

## **EFFECT OF PROBIOTICS ACTION ON GROWTH PERFORMANCE AND ENZYME ACTIVITY IN THE INTESTINE OF CATLA CATLA FINGERLINGS**

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### **ABSTRACT**

Effect of probionts for *Catla Catla* fingerlings based on growth performance, feed utilization (FCR) and digestive enzymes Protease, amylase and lipase activities was investigated. The photosynthetic bacteria, bacillus subtillus (water probiotics) and lactobacillus subtillus (feed probiotics). Isolated from common carp ponds were added to carp basal diets as the probiotics in two forms: 1g. kg water probionts (Bacillus subtillus) and feed probionts (lactobacillus subtillus) and their mix.six aquaria with replicates for treatment and control were used. After 60 days feeding experiment with probiotic supplemented and non-supplemented control diets, The diets supplemented with probiotics showed significantly better results of growth performance and FCR than those with the basal diets (control diet). The mean

digestive enzyme activities of all probiotics treatment groups were significantly different ( $P < 0.05$ ) with that of the control. The Protease activity was remarkably higher in the mix and Bacillus subtillus. Compared with experimental and control. However, there was no difference between the mix and Bacillus so. As for amylase and lipase, assays shows higher activity in the mix as compared to the rest. In conclusion it shows that probionts highly increased the growth performance and digestive enzyme activities, and decreased FCR. Further more, different probionts forms indicated different performance and the mix produced the results.

**KEYWORDS:** Probiotics; *Catla Catla* fingerlings; Growth performance; Protease; Amylase; Lipase.

## INTRODUCTION

Probiotics are micro -organisms used in aquaculture which controls diseases and supplement the nutrients. In the present study growth promoter was observed in fishes fed with diets containing the probiotic bacteria. Appropriate probiotic applications were shown to improve intestinal microbial balance, thus leading to improved food absorption (Parker, 1974; Fuller, 1989), digestive enzyme activities (Tovar-Ramirez et al., 2004) and reduced pathogenic problems in gastrointestinal tract (Lloyd et al., 1977; Pivnick et al., 1981; Cole and Fuller, 1984; Goren et al., 1984). With some trials, growth promotion was clearly demonstrated in poultry (Alder and Damassa, 1980) and pigs (Pollman et al., 1980) compared with control groups. Those results were most promising and gave confidence that further improvements in probiotic applications were possible. The application of probiotics in aquaculture as the environment friendly treatments was also increasing rapidly (Gatesoupe, 1999) and some experiments were associated with the effect of probiotics in fish (Mohanty et al., 1993, 1996; Sharma and Bhukhar, 2000).

All the fish and prawn species have very well developed intestine. Proteolytic, lipolytic and amylolytic activities take place in this particular organ in the presence of number of enzymes. The enzymes which act in the intestinal lumen are generally secreted from pancreas, D-amylase, lipase, trypsin, chymotrypsin, endo and exopeptidase are secreted from the intestine. The digestion of carbohydrates by enzymes secreted by the fish results in the production of monosaccharide. In almost all fish species the activity of d-amylase and other carbohydrates are less compared to that of land animals, so carbohydrates digestion in fish is comparatively less. This phenomenon in aquatic animals is due to the fact that the aquatic body contains less carbohydrate and more protein. Lipase activity in all fishes is very high. So the digestability of fat is more in case of fish and as cheap source energy in aquatic animals.

In human and agriculture application, probiotics research had enjoyed much more attention through history and several modes of action had been supported by unambiguous experimental data (Fuller, 1989). It was clear that the experience obtained with terrestrial animals has been used in aquaculture, especially with regard to the use of lactic acid bacteria. The modes of action were as follows: production of inhibitory compounds; competition for chemicals or available energy; competition for adhesion sites; enhancement of the immune response; improvement of water quality; interaction with phytoplankton; source of macro and micronutrients, enzymatic contribution to digestion (Verschuere et al., 2000). And there were

some experiments associated with several modes of probiotics action (Fredrickson and Stephanopoulos, 1981; Lemos et al., 1991; Pybus et al., 1994; Montes and pugh, 1993; Soderhall and Cerenius, 1998; Fukami et al., 1997). However, little had been done to incorporate probiotics into *Catla Catla* fingerlings based on growth performance and digestive enzyme activities. Thus, this study was designed to evaluate the use of *Bacillus* so, and their mix. as probiotics supplements in diets for *Catla Catla* fishes, which is one of the most economical fresh water fish in India.

## MATERIALS AND METHODS

### Test Species



### The systematic position of *Catla Catla* (Hamilton, 1822).

Kingdom	– Animalia
Phylum	– Chordata
Sub-Phylum	– Vertebrata
Class	– Actinopterygii
Order	– Cypriniformes
Family	– Cyprinidae
Genus	– Gibelion
Species	– <i>Catla Catla</i>

### Collection and Acclimatation of Experimental Fishes

*Catla Catla* fingerlings were collected from Govt. fish farm at Kalyanidam, tirupati, Andhrapradesh. Using cast net and maintained laboratory conditions in Sri Venkateswara University, Aquaculture department, used plastic tubs and acclimated in aerated tap water (temp 30±1°C, pH. 8.0; DO 7.0±0.3 mg/l) with continues aeration for 12 days prior experimentation. During the period, fishes were fed with known amount of fish feed. The proximate composition including crude protein, crude fat, crude ash, gross energy and moisture of basal diets was determined using the standard procedure of china according to

Zhang and Zhu (1998). Crude protein was determined using the Kjeltec Analyzer unit (2300, Sweden). Gross energy was determined with an adiabatic bomb calorimeter (PARR1281, USA). Weights of all collected *Catla Catla* fingerlings from aqua lab were determined at initial and the end during the 60 days experimental period, which treated as initial weight and final weight, respectively. At the same time, *Catla Catla* fingerlings based survival was also determined by counting the individuals in each experimental tub. The daily gain (g d<sup>-1</sup>) was calculated as:

$$\frac{\text{Final weight (g)} - \text{initial weight (g)}}{60 \text{ days}}$$

The relative gain rate (%) (RGR) used the following formula:

$$\frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Initial Weight (g)}} \times 100\%$$

And the feed conversation ratio (FCR) formula as

Total feed consumption (total feed casting -total feed residue) (g).

Total final weight (g) -total initial weight (g) +total mortality weight (g).

For enzymatic analysis, six *Catla Catla* fingerlings starved for 24 h were collected from each experimental tub at the end of the trails and anesthetized in diluted MS-222(ethyl 3-aminobenzoate methanesulfonate, Tricaine; Sigma) (1:2500) in order to study the effect of probiotics based on digestive enzyme activities. Dissection produced a crude mixture of intestine of each segment by operating at 4°C following the method of Hung et al. (1996, 1999). The samples of intestine were separated and rinsed with cold distilled water. Total intestine content was then homogenized in phosphate buffer (PH 7.5; PBS) (1 g per 10 ml) using a hand held glass homogenizer at 4 °C. The homogenate was then centrifuged at 4°C at 15000×g for 15 min. The supplement was then stored at 4°C prior to analysis. All enzymatic assays were conducted within 24 h after extraction.



**Experimental setup in indoor laboratory.**

Total protein content of supplement was assayed according to Bradford (1976) using bovine albumin as a standard. Protease activity was evaluated according to Lowry et al. (1951) using Folin -phenol reagent and amylase activity was measured according to Jiang (1982) and Worthington (1993) using iodine solution to reveal non -hydrolyzed starch. Lipase activity was determined based on measurement of fatty acids release due to enzymatic hydrolysis of triglycerides in stabilized emulsion of olive oil (Borlongan, 1990; Jin, 1995). Enzyme activities including Protease and Amylase were both expressed as specific activity ( $\mu$  mg-1 protein) and lipase activity was expressed as  $\mu$  g-1 intestine content.

Statistical analysis using one -way ANOVA (Ming, 2002; statistical Analysis system, SAS, Version 6.03) was performed to find significant difference on various parameters between treated and control trials. A significance level of  $P < 0.05$  was used.

## RESULTS

All fishes and prawn species have very well defined intestines. Proteolytic, lipolytic and amylolytic activities take place in the intestine in the presence of a number of enzymes. The enzymes which act in the intestinal lumen are generally secreted from the pancreas.  $\alpha$ -amylase, lipase, trypsin, chymotrypsin, endo and exopeptidase are secreted from the intestine. The digestion of carbohydrates leads to synthesis of monosaccharides in fish. In almost all fish species the carbohydrate digestion is relatively less. However lipase activity is relatively high in all fish species and lipid available as a cheap source of energy in all aquatic animals, similar is the case Protease. In the lumen of intestine, products of protein digested into free amino acids and oligopeptides, which are absorbed by epithelial cells of the small intestine.

There was no obvious effect of probiotics on the water quality in the four feed treatments.

Total ammonium (0.02 mg/l), nitrite (0.01 mg/l) and pH (7.0-7.4) were stable and within acceptable ranges (Boyd and Tucker, 1998). The *Catla Catla* carps survival rate of all the feed treatments was 100% after 60 days culture and there was no difference ( $p>0.05$ ) between trails 1 and 3 treated with the probiotics and control.

The activity levels of amylase, Protease and lipase have been determined in the intestine of *Catla Catla* fingerlings fed for 30 days 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 *Catla Catla* 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 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 (Fig. 1). For instance E1 and E2 diets increased intestinal Protease activity by 14% and 18% respectively on 20 day and by 15% and 20% respectively on 30 day compared to the respective C1 and C2 diets. However there are no significant difference in the intestinal Protease activity between E1 and E2 diets on 1 day and 10 day (Fig. 2). It is interesting to note that the E2 diet has increased intestinal Protease activity much more than E1 diet.

Figures 3 and 4 represent that, on the activity levels of amylase in the intestine of *Catla Catla* fingerlings fed on control (C1,C2) and experimental diets (E1,E2) that there is a significant increase ( $P<0.001$ ) in the intestinal amylase activity with increase in rearing time from 1 to 30 day both in control and experimental groups. However the magnitude of increased amylase activity in fingerlings fed on E1 and E2 diets compared to those fed on C1 and C2 diets (Fig 3). Obviously E1 and E2 diets enhanced intestinal amylase activity by 10% and 15% respectively on 10 day by 11% and 14% respectively on 20 day and by 14% and 19% respectively on 30 day compared to the respective C1 and C2 diets (Fig 4).

However there are no significant difference 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 *Catla Catla* fingerlings fed on control (C1, C2) and experimental (E1,E2) diets are presented in figures 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 30 day both in control and experimental groups with the magnitude of increase being more in fingerlings fed on E1 and E2 diets than in those fed on C1, C2 diets (Fig 5). Apparently E1 and E2 diets caused on increase in the intestinal lipase activity by 13% and 16% respectively on 10 day; by 18% and 20% respectively on 20 day and 15% and 20% respectively on 30 day 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 30 days.

## DISCUSSION

Enzymes are the biocatalysts which catalyze biochemical reactions releasing energy and produce new products. Enzymes are both catabolic and anabolic for example digestive enzymes breakdown macronutrients into micro nutrients. The production of macro and micro nutrients depending upon the situation Protease, amylase and lipase catalyze reactions which break down 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 a an organism. In addition enzymes are responsible for releasing energy which is used for various body functions.

The results of this study shows that the probiotics supplemented diets have enhanced the digestive activities in *Catla Catla* 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 (Fig. 1) However E1 and E2 diets have a more augmenting effect on the intestinal Protease activity than C1 and C2 diets are reflected from the present change in figure 2. It is further interesting to note that the present increase in the intestinal Protease activity at the end 30 day is 18% and 19% in the fingerlings fed on E1 and E2 diets respectively.

These figures suggest that the probiotics added as commercially formulated feed supplement to E1, E2 diets may be responsible for a significant increase in the Protease activity. It has

been reported earlier (Bairagi et al 2002, Panigrahi et al 2009) that probionts supplemented diets are known to enhance digestive enzyme activities in fresh water culture fish.

Austin and Austin (1993) reported that probionts when added to fish feeds has not only increased digestive enzyme activities but also enhanced the growth of the fish. Moreover, unlike in terrestrial animals where carbohydrate is the main source of energy, Proteins act as main source of energy in fish and thus an increase in protease activity resulting in accumulation of protein.

The digestion of carbohydrates 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-glycoside bonds to produce dextrin's and maltose. Salivary amylase gets inactivated by high acidity (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 ( $\beta$ -amylase) acts on starch and continues the digestion process to produce disaccharides and oligosaccharides to monosaccharides primarily occurs at the mucosal lining of upper jejunum. The monosaccharides get absorbed and assimilated. Thus  $\alpha$ -amylase and  $\beta$ -amylase play an important role in converting complex sugars in to simple sugars.

Present results shows in figures 3 and 4 clearly shows that there is a significant increase in the intestinal amylase activity of *Catla Catla* 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 30 day rearing periods as shown in figure 4 interestingly the present increase in the intestinal amylase activity is 32% and 22% respectively in the fingerlings fed on C1 and C2 diets compared to 50% and 45% respectively in those fed on E1 and E2 diets are more effective in augmenting amylase activity than C1 and C2 diets. The results clearly demonstrate the probionts supplemented feeds of fish diet is highly beneficial to the fish because it enhance the intestinal amylase activity leading to accumulation of carbohydrates and protein sparing effect.

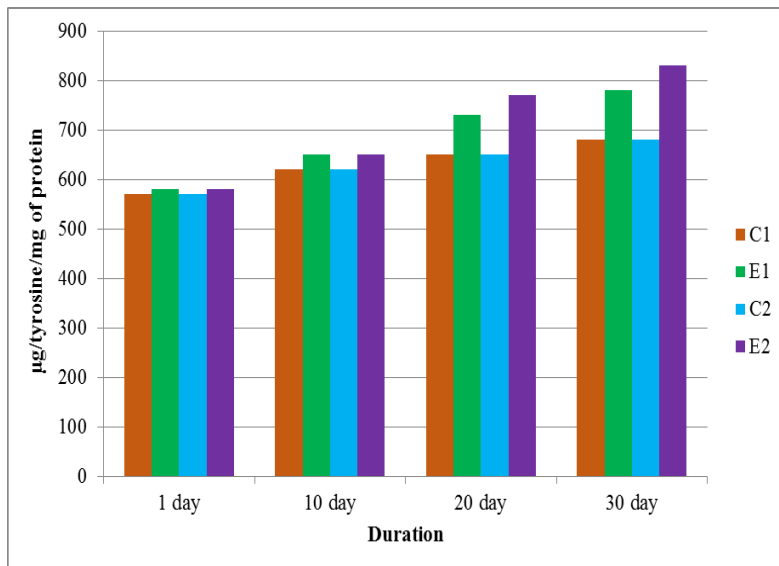
Ingested lipids normally comprises more than 90% fat but the rest of the dietary lipid is made up of phospholipids, cholesterol, esters and free fatty acids. The digestion of lipid mainly occurs in the stomach through the action of gastric lipase which degrades fat into the fatty



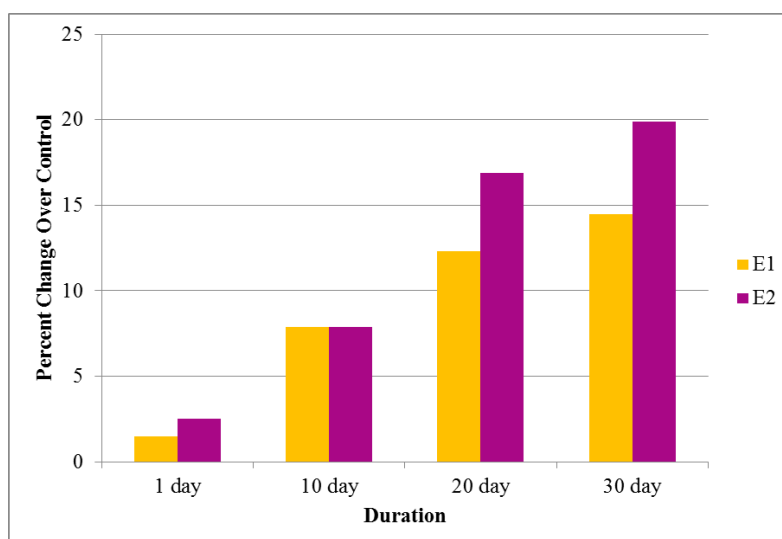
acids at neutral pH. The emulsification of lipid is essential which is a process of breaking down lipid molecules into smaller droplets because enzymes can act only on the surface of a lipid droplet.

The results presented in figures 5 and 6 that there is a significant increase in total lipase activity of *Catla Catla* 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 throughout the 30 day rearing period as shown in figure 5. When measured at the end of 30 day rearing period. The present increase in Lipase activity of fingerlings is much more significant with E1 (47%) and E2 (51%) diets than with C1 (26%) and C2 (25%) diets suggesting that E1 and E2 diets are more effective in enhancing lipase activity than C1 and C2 diets.

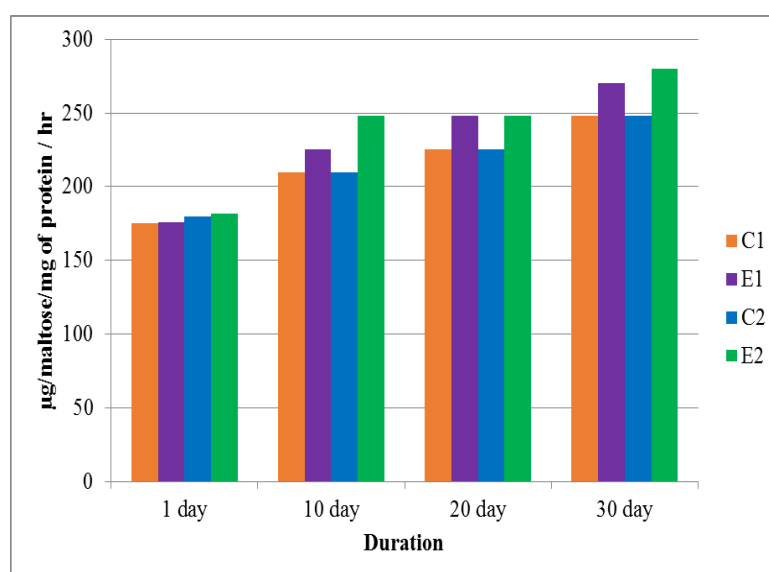
Similar results have also been reported in other fish species fed on probiotic diets (Geldzwis 2007 and Senole et al 2005). Appropriately feed and water probionts feed supplement in E1 and E2 diets might have either enhanced lipase concentration there by increasing in activity or created a conducive environment for the enzyme to express its activity more efficiently.



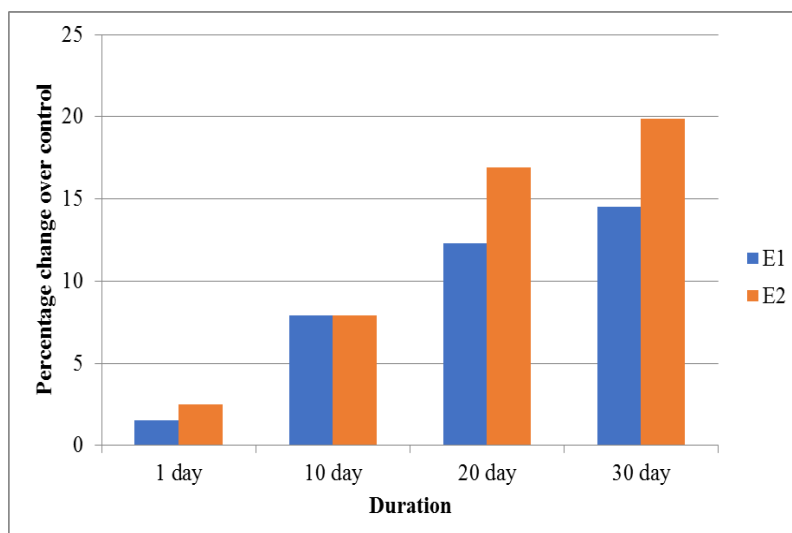
**Fig. 1:** Changes in the levels of protease  $\mu\text{g/tyrosine/mg of protein/hr}$  in intestine of fingerlings of *catla catla* fed with different diets (C1,C2,E1,E2) at the end of 1 day,10 day, 20 day and 30 day.



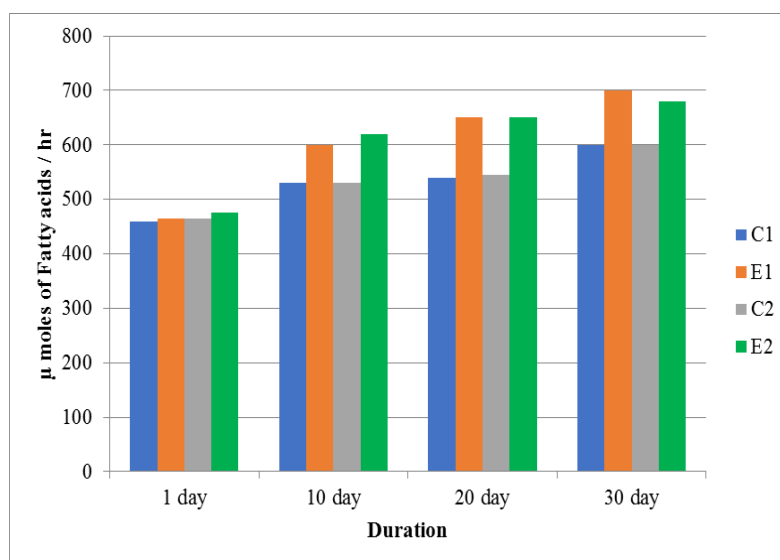
**Fig. 2:** Percent changes in the levels of protease  $\mu\text{g}/\text{tyrosine}/\text{mg}$  of protein/hr in intestine of fingerlings of *Catla Catla* fed with different diets (C1,C2,E1,E2) at the end of 1 day, 10 day,20 day and 30 day.



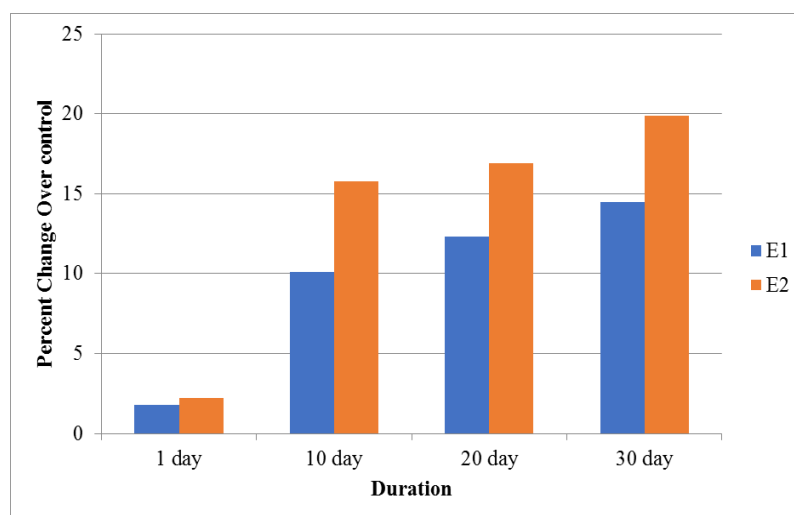
**Fig. 3:** Changes in the levels of amylase  $\mu\text{g}/\text{maltose} / \text{mg}$  of protein /hr in intestine of fingerlings of *Catla Catla* fed with different diets (C1,C2,E1,E2) at the end of 1day,10 day,20 day and 30 day.



**Fig. 4:** Present changes in the levels of Amylase  $\mu\text{g}/\text{maltose} / \text{mg}$  of protein /hr in intestine of fingerlings of catla fed with different diets (C1,C2,E1,E2) at the end of 1day,10 day,20 day and 30 day.



**Fig. 5:** Changes the levels of lipase  $\mu$  moles of fatty acids/hr in intestine of fingerlings of *Catla Catla* fed with different diets (C1,C2,E1,E2) at the end of 1day,10 day,20 day and 30 day.



**Fig. 6: Present changes in the levels of lipase  $\mu$  moles of fatty acids /hr in intestine of fingerlings of *Catla Catla* fed with different diets (C1,C2,E1,E2) at the end of 1 day, 10 day, 20 day and 30 day.**

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