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Perspectives on the utilization of Azolla-Anabaena system as feed supplement

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ABSTRACT

The nitrogen fixing aquatic pteridophyte *Azolla* is one of the fastest growing nitrogen-fixing plants and it is used as a potential source for high rate biomass production. *Azolla* has the ability to fix atmospheric nitrogen at cheaper and faster rates due to the presence of a symbiotic cyanobacterium *Anabaena azollae*. Therefore, the ability to fix atmospheric nitrogen is important from an agricultural perspective. However, *Azolla* is gaining popularity as feed supplement for cattle, poultry and fish. Further, the ease of cultivation and favourable nutrient composition make *Azolla* an important feed supplement. This review focuses on the perspectives of *Azolla* as feed supplement.

Keywords: *Azolla*, Biomass, Feed supplement, Nitrogen fixation, Protein

The aquatic nitrogen fixing pteridophyte *Azolla* has become increasingly popular due to its high rate of biomass production (Muradov *et al.* 2014). *Azolla* grows luxuriantly in ditches, fresh water ponds and paddy fields and is found in both temperate and tropical regions. This fast growing macrophyte has a doubling time of 5–15 days and multiplies by vegetative means by breaking up to form separate plants. However, it also reproduces sexually through large female spores and tiny male spores. The presence of the endophytic cyanobacterium, *Anabaena azollae* as symbiont allows *Azolla* to grow efficiently even in the absence of nitrogen (Calvert and Peters 1981, Zheng *et al.* 2009, Pereira and Vasconcelos 2014). Wagner (1997) reported that in 30 days the *Anabaena azollae* strains can fix 30–60 kg N/ha. In addition to supplying nitrogen to crops, it also provides other nutrients such as vitamins and growth promoting substances. *Azolla* also improve the structure and general fertility of the soil by increasing the organic matter in soil. The agronomic potential of *Azolla* has already been demonstrated by Singh (1989). Extensive use of *Azolla* has been reported in several countries (Shao *et al.* 2011, Ali *et al.* 2014). China has a long history of utilizing non-conventional feed resources such as *Azolla* (Coughenour and Makkar, 2012). Traditionally, the *Azolla-Anabaena* system has been used as biofertilizer for rice (Joshi *et al.* 2012). Wagner (1997) listed several other uses of the *Azolla–Anabaena* system and because of the multifaceted uses it has been aptly referred to as "green gold mine". Antimicrobial compounds of plant origin have been reported in *Azolla*. Abraham and Aeri (2012) studied the phytochemical composition of *Azolla microphylla* Kaulf. Abraham *et al.* (2015) investigated the antimicrobial potential of the extract of *A. microphylla* against plant pathogenic bacteria.

Aquatic plant species like *Azolla* offer great potential as source of protein for animals due to ease of cultivation, productivity and nutritive value. Gouri *et al.* (2012) and Yadav *et al.* (2014) recently reviewed the importance of *Azolla* as a sustainable feed for livestock and poultry. There will be chronic shortage of feed in Asian countries by 2020, and India is expected to need 526 million tonnes of dry matter, 56 million tonnes of concentrate feed and 855 million tonnes of green fodder (Dikshit and Birthal, 2013). If the scenario is not carefully managed it will lead to import of feed material and involvement of foreign exchange. Therefore, plants with high nutritional value and palatability are adapted to local harsh conditions can be used as alternate feed resources. Feeding trials conducted elsewhere indicated that *Azolla* could be included in diets at rates of 10% for poultry, 15% for sows and 25% for tilapia fish (Becerra *et al.* 1995; Alalade and lyayi, 2006; Abdel-Tawwab, 2008). Devendra and Leng (2011) suggested the use of fresh dried or ensiled *Azolla* as food supplement for pigs, rabbits, chickens, ducks, and fish. This review outlines the utilization of *Azolla* as feed supplement for cattle, poultry and fish.

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General features of Azolla

The aquatic pteridophyte *Azolla* grows luxuriantly in ditches, fresh water ponds and paddy fields and is found in both temperate and tropical regions. It is tiny and has a horizontal rhizome of 0.5 to 7 cm in diameter with branches having densely arranged and overlapping leaves. An individual leaf consists of a thick dorsal lobe and a thin ventral lobe. The dorsal lobe of the leaf harbours the symbiotic cyanobacterium in a mucillage filled cavity (Peters and Mayne, 1974). Generally, *Azolla* requires 25–50% full sunlight for its normal growth. *Azolla* can survive a water *p*H ranging from 3.5–10, with optimum growth occurring at *p*H 4.5–7.0. *Azolla* belongs to the monotypic family *Azollaceae* and there are seven extant species of *Azolla* (Hills and Gopal 1967, Konar and Kapoor 1972). Further, the two sub genus of *Azolla* Euazolla and Rhizosperma are characterized by the presence of number of floats of megaspores. The sub genus Euazolla having three floats of megasporocarps consists of species such as *A. caroliniana*, *A. filiculoides, A. mexicana, A. rubra* and *A. microphylla*. On the other hand, species such as *A. pinnata* and *A. nilotica* belong to the sub genus Rhizosperma which is characterized nine megaspore floats. Since many accessions do not form sporocarps under culture conditions, the taxonomic assignment of *Azolla* is difficult. Identification of the organism at the species level is based on the trichomes (Lumpkin and Plucknett 1982, Nayak and Singh 1988). Morphological, vegetative and molecular biology tools are important in the taxonomy of *Azolla* (Pereira *et al.* 2011). Abraham *et al.* (2013) developed species specific sequence characterized amplified region (SCAR) markers for the identification of different species of *Azolla*. The somatic chromosome number of the species varies from 44 to 66 (Nayak and Singh 1988). *Azolla* reproduces mainly by vegetative means and the sexual reproduction is through the formation of sporocarps.

Mass multiplication and productivity of Azolla

Azolla can be produced in plots, ponds, ditches, canals, concrete tanks and polythene lined pits. *Azolla* is a fast growing plant with the ability to produce adequate quantity of biomass (Costa *et al.* 1999, Miranda *et al.* 2016). In natural ecosystems, such as rivers, lagoons and irrigation channels *Azolla* can bloom with growth rates up to 300 g/m^2 day of fresh biomass (Van Hove *et al.* 1987). Dawar and Singh (2002) observed optimal growth and productivity of *Azolla* maintained as soil and nutrient based cultures. Arora and Singh (2003) compared the productivity of different species of *Azolla* under polyhouse conditions and based on the biomass production potential selected *Azolla microphylla* for mass production. (Brouwer *et al.* (2017) observed an annual productivity of 32.8–35.5 t dry weight ha^{-1} per year in a fully controlled indoor production system. The ease of cultivation, productivity and nutritive value makes *Azolla* a potential source of protein for animals (Prabha and Kumar, 2010). Fig. 1 depicts the high rate mass multiplication by *Azolla microphylla* in cemented tanks out door.

Fig.1. Mass multiplication of *Azolla* in cemented tanks.

Nutrient content of Azolla

The chemical nature of the constituents of *Azolla* is such that it has all the properties as feed source for the animals (Cohen *et al.* 2002, Lejeune *et al.* 2000). The nutrient and chemical composition of the *Azolla* biomass is depicted in Table 1. Various workers reported the protein content of *Azolla* biomass as 200–400 g/kg dry weight (Alalade and Lyayi 2006, Leterme *et al.* 2010). In *A. Africana*, the crude protein content of leaf protein concentrate and residual pulp fibre constitute 71.3 and 12.6%, respectively (Fasakin 1999, Brouwer *et al.* 2016). *Azolla* has sufficient protein content and is easily digested by poultry (Parashuramulu *et al.* 2013). Protein content of the biomass also varies depending upon the species (Sanginga and Van Hove, 1989). *Azolla* is a rich source of protein and contains essential amino acids, vitamins (vitamin A, vitamin B_{12}, and beta-carotene), growth promoter intermediaries and minerals (Pillai *et al.*

Table 1. The nutrient and chemical composition of *Azolla*

Nutrient	% dry matter basis
Crude protein	22.48
Crude fibre	14.70
Ether extract	4.50
Total Ash	17.34
NFE	40.97
Cell wall fraction	
Neutral detergent fibre	54.85
Acid detergent fibre	36.57
Mineral	
Calcium	1.64
Total phosphorus	0.34
Potassium	2.71
Trace mineral	(ppm)
Manganese	2,418.00
Zinc	325.00
Copper	16.74
Iron	1,569.00
Boron	31.00

Source: Anitha *et al.* (2016).

Table 2. Amino acid composition of *Azolla*

Amino acid	Values as % of dry matter	Values as % protein	Chemical score (%)
Lysine	0.98	4.58	130.9
Methionine	0.34	1.59	45.4
Cystine	0.18	0.84	24.0
Threonine	0.87	4.07	116.3
Tryptophan	0.39	1.82	52.0
Arginine	1.15	5.37	153.4
Isoleucine	0.93	4.35	124.3
Leucine	1.65	7.71	220.3
Phenylalanine	1.01	4.72	134.9
Tyrosine	0.68	3.18	90.9
Glycine	1.00	4.60	131.4
Serine	0.90	4.21	120.3
Valine	1.18	5.51	157.4

Source: Alalade and Iyayi (2006).

2002). Amino acid composition of *Azolla pinnata* was found to be in agreement with the FAO standards and requirement of chicks (Dewanji 1993). Amino acid content of the *Azolla* biomass is shown in Table 2. Compared to soybean, the *Azolla* biomass contain high levels of protein and favorable amino acid content (Brouwer *et al.* 2018). The biomass is also rich in lipids (79–160 g/kg) and may contribute to the gross energy of whole *Azolla* feed (Paoletti *et al.* 1987; Bravo, 1998). The fatty acid composition in four different species of *Azolla* evaluated by Bhaskaran and Kannappan (2015) showed ideal levels of various fatty acids. Saturated fatty acids (SAFA) and polyunsaturated fatty acids (PUFA) were found to be the major fatty acids in *Azolla* species (Miranda *et al.* 2018). Dried *Azolla* biomass can also be utilized as feed supplement. Anitha *et al.* (2016) observed that sun dried *Azolla* is rich in crude protein, trace minerals and vitamins and can be used as livestock feed. Increase in the demand for milk and meat have generated considerable increase in animal husbandry and dairying as profitable occupation. However, the feeding cost is a major component in dairy and reduction in the feeding cost makes dairying profitable. There is substantial decrease in the availability of fodder due to shrinking in area of forests and grass lands. The crop residues available for feed is also declining. It is in this context, the aquatic fern *Azolla* without any requirement for arable land and fertilizer input has an added advantage over other crops.

Azolla *as feed supplement for poultry*

Banerjee and Matai (1990) suggested the utilization of *Azolla* as animal feed for better productivity of livestock. The cost of the feed alone accounts for nearly 75% of the total cost of poultry production. Therefore, to reduce the cost of poultry production, unconventional and easy to produce feed stuff may be used to enhance the profit. Singh and Subudhi (1978a) observed that fresh *Azolla* biomass was preferred by the birds as compared to dried form. Incorporation of fresh *Azolla* biomass resulted in the replacement of about 20–25% of the commercial feed (Subudhi and Singh, 1978b). Further, the incorporation of *Azolla* meal in broiler ration resulted in improvement of live weight, production number, and protein efficiency (Basak *et al.* 2002). Experiments conducted by Shamna *et al.* (2013) on Japanese quails resulted in increase in body weight and feed conversion efficiency due to incorporation of *Azolla*. In poultry fed on *Azolla*, significant increase in the body weights and consequent increase in the net return was observed (Rai *et al.* 2012). Studies conducted by Saikia *et al.* (2014) observed that replacement of the conventional feed with *Azolla* resulted in significant body weight gain, feed consumption, and feed efficiency as compared to control group without *Azolla*. Growth and body weight values of broilers fed with *Azolla* was fund to be similar to those birds fed on mustard oil cake meal (Ashraf *et al.* 2015). *Azolla* in fresh form is an excellent poultry feed with no side effects (Singh and Subudhi, 1978a).

Azolla *as feed supplement for fish*

Feeding experiments conducted earlier using *Azolla* did not show encouraging results (Antoine *et al.* 1986; El-Sayed, 2008). However, Basudha and Vishwanath (1997) observed that integration of *Azolla* powder in the fish meal enhanced the weight of the carp *Osteobrama belangeri* and resulted in better feed conversion efficiency and protein efficiency ratio. *Azolla* was used as protein supplement for the fish *Tilapia mossambica* leading to increase in feeding, absorption and growth rate (Sithara and Kamalaveni, 2008). *Azolla* meal was found to partially substitute for fish meal and significantly increased the growth of *Labeo rohita* (Maity and Patra, 2008). Abou *et al.* (2012) observed that up to 30% of fish meal based diet fed to Nile tilapia could be successfully replaced with dried *Azolla* meal. It was observed that *Labeo fimbriatus* fed with feed containing dried *Azolla* (40%) was effective in reducing the feeding cost by 25% without compromising the yield (Gangadhar *et al.* 2015). Specific growth rate of *Labeo rohita* was found to increase when the fish feed containing up to 28.3% crude protein was replaced with 40% *Azolla* (Panigrahi *et al.* 2014). Kumari *et al.* (2017) reported better growth performance of Rohu fingerlings when fed on *Azolla* supplemented diet. Supplementation of *Azolla* resulted in positive effect on the growth performance of fish and reduced the cost of feeding (Mosha, 2018).

A culture system consisting of rice-*Azolla*-fish was found to be successful in Fujian, China in increasing freshwater fish yield (Lu and Li 2006). Apart from enhancing the fish yield, this system was effective in eradicating weeds and harmful insects, loosening soils, increasing dissolved oxygen, and improving the fertility of paddy fields. This practice is low cost, effective and resulted in better economic returns besides acting as an additional source of food and income in rural areas (Kangmin 1988). Das *et al.* (2018) examined the effect of fresh *A. pinnata* as substitution of commercial fish feed (CFF) for Thai silver barb *Barbonymus gonionotus.* Although, complete replacement

of the commercial fish feed with *Azolla* yielded negative results, substituting the commercial fish feed with fresh *A. pinnata* (25%) resulted in beneficial effects and a higher profit margin. Rice–fish–*Azolla* integration however, showed increase in fish yield (Cagauan and Nerona 1986, Shanmugasundaram and Balusamy 1993). This has also resulted decrease in need for inorganic fertilizers and pesticides, besides improvement in soil fertility. Therefore, the supplementation of *Azolla* shows positive effect on the growth performance of fish and reduced the cost of feeding. These studies also showed that, excess amount of *Azolla* incorporated in the fish diet will decrease growth performance and food conversion efficiency.

Azolla *as feed supplement for livestock*

Azolla is being promoted in India for dairy production since long (Pillai *et al.* 2002). *Azolla* meal is a potential and unconventional source of protein for buffalo calves (Indira *et al.* 2009). Cherryl *et al.* (2014) realized the importance of *Azolla* as pig feed as the results were found to be at par with the expensive soybean-based meal. Increase in the milk yield due to feeding of *Azolla* has been reported in buffaloes (Mathur *et al.* 2013; Singh *et al.* 2017). Kololgi *et al.* (2009) observed increase in milk yield by 10% in lactating buffaloes. Ambade *et al.* (2010) observed that incorporation of *Azolla* in the diet of dairy cows increased milk yield by 15 to 20%. *Azolla* meal (in dried form) was found to be ideal for growing Osmanabadi goats at the rate of 15% of total concentrate requirement (Ghodake *et al.* 2012). Chatterjee *et al.* (2013) observed that *Azolla* resulted in improvement of productivity in the crossbred cattle in terms of growth, milk, meat etc. Feeding fresh green *Azolla* with cotton seed cake enhanced the milk production in buffaloes and the animal showed excellent signs of health (Meena *et al.* 2017). Several farmers have realized the potential of *Azolla* and have initiated feeding their livestock with *Azolla*. *Azolla* as livestock feed has resulted in appreciable increase in milk production and meat by weight in milch animals and goats (Kumar and Chander, 2017). Feeding of *Azolla* to milch animals resulted in increase in milk yield and improved the mean returns from single cow (Roy *et al.* 2018).

Future perspectives

The feed costs accounts around 60% of total recurring cost. Shortage of fodder resources due to decreasing land area enhanced greater dependence on commercial cattle feed, resulting in increased costs of milk production. Further, the low quality and adulterated cattle feed has also resulted in reduced longevity of the livestock. All these have necessitated the use of alternative and cheap sources of cattle feed to make the maintenance of livestock production viable. *Azolla* is a potential candidate to be exploited as feed supplement for cattle, poultry and fish owing to its ideal nutritional composition. However, systematic studies have not been carried out to assess the biomass production and the factors influencing the biomass production by different species of *Azolla*. Scientific evaluation of the performance of the birds/animals/fishes must be conducted using appropriate control and treatment group. The effectiveness of fresh versus dried *Azolla* as feed supplement also needs to be evaluated to enhance the utility of the biomass. Value addition of the biomass as feed block/pellet form could be attempted to make the feeding more effective. It is also important to work out the economics of the mass multiplication of *Azolla* so that it could be promoted among the marginal farmers.

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