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Research into Alternative Resources to Improve Animal Feed: The Case of *Azolla*

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Abstract: In countries where the animal feed are mainly imported, the search for alternative resources is a key way of reducing these costs. We have chosen to use the *Azolla filiculoide* plant. It is an aquatic fodder plant that is rich in protein. Its cultivation is simple. For the assessment of its development and yield, *Azolla* has been grown in two different environments. The first experiment was carried out in two open-air ponds with a surface area of 02 m^2. The water depth was 3 cm and 5 cm, respectively. The second was carried out in the laboratory, using a system of small caissons arranged on shelves. Water was added to these caissons at different depths. In both experiments, nutrient solution consisting of cow dung and some NPK was added. After 20 days of

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growing, the outdoor experiment showed that *Azolla filiculoide* could tolerate depths of 3 and 5 cm and could multiply easily. Daily production reached a yield of 305 g/m^2. This corresponds to about 10 kg per month. On the other hand, the laboratory trial was a failure. The *Azolla* gradually deteriorated over the course of the trial until it died. The reason for this was the inefficiency of the lighting system due to the use of ordinary fluorescent lamps that are unsuitable for *Azoll*a cultivation. We recommend the use of fluorescent lamps specially designed for this purpose. It is possible to grow *Azolla* in a closed environment as long as the environmental parameters are well controlled. These promising results suggest that *Azolla* has interesting potential as an alternative source of protein to feed livestock. However, further research will be necessary in order to improve yields and optimize growth conditions.

Keywords: *Azolla filiculoide*, Animal feed, Alternative resource, Protein source, *Azolla* cultivation, Agricultural engineering

Introduction

Worldwide demand for animal protein is constantly increasing due to population growth. Animals need to consume more energy and plant proteins than they produce for human consumption (Laisse et al., 2019). In the new forms of livestock farming, which are often more intensive, field crops (cereals, oilseeds, protein crops) and their co-products (oilcake, bran and milling waste) are widely used throughout the world. There is competition for arable crop resources between humans and animals, as well as competition for available farmland (Dronne, 2018).

Current production methods certainly provide higher yields, but at the high cost of some raw materials, particularly soya meal (Adouko et al., 2021). The use of local, non-conventional feedstuffs appears to be an endogenous solution to the high cost of imported raw materials (Adouko et al., 2021). These resources are an alternative to supplementation with concentrates and intensification of fodder production (Geoffroy et al., 1991).

Alternative or non-conventional food resources are foods of plant, animal or mineral origin that do not compete with human food. They include seeds (*Mucuna spp., Lablab purpureus, Canavalia ensiformis, sesame*), leaves (*Moringa oleifera, Leucaena leucocephala, Azolla pinnata*), tubers and various animal products (Dahouda et al., 2009). Introducing these resources into animal feed has become the only way of reducing the cost of the ration supplemented with concentrate without affecting herd productivity (Lassoued et al., 2011).

In the case of this study, the interest is focused on the fern *Azolla filiculoide*, an inexpensive alternative that offers an excellent protein supply. *Azolla* can be a valuable protein supplement for many animal species, including ruminants, poultry, pigs and fish (Ouedraogo et al., 2021).

Method

To test the *Azolla filiculoide* plant, two systems were set up in order to grow this plant. The first set-up consisted of two basins, each measuring 02 m2 and dug into the soil with a water depth of 03 and 05 cm respectively, set up in the open air. The second system consists of 06 small caissons of different volumes and depths installed on a shelf and set up in a laboratory, where the environmental parameters (water pH, H°, air and water T°) are controlled. The volumes and depths were as follows: 02 caissons A1 (volume 0.025 m³, depth 5 cm); 02 caissons A2 (volume 0.035 m³, depth 7 cm); 02 caissons A3 (volume 0.045 m³, depth 9 cm). For each depth, there were two repetitions. 18W neon lamps were superposed on each tank and operated for 20 hours per day using a timer.

The test lasted 20 days, after a 10-day adaptation period. It began on 18 May 2023. All the caissons were filled with water. The laboratory basins were covered by a thin layer of soil from the open-air basin. A nutrient solution was prepared and left to stand for 10 days. It consists of 10 kg of cow dung + 40 litres of water + 80 g of NPK. For the open-air tank, 02 litres of nutrient solution are added each week. For each laboratory tank, a volume of 500 ml / week is added (figure 1).

Azolla was planted on the same day for all the basins (in the lab and outdoors). The quantity of *Azolla* planted in the laboratory caissons was 250 g/0.5 m2 of water surface. In the open-air basins, which each had a water surface area of 02 m2, an area of 0.5 m2 was marked off with a board, where the quantity of *Azolla* planted was 250 g.

Figure 1. Experimental device used

Results and Discussion

The Development of *Azolla filiculoide* in Open-Air Basins

Air temperature monitoring recorded an average of 19.3°C, with values ranging from 12.8 to 26°C. For *Azolla*, ambient temperature is the main factor that has a direct influence on the plant. According to Van Hove (1989), the temperature that favours plant growth is between 20 and 30°C (figure 2). The water temperature varied between 13 and 21°C, with an average of 16.2°C. The drop in temperature was due to the *Azolla* mat on the surface of the water, which prevented the sun's rays from penetrating the two pools.

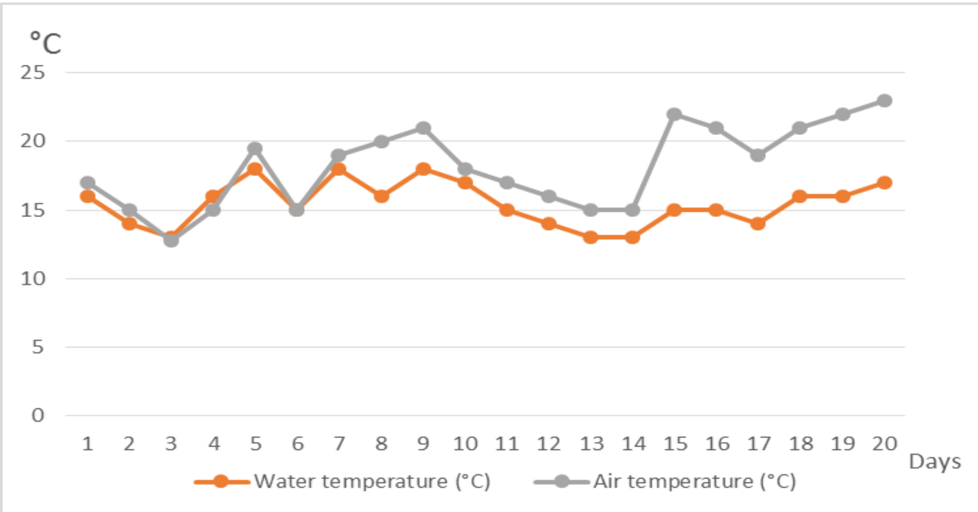

Figure 2. Evolution of water and air temperatures in the two open-air basins.

The pH of the water varies between 6.5 and 6.8 (Figure 3). It is basic and is suitable for the proper development of the plant, and encourages its growth and multiplication. The adequate pH for growing *Azolla* varies between 4.5 and 7 (Peters et al., 1980; Lumpkin and Plucknett, 1980). The average humidity level recorded during the trial was 67.3%. It varied between 48% and 92%. The optimum humidity for the growth of this plant varies between 85 and 90% (Rajesh, 2020). Although the humidity level recorded over the days varied, this did not have a negative influence on the plant's development (figure 3). Even when the humidity level was below 60%, the growth and multiplication of the *Azolla* were not affected, and we observed a positive evolution of the plant in both cultivation basins.

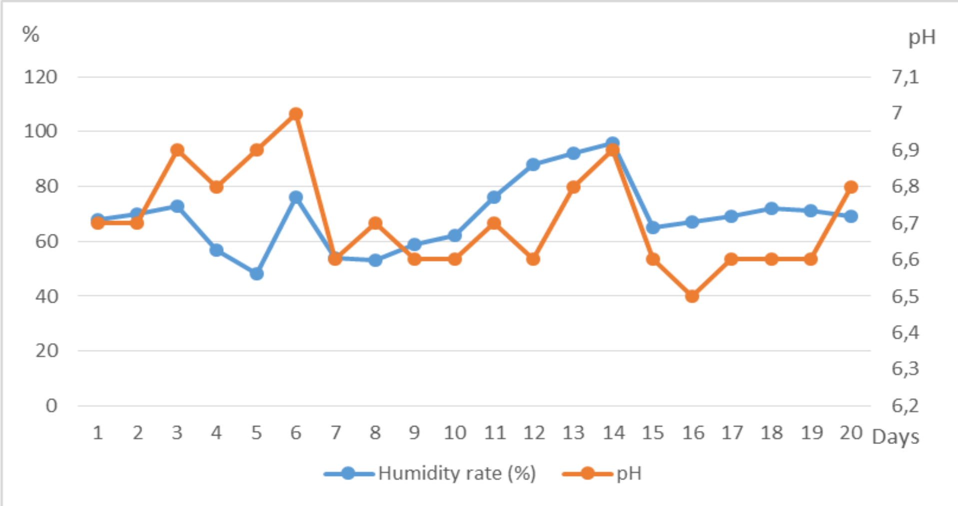

Figure 3. Evolution of humidity levels and water pH in the two open-air basins

Azolla Harvest and Yield in the Open-Air Basins

Once the plant had matured and adapted, it was found that the *Azolla* could resist and grow without difficulty in open-air basins at depths of 03 and 05 cm. The first harvest was made 20 days after planting the fern (figure 4).

Figure 4. Evolution of *Azolla* development in open-air basins

The second harvest took place on day 28. For basin 1 (5 cm deep), a quantity of 3350 g was harvested for the first time, taking into account the need to leave an initial quantity for later multiplication (the entire basin was still covered with *Azolla* after harvesting).

The second harvest was carried out 08 days after the first. The quantity harvested was 3110 g of *Azolla*. In basin 2 (3 cm deep), the first harvest was 3240 g, and the second was 3040 g (Table 1). *Azolla* dries by spreading it out on a ventilated surface. It can take up to 48 hours. The following table shows the weight differences between the fresh and dried states of the plant.

Table 1. Weight of fresh, dry *Azolla* in both basins.

	Fresh *Azolla*	Dry *Azolla*
	Basin 1 (05 cm depth)	
Harvest 1	3350 g	301,5 g
Harvest 2	3110 g	280,9 g
	Basin 2 (03 cm depth)	
Harvest 1	3240 g	298,08 g
Harvest 2	3040 g	273,6 g

After drying, *Azolla* loses almost 91% of its initial weight. Dry matter only represents nearly 9%. This result is close to that of Parashuramulu et al. (2013), who indicated a rate of 8.7% dry matter. Despite this considerable loss of weight, the process has little effect on the nutritional value of the plant (Van Hove, 1989). It should also be noted that there are no moulds, which gives the plant a good health quality. To estimate the yield of *Azolla* per m2, the quantity reproduced was estimated using the following formula:

Quantity multiplied = total quantity harvested - initial quantity

If we take into account the second harvest, which took place on day 28, giving us 08 days of cultivation, we get the following figure:
For basin 1 (05 cm deep) :
3110 g - 250 g = 2860 g /2 m^2. i.e. production of 357.5 g/day/2 m^2.
i.e. a production of 178.75 g / day / m2. The 2 m^2 basin can produce 10.7 kg of *Azolla* / month.
For basin 2 (03 cm deep) :
3040 g - 250 g = 2790 g/2 m^2. i.e. production of 348.75 g/day/2 m^2.
i.e. a production of 174.37 g / day / m^2. The 2 m2 basin can produce up to 10.4 kg of *Azolla* / month.
On average, a 2 m^2 basin will produce 10.55 kg of *Azolla*. The plant multiplies very quickly. This is one of its advantages. This is also reported by Kumar and Chander (2017).

Azolla Developement in the Laboratory Set-up

Given the three basins (with repetition) were subjected to the same environmental conditions, we measured, for the three parameters, the average of all the basins. In the laboratory, near the device, the air temperature varied between 19 and 26°C during the test period. This temperature range is in line with standards. According to Van Hove (1989), the temperature that favours plant growth is between 20 and 30°C. The temperature inside the caissons varied between 16 and 23°C (figure 5).

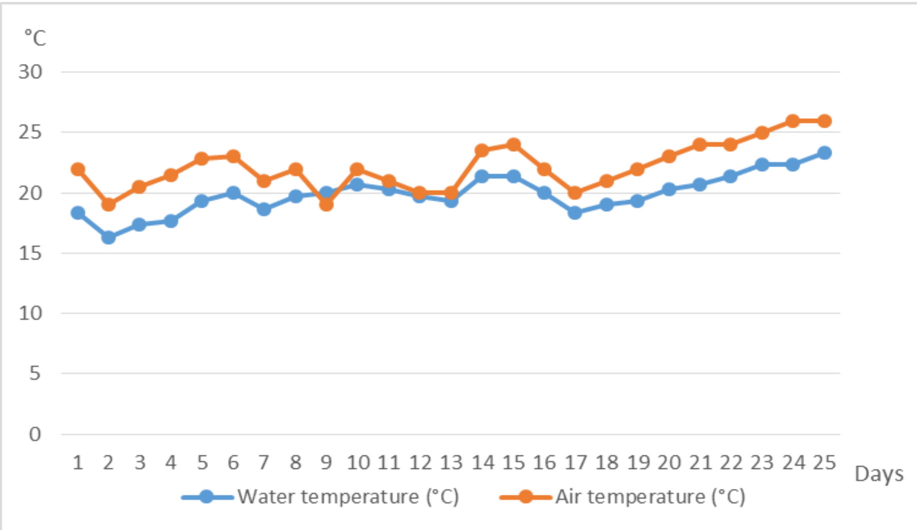

Figure 5. Evolution of water and air temperatures in the laboratory set-up basins

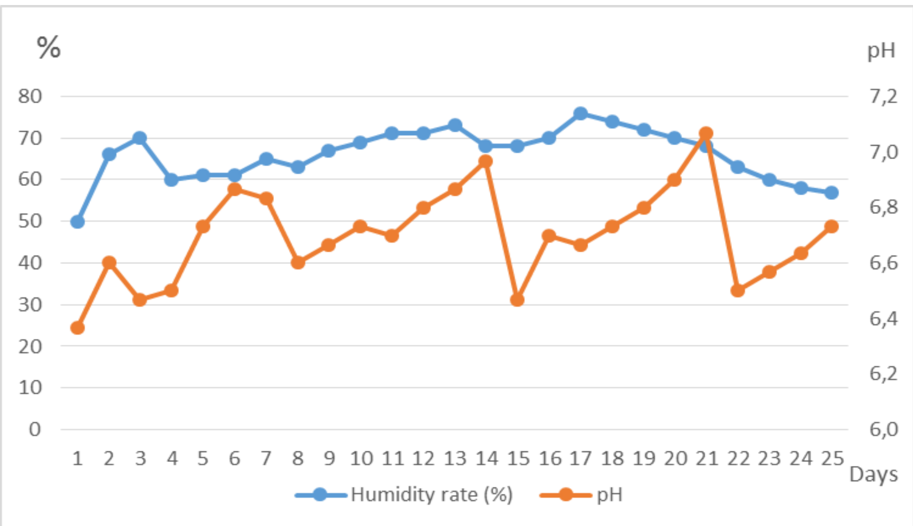

Figure 6. Evolution of the humidity level and pH of the water in the basins of the laboratory set-up

Humidity levels varied between 50 and 76% (figure 6). This is thought to be due to the variations in temperature between mid-May and mid-June during the trial period. While the pH varied between 6.4 and 7.1, *Azolla* is particularly tolerant of environmental pH, surviving in a range from 3.5 to 10 (Peters et al., 1980; Lumpkin and Plucknett, 1980).

Monitoring the Development of Azolla

The *Azolla filiculoide* developed normally until the 5th day, and its density was increasing. From day 8 onwards, the roots take a long time to develop. They become fragile, and when they reach a certain stage of growth, they detach from the frond and new regrowth appears. On the other hand, the fern continued to grow and an increase in its density was observed.

On the 10th day, the end of the adaptation phase, the density had increased. However, the roots had not developed and had not reached maturity (figure 7). On day 15, a deterioration in the condition of the plant was observed, with a significant decrease in density and the absence of roots. On day 20, there was no improvement and the plant continued to deteriorate despite the addition of artificial light and NPK fertiliser (20%).

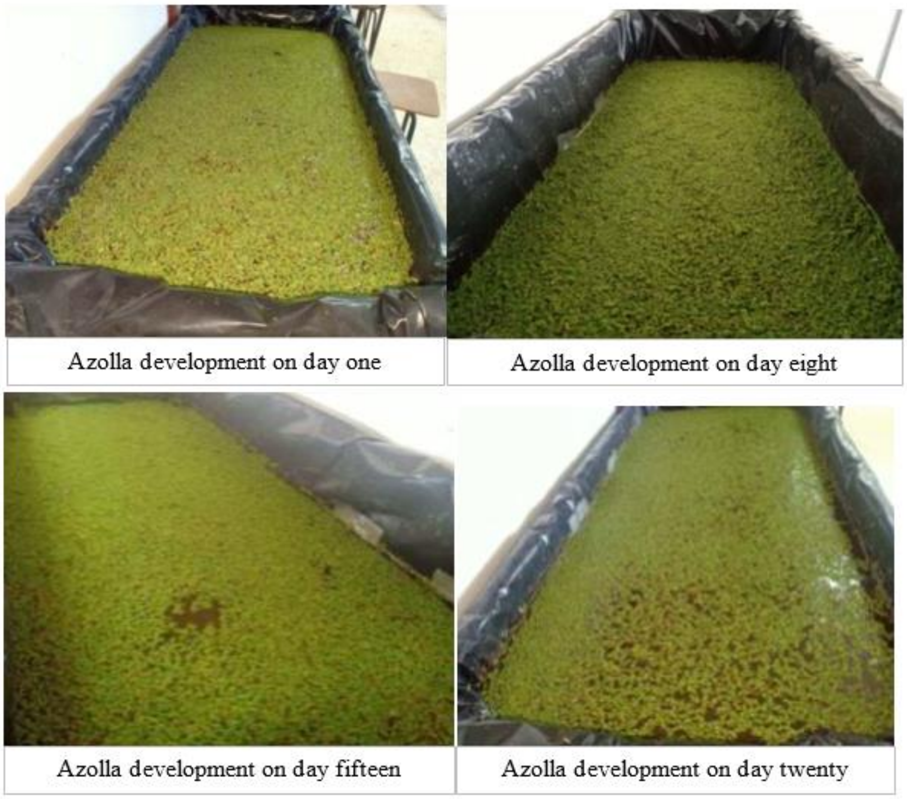

Figure 7. Evolution of *Azolla* development in the laboratory caissons

The laboratory device did not give positive results. There was a lack of light. The neon lights placed in the boxes did not provide enough light for the *Azolla* to develop. According to Lumpkin and Plucknett (1980), *Azolla* growth declines rapidly if light levels are below 1500 lux.

Conclusion

The aim of growing *Azolla filiculoide* in two different environments (in the open air and in a closed environment) was to assess the plant's profitability in the field of animal nutrition. In the open-air trial, the fern showed very promising performance; a 2 m2 basin can produce up to 10.4 kg of *Azolla* per month. *Azolla* can grow in water depths of between 3 and 5 cm. *Azolla* loses up to 90% of its weight when it dries out. In the laboratory test, there was a lack of light. The *Azolla* deteriorated over the last few days, until it disappeared. To improve this system, it would be necessary to carefully control the level of light (in Lux) required for the *Azolla* to develop.

Recommendations

Azolla filiculoide is an alternative source that we strongly recommend for animal feed because of its high protein content, its increasing multiplication and its less expensive production, which requires less water and cultivation area. The laboratory set-up we have proposed could increase *Azolla* productivity and reduce production costs by improving the amount of light needed for *Azolla* development.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM Journal belongs to the authors.

Acknowledgements or Notes

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