research papers reviewed, *Azolla* seems to be good replacer of protein from expensive sources such as fish meal. Among reviewed papers, suggest that Nile tilapia and *T. mozambicuss* can perform better in a range of 20% to 42% of *Azolla* inclusion diet [38,68]. Some literatures suggest positive growth even in higher inclusion level of *Azolla* up to 50% [45,69]. However, young Nile tilapia have been reported to efficiently utilize sun-dried *Azolla* more than adults [44,69,70]. The reason might be due to highly presence of enzymes in the gut which can effectively digest *Azolla* which have a relatively low fibre content and no ant-nutrient factors or a deficiency in amino acids and phosphorus [71].

In recent biochemical studies on *Tilapia mossambica* reported that, the increased protein, carbohydrate and lipids content in liver when fish fed with *Azolla* diet [48]. The significant increases of the biochemical parameters in various fish's tissues revealed that the protein conversion ratio, mobilization and utilization of glycogenic amino acids are very high, in fish fed with *Azolla* diet. While, the increased lipid content suggests the fewer uptakes of lipid components by tissues for utilization [48]. In contrast, several studies conducted by Almazan et al. [69] (with *O. niloticus*), Antoine et al. [72] (with *O. niloticus* and *Cichlasoma melanurum*), Micha et al. [46] (with *O. niloticus* and *Tilapia rendalli*) and Joseph et al. [70] (with *Etroplus suratensis*) revealed lowering of growth performance and food conversion with increasing *Azolla* incorporation in the diet. The reason could probably be due to the lower protein digestibility of this fern, as mentioned by Leonard et al. [73] and, Micha and Leonard [74] in *Oreochromis aureus* Steindachner and in *O. niloticus* respectively.

In addition, *Tilapia zillii* reported to have poor growth performance in *Azolla* meal (Abdel-Halim et al., 1998) despite being a microphagous omnivore fish [75-77]. Similarly, Micha et al. [46] reported a decreased growth of both *O. niloticus* and *T. rendalli* when *Azolla* was incorporated in their diets. The reasons might be due to the low protein content of *Azolla* diets (about 20%) while protein requirement for tilapia fry is 35% [78,79] and may be as much as 40% [80]. Also, the poor performance of *T. zillii* fed high levels of *Azolla* meal on diets might be contributed with the deficiency of some essential amino acids especially methionine, lysine and histidine, as well as the high neutral detergent fiber of *Azolla* and possibly adenine limits the usefullness of *Azolla* as a food ingredient for simple-stomach animals [81].

On the other hand, fish belong to family *Cyprinidae* reported to have different ranges of *Azolla* inclusion levels in the diet. Most studies reviewed reported improved feed utilization and increased growth in Rohu at 10-50% *Azolla* inclusion level in the diet [6,11,82]. While Orange fin labeo [55], Catla [35], silver carp and mrigal [82], grass carp [83], and Thai Silver barb [65], reported to have a range between 10-25% *Azolla* inclusion level in the diet [1]. Similar results have been reported by several researchers with other aquatic plants supplemented diet such as Duck weed [84,85]. The reasons for the different inclusion levels might be due to the presence of ω-6 fatty acids [52], nutrients value of the plants such as the gross energy content of the diet and the dietary protein [86,87] which assimilated differently, depends on feeding habits of the species (example, Calta vs Ruhu). Also, due to different enzymes in the fish gut play an important role in the digestion and utilization of feed [88].

In contrast, among published papers reviewed, only one publication has been reported insignificant differences in the growth parameters of *Labeo fimbriatus* fry receiving *Azolla*-incorporated diets (up to 40% inclusion level) [57]. The reason could be due to differences in energy contents of the experimental diets [89].

Conclusion

Azolla seems to be good replacer of protein from expensive sources such as fish meal depending on feeding habits of the species. This is due to proper corroboration between the activity pattern of the digestive enzymes in fish and the essential nutrients such as ω-6 fatty acids from *Azolla* diet. Also, the dietary *Azolla* supplementation shows to have a positive effect on growth performance of fish and reduce the cost of feeding from fish meal and fish oil diet. In addition, due to increase of the biochemical parameters in various fish's tissues revealed that the protein conversion ratio, mobilization and utilization of glycogenic amino acids are very high, in fish fed with *Azolla* diet. However, too much *Azolla* incorporation in the diet will decrease fish growth performance and food conversion, probably due to low protein digestibility and high fiber contents. Therefore, this review suggests that, 10-45% of *Azolla* inclusion level can be incorporated in the diet for Tilapia species, except for *T. zillii* which requires more than 40% protein contents. While in fish belong to the family *Cyprinidae*, the inclusion level should be 10-50% and 10-25% for *L. rohita* and the rest of family members respectively, except *Labeo fimbriatus* which didn't shows any effect up to 40% *Azolla* inclusion level in a diet.

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