Full Length Article

Evaluation of *Medicago sativa* leaf powder as supplementary feed for the growth of Fish, C*irrhinus mrigala*

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Abstract

Presently, aquaculture has been the fastest growing food-producing sector in the world. The expansion of aquaculture production has been accompanied by rapid growth of aqua - feed production. As the increased prices and scarcity of traditional fishmeal, it is essential to search alternatives of fishmeal with low economical input. The global market requires enhanced efforts to thoroughly evaluate reasonable alternatives such as various plant feedstuffs. Viable utilization of plant feedstuffs to formulate aqua-feeds for the production of various fish species is an essential requirement for future development of aquaculture. There is a scope to formulate fish diets containing locally available plant protein sources. In the present study, fishmeal is replaced by a plant protein product to achieve fish growth. *Medicago sativa* is the plant used in the study for fish *Cirrhinus mrigala*. Fish shows good growth results to plant protein diet as compared to control.

INTRODUCTION

In fish farming, nutrition is critical because, feed represents 40-50% of the production costs. Fish nutrition has advanced dramatically in recent years with the development of new, balanced commercial diets that promote optimal fish growth and health. Fish meal represents an ideal nutritional source of dietary protein and lipid for fish. It is the most important protein source for aquaculture industry because of its high biological value, but this is a limited food resource and there is serious concern on the long-term availability of this feed stuff for use in fish diets (Hardy, 1996). The aquaculture industry depends worldwide on the availability of low cost, high quality feed. Continuous efforts are being made by the nutritionist to reduce the feed cost as a strategy to sustainable aquaculture. Over several decades, fish intuitionists have evaluated alternatives sources of plant origin protein in fish diets as partial or total fishmeal replacement (Ramchandran and Ray, 2004).

In order to reduce the cost of balanced fish diet, locally available ingredients such as agricultural byproducts and plant proteins should be included in the diet or substituted for expensive animal, plant protein sources. Research interest has been focused on different leaf meals as protein sources in animal feed. Fish nutritionists have evaluated alternative sources of plant origin protein in fish diets as partial or total fishmeal replacement (Ramachandran and Ray, 2004; Goda et. al., 2007).

Medicago sativa (Alfalfa) plant has been widely used in terrestrial animal feeds, as it is rich in protein with a balanced amino acid profile, vitamins, and carotenoids. However, attempts to incorporate alfalfa products in fish feeds have been met with mixed success (Chatzifotis et. al., 2006). However, complete replacement of fish meal with individual plant protein has generally resulted in a decrease in fish growth performance (Sklan et. al., 2004). The present study was undertaken to assess the partial replacement of fish mealprotein with plant source, *Medicago sativa* for selected

fish species, *Cirrhinus mrigala*.

Materials and Methods

The feeding experiment was conducted in triplicate for 90 days. Fingerlings of *Cirrhinus mrigala* weighing 2.00 to 2.50 g were used for the experiment. Four types of pelleted feeds were formulated using different ingredients such as rice bran, groundnut oilcake, fishmeal, guar gum binder, Vitamin – Mineral mixture, fine powder of *Medicago sativa* leaf powder in different proportions (Table 1). Experimental diets were analyzed for proximate composition such as moisture, crude protein, crude fibre and total ash.

Table 1: Percentage and proximate composition of different ingredients used in formulated pelleted diets (per 100 gm):

Ingredients	Control	30% Diet	40% Diet	50% Diet
Rice bran	52	20	17	15
Groundnut oilcake	37	40	34	26
Guar gum Binder	10	09	08	08
Mineral – Vitamin mixture	01	01	01	01
M. sativa leaf Powder	00	30	40	50
Proximate composition				
Moisture (%)	7.05	5.19	6.79	5.84
Total Ash (%)	12.13	9.39	9.48	9.54
Crude Protein (%)	19.24	27.45	29.77	26.94
Crude Fiber (%)	16.54	10.05	10.85	12.51

Fishes were fed at the rate of 5% body weight in two equal rations daily. At fortnightly intervals a minimum of 50% of fishes were sampled to record the growth. At the end of experiment the growth performance of experimental fishes was determined in terms of final individual fish weight (g), Specific Growth Rate (SGR, % per day), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Feed Efficiency Ratio (FER) and Net Protein Retention (NPR).

Fishes were sacrificed; liver and muscle tissues were dissected out as quickly as possible and stored to analyze total glycogen, total protein and total lipid contents of liver and muscle tissues. These tissues were weighed and used for the estimation of biochemical components like protein (Lowry et. al., 1951), Glycogen (De Zwaan and Zandee, 1972) and lipids (Barnes and Blackstock, 1973).

Results and Discussion:

Growth performance:

The growth, gain in weights, feed conversion efficiency, specific growth rate, protein efficiency ratio, net protein retention data of fish fed with various test diets containing different levels of *Medicago sativa* plant leaf powder meal are summarized in table 2.

Table 2: Growth performance and feed utilization of *Cirrhinus mrigala* with different experimental diets:

	Control	30 % Diet	40 % Diet	50 % Diet
Initial average weight (gm)	2.15 ± 0.02	2.4 ± 0.03	2.2 ± 0.03	2.2 ± 0.02
Final average weight (gm)	12.6 ± 0.05	13.7 ± 0.06	13.3 ± 0.05	12.1 ± 0.05
Total weight gain	10.45± 0.03	11.3 ± 0.04	11.1 ± 0.04	9.9 ± 0.03
Specific growth rate (SGR)	0.730± 0.02	0.714± 0.01	0.747± 0.02	0.743± 0.02
Food conversion ratio (FCR)	0.047± 0.01	0.044± 0.01	0.042± 0.02	0.045± 0.02
Protein efficiency ratio (PER)	20.9± 0.12	22.6± 0.17	23.6± 0.21	22.2± 0.19
Net protein retention (NPR)	88.35± 0.68	91.07± 0.79	100.77± 0.95	89.08± 0.91
Feed efficiency ratio (FER)	23.75± 0.12	28.25± 0.23	31.89± 0.21	41.11± 0.17

The highest weigh gain was seen in 30% plant diet group, while 50% plant diet showed least growth among all the feeds. All feeds groups showed better growth results as compared to control.

Biochemical alterations:

The proximate composition of liver and muscle tissues of fish fed with different plant protein incorporated diet was shown in table 3 and 4. The 40% plant diet shows highest protein content in both liver and muscle tissues, whereas the 50% plant diet shows related lowest protein content. As compared with control diet, all plant protein based diets showed better protein content increase. The lipid content of liver was higher in control diet, whereas 30% and 50% plant diet showed highest lipid content in muscle. The glycogen content was very less in both the tissues. The highest activity was observed in 40% plant diet for both the selected tissues.

Table 3: Biochemical alterations in liver tissues from the fish *C. mrigala* fed with *M. sativa* plant diet:

	Total Protein	**Total Lipid**	**Total Glycogen**
Control	17.53 ± 0.37	17.19 ± 0.56	0.9 ± 0.04
30% Diet	17.49 ± 0.88 NS	15.53 ± 0.35 NS	1.63 ± 0.21 **
40% Diet	18.59 ± 0.49 NS	12.60 ± 0.20 *	0.77 ± 0.02 NS
50% Diet	11.83 ± 0.57 ***	14.16 ± 1.15 NS	0.65 ± 0.05 NS

(Value expressed in mg/100mg wet tissue; ±: SE)
*P<0.05, **P< 0.01, ***P< 0.001, NS – Non Significant

Table 4: Biochemical alterations in muscle tissues from the fish *C. mrigala* fed with *M. sativa* plant diet:

	Total Protein	**Total Lipid**	**Total Glycogen**
Control	22.34 ± 0.40	6.16 ± 0.14	0.16 ± 0.01
30% Diet	32.36 ± 0.78 ***	8.73 ± 0.16 **	0.48 ± 0.01 ***
40% Diet	35.16 ± 0.54 ***	4.68 ± 0.30 NS	0.47 ± 0.05 ***
50% Diet	25.82 ± 0.33 *	8.70 ± 0.37 **	0.34 ± 0.02 **

(Value expressed in mg/100mg wet tissue; ±: SE)
*P<0.05, **P< 0.01, ***P< 0.001, NS – Non Significant

In the present study, it is observed that, the growth of fish was increased up to a certain limit depending upon the incorporation of plant protein in the feed. Above the optimum level of inclusion of plant proteins, the retarded growth was observed. In the present study, the experimental fish, *C. mrigala* showed good and increased growth upto 40% inclusion level and suddenly shows decreased growth for 50% inclusion. Determination of palatability of a feed ingredient is an important criterion in the evaluation of that ingredient for fish. The growth of fish depends upon the ingredients and its percentage in the formulated feed (Glencross et. al., 2007).

A progressive decline in the NPR and PER values with an increasing level of raw *M. sativa* meal in diets was recorded in the present investigation. Similar declining trends in NPR and PER values have also been reported with higher levels of inclusion of copra meal (Mukhopadhyay and Ray, 1999) and grass pea seed meal (Ramachandran et. al., 2005) in carp diets.

Feeding trials, under laboratory conditions on *C. mrigala* have revealed that weight gain and biochemical alterations was significantly high in the fish groups fed on formulated feed. These results are in agreement with Sadiku and Jauncey (1998) and Vhanalakar and Muley (2014). The proximate carcass composition of the fish at the end of the feeding trial showed significant increase in protein and lipid in comparison to the initial value in all dietary treatments. The values of carcass protein and lipid contents were found to be higher in the fish fed diets with 40% plant meal. The study results are in agreement with previous findings where similar trends were noted with higher levels of fermented sesame seed, leaf meals and grass pea seed meals in carp diets (Mukhopadhyay and Ray, 1999; Bairagi et. al., 2002). The trend of results

obtained for carcass composition in this study agreed with similar results reported in dehydrated alfalfa and salt bush, Atriplex (Yousif et. al., 1994) in *Oreochromis aureus* L. diets and *Azolla pinnata* (El-Sayed, 1992) in *O. niloticus* L. diets.

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