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

GERARD ABRAHAM1,2, PRANITA JAISWAL1, YUDH VIR SINGH1, RAVINDRA KUMAR YADAV2, RAVINDRA KUMAR3, VISHAL MUDGAL4 and PAWAN KUMAR SINGH5

Centre for Conservation and Utilization of BGA, ICAR-Indian Agricultural Research Institute, New Delhi 110 012 India

Received: 24 January 2020; Accepted: 17 March 2020

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 Kauf. 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 due 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 Iyayi, 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.
**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 mucilage filled cavity (Peters and Mayne, 1974). Generally, *Azolla* requires 25–50% full sunlight for its normal growth. *Azolla* can survive a water pH ranging from 3.5–10, with optimum growth occurring at pH 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/m² 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.

**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 Lyaiy 2006, Letterme 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 B₁₂, and beta-carotene), growth promoter intermediaries and minerals (Pillai et al. 2010). The nutrient and chemical composition of *Azolla* is selected

| 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).
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 (Maiti 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).

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 Shamma 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 (Maiti 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 Barbunymus gonionotus. Although, complete replacement

---

**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 Iyai (2006).*
incorporation of
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). Cherry et al. (2014) realized the importance of Azolla as pig feed as the results 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.

ACKNOWLEDGEMENTS

We acknowledge the financial assistance (IARI: CRSCIRISIL 2014030262) received from the authorities of ICAR-Indian Agricultural Research Institute, New Delhi. Financial assistance from HRDG, Council of Scientific and Industrial Research, New Delhi (No.38 (1319)/12/EMR-II) is also gratefully acknowledged.

REFERENCES

Abdel-Tawwab M. 2008. The preference of the omnivorous macrophagous, Tilapia zillii (Gervais) to consume a natural free floating fern Azolla pinnata. Journal of the World Aquaculture Society 39(1): 104–12.

Abou Y, Saidou A, Mama D, Diogbé E D and Micha J C. 2012. Evaluation of nitrogen and phosphorus wastes produced by Nile Tilapia (Oreochromis niloticus L.) fed Azolla-diets in earthen ponds. Journal of Environmental Protection 3(6): 502.

Abraham G and Aeri V. 2012. A preliminary examination of the phytochemical profile of Azolla microphylla with respect to season. Asia Pacific Journal of Tropical Biomedicine S1392-S1395.

Abraham G, Pandey N, Mishra V, Chaudhary A A, Ahmad A, Singh R and Singh P K. 2013. Development of SCAR based molecular markers for the identification of different species of Azolla Indian Journal of Biotechnology 12: 489–92.

Abraham G, Yadav R K and Kaushik G K. 2015. Identification of potential antimicrobial compounds from the aquatic fern Azolla microphylla. Indian Journal of Experimental Biology 53(4): 232–35.

Alalade O A and Iyaiy E A. 2006. Chemical composition and feeding value of Azolla (Azolla pinnata) meal for egg type chicks. International Journal Poultry Science 5: 137–41.

Ali M A, Sattar M A, Islam M N and Inubushi K. 2014. Integrated effects of organic, inorganic and biological amendments on methane emission, soil quality and rice productivity in irrigated paddy ecosystem of Bangladesh: field study of two consecutive rice growing seasons. Plant Soil 378: 239–52.

Ambade R B, Jadhav S N and Phalke N B. 2010. Impact of Azolla as a protein supplement and its influence on feed utilization in livestock. Livestock line 4(4): 21–23.

Anitha K C, Rajeshwary B Y, Prasanna S B and Shilpa S J. 2016. Nutritive evaluation of Azolla as livestock feed. Journal of Experimental Biology and Agriculture Science 4: 670–74.

Antoine T, Carraro S, Micha J C and Van Hove C. 1986. Comparative appetency for Azolla of Cichlasoma and Oreochromis (Tilapia). Aquaculture 53: 95–99.

Arora A and Singh P K. 2003. Comparison of biomass productivity and nitrogen fixing potential of Azolla spp. Biomass and Bioenergy 24: 175–78.
Ashraf H, Matto F A, Ganai A M, Reshi I M and Sheikh F A. 2015. Effect of replacement of mustard oil cake with Azolla (Azolla pinnata) meal on growth performance of broilers and economics of feeding under temperate conditions. Indian Journal of Animal Nutrition 32(3): 325–28.

Banerjee A and Maiti S. 1990. Composition of Indian aquatic plants in relation to utilization as animal forage. Journal of Aquatic Plant Management 28: 69–73.

Basak B, Pramanik A H, Rahaman M S, Tarafdar S U and Roy B C. 2002. Azolla (Azolla pinnata) as a feed ingredient in broiler ration. International Journal of Poultry Science 1: 29–32.

Basudha C and Vishwanath W. 1997. Formulated feed based on aquatic weed Azolla and fish meal for rearing medium carp Osteobrama belangeri (Valenciennes). Journal of Aquaculture in the Tropics 12(3): 155–64.

Becerra M, Preston T R and Ogle B. 1995. Effect of replacing whole boiled soya beans with Azolla in the diets of growing ducks. Livestock Research for Rural Development 7(3): 32–38.

Bhaskaran S K and Kannapan P. 2015. Nutritional composition of four different species of Azolla. European Journal of Experimental Biology 5(3): 6–12.

Bravo L. 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutrition Reviews 56(11): 317–33.

Brouwer P, Bräutigam A, Buiks V A, Tarelaar A O E, van der Werf A, Schütler U, Reichart G J, Bolger A, Usadel B, Weber A P and Schluepmann H. 2017. Metabolic adaptation, a specialized leaf organ structure and vascular responses to diurnal N₂ fixation by Nostoc azollae sustain the astonishing productivity of Azolla ferns without nitrogen fertilizer. Frontiers in Plant Science 8: 442.

Brouwer P, Schluepmann H, Nierop K G, Eldersron J, Bijl P K, van der Meer I, de Visser W, Gert Jan Reichart, Smeekens S and van der Werf A. 2018. Growing Azolla to produce sustainable protein feed: the effect of differing species and CO₂ concentrations on biomass productivity and chemical composition. Journal of the Science of Food and Agriculture 98(12): 4759–68.

Brouwer P, van der Werf A, Schluepmann H, Reichart G J and Nierop K G. 2016. Lipid yield and composition of Azolla filiculoides and the implications for biodiesel production. BioEnergy Research 9(1): 369–77.

Cagauan A G and Nerona V C. 1986. Tilapia integrated rice-fish farming practises for increased productivity. International Journal of Aquaculture and Fisheries Management 26(12): 317–29.

Calvert H E and Peters G A. 1981. The specialized leaf organ structure and vascular responses to diurnal N₂ fixation by Nostoc azollae sustain the astonishing productivity of Azolla ferns without nitrogen fertilizer. Frontiers in Plant Science 8: 442.

Coughenour M B and Makkar H P. 2012. Conducting national feed assessments (No. 15). Food and Agriculture Organization of the United Nations (FAO). Rome, Italy.

Das M, Rahim F I and Hossain M A. 2018. Evaluation of Fresh Azolla pinnata as a Low-Cost Supplemental Feed for Thai Silver Barb Barbonymus gonionotus. Fishes 3(1): 15

Devendra C and Leng R A. 2011. Feed resources for animals in Asia: issues, strategies for use, intensification and integration for increased productivity. Asian-Australasian Journal of Animal Science 24: 303–21.

Dewanjii A. 1993. Amino acid composition of leaf proteins extracted from some aquatic plants. Journal of Agricultural and Food Chemistry 41(8): 1232–36.

Dhikshit A K and Birlath P S. 2013. Positive environmental externalities of livestock in mixed framing systems of India. Agricultural Economics Research Review 26(1): 21–30.

El-Sayed A F M. 2008. Effects of substituting fish meal with Azolla pinnata in practical diets for fingerling and adult Nile tilapia, Oreochromis niloticus (L.). Aquaculture Research 39: 167–73.

Fasakin E A. 1999. Nutrient quality of leaf protein concentrates produced from water fern (Azolla africana Desv) and duckweed (Spirodela polyrrhiza L. Schleiden). Bioresource Technology 73(2): 185–87.

Gangadhar B, Sridhar N, Saurabh S, Raghavendra C H, Hemaprasanth K P, Raghunath M R and Jayasankar P. 2015. Effect of Azolla-incorporated diets on the growth and survival of Labeo fimbriatus during fry-to-fingerling rearing. Cogent Food and Agriculture 1(1): 1055359.

Ghodake S L S, Fernandes A P, Darade R V and Zagade B G. 2012. Effect of different levels of Azolla meal on growth performance of Osmanabadi kids. Research Journal of Animal Husbandry and Dairy Science 3(1): 13–16.

Gouri M D, Sanganal J S, Gopinath C R and Kalibavi C M. 2012. Importance of Azolla as a sustainable feed for livestock and poultry. Agricultural Reviews 33(2): 93–103.

Hills L V and Gopal B. 1967. Azolla prasinaea and its phylogenetic significance. Canadian Journal of Botany 45(8): 1799–91.

Indira D, Sarjan R K, Suresh J, Venugopal N K and Ravi A. 2009. Azolla (Azolla pinnata) as feed supplement in Buffalo calves on Growth performance. Indian Journal of Animal Nutrition 26(4): 345–48.

Joshi L N, Rana A and Shivay Y S. 2012. Evaluating the potential of rhizo-cyanobacteria as inoculants for rice and wheat. Journal of Agricultural Technology 8(1): 157–71.

Kangmin L. 1988. Rice-fish culture in China: A review. Aquaculture 71(3): 173–86.

Kololgi S D, Hosamani S V, Malshet Karuna and Nagaraj M S. 2009. Azolla an organic feed supplement feed for livestock. National symposium on Organic Livestock Farming-Global issue, trends and challenges, 26–28 Feb, Kolkata, pp. 35.

Konar R N and Kapoor R K. 1972. Anatomical studies on Azolla pinnata. Phytomorphology 22: 211–23.

Kumar S and Chander H. 2017. A Study on the Potential of Azolla pinnata as Livestock Feed Supplement for Climate Change Adaptation and Mitigation. Asian Journal of Advanced Basic Sciences 5(2): 65–68.

Kumari R, Ojha M L, Saini V P and Sharma S K. 2017. Effect of Azolla supplementation on growth of rohu (Labeo rohita) fingerlings. Journal of Entomology and Zoology Studies 5(4): 
1116–19.
Lejeune A, Penga J, Le Boulenge E, Larondellec Y and Van Hove C. 2000. Carotene content of Azolla and its variations during drying and storage treatments. *Animal Feed Science and Technology* 84(3–4): 295–301.
Leterme F, Longoño A M, Ordóñez D C, Rosales A, Estrada F, Bindelle J and Buldgen A. 2010. Nutritional value and intake of aquatic ferns (Azolla filiculoides Lam. and Salvinia molesta Mitchell.) in sows. *Animal Feed Science and Technology* 155(1): 55–64.
Lu J and Li X. 2006. Review of rice–fish-farming systems in China-one of the globally important ingenious agricultural heritage systems (GIAHS). *Aquaculture* 260: 106–13.
Lumpkin T A and Plucknett D L. 1982. Azolla as a green manure; use and management in crop production. *West view Press Boulder Colorado* USA 15: pp. 230.
Mainty J and Patra B C. 2008. Effect of replacement of fishmeal by Azolla leaf meal on growth, food utilization, pancreatic protease activity and RNA/DNA ratio in the fingerlings of *Labeo rohita* (Ham). *Canadian Journal Pure and Applied Sciences* 2(2): 323–33.
Mathur G N, Sharma R and Choudhary P C. 2013. Use of Azolla (*Azolla pinnata*) as Cattle Feed Supplement. *Journal of Krishi Vigyan* 21(1): 73–75.
Meena G S, Dhaka B L, Singh B, Meena R K and Meena K C. 2017. Effect of *Azolla* as Feed Supplement on Milk Yield in Buffaloes. *International Journal of Current Microbiology and Applied Sciences* 6(12): 3490–94.
Miranda A F, Biswas B, Ramkumar N, Singh R, Kumar J, James A, Roddick F, Lal B, Subudhi S, Bhaskar T and Mouradov A. 2016. Aquatic plant Azolla as the universal feedstock for biofuel production. *Biotechnology for Biofuels* 9.
Miranda A F, Liub Z, Rochfort S and Mouradov A. 2018. Lipid production in aquatic plant *Azolla* at vegetative and reproductive stages and in response to abiotic stress. *Plant Physiology and Biochemistry* 124: 117–25.
Mosha S S. 2018. A review on significance of *Azolla* meal as a protein plant source in finfish culture. *Journal of Aquaculture Research and Development* 9: 544.
Muradov N, Taha M, Miranda A F, Kadali K, Gujar A, Rochfort S, Stevenson T, Ball A S and Mouradov A. 2014. Dual application of duck weed and *Azolla* plants for waste water treatment and renewable fuels and petrochemical production. *Biotechnology for Biofuels* 7(30): 1–17.
Nayak S K and Singh P K. 1989. Cytological studies in the genus *Azolla*. *Cytologia* 54: 275–86.
Panigrahi S, Choudhury D, Sahoo J K, Das S S and Rath R K. 2014. Effect of dietary supplementation of *Azolla* on growth and survivability of *Labeo rohita* fingerlings. *Asian Journal of Animal Sciences* 9: 33–37.
Paoletti C, Bocci F, Lercker G, Capella P and Materassi R. 1987. Lipid composition of *Azolla caroliniana* biomass and its seasonal variation. *Phytochemistry* 26: 1045–47.
Parasharumulu S, Swain P S and Nagalakshmi D. 2013. Protein fractionation and *in vitro* digestibility of *Azolla* in ruminants. *Online Journal of Animal and Feed Research* 3 (3): 129–32.
Pereira AL, Asapagor and Vasconcelos V. 2014. Classification and phylogeny of the cyanobiont *Anabaena azollae* Strasburger: an answered question? *International Journal of Systematic and Evolutionary Microbiology* 64(6): 1830–40.
Pereira A L, Martins M, Olivia M M and Carrapico F. 2011. Morphological and genetic diversity of the family Azollaceae inferred from vegetative characters and RAPD markers. *Plant Systematics Evolution* 297(3–4): 213–26. Petersburg A and Mayne B C. 1974. The *Azolla-Anabaena azollae* relationship I. Initial characterization of the association. *Plant Physiology* 53: 813–19.
Pillai P K, Premalatha S and Rajamony S. 2002. *Azolla*—A sustainable feed substitute for livestock. *Leisa Magazine India* 3: 15–17.
Prabha B J and Kumar K. 2010. Dried *Azolla* as a nutritionally rich cost effective and immuno-modulatory feed supplement for broilers. *Asian Journal of Animal Sciences* 5(1): 20–22.
Rai B R, Dhama K, Damodaran T, Hamid A, Rai, Singh B and Bhatt P. 2012. Evaluation of *Azolla* (*Azolla pinnata*) as a poultry feed and its role in poverty alleviation among landless people in northern plains of India. *Veterinary Practitioner* 13(2): 250–54.
Roy P S, Roy A, Pathak P K, Mandal N, Soren S and Kumar S. 2018. Effect of *Azolla* supplementation on milk yield and economics under farmers’ field. *Journal of Crop and Weed* 14(2): 77–80.
Sakia N, Sapcota D and Hazarika R. 2014. Effect of feeding *Azolla* (*Azolla pinnata*) meal to broilers: A field study in Assam. *Indian Journal of Poultry Science* 49 (1): 113–14.
Sangina N and Van Hove C. 1989. Amino Acid Composition of *Azolla* as affected by strains and population density. *Plant and Soil* 117: 263–267.
Shamma T F, Peethambaran P A, Jalaludeen A, Leo J and Muhammad A M K. 2013. Broiler characteristics of Japanese quails (*Coturnix coturnix japonica*) at different levels of diet substitution with *Azolla pinnata*. *Animal Science Reporter* 7(2): 75–80.
Shamugasundaram V S and Balusamy M. 1993. Rice-Fish-Azolla: a sustainable farming system. *NAGA*, The ICLARM Quarterly 16(2–3): 23.
Shao L S, Wang J H, Yan J B, Pang X, Wang R G, Liang H Y and Cheng Y G. 2011. Research and application of integrated cultivation technology for pollution free cucumber production with biogas dregs [J]. *Journal of Shansi Agricultural Sciences* 10: 012.
Singh B, Meena G S, Meena K C, Meena R K, Singh B and Indoria D. 2017. Effect of a Wonder herb *Azolla* on Buffaloes Milk Yield. *International Journal of Current Microbiology and Applied Sciences* 6(11): 1059–66.
Singh P K and Subudhi B P R. 1978a. Utilization of *Azolla* in poultry feed. *Indian Farming* 27: 37–39.
Singh P K. 1989. Use of *Azolla* in Asian Agriculture. *Applied Agricultural Research* 4(3): 149–61.
Sithara K and Kamalaveni K. 2008. Formulation of low-cost feed using *Azolla* as a protein supplement and its influence on feed utilization in fishes. *Current Biotica* 2(2): 212–19.
Subudhi B P R and Singh P K. 1978b. Nutritive value of water fern *Azolla pinnata* for chicks. *Poultry Science* 57(2): 378–80.
Van Hove C, Bailonville T W, Diara H F, Godard P, Kodomi Y M and Sangima N. 1987. *Azolla* collection and selection. Workshop on *Azolla* Use International Rice Research Institute, Fuzhou, Fujian, China, pp. 77–87.
Wagner G M. 1997. *Azolla*: A review of its biology and utilization. *The Botanical Review* 63(1): 1–26.
Yadav R K, Abraham G, Singh Y V and Singh P K. 2014. Advancements in the Utilization of *Azolla-Anabaena* system in Relation to Sustainable Agricultural Practices. *Proceedings of Indian National Science Academy* 80(2): 301–16.
Zheng W, Bergman B, Chen B, Zheng S, Xiang G and Rasmussen U. 2009. Cellular responses in the cyanobacterial symbiont during its vertical transfer between plant generations in the *Azolla microphylla* symbiosis. *New Physiologist* 181(1): 53–61.