INFLUENCE OF FORMULATED DIETS WITH VARYING PROTEIN LEVELS ON GROWTH AND AMMONIA EXCRETION IN THE FRY OF INDIAN MAJOR CARP, *CIRRHINA MRIGALA*

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ABSTRACT

The influence of formulated isocaloric diets of different protein levels (30, 35, 40, 45 and 50%) on the growth and ammonia excretion of the Indian major carp *Cirrhina mrigala* fry was studied for a rearing period of four weeks in the laboratory. Fishmeal, groundnut oilcake and silkworm pupae formed the source of protein in all the diets. As the dietary protein level increased from 30 to 40%, the growth and conversion efficiency increased significantly. Further increase in the protein level resulted in decrease in growth and conversion efficiency. Growth rate, weight gain (%), and gross and net feed conversion efficiencies were maximum at 40% dietary protein level. Ammonia excretion was directly proportional to the level of protein in the diet.

Keywords : *Cirrhina mrigala*, formulated diet, ammonia, dietary protein

INTRODUCTION

In fish culture, besides utilising the natural food items such as plankton, insect larvae, copepods, etc., supplementary feeding in the form of artificial feed is a usual practice to enhance fish production. Feed alone accounts for 60% of the production cost (Pandian, 2001). Hence, the artificial feed should be prepared from low-cost by-products.

It has been established that growth is directly affected by the amount and quality of protein fed to fish (Ogino, 1980; Pandian, 1989, 2001). The optimum protein level in feed is important because either a low or a high protein level may result in poor growth and increased susceptibility to diseases (Chuapoehuk, 1987). Additionally, excessive protein in fish diet is wasteful and expensive. Several workers have studied the protein requirements for better growth in fishes (Dabrowski, 1977; Lim *et al.*, 1979; Ogino, 1980; Jauncey, 1982; Pandian, 1989, 2001; Ayyappan and Jena, 1998; Mukhopadhyay, 1998; James and Sampath, 2003). But the studies on the protein requirement of Indian major carps are meagre.

For this work, *Cirrhina mrigala* was selected as it is an important species in composite fish culture, locally available throughout the year and widely consumed by the local people. It is a bottom dweller, feeding on decaying organic debris, and has an effective role in scavenging waste materials in aquaculture ponds.

Halver (1972), Mazid *et al.* (1979) and Gatlin *et al.* (1986) reported that the caloric value of food plays a major role in the growth of fish. Of the net energy used by fish, about 25% is utilized for growth (Halver, 1972). The best growth and food conversion ratio (FCR) were obtained with an average of 379.72 kcal/100 g at 41% protein diet. Increased allowance of energy and protein is utilized for tissue building and the enhancement of growth. Better growth is observed with the increase in the dietary protein levels of isocaloric diets (Sethuramalingam and Hanifa, 2001). It has become necessary to have isocaloric feeds which possess different protein contents for finding out the optimum protein requirement (Mazid *et al.*, 1979). So, in this study, isocaloric feeds with varying protein levels were used.

The major constraint in aquaculture production is the accumulation of ammonia from protein metabolism (Armstrong and Boyd, 1982). The accumulation of ammonia limits the growth of fish to some extent. Though some studies (Sadler, 1981; Soderberg *et al.*, 1983; Degani *et al.*, 1985; Arunachalam, 1986; Chakraborthy *et al.*, 1992) on the effects of ammonia on growth are available with reference to fishes, studies on the effect of ammonia excretion on growth in *C. mrigala* is lacking. Hence, an attempt was made to investigate the protein requirement, and interaction between the dietary protein levels and ammonia excretion on the growth of *C. mrigala*.

MATERIAL AND METHODS

Collection and Acclimation

Fry (1-2 g) of *C. mrigala* were collected from the fish farm at Aliyar Dam (Near Pollachi in Tamil Nadu). The fish were acclimated in the laboratory in rectangular glass aquaria for 15 days with conventional pelleted feed (groundnut oilcake, rice bran, fishmeal and tapioca flour in the ratio of 1.0:1.0:0.5:0.5). After a period of 15 days, these fish were used for the experiments. Feeding was stopped one day prior to the start of the experiments.

Feed Preparation

Isocaloric feeds containing different levels of protein (30, 35, 40, 45 and 50%) were prepared following the method described by Ali (1980), and Jayaraman and Shetty (1981). The required quantities of the various ingredients (silkworm pupae, fish meal, groundnut oilcake, rice bran and tapioca flour) were dried, powdered and mixed as shown in Table 1. The ingredients were thoroughly mixed with water to form soft dough. The dough was then

Table 1: Composition (% dry weight) of isocaloric fish feeds

Ingredient	Protein level	Diet				
		1	2	3	4	5
Tapioca flour	2.4	20.0	20.0	19.5	19.5	12.5
Rice bran	10.4	25.0	14.5	9.5	--	--
Fishmeal	57.4	29.5	34.5	55.0	69.5	84.5
Groundnut oilcake	44.2	14.5	10.0	5.0	9.5	1.5
Silk worm pupae	45.3	9.5	19.5	9.5	---	---
Vitamin-mineral mix		1.5	1.5	1.5	1.5	1.5
Calculated chemical composition						
Protein content of diet		30.0	35.0	40.0	45.0	50.0
Caloric content (kcal/g)		4.752	4.754	4.751	4.754	4.750

Vitamin mix (mixed in 1 kg of fish feed): Vitamin A - 2500 IU, Vitamin D3 - 500 IU, Vitamin D2 - 1 mg, Vitamin E - 40 IU, Vitamin K - 0.50 mg, Vitamin B12 - 0.05 mg; Calcium pantothenate - 1.25 mg, Nicotinamide - 5.00 mg

Mineral mix (mixed in 1 kg of fish feed): Choline chloride - 75 mg, Calcium - 375 mg, Manganese – 14.0 mg, Iodine - 0.5 mg, Iron - 4.0 mg, Zinc - 7.5 mg, copper - 1.0 mg, cobalt - 2.5 mg

cooked for 30 minutes in a pressure cooker. The vitamin and mineral mixture (1.5%) was thoroughly mixed in the dough after cooking. Finally, it was pressed through a hand pelletizer having a perforated 2-mm die. The pellets produced were air-dried and broken into pieces of about 2-mm length. Air-dried pellets were stored and used for this study. The micro-Kjeldahl method was followed for the estimation of the protein content in the ingredients used. The caloric value of the various ingredients was determined using a micro-bomb calorimeter (Toshniwal, India).

Experimental Design

After thorough acclimation, fry were sorted out from the stock and divided into five groups with three replicates with four fish in each replicate. Each group of fish was reared in glass aquaria (13 x 15 x 20 cm). Experiments were conducted for four weeks at the room temperature of 29 ± 1 °C at the dissolved oxygen concentration of 7-9 mg/l and the pH of 7.0-7.8. The photo period was 12 D:12 N and the survival percentage was 100 in all the test groups.

Feeding Schedule

The experimental fish were fed *ad libitum* twice a day at 08.00 and 18.00 hours with test feeds for a period of four weeks. Food remains were collected one hour after feeding using an aspirator, and dried in an oven (85 to 95C for one day) and weighed. Faecal strings were collected from the aquarium by filtering the entire aquarium water during water change. Water was changed daily. The faeces were also dried in an oven and weighed. The fish were weighed daily at 08.00 hours before feeding and this procedure was maintained till the last day of the experiment.

Excretory ammonia in water was estimated by nesslerisation using a spectrophotometer. For the estimation of ammonia, samples were taken daily before the water exchange. The various energetic components were estimated using the international biological programme (IBP) formula (Petrusewicz and Macfadyan, 1970) as modified by Arunachalam and Palanichamy (1982).

Growth Parameters

In this work, the IBP formula (Petrusewicz and MacFadyan, 1970) $C = F + U + P + R$, where C is the food consumed, F the amount of faeces egested, U the nitrogenous excretory waste, P the growth and R the metabolism (respiration) was modified. Here, both R and U are expressed as metabolism (M). Hence, the IBP formula was modified as $C = F + M + P$ (Arunachalam and Palanichamy, 1982). Food absorbed (A) was calculated by subtracting the quantity of faeces (F) from that of food consumed (C) as $A = C - F$. Growth was estimated as the differences between the initial and final weights of test individuals.

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry weight of the food consumed}}{\text{Gained wet weight of fish}}$$

Gross conversion efficiency (GCK) = Growth (g) / Food consumed (g) x 100

Net conversion efficiency (NCE) = Growth (g) / Food absorbed (g) x 100

$$\text{Weight gain (\%BWI)} = \frac{\text{Final wet weight of fish - Initial wet weight fish (g)}}{\text{Initial wet weight of fish (g)}} \times 100$$

Analysis of Data

The data were subjected to the Student's t test, correlation and regression, and analysis of variance (one way) following Zar (1984).

RESULTS AND DISCUSSION

It was observed that the feeding rate and absorption rate were similar in all the treatment groups. The statistical analysis of data on the feeding rate and absorption rate at different dietary protein levels indicated that the feeding rate ($p > 0.05$; ANOVA) and absorption rate ($p > 0.05$) did not vary significantly as a function of dietary protein levels.

The conversion rate (growth) increased gradually as the protein level increased up to 40% and after that, decreased gradually (Table 2). The conversion rate varied significantly ($p < 0.05$, ANOVA) with the different protein levels. In all the tested groups, fish reared at 40% dietary proteins showed the maximum conversion (Table 2). The FCR was low at 40% protein diet. The low FCR (2.67) for 40% dietary protein suggests that 40% protein is best suited for fast growth in *C. mrigala*. Similar result of low FCR was observed in *Labeo rohita* by Sethuramalingam and Hanifa (2001). The fish fed with 40% protein diet exhibited significantly ($p < 0.05$) higher gross and net conversion efficiencies when compared to those fish fed at other protein levels. The weight gain (%) was the highest at 40% protein diet (Table 2).

Dietary protein requirements have been studied for a few species of fishes. *Oreochromis niloticus* exhibits the best growth at 30% dietary protein (Siddique *et al.*, 1988). The best growth was recorded at protein level of 40% in milkfish, *Chanos chanos* (Lim *et al.*, 1979), and at 45% in grass carp, *Ctenopharyngodon idella* (Dabrowski, 1977). The literature indicates that the optimum protein level for fishes vary from 30 to 45% (Pandian, 1989). Being an omnivorous fish, *C. mrigala* requires more protein in the diet. Hence, 40% dietary protein is suggested as the optimum dietary protein level for *C. mrigla*.

The growth and conversion efficiencies of fish reared with 50% protein diet decreased (Table 2). The reduction in the growth and conversion efficiencies may be due to the excretion of excess amino acid from high-protein diet or the production of excess ammonia which affects the uptake of food and conversion efficiency. The high concentration of ammonia in fish induces the fish to switch over to urea excretion, which in turn, imposes high-energy demand (Brett and Groves, 1979). In the present study, ammonia excretion was directly proportional to the level of protein in the diet. This observation agrees with the above suggestions and a previous finding by Degani *et al.* (1985) who observed that ammonia production is lower in the group fed on pellets containing 25% protein and higher in the groups receiving higher (50%) protein diet. The accumulation of ammonia in the

Table 2: Effect of dietary protein levels (%) on food utilization and ammonia excretion in *C. mrigala*

Parameter	Protein level				
	30	35	40	45	50
Feeding rate (mg/g/d)	34.31±0.33^{a}	35.81±1.28^{a}	38.99±3.21^{a}	35.74±0.76^{a}	31.58±5.09^{a}
Absorption rate (mg/g/d)	27.08±0.45^{a}	28.24±0.87^{a}	30.76±2.89^{a}	28.23±0.60^{a}	26.02±4.51^{a}
Conversion rate (mg/g/d)	1.76±0.18^{b} (3.70)	2.08±0.11^{b} (3.25)	2.76±0.14^{bc} (2.67)	2.09±0.15^{b} (3.62)	1.52±0.05^{b} (3.95)
Gross conversion efficiency (%)	5.14±0.56^{b}	5.81±0.38^{b}	7.09±0.23^{bc}	5.87±0.53^{b}	4.89±0.65^{b}
Net conversion efficiency (%)	6.50±0.75^{b} (23.39)	7.38±0.52^{b} (27.44)	9.00±0.40^{bc} (36.42)	7.43±0.66^{b} (27.57)	5.95±0.85^{b} (20.09)
Ammonia excretion (mg/g/l/d)	0.056±0.011	0.075±0.005	0.093±0.022	0.113±0.007	0.142±0.006

Each value is the average (mean ± SD) performance of three groups of fish observed for four weeks at 29±1°C.

Figures in each row having the superscript ‘a’ are not significantly different ($p > 0.05$).

Figures in each row having the superscript ‘b’ are significantly different ($p < 0.05$).

Figures in 40% protein level column having the superscript ‘c’ exhibited best results when compared to those of fish fed with other protein diets.

Figures in parentheses in conversion row are FCR.

Figures in parentheses in net conversion efficiency row are weight gain (%).

culture medium also produces toxic effect which affects fish growth (Arunachalam, 1986). The toxicity of ammonia is mainly due to the unionised form (NH_3) which passes through cell membrane more rapidly than the ionic form NH_4^+ (Hampson, 1976). Due to the above reasons, growth and conversion efficiencies were poor in the group of fish, which were reared on diets containing higher level of protein.

The growth of fish is also influenced by the caloric value of food (Halver, 1972). In the present study, diets containing the same caloric value (4.75 kcal) were used. So, the observed growth difference among the tested groups could be attributed to the influence of dietary protein.

The quantities of ammonia excreted by fish fed with 30, 35, 40, 45 and 50% protein diets were 0.056, 0.075, 0.093, 0.113 and 0.142 mg, respectively, per gramme body weight of fish per litre of water in the aquarium per day. It was found that ammonia excretion was directly proportional to the level of protein in the diet. A higher correlation (r^2) of 0.9870 was found between protein levels in the diet and ammonia excretion. The regression equation $Y = 0.0036\,x - 0.491$ has been found for the two parameters.

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