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GROWTH PERFORMANCE OF COMMON CARP, *CYPRINUS CARPIO* FED VARYING LIPID LEVELS THROUGH LOW PROTEIN DIET, WITH A NOTE ON CARCASS COMPOSITION AND DIGESTIVE ENZYME ACTIVITY

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Protein sparing by lipid has been demonstrated in certain cultivable species of fish. This study was carried out using four low protein isonitrogenous diets (24% crude protein) formulated by supplementing varied levels of fish oil (0, 3, 6, and 9%). The diets were fed for 120 days at 5% body weight to triplicate groups of common carp (av. wt. 2.13–2.21 g) stocked at 1 per m^2 in mud bottomed cement tanks (18 m^2), fertilized with poultry manure. The growth of fish was the highest ($P < 0.05$) with the diet containing 6% fish oil, followed by 3, 9, and 0%. Food conversion ratio and protein efficiency ratio improved with increasing dietary lipid level. Dietary lipid had a positive impact on carcass lipid level ($P < 0.05$). Moisture and crude protein did not vary ($P > 0.05$) from that of the control. Survival ranged from 96.29 (T_1) to 100% (T_0) without any significant ($P > 0.05$) difference among the treatments. While there was a general increase in amylase activity in the treated fish, protease activity showed a reduction with increase in oil supplementation. No difference ($P > 0.05$) in lipase activity was observed between the different treatments. The results indicate the beneficial effects of incorporating fish oil in the diet of common carp.

Key words: growth, common carp, *Cyprinus carpio*, carcass composition, digestive enzymes

INTRODUCTION

The amount of non-protein energy in the diet is one of the factors influencing the quantitative dietary protein requirement of a fish species (Jauncey 1982). This is because part of the dietary protein may be utilized as an energy source, if the diet is deficient in

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non-protein energy. Since the use of protein for energy is wasteful from nutritional, economic, and ecological points of view when compared to lipids and carbohydrates, it is worthwhile supplying as much of the required energy as possible as lipid and carbohydrate (Peres and Oliva-Teles 1999). Both carbohydrate and lipid have good food value for carp (Omar et al. 1986). However, dietary lipid exerts greater protein sparing action than carbohydrate as observed in juvenile red drum, *Sciaenops ocellatus* by Ellis and Reigh (1991). Protein sparing effect of lipid has been taken advantage of in feed manufacture to develop diets that maximize nutrient retention and minimize nutrient loss (Tacon 1997). Most of the dietary lipid supplementation studies have been conducted in unmanured culture systems and a level of 5–12% has been shown to be optimal for carps (Bazaz and Keshavanath 1993, Jafri et al. 1995, Hasan et al. 1996, Gangadhar et al. 1997). In India, carps are generally grown by providing fertilizers and a 1 : 1 mixture of groundnut cake and rice bran as supplementary feed, containing about 25% protein. An earlier study (Keshavanath et al. 2002) indicated that common carp performs well with low protein diets in manured tanks. This investigation was therefore, undertaken to study the effect of low protein diet with varying lipid levels on growth, body composition, and digestive enzyme activity of common carp, *Cyprinus carpio* in fertilized tanks.

MATERIAL AND METHODS

Diets

Four nearly isonitrogenous low-protein (24%) test diets were formulated (Varghese et al. 1976) procuring all the ingredients (Table 1) locally. Commercial fish meal available locally is prepared by drying low quality fish on beaches and powdering them. With the result, its quality is poor as it contains low amount of protein and high level of ash (Table 1). Dietary ingredients (fish meal, ground cake, rice bran, and maize) were dried, pulverised, and sieved to obtain uniform particle (400 mm) size. Fish (sardine) oil was substituted by weight at 3, 6, and 9% levels in diets T_1, T_2, and T_3 respectively, by reducing the quantity of maize. Diet T_0 without oil supplementation served as the control. Oil incorporation to the diets was done by adding requisite amount of oil to 250 ml of water containing a few drops of Tween-80 (Polysorbate-80, Himedia Laboratories, Mumbai, India), mixing thoroughly with the help of a glass rod and using the suspension along with additional 550 ml of water per kg of ingredient. The diets were prepared following the method described by Jayaram and Shetty (1981) to obtain pellets of 3 mm diameter. The pellets were dried in a thermostatic oven at a temperature of 40°C and packed in heavy-duty plastic bags.

Table 1

Diet formulation and proximate composition of diets and ingredients ($\bar{x}$ ±s.e.)

Ingredients (%)	Diets			
	T_0	T_1	T_2	T_3
Fish meal	10	10	10	10
Groundnut cake	25	25	25	25
Rice bran	24	24	24	24
Maize	40	37	34	31
Vitamin and mineral mixture*	1	1	1	1
Sardine oil	0	3	6	9
Proximate composition of diets (%)				
Moisture	7.23 ±0.21	7.14 ±0.17	7.23 ±0.32	7.39 ±0.17
Crude protein	24.84 ±0.38	24.32 ±0.25	24.03 ±0.25	23.85 ±0.13
Fat	6.85 ±0.13	9.09 ±0.10	11.42 ±0.03	13.01 ±0.07
Ash	13.00 ±0.18	12.98 ±1.62	13.38 ±1.15	13.09 ±1.21
Crude fibre	8.05 ±0.32	7.94 ±0.41	7.83 ±0.25	7.64 ±0.36
NFE	40.03	38.53	36.11	35.02
Energy ($kJ \cdot g^{-1}$)	1.45	1.49	1.54	1.57
Proximate composition of ingredients (%)				
	Fish meal	Groundnut oil cake	Rice bran	Maize
Moisture	8.16 ±0.38	9.46 ±0.41	8.92 ±0.24	8.43 ±0.21
Crude protein	58.52 ±0.40	39.62 ±0.27	8.17 ±0.24	8.62 ±0.21
Fat	6.48 ±0.13	8.02 ±0.02	7.26 ±0.19	3.66 ±0.03
Crude fibre	—	3.42 ±0.33	24.07 ±0.31	3.54 ±0.82
Ash	18.27 ±0.35	5.74 ±0.18	17.49 ±1.20	1.31 ±0.15
NFE	8.85	33.74	34.09	74.44
Energy ($kJ \cdot g^{-1}$)	16.11	16.94	10.08	15.65

* Supplevite-M (Sarabhai Chemicals, Baroda, India)

Experimental set up

The experiment was carried out over a period of 120 days in 12 cement tanks of 18 m^2 each, with a 15 cm thick soil base. The tanks were cleaned and dried, limed at 400 $kg \cdot ha^{-1}$ (0.72 kg per tank), and initially fertilized with poultry manure at 2000 $kg \cdot ha^{-1}$ (3.6 kg per tank), while subsequent fertilization was done at 5% of the initial dose at two-week intervals. The manure contained 2.51% nitrogen, 2.72% phosphorus, 1.95% potassium, and 2.30% calcium. Ground water was used to fill the tanks, maintaining a depth of 90 ±5 cm throughout the experimental period. Advanced fry of common carp (av. wt. 2.13–2.21 g) were stocked at a density of 1 per m^2 (18 per tank) as practiced by Indian aquafarmers. The four diets were fed to triplicate group of fish every day once in the morning at 5% body weight as per Varghese et al. (1976), using trays suspended into the tanks 50 cm below the water surface. A minimum of 50% of the stocked fish from each tank was collected every fortnight for measuring growth. The quantity of feed given was readjusted after each fish sampling, taking into

consideration the weight of the fish. On termination of the experiment, the surviving fish were weighed, based on which the following parameters were calculated.

$$\text{Specific growth rate } (SGR) = \frac{\text{ln final weight} - \text{ln initial weight}}{\text{experiment duration}} \times 100 \text{ (\% per day)}$$

$$\text{Feed conversion ratio } (FCR) = \frac{\text{dry weight of feed given}}{\text{wet weight gain}} \text{ (g)}$$

$$\text{Protein efficiency ratio } (PER) = \frac{\text{wet weight gain}}{\text{amount of protein fed}} \text{ (g)}$$

Water quality

Water samples were collected at two-week intervals between 0700 and 0830 hours for the analysis of temperature, dissolved oxygen, pH, free carbon dioxide, and total alkalinity. Water temperature was recorded using a thermometer. pH was measured with a digital pH meter (LI-120, ELICO, India). Dissolved oxygen, total alkalinity, and free carbon dioxide were determined following standard procedures (Anonymous 1992). Plankton samples were also collected on fish sampling days, using a net made of No. 30 bolting silk cloth having 60 μm mesh size, by filtering 100 l of water from different locations of each experimental tank. Dry weight of plankton was determined by drying the samples in a hot air oven at 100°C till a constant weight was obtained. Quantitative estimation of plankton was done by the direct census method using a Sedgewick rafter cell having 100 equal squares (Jhingran et al. 1969). The planktonic organisms were identified up to the generic level.

Biochemical composition

Proximate composition of ingredients, diets, and fish carcass was analysed in triplicate. Three fish from each treatment were used for carcass analysis. Protein was determined by Kjeltec (Tecator-1002), lipid by Soxtec (Tecator-1043) and fibre by Fibretec (Tecator-1017). Ash was analysed by incineration (Anonymous 1975) and NFE by the difference method (Hastings 1976). The energy content of the feed ingredients and diets was calculated using values of 22.6 $kJ \cdot g^{-1}$ for protein, 38.9 $kJ \cdot g^{-1}$ for lipid, and 17.2 $kJ \cdot g^{-1}$ for carbohydrate as NFE (Mayes 1990).

Enzyme assay

The activity of digestive enzymes—amylase, protease, and lipase in the intestine and hepatopancreas of the experimental fish was analysed in triplicate on termination of the experiment by the methods of Bernfeld (1955), Kunitz (1947), and Bier (1955) respectively. Six fish from each treatment were used to collect the tissues for enzyme assay.

Statistical analysis

Comparison among different dietary treatments was done by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test at $P < 0.05$ (Duncan 1955, Snedecor and Cochran 1968).

RESULTS

The water quality parameters monitored, were within the tolerable limits for common carp. Water temperature ranged from 19.2 to 22.4°C; pH, from 7.05 to 8.35; dissolved oxygen, from 7.24 to 8.72 ppm; free carbon dioxide, from 0 to 1.33 ppm; and alkalinity ($CaCO_3$), from 53.12 to 76.40 ppm. The average dry weight of plankton in T_0, T_1, T_2, and T_3 varied from 2.36 to 51.06, from 1.92 to 58.8, from 2.03 to 67.94, and from 1.84 to 74.35 mg·100 l^{-1} respectively. Plankton dry weight showed a peak in the middle of the experiment (60th day).

Among blue-green algae (Cyanophyceae), *Anabaena* sp., *Microcystis* sp., *Oscillatoria* sp., were dominant. The green algae (Chlorophyceae) were represented by *Closterium* sp., *Coelastrum* sp., *Pediastrum* sp., *Pandorina* sp., *Mougeotia* sp., *Scenedesmus* sp., *Eudorina* sp., *Ulothrix* sp., and *Volvox* sp. The dominant diatoms (Bacillariophyceae) were *Synedra* sp., *Melosira* sp., and *Navicuala* sp. The important zooplankton encountered in the various treatments belonged to the groups Rotifera, Cladocera, Copepoda, Ostracoda, and larval forms. Rotifers were found to be the most dominant group in all the treatments, mainly represented by *Brachionus* sp., *Keratella* sp., *Hexarthra* sp., *Ascomorpha* sp., *Asplanchna* sp., and *Monostyla* sp. The important Copepod species were *Cyclops* and *Diaptomus*. Cladocerans mainly consisted of *Moina* sp. Ostracods were encountered to a lesser extent.

Common carp gained highest ($P < 0.05$) weight in treatment T_2 (68.78 g), followed by T_1 (63.12 g), T_3 (60.64 g), and T_0 (53.26 g) treatments; specific growth rate (% per day) followed the same trend (Table 2). *FCR* and *PER* improved with oil supplementation. Survival ranged from 96.29 (T_1) to 100% (T_0) and did not show any significant ($P > 0.05$) difference among the treatments. A marginal reduction ($P > 0.05$) in carcass moisture was recorded with supplemental oil. Dietary fish oil did not affect ($P > 0.05$) carcass protein content; but lipid level increased proportionately ($P < 0.05$), the highest being in T_3 treatment (4.63%).

Digestive enzyme activity was influenced by oil supplementation (Table 3). A reduction in protease activity and an increase in amylase activity (except in hepatopancreas of carp fed diet T_3) was recorded with increase in fish oil supplementation. However, no difference ($P > 0.05$) in lipase activity was observed in either tissue of fish from different treatments.

Table 2

Growth parameters and carcass composition of common carp from different treatments ($\bar{x}$ ±s.e.)

Parameters	Treatments			
	T_0	T_1	T_2	T_3
Mean initial weight (g)	2.21 ±0.02^a	2.20 ±0.03^a	2.13 ±0.06^a	2.21 ±0.03^a
Mean final weight (g)	53.26 ±0.42^c	63.12 ±1.64^b	68.78 ±0.67^a	60.64 ±1.02^b
Specific growth rate (%)	1.15 ±0.00^d	1.21 ±0.01^b	1.25 ±0.01^a	1.19 ±0.00^{bc}
Feed conversion ratio	2.64 ±0.02^a	2.54 ±0.06^a	2.49 ±0.01^{ab}	2.33 ±0.04^b
Protein efficiency ratio	1.39 ±0.05^b	1.43 ±0.04^b	1.49 ±0.02^b	1.67 ±0.03^a
Survival (%)	100.00 ±0.00^a	96.29 ±1.85^a	98.14 ±1.85^a	98.14 ±1.85^a
Carcass proximate composition (%)				
Moisture	80.37 ±0.09^a	79.41 ±1.81^a	78.31 ±1.17^a	77.73 ±1.23^a
Protein	14.25 ±0.27^a	13.92 ±0.11^a	14.21 ±0.32^a	14.28 ±0.37^a
Fat	1.69 ±0.03^c	3.54 ±0.07^b	4.51 ±0.12^a	4.63 ±0.31^a
Ash	2.63 ±0.01^a	1.97 ±0.03^c	2.36 ±0.02^b	2.52 ±0.04^a
NFE	1.06	1.16	0.61	0.94

Values with the same superscript in each row are not significantly different ($P > 0.05$).

Table 3

Digestive enzyme activity in intestine (I) and hepatopancreas (H) of fish from different treatments ($\bar{x}$ ±s.e.)

Enzyme		Treatments			
		T_0	T_1	T_2	T_3
Protease	I	0.244 ±0.022^a	0.150 ±0.003^b	0.078 ±0.004^c	0.051 ±0.010^c
	H	0.101 ±0.003^a	0.086 ±0.005^b	0.053 ±0.004^c	0.030 ±0.004^d
Amylase	I	0.146 ±0.012^d	0.229 ±0.008^c	0.374 ±0.029^b	0.593 ±0.011^a
	H	0.321 ±0.022^c	0.422 ±0.023^b	0.490 ±0.017^a	0.323 ±0.012^c
Lipase	I	0.077 ±0.006^a	0.088 ±0.017^a	0.108 ±0.026^a	0.078 ±0.006^a
	H	0.076 ±0.008^a	0.089 ±0.007^a	0.080 ±0.006^a	0.080 ±0.005^a

Enzyme activity is expressed in µmoles of product liberated per minute per mg of tissue protein at 28°C.

Values with the same superscript in each row are not significantly different ($P > 0.05$).

DISCUSSION

Diet containing 24% protein and 6% fat was used as the basal diet (T_0) in the present study. The total lipid content of the diet (T_2) that induced the best growth of common carp was 11.42%. Hence, a level of around 11.5% could be considered as optimum for common carp grown in manured tanks when the dietary protein content is around 24%. According to Dias et at (1998), the beneficial effects of an increase of dietary lipid level in European seabass (*Dicentrarchus labrax*) were significant only with a low protein diet, but not with a high protein diet. The growth of fish with diet T_3 which had the highest lipid level (14.2%) was inferior to that of T_2, reflecting a

negative impact of dietary fat beyond the optimum level. This could be due to the imbalance in protein : fat ratio as reported earlier by Berge and Storebakken (1991) and De Silva et al. (1991), reflecting inability of the fish to utilize lipid above a certain threshold level. Nonetheless, all fish receiving supplemental oil performed better than the control in the present study, indicating protein sparing by lipid. In a number of fish species, protein sparing by lipid has been demonstrated (De Silva et al. 1991, Bazaz and Keshavanath 1993, Vergara et al. 1996, Gangadhar et al. 1997, Weatherup et al. 1997), while in a few species no such effect has been observed (Andersen and Alsted 1993, Trono-Legiralde 1996, Peres and Oliva-Teles 1999).

Gangadhar et al. (1997) reported that the growth of rohu (*Labeo rohita*) fingerlings fed a diet containing 25% protein and 9% fat was comparable with those fed 30% protein and 6% fat. Their results indicated that growth induced by 3% dietary oil is comparable to that produced by 5% dietary protein. Jafri et al. (1995) found 5–7% lipid diet to be optimum for mrigal (*Cirrhinus cirrhosus*) fingerlings, when the diet contained 35% protein. It may be presumed that with decreased dietary protein levels, optimal lipid requirement increases. Moreover, the difference in optimal lipid requirement could also be species specific. Eventhough the protein content of the diets employed in the present study was 24%, the protein contribution by natural food has to be taken into account. Lovell (1975) observed that natural food plays a key role in the determination of dietary protein requirements of fish under pond conditions. When mirror carp was grown with both natural food and a high protein supplemental feed, fish growth and specific growth rate were positively correlated by the presence of natural food (Lam and Shephard 1988). According to Albrecht and Breitsprecher (1969), the mean protein, carbohydrate, and lipid contents of fish food organisms are 51.1, 27.3 and 7.7% respectively, the calorific value ranging from 6.7 to 23.8 $kJ \cdot g^{-1}$. The work of Varghese et al. (1976) indicated good growth of common carp with a fish meal based 31% protein diet in the absence of natural food. Since all the experimental tanks of the present study, including the control were fertilized similarly, nutrients derived by fish from natural food in all the treatments could be presumed to be equal.

Supplementation of diet with sardine oil improved *FCR* and *PER* as found earlier by Chou and Shiau (1996) and Lim et al. (2001). Jafri et al. (1995) recorded the best *FCR* and *PER* in mrigal receiving 5–7% dietary fat, when fed lipid levels ranging from 3 to 13%, through a 35% protein diet. De Silva et al. (1991), Keshavanath and Jagadeesh (1994), and Vergara et al. (1999) reported that increasing dietary lipid level increased protein retention, enhancing the proportion of dietary protein utilized for growth. No effect of dietary fat was observed on the survival of common carp in the present study. This is in agreement with the results of Trono-Legiralde (1996) with bighead carp (*Aristichthys nobilis*).

Feeding oil-supplemented diets resulted in higher levels of carcass fat in common carp. Earlier studies have shown a significant positive correlation between fish weight and carcass lipid levels, as found in the present study (Hemre and Sandnes 1999,

Torstensen et al. 2001). Increase in carcass/muscle lipid with increasing dietary lipid has been reported for most species investigated (De Silva et al. 1991, Silver et al. 1991, Bazaz and Keshavanath 1993, Anwar and Jafri 1995, Trono-Legiralde 1996, Vergara et al. 1996, Gangadhar et al. 1997, Erfanullah and Jafri 1998, Refstie et al. 2001). Dietary lipid incorporation has resulted in varied carcass protein response in different species. Hanley (1991) observed that changes in carcass protein content were not significant in Nile tilapia (*Oreochromis niloticus*) fed increasing levels of fat, as is the case in the present study. However, Bazaz and Keshavanath (1993) and Gangadhar et al. (1997) observed an increase in carcass protein with increase in dietary lipid in Deccan mahseer (*Tor khudree*) and rohu, respectively. On the contrary, Silver et al. (1991) reported an inverse relationship between carcass protein and dietary lipid in chinook salmon (*Oncorhynchus tshawytscha*). Apparently, the additional energy is stored in the form of fat and not protein in common carp. Though not significant ($P > 0.05$), an inverse relationship between carcass moisture and dietary lipid level was found in the present study as reported by Anwar and Jafri (1995) and Jafri et al. (1995).

Protease activity was higher in the intestinal tissue compared to hepatopancreas. Higher and lower proteolytic activities in common carp pancreatic and intestinal extracts, respectively have been observed by Bondi and Spandorf (1954). Bazaz and Keshavanath (1993) also recorded greater protease activity in the intestinal segments than in liver of mahseer fed sardine oil supplemented diets. The reduction in protease activity with an increase in dietary lipid observed in the present study corroborates the findings of Gangadhar et al. (1997) in rohu. The increased amylase activity in common carp receiving lipid incorporated diets could be attributed to effective utilization of carbohydrate from the diet. Carps are known to utilize carbohydrate preferentially over fat due to high amylolytic activity (Jafri et al. 1995). Though common carp is known to digest fat better than north African catfish, *Clarias gariepinus* (cf. Degani and Revach 1991), dietary lipid did not influence lipase activity in the present study. In contrast, increased lipase activity/lipid digestibility was observed in mahseer (Bazaz and Keshavanath 1993), rohu (Gangadhar et al. 1997), and European seabass (Peres and Oliva-Teles 1999) fed increasing levels of dietary lipid.

Thus, dietary fat supplementation influenced growth, food conversion, protein efficiency, carcass lipid content, and digestive enzyme activity, but not survival. Among the levels tested, 6% additional fat proved more effective, inducing the best growth. The results clearly show that even in common carp that is known to utilize carbohydrates well, growth can be augmented with low protein diets, if supplemented with fish oil.

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