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Azolla cultured using different manure as a fish feed ingredient

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ABSTRACT

Application of locally available organic fertilizers on the growth and protein composition of the aquatic plant *Azolla pinnata,* and its significance as a protein source of fish feed in the culture of tilapia strain Chitralada (*Oreochromis niloticus*) is evaluated. An experiment was conducted to optimize the growth of *Azolla pinnata* in eight different organic fertilizers (fresh cow dung, dried cow dung, jeevamrutham, biogas slurry, vermicompost, poultry manure, and urine of cow). The culture of Azolla was carried out for 6 months, along with the estimation of biomass, relative growth rate (RGR), doubling time, protein-lipid composition, and parameters like pH, and temperature in the culture media. To assess the growth performance in the Chitralada strain of tilapia, the percentage of fish meal was replaced with dried and ground azolla in the formulated diets. In both experiments, the results showed that the culture of azolla resulted in a good yield in the pit fertilized by poultry manure and with the highest biomass (224 g) and the fastest doubling time (8.24 days). Meanwhile, the highest protein-lipid composition was found in azolla cultured in fresh cow dung (27.7 and 0.9 g/100g). In feeding experiments conducted in tilapia strain Chitralada, the body length was found to be increased up to 12.50±0.13 cm, and that of body weight 35.10±0.15 g on the 50th day. These results suggest that the combination of azolla meal along with fish meal demonstrated better growth performance. Azolla cultured in different organic manures, particularly poultry manure followed by cow dung and vermicompost, were found to be the best organic fertilizers that enhance the growth and protein composition of azolla.

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1. Introduction

The free-floating azolla is an aquatic pteridophyte commonly known as mosquito fern. These plants can grow in a nitrogen-deficient medium due to their nitrogen fixation ability with symbiont *Azollae anabaena*. The high level of crude protein and high concentration of amino acids such as lysine made Azolla a good source of protein for fish feed. In recent years, Azolla has been used as a natural animal feed supplement. They need only a minimum quantity of water for their complete propagation and can multiply rapidly within few days. Their ability to fix nitrogen makes them a highly productive plant. They can also be used as fish feed ingredients due to their high protein content. Azolla production can be considered as one of the cheapest ways to increase fish production. Prabha and Kumar (2010) reported that azolla has great potential as a protein source due to its ease of cultivation, rapid multiplication, nutritional value, and productivity.

Several recent studies have underscored the promising potential of Azolla as a valuable component in tilapia feed formulations, presenting cost-effective and nutritionally advantageous opportunities. Research by Sithara and Kamalaveni (2008) indicates that Azolla incorporation into the feed can significantly reduce overall feed costs, especially when mixed with agricultural by-products like wheat bran and rice bran, thereby enhancing feed digestibility and quality. Moreover, Santiago et al. (1988) and Micha et al. (1988) reported enhanced growth performance in Nile tilapia fry and Tilapia rendalli fingerlings when Azolla was integrated into their diets. Fiogbe et al. (2004) found favorable results with Azolla replacing up to 45% of control diets in young *Oreochromis niloticus*, with juveniles displaying enhanced efficiency in Azolla utilization. However, some studies, such as those by Abdel Halim et al. (1998) and Micha et al. (1988), noted negative growth responses in certain tilapia species when Azolla was included in their diets. Despite these discrepancies, previous research indicates that optimizing feed utilization, as demonstrated by Sithara and Kamalaveni (2008), results in increased protein, carbohydrate, and lipid content in fish tissues. Overall, Azolla holds promise as an efficient feed supplement, amino acids, and proteins, rich in vitamins and minerals, making it a potential ingredient for various animal species. In this context, our present research aims to assess the efficiency of Azolla in different fertilizer mediums and explore its significance as a fish feed ingredient, ultimately contributing to sustainable and eco-friendly aquaculture practices.

2. Materials and Methods

A coconut farm field of 300 ft^2 was selected for the preparation of the Azolla fodder plot. The plot is divided into 8 pits with a uniform size of (1.4m × 1.5 m) and a depth of 0.3 m. A polythene sheet of 150 GSM was spread over the pit, and 20 kg of sieved soil was spread over the bed. Sufficient water was added up to 20 cm from the soil level. The seven organic manures for the Azolla culture were collected from a local market at Kayamkulam. About 300 g of fresh cow dung dissolved with water was added to the first pit, followed by 250 g of dried cow dung in the second pit, 350 g of vermicompost in the third pit, and 400 g of biogas slurry in the fourth pit. The fifth and sixth pit is filled with 500 ml Jeevamrutham and 250 ml urine of cow. The last two pits were filled with 300 gm of poultry manure and control, devoid of organic manure. The culture of Azolla was carried out for two weeks with the estimation of relative growth rate, biomass, and doubling time, along with the estimation of lipid and protein composition.

From the proximate analysis, a culturing medium having Azolla with the highest protein composition was selected and used as a replacement for protein sources in fish feed. The experimental diet with 40 % crude protein was prepared from Azolla (27.7%), fish meal (58.32%), rice bran 12.80%, and groundnut oilcake (42%) and was balanced using the square method. 12 juveniles of Chitralada tilapia (*Oreochromis niloticus*) of initial length 6.50±0.12 cm and weight of 7.10±0.15 g were fed with these diets twice a day (10 g) for 60 days. Growth performance and proximate composition of fish was estimated after the feeding experiment.

3. Results and Discussion

3.1 Growth parameters of azolla

In general, Azolla grown in seven organic manures showed an increase in biomass and relative growth rate compared with control. The highest values were observed in poultry manure, followed by vermicompost and cow dung (Table 1). Fig. 1 depicts the decrease in doubling time of Azolla grown in organic culturing media (8 – 11 days) compared to control, which requires more than 11 days. The highest biomass values (212±7.63 gm^{-2}) and relative growth rate (0.035±0.0012 g/g/d) were observed in the poultry manure, and the lowest biomass (123.25±5.52 gm^{-2}) and relative growth rate (0.025±0.0010 g/g/d) were observed in control. So, all the organic culturing media showed a significantly higher value of growth than the control ($P<0.05$).

Utomo et al. (2019) and Menéndez (2002) reported similar results on azolla culture in organic and inorganic media. They suggested that phosphorous and nitrogen-contained organic fertilizers have the ability to increase biomass on microalgal tissues. Similarly, Ehab et al. (2020) reported that the Azolla grown in organic treatment ponds has a higher capacity to increase their fresh weight, dry weight, biomass, and relative growth rate.

Indira et al. (2014) reported that poultry and cattle manures have high efficiency in Azolla production. Goliya et al. (2017) also checked the comparative efficiency of different organic manures in Azolla and zooplankton production and reported better yield in poultry manure, cow dung, and vermicompost.

3.2 Nutrient composition of azolla

Azolla has a high protein composition and can be categorized as a highly productive plant. In the present experiment, Azolla grown on cow dung showed the highest amount of protein (27.7±0.46 %). Vermicompost (26.20±0.19 %), biogas slurry (25.90±0.42 %), poultry manure (25.10±0.19 %) and control (23.13±0.39 %) grown Azolla also comprise rich protein. But in the case of lipids, biogas slurry (1.2±0.03 %) has comparatively more efficiency in lipid production than other culturing mediums. While compared with control, the proximate composition of Azolla in culturing mediums like cow dung, vermicompost, and biogas slurry is significantly higher than control ($P<0.05$).

Fujiwara et al. (1947); Singh (1980); Subudhi and Singh (1977); Singh and Subudhi (1978); Sreemannaryana et al. (1993); Van Hove (1989); Kumar et al. (2012); Kathirvelan et al. (2015) estimated the composition of protein in Azolla was between 20-37%. Whereas the crude fat in Azolla is estimated to be between 0.8-6.7% along with 13-27% polyunsaturated fatty acids (PUFA) and essential amino acids, Buckingham et al., (1978); Querubin et al., (1986); Sreemannaryana et al., (1993); Ali and Lesson, (1995).

Since the Azolla has nitrogen fixation capacity due to the symbiotic relationship between the *Azolla and anabaena*, they can produce nitrogen and convert it into protein form (Handajani, 2012). So, these aquatic ferns always prefer the medium that constitutes a low level of nitrogen and a high level of phosphorous Ehab (2020). In the present experiment, culturing mediums with a high phosphorous level maintain a higher protein level (Cow dung, vermicompost, poultry manure, and biogas slurry). Hazary (2015) also reported the level of protein in Azolla increases with an increase in the phosphorous in the culturing medium. In lipid composition, Azolla has better results in biogas slurry; this may be due to phosphorous and iron in the biogas medium. The findings of Ralph et al. (2018) and Brouwer et al. (2017) supported this assumption. They reported that an additional supply of Fe in the culturing medium might enhance the efficiency of the Azolla plant in growing in a nitrogen-deficient medium. Our results also depict the dynamic response of Azolla in the phosphorus-rich medium; the addition of Fe also improves the productivity and composition of Azolla. However, the productivity becomes saturated or limited depending upon the level of nitrogen in the medium.

3.3 Growth parameters of the fish (*Oreochromis niloticus*)

The experiment results indicate that Azolla incorporated experimental feed has significant effects on the growth rates and feed utilization of Nile tilapia (*O. niloticus*). The highest survival rate of the fish was 91.6%, and the absolute growth rate was (21.175±0.76 g). The specific growth rate (0.982±0.04% day-1) and daily growth rate (0.325±0.012 g/fish/day) were also observed. The growth and production performance of fishes gradually increase with the supplementation of Azolla. The survival rate is not significantly affected by the replacement of Azolla with protein supplementation of fish meal.

The results from the growth parameters are comparable with several studies. Hossain et al.;(2002) and Fasakin et al. (2001) conducted the same studies on the partial replacement of fish meal with plant protein in Nile tilapia and Sudaryono (2006) reported the substitution of soyabean meal with Azolla pinna decreases the growth rate in black tiger shrimp (*Penaeus monodon*). The SGR and DGR were

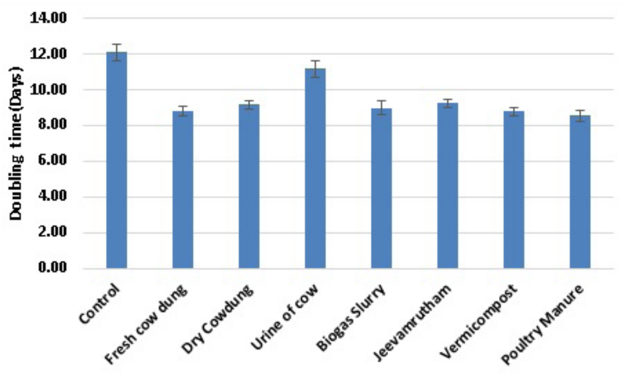

Fig. 1. Doubling time of azolla (days)

Table 1. Analysis of variance (One Way ANOVA) of different parameters comparing 8 groups

Parameter	Group	Mean	± SD	F value
Biomass	Control	123.25[a]	5.52	121.652**
	Fresh cow dung	201.00[d]	8.69	
	Dry cow dung	188.25[cd]	5.20	
	Urine of cow	138.50[b]	6.78	
	Biogas Slurry	196.00[de]	13.50	
	Jeevamrutham	186.25[c]	7.91	
	Vermicompost	202.50[d]	8.02	
	Poultry Manure	212.00[e]	7.63	
RGR	Control	0.025[a]	0.0010	102.453**
	Fresh cow dung	0.034[d]	0.0010	
	Dry cow dung	0.033[c]	0.0008	
	Urine of cow	0.027[b]	0.0011	
	Biogas Slurry	0.034[cd]	0.0014	
	Jeevamrutham	0.033[c]	0.0008	
	Vermicompost	0.034[d]	0.0010	
	Poultry Manure	0.035[d]	0.0012	

a, b, c, d, e: Means with same superscript do not differ each other (Duncans Multiple Range Test)

Table 2. Analysis of variance (One Way ANOVA) of different parameters comparing 8 groups

Parameter	Group	Mean	± SD	F value
Protein	Control	23.13[c]	0.39	1965.805**
	Fresh cow dung	27.70[f]	0.46	
	Dry Cow dung	11.93[a]	0.60	
	Cow Urine	11.60[a]	0.27	
	Biogas Slurry	25.90[e]	0.42	
	Jeevamrutham	18.60[b]	0.28	
	Vermicompost	26.20[e]	0.53	
	Poultry manure	25.10[d]	0.19	
Lipid	Control	0.90[a]	0.07	21.635**
	Fresh cow dung	0.90[a]	0.06	
	Dry Cow dung	0.80[a]	0.02	
	Cow Urine	0.80[a]	0.06	
	Biogas Slurry	1.20[d]	0.19	
	Jeevamrutham	0.80[a]	0.02	
	Vermicompost	1.10[c]	0.14	
	Poultry manure	1.00[b]	0.03	

a, b, c, d, e: Means with same superscript do not differ each other (Duncans Multiple Range Test)

found to be high, using 75% azolla as protein substitution in fish feed (Kohinoor et al. 1999 Shah et al.1998).

3.4 Proximate composition of the feed and fish.

The proximate composition of experimental feed and fish was represented in Fig. 2. In this study, the protein, carbohydrate, and lipid composition of the Azolla incorporated experimental feed were 35.36%, 10.79%, and 1.76%, respectively. The chemical composition of the feed was comparable with previous reports done by Bolka et al. (2011), Kumar et al. (2012), Kumarasinghe et al. (2012), and Cherryl et al. (2014). They observed that the crude protein content of azolla meal is found between 21.4 - 25.78%. On the contrary, Parthasarathy et al. (2001) endowed that the NFE content of Azolla meal is 38.85%. Both values are comparable with results obtained because the response of Azolla to environmental conditions such as light intensity, temperature, and soil nutrients affect the morphology and nutrient composition of the Azolla meal.

Biochemical studies of the fish were done after feeding

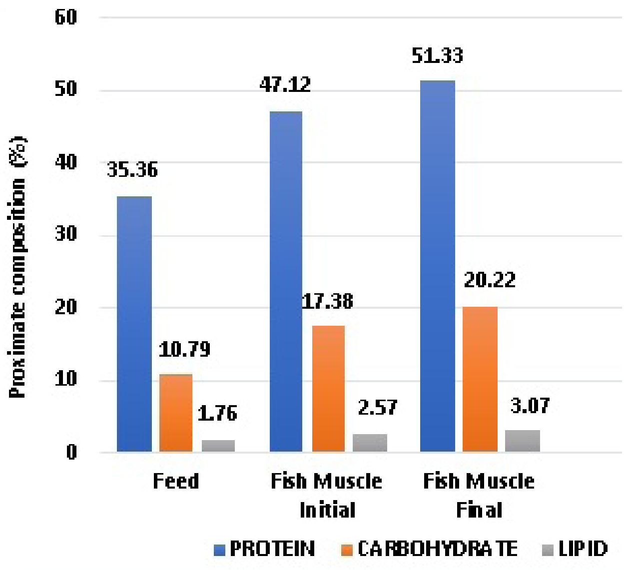

Fig. 2. Proximate composition of feed and fish

to understand the muscle tissues' changes in protein, carbohydrate, and lipid composition. Results indicated a significant change in the muscle tissues, and the influence of feed is depicted in Fig. 2. The initial composition of protein was 47.12%, and that of carbohydrate was 17.38% and lipid 2.57%. The proximate composition of fish increases after feeding with an azolla diet; protein level changes to 51.33%, carbohydrate 20.22%, and lipid 3.07%. This significant increase was already reported by Micha et al. (1988). He observed that the protein conversion ratio is always high in fishes fed with the Azolla diet. Kitchwell and Windell (1997), Fisher (1973), and Mathavan and Christopher (1980) suggested the effects of feeding plant diets made a positive impact on the proximate composition of omnivorous fish.

4. Conclusion

Azolla has a high yield when grown with poultry manure, cow dung, and vermicompost. Cow dung and biogas slurry have more efficiency in enhancing the protein-lipid composition of Azolla. The optimum conditions for maximum yields of Azolla were a pH ranging from 6 -7 and a temperature of below 29°C.

The present study indicates that Azolla can be considered an attractive ingredient in formulating low-cost feed. They can increase the protein-lipid composition of omnivores and phytophagous fish *Tilapia.* Therefore, Azolla can be used as an alternative option for protein replacement in tilapia production and can offer high-profit margins.

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