Indian J. Fish., 63(4): 96-103, 2016
DOI: 10.21077/ijf.2016.63.4.52678-15

Growth response of *Catla catla* (Hamilton, 1822) raised in manured tanks on low fishmeal diets, with a note on carcass composition and digestive enzyme activity

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ABSTRACT

Low fishmeal diets (10%) with varied levels of maize (40-31%) and sardine oil (0-9%) were fed for 120 days to triplicate groups of catla (*Catla catla* Hamilton,1822) fingerlings (average weight 1.84-1.90 g) stocked @ 1 fish per m^2 in cement tanks with soil base fertilised with poultry manure. Fish fed on diet containing 6% oil and 34% maize (T_2) showed the highest ($p<0.05$) growth, followed by those received 9/31 (T_3), 3/37 (T_1) and 0/40% (T_0) oil/maize supplemented diets. Food conversion ratio improved due to oil supplementation, while protein efficiency ratio was not affected significantly. Dietary lipid had a positive impact on carcass protein and lipid levels ($p<0.05$). Fish survival ranged from 90.73% in all the treatment groups to 92.58% in the control, without any significant ($p>0.05$) difference among them. Net fish production on termination of the experiment was lowest (820 g) in the control and the highest (1017.85 g) inT_2 treatment. Viscerosomatic index (VSI) varied from 3.59 (T_2) to 4.65% (T_0) and hepatosomatic index (HSI) from 1.13 (T_0) to 1.91% (T_3). RNA/DNA ratio was highest (3.05) in T_2 and lowest (1.84) in T_0. An increase in intestinal amylase activity was observed in the treated fish, while intestinal protease and lipase activity showed increase only with higher levels of oil supplementation (6 and 9%). No difference ($p>0.05$) in enzyme activity was observed in the hepatopancreas of the control and treated fish. The results indicated beneficial effects of incorporating maize and fish oil in low fishmeal diet for catla.

Keywords: Carcass composition, *Catla catla,* Digestive enzymes, Fishmeal, Growth response, Maize, Sardine oil

Introduction

Fishmeal is generally added to carp diets to increase feed efficiency and growth as it helps to enhance feed palatability, nutrient uptake, digestion and absorption. The balanced amino acid composition of fishmeal complements other animal and vegetable nutrients to provide synergistic effects that promote faster growth (Mile and Chapman, 2005). Furthermore, fishmeal has low fibre content and is also a valuable source of vitamins B1, B2, B6 and B12, in addition to calcium, phosphorous, magnesium, potassium and trace elements. However, due to uncertainty in availability, the high cost and environmental impact, it is advisable to keep the fishmeal component of fish diets low. This is generally done by replacing part of the fishmeal component with cheaper plant protein sources (Mbahinzireki *et al*., 2001) that have fairly good amount of protein and high levels of carbohydrate. In such diets, the energy content can be enhanced through the addition of oil.

Although carps can utilise carbohydrates efficiently, lipids are considered as important energy sources in carp diet (Steffens, 1996). Carbohydrates improve the pelleting quality and nutrient value of diets (Lovell, 1989) while lipids play important physiological roles in providing energy, essential fatty acids and fat soluble nutrients for normal growth and development of fish. Deficiency of dietary lipid may increase the use of protein for energy and result in increased ammonia excretion leading to water pollution (Kaushik and Cowey, 1991). Carbohydrate and oil have been demonstrated to spare protein in fish and crustaceans (Nankervis *et al*., 2000; Keshavanath *et al*., 2002; Ovie *et al*., 2005; Singh *et al*., 2006; Mohseni *et al*., 2011; Wang *et al*., 2014).

Knowledge of the optimal level of protein and the protein sparing effects of non-protein nutrients such as lipids or carbohydrates can be used effectively in reducing feed costs (Shiau and Lin, 1993). The aim of this study was to examine the growth response of *Catla catla*

(Hamilton,1822) to diets containing low level of fishmeal with varying levels of maize and sardine oil, when grown in manured tanks. Catla is a fast growing Indian major carp, feeding mainly on zooplankton at the surface (Jhingran, 1991). It accepts artificial diets and therefore, is a popular species for polyculture in India.

Materials and methods

Diets

Four low-protein diets were formulated (Varghese *et al*., 1976) to contain 24% protein (Table 1). The feed ingredients (fishmeal, groundnut oil cake, rice bran and maize) were dried, pulverised and sieved to obtain uniform particle size (400 µm). 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 to that extent. Diet T_0 without oil supplementation served as the control. Oil incorporation to the diets was done by adding the 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 2 mm dia. The pellets were dried in a thermostatic oven at a temperature of 40°C and packed in heavy duty plastic bags until use.

Experimental set up

The experiment was carried out over a period of 120 days in 12 cement tanks of 18 m^2 each, with 15 cm thick soil base. The tanks were cleaned and dried, limed at 400 kg ha^{-1} (0.72 kg per tank) and initially fertilised with poultry manure at 2000 kg ha^{-1} (3.6 kg per tank), while subsequent fertilisation was done at 5% of the initial dose at fortnightly 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. Catla fry obtained from B. R. Project Fish Farm, Shimoga were acclimatised for a week and were stocked at a density of 1 per m^2 in the experimental tanks (18 per tank) as practiced by Indian aquafarmers (Jhingran, 1991). Their initial average weight in the different treatments ranged from 1.84 to 1.90 g. The four test diets were fed to triplicate groups of fishes every day once in the morning at 5% body weight as per Varghese *et al*. (1976), using plastic trays suspended into the tanks 50 cm below the water surface. A minimum of 50% of the stocked fish from each tank was sampled every fortnight for assessing growth. The quantity of feed given was adjusted 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:

Specific growth rate (SGR) = [(ln Final weight - ln Initial weight/ Experimental duration in days)] × 100

Feed conversion ratio (FCR) = Feed consumed (g) / Weight gain (g)

Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g)

$$\text{Condition factor (K)} = \frac{W \times 100}{L^3}$$

where, W = weight of fish (g), L= length of fish (cm)

Yield (g) = Mean body weight (g) x Total number of live fish at harvest

Viscerosomatic index (VSI) (%) = Weight of viscera (g)/Weight of fish (g) x 100

Hepatosomatic index (HSI) (%) = Weight of liver (g)/Weight of fish (g) x 100

Table 1. Ingredient proportion and proximate composition (%, mean±SD) of the experimental diets

Ingredient (%)	Diets			
	T_0	T_1	T_2	T_3
Fishmeal	10	10	10	10
Groundnut oil cake	25	25	25	25
Rice bran	24	24	24	24
Maize	40	37	34	31
Sardine oil	0	3	6	9
Vitamin and mineral mixture	1	1	1	1
Proximate composition (%)				
Moisture	7.41±0.15	8.00±1.02	7.92±0.92	7.88±0.62
Crude protein	24.54±0.15	24.08±0.56	23.78±0.40	23.84±0.52
Lipid	7.01±0.11	9.23±0.08	11.56±0.19	13.19±0.22
Ash	13.12±1.02	13.20±0.91	13.01±0.68	12.86±0.91
Crude fibre	8.12±0.53	7.95±0.61	7.76±0.42	7.51±0.16
NFE	39.80	37.54	35.97	34.72
Total energy (kJ g^{-1})	15.12	15.49	16.06	16.49
CHO: L ratio	5.68	4.01	3.11	2.63

Water quality

Water samples were collected at 15 day intervals between 07.00 and 08.30 hrs for analysis of temperature, dissolved oxygen, pH, free carbon dioxide and total alkalinity. Water temperature was recorded using a thermometer and pH was measured with a digital pH meter (ELICO, India). Dissolved oxygen, free carbon dioxide and total alkalinity were determined following standard procedures (APHA, 1992). Plankton samples were also collected by filtering 100 l of water from different locations of each experimental tank using a net made of bolting silk (No. 30) having 60 µm mesh size. 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).

Biochemical composition

Proximate composition of feed ingredients, diets and fish carcass was analysed in triplicate. Three fish from each tank were used for carcass analyses. Protein was determined by Kjeltec (Tecator-1002), lipid by Soxtec (Tecator-1043) and fibre by Fibretec (Tecator-1017). Ash was analysed by incineration (AOAC, 1995) and nitrogen free extract (NFE) by the 'difference method' of Hastings (1976). Energy content of the feed ingredients and diets was calculated using values of 22.6 kJ g^{-1} for protein, 38.9 kJ g^{-1} for lipid and 17.2 kJ g^{-1} for carbohydrate as NFE (Mayes, 1990).

Enzyme assay

On termination of the experiment, the activity of digestive enzymes, amylase, protease and lipase in the intestine and hepatopancreas of the experimental fish was analysed in triplicate as per the methods of Bernfeld (1955), Kunitz (1947) and Bier (1955) respectively. Pooled tissues from six fishes per treatment were used for enzyme assay.

Estimation of muscle DNA and RNA

Nucleic acids from the experimental fish muscle were extracted using perchloric acid (Burton 1956). DNA was determined by the diphenylamine method of Giles and Myres (1965) and RNA as described by Ceriotti (1955).

Statistical analysis

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

Results and discussion

Water quality parameters recorded in the tanks during the experimental period were: water temperature - 20.0 to 22.8°C; pH - 7.46 to 8.42; dissolved oxygen - 4.56 to 8.62 ppm; free carbon dioxide - 0 to 2.40 ppm and alkalinity ($CaCO_3$) - 55.44 to 98.28 ppm, which were within the tolerable limits for catla. The diets (T_0-T_3) used in this study had varying levels of maize (decreasing from 40 to 31%), a rich carbohydrate source and sardine oil (increasing from 0 to 9%), a major lipid source and their fishmeal content was constant at 10%. The crude protein content of diets varied between 23.24% (T_3) and 24.54% (T_0), while lipid content ranged from 7.01% (T_0) to 13.19 (T_3), increasing with oil supplementation, but not exactly reflecting the added oil percentage. The carbohydrate:lipid (CHO:L) ratio was found decreasing with increase in fish oil addition, with the lowest value in diet T_3 (2.63), highest in T_0 (5.68), 4.01 in T_1 and 3.11 in T_2. Fish fed diet T_2 containing 34% maize and 6% added oil (NFE 35.97% and fat 11.56%) showed the best growth (62.33 g); this probably indicates the optimum level of carbohydrate and fat required by catla fingerlings when fed a diet having 24% protein and grown under pond conditions. Best growth of catla was recorded in T_2 treatment, followed by T_3 (57.29 g), T_1 (55.21g) and T_0 (49.22 g), growth being significantly ($p<0.05$) higher in all the treatment groups compared to the control (T_0). Specific growth rate (% per day) followed the same trend (Table 2). Based on the performance of fish, CHO:L ratio of 3.11 may be considered as optimal for catla. Erfanullah and Jafri (1998) recorded maximum weight gain, SGR, FCR, PER, protein retention and energy retention in catfish, *Clarias batrachus* fed a 35% protein, 27% carbohydrate and 8% lipid diet, corresponding to a CHO:L ratio of 3.38.The optimum CHO:L ratio found in the present study is close to that of catfish, even though the protein content of the diets used was lower at 24%, an indication of lower protein requirement by catla. Nonetheless, it is pertinent to consider the protein contribution by natural food, since the present experiment was conducted in manured tanks. In systems where fertilisation is used to enhance natural feed production, a part of the nutritional requirement of the fish is met by the natural food (Priyadarshini *et al*., 2011). According to Albrecht and Breitsprecher (1969), the mean protein, carbohydrate and lipid contents of natural food are 51.1, 27.3 and 7.7% respectively, with calorific value ranging from 6.7 to 23.8 kJg^{-1}. 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 natural food. Natural food also improves the utilisation of artificial diets by supplying certain digestive enzymes (Jhingran, 1991). The mean

Table 2. Growth parameters and carcass composition (mean±SD) of catla from different treatments

Parameter	Treatments			
	T_0	T_1	T_2	T_3
Initial weight (g)	1.88±0.04[a]	1.84±0.03[a]	1.86±0.05[a]	1.90±0.03[a]
Final weight (g)	49.22±1.10[c]	55.21±0.52[b]	62.33±1.09[a]	57.29±1.26[b]
Initial length (cm)	5.42±0.02[a]	5.44±0.07[a]	5.43±0.02[a]	5.43±0.03[a]
Final length (cm)	13.58±0.04[c]	14.50±0.13[b]	15.29±0.23[a]	14.70±0.06[b]
Net weight gain (g)	47.34±0.81[c]	53.37±0.51[b]	60.47±1.11[a]	55.39±1.27[b]
SGR (%)	1.18±0.02b[c]	1.22±0.01[b]	1.27±0.01[a]	1.23±0.01[b]
FCR	2.18±0.05[a]	2.08±0.02[b]	2.09±0.04[ab]	2.10±0.05[ab]
PER	1.60±0.04[a]	1.71±0.01[a]	1.70±0.03[a]	1.67±0.04[a]
Survival (%)	92.58±1.85[a]	90.73±1.85[a]	90.73±1.85[a]	90.73±1.85[a]
Condition factor	1.96	1.81	1.74	1.80
VSI	4.65±0.17[a]	4.44±0.07[a]	3.59±0.29[b]	4.55±0.16[a]
HSI	1.13±0.14[a]	1.47±0.13[b]	1.59±0.07[b]	1.90±0.18[c]
RNA/DNA ratio	1.84	2.42	3.05	2.73
Carcass composition (%)				
Moisture	80.58±0.54[a]	78.97±0.86[a]	77.35±0.24[b]	79.00±0.27[ab]
Crude protein	12.11±0.16[c]	13.64±0.08[a]	13.98±0.05[a]	12.83±0.09[b]
Fat	2.07±0.07[c]	2.70±0.03[b]	3.06±0.07[a]	3.54±0.12[a]
Ash	3.10±0.02[a]	2.68±0.33[a]	3.13±0.02[a]	2.99±0.28[a]
NFE	2.14	2.01	1.94	1.64

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

plankton density (no l^{-1}) recorded in different treatments over the experimental duration is given in Table 3. The average dry weight of plankton in T_0, T_1, T_2 and T_3 treatments varied from 2.39 to 70.44, 2.01 to 65.48, 2.45 to 50.14 and 2.67 to 71.25 mg 100 l^{-1} respectively over the experimental duration. The mean phytoplankton density showed a steady increase till the 60th day of the experiment and a decline thereafter. Phytoplankton belonging to Chlorophyceae, Cyanophyceae and Bacillariophyceae were encountered. Chlorophyceae consisted of *Closterium* sp., *Pediastrum* sp., *Pandorina* sp., *Eudorina* sp., *Volvox* sp., *Ulothrix* sp. and *Scenedesmus* sp. Among Cyanophyceae, *Anabaena* sp., *Microcystis* sp. and *Spirulina* sp. were conspicuous. The dominant Bacillariophyceae were *Synedra* sp., *Melosira* sp., *Fragilaria* sp. and *Navicuala* sp. No clear cut trend was observed in mean zooplankton density. The important zooplankton belonged to the groups Rotifera represented by *Brachionus* sp., *Keratella* sp., *Asplanchna* sp., *Polyarthra* sp. and *Filinia* sp. Copepoda with *Cyclops* sp. and *Diaptomus* sp. and Cladocera consisting of *Moina* sp. The increase in mean plankton density might be due to the effect of poultry manure used as well as the fertilising effect of the fish fecal matter and the decline thereafter is attributable to effective grazing by the growing fish whose protein requirement would be higher. 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 with the natural food (Lam and Shephard, 1988).

The use of protein energy from diet is wasteful from the nutritional, economic and ecological points of view when compared to lipids and carbohydrates and therefore, it is worthwhile supplying much of the required energy as possible through lipid and carbohydrate (Peres and Oliva-Teles, 1999). Watanabe *et al*. (1987) observed that it would be advisable to use high lipid (15%) with low protein (30%) in the diet of common carp in order to reduce nitrogen excretion and pollution of the environment, without hampering fish growth. Nandeesha *et al*. (1999) reported improved SGR and food conversion in common carp fed a 30% protein diet, with increasing level of sardine oil incorporation. Gangadhar *et al*. (1997) reported that the growth of rohu (*Labeo rohita*) fingerlings fed a diet containing 25% protein and 9% lipid was comparable with those fed 30% protein and 6% lipid. Their results indicate that growth induced by 3% dietary oil is comparable to that produced by 5% dietary protein. It may be presumed that with decreased dietary protein levels, optimal lipid requirement increases. All fish groups receiving supplemental oil in the present study performed better than the control, indicating protein sparing by the three supplemented oil levels tested, through diets that had different levels of maize. Protein sparing by lipid has been demonstrated in a number of fish species (Gangadhar

Table 3. Mean plankton density (no. l^{-1}) in different treatments

Plankton	Treatment	Days								
		0	15	30	45	60	75	90	105	120
Phytoplankton	T_0	169^{b}	1218^{b}	4489^{b}	9286^{a}	10779^{a}	8474^{a}	5417^{a}	7248^{a}	3875^{b}
	T_1	212^{a}	1674^{a}	3039^{d}	7605^{c}	9574^{b}	7185^{b}	5144^{a}	4354^{b}	3569^{b}
	T_2	168^{b}	1122^{b}	3447^{c}	6626^{d}	10587^{a}	6500^{c}	4292^{b}	3933^{bc}	2016^{c}
	T_3	190^{a}	1675^{a}	5902^{a}	8359^{b}	11975^{a}	6833^{bc}	3500^{c}	3454^{c}	5166^{a}
Zooplankton	T_0	55^{a}	27^{b}	117^{a}	228^{a}	372^{a}	389^{a}	290^{a}	187^{a}	186^{a}
	T_1	16^{b}	15^{c}	87^{ab}	130^{b}	150^{b}	150^{b}	185^{b}	181^{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 the same superscript in each column are not significantly different ($p>0.05$)

et al., 1997; Weatherup *et al*., 1997; Mongile *et al*., 2014). However, excessive dietary lipid reduces feed intake and growth performance of fish (Regost *et al*., 2003; Kim *et al*., 2006).

FCR was the best in T_2 treatment, being significantly different from that of the control. Though PER improved with oil supplementation, there was no significant ($p>0.05$) difference between treated groups and the control. No effect of dietary lipid/CHO:L ratio was observed on the survival of catla which varied from 90.73% in all the treated groups to 92.58% in the control. Net fish yield ranged from 820 g (T_0) to 1017.85 g (T_2), it being 901.58 g and 935.55 g in tanks of 18 m^2 for 120 days in T_1 and T_3 treatments. The values of condition factor 'K' were 1.96, 1.81, 1.74 and 1.80 in T_0, T_1, T_2 and T_3 respectively. Condition factor is vital to culture system management, because it reflects the specific condition under which organisms grow (Araneda *et al.,* 2008). Higher condition factors indicate good health with an isometric growth, which is desirable in fish farms (Ayode, 2011). Lee and Kim (2009) observed significant influence of dietary CHO:L ratio on condition factor, as well as VSI and HSI of grower rockfish. VSI of catla from T_2 treatment was significantly lower than the rest of the treatments and the control, whereas HSI showed significant increase with every level of oil supplementation (Table 2). This reflects better utilisation of mesenteric fat under T_2 and an increase in liver glycogen/fat of treated fish with increasing dietary lipid level. Kim *et al*. (2012) found that varied lipid levels and sources significantly increased HSI in the olive flounder (*Paralichthys olivaceus*), but did not affect VSI; and they attributed changes in HSI to changes in lipid composition of the diets. VSI and HSI are important indicators of digestion and absorption; synthesis and secretion of digestive enzymes as well as carbohydrate metabolism (McLaughlin, 1983). Ighwela *et al*. (2014) used these indices for the measurement of condition in *Oreochromis niloticus* fed varying dietary maltose levels. The RNA/DNA ratio was highest (3.05) in T_2 treatment and lowest (1.84) in T_0, reflecting the trend of fish growth. RNA concentration and the RNA/DNA ratio have usually been related to the tissue growth rate (Perago´n *et al*., 2000), changes in RNA/DNA ratio reflecting recent changes in growth rate (Bulow, 1987). The increasing RNA/DNA ratio and the fish growth recorded with all the test diets in this study can be correlated with increased protein synthesis. Organisms in good condition tend to have higher RNA/DNA ratios than those in poor condition (Chicharo and Chícharo, 2008).

Effect of the test diets on the chemical composition of carcass was found to be significant. Significant ($p<0.05$) reduction in carcass moisture content in fishes was recorded in T_2, while in the other two treatments the reduction was marginal. Protein and lipid contents were higher ($p<0.05$) in all fish groups receiving oil supplement, the highest being in T_2 (13.98%) and T_3 (3.54%) treatments respectively (Table 2). 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; Ghanawi *et al*., 2011). Increase in carcass/muscle lipid with increasing dietary lipid has been reported for most species investigated (Bazaz and Keshavanath, 1993; Gangadhar *et al*., 1997; Erfanullah and Jafri, 1998; Nandeesha *et al*., 1999; Refstie *et al*., 2001; Jan *et al*., 2013).

Digestive enzyme activity was influenced by the test diets (Fig. 1). Significant ($p<0.05$) increase in intestinal amylase, protease and lipase activity was recorded in the treated fish, with the exception of the latter two in T_1. However, no difference ($p>0.05$) in the activity of these enzymes was observed in the hepatopancreatic tissue of fish from control and treatment groups.The increased amylase activity in the treated fish could be attributed to effective utilisation of carbohydrate from the diet. Carps are known to utilise carbohydrate preferentially over lipid due to high amylolytic activity (Jafri *et al*., 1995). Higher protease activity in the treated fish can be correlated with higher carcass protein. De Silva *et al*. (1991), Keshavanath and Jagadeesh (1994) and Vergara

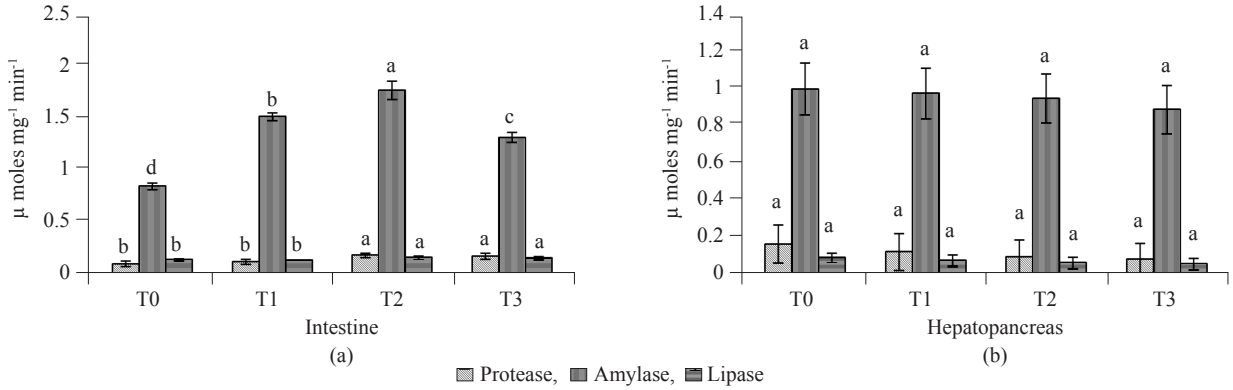

Fig. 1. Digestive enzyme activity (mean±SD) in catla from different treatments. Different alphabets for the same enzyme for a given tissue indicate significant difference ($p<0.05$). Digestive enzyme activity is expressed as μ moles of product liberated per min per mg tissue protein at 28°C

et al. (1999) reported that increasing dietary lipid level increased protein retention, enhancing the proportion of dietary protein utilised for growth. Increased lipase activity/lipid digestibility was found 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.

Incorporation of maize and sardine oil into the diets influenced growth, food conversion, protein efficiency, carcass protein and lipid contents and digestive enzyme activity, but not fish survival. Among the levels tested, 34% maize and 6% additional oil proved more effective, inducing the best growth. The results clearly show that in manured tanks, low protein diet supplemented with maize and sardine oil augments the growth of catla.

Acknowledgements

Authors thank the Dean College of Fisheries, Mangalore for providing facilites.

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Date of Receipt : 14.10.2015
Date of Acceptance : 08.12.2016