Effect of probiotic bacteria on biochemical variables of muscle tissue of *Catla catla* fingerlings

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Abstract

The development of fish culture as a commerce has accelerated above the past decades; this has resulted in conservational damages and low efficiency of various carps. The necessity for amplified disease resistance, development of aquatic organisms, and feed competence has brought about the use of probiotics in aquaculture practices. The first application of probiotics occurred in 1986, to test their ability to increase growth of hydrobiotics (organisms that live in water). Later, these probiotics were used to recover water quality and control of bacterial contaminations. Nowadays, there is documented evidence that probiotics can improve the digestibility of nutrients, upsurge tolerance to stress, and encourage reproduction. Presently, there are many commercial probiotic products prepared from various bacterial species such as *Bacillus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Carnobacterium* sp., and the yeast *Saccharomyces cerevisiae* among others. The use of these probiotics is regulated by careful supervision recommendations. The present paper shows the recent knowledge of the use of probiotics in aquaculture, and its effect on the biochemical variables of muscle tissueof Catla catla fingerlings for a period of 30 days. The action of probiotics were significantly (P<0.01) higher in probiotics incorporated diet (E1 and E2) fed fish when compared with control deits (C1and C2). All the biochemical variables were also recorded to be better with diets E1 and E2 compared to Control diets C1 and C2. This demonstrates the efficient utilization of feed by fishes. The present study conclude that probiotics incorporated feed was the best feed concentration food for the freshwater fish *C.catla*.

Keywords: accelerated, probiotics, digestibility, biochemical, significant.

Introduction

Aquaculture culture is being recognized as a growth area of economic importance in countries and is attracting the attention of both public and private sectors. Fish plays a very significant role in human nutrition all over the world. Good and sufficient nutrition plays a very vital role in the expression of mental, physical and intellectual potentials in humans. To ensure access to the nutritionally rich food for the improvement in the quality of diet of a poor person in the society, fish is the solitary medium which can serve the very purpose. They have the ability to reduce blood lipid level, particularly serum triglycerides (Boberg, 1990) and also acts as good source for human nutrition due to their therapeutic role in reducing certain cardiovascular disorders (Stickney and Hardy, 1989; Ahmed and Garnett, 2011).

India is the second largest producer of farmed fish in the world with 95% of total fish production originating from inland aquaculture (4.6 million tons) (FAO, 2013). Owing to the swift growth of the aquaculture sector, the per capita intake of fish has increased from 5 kg to 16 kg throughout the last five decades (Ayyappan and Biradar, 2004). Rich quality feed with a protein content of 30-40% is important for proper growth of fish and its health. In addition to the vital nutrients, balancing additives are also needed to enhance the growth and survival of fish. Some of the common growth endorsing feed additives used in aquaculture include antibiotics, hormones, ionophores and some salts (Fuller, 1992; Gongora, 1998). The main aim of aquaculture is to achieve maximum sustainable production per unit water area.

Due to the misuse of antibiotics in animal growth promoters, antibiotic resistance has become a common characteristic in microorganisms [Das et.al, 2013 and Kumaree et.al, 2015] thus caused serious problems in microbial infectious treatments [Saarela et.al, 2000]. The use of "probiotics", which are beneficial microorganisms or their products with the benefit effects to the hosts, have been used in aquaculture in order to control disease, as supplements for improving growth and in some cases as a mean of replacing antimicrobial compounds.

Lilly and Stillwell 1965 referred to probiotics as "Microorganisms promoting the growth of other microorganisms". Probiotics, as feed supplements benefit the host by improving the feed value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth promoting factors and increasing immune response [Verschuere et.al, 2000 and Harikrishnan et,al. 2010].

Fish proteins are essential and critical components of human diet in some densely populated countries where the total protein intake level may be low. Presently fish accounts for more than, or close to 50% of the total animal protein consumed in most countries of the world. Per capita fish supply based on reported production has increased dramatically over the last 20 years indicating the growing importance of fish as food (FAO, 2000). World aquaculture production will surely increase to meet the increasing demand for animal protein. The growth of the human population has led to an intensified search for methods of producing animal protein, other than conventional animal livestock and capture fisheries, as both fact limits in production. The potential of aquaculture as a supplementary producer of protein is attracting more interest than ever.

After proteins, carbohydrates represent the most abundant group of organic compounds in the animal body. The primary focus of nutrition research has been to evaluate diet quality in terms of growth potentials of cultured organisms (Akiyama, 1992). Carbohydrates are the main energy sources for general body metabolism. But, actually carbohydrates yield almost same energy as that released by protein but utilized for routine metabolism and day to day energy requirements.

The quality of the lipids for the fish is presumed to be related to the types and content of essential fatty acids. Fishes and prawns have a limited capacity to synthesize polyunsaturated fatty acids which have to be added to the diet. The use of lipids is desirable because lipids are a highly digestable source of concentrated energy. Lipids supply energy equal to that of carbohydrates, supply essential fatty acids, serve as vehicles for absorption of fat soluble vitamins, increase feed patability, serve as precursors for steroid hormones and other compounds. The fingerlings of carps require 6-8% lipid contents in their feeds.

Dietary probiotics are considered environment-friendly and can be beneficial for the aquatic animals through: improving their disease resistance and reducing malformations; increasing their growth performance, body composition; improving their gut morphology as well as the microfloral balance (Suzer et al. 2008, Merrifield et al. 2010, Ekici et al. 2011) The live culture of natural and beneficial Bacilli can be introduced into the gut of animal in massive number to supplement those already present. Pro-biotics are of two types. They are

(i) Feed probiotics which are administered through artificial pelleted feed.

(ii) Water probiotics which are applied to water surface as a part of water quality management

Probiotics used in aquaculture intensify the growth rate, helps to maintain the water quality, increases the survival rate during the hatchery operation, stimulate non-specific immunity by promoting production of haemocytes thereby increasing the host's resistance to various diseases and suppress the growth of harmful bacteria.

The present work is oriented towards understanding the changes in biochemical constituents in muscle tissue of Indian major carp *Catla catla* under the influence of probiotics. This trial was carried out for 30 days under laboratory environments.

Material and Methods

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.

Bio chemical Constituents:

Fish from different groups were done to death by a strong blow over the head. Then the body was cut open and required muscle tissue was isolated, quickly, transferred immediately into prechilled small glass vials placed on the ice cubes. These tissues were used for the biochemical estimations.

Estimation of Total Proteins:

The total protein content of the tissues was estimated by the method of Lowry et.al. (1951). 1% homogenate of tissues was prepared in distilled water. To 1ml of the homogenate 2ml of 10% TCA was added and centrifuged. The residue was dissolved in 1ml 1N NaoH. 0.2 ml of this solution was added to 4 ml alkaline copper reagent followed by folin phenol reagent (1:1 folin: water) and the color was measured at 600nm. The protein content was expressed as mg/g wet weight of the tissue.

Estimation of Total Carbohydrates:

The total carbohydrate content was estimated by the method of Carroll et.al. (1956). Tissues from control and experimental animals were isolated and homogenized separately in 10% TCA to prepare 5% homogenates were centrifuged at 3000rpm for 15 minutes. To 0.2ml (muscle) and 0.1ml (muscle) of TCA supernatants, 5 ml of anthrone reagent was added and boiled for 15 minutes. The tubes were cooled and the color developed was added and boiled for 15 minutes. The tubes were cooled and the color developed was read at 620 nm in a spectrophotometer using a blank prepared with TCA and anthrone reagent.

Estimation of Total Lipids:

Total lipid content was estimated by the method of Folch et al., (1957). A mixture of chloroform: methanol (1:1 W/V) was used as the homogenizing medium. The tissues were isolated, homogenized and centrifuged at 25,00 rpm for 15 minutes. To the supernatant, a small quantity of water was added and the contents were vigorously shaken. The aquatic layer was pipetted out from biphasic solution. The chloroform layer was taken in a small aluminum boat of known weight and evaporated at 50-60^{0}c. After complete evaporation the container was once again weighed and the difference between the initial and final weights represents the total lipid content. The values were finally expressed as mg of total lipid/g wet weight of tissues.

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

Animal proteins are easily digestible and contain all essential amino acids. The dietary protein requirement has a linear relationship with the specific growth rate utilization of dietary protein for new tissue growth is relatively constant in fish. The protein requirement of fish broadly ranges from 30 to 60 % carps need 30 to 47% protein in their diet for optimum growth. Protein requirement for fish is about 2-3 times higher than that of mammals. Protein levels in aquaculture feeds generally average 18 to 20% for marine shrimp, 28-32% for cat fish 32-38% for Tilapia 38-42% for hybrid striped bass.

At the end of experimental period both groups received probiotics supplemented diets, the concentration of total proteins (TP), total carbohydrate, and total lipid have been measured in the muscle tissue of "*Catla catla*"-fingerlings fed for 30 days on control and experimental diets on days 1, 10, 20 and 30 of the rearing period respectively. Results pertaining to the concentration of total protein in the muscle of "*Catla catla*" fingerlings fed on control and experimental diets are presented in figures 1 and 2.

Results pertaining to the concentration of total protein in the muscle 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 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 increases (P<0.01) is the total protein content of the muscle in the experimental groups (E1, E2)compared to control (C1, C2) groups. For instance E1 and E2 diets enhanced the protein content of the muscle by 16 and 18% respectively on day 10; by 26% and 27% respectively on day 20 and by 30% and 29% respectively on day 30 compared to the respective C1 and C2 diets (Fig.2). However there is no significant difference between the muscle protein content of the fingerlings fed on E1 and E2 on all the specified rearing periods.

Displayed 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 3 and 4 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 3). 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 by 17% and 20% respectively on day 30 compared to the respective C1 and C2 diets (Fig 4). However E2 diet increased total carbohydrate content of the muscle significantly than E1 on day 10, 20 and 30.

Results relating to the concentration of total lipid in the muscle of *Catla catla* fingerlings fed on control (C1, C2) and experimental (E1, E2) diets are presented in figures 5 & 6. 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.5) 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 by 20% and 24% respectively on day 30 compared to the respective C1 and C2 diets (Fig. 6). 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.

Discussion

Fish cultures are increasing to compensate for the storage 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 finger lings of *Catla catla* (Singh et al 1979, 1980). As a high level of 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.

After protein, carbohydrate and total lipid represent the third most abundant group of organic compounds in the animal body carbohydrates are the most economical source of dietary energy and are known for their protein sparing action. Carbohydrates play an important role in glycogen storage and formation of steroids and fatty acids in fishes. It has been reported that total carbohydrate can play a significant role in determining the quantitative requirement of protein and lipid while it can be utilized for metabolic energy needs of the animal protein (Pascual et al., 1983, Ali 1993) and lipid can be spared for more important and essential body functions and growth.

Body composition is a good indicator for the physiological condition of a fish but it is comparatively time consuming process. Proximate body composition is the investigation of carbohydrates, proteins, moisture and ash contents of fish. The proportion of water is good indicator of its relative contents of energy, proteins and lipids. The lower the percentage of water, greater are the lipids, protein contents and higher energy density present in the fish (Dempson *et al*., 2004). Fish requires energy and other nutrients for growth, reproduction and health. Growth is characterized primarily by an increase in protein, minerals and water. Energy yielding nutrients such as lipid and carbohydrate are important to support the growth process, and an adequate supply of vitamins is also required. These nutrients may come from natural aquatic organisms or prepared feed. However, in contemporary aquaculture prepared feeds from commercial foodstuffs are the primary source. Thus a familiarization with the nutrients and their sources, requirement and role in metabolism are necessary for successful aquaculture (Lovell, 1998). The total requirement for a given nutrient during growth must include the amount needed for maintenance as well as the amount required for the new tissue formed (Jauncey, 1998).

Nutritional requirements must be balanced so that the fish will have enough essential nutrients for optimum growth. All types of formulated fish diet must satisfy the nutritional requirements of the cultured species in terms of proteins (essential amino acids), lipids (essential fatty acids), energy, vitamins and minerals. Dietary protein is the most expensive ingredient in aqua feeds. Fish meal has been the major protein source in these diets due to its optimum amino acid and fatty acid profile, good palatability to fish and other desirable nutritional properties (Bassompierre *et al*., 1997: Watanabe 2002). A decline in the global production of fish meal, high prices and the need to reduce nitrogen and phosphorous loads are motivating researchers to identify alternative protein sources (Vegetable or animal origin) that can be efficiently utilized to optimize growth, reduce feed cost and lower ammonia and phosphorus outputs. The most common alternative protein sources being investigated are: full-fat and defatted soybean meal (at present the most cost effective alternative), canola, sunflower, cottonseed, corn gluten meal, squid, crab, shrimp and meat meal.

Dietary carbohydrate can be utilized as an energy source by fish. Omnivores are known to utilize carbohydrates as an energy source and create a protein- sparing effect, unlike carnivores (Morris, 2001 Hemre *et al*., 2002). The addition of carbohydrates to feeds to demuscle energy to fish and create a protein-sparing effect is being investigated. Several studies show that high energy diets and increasing carbohydrate concentrations (dextrin, starch, and wheat flour) increases muscle lipid and lipid content of the fillet. According to Hemre (2001), approximately 15% of the carbohydrate ingested will be stored as muscle glycogen (1% of total glycogen) and around 8-15% will be stored as muscle glycogen (8% or total muscle glycogen).

Dietary lipid provides essential fatty acids, phospholipids and energy to fish to promote growth, health, metabolic pathways and is the precursors of eicosanoids (physiological functions) (Sargent *et al*., 1999). Dietary lipid, just as dietary carbohydrate, can act as an energy source to spare protein utilization for energy and also increase palatability. The development of high-energy diets (high lipid content) to reduce nitrogen and phosphorus loads from high –protein diets and improve feed conversion ratios, among other factors, is rapidly gaining popularity (Rasmussen *et al*., 2000; Chaiyapechara *et al*., 2003).

However from whole study we can come to a conclusion that E1 and E2 diets seem to have a more potentiating effect on the total protein content of the muscle than C1 and C2 diets on days 10, 20 and 30. The fact that E1 and E2 diets have probionts as a component could be the reason which probably explains the significant increase in the total protein content of the muscle and muscle compared to control diets C1 and C2 without probionts. Similar results have been reported in other fresh water fish where use of probionts diets greatly enhanced the total protein content of the fish.

On the whole the results of the study clearly demonstrated the positive influence of probiotics with commercial formulated feed on the growth of *Catla catla* fingerlings. It is quite evident from the results that the protein content of the muscle and muscle of the fingerlings increased with increase in time from 1 to 30 days indicating the positive influence of the probiotic feeds on growth (Fig.1 to 6).

Fig.1: Changes in the levels of Total Protein (TP) mg/gm wet weight in muscle tissues of fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2) at the end of 1, 10, 20 and 30 days respectively.

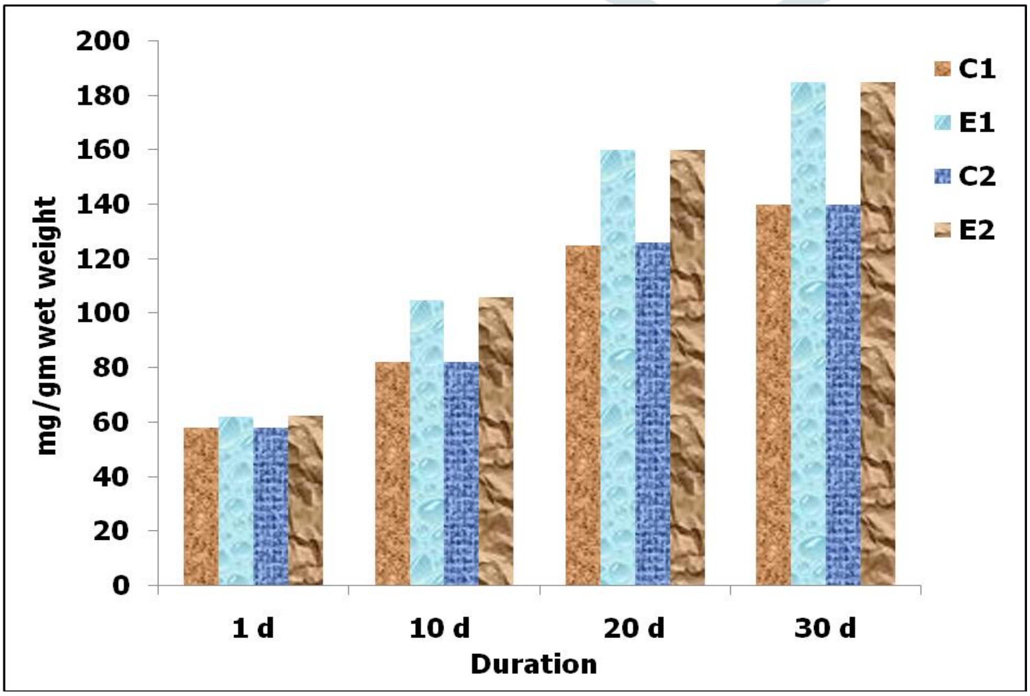


Fig.2: Percent change in the levels of Total Protein (TP) mg/gm wet weight in muscle tissues of fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2) at the end of 1, 10, 20 and 30 days respectively.

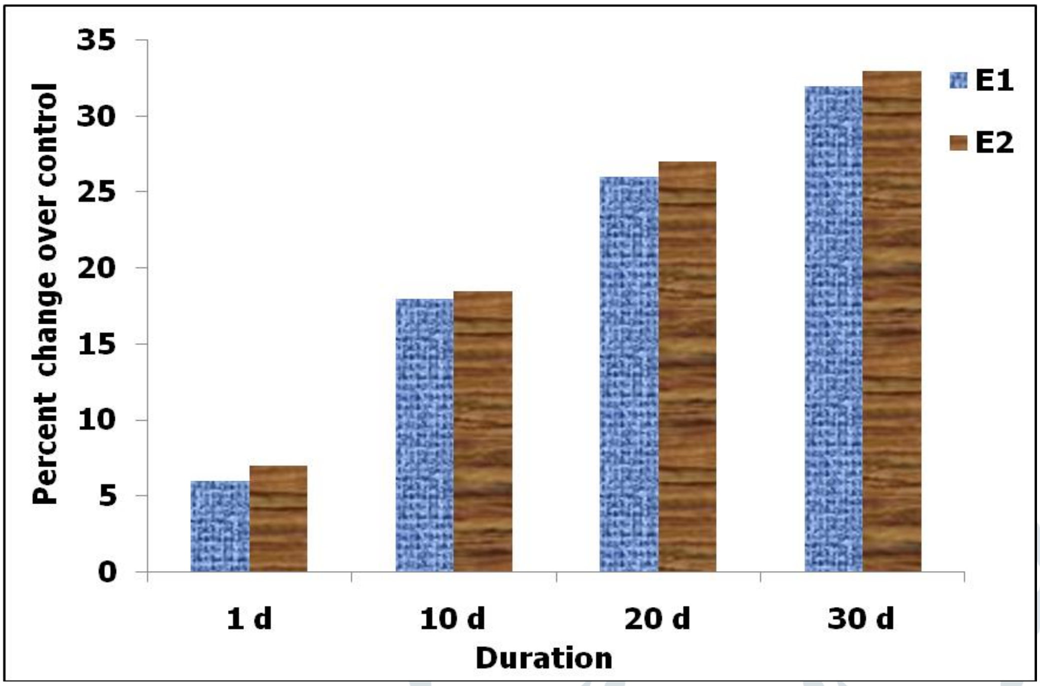


Fig.3: Changes in the levels of Total Carbohydrate (TCHO) mg/gm wet weight in muscle tissues of fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2) at the end of 1, 10, 20 and 30 days respectively.

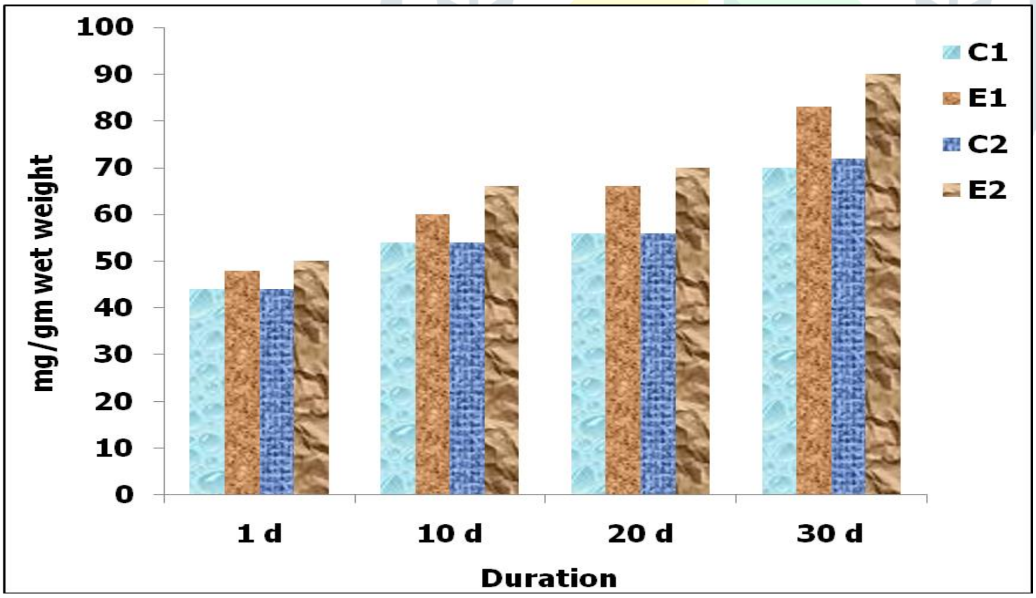


Fig.4: Percent changes in the levels of Total Carbohydrate (TCHO) mg/gm wet weight in muscle tissues of fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2) at the end of 1, 10, 20 and 30 days respectively.

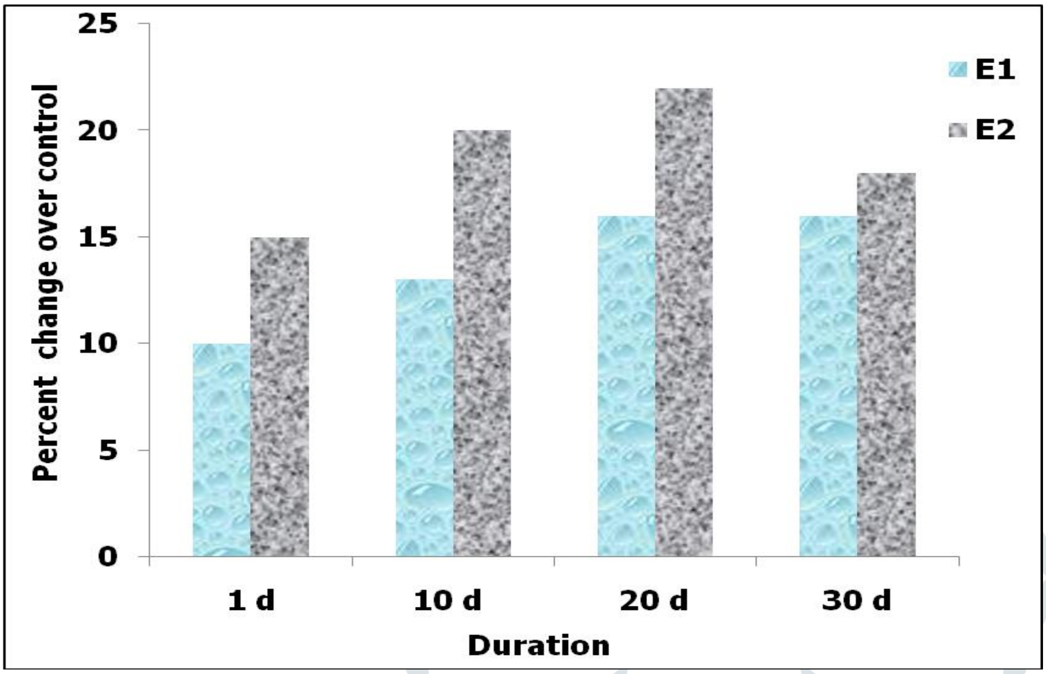


Fig.5: Changes in the levels of Total Lipids (TL) mg/gm wet weight in muscle tissues of fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2) at the end of 1, 10, 20 and 30 days respectively.

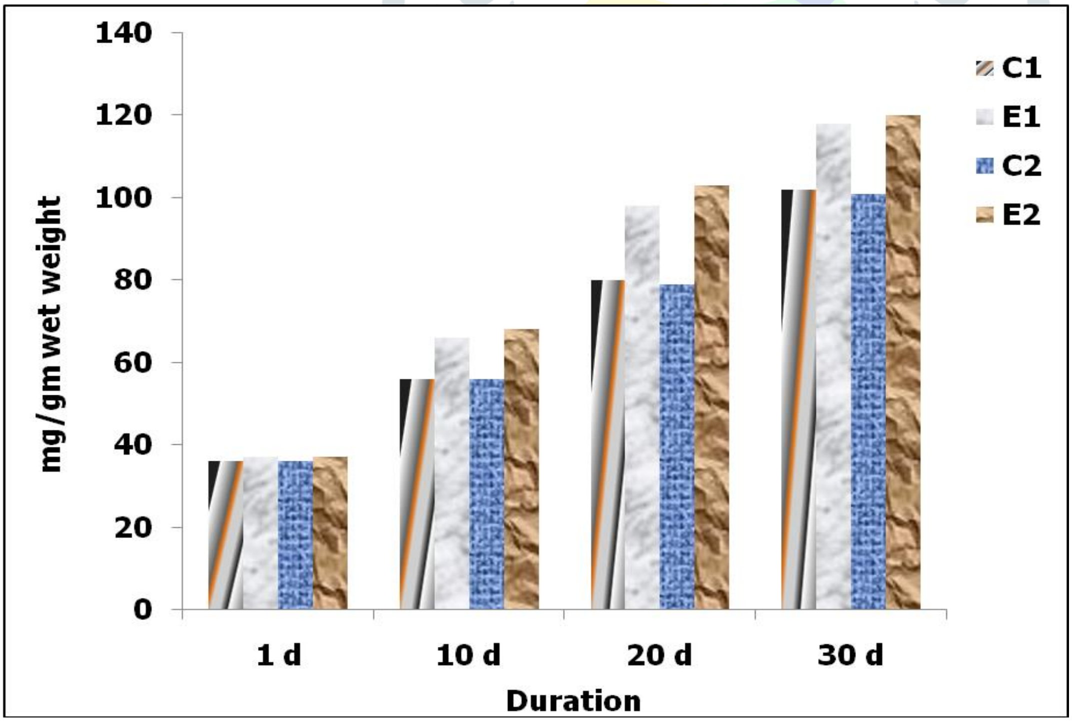
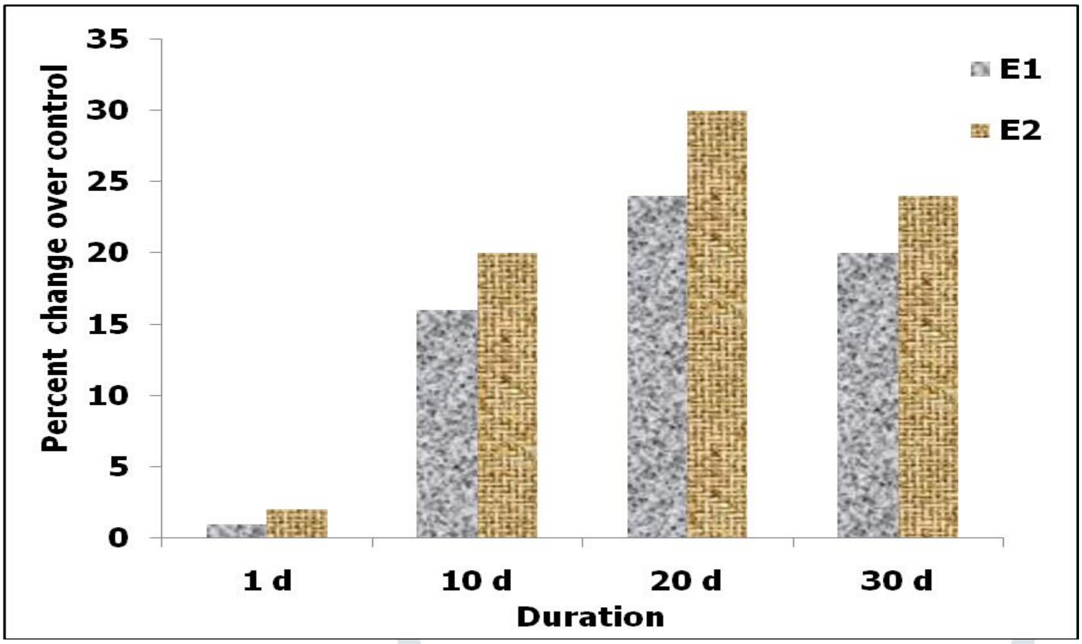


Fig.6: Percent changes in the levels of Total Lipids (TL) mg/gm wet weight in muscle tissues of fingerlings of *Catla catla* fed with different diets (C1, C2, E1, E2) at the end of 1, 10, 20 and 30 days respectively.



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