Dry Azolla Based Feed Formulation for Fresh Water Carp

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Abstract. Azolla pinnata is a macro alga rich in protein, essential amino acids, vitamins and minerals. Hence it can be used in sustainable animal feed production. In this study, fish feeds were formulated using wheat flour, fish meal, groundnut oil cake combined with either 25% or 30% of dried Azolla. Fish feeding experiments were conducted with Catla fries in tanks using commercial feed (C), 25% Azolla replaced (T1) and 30% Azolla replaced (T2) feeds. Catla fishes grown in Azolla replaced feed formulations showed higher growth with 2% increase in their body weight compared to control feed. In addition, survival rate and nutrient contents such as carbohydrate, and protein were also found higher in fishes grown in dry Azolla based feeds than commercial feed. This study substantiates that dry Azolla can be used as a cheap nutrient supplement in fish feeds.

Keywords: Azolla pinnata, Carp, Catla catla, Feed formulation, Proximate analysis.

1 Introduction

Fishing is an important occupation in India due to its vast coastal area. India is the third largest fish producer and ranks second in the production of farmed fishes. The state Tamil Nadu is the fifth highest producer in the Indian scenario (World Bank 2013). Fish accounts for 20% of the animal derived protein in low income, food deficit countries. Nearly 17% of the global protein intake comes from fishes. Expansion in aquaculture may help alleviate protein malnutrition especially in poor rural areas. In order to attain sustainability in fish production it is necessary to reduce the dependability on wild fishes and to intensify fish farming (Delgado et al 2002). By 2030, aquaculture will produce two third of the global food fish (World Bank 2013). Thus, the aquaculture industry is expected to grow significantly in the near future.

Carps are the most farmed fish species in India. They provide 26.2% of the total animal meat and are considered as the fast-growing food source in Asia (Delgado et al 2002). The three major carps are *Catla catla* (Catla), *Labeo rohita* (rohu) and *Cirrhinus mrigala* (mrigal). Preference for growing these fishes are mainly because of the ease of culture, rapid growth, attainment of large size, compatibility, keeping quality, consumer preference and hardness which makes it easy to handle and transport. *Catla* is also known as the major Indian carp, which is considered as the economically most important fish of South Asia. Fish farmers like to grow Catla because it attains 1 – 1.2 kg in the first year while rohu and mrigal attain 600 – 800 g. *Azolla* is a prominent ingredient used in diets of fish because of its high protein content and excellent amino acid profile. It is an aquatic fern which can be grown in ponds, ditches

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and rice fields of warm-temperate and tropical regions throughout the world (Lumpkin and Plucknett 1980). It can be fed to livestock either in a fresh or dried form. It can be fed either directly or mixed with concentrates to livestock (Giridhar and Rajendran 2013). Soybean or fish meal can be partially substituted with *Azolla* (Gangadhara et al 2018). Thus, there will be reduction in the usage of high cost protein sources like fish meal and soya bean in fish feeds when *Azolla* is used. In this study, dry *Azolla* based fish feeds were formulated and evaluated in fresh water carp, *Catla catla* with an objective to estimate whether there is an increase in growth, and survival rate and nutrient content of fishes.

2 Materials And Methods

Azolla cultivation and drying

Fresh *Azolla* was cultivated in tubs which were filled with fertilizer, rock phosphate and cow dung slurry. Fresh *Azolla* was seeded in the tubs and harvested in 6-8 days when the tub was fully covered with grown Azolla (Figure 1A and 1B). *Azolla* harvested was shade dried, powdered and used in the feed.

Azolla cultivated in tub

Dried Azolla

Fig 1A. Fresh *Azolla* cultivated in tubs within a week for feed preparation.

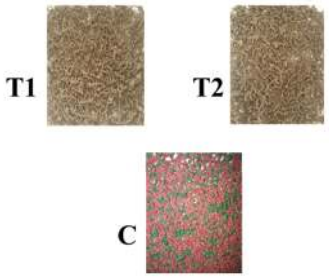

Fig 1B. Images of formulated fish feed for *Catla catla*. T1 feed, T2 feed and (C) control feed

Fish feed formulation

Along with dry *Azolla,* other ingredients like fish meal, wheat flour and groundnut cake were powdered and used as per the requirement of Catla feed farming studies. Feeds were prepared based on the ratio tabulated in Table1. For 100g of ground feed material 140 ml of water was used to make fine dough for extrusion. Dough was made to pellets using a kitchen extruder of pore size 2 mm. Then, the extruded pellets were dried in oven at 80°C for 36 hours. The nutrient content of the feeds was estimated by proximate analysis following the protocols for carbohydrate from (McCluer 1963) and protein, lipid, ash and moisture content from "Proximate analysis of feed stuff" (2009) which followed AOAC protocol.

Table 1: Fish feed composition

Ingredients	**Test feed 1 (T1) (%)**	**Test feed 2 (T2) (%)**	**Control feed (C) (%)**
Azolla	25	30	Nil
Fish meal	44	40	✓
Groundnut oil cake	11	10	Nil

Wheat Flour	20	20	✓
Soya bean meal	Nil	Nil	✓
Corn meal	Nil	Nil	✓
Yeast	Nil	Nil	✓

Fish feeding experiments

Fish feeding experiments were carried inside a poly house in polyethylene fish tanks at PSG College of Technology, Coimbatore, India during November 2018 to April 2019. Tanks were swabbed well; three-fourth of the tank was filled with tap water and then seeded with 150 fish fries in each tank. The tanks were cleaned once a week. The fish fries were fed with 1/3rd of their average body weight once a day every morning. Dry Azolla based fish feed formulations T1-25% Azolla replaced, T2-30% Azolla replaced and the C-control is a commercial feed.

Data collection and Analyses

Wet weight of the fry was measured using an electronic analytical balance, taking random sample (n=15) from each tanks and count of live fishes was also taken with monthly intervals. Percentage increase in weight of fishes was also calculated using current weight comparison to the original weight of the fish fries. Survival rate of fishes for each feed was also calculated.

% increase in weight gain = ((final weight – initial weight) / initial weight) *100

Survival % = (No. of fishes survived / No. of fishes initially introduced) *100

After a growth period of five months, nutrient content in fishes such as carbohydrate, protein and fat were analysed in fish samples. Cost of feed ingredients was noted and cost analyses were done for each feed.

Statistical analysis

The statistical analysis was done using one-way ANOVA in Excel. The p value and F value were calculated to measure the significance of the results obtained.

3 Results

Comparison of fish feeds

Nutrient composition of dry Azolla based feed formulations (T1 and T2) and control feeds are compared in Fig. 2. The test feeds were found to have matching levels of protein and carbohydrate with the Control feed. Lipid, moisture and ash levels were found to be low in the control feed (C). The lipid contents were 4.5 % and 6% in dry Azolla replaced Test feeds T1 and T2 respectively. Moisture content was 25% in the formulated feeds whereas it was only 10%, much lower in the control feed.

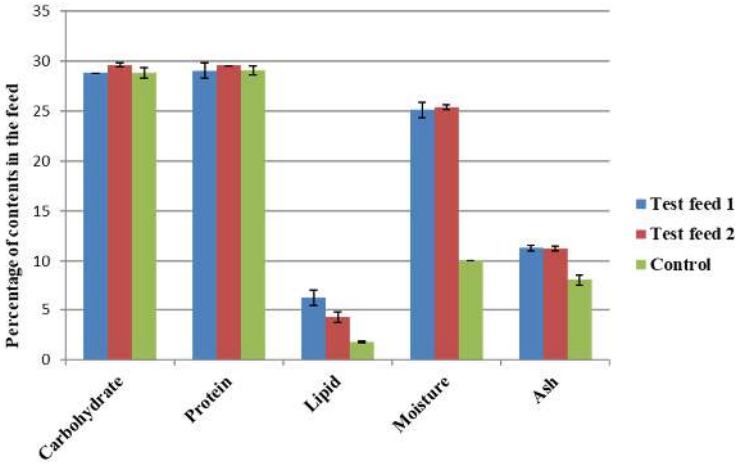

Fig 2. Graphical representation of quantitative analysis of test feeds and control feeds

Survival and Growth rate

The number of fish lings survived over a period of five months' time fed with test feeds T1, T2 and control were represented in figure 3. The mortality was higher in control feed compared to test feeds.

Growth rate of fish was measured in terms of weight gain in fishes (Fig. 4) and increase in the length of fishes (Fig.5). Overall, there was only a marginal weight gain difference of 2% (90-92%) observed between treatments. Test feed which had 30% replacement Azolla had the highest weight gain and control feed the lowest.

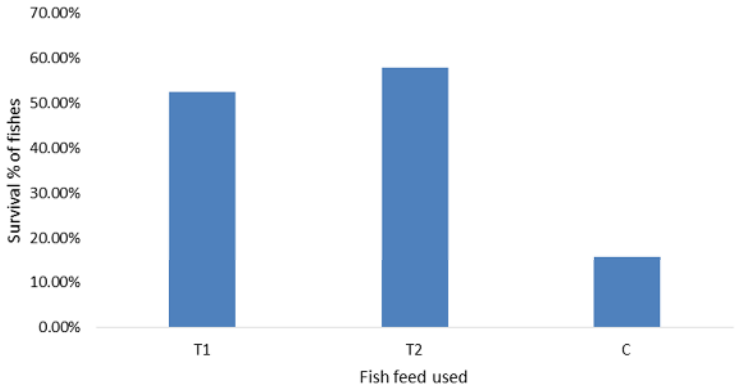

Fig 3. Graphical representation of survival of fishes in the growth period

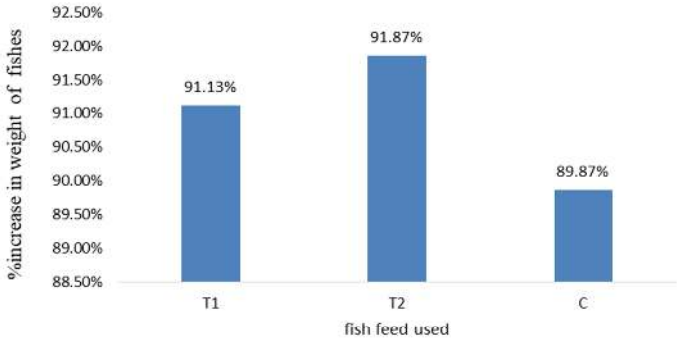

Fig 4. Graphical representation of percentage increase in weight of fish lings

A **B** **C**

Fig 5. Images of fish lings grown after five months

A) Fishes fed with T1 feed B) Fishes fed with T2 feed C) Fishes fed with C1 feed.

Nutrient content of fishes

The test feeds in which the protein source is replaced with Azolla but matched to the requirement of Catla fishes were given to them along with a commercially available fish feed. The nutrient status of the five month grown fishes were analysed by measuring the carbohydrate (Fig. 6), protein and lipid (Fig. 7). The results indicate that T2 feed with 30% Azolla replacement fed fishes had the highest carbohydrate, protein and lipid content. Fishes fed with the control feed had the lowest amount of carbohydrate and protein. The lipid content was found to be least in the fishes fed with T1feed. All the observed values of test feed and values for the fishes fed with test feed were significant ($P<0.05$) compared to the control feed and fishes.

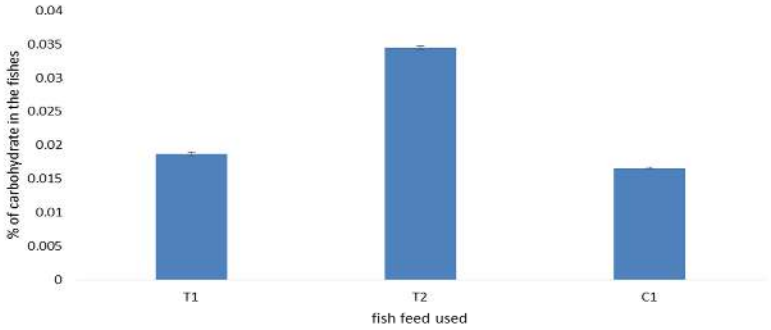

Fig 6. Graphical representation of percentage of carbohydrate in the fishes fed with different feeds

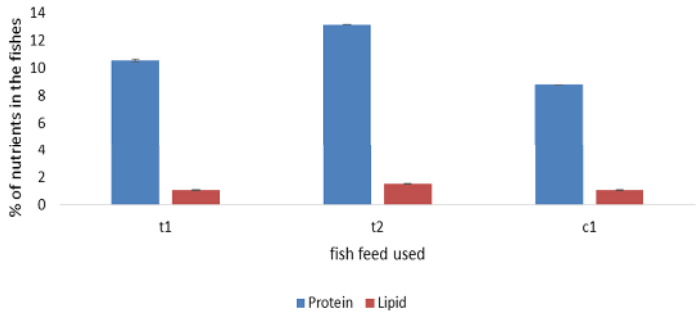

Fig 7. Graphical representation of percentage of protein and lipid in the fishes fed with different feeds

Cost analyses of feed

Azolla, a cheap source of protein was replaced to a tune of 25 and 30% in fish feed formulations to reduce the cost of fish feed. A cost comparison of ingredients used in three feeds used in the study is given in table 2. Fish meal was the high cost component. The test feed T2 which had the highest amount of Azolla had the lowest cost among the three feeds. The commercial feed price is the highest.

Table 2: Estimated cost of production of feeds used (for 1 kg of feed)

Ingredients	T1 (in rupees)	T2 (in rupees)	C (in rupees)
Dry *Azolla* powder	25	30	Nil
Fish meal	396	360	504
Groundnut oil cake	4.4	4	6
Wheat flour	16	16	23.2
TOTAL	441.4	410	533.2

4 Discussion

Dry Azolla replacement for protein in fish feeds of about 25% and 30% were evaluated in this study. Wheat flour, groundnut oil cake and fish meal along with Azolla were used to bring the required protein content. Arable land available for cultivation is reducing, hence aquatic fern that can be cultivated easily with protein accumulation of up to 176-208 g kg^{-1} DW (Brouwer et al 2018) is a suitable alternate protein feedstock for fish feeds. Protein content in fish feeds were matched to the requirement of Catla fish; on doing that fat and ash content were found to be higher than control feed (Fig.2). The lipid content in the formulated feed was in the range of 4.5 to 6%. The results coincided with the report of (Anitha et al 2016) who reported 4.5% lipid content in their study. The ash content in test feeds was higher than control (Fig. 2) since Azolla is a fern rich in minerals and hence the ash content is expected to be higher (Roy et al 2016). The quantitative analysis of feeds coincided with the findings for *Azolla* formulated feeds in ICAR (2006).

Evaluation of the formulated feed with the *Catla* fish lings indicated that 30% *Azolla* showed higher survival and increased body weight. Similarly, fresh water prawn showed high growth and good feed efficiency with diets containing 17% Azolla supplemented with or without Digestin enzyme (Goda et al 2018). In contrast, growth of carp was found to be poor when Azolla replacement was 15% and 25% compared to 5% (Abdalbakee and Mohammed 2019). Similarly, complete protein supplementation with soybean has shown reduced growth compared to 65% fishmeal silage in the growth of red tilapia fishes (Moharram and Raky 2007). Azolla replacement in poultry feed up to 10% in nera brown chicks has shown weight gain whereas, a 15% replacement showed reduced feed intake and weight gain (Alalade and Iyayi 2006). This study suggests that fishes prefer Azolla better than chicks.

Better growth performance in 30% Azolla replaced feed of fishes meant better digestion, absorption and feed conversion of nutrients to flesh. The protein content in fishes increased as the Azolla content increased (Fig. 7) in the feed suggesting a good nutrient utilization. A slight increase in lipid and carbohydrate levels (Fig. 6 and 7) were observed in fishes fed with 30% Azolla replaced feed. Similar observation, in fresh water prawn fed with 17% Azolla replaced diet was observed by (Goda et al 2018) in lipid content. However (El-Syed 1992) have reported that fresh and dry Azolla replacement in feed is negatively correlated to body protein and lipid contents in Nile tilapia. The cost of fish meal is ten times higher than the other components. Reducing the fish meal in feed will reduce the cost. The cost of Azolla based feed is lower due to the reduction in the fish meal component. Azolla is rich in calcium, phosphorous, potassium, ferrous, copper, magnesium, and several vitamins (Gouriet al 2012). The components present in Azolla are comparable to fish meal. Azolla can be increased up to 45% in fish feed (Mosha 2018) hence further reduction in fish meal can be evaluated in future.

5 Conclusion

In this study, it is observed that dry Azolla can be replaced to a tune of 30% in fish feeds without causing any adverse effect on growth and survival rate of Catla fishes. In addition, improved protein and marginal increase in fat content in Catla fishes were also observed. The cost of this unconventional feed ingredient is lesser than the traditional high

protein feed stocks. In future, dry Azolla can be used as a cheap protein source for growing fresh water carps.

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