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Nutritive Value of Azolla as Poultry Feed-A Review

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ABSTRACT

Azolla has great prospective as poultry feed due to its high content of proteins, essential amino acids, vitamins (vitamin A, vitamin B_{12}, â-Carotene), growth promoter intermediaries and minerals. Azolla contains (on DM basis) 20.4-28.5 % crude protein, 2.37-6.70 % ether extract,12.6-17.5 % crude fibre, 15.7-19.9 % total ash, 30.0-47.0% nitrogen free extract,48.2-54.8 % neutral detergent fibre, 36.5-37.1 % acid detergent fibre,0.80-2.22% calcium and 0.35-1.39% total phosphorous. It contains 9.10-26.2 ppm copper, 83.9-2418 ppm manganese, 30.0-225 ppm zinc, 283-1569 ppm iron and 206-632 ppm carotene. The total flavonoid and total phenolic contents (µg/ml) in methanolic extract are 56.2 and 87.3, respectively. It has good potential for imparting rich colour to egg yolk due to presence of â-carotene pigment. It has higher potential to proliferate without inorganic nitrogen fertilization and high rate of growth in water. Azolla on meal basis can be included in broiler ration and pullet chick ration up to a level of 5 and 10%, respectively without any adverse effect on their performance. Fresh azolla at a rate 200g/duck/day in White Pekin duck feeding was economical. Higher levels of inclusion of azolla limit its beneficial effect due to decrease in feed consumption owing to higher level of fibre comprising mostly of ADF and lignin in azolla. Azolla production through low cost technology can be popularised and supplemented in the diets chicken and ducks either in fresh or dried form for economic production of meat and egg.

Key words: Azolla, Duck, Feed, Poultry,Review

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INTRODUCTION

India ranks 3rd in poultry egg production and 5th in poultry meat production (Singh, 2022). Poultry industry plays an important role in agricultural sector which provides wholesome meats and eggs for utilization in human beings. However, supply of quality feed at a rational price can make the poultry production more remunerative (Basak et al., 2002). The per capita availability of egg is about 74 and meat is 2.8 kg against annual requirement of eggs and meat of 180 eggs and 11 kg poultry meat, respectively in the year 2018 recommended by Indian Council of Medical Research (ICMR)(Kumar and Pandey, 2019); and thus, there is a wide gap between the demand and supply. The main constraints in the production of poultry is the exorbitant cost of feed ingredients, particularly of maize and soybean meal due to the competition between human beings and livestock for these commodities. Hence, alternatives have to be thought to reduce the use of these conventional feed ingredients to minimise the competition with human beings and to optimise feed cost to make the poultry products competitive in the market. Food and Agricultural Organization also focuses on expanding the feed base through increased use of locally available feed resources in developing countries (Sansoucy, 1993).

The green plants are the most prospective and cheapest protein sources due to their ability of amino acids synthesis (Fasuyi and Aletor, 2005).Further, aquatic plant species are better protein sources than tree leaves as they do not appear to accumulate secondary plant metabolites (Bacerra et al., 1995). Among the various aquatic plant species, azolla

(*Azolla pinnata*) provides a sustainable feed for poultry.

TAXONOMY AND MORPHOLOGY

The name *Azolla* is imitative from two greek words, Azo (to dry) and Ollyo (to kill), which means it is killed without water. It is otherwise known as mosquito fern, duckweed, fairy moss or water fern (Lumpkin and Plucknett, 1982). *Azolla* (Lumpkin and Plucknett, 1982) belongs to Kingdom: Plantae, Division: Pteridophyta, Class: Pteridopsida, Order: Salviniales, Family: *Azollaceae,* Genus: *Azolla.* The genus azolla consist of two subgenera and six living species. Subgenus Euazolla include four species: *Azolla filiculoides, Azolla caroliniana, Azolla microphylla, Azolla mexicana*. The Subgenus Rizosperma include two species: *Azolla pinnata* and *Azolla nilotica. Azolla* consists of a short, branched, floating stem, bearing roots which drooped in the water (DeFrank, 1995). The leaves are arranged alternately and each consists of a thick aerial dorsal lobe possessing green chlorophyll and a ventral lobe which is thin, colourless and slightly larger in size. Sometimes, fern is reddish-brown colour due to presence of an anthocyanin pigment. The plant diameter varies from 1-2.5 cm (*Azolla pinnata)* to 15 cm or more (*Azolla nilotica)*. Azolla are triangular or polygonal in shape, and hang on the water surface either individually or in the form of mats. The most important characteristic of azolla is its symbiotic relationship with the nitrogen-fixing blue-green algae (cyanobacterium) *Anabaena azollae*. Azolla supplies nutrients and a protective cavity in each leaf to *Anabaena* colonies in lieu of fixed atmospheric nitrogen and perhaps other growth-promoting substances (Lumpkin and Plucknett, 1980).

DISTRIBUTION

Natural sources of water which favours of growth of Azolla are still water in drains ponds, rivers, canals, rice fields, water bodies and soggy lands in humid regions with temperature range of 15-35^{0}C. Every species of azolla has a distinctive geographic range of distribution; *viz* eastern North America and the Caribbean hold the native soil for *Azolla caroliniana* ; *Azolla filiculoides* are found in southern South America through western North America including Alaska; *Azolla microphylla* are native to tropical and subtropical America; *Azolla Mexicana* belongs to northern South America through western North America; *Azolla nilotica* found in upper reaches of the Nile to Sudan; The native soils for *Azolla pinnata* are found in most parts of Asia and the coastal region of tropical Africa (Lumpkin and Plucknett, 1980). In early 1960s, the use of azolla was promoted in China and Vietnam ensuing in its fast growth in these countries. It has been identified as a useful plant in southern China and northern Vietnam and exploited for use as a biofertilizer and green manure in enriching rice crop by virtue of its N-fixing abilities (Van Hove and Lejeune, 1996). However, in 1980s, azolla production was decreased, due to constraints like water unavailability, problems in safeguarding and handling, high labour needs and inadequate familiarity on the precise needs of each azolla species (Van Hove and Lejeune, 1996). The perception of azolla was not always positive as it was often considered as harmful weed by the farmers (Lumpkin and Plucknett, 1980). Azolla have important qualities *viz.* the ability to fix atmospheric nitrogen; high productivity in ample environments; high protein content; malherbicide effect and the ability to reduce N-fertilizer volatilization. These beneficial reasons enables azolla to attract interest again in the late 1990s, particularly as a constituent of integrated farming systems such as rice-fish-azolla, rice-duck-azolla, rice-duck-fish-azolla or pig-fish-azolla (Van Hove and Lejeune, 1996).

POTENTIAL OF AZOLLA AS LIVESTOCK FEED

Investigation and support on azolla production as a livestock feed has been increasing due to its higher protein content (19-30%) and essential amino acid composition for animal nutrition (particularly lysine) (Hasan and Chakrabarti, 2009). Azolla has huge prospective as a livestock feed because it contains high amount of proteins, essential amino acids, vitamins (vitamin A, vitamin B_{12}, â-carotene), growth promoters and minerals. It has ability to

grow without inorganic nitrogen fertilization with a faster growth rate in water without the need to relocate existing crops or natural environmental systems. Azolla has been exploited as a potential feed resource for pigs, ducks, chickens, cattle, fish, sheep and goats and rabbits throughout Asia and part of Africa.

Because of its easiness of farming, yield and nutritive value (Lumpkin and Plucknett, 1982; Van Hove and Lopez, 1983); the use of Azolla as a feed resource for fish, swine and poultry had been experienced with positive results (Castillo et al., 1981; Maurice et al., 1984; Becerra et al., 1995; Basak et al., 2002; Alalade and Iyayi, 2006; Sujatha et al., 2013). Becerra et al. (1995), Lumpkin and Plucknett (1982) and Van Hove & López (1983) opined that *Azolla* is generally a gifted aquatic plant for livestock feed due to its ease of farming, yield and good nutritive value. The use of Azolla as a feed for fish, swine and poultry was also suggested by Alcantara and Querubin (1985). Studies conducted by Dao and Tran (1979) suggested that one hectare of Azolla can generate 540-720 kg of protein per month. Cultivation of Azolla and its feeding to poultry can reduce feed cost, mortality rate and off smell and housefly menace in poultry shed (Mahantesh et al., 2018).

FACTORS AFFECTING THE GROWTH OF AZOLLA

The important factors that affect the growth of azolla are water, salinity (pH), temperature, sunlight, humidity and phosphorus fertilizer. Water is the primary necessity for the growth and development of Azolla. Azolla prefer a free-floating state even though it can grow on wet mud surfaces. The pH range of Azolla is 3.5-10; however, it has most favourable growth in the p^H range of 4.5-7. The salinity tolerance of Azolla depends on the species. The growth rate of *Azolla pinnata* declined at a salinity level of above 380 mg/l. The most salt-tolerant species was *Azolla filiculoides*. Azolla declines to grow and begins to die at temperatures less than 5°C and beyond 45°C. The most advantageous temperature for growth and nitrogen fixation of azolla depends on the species; and is typically in the range of 20-30°C. on the other hand, *Azolla mexicana* is tolerant of temperatures more than 30°C. *Azolla filiculoides* could survive at temperatures as low as -5°C. During phosphorous deficient field condition, azolla may look reddish due to changes in the photosynthesis process. Azolla shows optimum growth at 25-50% sunlight and 85-90% relative humidity. At relative humidity of less than 60 %, Azolla becomes dry and fragile (Hasan and Chakrabarti, 2009; Lumpkin and Plucknett, 1980). Application of phosphorous fertilizer (0.5 to 1.0 kg P/ha/week) optimises azolla production (Lumpkin and Plucknett, 1985).

PRODUCTION AND MANAGEMENT OF AZOLLA

The production and management of Azolla is well described (Anonymous, 2014-15 and 2015-16). A water body is made under shade using a silpauline sheet. Silpauline is a polythene tarpaulin which is resistant to ultraviolet radiation. A pit size of 2m x 2m x 0.2m is dug and plastic gunny bags are spread over it to avoid the roots of nearby trees damaging the silpauline sheet. The silpauline sheet can be extended over the gunnies. An area under shade of tree with ample sunlight should be selected for the azolla production. The production pit can be covered with a green net or plastic net to prevent falling of tree leaves in the pit. Care should be taken to maintain uniform water level throughout the pit. Approximately 10 kgs of sieved fertile soil is homogeneously spread covering the entire area of the silpauline sheet. A mixture is made from 2kg 1-2 days old cow dung and 30 gms of super phosphate dissolved in 10 litres of water and poured over the sheet. The water level is maintained at 10 cm level by adding extra water to the pit. Nearly 500 gms of unadulterated culture of azolla was inoculated in to the pit. In this way azolla will grow fast to fill up the pit within a period of 10-15 days. Afterwards, about 500-600gms of azolla can be harvested daily. Azolla biomass @ 300-350 gm per square metre can be removed daily to evade overloading and for keeping the fern at faster growing phase. A mixture

of 1kg of cow dung and 20 gms of superphosphate should be added once in a week to keep the azolla in speedy growth phase. The mineral content of azolla can be increased by adding micro nutrient mixture periodically. Appropriate nutrients should be added to the pit as and when, nutrient deficiency is noticed. Approximately 5 kg of used soil in the pit should be replaced with fresh soil once in 30 days to prevent accumulation of excess nitrogen and to avoid paucity of micro nutrient. Replacement of 25-30 percent of water with fresh water is essential within an interval of 10 days to avoid accumulation of nitrogen in the bed. After every six months, soil and water should be replaced with inoculation of azolla afresh. If there is contamination of the azolla pit due to pest and diseases, a fresh bed can be prepared with inoculation of wholesome culture of azolla. In this method of production, the cost of production of azolla is about 65 paise/ kg. About 750 tonnes of azolla can be harvested from one acre area of production. Azolla is a highly prolific in nature. The biomass of azolla can be doubled in 3-10 days depending on situation and yield of up to 8-10 t /ha is recorded in rice fields of Asia region. In India 37.8 t fresh weight/ha (2.78 t DM/ha dry weight) has been reported for *Azolla pinnata* (Hasan and Chakrabarti, 2009).

NUTRITIVE VALUE OF AZOLLA

Azolla is rich in proteins, essential amino acids, vitamins (vitamin A, vitamin B_{12}, â Carotene), growth promoter mediators and minerals including calcium, phosphorous, magnesium, potassium, iron and copper. Azolla contains 4.70-6.60% dry matter; 20.4-28.5% crude protein; 11.2-15.9% crude fiber; 2.37-4.60 % ether extract; 12.3-19.9% total ash; 30.0-47.0% nitrogen free extract; 0.80-2.22 % calcium; and 0.35-1.29 % total phosphorous (Bacerra et al., 1995; Basak et al., 2002; Parthasarathy et al., 2002; Alalade and Iyayi, 2006;Indira et al., 2009; Sujatha et al., 2013; Rathod et al.,2013;Saikia et al., 2014; Acharya et al., 2015; Ashraf et al., 2015;Kavya et al., 2015; Anitha et al., 2016; Swain et al., 2018 and Shukla et al., 2018).

Besides *Azolla* is rich in iron (283–1569 ppm dry weight), copper (7.33–16.7 ppm dry weight) manganese (83.9–2418 ppm dry weight), zinc (46.7-325 ppm dry weight) and carotenes (206-632 ppm dry weight) (Bacerra et al.,1995; Lejune et al., 2000; Alalade and Iyayi, 2006;Mandal et al.,2012; Sujatha et al., 2013; Acharya et al., 2015;Kathirvelan et al., 2015 and Anitha et al., 2016) . A study on phytochemicals present in *Azolla pinnata* conducted by Mithraja et al.(2011) revealed that flavonoid is present in aqueous extract of *A.pinnata* and phenol is present in all extracts i.e. aquesous, acetone, benzene, chloroform and ethanol. Tannins were present in aqueous, benzene and ethanol extract and saponins were present in benzene and chloroform extracts.

Table 1. Chemical Composition of Azolla on DM basis

Sr. No.	Attributes	Range Values (%)
1	Crude protein	20.4-28.5
2	Ether extract	2.37-6.70
3.	Crude fibre	12.6-17.5
4.	Total ash	15.7-19.9
5.	NFE	30.0-47.0
6.	ADF	36.5-37.1
7.	NDF	48.2-54.8
8.	Calcium	0.80-2.22
9.	Total Phosphorus	0.35-1.29
10.	Copper(ppm)	9.10-26.2
11.	Manganese(ppm)	83.9-2418
12.	Zinc(ppm)	30.0-325
13.	Iron(ppm)	283-1569
14.	Carotene (ppm)	206-632
15.	Total flavonoid content in methanolic extract(µg/ml)	56.2
16.	Total phenolic content in methanolic extract (µg/ml)	87.3

Querubin et al., 1986; Bacerra et al., 1995; Lejune et al., 2000; Thiripurasundari and Padmini, 2018; Srinivas et al., 2012; Mandal et al., 2012; Sujatha et al., 2013; Chatterjee et al., 2013; Rathod et al.,2013;Saikia et al., 2014; Acharya et al., 2015;

Ashraf et al., 2015; Swain et al., 2015; Gowda et al., 2015; Anitha et al.,2016; Yadav and Chhipa,2016; Rana et al., 2017; Gupta et al., 2018; Swain et al.,2018; Shukla et al., 2018

USE OF AZOLLA AS POULTRY FEED

Fresh azolla is used as feed for poultry, wild fowls, domesticated ducks and domestic fowl in different countries (Dao and Tran, 1966).

Chicken

Subudhi and Singh (1978) concluded that about 20% of commercial feed can be replaced by fresh Azolla in the diet of young chickens. Approximately 9 kg fresh azolla is required for 100 chickens daily, which can be obtained by culturing azolla in a pond measuring 60 m^2 area. The growth of White Leghorn female chicks given the diet with 5% azolla was faster than the control group. However, the growth was slightly slower in those given the diet with 12.5% dried azolla. On the other hand, significant reduction in growth rate was observed when azolla was included in the diet at a level of 16 % (Singh and Subudhi, 1978).

Maize and soybean meal can be replaced by 10% dried azolla on an equal digestible protein basis in the diet of 14 day old broilers (Ali and Lesson, 1995). Depressed growth and protein efficiency ratio was observed with no effect on survivability at 10-20 % level of inclusion of azolla (Parthasarathy et al., 2002). Improvement in live weight, feed conversion ratio, protein and energy efficiency, dressing percentage and profitability was reported in broilers (7-42days) fed diet with 5 % azolla (Basak et al. 2002). Formulation of chick's diet with addition of azolla meal (AZM) up to 10% resulted in best performance in terms of Feed conversion ratio (FCR) and efficiency of feed utilization (Alalade and Iyayi, 2006). An experiment was conducted in Nera brown pullets (2 weeks) with 0 to 15% dried azolla in the diet and it was concluded that Azolla could be included in the diet up to 10% for best performance (Alalade and Iyayi, 2006). Namra et al. (2010) reported that when commercial diet was offered to broilers from 2-16 weeks at 15-45%, supplemented with fresh azolla given *ad libitum* led to reduction in performance and slaughtering parameters and the group fed fresh azolla *ad libitum* supplementing the diet restricted at 45% exhibited optimal economical efficiency. The performance of broilers was maintained up to a level of inclusion of 7.5% dried azolla in their diet (Prabina and Kumar, 2010). Earlier workers (Balaji et al., 2009; Balaji et al., 2010) reported that addition of 4.5% azolla in the diet of broilers resulted in decreased cholesterol content in serum and meat without any undesirable effect on Production performance. Inclusion of dried azolla up to 5 % by replacing soybean meal had no adverse effect on palatability, efficiency of feed utilization and carcass quality of broilers (6 week of age) and was profitable (Dhumal et al., 2009). A trial conducted by Naghshi et al. (2014) in Cobb broilers (1-42d) using 0-15% azolla in powder form in corn-soybean meal diet revealed that 5 % azolla dietary group had lowest feed intake, highest weight gain and best feed conversion ratio. The carcass efficiency percentage was significantly higher in broilers belonging to azolla dietary group. The group fed 5% azolla had the least feed cost per kg body weight. Supplementation of azolla meal at a level of 5% replacing sesame meal of the diet improved the body weight gain of broilers at 6 week of age (Basak et al., 2002).Inclusion of azolla at a level of 5-10 % by replacing fish meal of the diet improved the performance in terms of increased body weight and net returns per bird(Joysowal et al.,2018).

Inclusion of azolla in the diet of broilers more than 5% depresses nutrient utilization and performance (Parthasarathy et al., 2002; Basak et al., 2002). Safe level of inclusion of azolla is up to 10% in pullet chicks (Alalade et al., 2007). Commercial diet can be replaced by fresh azolla at a level of 20% or more (Subudhi and Singh, 1978; Namra et al., 2010). The dressing %, carcass weight of thigh, breast and wing were not affected by inclusion of 5-10 % azolla meal in the diet of broilers (Abdelatty et al.,2020). However, the redness and juiciness of breast meat significantly increased in broilers fed 5% azolla meal. These workers also

observed that the breast meat cooking loss decreased linearly with inclusion of 5-10 % azolla meal in the diet. The positive effect of azolla meal on meat quality might be due to the stimulation of muscle protein synthesis through activation of mTOR/S6 kinase signalling pathway. Rana et al.(2020) reported that broilers fed 5 % azolla meal had significantly (PÂ0.05) higher overall acceptability of cooked meat, breast meat yield and cooking yield. However, there were no significant differences in proximate composition of broiler meat of control group and the azolla meal fed group. Addition of 30% (w/w) azolla and 70% commercial feed improved the average body weight gain and FCR whereas the mortality and cost of production were reduced compared to those birds fed 100 % (w/w) commercial feed (Mahanthesh et al., 2018). The FCR, performance index,egg weight,egg production, egg shape index and yolk color were improved in laying geese fed azolla-based diets (Riaz et al.,2022).

Inclusion of dried azolla at a level of 15% in the diet of commercial layers in a 16 week study could not affect the performance of the laying hens in terms of egg production (Khatun et al., 2008). Boitai et al. (2018) reported that Vanaraja laying hens fed diets incorporated with 10 % azolla meal during a period of 8 weeks did not show any adverse effect egg production and egg quality. These workers also reported that dietary inclusion of 10% azolla meal had no influence on egg quality indices like albumen,yolk, shell percentage and egg qualities like haugh unit score and shell thickness, Feed consumprion of birds decreases due to the increase in the inclusion level of azolla up to 15 % in the diet of poultry birds which might be due to the reduced palatability Sreemanarayana et al.,1993) and increased bulkiness of azolla (Bacerra et al.,1995) which reduces its utilization.

Ducks

Alejar and Aragones (1989) observed comparable egg production and darker yolk color in Mallard ducks by replacing rice grain-snail-shrimp diet partially (20%). Gavina (1994) concluded form a study that feeding of fresh azolla was detrimental to growth performance in native ducks when fed fresh azolla + 40 or 60% of chicken grower mash fed to them. Fresh azolla can partially replace Whole soybeans can be partially replaced by fresh azolla up to a level of 20% of the total crude protein in diets of meat type ducks based on sugar cane juice with no undesirable effect on growth rate or health with lowest feed cost per kg weight gain and highest net profit per bird. However, at higher level of replacement than the above, poor growth rate and feed conversion efficiency were recorded (Bacerra et al., 1995). Liu Xiang et al. (1998) supplemented fresh azolla to a formulated feed and observed that duckling (2-8 weeks) exhibited higher growth rate with feeding of azolla had compared to the ducks of control group. Sujatha et al. (2013) conducted experiment in indigenous layer ducks and observed comparable performance by replacing 0-20% commercial layer feed with 200g fresh azolla per duck per day with daily saving of approximately Rs. 1/- per duck, increase in Roche fan colour score and yolk colour score (6.0 vs 7.4), which may be due to the enrichment of azolla with â-carotene pigment.

A sequence of feeding experiments were carried out by Escobin (1987) to find out the influence of feeding fresh azolla by partly replacing traditional paddy rice-snail-shrimp (PSS) based diet for laying Mallard and growing Muscovy ducks. It was inferred from the study that there was no significant difference among the various dietary treatments containing 0, 20, 30 and 40% azolla replacing matching levels of PSS, both in Mallard ducks for egg production and Muscovy ducks for meat production in terms of production efficiency. A study conducted by Sujatha et al. (2013) revealed that a backyard duck farm with a capacity of 50 ducks will need about 10 kg of fresh azolla per day (200g fresh azolla/duck) to substitute 30 percent of commercial feed. Azolla cultivation in a shallow pond of dimension 68 m^2 or 17 superficial tanks each of 4 m^2 with 30 cm in deepness will produce sufficient azolla daily to meet the daily requirement of 50 ducks. Approximately Rs50/- can be saved on feed cost daily. These workers also observed that the

Roche fan colour score improved with inclusion of azolla in the diet. The yolk colour score of 6.0 recorded in the eggs of control group ducks increased to 7.4 when azolla was included in the diet. The pigmenting ability of azolla clearly established by its high Roche fan colour score which might be due to the presence of more quantity of â-carotene pigment in azolla. The change in yolk colour due to azolla supplementation of diet has also been demonstrated by Bastian (1987).

Elevated levels of ADF and lignin have been indicated as the major factor restricting the proper utilization of azolla meal (AZM) by monogastric animals (Bukingham et al., 1978 and Tamany et al., 1992). There are contradictory reports regarding the feed consumption in poultry fed on azolla incorporated diets. Feed intake was significantly affected in chicks fed on 10 and 15 % AZM diets (Cambel, 1984; Bhuyan et al., 1998). The feed consumption of broilers was not affected due to supplementation of azolla meal in the diet (Castillo (1983) and Basak et al (2002). However, it was clarified that the decrease in dry matter intake might be due to reduction in intake of bulky AZM based diets by the birds (Bacerra et al. (1995). The feed intake was considerably low in laying ducks (153 g/duck) with azolla supplementation (200g fresh azolla/duck/day) as against control group (219 g/ duck) (Sujatha et al.2013).

CONCLUSIONS

Azolla has been a very popular source of green feed because of it's high nutritional value in terms of protein, essential amino acids, vitamins and minerals; good potential for imparting rich colour to egg yolk due to presence of â-carotene pigment; ease of cultivation and high productivity in spite of land constraint situation faced by the farmer. Dried azolla can be incorporated in the diet of chicken and ducks at a level of 5-10 % in order to improve their performance and to reduce the cost of feed. Azolla production through low cost technology can be popularised and supplemented in the diets chicken and ducks either in fresh or dried form for economic production of meat and egg.

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