



## The Effect of isolated Gut Probiotic Bacteria *Bacillus licheniformis* on biochemical changes in the freshwater fish *Cyprinus carpio* (L)

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### Abstract

The present study aims to find out the influences of isolated gut probiotic bacteria *Bacillus licheniformis* on biochemical changes of *C. carpio*. The fish in the control tank were fed only with supplementary fish feed without any probiotics and the fish in the Experimental tank were fed with supplementary fish feed mixed with the probiotic bacteria *Bacillus licheniformis* isolated from the gut of Indian major carp *Labeo rohita* based on biochemical test and 16s rRNA profile (GATTGGGGTGAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCG) (Seq.id.NR.118996.1). Fish in the both tanks were fed twice a day (3% body wt of fish) regularly. The experiment was carried out for 75 days and the samples were taken from control and experimental fish on 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, 60<sup>th</sup> and 75<sup>th</sup> day of the experimental period. The present result shows that significantly increased total protein and total carbohydrate content in the muscle of probiotic fed fish.

**Keywords:** *Bacillus licheniformis*, *C. carpio*, total proteins, total carbohydrates

### Introduction

Biochemical variables are reflects the physiological condition of fish (Mohapatra *et al.*, 2014). The biochemical analyses often provide vital information for health-assessment and management of cultured fish (Cnaani *et al.*, 2004; Rehulka *et al.*, 2004). The definition of a probiotic varies significantly relying upon the source however the first by acknowledged definition was proposed by Fuller (1989) as a live

microbial feed supplement beneficially affects the host animal by improving its microbial balance. The *Bacillus sp.* as putative probiotics that can be resistant to high temperature and pressure have been used extensively as fish culture feed additives. Probiotics are considered as organisms to boost the nutritive value of an animal feed. (El-Haroun *et al.*, 2006).

Fish species need a level of crude protein ranging between 25 to 55% (NRC 1993). There are two types of amino acids, they are essential and non-essential amino acids that are classified according to the body's ability to synthesize and to meet the metabolic necessities. The amino acid is one of the most important sources and the major substrates in the early formative phases for the synthesis of a large number of bioactive proteins (Finn and Fyhn, 2010). The microflora of fish from aquatic species has been studied as a source of protection against diseases (Sissons, 1989). The level of blood glucose and total protein are easily modified under the influence of external or internal factors (Elena *et al.*, 2015). However, studies have been carried out based on growth performance and digestive enzyme activities to incorporate probiotic effects on marine cultured fish *Sparus aurata* (Salinas *et al.*, 2005, 2006; Diaz-Rosales *et al.*, 2006; Suzer *et al.*, 2008; Avella *et al.*, 2010) freshwater carp species *Cyprinus carpio* (Wang and Xu, 2006) and *Ctenopharyngodon idella* (Wu *et al.*, 2012) and shrimp *Penaeus vannamei* (Wang, 2007; Zhou *et al.*, 2009) and limited on biochemical changes. Hence, the present study aims to find out the influences of isolated gut probiotic bacteria *Bacillus licheniformis* on biochemical changes in the freshwater fish *Cyprinus carpio* (L.).

## Materials and Methods

The healthy fresh water fish *Cyprinus carpio* (L) with (1.6 gm weight and 2.5cm length) were purchased from Sirago fish farm, Nerinjipet, Mettur, Tamil Nadu, India and acclimatized in nursery tank for a month and the fish were fed well with supplementary fish feed *ad libitum*. The probiotics strain *Bacillus licheniformis* were isolated identified based on biochemical test from the gut of Indian major carp *Labeo rohita* (Holt *et al.*, 1994). Selected strain of probiotics bacteria *Bacillus licheniformis* was mass cultured and the concentration of colony forming units were determined Supplementary feed Hipro (Aptimum Company, Thailand) contains ingredient such as crude protein (38 %), crude fat (4 %), crude fiber (3%), moisture content (12%) and crude ash content (12%). The proximate composition of organic feed ingredients were analyzed by AQAC method, (1995). Feed pellets were warmed to 60°C and blended with the molten agar containing fresh bacterial cells. The mixture was stirred well with sterile glass rods to have a uniform coating of the bacteria over the feed pellets (*B. licheniformis* live cells  $3.0 \times 10^8$  CFUg<sup>-1</sup>).

## Experimental design

The healthy *C. carpio* was divided into two groups introduced 100 fish into each control tank and experimental tank. The fish in the control tank was fed with Supplementary fish feed without probiotics and the fish in the experimental tank was fed with supplementary fish feed along with isolated gut probiotic bacteria *Bacillus licheniformis*. Feed was given as per body weight of fish (3% body wt of fish per day) twice a day that is early morning (5.30 am-6 a.m) and evening (5.30 pm-6 p.m) regularly. The experiment was carried out for seventy five days and total protein and total carbohydrates were analyzed from control group and experimental group on 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, 60<sup>th</sup> and 75<sup>th</sup> day of the experimental period. Biochemical parameter such as total muscle carbohydrate, total muscle protein were estimated by using standard method and the total muscle protein was estimated by the method of Lowry *et al.* (1951) and the total muscle carbohydrate was estimated by the method of Roe *et al.* (1955).

## Statistical analysis

The results are presented as Mean + SD, differences were analyzed by One way analysis of variance (ANOVA) and statistical analysis was carried out by using SPSS software (16 versions).

## Results and Discussion

The biochemical analyses often provide vital information for health-assessment and management of cultured fish (Cnaani *et al.*, 2004; Rehulka *et al.*, 2004). Probiotics make up a part of the resident microflora by adhering to the mucus, gastrointestinal tract, epithelial cells and other tissues, further contributing to the health or well-being of the host (Gatesoupe, 1999). The total protein content in the muscle of fish fed with supplementary feed without probiotic bacteria was recorded as  $7.07 \pm 0.06$  gm/dl on 15<sup>th</sup> day,  $7.10 \pm 0.10$  gm/dl on 30<sup>th</sup> day,  $7.17 \pm 06$  gm/dl on 45<sup>th</sup> day,  $7.42 \pm 09$  gm/dl on 60<sup>th</sup> day and  $7.57 \pm 06$  gm/dl on 75<sup>th</sup> day of the experimental period. The total protein content was significantly increased in the fish fed with isolated gut probiotic bacteria *B. licheniformis* as  $7.26 \pm 0.04$  gm/dl on 15<sup>th</sup> day,  $7.76 \pm 0.05$  gm/dl on 30<sup>th</sup> day,  $7.80 \pm 0.10$  gm/dl on 45<sup>th</sup> day,  $8.11 \pm 0.17$  gm/dl on 60<sup>th</sup> day and  $8.34 \pm 0.17$  gm/dl on 75<sup>th</sup> day of the experimental period. Significantly (P<0.05) increased total protein content was recorded in the muscle of the fish fed with isolated gut probiotic bacteria than

the fish fed with supplementary feed alone (Table 1 and Figure.1). 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 (Harikrishnan *et al.*, 2010;

Verschuere *et al.*,2000). Subramanian and Bala Subramanian (2014) reported that 1% combined diet results in significant increase in the carbohydrate, protein and lipid levels. Zhou *et al.* (2010) recorded higher total protein and globulin concentration of Tilapia blood serum in fish treated with *Lactococcus lactis* RQ 516 as water additives.

**Table.1. Changes in the Total protein content of the *C. carpio* fed with isolated gut probiotic bacteria *B. licheniformis*.**

Experimental period (Days)	Total proteins (gm/dl)	
	Control tank	Experimental tank
15	7.07±0.06	7.26±0.04
30	7.10±0.10	7.76±0.05
45	7.17±06	7.80±0.10
60	7.42±09	8.11±0.17
75	7.57±06	8.34±0.05

Values are in Mean±SD, Significant level at (P<0.05)

**Figure.1. Total protein content of the *C. carpio* fed with isolated gut probiotic bacteria *B. licheniformis*.**

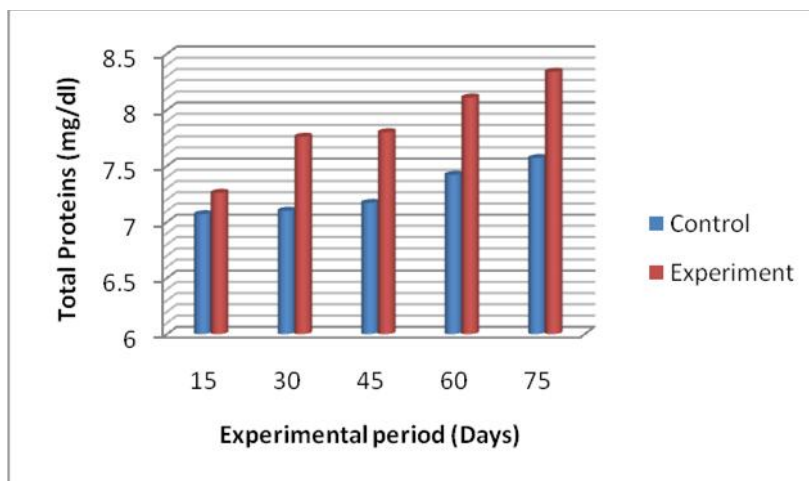


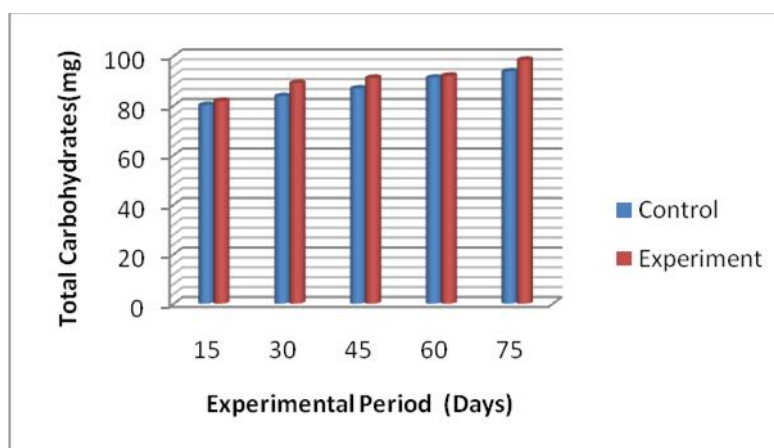
Table.2 and Figure.2 depicts the total carbohydrate in the muscle of fish fed with supplementary feed without probiotic bacteria and fish fed with isolated probiotic bacteria along with the supplementary feed. The total carbohydrate content was analyzed as 80.15±0.14 mg on 15<sup>th</sup> day, 83.74±0.64 mg on 30<sup>th</sup> day, 86.84±0.26 mg on 45<sup>th</sup> day, 91.22 ± 0.02 mg on 60<sup>th</sup> day and 93.75±0.64 mg on 75<sup>th</sup> day of the experimental period in the muscle of control

*C. carpio*, The total carbohydrate content was significantly increased in the muscle of the fish fed with isolated gut probiotic bacteria as 81.87 ± 0.08 mg on 15<sup>th</sup> day, 89.08 ± 0.28 mg 30<sup>th</sup> day, 91.13 ± 0.10 mg on 45<sup>th</sup> day, 92.02 ± 0.02 mg on 60<sup>th</sup> day and on 98.41 ± 0.17 mg on 75<sup>th</sup> day of the experimental period.

**Table.2. Changes in the total Carbohydrate content of the *C. carpio* fed with isolated gut probiotic bacteria *B. licheniformis*.**

Experimental period (Days)	Carbohydrates(mg)	
	Control tank	Experimental tank
15	80.15±0.14	81.87±0.08
30	83.74±0.64	89.08±0.28
45	86.84±0.26	91.13±0.10
60	91.22±0.02	92.02±0.02
75	93.75±0.64	98.41±0.17

Values are in Mean±SD, Significant level at (P<0.05)

**Figure.2.Total Carbohydrate content of the *C. carpio* fed with isolated gut probiotic bacteria *B. licheniformis*.**

Significantly (P<0.05) increased total carbohydrate was found to be more prominent in the muscle of the fish fed with isolated gut probiotic bacteria *B. licheniformis* than the control fish. Biogen® dietary supplementation was also reported to improve carcass protein deposition in Nile tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) respectively (EL-Haroun *et al.*, 2006 and EL-Haroun, 2007). Dietary supplementation with yeast extract powder also reported improved carcass protein content in rohu (*Labeo rohita*) fingerlings (Ghosh *et al.*, 2005). The higher carcass protein content can be attributed to the colonization of probiotics in the gut that produces protease enzymes for hydroxylation of complex protein molecules, facilitating their better digestion and absorption resulting ultimately in higher protein retention in body. In the present study, increased biochemical parameters such as total carbohydrate, total protein in the muscle of experimental fish may be due to the alterations in the synthesis and deposition rate of biochemical compounds in the muscle of fish (Abdel- Tawwab *et al.* (2006). The present results agreed with results of Subramanian and Bala

Subramanian (2014). From the present study, it is concluded that isolated gut probiotic bacteria *B. licheniformis* increased the muscle biochemical materials.

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