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Effect of *Nymphaea* meal incorporated diets on growth, feed efficiency and body composition in fingerlings of *Cyprinus carpio* L.

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Abstract: The effect of varying levels of *Nymphaea* leaf meal on the growth and survival of common carp, *Cyprinus carpio* was investigated. In a feeding trail of 45 days, three experimental diets containing *nymphaea* leaf meal at 300, 400 and 500g kg^{-1} level of incorporation were fed to triplicate groups of 10 fish each. The conventional feed used in India, consisting of a mixture of groundnut oil cake and rice bran in 1:1 ratio served as the control. Best growth in terms of weight gain (35.2g), specific growth rate (4.67), protein efficiency ratio (PER) (2.7), feed conversion ratio (FCR) (2.5) was obtained for the test diet with 400g kg^{-1} nymphaea meal inclusion level. However no statistical difference was observed between the three experimental diets. Digestive enzyme activity and digestibility studies also indicated the same pattern. Thus the results of the present study indicate that a diet of 300g kg^{-1} overall protein with *nymphaea* meal included at 400g kg^{-1} can elicit good growth response and survival in common carp.

Keywords: *Cyprinus carpio*, Digestibility, Enzyme activity, Growth, *Nymphaea* meal

INTRODUCTION

In fish farming, nutrition is critical because feed represents 60% - 71% of the production costs (De Silva, 1985), thus fish nutrition investigations are mainly directed towards reducing feed cost by manipulating the feed formulation. In contrast to quality commercial prawn feeds that are readily available in India, there is a acute paucity of nutritionally sound, cost-effective feeds for finfish in general, and for, in particular. The traditional feed mixture employed in the culture of Indian Major Carp (IMC) is unbalanced. There is, therefore, an urgent need to develop low-cost, nutritionally balanced IMC diets that can support increased production levels. The reduced availability as well as the escalating cost of fish meal has necessitated the need to identify suitable cost-effective alternatives to fish meal.

Consequently, identification and evaluation of alternative protein sources to fish meal is a top research priority in fish nutrition. Considerable attention has been devoted to the evaluation of plant protein such as soy bean meal, solvent extracted cotton seed meal, lupin meals, various legumes (cow pea, green mung bean, rice bran), leaf meals, and papaya or Canote leaf meal (Ramachandran and Ray, 2007, Latif *et al*., 2008, Lim and Lee, 2008, Tahir *et al.,* 2008, Dadgar *et al.,* 2009, Gut *et al.,* 2010, Pavan Kumar *et al.,* 2011) as ingredients in feeds of aquatic animals. The present study evaluates the possibility of utilizing *Nymphaea* leaf meal in diets for the common carp *Cyprinus carpio* by assessment of its growth response to varying levels of *Nymphaea* leaf meal in the diet.

MATERIALS AND METHODS

Fingerlings of *C. carpio* obtained from the Carp Seed Production Center, Kalyani Dam, Tirupati were used for the study. The weight of the individuals ranged from 1.50 to 1.55 g. The fish were acclimated to experimental conditions for a fortnight prior to the start of the trail. Fish were fed with conventional feed during acclimatization and feeding was suspended two days before the commencement of the experiment.

Four test diets designated as P30, P40, P50 and Control Diet (CD) were used. The three test diets had *Nymphaea* leaf powder incorporation at levels 300, 400 and 500g per kg respectively. The last diet which severed as the control was typical of the traditional diets used in India and contained groundnut oil cake and rice bran in a 1:1 ratio for 30 min. The crude protein levels of the four diets were 27.00 (CD), 28.01 (P30), 29.32 (P40) and 31.94 (P50) respectively. The percentage composition of the feed ingredients of the various test diets is presented in Table 1.

All the feed ingredients were dried and powdered. Ingredients were mixed with sufficient quantity of water to get the required soft consistency and hand kneaded. It was then cooked in a pressure cooker for 30 min. The cooked feed was cooled by spreading under the fan, vitamin mineral premix was added and mixed uniformly. The feed was sun dried and analyzed for proximate composition and stored in plastic containers.

Moisture content (Boyd, 1979), crude protein and crude fat (by using Tecator Kjeltec and Soxhlet apparatus respectively, AOAC, 1975): Crude fibre (Pearson, 1976)

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and carbohydrate (Nitrogen Free Extract - NFE) (Hastings, 1976) were analyzed. Total ash was determined by burning the sample for 6 hrs in Muffle furnace.

Trails were conducted for a period of 45 days in 80 lit cylindrical fiber glass tanks filled with filtered fresh water. Each treatment had three replications as per a completely randomized design. 10 carp fingerling were randomly distributed in each tank after recording the individual wet weight (g). The feed was given *ad libitum* once daily and the leftover feed was collected every day and weighted. Ten percent of the water was exchanged daily and aeration was provided. Growth was assessed by sampling fortnightly, wherein total weight of the sampled fish was recorded. At the end of the experiment, total weight and the number of surviving fish in each experimental unit were noted. Water quality parameters, viz. temperature, pH and dissolved oxygen (Classical Winkler method) were monitored fortnightly.

The evaluation criteria were percentage weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and percentage survival.

Weight gain = average final weight – average initial weight.

$$\% \text{ weight gain} = \frac{\text{Average final weight} - \text{average initial weight}}{\text{Average initial weight}} X\ 100$$

FCR = feed intake (g) / live weight gain (g).

PER = live weight gain (g) / Protein intake (g)

$$\text{SGR } \% = \frac{\text{Log}_e W_t - \text{Log}_e W_0}{T} X\ 100$$

Where, W_t is the weight (g) attained after specific period of time't' of experiment, W_0 the weight (g) at '0' day of experiment and't' the days of experiment.

% survival = (initial number stocked – number of dead) X 100/ initial number stocked.

At the end of the experiment, fish from each experiment unit were killed. The fish muscle devoid of bones was dried and analyzed for proximate composition. Digestive enzymes like amylase, protease and lipase were determined from different treatment groups at the termination of the experiment. Intestinal tract was isolated from the freshly killed fish and immediately transferred to chilled watch glass containing 0.65% cold saline. Intestinal segments were slit open longitudinally and thoroughly washed with cold saline to remove the debris. All the tissue segments were blotted dry, weighed, homogenized and centrifuged at 10,000 rpm for 10 min and the supernatant was used for the enzyme study. Enzyme concentrations were expressed as µ moles of product liberated mg^{-1} protein min^{-1}. Protein in the crude enzyme extract was measured by Lowry *et al.* (1951) using Bovine serum albumin as the standard. Total protease activity was measured by casein digestion method (Kunitz, 1947), Amylase activity (α-amylase was measured by the method of Bernfeld (1955) while Bier's titrimetric (Bier, 1962) was used for the estimation of lipase activity with minor modifications. Apparent nutrient digestibility was estimated by the method of Furukawa and Tsukuhara (1957).

Analysis of variance (ANOVA) was performed and transformation was obtained on percentage values before subjecting them to analysis (Snedecor and Cochran, 1968).

RESULTS

The proximate composition of the test diets P30, P40, P50 and CD in the study is presented in Table 2. The crude protein level varied from 27.00 to 31.94. The mean value of percentage weight gain, SGR, FCR, PER and survival rate are presented in Table 3. The maximum weight gain (35.2g) was observed in the P40 treatment group and lowest (21.7g) was recorded in the control treatment group that has a significantly lowest percentage weight gain than all the test diets. The SGR revealed the same pattern, where in the values were minimum (3.17) in control treatment group and maximum (4.67) in P40 diet group. The FCR in the control group significantly differed from all other test diets with the poorest conversion (3.1) being in the control and best (2.5) in the P40 treatment group. The PER was significantly different between each of the diets. The survival rate of P40 groups was not significantly different from that of the other groups.

The carcass composition of the fish fed with different test diets is presented in Table 4. The results revealed that the moisture content in the control diet group did not differ from other groups feed with the test diets, while the protein and the lipid contents differed significantly among the fish fed the test diets.

Intestinal protease and lipase activities were found to be directly related to the levels of dietary protein and lipids.

Table 1. Composition of feed ingredients in different feeds (in g per kg feed).

Ingredient	Control diet (CD)	Experimental diets		
		P 30	P 40	P 50
Rice bran	39	24	19	14
Ground nut oil cake	39	24	19	14
Soybean meal	20	20	20	20
Nymphaea leaf meal	-	30	40	50
Vitamin – mineral premix	2	2	2	2

Table 2. Proximate composition of test diets (g kg $^{-1}$).

Parameter	CD	Experimental diets		
		P 30	P 40	P 50
Moisture	9.02 ± 0.12	8.97± 0.33	8.93 ± 0.41	8.89± 0.86
Crude protein	27.00 ± 0.21	28.01± 0.54	29.32± 0.43	31.94± 0.78
Lipid	4.69 ± 0.43	4.67± 0.21	4.60± 0.86	4.56± 0.42
Crude fibre	11.08± 0.33	11.78± 0.69	11.95± 0.58	11.81± 0.92
Ash	9.04± 0.76	8.59± 0.91	8.13± 0.16	7.64± 0.37

Gut amylase activity increased with increase in dietary protein level. Higher digestibilities of protein and fat were found with the three experimental diets than the control diet, indicating the ability of common carp to digest *Nymphaea* leaf protein. Protein digestibility was found to increase with increase in dietary protein levels in the test diets. Reverse trend however was observed in lipid digestibility.

DISCUSSION

Results of the present study demonstrated that water quality parameters were within the optimal range of fish production on par with the study of Banarjee (1967) and indicated water quality did not produce any stress on fish during experiment. The results indicated the positive influence of the test diets in the fingerlings of common carp. Daily consumption was variable but the mean consumption did not differ significantly among the plant substituted diets. Growth was observed to increase with increase in *Nymphaea* leaf meal only up to the level of 40% inclusion (P30, P40) and the growth performance and feed utilization efficiency decreased thereafter with increase in *Nymphaea* leaf meal incorporation. This could be attributed to the reduced digestibility at higher percentage *Nymphaea* leaf meal incorporation due to higher carbohydrate content. It has been reported that reduced digestibility with increased carbohydrate content is related to actual reduction in gland stimulation, and enzyme reduction (Falge *et al.,* 1978). The PER, FCR and SGR observed in the present study are on par with the observations made by Ramachandran and Ray (2007), Tahir *et al.* (2008) and Pavankumar *et al.* (2011). Carcass composition (Table 4) also indicated that protein deposition was higher with lower incorporation level. Similar observations were made by Patra *et al.* (2000) in *Cirrhinus mrigala* with *Nymphoides* leaf meal where 30% incorporation was found to give better growth rate than 60% incorporation. Feed utilization in 45 day reared fingerlings was significantly higher in test diet fed groups compared to control fed groups. The poor FCRs recorded in all treatments except for P 30 diet. This could be related to high fibre content of *Nymphaea* leaf meal which might have played an important role in feed conversion efficiency that varied at different time intervals. Fischer (1972) reported that large grass pellets containing fairly large amounts of crude fibre affected digestibility and food conversion. Not only high fibre content but also high ash content (12%) of *Nymphaea* leaf powder in the test diets might have contributed to a better conversion. This observation derives support from the studies of Tan (1970) who fed several types of vegetation to grass carp (*Ctenopharyndon idella*) in pond and found *Hydrilla* to be an excellent food because of the soft nature of the plant (low fibre) and high mineral content.

Carcass quality is a matter of great importance from the perspective of consumer acceptance. Increase in muscle protein and lipid content of fish fed on test diets could be ascribed to the efficiency of common carp to digest plant ingredients as an energy supplement for growth.

Table 5 shows the gut enzyme activity of common carp. Intestinal protease activity increased with an increase in the *Nymphaea* meal inclusion up to 40% inclusion level, after that the protease activity decreased. A reduction in the activity of lipase was observed at higher levels of *Nymphaea* meal inclusion, while the amylase activity increased with increase in *Nymphaea* meal incorporation. The protein digestibility data support the growth trend (Table 5). While the protein digestibility of three test

Table 3. Growth parameters and survival of *Cyprinus carpio* fed different diets.

Parameter	CD	Experimental diets		
		P 30	P 40	P 50
Initial weight (g)	1.50 ± 0.2	1.51 ± 0.1	1.50 ± 0.1	1.50 ± 0.1
Final weight (g)	23.2 ± 0.1	31.01 ± 0.1	36.7 ± 0.2	35.1 ± 0.2
Weight gain (g)	21.7 ± 0.3	29.5 ± 0.2	35.2 ± 0.2	33.6 ± 0.3
SGR	3.17 ± 0.5	4.12 ± 0.8	4.67 ± 0.3	4.31 ± 0.9
FCR	3.1 ± 0.1	2.9 ± 0.03	2.5 ± 0.01	2.7 ± 0.1
PER	1.9 ± 0.001	2.1 ± 0.001	2.7 ± 0.01	2.4 ± 0.04

Table 4. Proximate composition of fish muscle of common carp fed different diets (percentage).

Parameters (%)	CD	Experimental diets		
		P 30	P 40	P 50
Moisture	61.8± 1.8	61.7 ± 1.4	61.7 ± 1.1	61.6 ± 1.8
Crude protein	19.0 ± 1.4	20.4 ± 1.7	21.5 ± 1.3	20.9 ± 1.9
Crude lipid	3.74 ± 0.1	3.67 ± 0.4	3.59 ± 0.4	3.58 ± 0.1
Ash	2.08 ± 0.3	2.22 ± 0.2	2.54 ± 0.7	2.35 ± 0.5
NFE	13.38 ± 0.7	12.01 ± 0.3	10.67 ± 0.1	11.57 ± 0.4

Table 5. Gut enzyme activity (µmol product liberated min^{-1} mg $protein^{-1}$ at 37^0C) and apparent digestibility (%) of the test diets.

Diets	Protease	Lipase	Amylase	Apparent digestibility	
				Protein	Lipid
CD	0.162	0.010	0.995	81.73	80.50
P30	0.187	0.015	1.082	87.93	84.31
P40	0.215	0.013	1.192	89.23	83.15
P50	0.199	0.011	1.212	88.45	81.67

diets did not differ significantly between the treatments. It was significantly better than the control diet. Digestibility evaluation studies with common carp showed that the maximum protein digestibility can be obtained at an incorporation level of 40% in a 30% protein diet. There is a great reduction in the fat digestibility at higher *Nymphaea* meal incorporation levels. The better digestibility of protein must be a result of better absorption since the protease activity showed the same pattern. Hepher (1988) reported rich intestinal bacterial also take part in other nutritional functions such as non-protein nitrogen utilization and synthesis of Vitamin B_{12} and Nicotinic acid (Teshima and Kashiwada, 1967; 1969). The higher growth rate of fish with the test diets could thus be attributed to protein sparing by carbohydrates. The present study clearly indicated that feeding fish with high levels of *Nymphaea* leaf meal (P 50 diet) has not yielded positive results. Only optimum levels of incorporation 40% yielded better results in terms of growth. Similar observations were made by Fynn-Aikins *et al.* (1992). It was further observed that better growth and production with *Nymphaea* leaf meal may be partly due to its fibre content, as the optimum level of crude fibre is beneficial in improving the utilization of certain nutrients (Steffens, 1981) and partly due to better utilization of the *Nymphaea* based diet.

Thus the present study suggests that *Nymphaea* leaf meal could be used as a source of dietary protein for common carp. This study also revealed that the fingerling of common carp grow well on the diets of 28% and 32% protein under laboratory probably as a consequence of optimum percentage of protein and also probably due to efficient utilization of diets.

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