

INFLUENCE OF PROBIOTICS [BACILLUS SUBTILLUS AND LACTOBACILLUS SUBTILLUS] ON THE GROWTH OF FINGERLINGS OF CATLACATLA [HAMILTON]

G. Hajarathaiah and Dr. P. Naga Jyothi*

Department of Fishery Science and Aquaculture, Sri Venkateswara University, Tirupati – 517
501 Andhrapradesh, India.

Article Received on
22 May 2018,

Revised on 12 June 2018,
Accepted on 02 July 2018,

DOI: 10.20959/wjpr201814-12864

***Corresponding Author**

Dr. P. Naga jyothi

Department Of Fishery
Science and Aquaculture,
Sri Venkateswara
University, Tirupati, A.P.,
India.

ABSTRACT

The effect of a commercially available probiotic product containing *Bacillus subtilus* and *Lactobacillus subtilus*, on the growth performance in *CatlaCatla* fingerlings was studied. An experiment was conducted to determine the growth performance of Indian major carp *CatlaCatla* fingerlings for period of 60 days. Two experimental groups of commercial pellet diet incorporated with two types of feed probiotics (*Bacillus subtilus* and *Lactobacillus subtilus*) and two control groups maintained separately for *CatlaCatla* fingerlings. The results evaluated that the *CatlaCatla* fingerlings showed maximum increase in length [29.06+0.74 mm], weight gain [343.39 mg], FCR [1.02] and SGR [0.89] similar trends were observed highest levels in

experimental group than that of control group

KEYWORDS: *Catla Catla* fingerlings, FCR, SGR, *Bacillus subtilus*, and *Lactobacillus subtilus*, growth performance and weight gain.

INTRODUCTION

Probiotics are organisms and substances which contribute to intestinal microbial balance (Fuller 1989). A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. The initial major purpose of using probiotics is to maintain or reestablish between friendly and pathogenic microorganisms that constitute the flora of intestinal skin muscle of fish. A successful probiotic is expected to have a few specific properties in order to certify a beneficial effect. Antagonism to pathogens is one such property of a probiotic bacteria. One sign of antagonism is that it is producing antimicrobial substances

like organic acids, hydrogen peroxide (Ringo and Gatesoupe,1998) or siderophores (Gram and Mel-Chiorsen 1996) in order to have a beneficial effect in the form of growth promotion or to protect fish against bacterial pathogens. Now a days probiotics are also becoming an important part of the aquaculture practices to obtain high production in aquaculture, probiotics have been used to control disease, enhance non-specific immunity, provide nutrients and enzymatic functions and improve water quality (Schaperclaus et al 1992, and Balcozar et al, 2006).

In this present study aims to assess the growth and biochemical parameters of Indian major carps *Catla catla* fingerlings with two different types of feed probiotics (*Bacillus subtilis* and *Lactobacillus subtilis*).

They were fed with commercial pellet incorporated with different nutritional supplements like probiotics. This study is useful to determine the suitable ratio of supplementary nutrition for weight gain and survival rate of the *Catla catla* fingerlings respectively.

MATERIALS AND METHODS



Fig. 1: The systematic position of *Catla catla* (Hamilton, 1822).

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

Collection and acclimatation of experimental fishes

CatlaCatla fingerlings were collected from Government 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 continuous aeration for 12 days prior to experimentation. During the period, fishes were fed with known amount of fish feed.

Experimental procedure and Feeding trails

CatlaCatla fingerlings having same size and weight were carefully selected. Rearing and feeding experiments were conducted with two different commercial probiotics (*Bacillus subtilis* and *Lactobacillus subtilis*) and control diet (micropelletized commercial feed). Feeding trails were started at a rate of 2% of the body weight in a feeding regime of four times 8:00am, 12:00pm, 16:00pm and 20:00pm was conducted for 60 days. The growth parameters weight gain (%), Feed conversion ratio (FCR) and specific growth rate (SGR) were calculated following the method of Aliyu-pakio *et al.* (2010). At the end of the experiment, analysis of body homogenate of *CatlaCatla* fingerlings was carried out for protein (Lowry *et al.*; 1951); carbohydrate (Carroll *et al.* 1956) and lipid (Folch *et al.*; 1957).

Growth parameters

The growth parameters of the *CatlaCatla* fingerlings were assessed by taking their body weight at 30 days of interval. The growth performance was assessed using the following formula Net weight gain = Net weight gain was calculated described by Mustafa and Ridzwan (2000).

$$\text{Percentage weight gain} = \frac{\text{Final body weight (g)} - \text{Initial body weight (g)}}{\text{Initial weight}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{\ln(\text{final weight}) - \ln(\text{Initial weight})}{\text{Experimental periods in days}} \times 100$$

$$\text{Food conversion ratio (FCR)} = \frac{\text{Feed given (dry Feeding weight)}}{\text{Body weight gain (wet weight of tissue)}}$$

Survival percentage

Survival percentage was calculated at the end of experiment by counting the number of fishes in each tub and is calculated as follows

$$\text{Survival (\%)} = \frac{\text{Total number of Animal harvested}}{\text{Total number stocked}} \times 100$$



Fig. 2: Experimental setup in indoor laboratory.

RESULTS

Probiotics are non – pathogenic and non –toxic organisms without undesirable side effects when administered to aquatic organisms (Farzanfar, 2006). Results of this study substantiate the fact of the probiotics have direct growth promoting effects on catlacatla fingerlings which is in accordance with the reports of Who *et al.*, (1994), Chaudhari and Razi (2007), there are various other benefits of using probiotics for fish aquaculture including that of carp such as reduction in culture cost of indian major carps (Ghosh *et al.*, 2003 and Swain *et al.*,1996) studies revealed enhancement of feeding efficiency in acipenser when fed with probiotics (Jafaryan and Bagherio 2008), enhanced immunity in catlacatla fingerlings after oral administration of probiotics *Bacillus subtilis* and *Lactobacillus subtilis* (Kumar *et al.*, 2008) and enhanced growth performance and feeding efficiency in catlacatla fingerlings (Chaudary and Razi (2007), this is in agreement with the present results where significance enhancement in growth performance and nutrient utilization like FCR and SGR when observed in catlacatla fingerlings fed with probiotics. Probiotics are live microbial cells that are administered to the gastrointestinal tract of the host as a feed supplement, improving its intestinal microbial balance health (Fuller, 1989), the addition of probiotics reduced culture cost of indian major carps (Swain *et al.*, 1996).

At the end of experimental period both groups received probiotics supplemented diets. The concentration of total proteins, carbohydrate and lipid have been measured in the muscle and liver tissue of “*CatlaCatla*” fingerlings fed for 30 days on control and experimental diets on days 1,10,20 and 30 of the rearing period. Results pertaining to the concentration of total protein in the muscle of “*CatlaCatla*“ fingerlings fed on control and experimental diets are

presented in figures 1 and 2. The results clearly show that there is significant increases in the protein content of the muscle with increase in rearing time both in control and experimental groups. Obviously within the treated /experimental groups, except in day 1 group. There are significant increase ($p < 0.01$) in the total protein content of the muscle in the experimental groups (E1, E2) compared to control (C1, C2) groups.

Show the figures 3 and 4 present results on the concentration of total protein in the liver of "Catla Catla" fingerlings fed on control (C1, C2) and experimental (E1, E2) diets. It is clear from the results that there is a significant increase ($P < 0.001$) in the total protein content of the liver with increase in rearing time both in control and experimental groups (fig 3). Apparently there are significant increased ($P < 0.001$) in the total protein content of the liver of *Catla Catla* fingerlings fed on experimental diets (E1, E2) compared to those fed on control diets (C1, C2) except the first group. Obviously E1 and E2 diets augmented total protein content of the liver by 32% and 34% respectively on day 10; by 32% and 44% respectively on day 30. Compared to the respective C1 and C2 Diets (fig 4). However E2 diet enhanced total protein content of the liver significantly than E1 a day 20 and opposite is true on 30 days.

Shown the results on the concentration of total carbohydrate in the muscle of fingerlings of *Catla Catla* feed on control and experimental diets are presented in figure 5 and 6 it is evident from the results that there is a significant increase ($p < 0.001$) in the total carbohydrate content of the muscle with increase in rearing time from 1 to 30 days both in control and experimental groups (fig 5). Obviously there are significant increases ($p < 0.001$) in the total carbohydrate content of the muscle of *Catla Catla* finger fed on experimental diet (E1, E2) compared to these fed on control diets (C1, C2) on day 10, 20 and 30 but not on day 1. Apparently E1 and E2 diets enhanced total carbohydrate content of the muscle significantly by 12% and 22% respectively on day 10; by 16% and 23% respectively on day 20 and 17% and 20% respectively on day 30 compared to the respective C1 and C2 diets (fig 6). However E2 diet increased total carbohydrate content of the muscle significantly than E1 on day 10, 20 and 30.

Show the results figure 7 and 8 present results on the total carbohydrate content of the liver of *Catla Catla* fingerlings fed on control (C1, C2) and experimental (E1, E2) diets. The results clearly show that there is a significant increase ($P < 0.001$) in the total carbohydrate content of the liver with increase in rearing time (1 to 30) days both in control and experimental group (fig. 7)

Apparently there are significant increase ($p < 0.001$) in the total carbohydrate content of the liver of fingerlings fed on E1 and E2 diets compared to those fed on C1 and C2 diets. The results show that E1 and E2 diets caused a significant increase ($P < 0.001$) in the total carbohydrate content of the liver by 22% and 33% respectively on day 10; by 24% and 27% respectively on day 20 and by 20% and 24% respectively on day 30 compared to the respective C1 and C2 diets (fig.8). Interestingly E2 diet enhanced the total carbohydrate content of the liver significantly than E1 diet on days 10 and 20 but not on day 30 (fig.8).

Results pertaining to the concentration of total lipid in the muscle of *CatlaCatla* fingerlings fed on control (C1,C2) and experimental (E1,E2) diets are presented in in figures 9&10. It is evident from the results that there is a significant increase ($P < 0.001$) in the total lipid content of the muscle with increase in rearing time from 1 to 30 days both in control and experimental groups (fig.9) clearly there are significant increase ($P < 0.001$) in the total lipid content of the muscle of fingerlings fed on experimental diets (E1,E2) compared to those fed on control diets (C1,C2) on days 10,20 and 30 but not on day 1.

Obviously E1 and E2 diets enhanced total lipid content of the muscle by 18% and 21% respectively on day 10 by 26% and 29% respectively on day 20 and 20% and 24% respectively on day 30 compared to the respective C1 and C2 diets (fig.10). It is further observed that E2 diet increased the total lipid content of the liver significantly than E1 diet on day 20 compared that on day 10 and 30.

Show the results on the concentration of total lipid in the liver fingerlings of *CatlaCatla* fed on control and experimental diets are presented in figures 11 and 12. The results clearly show that there is significant increase ($P < 0.001$) in the total lipid content of the liver with increase in rearing time from 1 to 30 days both in control and experimental group (fig.11). Evidently there are significant increase ($P < 0.001$) in the total lipid content of the liver of fingerlings fed on experimental diets (E1,E2) compared to these fed on control diets (C1,C2) on days 10,20 and 30 but not on day 1. It is clear that E1 and E2 diets enhanced total lipid content of the liver by 22% to 26% respectively on day 10; by 23% and 25% respectively on day 20 and by 28% and 30% respectively on day 30 compared to the respective C1 and C2 diets (fig 12), interestingly no significant difference were observed in the total lipid content of the liver as a function of treatment of fingerlings with E1 and E2 diets on day 10,20 and 30.

DISCUSSION

Fish culture are increasing to compensate for the shortage of animal protein all over the world. Protein is the major source of energy in fish feeds and it helps primarily in tissue buildup of fishes. It has been reported that the quality of protein feed is highly important for the growth of fingerlings of *Catla Catla* (Singh et al 1979,1980). As a high level protein of animal origin is the choice in the selection of commercial fish feed.

Catla Catla (Botcha) is an ideal species form as culture in fresh water ecosystem. Development of a balanced artificial diet is most essential for successful rearing of *Catla Catla* it has been reported that only those diets which are low in both protein and total energy have resulted in decreased weight gain (Garling and Wilson 1976).The optimum amount of dietary protein and energy therefore, play a key role in the growth of fish.

A recent trends use of feed probionts are live microbial feed suppelements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller,1989,1991) the potential benefits include improved nutrition and growth and prevention of various disorders.

On the whole the results of the study clearly demonstrated the positive influence of probiotics with commercial formulated feed on the growth of *CatlaCatla*fingerlings.It is quite evident from the results that the protein content of the muscle and liver of the fingerlings increased with increase in time from 1 to 30 days indicating the positive influence of the probiotics feeds on growth (fig.1 to 11).

However E1 and E2 Diets seem to have a more potentiating effect on the total protein content of the muscle and liver than C1 and C2 diets on day 10, 20 and 30. The fact that E1 and E2 diets have probiotics(*Bacillus subtillus* and *Lactobacillus subtilus*) as a component could be the reason which probably explains the significant increase in the total protein content of the muscle and liver compared to control diets C1and C2 without probionts.Similar results have been reported in other fresh water fish where use of probiotics diets greatly enhanced the total protein content of the fish.

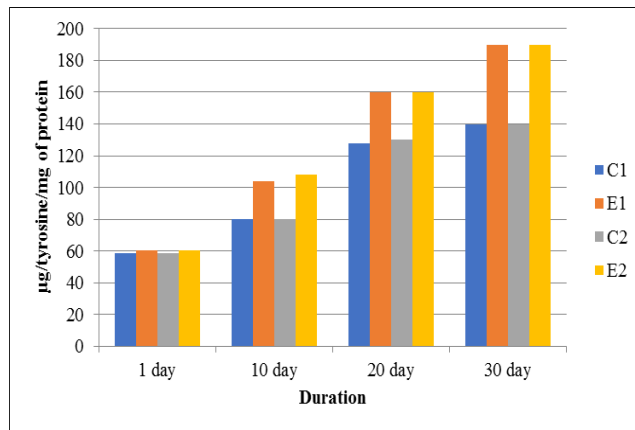


Fig. 1: Furnished the levels of total protein (TP) mg/gm wet weight in muscle tissues of fingerlings of *CatlaCatla* fed with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 day.

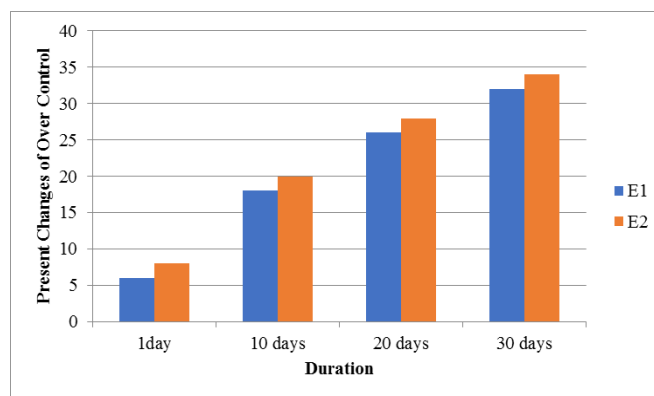


Fig. 2: Percent change in the levels of total protein (TP) mg/gm wet weight in the muscle tissue of fingerlings of *CatlaCatla* fed with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 days.

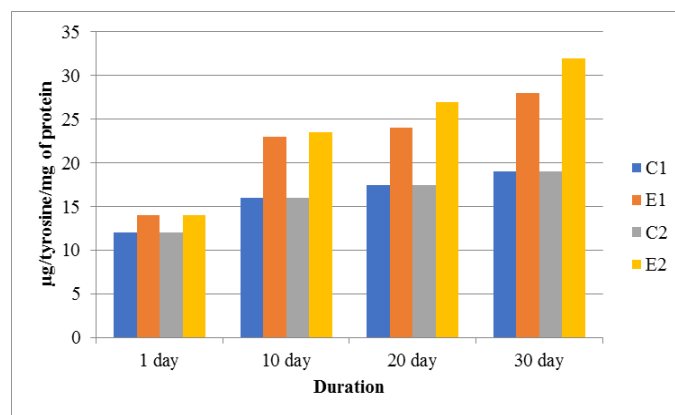


Fig. 3: Changes in the levels of total protein (TP) mg/gm wet weight in liver tissue of fingerlings of *CatlaCatla* with different diets (C1, C2, E1, E2) at the end of 1day, 10 day, 20 day and 30 days.

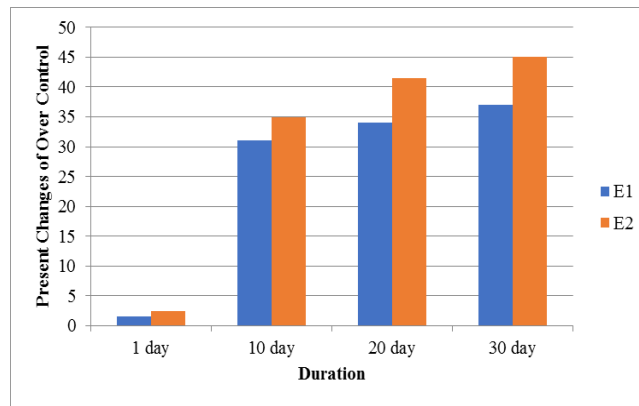


Fig. 4: Percent change in the levels of total protein (TP) mg/gm wet weight in the liver tissue of fingerlings of *CatlaCatlafed* with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 days.

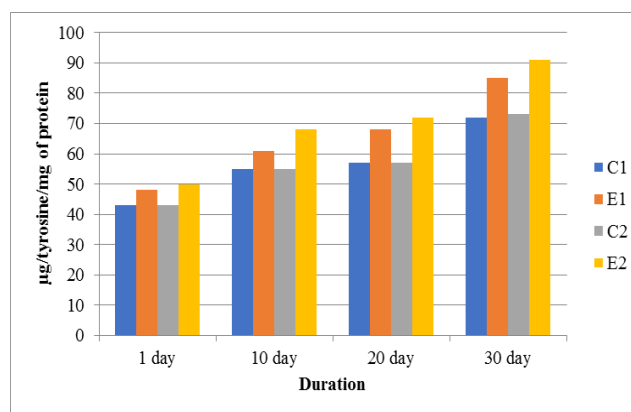


Fig. 5: Changes in the levels of total carbohydrate (TCHO) mg/gm wet weight in muscle tissue of fingerlings of *CatlaCatlafed* with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 days.

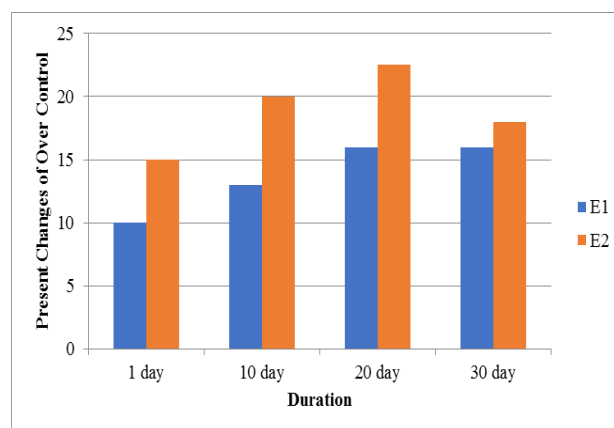


Fig. 6: Percent change in the levels of Total carbohydrate (TCHO) mg/gm wet weight in muscle tissue of fingerlings of *CatlaCatlafed* with different diets (C1, C2, E1, E2) at the end of 1day, 10 day, 20 day and 30 days.

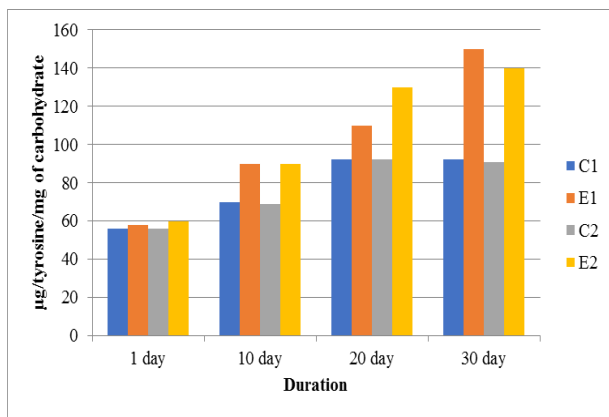


Fig. 7: Changes in the levels of total carbohydrate (TCHO) mg/g wet weight in liver tissues of fingerlings of *Catla* fed with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 days.

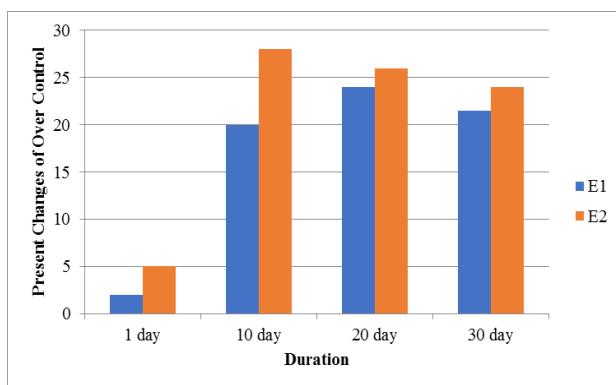


Fig. 8: Percent changes in the levels of total carbohydrate (TCHO) mg/gm wet weight in liver tissues of fingerlings of *Catla* fed with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 day.

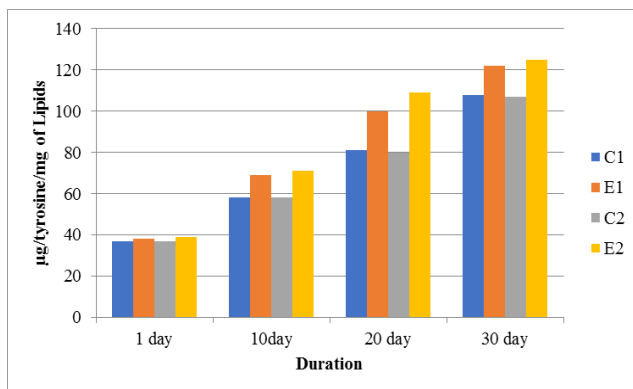


Fig. 9: Changes in the levels of total lipids (TL) mg/gm wet weight in muscle tissues of fingerlings of *Catla* fed with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 days.

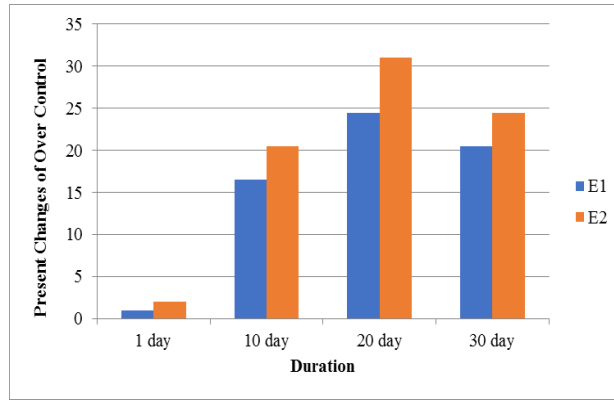


Fig. 10: Percent changes in the levels of total lipids (TL) mg/gm wet weight in liver tissues of fingerlings of *CatlaCatlafed* with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 days.

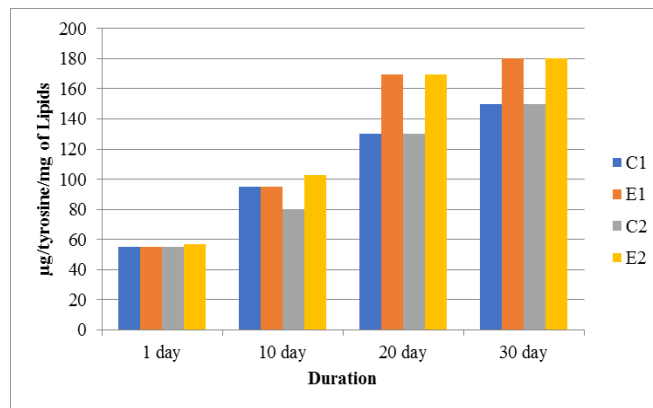


Fig. 11: Changes in the levels of Total lipid (TL) mg/gm wet weight in liver tissues of fingerlings of *CatlaCatlafed* with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 days.

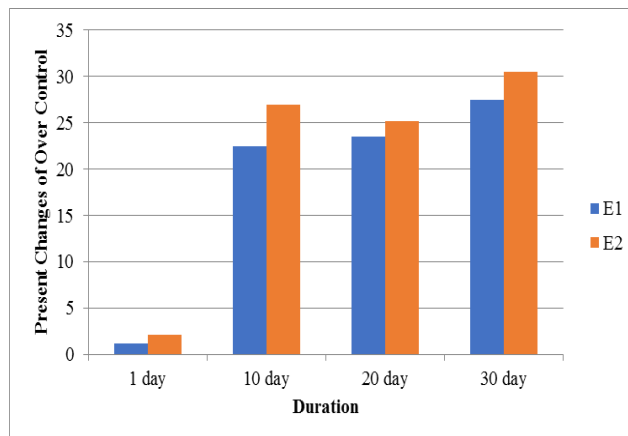


Fig. 12: Percent changes in the levels of total lipids (TL) mg/gm wet weight in liver tissues of fingerlings of fed with different diets (C1, C2, E1, E2) at the end of 1 day, 10 day, 20 day and 30 days.

REFERENCES

1. Fuller, R. Probiotics in man and animals. *J. Appl Bacteriol.*, 1989; 365-378.
2. Ringo and Gatesoupe, The use of probiotics in aquaculture. *Aquaculture*, 1998,1999; 180: 147-165.
3. Gram and Mel-chorsen 1996, in vitro antagonism of probiont *spseudomonos Huoresencesrain* against *Aeromonas Salmoncida* does not confer protein of salmon against furculosis Aghasnsthe 1991.
4. Schapreclaes et. All, and Ballcozar et all 2006. The role of probiotics in aguaculture. *Veterinary Microbiology* 1992; 114: 173-186.
5. Aliyu-Pakio, et. all Effect of the probiotics on growth performance,haematology parameters and immunoglobulin concentration in African catfish (*Clariasgariepinus*, Burchell 1822) fingerling. *Aquaculture Research* 2009, 2010; 40: 1642-1652.
6. Ridzwanet, all suitable marine Aquaculture recent developments with special reference to south east aciakotakinabalu, Malacia 2000.
7. Carroll.et.all Glycogen determination in liver and muscle by use of anthrone reagent, *J. Biol. chem*, 1956; 22: 583.
8. Folch.et all A simple method for the isolation and purification of total lipid from animal tissue *J. Bio. chem*, for use in Aqua feeds 1957; 226: 497509.
9. Garling and Wilson impaired collagen formation in the scrobustic channel fish *J.nutrol* 1976; 1359.
10. Roe, J.H.et all The determination of sugar in blood and spinal fluid with anthronereagent. *J. Biol. chem* 1955; 212,235-343.
11. FAO/WHO,Health and Nutritional properties of probiotics in food including powder milk with, 2001.
12. Lowry, O.H., Rosebrough, N.j., Farr, A.L., Protein measurement with the Folin –Phenol reagent.*J.Biol. Chem.* 1951; 193: 265-275.
13. Ghosh K, SenSK, Ray Ak. Supplementation of an isolated fish gut bacterium, *Bacillus circulans*, in formulated diets for rohu, Labiorohita, fingerlings.*Isr J AquacultBamideh* 2003; 55: 13-21.
14. Ghosh S, Sinha A, Sahu C. Dietary Probiotic supplementation in growth and health of live-bearing ornamental fishes. *AquacNutr*, 2008; 14: 289-299.
15. Swain, S.K., Rangacharyulu, P.V., Sarkar, S., Das, K.M., Effect of a probiotic supplement on growth, nutrient utilization and carcass composition in mrigalfry. *J. Aqua.* 1996; 4: 29-35.

16. Fuller, R. Probiotics in human medicine. *Gut*, 1991; 32: 499-442.
17. Chaudhary and Razi Influence of Probiotics *Pseudomonas Pseudocaligenes* Fermented feed on growth performance of Rohu (*Labeorohita*) Fingerlings Punjab Univ. J. 2001, 2007; 22(1-2): 41-56.
18. Jafaryan and A. Bagheri Effect of Probiotic Bacilli on the Enhancement of Feeding Efficiency, the proceedings of Aquaculture Europe., 2008.
19. Kumar at all., Study of digestive proteinase and proteinase inhibitors of *Daphnia carinata*, *Aquaculture*, 2005, 2007; 293: 367-372.
20. Who at all Effect of antibiotics enzyme, yeast culture and Probiotics on the growth performance of Israeli carp *Korean journal of Animal science*. 1994; 36: 480 -486.