EFFECT OF PROBIOTIC FEED ON GROWTH PARAMETERS OF *CATLA CATLA* FINGERLINGS

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ABSTRACT

With increasing demand for animal protein, aquaculture has become one of the fastest growing food sectors of the world. It has become the most viable and promising sector for providing nutritional and food security to human. But, sustainable production is effected in most of the aquaculture farms by factors such as poor water quality management, nutritionally imbalanced supplementary feed and the disease frequency. To control these diseases, indiscriminate use of antimicrobial drugs in aquaculture, lead to the emergence of antibiotic resistant bacteria. These are called Probiotics. Probiotics play a significant role in growth increment and stress mitigation in fish. In the present study two types of commercial probiotics were used as two experimental diets with two different control diets to know their significant effect on the growth parameters of *Catla catla*. The plastic tubs are stocked with catla (*Catla catla*), rohu (*Labeo rohita*), mrigala (*Cirrhinus mrigala*), silver carp (*Hypothalomisthy molitrix*), common carp (*Cyprinus carpio*) and grass carp (*Ctenipharyngodon idelta*) for a culture period of 30 days. The results evidently showed that probiotic supplemented diets have significantly enhanced the growth parameters of fingerlings compare with the control diets. Further the investigations reveal that growth improvement was obtained higher in probiotic treated ponds than that of control pond.

Keywords: nutrition, imbalance, antimicrobial, probiotics, enhance.

INTRODUCTION

Aquaculture is one of the easiest and fastest food producing sectors that provides nutritional and food security across the world. In India, fresh water fish production by aquaculture mainly consists of three major carps which have high market value and demand viz, catla (Catla catla), rohu (Labeo rohita), and mrigal (*Cirrhinus mrigala*). These have most important commercial fishes in India with a maximum market demand and acceptability as food by the consumers due to their taste and flesh. They are source of beneficial lipids and proteins (Swapna, Amit, Bhaskar & Sachindra, 2010). They contribute about 67% of total freshwater fish production (ICLARM, 2001). *Catla catla* contributes a major portion to the fresh water fish production in south India.

Feeding management plays a vital role in the success of fish culture. The existing trend in fish culture is towards increased intensification whereby, providing of feeds becomes necessary and increase in growth depends significantly on the availability of well-balanced, nutritionally complete and cost effective compounded feeds. Fish require ample nutrition in order to grow and survive. Nature offers a great diversity of food to fish including plants and animals. However artificial feed plays an important role in semi intensive fish culture where it is required to maintain a high density of fish than the natural fertility of the water can support (Jhingran, 1991). The role of artificial feed in intensive fish farming cannot be ignored as nutritional requirements we get from fish depends upon the feed supplied to it. The quantity and quality of feed consumed by fish have a distinct effect on growth rate, efficiency of feed conversion and chemical composition of fish (Hassan et al., 1996; Jena et al., 1998; Erfanullah and Jafri, 1998).

Another huge problem in Indian aquaculture is bacterial diseases. This is the major limitation in fresh water fish culture (Sahoo et al., 2011). During recent decades, prevention and control of diseases have led to a considerable increase in the use of antimicrobial drugs. This indiscriminate use of antibiotics to control diseases without awareness of dose and pharmacokinetic data on the fish poses serious ecological hazards (Baticados & Paclibare 1992). However, the use of antibiotics and chemotherapeutic can result in the emergence of drug resistant microorganisms, which can cause environmental hazards and food safety problems (Austin & Austin, 2016). To overcome these problems, use of probiotics having capability to enhance immune response can be used as a potential alternative for the prevention and control of disease outbreaks in aquaculture (Mohapatra et al., 2011).

Probiotics are live microorganisms that provide health benefits to the host by improving the feed value, digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth-promoting factors and increasing immune response (Harikrishnan, Balasundaram & Heo, 2010; Shane, 2008). Probiotics are beneficial microorganisms that protect the host from diseases. Fuller (1992) defined probiotics as ‘live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance’. Microbes play very important and critical roles in aquaculture systems. In aquaculture, persistent disease problems necessitate the use of bacteria as probiotics and as alternatives to antibiotics. There are several microbial strains used as probiotics in aquaculture systems. The most common probiotics reported for improving culturing of carp are Bacillus spp., Lactobacillus spp., Lactococcus spp. and Saccharomyces cerevisiae (Geng et al., 2012; Harikrishnan et al., 2010; Ramakrishnan et al., 2008; Ramesh, Vinothkanna, Rai & Vignesh, 2015).

The use of probiotic as feed enhancements has gained a considerable attention by feed manufactures as key element of improving livestock performance. Most of the studies connected with the effect of probiotics on cultured aquatic animals have emphasized a reduction in mortality and increased survival (Change and Liu, 2002), improved resistance against disease (villamil *et al*., 2003); enhance the ability to adhere and colonize the gut (vine *et al.*, 2004;Abo-State 2009); improved the ability to antagonize other organism (Burgents *et al*., 2004;Li *et al*., 2004; Brunt and Austin,2005).

The present study has been hypothesized to test the efficacy of water probiotic *Bacillus subtilis* and feed probiotic *Lactobacillus subtilis* on growth parameters of carp catla (*Catla catla*).

MATERIAL AND METHODS

Maintenance of Test Species

All experiments were carried out under laboratory conditions. The system consisted of 15 round shaped plastic tubs 30 liter capacity 40 cm diameter and 25 cm depth each filled with fresh water 6 replicates were maintained. The bottom of the rearing tanks was specifically made milky white to facilitate easy recognition and collection of faeces. The tubs are periodically aerated to provide enough oxygen to fish throughout the experimental period water in the system was replaced daily with fresh water to maintain water quality and to avoid accumulation of faeces and uneaten feed or any excretory products. The faeces were collected carefully after 3 hr from the morning feeding (maximum, shedding of faeces was observed to occur 3hr after the feeding) with pipette on to a piece of blotting cloth. Faeces were carefully transferred to a Petri dish and dried in the oven at 600C to constant weight. Collection of faeces was commenced one week after starting the feeding experiment and continued till the end of the experiment. The design and planning of the feeding experiment simultaneously for digestibility studies and growth evolution provided a longer period for faeces collection and to gather adequate quantity for chemical analysis. A constant photoperiod of 12 h light x 12 hr dark was maintained. Body mass water volume ratio was maintained at 1g/lit.

Control and Experimental Diets:

Control diet-1 is prepared by using Rice bran, Fish meal, Groundnut oil cake, and Soyabena cake.

Control diet-2 is prepared by using Rice bran, Fish meal, Groundnut oil cake, Soyabena cake and Prawn meal.

Experimental diet-1 is prepared by using Control diet-1 + water probiotics (*Bacillus subtillus*).

Experiemntal diet-2 is prepared by using Control diet 2+Feed probiotics (*Lactobacillus subtilus*).

The experimental duration is for 30 days. The size of fingerlings measured (2.5 gm to 5.5 grm). Six different replicants were used and six fishes in each replicate.

Probiotics and Feed Supplements:

Most widely used probiotics and feed supplements viz “Bharat lux Feed and Avanti fish Feed”(Bhimavaram, Andhra Pradesh, India) respectively were used in the present study.

Experimental Practices:

Acclimatization:

Fingerlings of fish were collected from local fisheries department as per the standard fish cultural procedure and were kept in cement tanks for a week with sufficient aeration and dechlorinated water to acclimatize them to laboratory conditions.

Fish Feeding:

Fish fingerlings were acclimatized to the experimental system using commercial control diet for two weeks before the start of experiment. During acclimatization weights were recorded. Fish were fed with 2% of their body weight per day which provides approximately the same amount of protein and energy in all the treatments. The stimulated diet was sub divided into four equal portions and the fish were fed 08:00, 12:00, 16:00 and 20:00 was daily taking care to provide small amounts of food at a time, to be sure that the fish at all the diet offered the experiment was conducted for 8 weeks and with six replicates per treatment.

Growth parameters

Determination of Total Body Weight:

Fish were taken out of the maintenance troughs, numbered by tagging and allowed to jump on a mat of filter papers in a trough to get rid of the adhered water. These fish then were transferred carefully and quickly into pre weighed small clean glass beakers. (Size of the beaker varies in accordance with the size of the fish) and the weight recorded to the nearest milligram in a sartorius balance. The difference between the beaker + fish and beaker alone gives the weight of the fish in gms.

Growth Parameters

The growth parameters of the *Catla catla* and other fingerlings were assessed by taking their body weights at 1, 10, 20 and 30 days respectively.. The growth performance was assessed using the following formulas:

Weight gain (g) = Mean final body weight (g) –Mean initial body weight (g).

Net weight gain was calculated described by Mustafa and Ridzwan (2000)

Feed Conversion Efficiency (FCE):

FCE is defined as the fish live weight gain per unit of food fed in dry weight. FCE was calculated as follows (Sambhu and Jayaprakash, 2001).

$$\mathbf{FCE} = \frac{\text{Live weight gain (g)}}{\text{Food fed (g) dry weight}}$$

Food Conversion Ratio (FCR):

Because feed is expensive, feed conversion ratio (FCR) or feed efficiency (FE) are important calculations for the grower. They can be used to determine if feed is being used as efficiently as possible.

FCR is calculated as the weight of the feed fed to the fish divided by the weight of fish growth (Mustafa and Ridzwan 2000).

$$\textbf{FCR} = \frac{\text{Total feed}}{\text{Wet Weight of tissue}}$$

Statistical analysis

The results are presented as means± SD, difference between parameters were analyzed by one way analysis of variance(ANOVA)and statistical significance was tested at $p < 0.05$ and $p < 0.001$ level. Statistical assessment of result was carried out using SSPS software.

RESULTS

The results showed significant change in *Catla catla* fingerlings with different feeding strategies. Basically aquaculture is intended to increase the weight of fish in the shortest possible time under artificial conditions through establishment of low cost feed. Supplementary feed accounts for nearly 40-60% of the total operational cost of intensive farming operations. Addition of probionts like *Bacillus subtilis, Lactobacillus substailus* etc. farm made feeds in an added advantage in the sense that it promotes fish growth in a holistic manner. The efficacy of such practice has been tested by measuring growth variables in the fingerlings of *Catla catla.*

Changes in the total body weight of fingerlings of *Catla catla* fed for 30 days with control (C1, C2) and experimental (E1, E2) diets are presented in figures 1 and 2. The results clearly shows that there is a significant increase ($P<0.001$) in the total body weight of fingerlings with increasing in rearing time up to 30 days (Fig.1) both in control and experimental groups with a magnitude of increase being more in fingerlings fed with E1, E2 diets than in those fed with C1, C2 diets. Obviously E1, E2 diets have enhanced the total body weight of fingerlings on 10th day by 49% and 50% respectively, by 58% and 63% on 20th day respectively and 56% and 60% on 30th day respectively when compared to C1 and C2 diets (Fig.2). However no significant difference was observed in total body weight between the fingerlings fed on control (C1, C2) and experimental (E1, E2) diets on day 1 signifying positive influence of probionts diets on the growth of fingerlings of *Catla-catla.*

Figures 3, 4 displays the results of changes in weight gain of fingerlings of *Catla catla* fed for 30 day on control (C1, C2) experimental (E1, E2) diets respectively. It is evident from the results that there is a significant increase ($P<0.001$) in the weight gain of *Catla catla* fingerlings with increase in rearing time fed with both control and experimental diet groups with the extent of increase being more in fingerlings fed on E1 and E2 diets than in those fed on C1 and C2 diets (Fig.3).

Visibly E1 and E2 diets augmented weight gain by 80% and 100% respectively on 10th day, by 88% and 94% respectively on 20th day and by 90% and 96% respectively on 30th day. However the current increase (E1-23% and E2-25%) is much less on day 1 than in other sampling periods (Fig.4). The results suggest that E1 and E2 diets are more effective in enhancing weight gain of *Catla-catla* fingerlings than C1 and C2 diets.

Variations in food conversion ratio of fingerlings of *Catla-catla* fed for 30 days under control (C1, C2) and experimental (E1, E2) diets are presented in figures 5 and 6. The results clearly illustrate that there is a significant decrease ($P<0.001$) in the food conversion ratio of fingerlings with increase in rearing time both in control and experimental diet fed groups with the magnitude of decrease being higher in fingerings fed on E1, E2 diets than in those fed on C1,C2 diets (fig 5).

Apparently E1 and E2 diets attenuated the food conversion ratio by 5% and 10% respectively on day 1, by 8% and 12% respectively on day 10; by 9% and 12% respectively on day 20 and by 9% and 19% respectively on day 20 and 9% and 18% respectively on day 30 with the magnitude of decrease being less on day 1 compared to other sampling periods (Fig.6). These results specify that E1 and E2 diets have effectively reduced food conversion ratio than C1 and C2.

Alterations in the food conversion efficiency of fingerlings of *Catla catla* fed for 30 day under control (C1, C2) and experimental (E1, E2) diets are presented in figures 7 and 8. It is clear from the results that there is a significant increase ($P<0.001$) in the food conversion efficiency of fingerlings with increase in rearing time both in control and experimental diet fed groups. However the quantum of increase is significantly higher in the fingerlings fed on E1, E2 diets than in those fed on C1, C2 diets throughout the 30 day period (Fig.7). Undoubtedly E1 and E2 diets enhanced the food conversion efficiency of *Catla catla* fingerlings than in those fed on C1, C2 diets throughout the 30 day period. Evidently E1 and E2 diets enhanced food conversion efficiency by 4% and 8% respectively on day 1; by 7% and 11% respectively on day 10; by 16% and 24% respectively on day 20 and by 19% and 28% respectively on day 30; however, the magnitude of percent increase is much less on day 1 and 10 than on days 20 and 30 (Fig.8). These results show that E1, E2 diets are more efficient in enhancing food conversion efficiency than C1, C2 diets.

DISCUSSION

Aquaculture is increasing day by day to compensate the scarcity of animal protein all over the world. A fish, under intensive culture environments will be badly affected and often fall prey to different microbial pathogens. These have been treated with chemotherapeutic substances of which antibiotics were intensively used. These therapeutic substances generate the problem of bacterial drug fastness on one hand and the public health hazards on the other hand (Robertson *et al*., 2000). These anticipated drawbacks enforced the fish pathologists to pursue for other alternatives; the use of natural immune stimulants in fish culture for the prevention of diseases is a promising new development and could solve the problems of immense antibiotic use. Natural immune drugs are biocompatible, biodegradable and safe for both the environment and human health. Moreover, they own an added nutritional value (Jessus *et al.,* 2002). The parallel use of organic products namely the probiotic is recently the objective of the disease bio control strategy in aquaculture as they improve the fish health and modify the fish related microbial community (Gibson and Roberfroid, 1995).

This study was planned to evaluate the effect of the probiotic on the growth parameters of the fish *Catla catla*. The results exposed that the fingerings showed maximum increase in length, weight gain. In general growth rates of fish are highly variable and in many cases seem to be limited by availability of food. Periods of feed restriction or feed deprivation resulting in growth relation or loss of weight do not appear to affect the ability of fish to grow when they are returned to full rations. Equally there is evidence to suggest that fish may show periods of rapid weight gain, commonly known as compensatory or catch-up growth following periods of feed restriction. The importance of feed utilization in relation to compensatory growth has been highlighted in several fish species (Bull and Metcalfe, 1997, Rueda et al., 1998). A number of investigators have suggested that compensatory growth could be exploited in the commercial production of fish to manipulate rates of weight gain or to improve growth efficiency with the conclusion of probiont ingredients in the commercial formulated feeds.

The results of the present study have clearly shown that probionts supplemented diets have significantly enhanced the total body weight of *Catla catla* fingerlings when compared to control diets (Fig.1, 2). Although both experimental diets increased total body weight over the 30 day period, the magnitude of increase was significantly higher in fingerlings fed on E1, E2 diets. It is intresting to note that the total body weight at the end of 30th day increased approximately 6 times in the fingerlings fed on C1, C2 diets where as 9 times in those fed on E1, E2 diets. These results suggest that use of probiotics supplemented diets might have contributed to optimistic growth by enhancing digestive enzyme activity and better food conversion efficiency (Shingran, 1991). Similar results have also been obtained in the present study the results also shows that there is significant increase in the net weight gain *Catla catla* fingerlings fed on E1, E2 diets than of fingerlings fed on C1, C2 diets (Fig.3, 4) on par with total body weight interestingly the total weight gain at the end of 30th day in approximately 20 times in fingerlings fed on C1, C2 diets against 36 times in those fed on E1, E2 diets.

Results of this study also shows that there is a significant drop in the food conversion ratio (Fig.5, 6) and a significant increase in the food conversion efficiency (Fig 7,8) of *Catla catla* fingerlings fed both on C1, C2 and E1, E2 diets. However the extent of changes both in food conversion ratio and in food conversion efficiency is significantly higher with E1, E2 diets when compared with C1, C2 diets. Similar results have been reported in other fishes also with regard to FCR and FCE Chaudhar; and Razi (2007). It is familiar that a lower FCR and higher FCE always promote growth in a fish species and the results of the present study are in acceptable with this general observation. In summary it can be said that probionts supplemented in the feed appears to influence the physiological process in such a way that *Catla catla* fingerings have shown much enhanced performance as far as growth is concerned.

Fig 1. Changes in the body weight (gm) in fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2)

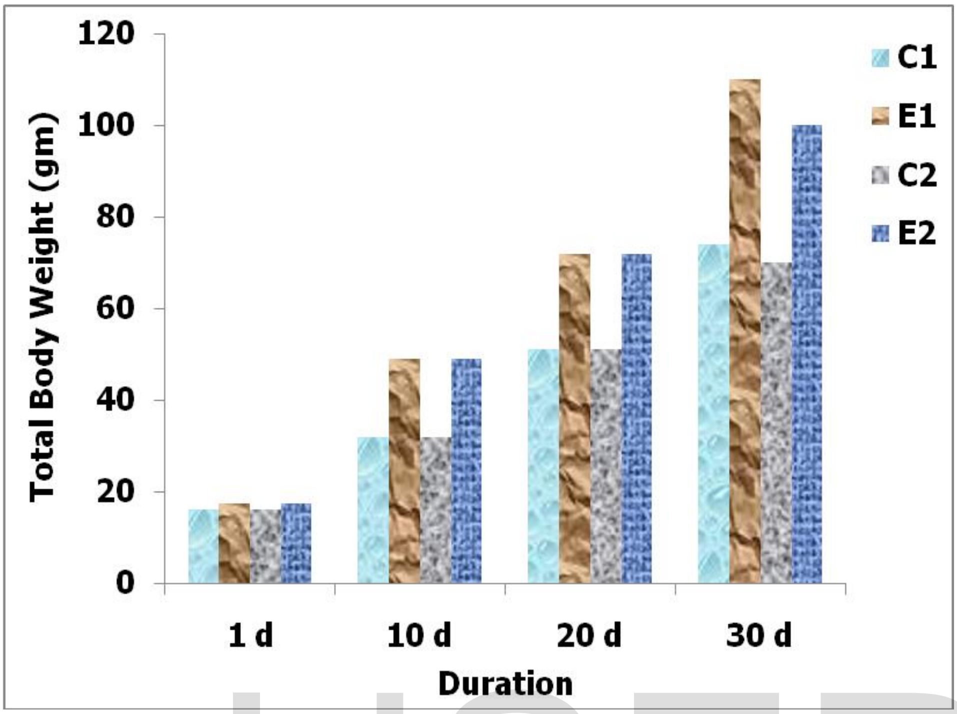


Fig 2. Percent changes in the body weight (gm) in fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2)

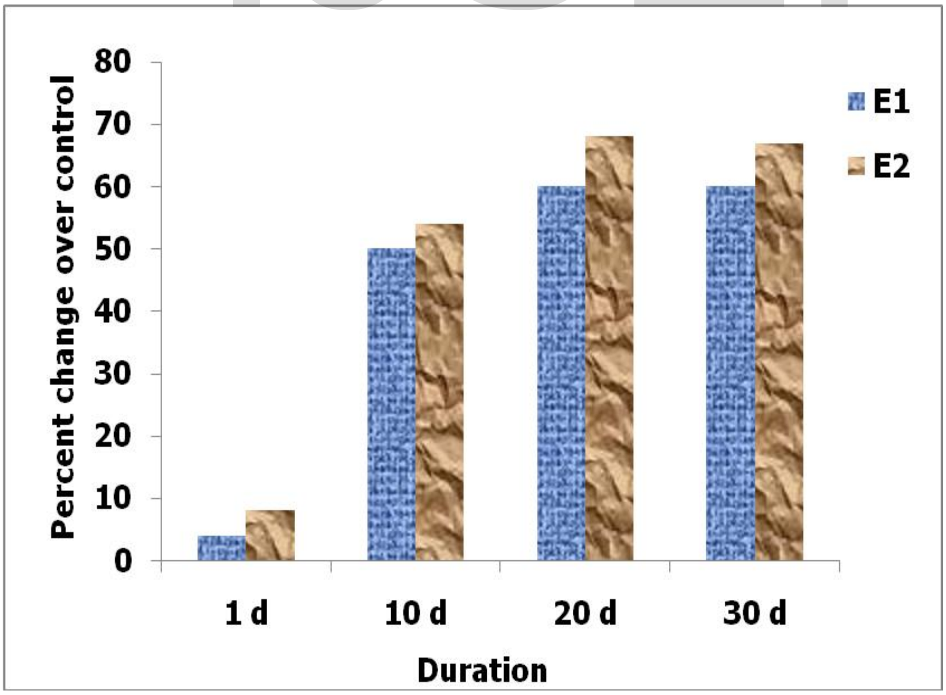


Fig 3. Changes in the weight gain in fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2)

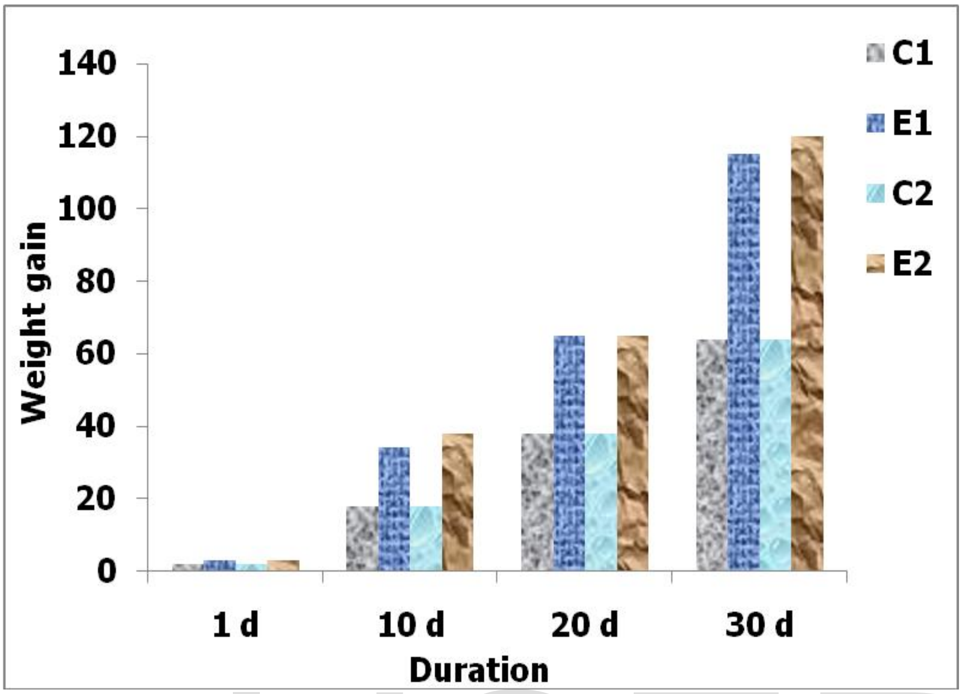


Fig 4. Percent Changes in the weight gain in fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2)

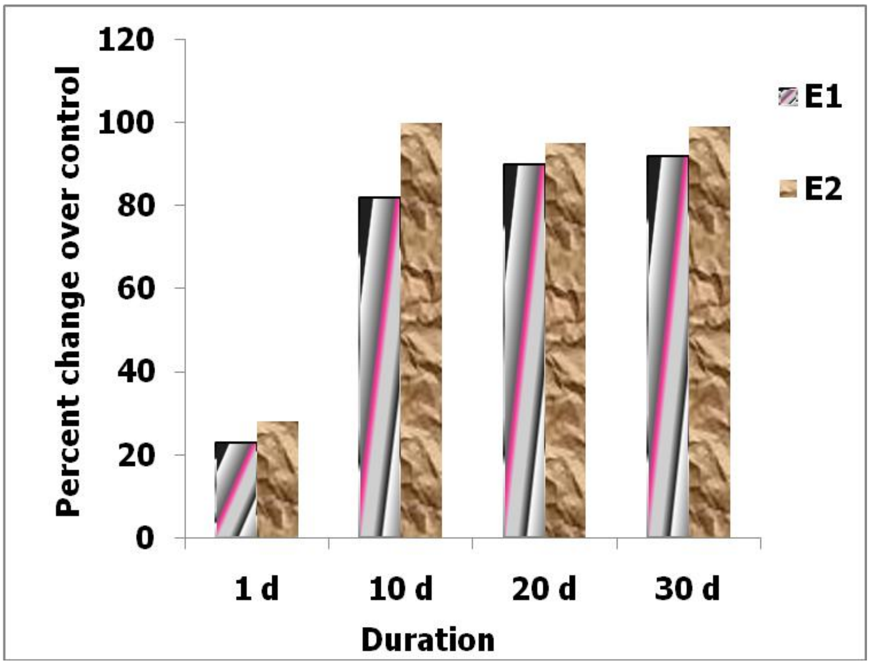


Fig 5. Changes in the Food Conversion Ratio (FCR) in fingerlings of *Catla catla* fed with different diets (C1, C2, E1, and E2)

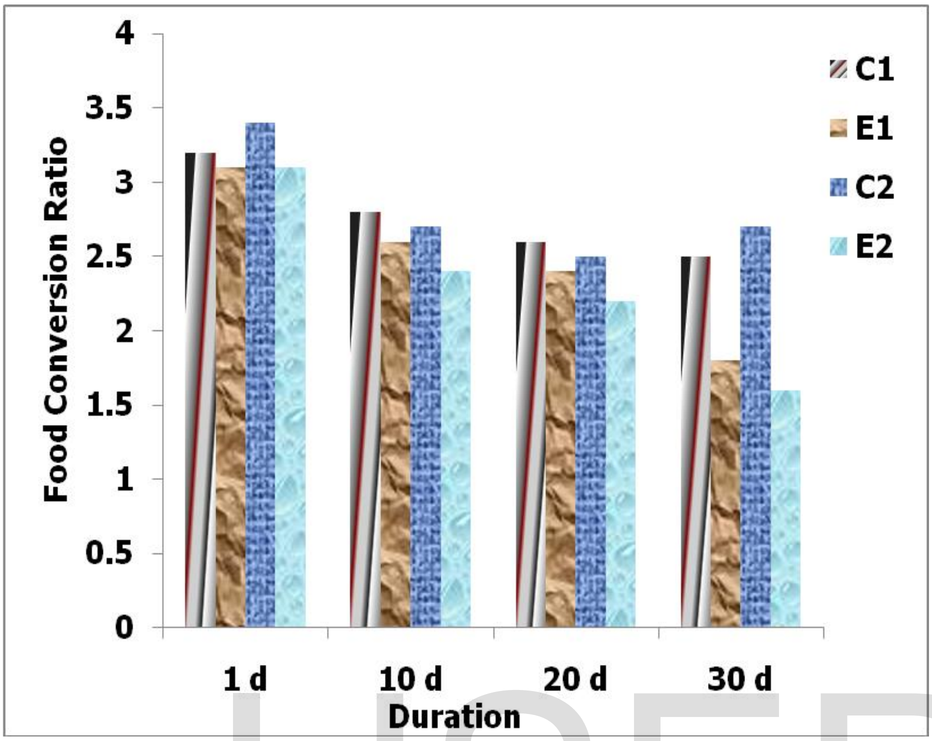


Fig 6. Percent changes in the Food Conversion Ratio (FCR) in fingerlings of *Catla catla* fed with different diets (C1, C2, E1, and E2)

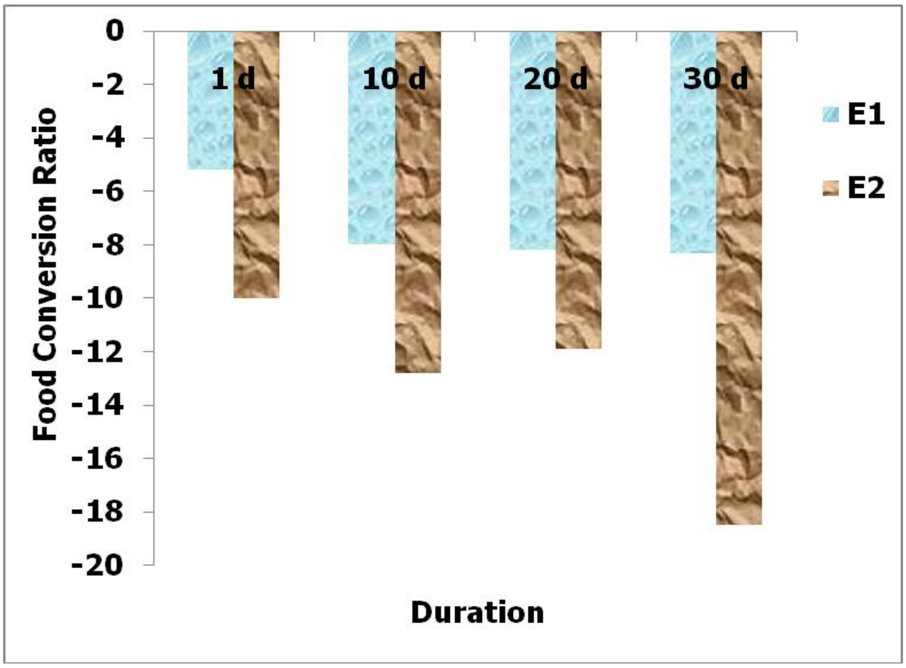


Fig 7. Changes in the Food Conversion Efficiency (FCR) in fingerlings of *Catla catla* fed with different diets (C1, C2, E1, and E2)

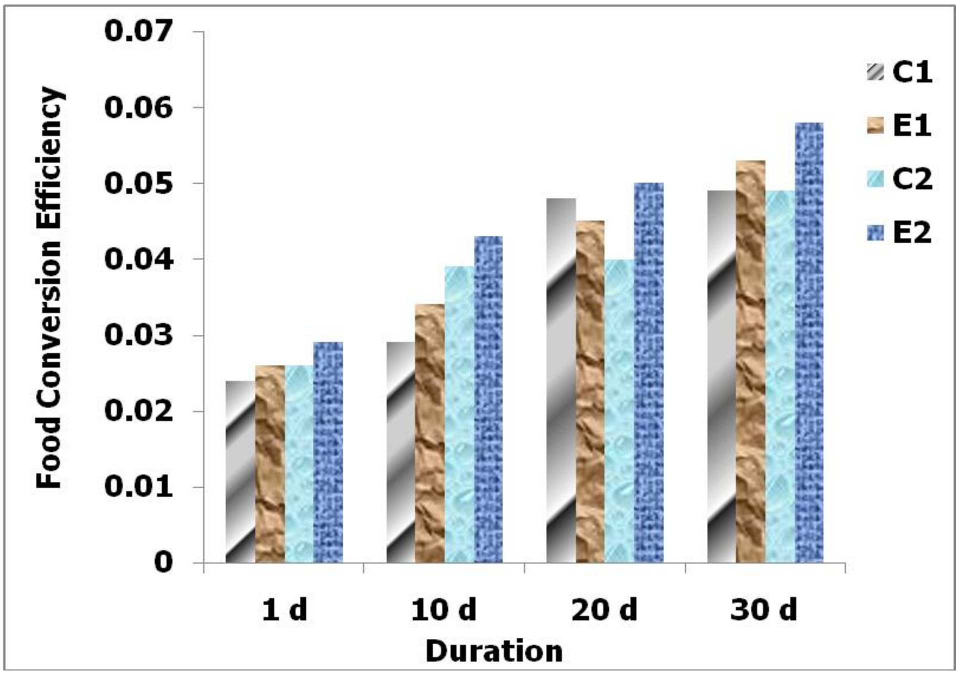
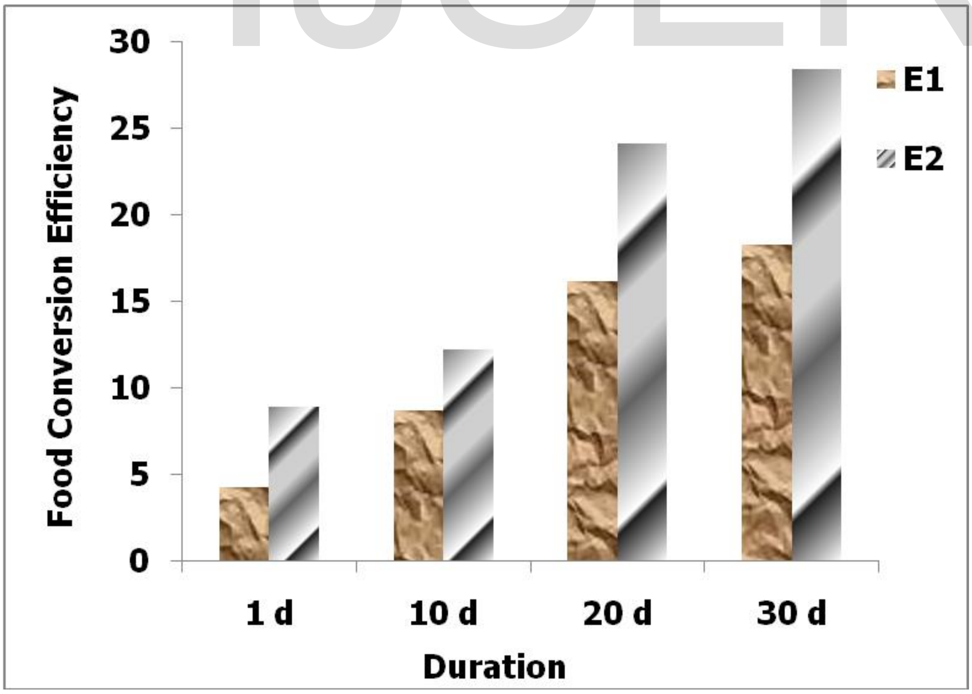


Fig 8. Percent changes in the Food Conversion Efficiency (FCE) in fingerlings of *Catla catla* fed with different diets (C1, C2, E1, and E2)



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