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EFFECT OF DIETARY SUPPLEMENTARY FEED OF SPIRULINA ON DIGESTIVE ENZYMES IN FINGERLINGS OF COMMON CARP (*CYPRINUS CARPIO*, L. 1758)

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ABSTRACT

Cyprinus carpio (Common carp) fingerlings, measuring 4.66±0.02g by weight and 4.2 ±0.22mm by length, procured from local fish ponds were brought to the laboratory and acclimatized to the laboratory conditions (12 hr: 12 hr L/D regime, continuous aeration, periodical exchange of non-chlorinated and non-polluted water) for a week. During acclimatization the fingerlings were fed on commercially available fish feed. The fingerlings were divided into four groups of ten each. Fish in group-1 (Control diet-1) are fed with feed containing rice bran, ground nut oil cake, soybean cake and fish meal (control diet-1). Fish in group-2 (Experimental diet-1) are fed with feed containing control diet-1+Spirulina (herbal feed). Fish in group-3 (control diet-2) are fed with feed containing rice bran, ground nut oil cake, soybean cake, fish meal, coconut oil cake and prawn meal (control diet-2). Fish in group-4 are fed with feed containing control diet-2+Spirulina. The fingerlings were fed @ 3% body weight at 8.00am and 8.00pm every day. Total protease, total amylase and total lipase were measured in the intestine of fingerlings on days 1, 10, 20 and 30. After the completion of 30 day period total protease, total amylase and total lipase of fingerlings were also recorded. It was observed that the intestinal enzyme activities increased with increase in time in both control and experimental groups; however the magnitude of increase or decrease respectively in the above variables is more pronounced in Spirulina fed fingerlings than in the control groups. Similarly the magnitude of increase in total body length and total biomass of Spirulina fed fingerlings is highly significant. The results highlight the importance of Spirulina as a feed supplement in enhancing the overall growth of fish.

KEYWORDS: *Cyprinus carpio*, Spirulina, supplementary feed, Digestive enzymes (protease, amylase and lipase) activites.

INTRODUCTION

All the fish and prawn species have very well defined intestine. Proteolytic, lipolytic and amylolytic activities take place in this particular organ in the presence of a number of enzymes. The enzymes which act in the intestinal lumen are generally secreted from pancreas. α-amylase, lipase, trypsin, chrmotrypsin, endo- and exopeptidases are secreted from the intestine.

In almost all fish species the activity of α-amylase and other carbohydrates are less compared to that of land animals and thus carbohydrate digestion in fish is comparatively less. Lipase activity in all fishes is very high because the digestibility of fat is more in case of fish and act as a cheap source of energy in aquatic animals.

The products of protein digestion in the lumen of the intestine are free amino acids and small (oligo) peptides. The later, enter the epithelial cells of the small intestine where they are hydrolyzed by specific protelytic enzymes into amino acids and get absorbed.

Digestion of proteins continue in the intestine also, and they are broken down in the alkaline medium by the action of the enzyme trypsin, secreted by the pancreatic tissue. The pH of the intestinal contents varies from 6.12 to 7.3 in several species like Rutilus, Gobio and Cyprinus (Al-Hussaini, 1949). Digestion of carbohydrates and lipids also take place in the intestine, and enzymes are present for this purpose. Amylase is produced in the pancreas which is the primary source of this enzyme. However, amylase has also been reported from the extracts of intestinal mucosa and pyloric caeca of several species. Maltase and lipase are also present in the intestinal extracts of several species. Possibly, the major source of all these enzymes (Trypsin, amylase, lipase, maltose), is the hepatopancreas. Pancreas exists in a compact from in a very few species of fishes. In most species, it is a diffuse gland, widely scattered and pancreatic acini invade the liver (forming the hepatopancreas), spleen and the mesenteries surrounding the intestine and pyloric caeca. The presence of certain enzymes in the extracts of the intestinal mucosa or pyloric caeca might be due to the extreme diffuse nature of the pancreas, which actually produces the enzymes.

Studies on the digestive physiology of fishes suggest the existence of a correlation between the enzymes and the diet of the animal. It has been found that the concentration of carbohydrates was highest in the predominantly herbivorous fish and lowest in the carnivorous while the concentration of proteases was found to be opposite to it. Herbivorous and omnivorous fishes which do not have a true stomach also lack pepsin as a low pH Proteolytic enzyme. However, omnivorous species have amylase activities in the intestine which is much higher than in the other species.

Compounds broken down by the action of pharyngeal teeth, gizzard and/or by the secretion of acid and enzymes, are finally absorbed through the intestinal wall. According to Kapoor and Khanna (1994), the alimentary canal of cyprinids can be divided into three functional zones: (¡) the rostral part of the gut including the intestinal bulb is fat absorptive zone, (ii) the posterior part of the intestine is a protein absorptive zone and (iii) posterior most part of the gut is for ion and water absorption.

It has been demonstrated previously that digestive enzymes in the common carp *Cyprinus carpio* fingerlings follow a pattern in which followed by protease, lipase and amylase later in development. The changes in the levels of digestive enzymes (protease, lipase and amylase) are tested in intestine of *Cyprinus carpio* fed with different diets.

Variations in several biological parameters associated with growth rate of *Cyprinus carpio* fed with formulated feeds with spirulina at 1d. 10d, 20d and 30day and also in combination of the above digestive enzymes at different levels were studied, in this study.

MATERIALS AND METHODS

Fingerlings of *Cyprinus Carpio* (Common carp L.1758) collected from government fish farm, Tirupati, near Chittoor Dist. A.P were brought to the laboratory and acclimatized to the laboratory conditions (12hr:12hr L/D regime continuous aeration) for a week. During acclimatization the fish were fed on herbal supplemented feeds and formulated feed. *Cyprinus Carpio* (Common carp Linnaeus, 1758) fingerlings measuring 1.66±0.2g by weight and 4.2±0.5cm length were used in all the experiments

The water quality parameters estimated contained Dissolved Oxygen - 4 to 8 ppm,Temperature-28 to 30^0 C, PH-7.4 to 7.8, Salinity-0.190 grams/liter,Chlorinity-0.110 grams/liter, Alkalinity-102 ppm, Hardness of water-112 ppm

ENZYME ACTIVITIES

COLLECTION OF TISSUES

At the termination of the growth experiment, fish were dissected four hours after the first feeding and the digestive tract (intestine) and liver were removed. After that, the attached fat had been removed. Intestine and liver were blotted dry with tissue paper and kept in small plastic bags, individually, before freezing at-60^0C until used for enzyme determination.

PREPARATION OF ENZYME SOURCE

After the experimental period (30 days) 6 test species from each water tub were removed and starved for 24 hours and sacrificed. The whole alimentary tract was dissected out in ice cold fish ringer solution and washed thoroughly externally. The tissue was then rinsed with cold distilled water, spilt open and washed thoroughly in fish ringer. The tissues were homogenized separately with distilled water using mechanical dispenser. The homogenate was centrifuged at 3000 rpm for 10 min. (Remi model K-11). The clear supernatant was used as the enzyme source for subsequent assay.

The amylase activity was estimated by the method of Bernfield (1955). The activity of protease was estimated by the method of Jany (1976). Lipase activity was measured by the Titrimetric method of Teiz and Friedrick (1966).

Table 1. Composition and proximate chemical analyses of the experimental diets.

Ingredients	Basal diet	Spirulina
Herring fish meal	9.10	
Soybean flour	52.57	
Corn flour	19.25	
Starch	7.00	
Corn oil	1.80	
Cod liver oil	1.98	
Vitamin premix (1)	2.00	
Mineral premix (2)	2.00	
α-cellulose	3.30	
Carboxy-methyl-cellulose	1.0	
Total	100	

Chemical Analysis (%)		
Dry matter	91.7	23.2
Crude protein	30.6	62.4
Ether extract	9.1	6.3
Ash	8.3	8.7
Nitrogen free extract (3)	52.0	22.6
Gross energy (kcal/g) (4)	4.72	5.04

RESULTS

The activity levels of amylase, protease and lipase have been determined in the intestine of *Cyprinus carpio* fingerlings fed for 30days on control (C1, C2) and experimental (E1, E2) diets on 1, 10, 20 and 30 days of the rearing period. Results pertaining to the activity levels of protease in the intestine of *Cyprinus carpio* fingerlings fed on control and experimental diets are presented in figures 1 and 2. The results clearly show that there is a significant increase ($P<0.001$) in the intestinal protease activity with increase in rearing time

both in control and experimental groups with the magnitude of increase being more pronounced in fingerlings fed on E1, E2 diets than in those fed on C1, C2 diets (Fug. 1). For instance E1 and E2 diets increased intestinal protease activity by 12% and 16% respectively on 20d and by 14% and 19% respectively on 30d compared to the respective C1and C2 diets. However there are no significant differences in the intestinal protease activity between E1 and E2 diets on 1d and 10d. (Fig. 2). It is interesting to note that the E2 diet has increased intestinal protease activity much more than the E1 diet.

Fig 3 and 4 present results on the activity levels of amylase in the intestine of *Cyprinus carpio* fingerlings fed on control (C1, C2) and experimental diets (E1, E2). It is clear from the results that there is a significant increase ($P<0.001$) in the intestinal amylase activity with increase in rearing time from 1 to 3o d both in control and experimental groups. However the magnitude of increase is much more pronounced in fingerlings fed on E1, E2 diets than in those fed on C1, C2 diets (Fig. 3). Obviously E1 and E2 diets enhanced intestinal amylase activity by 10% and 15% respectively on 10d; by 11% and 14% respectively on 20 day and by 14% and 19% respectively on 30day compared to the respective C1 and C2 diets (Fig. 4). However there are no significant differences in the intestinal amylase activity between E1 and E2 diets on day 1. Evidently E2 diet caused greater increase in amylase activity than E1 diet on 10, 20 and 30 days.

Results with regard to the activity levels of lipase in the intestine of *Cyprinus carpio* fingerlings fed on control (C1, C2) and experimental (E1, E2) diets are presented in fig.5 and 6. It is evident from the results that there is a significant increase ($P<0.001$) in the intestinal lipase activity with increase in rearing time from 1 to 30d both in control and experimental groups with the magnitude of increase being more in fingerlings fed on E1, E2 diets than in those fed on C1, C2 diets (Fig. 5). Apparently E1 and E2 diets caused an increase in the intestinal lipase activity by 13% and 16% respectively on 10d; by 18% and 20% respectively on 20d and by 15% and 20% respectively on 30d compared to the respective C1 and C2 diets (Fig. 6). However, no significant difference was observed in the intestinal lipase activity between E1 and E2 diets on day 1. Clearly E2 diet caused greater increase in intestinal lipase activity than either E1 diet or C1 and C2 diets throughout the rearing period from 1 to 30days.

DISCUSSION

Enzymes are the biocatalysts which catalyze biochemical reactions releasing energy and/or produce new products. Enzymes are both catabolic and anabolic. For example digestive enzymes breakdown macronutrients into micronutrients and synthetases promote the synthesis of macro and micronutrients depending upon the situation proteases, amylases and lipases catalyze reactions which breakdown protein, carbohydrate and lipid into their respective smaller units which can be absorbed and assimilated. Thus enzymes play a vital role in the breakdown and synthesis of substances reflecting the metabolic status of an organism. In addition enzymes are responsible for releasing energy which is used for various body functions.

The results of this study have clearly shown that the herbal supplemented diets have enhanced the digestive enzyme activities in *Cyprinus carpio* fingerlings. It is quite clear from the results that the intestinal protease activity of the fingerlings gradually increased from 1 to 30 days both in the control and experimental groups (Fig. 1). However E1 and E2 diets have a more augmenting effect on the intestinal protease activity than C1 and C2 diets as reflected from the percent changes in fig. 2. It is further interesting to note that the percent increase in the intestinal protease activity at the end of 30d is 19% and 20% in the fingerlings fed on C1 and C2 diets respectively compared to 37% and 41% in those fed on E1 and E2 diets respectively. These figures suggest that Spirulina added a on herbal supplement to E1, E2 diets may be responsible for a significant increase in the protease activity. As has been reported earlier (Machiels and Henken, 1985; Pantazis, 1999) herbal supplemented diets are known to enhance digestive enzyme activities in freshwater culture fish. Stefens (1989) reported that Spirulina when added to fish feeds has not only increased digestive enzyme activities but also enhanced the growth of the fish. In the present study also Spirulina was found to enhance intestinal protease activity which ultimately could have resulted in better conversion and accumulation of proteins. Thus it is obvious that E1 and E2 diets with Spirulina as a component are better diets than C1 and C2 diets because they enhance the intestinal protease activity and total body protein which ultimately results in faster growth of *Cyprinus carpio* fingerlings in a shorter time period. Moreover, unlike in terrestrial animals where carbohydrate is the main source of energy, protein is the main source of energy in fish and, thus, an increase in protease activity resulting in accumulation of protein is advantageous to fish.

The digestion of carbohydrate occurs briefly in the mouth and largely in the intestine. During the process of mastication salivary amylase acts on starch randomly and cleaves 1, 4-glycosidic bonds to produce dextrins and maltose. Salivary amylase gets inactivated by high acidity (low PH) in the stomach. Consequently the acidic dietary contents of the stomach on reaching small intestine are neutralized by bicarbonate produced by pancreas. The pancreatic amylase (β-amylase) acts on starch and continues the digestion process to produce disaccharides and oligosaccharides. The final digestion of di-and oligosaccharides to monosaccharides primarily occurs at the mucosal lining of upper jejunum. The monosaccharides get absorbed and assimilated. Thus α- and β-amylases play an important role in converting complex sugars into simple sugars.

Results presented in fig-3 and 4 clearly show that there is a significant increase in the intestinal amylase activity of *Cyprinus carpio* fingerlings fed on control (C1, C2) and experimental diets. E1 and E2 diets caused greater increase in the enzyme activity than C1 and C2 diets during the 30d rearing periods as shown in fig-4. Interestingly the percent increase in the intestinal amylase activity is 33% and 23% respectively in the fingerlings fed on C1 and C2 diets compared to 51% and 45% respectively in those fed on E1 and E2 diets at the conclusion of 30d rearing period further suggesting that E1 and E2 diets are more effective in augmenting amylase activity than C1 and C2 diets. Similar results have been reported in other carps fed on herbal supplemented diets (Morris, 2001; Hemre et al., 2002 Yang et al., 2003). Obviously Spirulina, which is a herbal supplement in E1 and E2 diets, might have promoted the enzyme synthesis *Per se* or created conditions where in the enzyme acted at a faster rate compared to fingerlings fed on C1, C2 diets which do not have Spirulina as an ingredient. Consequently the whole process leads to better conversion efficiently and accumulation of total carbohydrate in the muscle and liver. Obviously accumulation of total carbohydrate leads to protein sparing effect (Kikuchi and Takeda, 2001). The results clearly demonstrate that Spirulina, a herbal supplement of the fish diet, is highly beneficial to the fish because it enhances the intestinal amylase activity leading to accumulation of carbohydrates and protein sparing effect.

Ingested lipids normally comprise more than 90% fat (triacylglycerol) but the rest of the dietary lipid is made up of phospholipids, cholesterol, cholesterol esters and free fatty acids. The digestion of lipid mainly occurs in the stomach through the action of gastric lipase which degrades fat into fatty acids at neutral pH. For this to take place emulsification of lipid is essential which is a process of breaking down larger lipid molecules into smaller droplets because enzymes can act only on the surface of lipid droplets.

It is quite clear from the results presented in figures 5 and 6 that there is a significant increase in the total lipase activity of *Cyprinus carpio* fingerlings fed on both control (C1, C2) and experimental diets (E1, E2). E1 and E2 diets caused greater increase in lipase activity than C1 and C2 diets through out the 30 d rearing period as shown in figure 5. When measured at the end of the 30 d rearing period the percent increase in lipase activity of fingerlings is much more significant with E1 (47%) and E2 (50.0%) diets than with C1 (28%) and C2 (27%) diets suggesting that E1 and E2 diets are more effective in enhancing lipase activity than C1 and C2 diets. Similar results have also been reported in other fish species fed on herbal supplemented diets (Watanbe 1982; Hassan et al., 1995; Luzzana et al., 2002; Gaylord et al., 2003; Linet et al. 2004). Apparently Spriulina which is a herbal supplement in E1 and E2 diets might have either enhanced lipase concentration thereby increasing its activity or created a conducive environment for the enzyme to express its activity more efficiently. The net effect, probably, is a better conversion efficiency leading to greater assimilation and synthesis of lipid. Obviously an increase in lipase activity leads to greater accumulation of lipid in the body of *Cyprinus carpio* fingerlings which is beneficial for the fish in the sense that accumulated body lipid results in protein sparing effect in fish (Bazaz and Keshavanath, 1993; Hassan and Jafri, 1996).

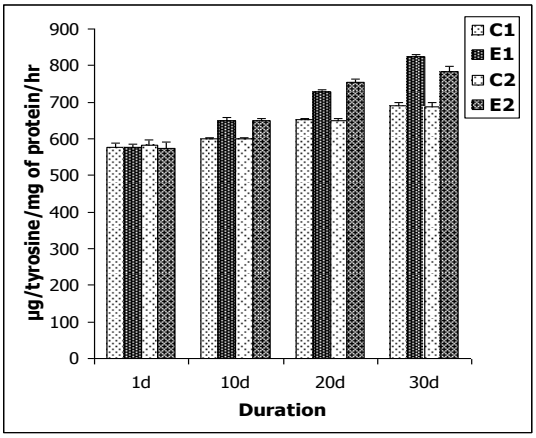

Fig.1: Changes in the levels of Protease µg/tyrosine/mg of protein/hr in intestine of fingerlings of C*yprinus carpio* fed with different diets (C1, C2, E1, E2) at the end of 1d, 10d, 20d and 30days the end of 1d, 10d, 20d and 30days

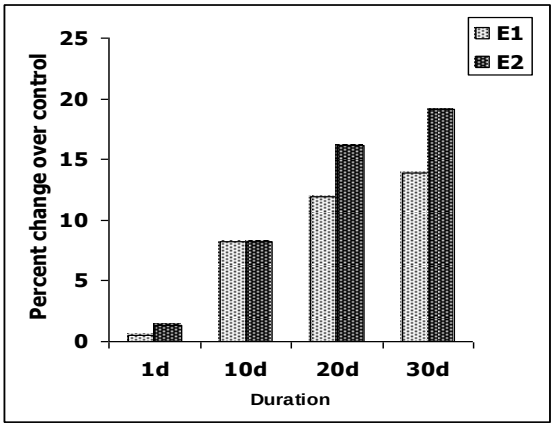

Fig.2: Percent changes in the levels of Protease µg/tyrosine/mg of protein/hr in intestine of fingerlings of C*yprinus carpio* fed with different diets (C1, C2, E1, E2) at the end of 1d, 10d, 20d and 30days

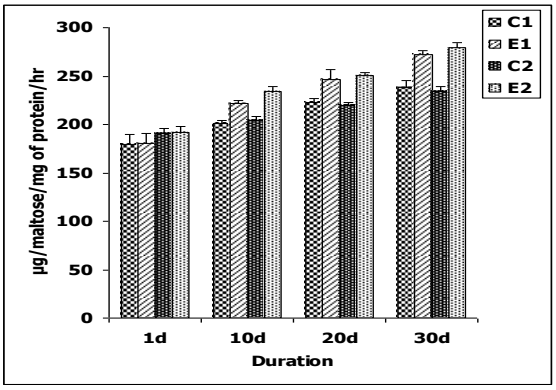

Fig.3 : Changes in the levels of Amylase µg/maltose/mg of protein/hr in intestine of fingerlings of Cyprinus carpio fed with different diets (C1, C2, E1, E2) at the end of 1d, 10d, 20d and 30days the end of 1d, 10d, 20d and 30days.

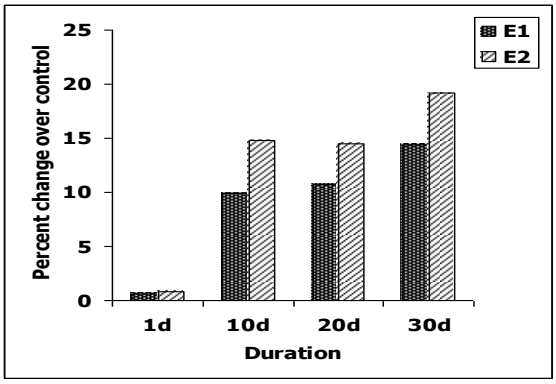

Fig.4: Percent changes in the levels of Amylase µg/maltose/mg of protein/hr in intestine of fingerlings of C*yprinus carpio* fed with different diets (C1, C2, E1, E2) at the end of 1d, 10d, 20d and 30days the end of 1d, 10d, 20d and 30days

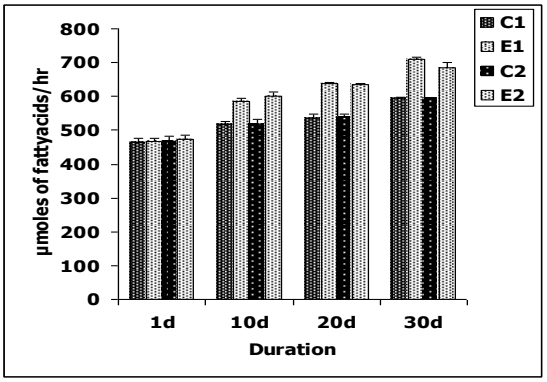

Fig.5: Changes in the levels of Lipase µ moles of fatty acids/hr in intestine of fingerlings of C*yprinus carpio* fed with different diets (C1, C2, E1, E2) at the end of 1d, 10d, 20d and 30days.

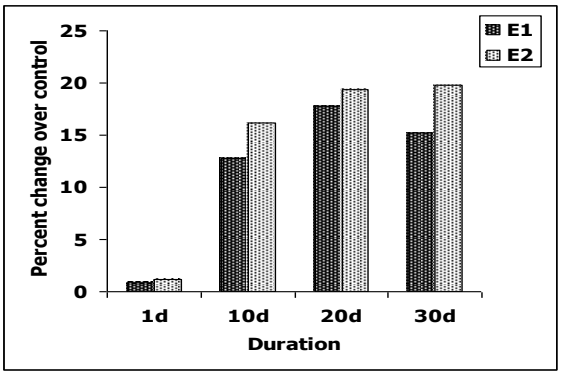

Fig.6: Changes in the levels of Lipase µ moles of fatty acids/hr in intestine of fingerlings of C*yprinus carpio* fed with different diets (C1, C2, E1, E2) at the end of 1d, 10d, 20d and 30days

Changes in the levels of Protease , Amylase and Lipase in intestine of fingerlings of Cyprinus carpio fed with different diets(C1, C2, E1, E2) at the end of 1d, 10d, 20d and 30days

		Protease				Amylase				Lipase			
Duration		**1d**	**10d**	**20d**	**30d**	**1d**	**10d**	**20d**	**30d**	1d	**10d**	**20d**	**30d**
Intestine	C1	577.94 ±10.13	599.91 ±4.359	651.06 ±5.561	691.08 ±6.57	177.93 ±10.45	201.49 ±2.903	222.23 ±4.746	234.21 ±7.524	467.50 ±11.982	517.333 ±8.703	538.636 ±9.476	593.86 ±3.225
	E1	576.59 ±8.37	649.29 ±8.909	729.05 ±5.47	823.12 ±7.33	178.09 ±10.43	231.35 ±3.809	246.13 ±10.50	271.24 ±4.589	473.108 ±11.007	601.012 ±11.449	634.487 ±3.713	701.213 ±4.950
	C2	582.55 ±13.408	599.04 ±4.707	649.50 ±5.68	688.62 ±10.06	190.46 ±5.55	203.79 ±4.196	219.13 ±3.806	237.99 ±4.496	463.858 ±14.667	518.182 ±14.138	529.937 ±8.603	593.68 ±2.418
	E2	574.94 ±17.49	648.11 ±6.565	754.49 ±8.12	784.45 ±12.55	191.04 ±5.570	234.01 ±4.970	251.01 ±2.357	272.20 ±5.276	466.056 ±11.170	584.148 ±10.947	638.123 ±4.368	684.241 ±16.066

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REFERENCES

1. Al-Hussaini. Quart, Tourn, Micros. Sci., London, 90: 109-129 Africa. *NAGA, World Fish Centre* Quarterly, 1949; 78(3), 4 July – December, 2005.
2. Bazaz, M. M. and Keshavanath, P. Effect of feeding different levels of sardine oil on growth, muscle composition and digestive enzyme activities of mahseer, *Tor khudree*. *Aquaculture*, 1993; 115(1-2): 111-119.
3. Bernfield, P. Amylases α and α, in methods in enzymology, edited by S. P. Colowick and N. O. Kalpan (Academic Press, New York),

149.containing different levels of *Spirulina platensis*. *Bioresour. Technol.*, 1955; (1)80: 117–120.
4. Jany, K.D. Studies on the digestive enzymes of stomachless bony fish *Carrasinus auratus* Gibelio (Block) Endopeptidases, *Comp. Biochem. Physiol*., 1976; 53: 31.
5. Kapoor, B.G. and Khannan, B. In Advances in Fish Biology (ed. H.R.Singh), 1994.
6. Keshavanath, P., Shivanna and B. Gangadhara, Evaluation of sugarcane by, 2006.
7. Kikuchi A and Takeda A, Onodera H. Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiology of Disease*, 2001; 2002; 9(2): 244–248.
8. Machiels, M.A.M. and Henken, A.M. Growth rate, feed utilization and energy metabolism of the African catfish, Clarias gariepinus (Burchell, 1822), as affected by dietary protein and energy content. Aquaculture, 1985; 44(4): 271-284.
9. Morris. N.M. A standard reference diet for crustacean nutrition research. V. Growth of fresh cray fish Cherax temnimamus. Journal of world Aquaculture, 2001; 20: 114-117.

a. Nandeesha, M.C., B. Gangadhara, J.K. Manissery and L.V.Venkataraman, growth performance of two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets product pressmud as a manure in carp culture. *Bioresour. Technol.*, 2001; 97: 628–634.

10. Remi model., Academic Press, Inc., London. 154-209.
11. Steffens, W. Principles of Fish Nutrition. Ellis Horwood Limited, Chichester, New York, Toronto, 1989; 384.
12. Teitz, N.W. and Friedrick, E.A. A specific method for serum II pase determination, AcTa Clinic Chem., 1966; 13: 302.
13. Watanabe, Lipid nutrition in fish. Comparative Biochemistsry and Physiology, 1997; 73(B): 3-15.
14. Watanabe, T., carbohydrate nutrition in fish. Comparative Biochemistsry and Physiology, 2002; 73(B): 49-78.