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STUDIES ON GROWTH AND SURVIVAL PERFORMANCE OF PROBIOTIC SUPPLEMENTED DIET ON ROHU (*LABEO ROHITA*) FRESH WATER FISH FINGERLINGS.

JOHNDEVADOSS GOBINATH, RAVICHANDRAN RAMANIBAI
Department of Zoology, Unit of Aquatic Biodiversity, University of Madras, Guindy Campus, Chennai-600 025, Tamilnadu, India

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Abstract: Microbial diseases are one of the major problems in fresh water aquaculture. There is an urgent need to develop microbial control strategies, since disease epidemics are recognized as import constraints to aquaculture production. This study reports the effect of the gram positive probiotic bacteria *Lactobacillus* sp on the freshwater fish rohu, *Labeo rohita* fingerlings against a pathogenic bacteria *Vibrio cholerae* of first feeding. Probiotic was administered through the diet at four different concentrations such as 312 x 10^{-4} (Group I), 126 x 10^{-5} (Group II), 75 x 10^{-6} (Group III) and 22 x 10^{-7} (Group IV) CFU/g of feed and fed at the rate of 3 % body weight for a period of 30 days. Control group was fed without probiotic incorporated diet. The growth was estimated by morphometric measurements for healthy and disease free fingerlings, franthus average body weight of 22.5 ± 1.5 g were a selected for biochemical, feed conversion ratio, feed conversion efficiency and protein efficiency. The results indicated that protein, lipid, carbohydrate content were high in group III fed fish than the control group. The FCR, FCE and PER ratio also showed significant increases when compared with other groups. These results clearly indicated the importance of probiotic feed to control *Vibrio cholerae* in freshwater aquaculture farms.

Keywords: Microbial Diseases, Probiotic, Diet

Corresponding Author: MR. RAVICHANDRAN RAMANIBAI

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INTRODUCTION

Probiotics are live microbial supplements when administered in adequate quantities, confer a beneficial effect on the health of the host by improving its intestinal microbial balance (FAO⁄WHO, 2001; Fuller, 1989). The wide use of antibiotics and chemotherapeutics to prevent and control bacterial diseases in fish farms has led to some problems like drug resistance (Alderman and Hastings, 1998; Teuber, 2001). These antibiotics may cause a inhibition of beneficial micro biota which is normally present in the digestive tract of the fish (Sugita *et al*., 1991). Moreover, vaccines cannot be used alone as a universal disease control measure in aquaculture (Amábile-Cuevas *et al*., 1995) due to their limited availability in few countries and their pathogen specific protective action for certain specific bacterial and viral diseases (FAO,2006). Hence, the use of probiotics as biological control agents or as dietary live microbial supplements in commercial fish culture to improve growth and immune function of the fish (Gatesoupe, 1999; Irianto and Austin, 2002; Kesarcodi-Watson *et al*., 2008).

Fish diseases are one of the major problems in the fish farm industry. Even though vaccines are being developed and marketed, they cannot be used as an universal disease control measure in aquaculture. The use of antibiotics to cure bacterial infection and prevent fish mortality in aquaculture is becoming limited as pathogens develop resistance to the drugs (Gonzalez et al., 2000; Gomez-Gil et al., 2000). Further, beneficial bacterial flora are killed or inhibited by orally administered antibiotics, leading to efforts to find an alternative disease prevention methods such as the use of non-pathogenic bacteria as probiotic bio control agents. The use of commercial probiotics in fish is relatively in effective as most commercial preparations are based on strains isolated from non-fish sources that are unable to survive or remain viable at high cell density in the intestinal environment of fish during the active growth phase of the fish (Gram et al., 2001). Hence, there is elegant logic in isolating putative probiotics from the host in which the probiotics intended for use. Such strains should perform better because they have already adhered to the gut wall of the fish and, thus, are well-adapted to complete with pathogens for nutrients. Presumably, strains that develop dominant colonies in the fish intestine are good candidates for preventing the adhesion of pathogens on the gut wall. In Asia, an average, almost 30 percent of total protein intake is derived from fish. Fish is a highly nutritious food, containing high amount of protein with high biochemical value for humans. Fish is a principal source of animal protein for over half of the global population. Probiotic are live microorganisms that are similar to beneficial microorganisms found in the human gut.

MATERIAL AND METHODS

Animal collection and maintenance

Fingerlings were collected from porur lake chennai Tamilnadu, India. The collected fingerlings were brought to the laboratory in plastic bags with oxygenated habitat water. Collected fingerlings were acclimatized to the laboratory conditions for 10 days in disinfected 1000 L circular FRP tanks. During acclimatization, the *Labeo rohita* fingerlings were fed with formulated diet. Healthy and disease free fingerlings, weighing average body Weight and length 22 ± 2g and 6 ± 0.3 cm were selected for further experiments (Fig. 1).

Biochemical analysis

The protein content in different tissues such as gill, liver, muscle and kidney of *Labeo rohita* fingerlings was estimated following the method of Bradford (1976).Total lipid content in different tissues such as gill, liver, muscle and kidney of fingerlings was extracted according to the procedure of Folch *et al.* (1957) and estimated according to the method of Barnes and Blackstock (1973). The carbohydrate content in different tissues samples of *Labeo Rohita* was estimated following the method of Roe (1955).

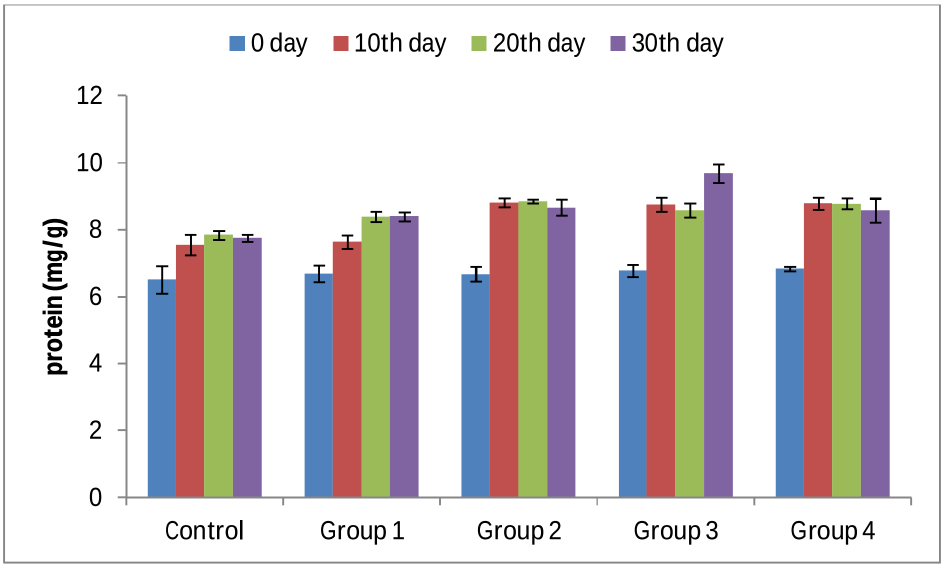
Results

The efficacy of the diets was studied in different tissues such as gills, muscle, liver and kidney of *Labeo Rohita* and compared with the fingerlings fed with control diet.

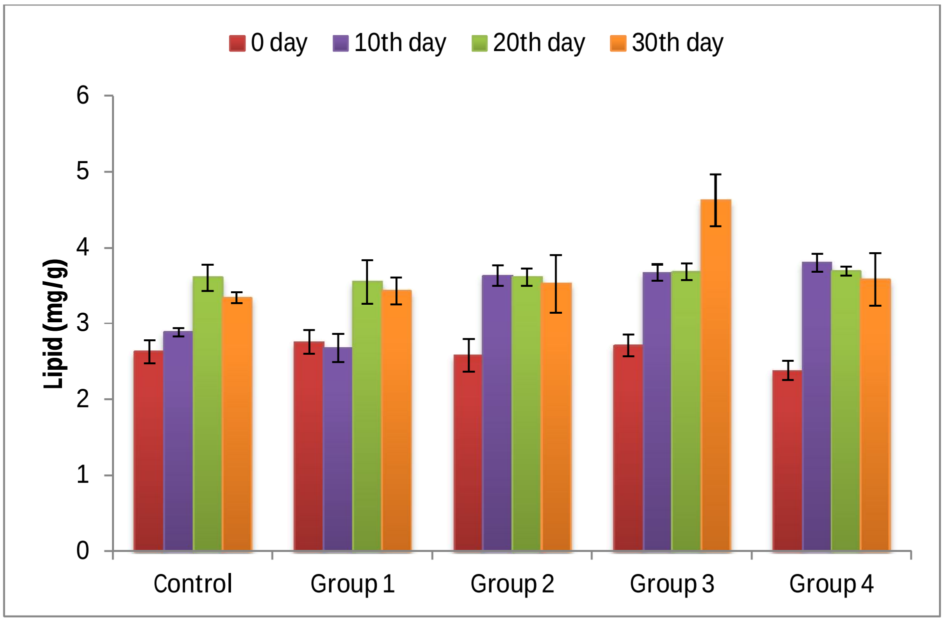
Gills

Protein content of gills in *Labeo Rohita* fingerlings fed with Group 3 (9.68 ± 0.27 However, other experimental diets showed a linear increase of protein content up to 30 days and Group 3 increased the protein content in gills. Lipid content of gills also varied in fingerlings fed with various experimental diets. Lipid content was high in fingerlings fed with Group 3 (4.62 ± 0.34 mg/g). Similarly carbohydrate content was maximum in fingerlings fed with Group 3 (3.72 ± 0.17 mg/g). In the experimental groups, Group 3 diets enhanced the carbohydrate content in gills.

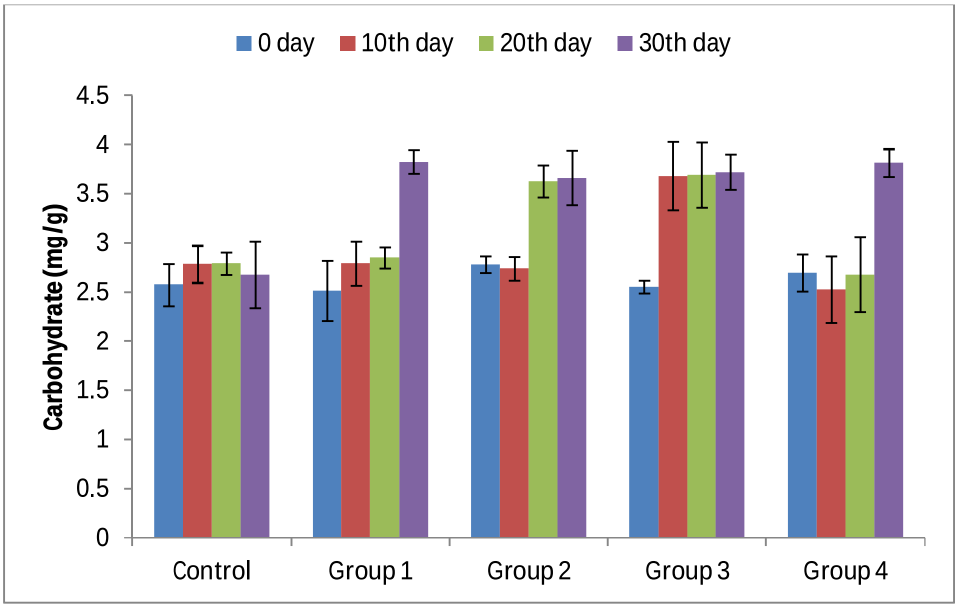
(Fig 1.) Protein content of gill



(Fig 2.) lipid content of gill



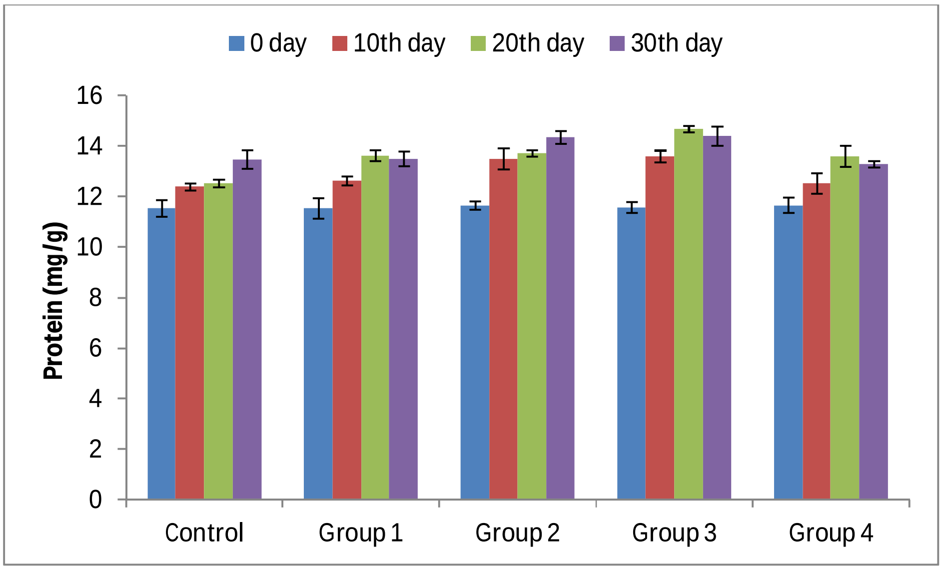
(Fig 3.) carbohydrate content of gill



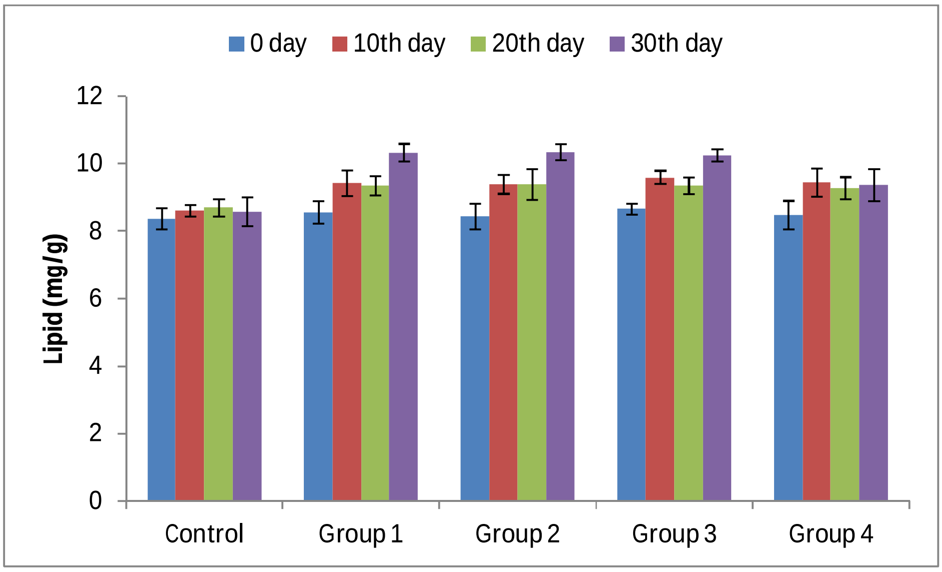
Liver

Protein content of Liver in *Labeo Rohita* fingerlings fed with Group 3 (14.40 ± 0.38 However, other experimental diets showed a linear increase of protein content up to 30 days and Group 3 increased the protein content in gills. Lipid content of gills also varied in fingerlings fed with various experimental diets. Lipid content was high in fingerlings fed with Group 3 (10.24 ± 0.18 mg/g). However carbohydrate content was maximum in fingerlings fed with Group 3 (7.28 ± 0.16 mg/g). In the experimental groups, Group 3 diets enhanced the carbohydrate content in gills

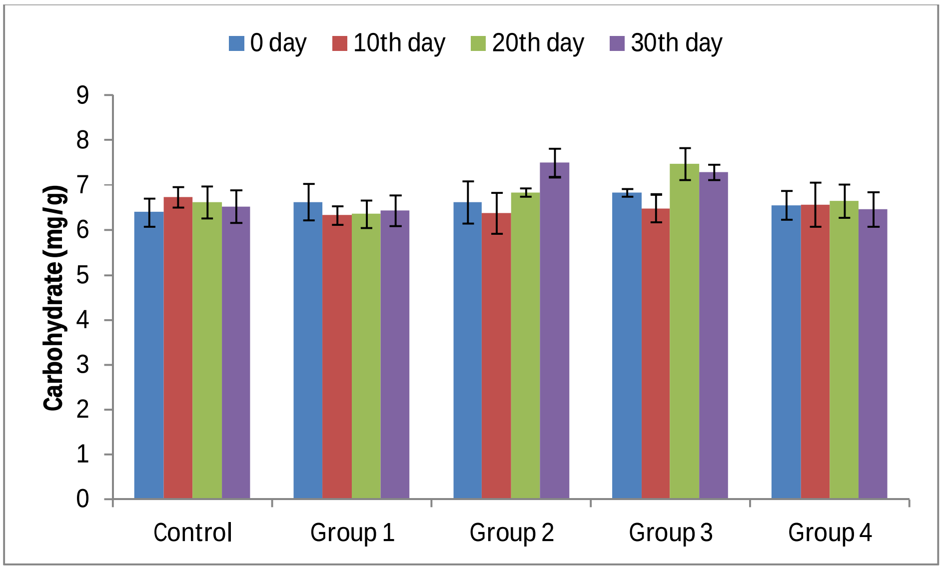
(Fig 4). Protein content of liver



(Fig 5). Lipid content of liver



(Fig 6). Carbohydrate content of liver



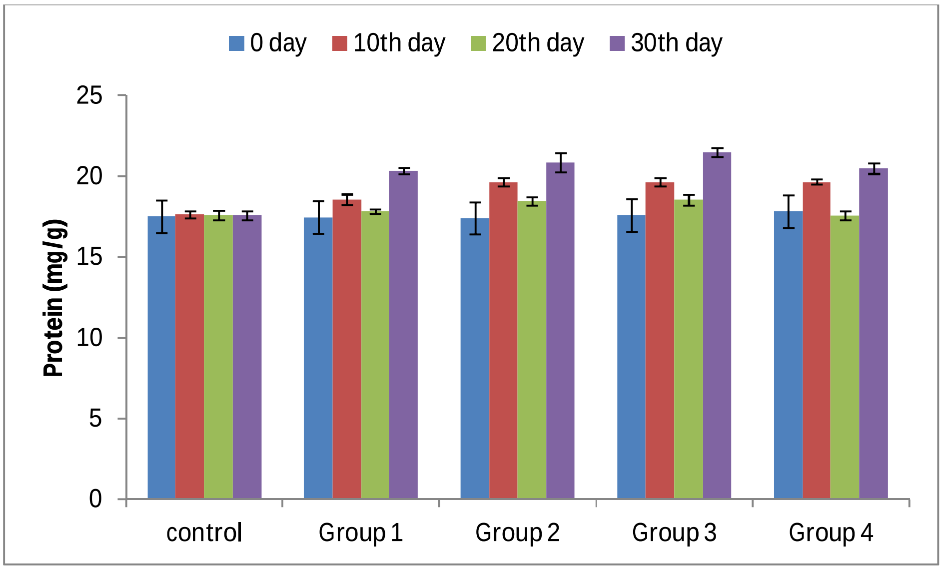
Muscle

Protein content was high in muscle of *labeo rohita* fingerlings fed with Group 3 probiotic (21.47 ± 0.26mg/g) control supplemented groups. Among the experimental groups, Group 3 diets increased the protein content of the muscle. The protein content of muscle varied significantly in fingerlings fed with different experimental diets compared to the control.

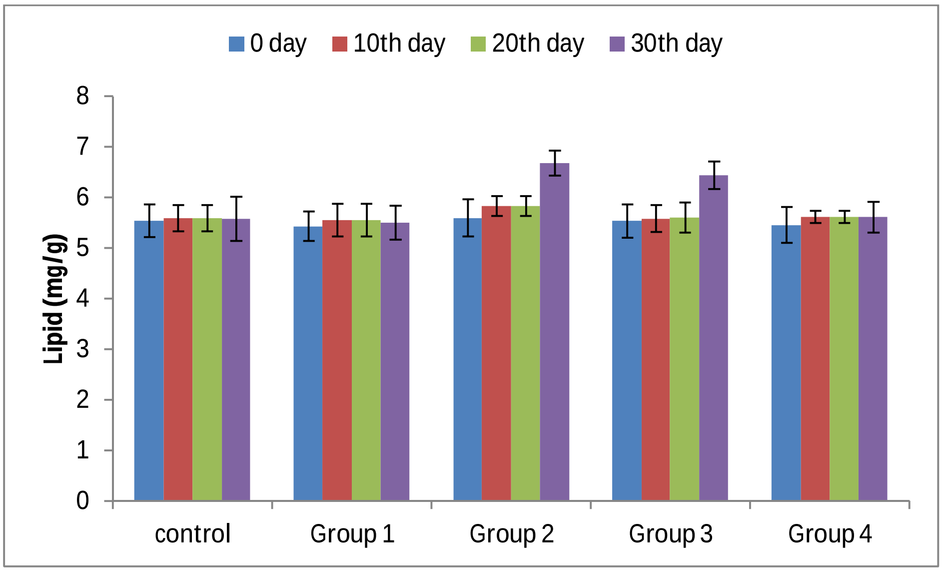
Lipid content was also high in muscle of fingerlings fed with Group 3 probiotic (6.44±0.27 mg/g) and low in on 30th day. Among the groups, Group 3 diets increased the lipid content of the muscle were significant in different experimental groups compared to the control .

Carbohydrate content of muscle was high in fingerlings fed with Group 3 probiotic (5.79 ± 0.18 mg/g) and control supplemented diets on 30th day. Among the experimental groups, Group 3 diets enhanced the carbohydrate of the muscle. Revealed that variations in the carbohydrate content of muscle were significant in the experimental groups compared to the control.

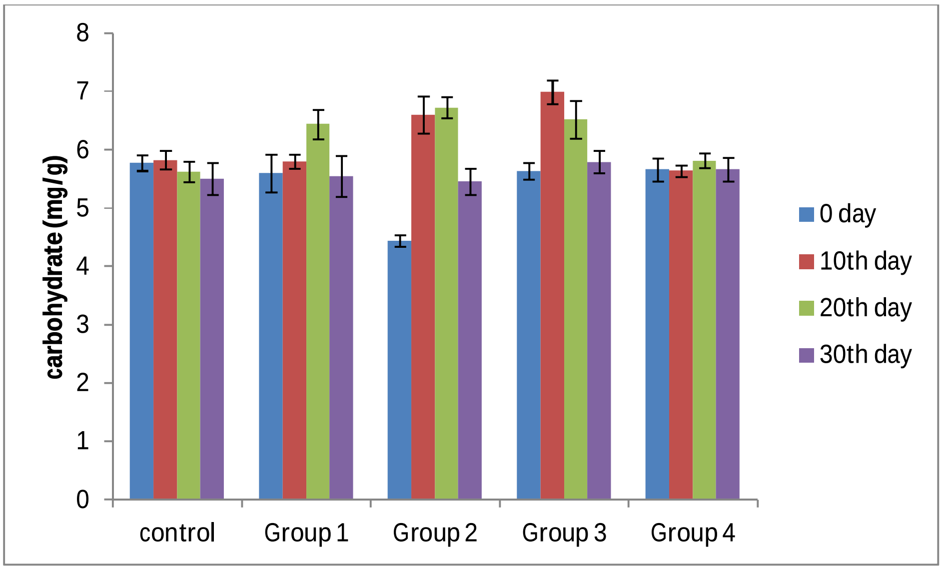
(Fig 7.) protein content of muscle



(Fig 8.) lipid content of muscle



(Fig 9.) carbohydrate content of muscle



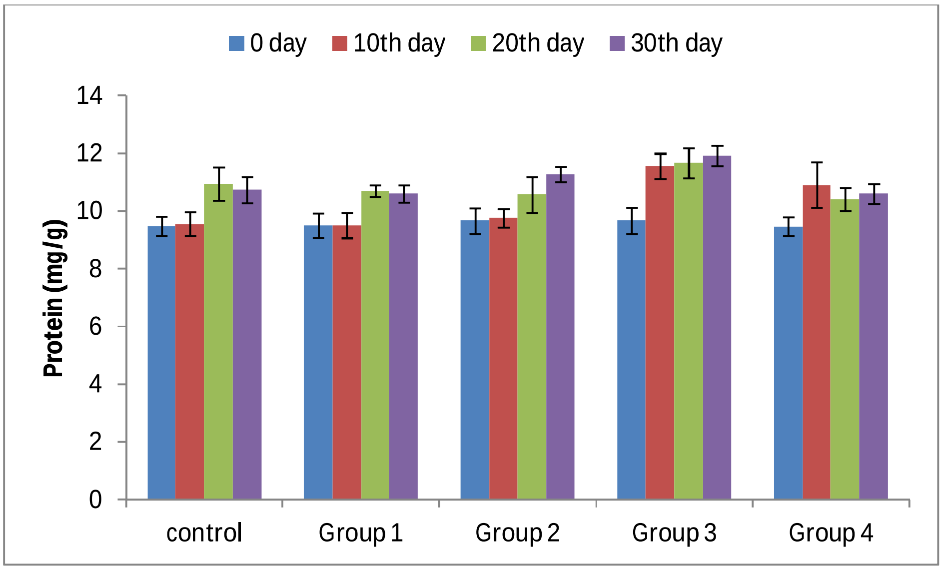
Kidney

Protein content of kidney in fingerlings fed with experimental diets varied among the groups studied. Protein content was high in fingerlings fed with Group 3 (11.92±0.35 mg/g) supplemented diets on 30th day. Among the experimental groups, Group 3 diets increased the protein content of the kidney.

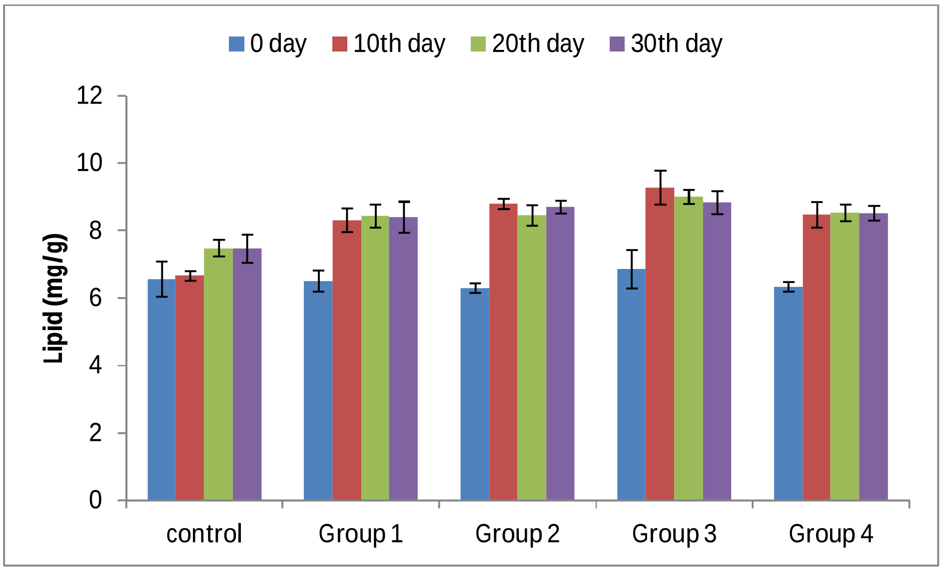
Lipid content of kidney in fingerlings fed with experimental diets showed significant changes among the groups studied. Lipid content was high in fingerlings fed with Group 3 (8.84±0.34 mg/g) on 30th day. Among the experimental groups, Group 3 diets enhanced the protein content of the kidney.

Carbohydrate content was maximum in Group 3 (5.93±0.15 mg/g) on the 30th day. Among the experimental groups, Group 3 diets enhanced the carbohydrate content of the kidney (Figs.).The carbohydrate content of the kidney varied significantly in the experimental groups compared to the control.

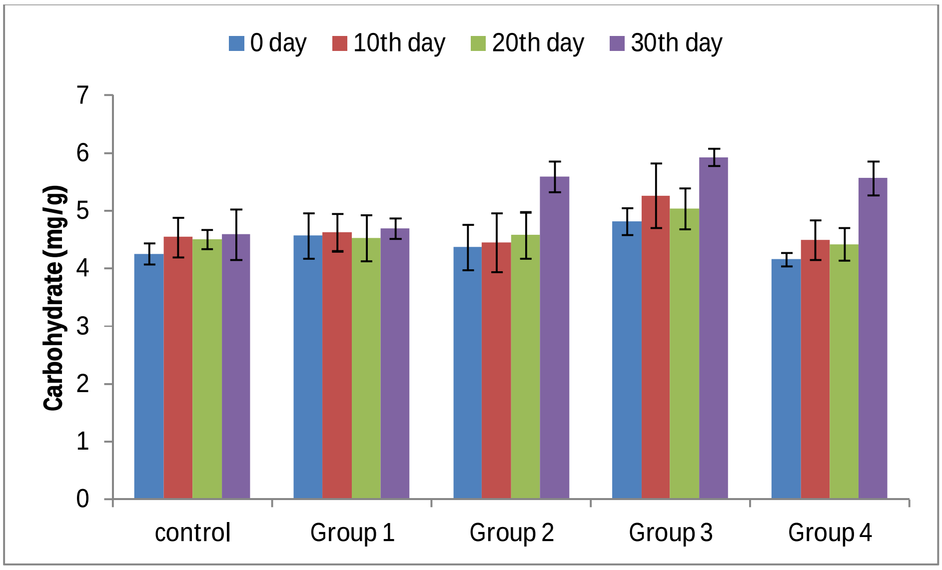
(Fig 10.) protein content of kidney



(Fig 11.) lipid content of kidney



(Fig 12.) carbohydrate content of kidney



4. DISCUSSION

Probiotic bacteria (*Lactobacillus* sp) act against *Aeromonas* virulence in *Oreochromis mossombicus* (Who et al., 1994). In the present study, efforts were to study the effect of *V. Cholarae* in the freshwater fish *L. rohita* fed with different concentration of probiotic bacteria *Lactobacillius* sp. supplemented diets. The results revealed that the length and weight of the fish fingerlings increase in diet III fed animals compared to other diets. (Fernandez, R., M, 2011) reported in an increase of 3% growth in *Labeo rohita* supplemented with 30 days. After the feeding trial experiments with probiotic supplementation, the morphometric data (final length and weight), survival and growth performance (SGR, FCE and PER) were significantly higher in supplemented diet (diet III) at the end of the experimental period of 30days.

Biochemical parameters serve as suitable indicators to assess the level of stress and iterations in metabolic cycles (Wang *et al.*, 2005). Hence, it is necessary to understand the significance of these biochemical changes in the candidate species *Labeo rohita* fingerlings, infected with *V. Choloreae*. The biochemical analysis revealed that variations in the protein, lipid carbohydrate content of gill, muscle, liver and kidney of infected fingerlings, fed with diet III, showed significant increase compared to the control and other groups. This increase may due to bacterial infecion (Acha and Szyfres, 2003). Hence it is suggested that the *Lactobacillus* sp. can be used as probiotic against *V. Choloreae* in the freshwater fish farming (especially *Labeo rohita)*

The protein content in gill, muscle, liver and kidney of *Labeo rohita* fingerlings varied in the supplemented groups compared to control. Among the different tissues studied, protein content in muscle increased in fingerlings fed with probiotic supplemented diets.

Lipids are essential organic constituents in animal tissue and play a key role in energy metabolism and their assessment serves as a tool for assessing the normal physiology (Palanivelu et al., 2005). Among the tissue studied, lipid content was maximum in liver tissue. The increase in the lipid content showed that the fish utilized energy derived from lipid metabolism to overcome the stress.

Carbohydrate is considered to be the first organic nutrient degraded in response to stress conditions imposed on animals. Carbohydrate content increased in gill, muscle, liver and kidney of *Labeo rohita* fingerlings fed with the probiotic Group 3 and supplemented diets. Probiotic supplemented diet fed fingerings was reduction in the carbohydrate content in all tissues.

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