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Effect of Different Feed Ingredients and Low Temperature on Diet Acceptability, Growth and Survival of Mrigal, *Cirrhinus mrigala*, Fingerlings

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Abstract.- Studies were conducted to investigate the possibility of partial or total replacement of fish meal with cheaper plant protein sources in the artificial diets for mrigal fingerlings. Second objective of these studies was to find if winter feeding could have an effect on growth of fish. There were four diet formulations. Diet 1 contained 45% fish meal and served as reference diet. Fish meal in the rest of the diets was substituted by sesame oil cake, mustard oil cake and blood meal. All the diets contained approximately 30% protein. In the first week of December mrigal fingerlings were collected from Hatchery ponds and weighed which averaged 3.897±5.516 - 4.267±3.559 g each. They were acclimatized to experimental environment for two weeks. Ten individuals were then randomly stocked in each fiber glass tank. Each dietary group had two replicates. Prepared feeds were offered @1.5% of fish wet biomass from December, 22 to February, 22. After two months of feeding, experiment was terminated. Tank water was completely drained. Fish were harvested and weighed individually. Growth of fish showed no significant differences among treatments. Rather group fed on sesame oil cake based diet grew comparatively better indicating strong possibility of total or major portion of fish meal replacement in mrigal diets. Secondly it was observed that fish did show growth during winter months and this growth increment can compensate its inherited slow growth capability, compared to other Indian major carps, to some extent, if fed properly during colder months.

Key words: *Cirrhinus mrigala,* artificial diets, Oil cakes.

INTRODUCTION

Cirrhinus mrigala is an important freshwater fish species cultured in Asia particularly in the Indian subcontinent (Khan *et al*., 2004). It is a bottom feeder and thrives on decaying organic matter and vegetable debris. Like its other family members, it is poikilothermic and its growth, food consumption, food conversion and other body functions are markedly influenced by water temperature (Andrews *et al*., 1978; Elliott, 1982; Hawkins *et al*., 1985; Houliham *et al*., 1993; Britz *et al*., 1997 and Azevedo *et al*., 1998). Its optimum growth and survival is only possible if it is reared in a water having a defined temperature (Gaddowski and Cadded, 1991) which normally ranges from 25-30°C (NRC, 1983). Therefore, it grows maximally at this temperature during the summer months. Onset of winter is followed by proportionate decrease in water temperature, sometimes below 10°C which influences the biological functions in fishes specifically food intake and growth rate. This state of affairs persists for four months (November to February) which shortens the growing season of fish by four months (Khan *et al*., 2004). During this stagnant period fish farmers stop feeding and start harvesting. Fish is therefore, starved and does not gain any weight at all.

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Supplementary feed in the form of single ingredient or mixture of two or more ingredients where fish meal serves an important animal protein source then becomes mandatory to enhance fish growth and to increase final out put. Among others fish meal is a widely used and expensive protein component (Carter and Hauler, 2000; Engin and Carter, 2005). It is well established that the partial or total replacement of fish meal in fish diets with cheaper and more reliable alternate protein sources without compromising growth has been a priority in aquaculture nutrition research (Morales *et al*., 1994; Hardy, 1996). Besides price and availability, the quality of the protein (availability and balanced amino acid profile) is an important determinant of the efficiency of deposition of dietary nitrogen within the body (Kaushik, 1996; Refstie *et al*., 1997). Research on many potential plant and animal proteins has been limited because of their unbalanced amino acid profiles and low protein content (Carter and Hauler, 2000). In addition, plant meals contain anti-nutritional factors including protease inhibitors and complex carbohydrates (oligosaccharides; phytates), which can impair growth performance and nutrient utilization in monogastric animals including fish (Wilson and Poe, 1985; Hernandez-Infante *et al*., 1998).

The present study was therefore, initiated to determine the economic aspects of feeding *Cirrhinus mrigala* during winter season and to evaluate the formulated diets for this fish in which the expensive and less available feed ingredient have been substituted with comparatively cheaper and readily available plant protein sources.

MATERIALS AND METHODS

Experimental design

Feeding trial was designed as One way Analysis of Variance (ANOVA).

Experimental procedure

Cirrhinus mrigala fingerlings obtained from Govt. Fish Hatchery, were acclimated to experimental conditions for two weeks and fed control diet only. Proper water quality (pH 7.5; DO≥ 5ppm) was maintained to avoid any unnecessary pre-experimental stress to fish.

Experimental system consisted of 8 fiberglass tanks (with water holding capacity of 600 L) 2 for each treatment. These tanks were divided into four groups. Forty fish (3.54 g each on the average) were randomly collected from the bulk and transferred into each rearing tank. These fish were starved for 24 hours. Five fish were then randomly taken out from each tank, anesthetized with MS 222 and weighed for initial biomass estimation. All the four diets were randomly assigned to a group of two fiberglass tanks interspersed with tanks of different dietary group. The diets were offered to respective group at 8.00, 12.00 and 18.00 hours. Every third day 1/3rd of total water volume was replaced by fresh tap water. Supplementary aeration was not provided due to low temperature and availability of sufficient oxygen (≥ 5ppm) for fish in each tank. Assessment of nitrogen, nitrite and nitrate level was not considered necessary due to regular water replenishment. Diet acceptability was regularly and closely observed in each group (Table II). Left over feed and fish feces accummulated at the tank bottom, was regularly removed to maintain proper water quality. There was no oily film on the water surface. Feeding trial lasted for nine weeks. At the termination of experiment fish were starved for 24 hours. Water was lowered to quarter of the total water volume and all the fish were harvested. Fish were counted from each tank to calculate survival rate and then weighed to estimate weight gain.

Preparation of diets

All the four diets were formulated from locally available feed ingredients. Fish meal based diet served as control. Sesame oil cake, mustard oil cake and blood meal based diet were fed to fish @ 1.5% of wet fish biomass to explore if they could replace fish meal, an expensive feed ingredient in practical fish diets (Table I). Individual ingredients were not chemically analysed. Formulation was based on literature values because differences in chemical composition of all the local varieties were negligible. Prepared diets were dried at 60^{o}C in an incubator up to constant weight. They were finely ground to match mouth gape of experimental fish for its easy intake. Final diets were proximately analysed.

Water quality parameters

Water from each dietary group was weekly analyzed in the morning and in the evening for temperature, dissolved oxygen (DO) and pH.

Chemical analysis

Proximate composition of diets was determined using standard methods (AOAC, 1995). A duplicate sample from each diet was dried overnight in convection oven at 105°C to determine dry matter contents. Protein was determined by microkjeldahl method. Total lipids were extracted in petroleum ether for quantitative estimation (AOAC, 1995). Ash contents were determined after incinerating the feed samples in a muffle furnace at 550°C (Table I).

Table I.- **Ingredient composition and proximate analysis of feed used for rearing of mrigal fingerlings during winter season. The values are given in percentage.**

Ingredient	Fish meal based diet	Sesame oil cake based diet	Mustard oil cake based diet	Blood meal based diet
Ingredient composition				
Fish meal	45	-	-	4
Sesame oil cake	10	50	-	-
Wheat flour	17	5	2	30
Molasses	4	4	4	4
Bone meal	5	-	-	2
Rice powder	17	4	3	15
Vitamin premix	1	2	2	2
Gelatin	1	1	-	1
Mustard oil cake	-	-	50	-
Egg shells	-	4	8	-
Corn gluten meal	-	30	30	10
Blood meal	-	-	-	30
α-cellulose	-	-	1	6
Total	100	100	100	100
Proximate analysis				
Moisture	8.8	9.09	9.0	5.6
Crude protein	30.8	29.8	29.81	30.81
Ether extract	7.25	11.03	5.35	4.31
Nitrogen free extract	32.2	45.26	43.64	30.03
Ash	7.23	5.05	4.22	4.3
Ca. P. fiber	13.72		7.98	24.68

Note: All the diets contained 30% protein in them.

Statistical analysis and weight increment calculations

One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range test was used to evaluate the statistical significance of differences among the treatments. Differences between the treatment means were considered significant at $p<0.05$ level. Biological performance of fish was assessed by the following mathematical formulae (Ricker, 1979). Digestibility studies were not attempted in this trial. While calculating FCR, loss of feed because of fish agitation and leaching was not taken into account.

Specific growth rate (SGR) $= (\log_e Y_2 - \log_e Y_1) \div (t_2 - t_1) \times 100$

Feed conversion ratio (FCR) = Feed consumed (g dry weight basis)÷Wet weight gain (g)

Survival $= N_t \times 100 \div N_o$

where Y_1 is initial weight of fish; Y_2, final weight of fish; t_1, initial time; t_2, final time; N_o, initial number of fish in each replicate; and N_t, final number of fish in each replicate.

RESULTS

Growth and survival

Fish in all the dietary treatments grew equally. Final weights ranged from 5.453±1.453 to 5.847±6.898 g. Differences among treatments were statistically insignificant (Table II). Other growth parameters showed same pattern and variability was not discernable (Tables III, IV). Mortality was highest (25%) in sesame oil cake fed group and lowest (18.75%) in mustard oil cake group (Table III). Apparently it seemed a big difference but when subjected to statistical analysis differences disappeared and were not distinguishable (Table III). Feed consumption was far less than normal hence feed remained excess in tanks. Leftover feed, however, did not foul water quality due to low temperature and regular water replenishment. Level of glucosinulates in plant ingredients and their effect on fish was not examined during these studies.

Table II.- **Acceptability score of different meal based diets fed to mrigal fingerlings.**

S.No.	Type of diet	Diet acceptability score
1.	Fish meal based diet	8
2.	Sesame oil cake based diet	10
3.	Blood meal based diet	8
4.	Mustard oil cake based diet	6

Diet acceptability

Sesame oil cake based diet was eagerly taken up and well accepted (Table II). Fish meal based and blood meal based diet were the second highest in acceptability, while mustard oil cake based diet was least accepted.

Water quality parameters

Differences were observed in pH and dissolved oxygen among the different treatment tank water but they were so minute that can not have any bearing on experimental results and not worth for record and any generalizations for future guidance. Little increase was observed in pH in the evening which might be due to leaching and accumulation of oil (fatty acids).

Table III.- Initial weight (g) and length (cm) of mrigal fingerlings fed on formulated feeds based on various animal and plant protein sources. The values are presented as X±SE. Values in the column followed by the same superscript letters are not significantly different from each other at p<0.05.

Treatment	Initial Mean weight(g)	Final Mean weight(g)	Initial Mean length(cm)	Final Mean length(cm)	Mortality(%)
F.M. based diet	4.165±4.616E-01[a]	5.633± 1.695[a]	7.367±3.670E-01[a]	9.083±6.338[a]	2.123±8.394E-01[a]
S.O.C. based diet	3.988±6.083E-01[a]	5.847±6.898E-01[a]	7.267±3.445E-01[a]	8.983±3.971E-01[a]	3.333±1.021E-01[a]
B.M. based diet	4.267±3.559E-01[a]	5.613±9.228E-01[a]	7.650±3.886E-01[a]	8.917±5.269E-01[a]	3.125±1.723[a]
M.O.C. based diet	3.897±5.516E-01[a]	5.453±1.453[a]	7.667±7.711E-01[a]	8.433±7.815E-01[a]	2.344±1.239[a]

Table IV.- Specific Growth Rate (% increase per day) and Feed Conversion Ratio of mrigal fingerlings over two month period.

Treatment	SGR	FCR
Fish meal based diet	0.50±0.23	2.55±0.34
Sesame oil cake based diet	0.63±0.19	1.93±0.27
Blood meal based diet	0.45±0.20	2.85±1.03
Mustard oil cake based diet	0.56±0.25	2.25±0.80

Where SGR, Specific growth rate, FCR, Feed conversion ratio.

Economics

When economics of winter feeding was calculated, it gave encouraging figures. It indicated that winter feeding is beneficial. Fish can give positive and beneficial response if fed properly. Findings have not been recorded in this paper because of its non-applicability to this situation.

DISCUSSION

Cirrhinus mrigala is a bottom feeder and poikilothermic fish means heavy dependency on temperature for efficient performance of all the biological activities. All the above it is a slow growing fish as compared to other members of polyculture system. March to October is its peak growth period due to higher temperatures which increase its metabolic needs, food consumption, conversion and growth rate (Harris and Bodaly, 1998; Drinkwater, 1998).

Results of our studies revealed that growth deficiency (800 g and 200 g) when compared with *Catla catla* and *Labeo rohita*, respectively) (Santhanam *et al*., 1990) of mrigal experienced during summer, peak growing months, can be compensated to some extent, if fed properly in a carefully managed rearing system during winter months. It has further proved that growth achieved during winter is economical and encouraging (Coutant, 1977; Scott, 1982; Khan *et al*., 2004). Onset of winter season and decrease in temperature depresses its growth (Boehlert and Yoklavich, 1983) and its competition with other species also intensifies. Winter feeding, therefore, was thought an option to add some weight to this slow growing fish, response of which was quite positive. This practice can also be applied to other species with beneficial results because farmers normally do not feed their fish during winter months. Winter feeding, therefore, will not only maintain but will also increase fish weight.

Our studies have further demonstrated that fish meal protein could be replaced by blood meal or plant protein like sesame oil meal and mustard oil cake without compromising growth in *Cirrhinus mrigala* fingerlings. Though 100% fish meal was replaced by plant/animal protein sources still all the fishes in each group performed equally well in growth and survival. Reasons of little mortality observed in sesame oil meal based diet are not clear at this moment. Level of unsaturated fatty acids in oil and their rancidity can be the possible reason but if it is, it should have affected growth first. Nevertheless, there is a lot of benefit in replacing fish meal by other cheaper plant proteins due to continuously increasing its demand and price. Better acceptability of sesame oil meal based diet by fish might be due to absence of endogenous anti-nutrtional factors like glucosinolates, phytic acid and tannin which were present in mustard oil cake (De Silva and Anderson, 1995).

Corn gluten meal has successfully replaced fish meal protein for several fish species because of its high protein and low fiber contents (Morales et al., 1994; Robaina *et al*., 1997; Watanabe *et al*., 1997; Kikuchi, 1999; Pereira and Oliva-Teles, 2003). Corn gluten, meat and bone meal with essential amino acid supplementation fed Nile tilapia performed equally well in growth and survival when compared with fish meal based diet group (Victor *et al*., 1999). Mbahinzireki *et al*. (2001) in their studies has replaced 50% fish meal by cotton seed meal without affecting growth and survival and causing no toxicity in *Oreochromis* sp. in re-circulating systems. Similarly Borgeson *et al*. (2006) in their studies has replaced unimaginably high concentrations (70%) of fish meal by complex mixture of plant ingredients in Nile tilapia (*Oreochromis niloticus*). Mukhopadhyay and Ray (1996) has replaced up to 30% fish meal by deoiled sal (*Shorea robusta*) and in following studies (1999) 40% with soaked copra meal in rohu (*Labeo rohita*). *Spirulina platensis* has completely replaced fish meal in *Catla catla* and *Labeo rohita* diets (Nanadeesha *et al*., 2001). Higher concentrations of detoxified castor bean oil, however, have negative effect on juvenile grass carp (*Ctenopharyngodon idellus*) (Cai *et al*., 2005). Hasan *et al*. (1997) did not get any difference in feed acceptability, survival, growth, feed conversion, protein utilization, protein digestibility, body composition and histopathological changes in plant ingredient based diet fed common carp (*Cyprinus carpio*) compared with fish meal based diet. El-Saidy *et al*. (2005) has successfully replaced 50% fish meal by cluster bean meal in common carp without any significant differences in weight gains, specific growth rates, feed conversion ratios, feed efficiency ratio, protein efficiency ratio and feed intake.

Replacement of fish meal by plant protein, however, has been a major problem in salmon feeds due to their relatively low protein content and tropical formulation to contain relatively high protein and oil contents (Carter and Hauler, 2000) but still Luo *et al*. (2006) successfully replaced 50% fish meal by plant protein in juvenile rainbow trout (*Oncorhynchus mykiss*) without affecting feed acceptability, digestibility and growth. Addition of soy protein in place of fish meal did not affect growth of rainbow trout, soy flour however did, probably due to poor carbohydrate digestibility (Kaushik *et al*., 1995). Zhou *et al*. (2005) has replaced up to 40% fish meal by defatted soybean meal in the diets formulated for juvenile cobia (*Rachycentron canadum*) without affecting its growth. De Francesco et al. (2007) has replaced up to 75% fish meal by plant protein in gilthead sea bream (*Sparus aurata*) with no difference in weight gain, specific growth rates and body composition from that of fish meal based diets. Previously Wilson and Poe (1985), Pongmaneerat and Watanabe (1993) and Carter *et al*. (1994), have replaced fish meal by soybean meal products in eels without affecting growth, however, it was not possible in 2 g eels without increased inclusion of fish or chicken meal. Garcia-Gallego *et al*. (1998) has attempted to replace fish meal by sunflower meal with added essential amino acids in European eels with sufficient success. Pham *et al.* (2007) has replaced 40% fish meal by mixture of cotton and soybean meal with methionine and lysine supplementation in juvenile Japanese flounder (*Paralichthys olivaceus*) without any significant difference in growth performance, feed utilization and whole body composition. Previously Eusebio *et al*. (2004) while working on juvenile grouper (*Epinephelus coioides* Hamilton) observed comparable apparent digestibility of white cowpea meal and defatted soybean meal, wheat flour and shrimp meal with those of fish meal diets (84-89%).

Comparison of investigative work on different fish species in above paragraphs shows that plant protein can replace fish meal partially in carnivorous fish species and almost totally in warm water species like Nile Tilapia, common carp, Catla and rohu. Our findings favourably corroborate with those of previous workers and further confirm that fish meal can be conveniently replaced by cheaper plant protein sources totally in herbivorous fish varieties while partially in carnivorous fish species. Nevertheless, it is much easier for *Cirrhinus mrigala* to utilize plant proteins and carbohydrates due to similar habitat, digestive morphology and physiology and feeding characteristics. Type and form/chemical nature of ingredient, however, drastically affect the fish performance and this varies from species to species which is very much evident in current studies, differences were obvious though a few ingredients were tested. Fish culturists/hatchery managers will be highly comfortable and fish farming will be very cost effective with the total elimination of fish meal from artificial diet formulations for locally culturable herbivorous fish varieties. Therefore, our studies are not only suggestive but can be conclusive in the inclusion of plant proteins in place of fish meal for mrigal in the formulation of cost effective artificial feeds.

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