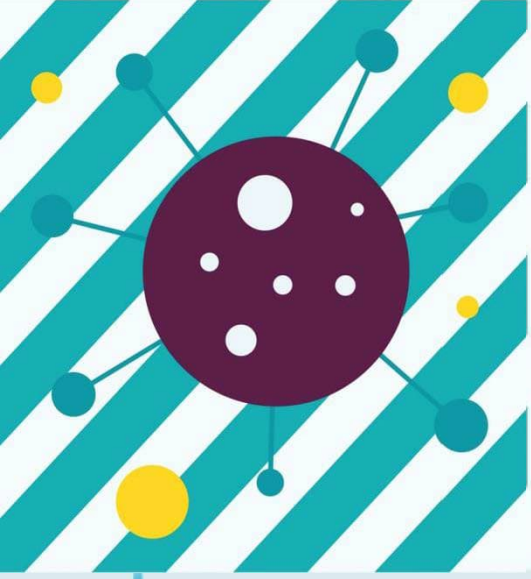

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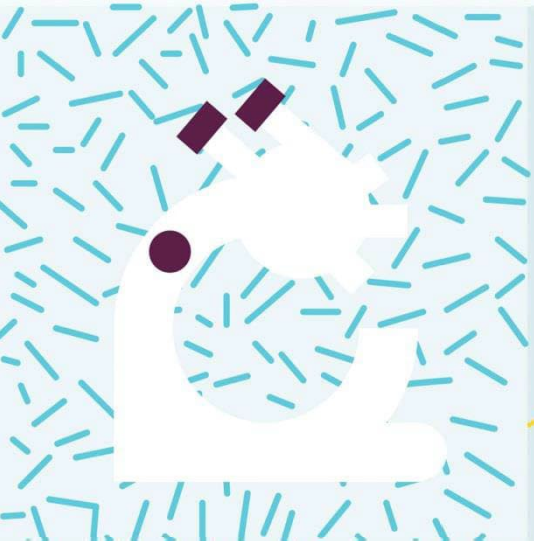

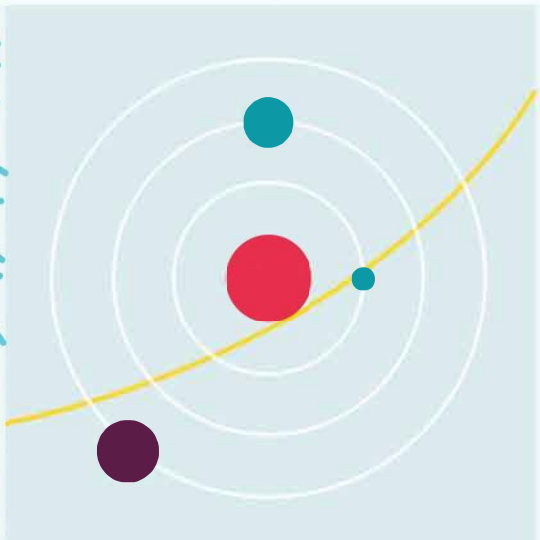

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Requirements For The Cultivation And Multiple Use Of Azolla

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A number of non-conventional feeds have the potential to be incorporated into animal feeds. One such feed is *Azolla*. This fern has been demonstrated to have a significant impact on the behaviour of the animals to which it is administered. It has been demonstrated that this species can enhance feed consumption and digestibility, thereby increasing the bioavailability of nutrients for the animals. Green *Azolla* (*Azolla pinnata*) is an incredible plant that can be used as an alternative to green fodder and as a supplementary protein feed because of its high flavor and increased yield. Its cultivation is considered the most promising due to its ease of cultivation, minimal water use, and high productivity and good nutritional value (Kathirvelan et al., 2015).

1. The Eco-physiology of *Azolla*

As outlined by Rahagarison (2005), *Azolla* necessitates a specific combination of environmental factors for the plant to thrive, develop, and grow. These factors include water, temperature, light and the pH of the water.

1.1. Water Requirements

As posited by Adzman et al. (2022), the optimal growth conditions for *Azolla* are characterized by the presence of a water layer with a depth ranging from 5 to 10 centimetres. This is advantageous for mineral nutrition, as it ensures proximity of the roots to the soil. Water is the fundamental requirement for *Azolla* growth and multiplication (Adzman et al., 2022). It is noteworthy that Azolla exhibits a high degree of susceptibility to water scarcity. It is therefore vital to maintain an optimal water level for its survival (Rajesh, 2020). The fern is unable to thrive in large lakes or turbulent waters, as the wave effect and turbulence of the water impede frond growth, leading to excessive fragmentation (Sabetraftar et al., 2013).

1.2. Humidity levels

As a purely aquatic plant, *Azolla* is unable to withstand humidity levels of less than 60%. The plant is highly susceptible to drought conditions and will perish within a few hours if the soil moisture content is reduced (Becking, 1979). The optimal range for relative humidity is 85-90%, as determined by Rajesh (2020).

1.3. Salinity

As a freshwater plant, *Azolla* can tolerate only a limited degree of salinity. Optimal growth occurs in solutions with a salinity level between 0.05% and 0.1% (Nandabalan and Kannaiyan, 1986). Growth is significantly inhibited at salinity levels above 1.3% (Sallam et al., 2024).

1.4. Temperature

The geographical distribution of the *Azolla* genus suggests that this plant can adapt to a wide range of climatic conditions (Rahagarison, 2005). Its optimal growth temperature ranges between 20 and 30°C, though certain strains exhibit temporary tolerance to extreme temperatures as low as -5°C or as high as 45°C (Rahagarison, 2005). However, some strains are notably sensitive to temperatures below 10°C, while others, like *Azolla pinnata*, can withstand temperatures above 35°C (Adzman et al., 2022). Notably, temperatures exceeding 37°C have been found to significantly hinder fern propagation (Rajesh, 2020).

1.5. Light

Azolla demonstrates optimal growth in conditions of full or partial shade, with a light intensity of between 25 and 50% of full sunlight. However, it has been observed that the growth rate declines precipitously in conditions of heavy shade (less than 1500 lux) and is significantly reduced in environments exceeding 50% of full sun, which impedes photosynthesis (Yáñez et al., 2021).

1.6. pH

Azolla demonstrates remarkable tolerance to fluctuations in environmental pH. It can survive within a broad pH range of 3.5 to 10, with minimal effects on growth. Furthermore, optimal performance and nearly identical growth characteristics are observed within the narrower pH range of 4.5 to 7 (Lumpkin and Plucknett, 1982 ; Da Silva et al., 2022).

1.7. *Azolla's* nutritional requirements

The mineral requirements of *Azolla* include the macronutrients phosphorus, potassium, calcium, and manganese, as well as the micronutrients iron (Fe), molybdenum (Mo), and cobalt (Co) (Becking, 1979; Lumpkin and Plucknett, 1982 ; Vroom et al., 2024). According to Becking (1979), deficiencies in these elements result in reduced growth rates (Table 7).

Table 7. Rate of decline in *Azolla* growth under deficiency conditions (Becking, 1979).

Nitrogen deficiency (N_2)							
Elements	Reference elements	P	K	Ca	Mg	Mn	Fe
Growth reduction rate	100% fresh weight - Minimum growth	22%	30%	5%	82%	23%	11%

2. Azolla cultivation process

According to Dhaker et al. (2021), the establishment of an *Azolla* fodder plot does not necessitate any particular expertise. Indeed, it is a relatively straight forward process that can be undertaken by farmers themselves. The following steps are recommended :

- It is recommended that the sides of the plots be raised to allow for the accumulation of stagnant water. In the event that the plot is situated in a backyard, it is necessary to level the area and cover it with bricks.
- An alternative approach involves installing the fodder plot in a pit approximately 0.2 meters deep, lined with a polyethylene sheet to retain a standing water depth of 10 cm. The bed width is maintained at 1.5 meters to facilitate access and management from both sides. The length of the plot can be adjusted according to specific fodder requirements. For instance, to meet 50% of the green fodder needs of two cows, two cultivation units—each measuring 2.5 meters in length and covering an area of approximately 8 m^2—are considered sufficient.
- Once the cultivation bed measuring 2.5 m × 1.5 m is prepared, approximately 15 kg of finely sieved soil is spread over the polyethylene lining to provide essential nutrients for *Azolla* growth. Around 5 kg of pre-decomposed cow dung (fermented for two days) is then mixed into the water, serving as a carbon source for the plant. A nutrient mixture—comprising 10 kg of rock phosphate, 1.5 kg of magnesium salt, and 500 g of muriate of potash—is subsequently added to the bed. This solution is further enriched with micronutrients in appropriate concentrations, ensuring that the nutritional requirements of the fern are met. As a result, the cultivated *Azolla* also provides enhanced nutritional value when used as livestock feed.
- The requisite volume of water is added to achieve a water level in the bed of 10 cm.
- The soil is then distributed uniformly across the base of the reservoir. The depth of the layer should be approximately 10 centimetres. The quantity of cow dung added is 1 to 1.5 kg per square metre of the tank surface area (equivalent to 2 to 3 kg of cow dung per tank).
- It is recommended that simple superphosphate (SSP) be added at a rate of 5 g per square metre of tank surface each week. (Ten grams of SSP per tank). The water tank should be filled to a level that allows for a 10 to 15 cm accumulation of water above the ground.
- Subsequent to this, the soil particles should then be permitted to settle. The fresh *Azolla* inoculum should be prepared by adding 2 g of carbofuran, which will prevent infestation by parasites. The layer of foam and scum that forms on the surface of the water should be removed, as this hinders the growth and penetration of Azolla roots. The tank should then be left to stand overnight.

On the following day, approximately 200 g of fresh *Azolla* inoculum should be spread on the surface of the water.

- It is estimated that the *Azolla* takes approximately two weeks to form a mat on the surface of the water. It is imperative to ensure that the water level in the tank remains constant, matching that of the reservoir, especially during the summer months.

The initial phase of the process is characterised by the rapid proliferation of *Azolla* across the entire surface of the bed, resulting in the formation of a dense mat within a period of seven days

(Fig. 1). The optimal result is the production of 10 kg of *Azolla* in seven days. It is imperative to maintain the water level throughout this period, which can be achieved by adding water each day.

Following a seven-day period, the *Azolla* can be harvested at a rate of 1.5 kg per day, and it is recommended that harvesting is conducted in plastic

Figure 1. *Azolla* spread over the surface of a pond (Tran, 2015).

- It is imperative that *Azolla* harvested for use in livestock husbandry be thoroughly rinsed with fresh water prior to provision. It is essential to subject the material to a washing process in order to eliminate the unpleasant odour associated with cow dung. The washings of *Azolla* can be utilised as a form of bio-manure for plants cultivated in close proximity.
- The addition of cow dung, mineral mix, soil and water is recommended at a frequency of once every seven days.
- It is recommended that the soil be removed from the bed every 60 days and 15 kg of fresh, fertile soil be added to prevent nitrogen build-up.

2.1. Precautions to be taken when carrying out the *Azolla* cultivation process

According to Dhaker et al. (2021), the following precautions should be observed during the cultivation of *Azolla*:

- It is of the utmost importance to maintain a pure crop, free from any contamination, in order to ensure a good yield.
- It is essential to harvest *Azolla* on a regular basis in order to prevent overcrowding.

The temperature is a significant factor influencing the growth process. The optimal temperature for optimal growth is approximately 35 degrees Celsius. In regions where temperatures are lower, it is advisable to cover the forage plot with a plastic sheet in order to mitigate the adverse effects of the cold.

Plots situated in areas with an abundance of direct sunlight are preferable, as shady locations tend to result in lower yields.

The pH of the environment should be maintained within the range of 5.5 to 7. The addition of appropriate nutrients, such as cow dung slurry and micronutrients, may be necessary to supplement the soil's nutritional profile.

3. The Different Systems Integrated into *Azolla* Cultivation

3.1. The Rice-Fish-*Azolla* System

One of the most successful applications of *Azolla* is as a fertiliser and/or feed in an integrated rice-fish-*Azolla* system. In such a system, the simultaneous development of rice, *Azolla* and different species of fish (planktivorous, macrophytophorous and polyphagous) is permitted. The interplay between these elements is critical to maintaining the system's equilibrium. The fish derive benefit from the *Azolla*, depending on the species. The fish waste encourages the proliferation of plankton, which is consumed by some of the fish and by others. Additionally, some fish consume the plankton, while the remainder fertilise the rice. The presence of polyphagous fish has been demonstrated to provide a protective function against a variety of harmful insects and molluscs (Hasan and Chakrabarti, 2009; Khumairoh et al., 2018).

3.2. The fish-*Azolla* system

In a fish-*Azolla* polyculture system (Fig. 2), diets containing 10-40% dried *Azolla* had no significant effect on water quality, except for nitrate and nitrogen content. All fish species exhibited significantly higher growth with diets containing up to 20% *Azolla* (Dhawan et al., 2010).

Figure 2. Fish-*Azolla* co-culture (www.theazollafoundation.org).

3.3. The canard-fish-*Azolla* system

In an integrated duck-fish-*Azolla* system (Fig. 3), Nile tilapia are stocked in ponds fertilized with a mixture of fresh pig and duck manure. This practice has been shown to lead to the complete disappearance of the fish population. In these ponds, *Azolla* is consumed at intervals of six to seven days (Gavina, 1994 ; Mansour et al., 2020).

Figure 3. The duck-fish-*Azolla* system / (www.theazollafoundation.org).

3.4. The rice-canard-*Azolla* system

In the rice-duck-*Azolla* system (Fig. 4), Becerra et al. (1995) conducted feeding trials to assess the effect of feeding *Azolla microphylla* on rice plants. The goal was to determine the impact of incorporating *Azolla microphylla* as a partial substitute for soybean protein. Additionally, the study aimed to evaluate the effectiveness of replacing protein in boiled soybeans with sugarcane juice-based diets for ducks and to assess the suitability of such diets for meat ducks. The results showed that fresh *Azolla* can replace whole soybeans at approximately 20% of the total crude protein in duck meat diets without any negative effects on growth rate or health. Moreover, this treatment resulted in the lowest feed cost per kg of weight gain and the highest net profit per bird (Nasir et al., 2022).

Figure 4. *Azolla*-rice-duck co-culture (www.theazollafoundation.org).

4. Utilization of *Azolla*

Azolla is considered one of the most nutritionally dense aquatic plants, owing to its high content of carotenoids and amino acids. Additionally, it can be incorporated into both animal feed and human food (Van Hove and Lejeune, 2002).

4.1. Use of *Azolla* in Human Nutrition

Azolla is consumed by humans, and it appears to be non-toxic, with some culinary preparations being quite palatable. However, the use of *Azolla* in human food is limited by the difficulty of removing impurities, particularly those associated with its root system (Bujak et al., 2024).

4.2. Utilization of *Azolla* in Animal Feed

Azolla can be provided to livestock either fresh or dried, and can be administered directly or incorporated into concentrates for cattle, poultry, sheep, goats, pigs, and rabbits. It is recommended that animals be gradually introduced to *Azolla* by feeding them concentrates for a few days to help them acclimatize to its taste. Thus, it is advisable to start feeding the concentrates during the initial stages. Additionally, when dung is used as fertilizer in *Azolla* ponds, it is crucial to thoroughly wash the plant with fresh water to remove any residual odor of dung (Giridhar and Rajendran, 2013).

4.2.1. Utilization of *Azolla* in Ruminant Feed

Most *Azolla* feeding trials for dairy cattle, growing buffaloes, sheep, and goats have been conducted in India since 2000 (Pillai et al., 2004). The production of *Azolla* has been shown to have the second-highest benefit/cost ratio, following the production of worms for vermicomposting (Deshmukh et al., 2013). *Azolla*, in both fresh and dried forms, can be incorporated into the feed of cattle, sheep, and goats (see Fig. 5). Despite its long-standing use in ruminant diets, there remains a lack of comprehensive data on its efficacy. Trials in India have demonstrated that fresh or dried *Azolla* can partially replace more conventional protein sources, such as groundnut meal (Tran, 2015).

Figure 5. A cattle fed with *Azolla* (Tran, 2015).

4.2.2. The use of *Azolla* in poultry feed

4.2.2.1. The Use of Azolla in Broilers and Pullets

The inclusion of *Azolla* in the diet of broilers should be limited to a maximum of 5%, as higher inclusion rates have been shown to reduce nutrient utilization and overall performance of the birds (Parthasarathy et al., 2002; Basak et al., 2002 ; Samad et al., 2020). In pullet chicks, Azolla can be safely included at levels up to 10% (Alalade and Iyayi, 2006). Fresh Azolla (Fig. 6) has the potential to replace 20% or more of the commercial broiler feed (Namra et al., 2010 ; Samad et al., 2020).

Figure 6. Poultry consuming *Azolla* (www.theazollafoundation.org).

4.2.2.2. The utilisation of Azolla in the diet of laying hens

The inclusion of dried *Azolla* in the diet of laying hens has been demonstrated to be a viable and sustainable practice. The incorporation of dried *Azolla* in the diet of laying hens has been shown to constitute up to 15% of the diet (**Alagawany et al., 2023**). The addition of *Azolla* to the diet has been shown to have a positive effect on yolk colour (Khatun et al., 2008).

4.2.2.3. The Utilization of Azolla in the Diet of Japanese Quail

Azolla pinnata has been shown to have potential as a feedstuff for Japanese quail due to its high nutrient content. However, incorporation rates exceeding 5% have been found to negatively impact growth performance and feed conversion (Sujatha et al., 2013).

4.2.2.4. The utilization of Azolla in the diet of geese

Azolla has been utilised as a green forage for geese. A study by Zhang Zhuang et al. (1987) found that the daily weight gain of geese fed on *Azolla* was comparable to that of geese fed vegetables. It is demonstrated in this study that the dietary supplementation of Egyptian geese with 8 and 16% Azolla during the fattening stage results in enhanced growth performance and significant fluctuations in most blood biochemical parameters (Ismail et al., 2023).

4.2.3. The utilization of Azolla in rabbit nutrition

Azolla appears to be a suitable foodstuff for rabbits. A trial was conducted in which 6-week-old rabbits were fed diets containing from 0% to 36% dried *Azolla*. The results of this trial revealed that growing rabbits could be safely fed rations containing 24% dried *Azolla* hay, which has beneficial effects on the health of the rabbit. Furthermore, this hay has been shown to have beneficial effects on most production traits (Abou-Zeid et al., 2001). In a subsequent trial conducted with breeding rabbits, the substitution of 25% of the soybean meal protein with sun-dried *Azolla* protein resulted in the maintenance of feed conversion, litter size at weaning and female body weight, as well as economic performance. However, a decline in the conception rate, litter size at birth and milk production was also observed (El-Deeb et al., 2021).

4.2.4. The utilization of *Azolla* in Fish Husbandry

A series of studies conducted in aquariums have shown that cichlids (specifically those from the *Oreochromis*, *Tilapia*, and *Cichlasoma* genera) as well as a herbivorous hybrid of carp and bighead carp exhibit a preference for *Azolla*. Several researchers have demonstrated a preference for *Azolla caroliniana* over other species of Azolla (Micha et al., 1988 ; Fiogbé et al., 2004 ; Yohana et al,., 2023).

4.3. Interests Associated with the Plant

4. 3. 1. Economic Benefits

Azolla is a nitrogen fixer due to its symbiotic relationship with other organisms. It is widely used as green manure in rice fields across various Asian countries. Additionally, *Azolla* has been observed to help regulate weed growth, reduce water loss through evaporation, and improve soil structure (Rahagarison, 2005 ; Akhtar et al., 2020).

4.3.2. Environmental Benefits

Azolla reduces the intensity of underwater light, inhibiting algal photosynthesis, which in turn prevents the subsequent increase in pH and the emission of NH_3. Since up to 50% of nitrogen fertilizer applied to rice fields is lost through volatilization, *Azolla* has the potential to reduce the amount of nitrogen fertilizer needed for rice crops (Yao et al., 2018). Additionally, *Azolla* has been shown to reduce the proliferation of mosquitoes (Ravi et al., 2020 ; Wilson et al., 2023).

5. Conclusion

In summary, the findings of these studies demonstrate the significance of the endeavours undertaken to cultivate this plant (*Azolla*). The objective is to establish this plant as a viable alternative food source for animals. The findings of these studies serve as a valuable repository of knowledge, which can be utilised to devise innovative solutions that enhance the utilisation of *Azolla* in animal nutrition. Consequently, this will contribute to the enhancement of food security in countries where the production of animal products is constrained by limited resources.

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