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Influence of Sardine Oil Supplemented Fish Meal Free Diets on Common Carp (*Cyprinus carpio*) Growth, Carcass Composition and Digestive Enzyme Activity

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ABSTRACT

Supplying much of the required energy through carbohydrates and/or lipids helps in avoiding protein utilization as an energy source. The present study was carried out to evaluate the effect of fish meal free diets, formulated with high levels of maize and supplemented with sardine oil, on growth, carcass composition and digestive enzyme activity of common carp, *Cyprinus carpio* when grown under a fertilization regime. Fingerlings of average weight (av. wt.) 2.21-2.24 g stocked at 1 per m^2 in mud bottomed tanks (18 m^2) were fed daily on one of the four fish meal-free (protein 17.73 to 18.91%, Nitrogen-free Extract (NFE) 40.44 to 47.40%) sardine oil supplemented (0, 3, 6 and 9%) diets in triplicate, over a period of 120 days. Highest weight gain and Specific Growth Rate (SGR) were recorded in fish fed the diet containing 9% added oil. Fish survival varied between 96.29 and 98.14%. Addition of oil resulted in lower ($p<0.05$) Feed Conversion Ratio (FCR). Carcass protein and fat increased, while ash content decreased in fish fed oil supplemented diets. Protease activity in the intestine as well as hepatopancreas of the treated fish showed a decrease ($p<0.05$). Amylase activity was high but did not differ among treatments ($p>0.05$). Increased lipase activity was observed with increasing level of oil addition only in the hepatopancreas. The results of the present study reveal the ability of common carp to perform well with low protein, oil supplemented fish meal free diets, 9% added oil inducing the best growth of fish when grown in fertilized tanks.

Key words: Fish meal-free diets, oil supplementation, *Cyprinus carpio*, growth, cacrass composition

INTRODUCTION

Fish meal and fish oil are still widely used as the main source of dietary protein and lipid, respectively, in fish feeds which leads to higher cost of production. Various approaches are being adopted to reduce the cost of cultured fish production. Prominent among these are the farm-made feeds with total substitution of fish meal and fish oil (Singh *et al*., 2006a; Shapawi *et al*., 2011) or development of diets with reduced dietary fish meal content (Sheeno and Sahu, 2006). The extent of fish meal reduction should not, however, affect the growth and quality of fish and at the same time maximize utilization of dietary protein for growth. Exploiting the synergetic interaction between natural food and supplemental feed would also be beneficial. According to Hasan *et al*.

(2007), the proportion of fish meal in aqua feeds will be substantially reduced by the increasing use of vegetable-based protein and by greater efficiencies in feeding. Protein utilization for growth is related to both the dietary protein level and the availability of non-protein energy sources. Supplying much of the required energy through carbohydrates and/or lipids helps in avoiding protein utilization as an energy source and improving protein utilization efficiency (Kaushik and Medale, 1994; Kim and Lee, 2005). Non-protein energy sources, such as lipids and carbohydrates, can be effectively used to reduce the requirement for protein (Watanabe, 2002). Protein-sparing effect of non-protein nutrients may be effective in reducing feed costs (Gumus and Ikiz, 2009). Studies carried out using non-protein dietary energy sources have shown positive impact on growth and body composition of fish (Martino *et al*., 2002; Skalli *et al*., 2004; Cho *et al*., 2005; Singh *et al*., 2006b; Hu *et al*., 2007; Mohanta *et al*., 2007; Schulz *et al*., 2008; Yoshii *et al*., 2010; Mohseni *et al*., 2011).

Common carp is an important bottom feeding species employed in carp polyculture, wherein natural productivity is enhanced through the use of fertilizers (Sarangi *et al*., 2004). This study was carried out with the major objectives of (1) evaluating the performance of common carp in fertilized tanks when fed sardine oil supplemented, low protein fish meal free diets, containing high levels of maize and (2) the protein sparing effect of the non-protein energy sources. Findings on fish growth, body composition and digestive enzyme activity are reported here.

MATERIALS AND METHODS

This work was conducted from December 2003 to March 2004 using common carp seed obtained from the fish farm of the Department of Fisheries, Karnataka.

Diets: Four fish meal free, low protein (17.73-18.91%) test diets (T_0, T_1, T_2 and T_3) were formulated (Varghese *et al*., 1976) incorporating high levels of maize (Table 1). Dietary ingredients (groundnut cake, rice bran and maize) procured locally were dried, pulverized and sieved to obtain uniform particles (400 mm size). Fish (sardine) oil was incorporated at 3, 6 and 9% levels in diets T_1, T_2 and T_3, respectively. 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(1:0.8 ratio). The diets were prepared following the method described by Jayaram and Shetty (1981) to obtain 3 mm diameter pellets that were dried soon after pelleting in a thermostatic oven at a temperature of 40°C and packed in heavy duty plastic bags.

Experimental set up: The experiment was carried out over a period of 120 days in 12 outdoor cement tanks of 18 m^2 each, with a 15 cm thick soil base. The tanks were cleaned and dried, limed at 400 kg ha^{-1} (0.72 kg $tank^{-1}$) and initially fertilized with poultry manure at 2000 kg ha^{-1}; subsequent fertilization was done at 5% of the initial dose of poultry manure every 15 days. Ground water was used to fill the tanks, maintaining a depth of 90±5 cm throughout the experimental period. Common carp fingerlings (av. wt. 2.21-2.24 g) were stocked at a density of 1 per m^2 (18/tank). The four diets were fed to triplicate group of fish once daily 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. The fish were sampled at 15-day intervals for measuring growth. The quantity of feed given was adjusted after each fish sampling, on the basis of new weight of the fish. On termination of the experiment, the surviving fish were weighed, based on which the following parameters were calculated.

Table 1: Ingredient proportion and proximate composition of diets and ingredients

Items	Diets			
	T_0	T_1	T_2	T_3
Ingredients (%)				
Groundnut oil cake	25	25	25	25
Rice bran	24	24	24	24
Maize	50	47	44	41
Fish (sardine) oil	0	3	6	9
Vitamin mineral mixture*	1	1	1	1
Cost (Rs. kg diet^{-1})	6.22	7.60	8.98	10.36
Proximate composition (%) of diets				
Moisture	6.92±(0.28)	7.19±(0.43)	7.23±(0.51)	8.04 ±(0.39)
Crude protein	18.91±(0.22)	18.63±(0.40)	18.09±(0.32)	17.73±(0.16)
Fat	5.48±(0.31)	8.33±(0.09)	11.61±(0.45)	13.28±(0.62)
Ash	12.89±(1.02)	13.11±(0.20)	12.98±(0.17)	12.43±(0.26)
Crude fibre	8.40±(0.31)	8.29±(0.18)	8.18±(0.25)	8.08±(0.43)
N-free extract	47.40	44.45	41.91	40.44
Energy (kJ g^{-1})	14.56	15.10	15.81	16.13
P/E ratio	1.29	1.23	1.14	1.09

Values are as Mean±SD, *Supplevite-M (Sarabhai Company Ltd., India)

$$\text{Specific Growth Rate (SGR) } (\% \text{ d}^{-1}) = \frac{\text{Ln final weight - Ln initial weight}}{\text{Experimental duration (days)}} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Dry weight of feed given (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Protein Effciency Ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Amount of protein fed (g)}}$$

Water quality: Water quality parameters viz. temperature, pH, dissolved oxygen, free carbon dioxide and total alkalinity were determined every 15 days, by collecting samples between 07.00 and 08.30 h. Water temperature was recorded using a thermometer while pH was measured with a digital pH meter (LI-120, ELICO, India). Dissolved oxygen, free carbon dioxide and total alkalinity were determined following standard procedures (APHA, 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 in each experimental tank. 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. Wet and dry weights of plankton were also determined.

Biochemical composition: Proximate composition of ingredients, diets and fish carcass was analyzed in triplicate. Three fish from each treatment were randomly selected for carcass analysis. Protein was determined by Kjeltec (Tecator-1002), Lipid by Soxtec (Tecator-1043) and fibre by

Fibretec (Tecater-1017) systems. Ash was analyzed by incineration (AOAC, 1995) and NFE was computed by the difference method (Hastings, 1976). The energy content of the feed ingredients and diets was calculated using values of 22.6 kJ g^{-1} for protein, 38.9 for lipid and 17.2 kJ g^{-1} for carbohydrate as NFE (Mayes, 1990).

Enzyme assay: The activity of digestive enzymes viz. amylase, protease and lipase in the hepatopancreas and intestine of the experimental fish was analyzed on termination of the experiment by the methods of Bernfeld (1955), Kunitz (1946) and Bier (1962), respectively, using six fish from each treatment. Enzyme activity is expressed in μm of product liberated per min per mg of tissue protein.

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 ranged as follows: water temperature 17.4-23.5°C, pH 7.45-8.36, dissolved oxygen 6.30-8.83 mg L^{-1}, free carbon dioxide 0-2.4 mg L^{-} and total alkalinity ($CaCO_3$) 55.84-89.12 mg L^{-1}.

Among the feed ingredients, groundnut cake had the highest crude protein and fat (39.62 and 8.02%), rice bran the highest crude fibre (24.07%) and maize the highest NFE (74.44%) (Table 2). The control diet (T_0) had the highest protein and NFE (18.91and 47.40%) values while they were the lowest in diet T_3 (17.73 and 40.44%). In contrast, fat content was the least in diet T_0 (5.48%) and highest in diet T_3 (13.28%) (Table 1).

The phytoplankton in the different treatments belonged mainly to Cyanophyceae (*Microcystis* sp.; *Spirulina* sp.; *Oscillatoria* sp.), Chlorophyceae (*Eudorina* sp.; *Scenedesmus* sp.; *Spyrogyra* sp.; *Volvox* sp.; *Ulothrix* sp. and *Pondorina* sp.) and Bacillariophyceae (*Melocera* sp.; *Navicula* sp.; *Synedra* sp. and *Fragilaria* sp.). The important zooplankton encountered belonged to the groups Rotifera (*Brachionus* sp.; *Asplanchna* sp. and *Filina* sp.), Copepoda (*Cyclops* sp. and *Diaptomus* sp.), Cladocera (*Moina* sp.) and larval forms (crustaceans). Some small protozoans were found occasionally. Table 3 summarizes the number of phytoplankton and zooplankton encountered in the water samples from different treatments. Plankton density in the different treatments ranged from 190 No. L^{-1} (T_2, Day 0) to 8292 No. L^{-1} (T_2, Day 75) during the experimental period. It was generally higher in the first

Table 2: Proximate composition (%) of ingredients

Proximate composition (%)	Groundnut oil cake	Rice bran	Maize
Moisture	9.46±0.41	8.92±0.24	8.43±0.21
Crude protein	39.62±0.27	8.17±0.09	8.62±0.26
Fat	8.02±0.21	7.26±0.19	3.66±0.03
Crude fibre	3.42±0.33	24.07±0.31	3.54±0.82
Ash	5.74±0.18	17.49±1.20	1.31±0.15
N-free extract	33.74	34.09	74.44
Energy (kJ g^{-1})	17.88	10.53	16.18

Values are as Mean±SD

half of the experiment, reaching peak values on the 75th day; thereafter, a decline was noticed (Table 3).

The mean final weight of common carp under T_2 (64.42 g) and T_3 (74.72 g) treatments was significantly ($p<0.05$) higher compared to that of the control (57.63 g). Specific growth rate (%) and FCR were the best ($p<0.05$) under T_3 treatment. FCR improved ($p<0.05$) with all levels of oil

Table 3: Mean plankton density (No. L^{-1}) in different treatments

	Days								
Treatments	0	15	30	45	60	75	90	105	120
Phytoplankton									
T_0	201^{a}	758^{a}	3622^{bc}	4462^{b}	4518^{a}	4873^{c}	3473^{c}	3032^{a}	1523^{b}
T_1	200^{a}	621^{a}	3446^{bc}	1339^{d}	1848^{d}	5691^{b}	5478^{a}	3159^{a}	1328^{bc}
T_2	190^{a}	371^{a}	5336^{a}	5165^{a}	4052^{b}	8292^{a}	4762^{b}	2331^{b}	1873^{a}
T_3	221^{a}	336^{a}	3715^{b}	3423^{c}	3414^{c}	4706^{c}	2991^{c}	2223^{b}	1233$^{c\,d}$
Zooplankton									
T_0	55^{a}	27^{b}	137^{a}	228^{a}	372^{a}	389^{a}	290^{a}	187^{a}	186^{a}
T_1	16^{b}	15^{c}	87^{b}	130^{b}	150^{b}	187^{b}	185^{b}	150^{a}	105^{bc}
T_2	15^{b}	84^{a}	60^{b}	132^{b}	61^{c}	102^{c}	44^{d}	54^{c}	129^{b}
T_3	17^{b}	35^{b}	66^{b}	93^{bc}	81^{c}	104^{c}	91^{c}	84^{b}	182^{a}

Values with same superscript in each column are not significantly different ($p>0.05$)

Table 4: Growth parameters and carcass composition of common carp in different treatments

	Treatment			
Parameter	T_0	T_1	T_2	T_3
Mean initial weight (g)	2.24±0.02^{a}	2.22±0.02^{a}	2.23±0.03^{a}	2.21±0.02^{a}
Mean final weight (g)	57.63±1.29^{c}	60.05±1.92^{bc}	64.42±1.47^{b}	74.72±1.37^{a}
Mean net weight gain (g)	55.39±1.21^{c}	57.83±1.91^{bc}	62.19±1.46^{b}	72.51±1.38^{a}
Specific growth rate (%)	1.16±0.01^{b}	1.17±0.01^{b}	1.18±0.02^{b}	1.27±0.01^{a}
Food conversion ratio	2.12±0.02^{a}	1.36±0.03^{c}	1.41±0.04^{c}	1.63±0.03^{b}
Protein efficiency ratio	2.47±0.05^{a}	2.55±0.08^{a}	2.46±0.05^{a}	2.24±0.04^{a}
Survival (%)	96.26^{a}	98.14^{a}	98.14^{a}	98.14^{a}
Carcass composition (%)				
Moisture	80.53±0.30^{a}	78.69±0.63^{ab}	76.86±0.39^{b}	78.64±0.60^{ab}
Crude protein	13.21±0.04^{c}	14.41±0.12^{b}	15.51±0.12^{a}	14.40±0.0^{b}
Fat	2.09± 0.05^{d}	3.39±0.02^{c}	4.58±0.12^{c}	4.58±0.01^{a}
Ash	2.66±0.02^{a}	2.30±0.02^{c}	2.50±0.03^{b}	2.36±0.06^{c}

Values in parentheses indicate standard error, Values with same superscript in each row are not significantly different ($p>0.05$)

Table 5: Digestive enzyme activity in common carp from different treatments

	Protease		Amylase		Lipase	
Treatment	Intestinal	Hepato[1].	Intestinal	Hepato.	Intestinal	Hepato.
T_0	0.165±(0.018)^{b}	0.054±(0.003)^{a}	0.807±(0.045)^{a}	2.166±(0.117)^{a}	0.082±(0.029)^{a}	0.024±(0.005)^{bc}
T_1	0.180±(0.004)^{a}	0.037±(0.001)^{b}	0.860±(0.137)^{a}	2.387±(0.304)^{a}	0.063±(0.011)^{a}	0.026±(0.002)^{bc}
T_2	0.143±(0.014)^{c}	0.029±(0.006)^{bc}	0.949±(0.093)^{a}	2.697±(0.195)^{a}	0.054±(0.005)^{a}	0.036±(0.010)^{ab}
T_3	0.095±(0.003)^{d}	0.017±(0.006)^{c}	1.216±(0.217)^{a}	2.770±(0.357)^{a}	0.056±(0.009)^{a}	0.041±(0.006)^{a}

[1]Hepatopancreatic, Values in parentheses indicate standard error, Enzyme activity is expressed in μ moles of product liberated per minute per mg of tissue protein at 28°C. Values with same superscript in each column are not significantly different ($p>0.05$)

supplementation. PER values were only marginally different ($p>0.05$) among treatments. The survival of fish ranged from 96.26 to 98.14% (Table 4).

Carcass protein, fat and ash content were affected by dietary oil supplementation ($p<0.05$). Fat increased with increasing dietary lipid level, the highest being 4.58% in T_2 and T_3 treatments. Fish from T_2 treatment had the highest carcass protein content of 15.51%. Fish under the different treatments had significantly ($p<0.05$) lower ash than that of the control (Table 4).

Protease activity in the treated fish showed a decrease in the hepatopancreas as well as intestine ($p<0.05$). Amylase activity was high but did not differ among treatments ($p>0.05$). Increased lipase activity was observed in the hepatopancreas with increasing level of dietary oil supplementation (Table 5).

DISCUSSION

Sardine oil supplementation of the diets led to an increase in energy and a decrease in protein content and P/E ratio (Table 1). An optimal P:E ratio is considered a crucial factor for protein use efficiency. Nonetheless, growth of fish receiving oil supplemented diets increased significantly over that of the control. This could be related to optimization of the available dietary protein for somatic growth while the lipid adequately meets the energy needs (Caballero *et al*., 1999; Satpathy *et al*., 2003). In an earlier study conducted under identical conditions using diets containing 10% fish meal (24% protein) and supplemented with sardine oil, common carp harvest weight ranged from 53.26 g on control diet with no oil addition to 68.78 g on diet with 6% supplemented oil (Manjappa *et al*., 2002). In contrast, in the present study, the best growth (74.72 g) was obtained with the 9% oil supplemented diet containing no fish meal (17.73% protein) as against the mean weight of 57.63 g of the control fish. This performance reflects the ability of common carp to grow well on a diet with no fish meal, when cultured in the fertilized system. The protein requirement of carps varies between 25-35%, depending upon age (Hossain *et al*., 1997). Since the diets employed in the present study had low protein (17.73 to 18.91%), it may be presumed that natural food has contributed to fish growth. The plankton number showed an increase till the 75th day of the experiment and then declined in all the treatments. Regular fertilization facilitated plankton multiplication while higher grazing with greater fish biomass and increase in energy demand as the experiment progressed would have led to the decrease noticed. Lovell (1975) observed that natural food plays a key role in the determination of dietary protein requirements of fish under pond conditions. Common carp being an omnivore must have been able to utilize some protein from detritus also, apart from plankton. When mirror carp was grown with both natural food and a high protein supplemental feed, fish growth and specific growth rate were positively correlated with the density of natural food (Lam and Shephard, 1988). Schroeder (1983) stated that the dietary protein level and also percent protein energy level in the diet of tilapia could be reduced in the presence of natural food as it can correct the deficient nutrient in the diet. Hepher (1988) reported 18-35% protein, 7-10% lipid and 27-48% ash content (dry matter basis) for planktonic algae in ponds which indicates the nutritive value of the natural food.

Higher growth of fish fed the oil supplemented diets in the present study in comparison with that of the control indicates protein sparing by lipid. Increasing the energy density of diets has been suggested as a strategy to spare protein and limit ammonia production for several fish species, including common carp (Steffens, 1996). If the growth of common carp obtained in this study is compared with that reported by Manjappa *et al*. (2002), 3% additional sardine oil supported growth

surpassing that by 10% dietary fish meal. Gangadhara *et al.* (1997) found 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, indicating that growth induced by 3% dietary oil is comparable to that produced by 5% dietary protein. Kheir and Saad (2003) who determined the suitable lipid level which spares dietary protein for maximum growth in three fish species viz. *Oreochromis niloticus*, *Sarotherodon galilaeus* and *Hypophthalmichthys molitrix*, found that a low protein level of 20% with a high lipid level of 6% produced nearly equal growth and FCR similar to that of high protein (30%) and low lipid (2%). They concluded that fish are able to store considerable quantities of lipid in their tissues for utilization as energy source in order to improve growth and feed utilization. Skalli *et al.* (2004) observed that increasing dietary lipid from 10.6 or 13.5 to 19.7% allowed the protein level to be decreased from 53 or 57 to 50% without affecting the SGR and FCR, suggesting that energy from lipid spares protein in fingerling *Dentex dentex*. Protein sparing by lipid has also been demonstrated in other fish species such as *Rhamdia quelen* (Meyer and Fracalossi, 2004), *Epinephelus coioides* (Luo *et al.*, 2005) and *Sander leucioperca* (Schulz *et al.*, 2008). More than one-third of dietary protein could be spared by replacing protein with lipid in the diet of *L. rohita* (Satpathy *et al.*, 2003). The beneficial effects of increased dietary lipid are associated with low protein diets rather with high protein diets (Dias *et al.*, 1998). Hu *et al.* (2007) reported maximum growth of yellowfin seabream at a dietary lipid level of 13.63%, a level close to that of 9% oil supplemented diet used in the present study (13.28%).

FCR of diets improved with oil supplementation which indicates that oil addition improved diet utilization. Similar observations have been made by Satpathy *et al.* (2003) in *L. rohita* and Cho *et al.* (2005) in *Schophthalmus maximus* and El-Marakby (2006) in *Oreochromis niloticus*. Addition of fish oil did not affect fish survival; it was 98.14% in all the treatments as against 96.26% of the control. Dietary oil supplementation enhanced carcass lipid content as has been reported in *C. carpio* fingerlings by Abbass (2007) and other species viz. *Pseudoplatystoma coruscans* (Martino *et al.*, 2002), *L. rohita* (Satpathy *et al.*, 2003), *S. maximus* (Cho *et al.*, 2005), *E. coioides* (Luo *et al.*, 2005), *Sparus latus* (Hu *et al.*, 2007), *E. malabaricus* (Tuan and Williams, 2007), *S. leucioperca* (Schulz *et al.*, 2008), *E. bruneus* (Yoshii *et al.*, 2010) and also the protein content, *Tor khudree* (Bazaz and Keshavanath, 1993), *L. rohita* (Gangadhara *et al.*, 1997) and *S. maximus* (Cho *et al.*, 2005).

High amylase activity recorded in fish from different treatments, including control, in this study could be attributed to the high maize content of the diets. Warm water herbivorous or omnivorous fish utilize high levels of carbohydrate and the efficiency of dietary carbohydrate utilization is better in common carp (Wilson, 1994). Jafri *et al.* (1995) opined that carps utilize carbohydrate preferentially over fat due to high amylolytic activity. Increased lipase activity was observed in the hepatopancreas with the increasing level of dietary oil addition. Higher lipase activity has been reported in *T. khudree*, *L. rohita* and *Dicentrarchus labrax* fed oil supplemented diets (Bazaz and Keshavanath, 1993; Gangadhara *et al.*, 1997; Peres and Oliva-Teles, 1999). Protease activity of the treated fish showed a decrease in the intestine as well as hepatopancreas. This could be because of the decreased dietary protein content following oil addition. Nonetheless, treated fish had higher carcass protein, reflecting protein synthesis and increased tissue production (Fafioye *et al.*, 2005).

CONCLUSIONS

It is concluded that common carp grown in the fertilized system can perform well on a low protein fish meal free diet when the energy content of the diet is enhanced through oil

supplementation. Further, sardine oil spares protein for growth and common carp is capable of effectively utilizing high amount of dietary maize.

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