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Partial Replacement of Protein Using Microfloc Meal for the Diet of Mrigal, *Cirrhinus mrigala* Fingerlings

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A B S T R A C T

The experiment was conducted in Aquaculture lab of CIFE (New campus) and fingerlings (5-6cm in size) of mrigal were brought from carp hatchery. Four compositions of experimental diet were prepared by using microfloc with fishmeal in different ratio and control without microfloc. For the preparation of biofloc Ammonium chloride (NH_4Cl_2) used as a nitrogen source (100g) and Sucrose (100g) as a carbohydrate source. Three treatments with each of triplicate were used to get the maximum accuracy in result. Fingerlings were fed with experimental diet with 5% of body weight (BW). Sampling was done at 10 days interval. At the end of the experiment, the various parameters of fingerlings were determined using best statistical methods. Apart from four treatments (T) the mean weight gain in control (0.87±0.20), T_1(1.05±0.18), T_2(1.30±0.21)and T_3(1.62±0.09), maximum mean weight gain *i.e.* 1.62±0.09gm, was observed in case of fingerlings fed with 1:1 ratio of microfloc and fish meal respectively. Similar results were seen in case of Specific Growth Rate (SGR) where it was 0.31±0.02gm for T_3. The overall experiment shows the fingerlings fed with microfloc gives better specific growth and mean weight gain.

Introduction

India ranks second in the world in total fish production and carps being the mainstay of Indian aquaculture. Carps contribute more than 87% in freshwater aquaculture production. (Naylor *et al.,* 2000) Among these, mrigal is the third most produced Indian major carps. Among carps, mrigal is mainly cultured for meat, due to its taste and quality protein (Rao *et al.,* 2013). Mrigal mainly feeds on dead and decaying organic matter in the adult stage but during its juvenile stage it feeds on planktonic organisms. In aquaculture feed contribute 60% of the production cost and presently the major problem in aqua feed is availability of ingredients especially fishmeal. Fishmeal is one of the important feed ingredient as it is an excellent source of essential nutrients such as protein and indispensable amino acids, essential fatty acids, cholesterol, vitamins, minerals, attractants and unidentified growth factors (Swick *et al.,* 1995; Samocha *et al.,* 2004). However, major problem is its availability and high price compared to other protein sources because of its high demand. Therefore, the recent researches are aimed to produce the quality feed with partial or full replacement of fishmeal with other available protein rich ingredients. A recently developed biofloc technology provides ingredient that

has potential for use in feed is microbial floc meal MFM (Kuhn *et al.*, 2009, 2010). According to Kuhn *et al.*, (2010), the use of MFM as an ingredient in aqua feed if implemented successfully, could be a sustainable alternative to the use of fishmeal. Thus, the conversion of effluents from biofloc to MFM can offer the aquaculture industry an alternative source which reduce the protein content required in feed, improve feed conversion rates by minimizing potential environmental problems. This study aims to analyse the effects of the dietary replacement of fishmeal with MFM on the performance of *cirrhinusmrigla* fingerlings to optimise the inclusion level of microbial protein in mrigal fingerlings feed with fishmeal replacement

Materials and Methods

Diet formation and preparation

The produced biofloc was dried and mixed with feed as an ingredient and fed to fishes. The microbial protein added to that different composition with the replacement of fishmeal ingredient. The ingredients were triturated before diet preparation. The pre-weighed ingredients were mixed and the mixtures were pelleted using a meat grinder to form pellets were dried at 60 °C for 24 h. Finished diets were stored in plastic bags at −18 °C until use. Diets contained the essential amino acid requirements for fingerling to fulfil the desired amount Upon termination of the 4-week growth trial, final weights of the Fishes were obtained. Survival (final number of fish/initial number of fish)×100; specific growth rate (100%×[ln final weight−ln initial weight]/trial duration) and for each dietary treatment were determined. Dissolved oxygen and temperature, pH were measured.

Composition of experimental feed

The ingredients used for feed preparation were Fish Meal; Soya bean Flour, Ground Nut Oil Cake, Wheat Bran, Corn Flour, Oil Mix, Vitamin Mineral Pre mixture, CMC and BHT. Before going to prepare the feed composition of feed was predetermined according to treatments.

Ingredients are taken based to their composition and kept separately according to their treatments. Except BHT, Vitamin Mineral Premix and Oil mix the remaining ingredients were mixed thoroughly and cooked in pressure cooker for 30 minutes. Cooking is done to improve the digestibility of the ingredients. Then it was too with remaining ingredients and made in to balls. Then it was pelletized using hand pelletizer and dried in room temperature.

Experimental system

The experiment was performed at central institute of fisheries education (CIFE- New Campus) over a 30-day period. The treatment consisted of four diets with 0, 30, 40, and 50% replacement of fishmeal with MFM. Each diet was replicated three times, and replicates were distributed randomly. The experiment was conducted in a static indoor rearing system consisted of a series of glass aquaria. The fishes are kept in aquarium having $3/4^{th}$ of water. The glass aquaria tanks were properly washed and rinsed with clean water.

They were filled with bore well water and aerated using air pumps to ensure proper oxygenation and continual aeration. Natural photoperiod of 12 h light and 12 h dark was maintained throughout the experiment. Water quality parameters such as DO, CO2, pH, ammonia, nitrate and nitrite were maintained within acceptable ranges for mrigal throughout the experimental period uneaten feed and faeces removal, regular partial water change and proper care. The experimental fish *Cirrhinus mrigala* fingerlings were collected from hatchery.

Experimental procedure and feeding trial

The experimental fish were randomly distributed at a stocking density of 10 fingerlings per aquarium tank in triplicates. They were fed at 5% body weight twice daily morning and evening at equal ration. Sampling was done weekly using a sensitive electronic balance to determine the average weight of the fish and adjust the feed accordingly. The study was conducted for 30 days. Water temperatures was monitored daily with a standardized mercury thermometer while dissolved oxygen with Winkler method and PH was determined using Digital PH meter and Ammonia, nitrite, nitrate by using spectrophotometer respectively

Statistical analysis

Data were subjected to analysis of variance (ANOVA) to compare the result using SPSS16.0 software. Statistical tables were used to evaluate the difference between means for individual diets at 5% (0.05) significance level.

Results and Discussion

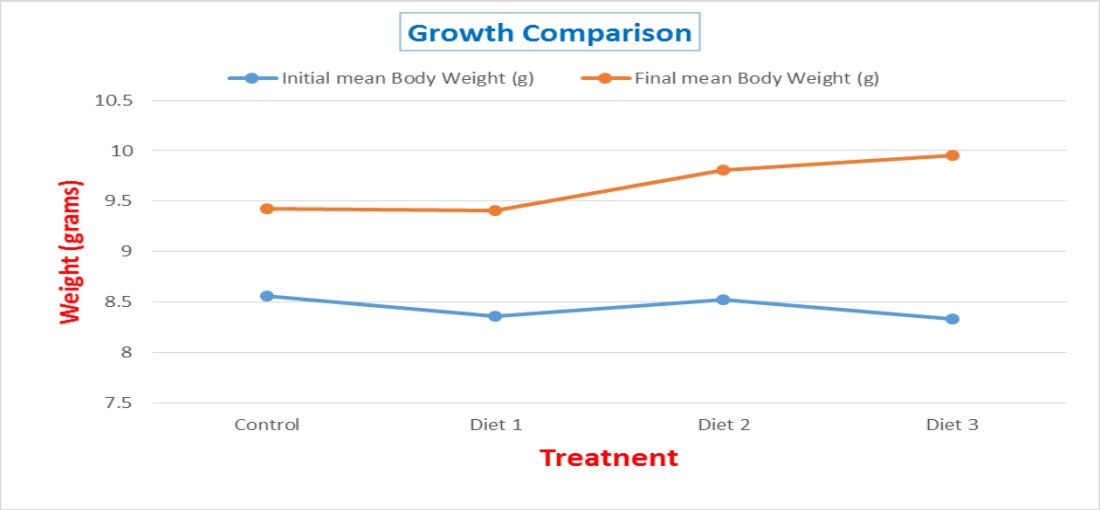
The three-inclusion level of microfloc meal in the experimental feed supported the growth for *Cirrhinus mrigala* (Table 1). No mortality of fishes were observed during the entire period of the experiment. The table 2 shows water quality parameters of the experiment.

The concentration of dissolved oxygen was highest 7.3 mg/L in control at pH 8 and temperature was 27 °C. Total ammonia, nitrite and nitrate were highest 0.018 mg/l, 0.54 mg/Land 0.15 mg/L, Diet 3 due to higher concentration of microfloc meal in feed respectively, Survival exceeded 100% for all treatments.

The average values of final weight, weight gain, and specific growth ratio are presented in Table 3. The gradual increase in average final weight of fish with time indicates that the fish belonging to different dietary groups fed actively.

The results of the present study indicate that microfloc meal can be a promising ingredient that could substitute fishmeal in the diet of *C. mrigala* fingerling up to 50%.

The fishes fed with diet 3 (50% microfloc) having highest weight gain compare to the fishes fed with other feeds. The table 3 shows fishes fed with diet 3 having more Specific Growth Rate (0.31) compare to other treatments.



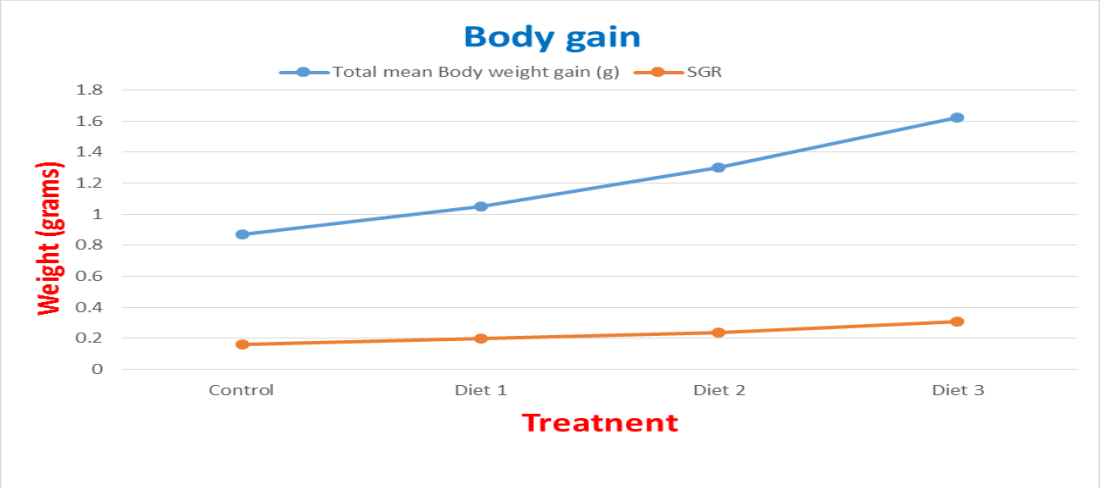


Table.1 Four different feeds having different ratios in fishmeal and biofloc

Treatment	Percentage inclusion level of Microfloc meal
T1	70% fish meal and 30% microfloc
T2	60% fish meal and 40% microfloc
T3	50% fish meal and 50% microfloc
C	No biofloc

Table.2 Water quality parameters of the experiment

		Water Quality				
Sl. No	**Treatments**	**DO (ppm)**	**CO2 (ppm)**	**NH_3 (ppm)**	**NO_2 (ppm)**	**NO3 (ppm)**
1.	Control	7.3	Nil	Nil	0.12	0.05
2.	Diet 1	6.8	Nil	0.008	0.20	0.09
3.	Diet 2	6.6	Nil	0.013	0.33	0.11
4.	Diet 3	6.2	Nil	0.018	0.54	0.15

Table.3 Growth responses of the experimental diets

Sl. No	Treatments	Initial mean Body Weight (g)	Final mean Body Weight (g)	Total mean Body weight gain (g)	Specific growth rate (SGR)
1.	Control	8.56±0.23	9.43±0.15	0.87±0.20	0.16±0.06
2.	Diet 1	8.36±0.22	9.41±0.20	1.05±0.18	0.20±0.02
3.	Diet 2	8.52±0.24	9.81±0.23	1.30±0.21	0.24±0.04
4.	Diet 3	8.33±0.18	9.95±0.17	1.62±0.09	0.31±0.02

The following graphs shows comparison of growth and weight against the treatments. The results showed that partial replacement of fishmeal with microfloc gives better growth rate in fish. It indicates that inclusion of microfloc in feed has direct relationship with fish growth. Therefore, the increment of microfloc feed increases the growth of fish. However, the problem with microbial protein it contains more protein (33-55%) which

leads to more addition of nitrogenous waste to the environment. Therefore, it can be avoided future by selecting the heterotrophic bacteria having probiotic activity, which helps in better digestion of protein, and reduce the release of waste into the environment. Several studies have reported complete replacement of fishmeal with plant-based ingredients in diets for omnivorous fish and Prawn (Tidwell *et al.*, 1993; Zhao *et al.*, 2010). Several studies report an increase in growth rates, general welfare and survival of shrimps reared in microbial floc based systems (Burford *et al.*, 2004; Moss, 2000; Tacon *et al.*, 2002; Wasieleskyetal, 2006), but few studies have used these microfloc as ingredients in aquaculture diets. According to Lim *et al.*, (1997b), live flagellates and ciliates contain16–25% of highly unsaturated fatty acids, and then they can be used as an interesting source of lipids. In that way, Amaya *et al.*, (2007) suggest that the success of replacing animal protein in diets with alternative sources is due in part to the ability of organism to use natural productivity as a food supplement. Thus, it is necessary to evaluate the use of these experimental diets in large-scale systems of commercial ponds under conditions of natural productivity. The results obtained for *C. mrigala* performance confirm that, under experimental conditions, this species can be fed with sources alternative to fishmeal (50% MFM), thereby excluding the need for fishmeal as the main protein source in fish diets. MFM can be a sustainable ingredient for aquaculture industry, with great potential to reduce the pressure on natural fish stocks and the problem of effluent disposal. The search for renewable replacements for fishmeal is and environmentally friendly and socially responsible method of achieving sustainability in aquaculture. In the present study, the fish readily accepted the experimental diets; the results showed improvement of fish growth by replacing 50% FM protein with MFM. In conclusion, the beneficial effects of MFM supplementation for FM replacement were demonstrated on growth performance, feed utilization and non-specific immune response of mrigal.

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