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Evaluation of Mulberry (*Morus alba*, Linn.) leaf meal as a complete diet for sting fish (*Heteropneustes fossilis*, Bloch.)

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Abstract

An outdoor experiment was conducted to evaluate the suitability of mulberry (*Morus alba*) leaf meal as a total substitute (100%) for dietary fish meal for sting fish (*Heteropneustes fossilis*), having weight 5.0±1.0 g and length 7.0±0.5 cm. Three isonitrogenous (25 – 27%) and isocaloric (2250 – 2300 kcal kg^{-1}) feeds were used to monitor a 92-days old trial in small domestic ditches (4.5 m × 3 m × 1.2 m) near Balarampur, Paschim Medinipur, West Bengal, India. The feed prepared from mulberry leaf was proved to be the best in terms of survival rate, feed conversion ratio (FCR) and specific growth rate (SGR) than other two feeds prepared from groundnut oil cake and dried earthworms. A significant ($P<0.01$) difference was observed in total weight gain of the leaf meal administered to fish over other treatment series. The leaf meal was not only superior in stimulating growth but also showed increase in survival rate and disease prevention hen compared with other two feeds.

Key-Words: Mulberry (*Morus alba*), Leaf, Fish (*Heteropneustes fossilis),* Diet

Introduction

In Asia, fisheries play a vital role in the food and nutritional security of people, especially in rural areas (Sugunan, 2002). But the production of inland fish is far less than the demand and naturally the cost of the fish is high in this continent. So, there is a necessity for augmentation of inland fish production. For optimum production of fish, supplementary diet is required in addition to natural food present in the aquatic environment. Market available fishmeal is quite expensive and is in short supply. Alternative resources, such as squilla meal, shrimp/prawn meal (Mohammed, 1977), meat and bone meal, hydrolyzed feather meal, fleshing meal and blood meal (Paul et al., 1997), dried fish and chicken viscera (Giri et al., 2000) have been tried in diets replacing fishmeal either partially or fully, but even these pooled meals of various animal sources are not sufficient to meet the growing demands of fish raising industry and the continued dependence on traditional feed ingredients like rice bran, oil cakes and fish meal has led to increase in the prices of those components (Kumar, 2000).

Hence, there is need to find out good quality, cheaper and readily available alternative resources as artificial fish feed. Mulberry (*Morus alba*, Linn.) plant is cultivated in sericulture industries and their leaves contain a good quantity protein (21.1%) which can be used as a total substitute (100%) for dietary fish meal. This plant is resistant to drought and can. It is commonly cultivated for leaves in sericulture industry for feeding the silkworm (*Bombyx mori).* Excess leaves can be obtained at a cheaper cost from any sericulture farm for the preparation of fish feed. Sting fish (*Heteropneustes fossilis*), a hardy air breathing fish was selected in this experiment because it can withstand to different unfavorable conditions of water and can be

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cultured in small ditches, which has also a high demand in market as food fish.

The main objective of this study is to formulate a balanced low cost feed for sting fish from mulberry leaves which enhances the interest of fish farmers for farming sting fish and also to guide the fish farmers to make a low cost feed easily.

Material and Methods

Fish and Maintenance

The present trial was conducted at Balarampur, about 5 km away from IIT-Kharagpur premises at Kharagpur, Paschim Medinipur, West Bengal, India for a period of 92 days (as it replication) during June-August in the year 2010. Seven hundred and fifty number *Heteropneustes fossilis* fish (20±5 day old, 5.0±1.0 g wet weight; 7.0±0.5 cm length) were procured from local fish merchants. Each group comprised of 250 individuals, reared in domestic ditch containing 5400±100 lit. static water in triplicate.

The clean unchlorinated tube well water (temperature 32±4°C, pH 7.3 ± 0.05, available nitrogen 0.7±0.05, hardness 140±15 mg.l-1, ammonia 0.81±0.12 mg.l-1 and DO 6.04 mg.l-1) was monitored biweekly. A constant water level was maintained by adding tube well water at 5-days interval. These ditches were also fed with rain water from similar catchment area during the trial season.

Preparation of Feed

Preparation of Fish Feed was done such that feeds turn into iso-nitrogeneous (25 – 27%) and iso-caloric (2250 – 2300 kcal kg-1) using Peerson square board.

Mulberry Leaf Feed (ML): Excess mulberry leaves were collected from sericulture farm near Bethuadahari. Leaves were dried and ground properly. Then feed was prepared by mixing wheat flour with it (Mulberry leaf: 70% + Wheat flour: 30%).

Dried Earthworm Feed (DE): Excess older earth worms were segregated from vermicompost pit and released in to water for complete evacuation of food consumed by them. Then those worms were chopped, dried and ground to dust. Then feed was prepared mixing wheat flour as binder. (Dried earthworm: 53.85% + Wheat flour: 46.15%).

Groundnut Cake Feed (GC): Groundnut cake was collected from local market. Cakes were cleaned, dried and lastly dust. The feed was prepared by mixing wheat flour with it. (Groundnut cake: 60.86% + Wheat flour: 39.14%)

Feeding

Feed was weighed and given ad libitum in a feeding bag at 10:00 AM for two hours daily. The feed was given in a feeding bag at 10:00 AM for two hours duration in four fixed places hanging from an iron rod. Unconsumed feed was taken out and dried in a hot air oven at 100 °C. Feed consumption was estimated by subtracting the weight of the unconsumed feed from the weight of the offered feed. The feed conversion ratio (FCR) was computed as the amount of feed consumed / body weight gain. Fish, feed samples, and unconsumed feeds were weighted in a digital top pan balance with an accuracy of 0.1g.

Growth Calculations

Somatic Growth

Prior to the experiment, a few representative fish were measured for calculating average weight and length. Three fish from the stock were sacrificed to calculate the water content (Maynard and Loosli, 1962) and determined the initial dry weight of the fish. The fish in each ditch were collected and weighed on 32, 62 and 92 days (table 2) and the dry weight was calculated by using the percent water content of the fish sacrificed at the beginning of the experiment. Growth was calculated by subtracting the initial weight from the final weight. The specific growth rate was calculated using the following formula.

Specific growth rate: final weight of fish (g) - initial weight of fish (g)/ number of days

Gonad Estimation

Gonad weight was measured from day 70 (where as the gonad development observed on day 75 during the culture period) to day 92. Females from each treatment were sacrificed to study the appearance of ovary. The ovaries were removed and weighted in a digital top pan balance and the Gonad Somatic index (GSI) was computed according to the following formula given by Dahlgren (1979).

Gonadosomatic index: gonad weight of fish/fish body weight

Feed Analysis

Moisture content was analyzed by drying the feed in an electric hot air oven at (100±2˚C) (AOAC, 1995). The protein and lipid contents of the experimental diets were determined in a spectrophotometer following methods by Lowry et al. (1951) and Bragdon (1951) respectively.

Statistical Analysis

ANOVA (one-way) was used to find differences among the treatments.

Results and Discussion

Protein content was almost analogous (26%) (Table 3) in the three test feeds (ML, GC and DE). But the fish group fed with ML showed the highest somatic growth followed by DE fed and GC fed fishes respectively. Feed type contributed significantly to increase in gonad weight and GSI. The development of gonad first started on day 75 irrespective of any treatment and the weight gradually increased in all the treatment series. Gonado somatic index was highest (1.89) at ML fed fish followed by DE (1.23) and GC (1.01) fed fish. The highest gonad weight (0.85 g) was showed by ML fed fish. The FCR was lowest in ML followed by DE and GC (Table 2). Both the somatic and reproductive growth were significantly ($p>0.01$) higher in ML administered series. The value of FCR was also higher in ML treated series.

Limnological Parameters

The study of limnological parameters showed that the temperature and pH remained more or less identical in all the treatments. But highest values of DO (8.0±1.0) was found in ML treated ditches, where as other feed treated ditches (DE and GC) showed less DO content of the water during the course of the experiment. The values of TDS, specific conductance was lower in the mulberry treated series. The secchi disc transparency was moderate in ML treated ditches. The values of available nitrogen and phosphorus were comparable in the different treatment series. Though the values of BOD and COD were higher in GC and DE administered series, the differences were negligible and were statistically insignificant (Table 1).

Disease Resistance

Significant difference was observed ($p<0.05$) in the number of fish showing open sores, tail and fin rot diseases in the treatment series as the numbers were less in the ML treated series when compared with other two treatments.

Mortality Rate

The lowest mortality was found in ML treated series (Table 4).

The FCR was low in treated fishes ML, indicating a higher acceptance of feed by the test fish and the higher GSI indicates that the leaf meal is very good food supplement in promoting reproduction as well as somatic growth in the sting fish. The higher occurrences of open sores were observed in the sting fish cultured in ditches where GC and DE were applied. This indicates that ML feed provided necessary ingredients to body for protection from diseases.

The limnological results indicate a favorable condition in ML treated series as the level of DO was higher as well as other physico-chemical parameters were within very good ranges in the ML treated ditches. From the studies it appears that mulberry leaf treated ditches produced higher amount of fish as well as increased efficiency in development of gonad as well as disease resistance.

The increasing price of feed is considered one of the most important factors that limit profitability in aquaculture systems, caused mainly by the cost of fish meal used as a primary source of protein (McCoy, 1990). As a result, there is a constant search for alternative protein sources for aquaculture diets. Plant proteins are considered among the most viable alternatives to replace fish meal, since their production is only limited by the availability of land, assuming that the environmental conditions for the crops are adequate.

The nutritive value of different plant materials as fish feed ingredient has been studied widely (Boyd, 1962; Varghese et al., 1976; Jayaram and Shetty, 1980; Edward et al., 1983; De Silva and Gunasekara, 1989; Shetty and Nandesha, 1990; Ray and Das, 1992). There are also reports on the practical use of different plant materials as fish feed ingredients such as algal meal (Ahamad, 1966; Appler and Jauncey, 1983; Mustafa et al., 1994; Harish and Gajaria, 1995; Nandesha et al., 2001), Azolla meal (Fasakin et al., 2001), alfalfa meal (Krichen, 2007) tomato by-product meal (Azaza et al., 2006), ulva meal (Azaza et al., 2008). For catfish diets, other products have been considered, including palm kernel meal (Ng and Chen, 2002), cottonseed meal (Barros et al., 2002) and roquette seed meal (Fagbenro, 2004) as alternative of SBM.

But reports are scanty on the use of mulberry leaf as fish feed. However the present studies indicate that mulberry leaf can be considered as an essential ingredient in feeds for carnivorous fish species. The use of such meal as a complete diet of sting fish is expected to bring down the cost of fish production.

ML enhances feed efficiency and GSI. ML keeps most favourable limnological condition suitable for culture of sting fish. It is also transpired that ML increases the immunity power against common fish diseases.

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Table 1: Limnological parameters studied in the experiment

S/No.	Limnological parameters	ML	GC	DE
1.	Temperature (°C)	32.66±3.3	32.66±3.2	32.66±3.4
2.	pH	7.33± 0.6	7.36± 0. 5	7.46± 0.55
3.	Specific conductance (µ cm-1)	180±15	250±17	220±14
4.	Sechi disc transparency (m)	0.8±0.06	0.9±0.08	1.0±0.07
5.	DO (mg.l-1)	8±0.9	5±0.7	6±0.5
6.	CO2 (mg.l-1)	0.4±0.05	0.5±0.03	0.7±0.09
7.	TDS (mg.l-1)	270.6±25	375.6±29	331±32
8.	Available Nitrogen (mg.l-1)	0.8±0.06	0.8±0.09	0.7±0.05
9.	Available Phosphate (mg.l-1)	0.15±0.011	0.2±0.015	0.2±0.019
10.	COD (mg.l-1)	23±2	28±2.5	26±2.2
11.	BOD (mg.l-1)	5±0.7	8±0.5	7±0.9

Table 2: Growth parameters studied

Type of feed	Initial Weight (g)	Weight at 32 days (g)	Weight at 62 days (g)	Weight at 92 days (g)	FCR	SGR (% day-1)	Initiation of ovary development (day)	Body weight at the time of ovary development	GSI (In %) (at 92days)
ML	5	15	25	45	1.32	43.47	75	45	1.89
GC	5	9	13	25	1.53	21.73	75	25	1.23
DE	5	10	14	26	1.48	22.8	75	26	1.01

Table 3: Chemical composition of prepared feeds, % DM basis

Prepared feed	Crude protein	Carbohydrates	Lipids	Ash
ML	26	14.2	4.5	7
GC	25.9	12.8	3.5	7.2
DE	26.1	11.5	3.6	6.1

Table 4: Occurrence of diseases and mortality rate

Feed provided	Total No. of fish Stocked	Total No. of fish harvested	Number reduced by death	Mortality rate	Open sore	Tail and fin rot disease
ML	250	214	36	14.4	05	01
GC	250	187	63	25.2	19	05
DE	250	201	49	19.6	10	03