Evaluation of growth of Azolla (*Azolla pinnata*) using different media and as a dietary supplementation for poultry and livestock

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ABSTRACT

The present study on evaluation of different media for Azolla production and use of Azolla as a dietrary supplementation for poultry and livestock was conducted at Kumaraguru Institute of Agriculture, Tamil Nadu in 2019. It was concluded on 7th and 21st day after inoculation of Azolla, treatment comprising red soil + cow dung + super phosphate resulted in maximum root length (1.77 cm and 2.30 cm respectivley) as compared to all other treatments. The number of roots per Azolla was statistically maximum in this treatment on 7th (17.67), 14th (17.67) and 21st (18.17) respectively. On 7th, 14th and 21st day after inoculation, it resulted in maximum leaf length of 1.70, 1.70 and 2.20 cm. On 14th day, the same treatment resulted in maximum leaf breadth (2.20 cm). Highest chlorophyll a (153.65 µg/g f wt), chlorophyll b (79.50 µg/g f wt) and total chlorophyll (17.92 µg/g f wt) was also recorded in the same treatment. There was no significant difference in weight of birds due to addition of Azolla to the concentrated feed. There was an increase of 500 ml in milk yield per animal when fed on concentrated feed (2 kg/cow/day) + Azolla (2 kg/cow/day) after a week as compared to 300 ml in animals fed only on concentrated feed (2 kg/cow/day). The maximum net return and B-C ratio were also recorded in the treatment red soil + cow dung + super phosphate.

Keywords: Azolla; growth parameters; economics; milk yield

INTRODUCTION

Due to increasing population and limited resources, it is necessary to develop a comprehensive economic programme for food production. The increase in demand for meat and milk creates opportunities for animal husbandary occupation. The shortage of fodder is compensated with commercial feed resulting in increased costs in meat and milk production. Moreover, as commercial feed is mixed with urea and other artificial milk boosters, it has a negative effect on the quality of milk and the health of the livestock. In this regard, Azolla holds the promise of providing a sustainable feed for livestock (Kumar et al 2020a)

Azolla is an aquatic fern which resembles algae. It is very rich in proteins, essential amino acids, vitamins, potassium, ferrous, copper, magnesium, zinc etc. On a dry weight basis, Azolla consists of 25-35 per cent protein, 10-15 per cent minerals and 7-10 per cent amino acids and bio-active substances and bio-polymers. Carbohydrate and oil content in Azolla is very low (Kathirvelan et al 2015). All these biochemical constituents, along with rapid multiplication rate, make Azolla ideal organic feed substitute for livestock. Livestock can easily digest Azolla due to high protein content and low lignin content in it. Trials on dairy animals have shown overall increase of milk yield by 15-20 per cent when 2-3 kg of Azolla was combined with regular feed introduction and 15-20 per cent of commercial feed can be replaced with the same quantity of Azolla on dry weight basis, without affecting milk production. Azolla feeding also improved the health and milk quality of livestock (Biswas and Sarkar 2013). Adzman et al (2022) reported that Azolla can be used as a sustainable biomass source to produce animal feed (high protein content) and bioenergy (high fibre content).

However, there is very limited research published regarding nutrition value and use of Azolla in broiler diets and few studies were conducted regarding the use of Azolla in livestock as well as in poultry. AzolIa can be considered as a moderate source of protein in

commercial broiler livestock. Keeping it in view, the present study was carried out to evaluate the growth parameters of Azolla using different media and economics of Azolla production.

MATERIAL and METHODS

A field experiment was conducted at Kumaraguru Institute of Agriculture, Erode, Tamil Nadu to examine the growth parameters of Azolla using different media and its economics of cultivation during Feb-April and June-August 2019. The experimental farm was situated in western agro-climatic zone of Tamil Nadu at 11°49' N latitude, 77°56' E longitude with an altitude of 200 m amsl. The average minimum and maximum temperature in the area ranged from 32 to 34°C and relative humidity from 65-80 per cent during the cropping period. The experiment was laid out in randomised block design with three replications. The treatments used were T_1 (Red soil + cow dung + super phosphate), T_2 (Red soil + cow dung + DAP), T_3 (Red soil + vermicompost + super phosphate), T_4 (Red soil + cattleshed waste water + super phosphate). *Azolla pinnatta* was the species cultivated.

The pits of 2 m x 2 m x 0.2 m were dug under the shade of trees (Plate 1) and lined with silpauline sheet (Plate 2). All corners of the pits were at the same level so that a uniform level of water could be maintained. The pits were covered with plastic gunny bags to prevent the roots of the nearby trees piercing the silpauline sheet. About 10-15 kg of sieved fertile soil was uniformly spread over the silpauline sheets. Slurry, made of 10 kg cow dung mixed in 20 litres of water, was poured on to the sheets.

Super phosphate (100 g/pit) was added in the slurry as a basal dose. Additional water was added to raise the water level to 10 cm. About 0.5-1 kg of fresh and pure culture of Azolla was placed in the water (Plate 3). This grew rapidly and filled the pits within 10-15 days (Plate 4). From then onwards, 500-600 g of Azolla could be harvested daily from each pit. To each pit, one kg cow dung was added once every 5 days.

To maintain the daily yield of 500 g, 100 g super phosphate was top dressed on 4th and 8th day in order to maintain rapid multiplication of Azolla. After harvesting, Azolla was washed thoroughly with clean water. The growth parameters like length of roots, number of roots per Azolla, number of main shoots, number of sub-branches, number of leaves per branch and leaf length and breadth were recorded on 7th, 14th and 21st day after inoculation.

To find out the utility of Azolla as a feed supplement to poultry broiler birds, fresh Azolla fronds to 20 days old birds were fed @ 100 g per bird per day as single dose along with concentrated feed of standard composition (Plate 5). A control batch of one bird of same age was maintained which was fed with only concentrated feed. The feed intake and feed left over were recorded periodically in both the batches and their weight was recorded.

For testing the effect of Azolla supplementation on the milk production in the lactating cows (Plate 6), treatment group of 5 cows were fed concentrated feed (2 kg/cow/day) + Azolla (2 kg/cow/day) and only concentrated feed (2 kg/cow/day) was fed to control group of 5 cows. The milk production was recorded after a week.

Total carotenoids and chlorophylls a and b were determined following the method of Lichtenthaler and Wellburn (1985).

RESULTS and DISCUSSION

Growth parameters

Growth parameters of Azolla viz length of roots, number of roots, number of main shoots, number of sub-branches and leaves per branch were recorded on 7th, 14th and 21st day after inoculation and the data are presented in Table 1.

Root length: On 7th day after inoculation, treatment T_1 (Red soil + cow dung + super phosphate) resulted in maximum root length (1.77 cm) as compared to all other treatments. On 14th day after inoculation, T_1 (1.77 cm) was statistically at par with T_2 (Red soil + cow dung + DAP) (1.53 cm). However, on 21st day after inoculation, T_1 (2.30 cm) proved to be superior to all other treatments.

Number of roots per Azolla: The number of roots per Azolla was statistically maximum in T_1 on 7th (17.67), 14th (17.67) and 21st (18.17) day as compared to all other treatments.

Number of main shoots per Azolla: On 7th day after inoculation, number of main shoots per Azolla

Plate 1. Preparation of pits

Plate 2. Silpauline pits with fertile red soil

Plate 3. Growth of Azolla after 7 days of inoculation

Plate 4. Growth of Azolla after 21 days of inoculation

Plate 5 . Poultry birds feeding on Azolla

Plate 6. Cattle feeding on Azolla

was maximum in T_1 (4.67) and T_2 (4.33), which were at par. On 14th day, however, there were no significant differences among the treatments in number of main shoots per Azolla. On 21st day, again T_1 (5.83) and T_2 (4.83) were found statistically at par for this trait.

Number of sub-branches per Azolla: On 7th and 14th day after inoculation, statistically there were no differences among the treatments for number of sub-branches per Azolla. However, on 21st day after inoculation, treatments T_1, T_2 and T_3 resulted in 4.17, 3.83 and 3.17 sub-branches per Azolla respectively, which were at par, where T_3 (Red soil + vermicompost + super phosphate) was at par with T_4 (Red soil + cattleshed waste water + super phosphate) (2.83).

Number of leaves per branch: On 7th day after inoculation, T_1 (9.00), T_4 (8.00) and T_2 (7.67) resulted in maximum number of leaves per branch, which were at par. However, on 14th and 21st day, there were no significant differences among the treatments for this trait.

Table 1. Effect of different media on Azolla growth

Treatment	Parameter						
	Root length (cm)	Number of roots/Azolla	Number of main shoots/ Azolla	Number of sub-branches/ Azolla	Number of leaves/branch	Leaf length (cm)	Leaf breadth (cm)
7th day after inoculation							
T_1	1.77	17.67	4.67	3.33	9.00	1.70	1.20
T_2	1.53	14.00	4.33	3.33	7.67	1.33	1.10
T_3	1.07	10.33	3.67	2.67	7.00	0.73	0.43
T_4	1.30	12.33	3.67	2.33	8.00	1.20	0.97
SEm(±)	0.08	1.01	0.40	0.72	0.78	0.12	0.09
$CD_{0.05}$	0.17	2.21	0.87	1.57	1.69	0.26	0.19
14th day after inoculation							
T_1	1.77	17.67	4.67	3.33	9.00	1.70	2.20
T_2	1.53	14.00	4.33	3.33	7.67	1.33	1.10
T_3	1.07	10.33	3.67	2.67	7.00	0.73	0.43
T_4	1.30	12.33	3.67	2.33	8.00	1.20	0.97
SEm(±)	0.12	1.23	0.47	0.53	0.94	0.14	0.09
$CD_{0.05}$	0.27	2.68	1.03	1.16	2.05	0.30	0.19
21st day after inoculation							
T_1	2.30	18.17	5.83	4.17	9.50	2.20	1.70
T_2	2.03	14.50	4.83	3.83	8.17	1.83	1.60
T_3	1.57	10.83	4.17	3.17	7.50	1.23	0.93
T_4	1.80	12.83	4.17	2.83	8.50	1.70	1.47
SEm(±)	0.12	1.00	0.51	0.51	0.94	0.14	0.09
$CD_{0.05}$	0.26	2.18	1.12	1.12	2.05	0.30	0.19

T_1 (Red soil + cow dung + super phosphate), T_2 (Red soil + cow dung + DAP), T_3 (Red soil + vermicompost + super phosphate), T_4 (Red soil + cattleshed waste water + super phosphate)

Leaf length: On 7th, 14th and 21st day after inoculation, T_1 resulted in maximum leaf length of 1.70, 1.70 and 2.20 cm respectively as compared to all other treatments.

Leaf breadth: On 7th day after inoculation, T_1 (1.20 cm) and T_2 (1.10 cm) resulted in maximum leaf breadth and were at par with each other. On 14th day, however, T_1 resulted in maximum breadth (2.20 cm) as compared to all other treatments. Again on 21st day, T_1 (1.70 cm) and T_2 (1.60 cm) exhibited maximum breadth and were at par.

These results are in close agreement with the work of Basak et al (2002) who reported that the fern produced more sporangia, indicating a healthy growth condition of root which was significantly higher due to heavy transfer of nutrients by the roots from the nutrient-rich media which in turn increased the number of branches and number of leaves per branch in Azolla.

Phytochemical analysis

Chlorophylls a and b and carotenoid content: Data given in Table 2 show that highest chlorophyll a content (153.65 µg/g f wt) was recorded in T_1 followed by T_2 (128.71 µg/g f wt) and T_4 (114.60 µg/g f wt) and the minimum in T_3 (68.02 µg/g f wt). The chlorophyll b content was highest in T_1 (79.50 µg/g f wt) followed by T_2 (60.90 µg/g f wt) and T_4 (57.62 µg/g f wt) and the minimum in T_3 (39.22 µg/g f wt). In overall, maximum total chlorophyll content was recorded in T_1 (17.92 µg/g f wt) followed by T_2 (11.95 µg/g f wt) and T_3 (11.37 µg/g f wt) and the minimum in T_4 (10.22 µg/g f wt). Maximum carotenoid content was recorded in T_2 (0.08 nm), followed by T_1 (0.07 nm), T_3 (0.06 nm) and least in T_4 (0.03 nm).

Effect Azolla feeding on poultry birds

Data given in Table 3 depict that the weight of bird was 750, 745 and 747 g on 20th day; 1,400, 1,350 and 1,420 g on 40th day and 1,920, 1,890 and 1,905 on

Table 2. Effect of different media used for Azolla cultivation on chlorophyll and carotenoid content

Treatment	Chlorophyll a (µg/g f wt)	Chlorophyll b (µg/g f wt)	Total chlorophyll (µg/g f wt)	Carotenoid (510 nm)
T_1	153.65	79.50	17.92	0.07
T_2	128.71	60.90	11.95	0.08
T_3	68.02	39.22	11.37	0.06
T_4	114.60	57.62	10.20	0.03

T_1 (Red soil + cow dung + super phosphate), T_2 (Red soil + cow dung + DAP), T_3 (Red soil + vermicompost + super phosphate), T_4 (Red soil + cattleshed waste water + super phosphate)

60th day due to feeding of concentrated feed (100 g/chick/day), concentrated feed (100 g) + Azolla (1:1)/chick/day and concentrated feed (100 g) + Azolla (1:2)/chick/day respectively. The total gain in weight was 1,170, 1,145 and 1,158 g due to feeding of concentrated feed (100 g/chick/day), concentrated feed (100 g) + Azolla (1:1)/chick/day and concentrated feed (100 g) + Azolla (1:2)/chick/day respectively. This shows that there was no significant difference in weight of birds due to addition of Azolla to the concentrated feed.

Basak et al (2002) reported that Azolla meal up to 5 per cent in the broiler ration was found to improve performance and may be used in broiler diet as a safe level. Azolla meal had no deleterious effect on the palatability of broiler diets. Khursheed et al (2019) reported that Azolla may be used as an unconventional feed in poultry diet due to its high protein content.

The result from the work of Samad et al (2020) showed that 15 per cent of *Azolla* spp had significantly highest overall body weight gain of broiler checken. The study concluded that the inclusion of *Azolla* spp up to 15 per cent in broiler chicken feed ration improved growth of body performance and showed no adverse effect on the growth performance and nutrient digestibility of the birds. Sharma et al (2020) found that dried Azolla meal incorporation up to 5.5 per cent of diet was a good source of nutrients and as an alternative sustainable feed ingredient for broilers without affecting performance.

Effect of Azolla feeding on the milk yield of dairy animals

The data given in Table 4 show that there was an increase of 500 ml in milk yield per animal after a week when fed on concentrated feed (2 kg/cow/day) + Azolla (2 kg/cow/day) as compared to 300 ml in animals which were fed only on concentrated feed (2 kg/cow/day).

In a study conducted by Bhutia et al (2020), it was found that the milk yield showed increasing trend and increased to 6.5 to 7.8 l per day per cattle and fat from 3.4 to 3.51 per cent after 90 days of feeding 1.5 kg fresh Azolla per day to dairy cattle. In an experiment on crossbred cows conducted by Kour et al (2020), it was found that significantly higher mean milk yield (8.30 + 0.41 kg/day) in treatment group which were fed fresh Azolla supplementation 500 per g per day was recorded in comparison to control (7.17 + 0.53 kg/day).

Singh et al (2017) conducted on farm trail to study the effect of azolla on buffaloes milk yield. The control group buffaloes were fed wheat straw with mustard oilseed cake, whereas, the treatment group was fed wheat straw and mustard oilseed cake with supplementation of 2 kg fresh green *Azolla pinnata*. The milk yield was higher in buffaloes fed Azolla with mustard oilseed cake in comparison to control group fed only mustard oilseed cake. The milk yield showed increasing trend and it increased to 10.5 l per day from 8.8 l per day after 90 days of feeding 2 kg Azolla per day with conventional feed mustard oilseed cake. On an average milk yield increased by 1.70 l per day.

Kumar et al (2020b) undertook a study to know the effect of feeding Azolla on milk yield of lactating cows and found that the milk yield increased to 9.90 l/day from 8.30 l/day after 90 days of feeding of Azolla per day with conventional feed. There was 19.51 per cent increase in milk yield.

Kololgi et al (2009) recorded 10 per cent increase in milk yield in lactating buffaloes due to Azolla feeding. Various studies have shown that Azolla could

Table 3. Effect of Azolla on weight of poultry birds

Feeding	Weight of poultry birds (g)			
	On 20th day	On 40th day	On 60th day	Total gain
Concentrated feed (100 g/chick/day)	750	1,400	1,920	1,170
Concentrated feed (100 g) + Azolla (1:1)/chick/day	745	1,350	1,890	1,145
Concentrated feed (100 g) + Azolla (1:2)/chick/day	747	1,420	1,905	1,158

Table 4. Effect of Azolla on quantity of milk of dairy animals

Feeding	Milk production/day (ml)		
	Before treatment	After treatment	Total increase
Concentrated feed (2 kg/cow/day)	5,800	6,100	300
Concentrated feed (2 kg/cow/day) + Azolla (2 kg/cow/day)	6,000	6,500	500

Table 5. Economics of Azolla production on different media

Treatment	Capital expenditure (Rs)	Recurring expenditure (Rs)	Total expenditure (Rs)	Yield/two months (kg)	Gross return/two Months (Rs)	Gross return/year (Rs)	Net return (Rs)	B:C
T_1	1,010.00	348.00	1,358.00	54	540.00	3,240.00	1,882.00	2.38
T_2	1,010.00	360.00	1,370.00	50	500.00	3,000.00	1,630.00	2.18
T_3	1,010.00	390.00	1,400.00	48	480.00	2,880.00	1,480.00	2.05
T_4	1,010.00	330.00	1,340.00	40	400.00	2,400.00	1,060.00	1.79

T_1 (Red soil + cow dung + super phosphate), T_2 (Red soil + cow dung + DAP), T_3 (Red soil + vermicompost + super phosphate), T_4 (Red soil + cattleshed waste water + super phosphate)

be fed to these animals without any adverse effects on them.

Economics of Azolla production on different media

The cost of Azolla produced was calculated based on the recurring expenses like cost of inputs used, labour charges and yield of Azolla biomass. Azolla was harvested at the end of the week and the yield of Azolla biomass was 54, 50, 48 and 40 kg from 15 m^2 plot size in T_1, T_2, T_3 and T_4 respectively as depicted in Table 5. Thus the average yield was 0.80 kg per m^2 from each pit. The net return obtained was Rs 1,882.00, 1,630.00, 1,480.00 and 1,060.00 from T_1, T_2, T_3 and T_4 respectively and thus B-C ratio was 2.38, 2.18, 2.05 and 1.79 in T_1, T_2, T_3 and T_4 respectively. Thus maximum net return and B-C ratio were recorded in the treatment T_1.

Sinha et al (2018) reported that the feeding of Azolla resulted in mean average change in the body weight of 446.95 g which was 161.85 g more than the farmers' traditional practice ie foraging. They concluded that feeding of Azolla 100 g per day per bird to poultry birds under backyard condition might be highly effective to get more profit.

Earlier Cherryl et al (2013) reported average yield per day of Azolla of 0.38 kg per m^2. The total capital expenditure required for Azolla production from single pit of 20 m^2 was Rs 1,058.00 and the recurring expenditure was Rs 35.96 for a week.

CONCLUSION

From the study, it can concluded that the medium comprising red soil + cow dung + super phosphate was superior over all treatments for Azolla yield and also recorded highest net return and B-C ratio. The supplement of Azolla 2 kg per day in lactating

cows increased the milk yield to 500 ml per day in comparison to 300 ml per day in cows who were fed only on feed concentrate (2 kg/cow/day). However, there was no significant difference in weight of birds due to addition of Azolla to the concentrated feed.

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