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THE INFLUENCES OF ISOLATED GUT PROBIOTIC *BACILLUS LICHENIFORMIS* ON THE GROWTH PERFORMANCE OF *CYPRINUS CARPIO* (L).

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Abstract: **The present study aims to find out the influences of isolated gut probiotics on growth performance of the fresh water fish *Cyprinus carpio* (L.). The fish in the control tank were fed only with supplementary fish feed without probiotic bacteria and the fish in the experimental tank were fed supplementary fish feed along with the isolated gut probiotic bacteria *Bacillus licheniformis*. The experiment was carried out for 75 days and the weight and length was measured on 15th, 30th, 45th, 60th and 75th day of the experimental period. Significantly increased weight and length were recorded in the probiotic fed fish than the control fish.**

***Keywords: C.carpio, B.licheniformis*, Growth, gut probiotics.**

1. INTRODUCTION

Nowadays, World aquaculture has grown very fast way in last 50 years [1]. Aquaculture has turned into a huge financial movement in some nations. Aquaculture is an important food producing sector for ever increasing global human population and developed due to intensified fish culture methods [2]. Probiotics are live microorganisms that confer a beneficial effect on the host when administered in proper amounts .The probiotics have been suggested as way to step into a more environment friendly by reducing the use of chemicals and antibiotics [3,4]. Probiotics are harmless bacteria that help the cell being of host animal and contribute directly or indirectly to protect the host animal against harmful bacteria pathogen [5]. Probiotic bacteria used as dietary additives seem to offer an attractive choice including overall health benefits to the host organism[6].The impact of probiotics on *Cyprinus carpio* and other aquatic organisms based on growth development and feed usage [7,8]. The study on the effect of isolated gut probiotics *Bacillus licheniformis* on improving water quality and growth performance is very rare. Hence, the present study aims to find out the effect of isolated gut probiotics on the growth performance of the fresh water fish *Cyprinus carpio* (L.).

2. MATERIALS AND METHODS

The healthy fresh water fish *C. 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. The fish were fed well with supplementary diet *ad libitum*.The proximate composition of organic feed ingredient was observed by the method of AQAC [9]. Selected strain of probiotics (*Bacillus licheniformis*) was mass cultured and the concentration of colony forming units were determined. Supplementary feed (Hipro, Aptimum Company, Thailand) contains crude protein (38%), crude fat (4 %), crude fiber (3%), moisture content (12%) and crude ash content (12%).Supplementary feed were blended

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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 (3.0×10^8 CFU g^{-1}).

1. Experimental Design

The healthy *C.carpio* (1.6 g weight and 2.5 cm length) was divided into two groups namely control group and experimental group. The fish in the control tank were fed with supplementary fish feed without any probiotics and the fish in the experimental tank were fed with supplementary fish feed blended with isoloated gut probiotic bacteria *Bacillus licheniformis*. Feed were given twice a day that is early morning (6.00 am-7a.m) and evening (5.00 pm- 6.00 p.m) regularly (3% body wt of fish per day). The experiment was carried out for 75 days and the weight of the freshwater fish *Cyprinus carpio* from control and experimental tank were measured on 15th, 30th, 45th, 60th and 75th day of the experimental period by using electronic balance [10]. Length of fish was measured by using graduated steel scale from the snout tip of the fish to the posterior point of caudal fin**.** The growth parameters were calculated according to the methods of Brown [11] and [12].

Weight Gain (WG) = Mean final body weight (g) – Mean initial body weight (g)

$$\text{Weight Gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \text{ X } 100$$

Length (cm) =Final length(cm)-initial length(cm)

$$\text{Length gain \%} = \frac{\text{Final length} - \text{Initial length}}{\text{Initial length}} \text{ X } 100$$

2. 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).

3. RESULTS

The experiment was conducted in two cement tanks (4 ft, 3 ft) for the period of 75 days. The growth performance such as weight and length was observed on 15th, 30th, 45th, 60th and 75th day of the experimental period, calculated weight gain and length gain and results were presented in figures. Significantly ($P<0.05$) increased weight was observed in the fish fed with isolated gut probiotic as 2.13 ± 0.05g on15thday, 3.43±0.05g on 30th day, 4.23±0.05g on 45th day, 6.83±0.05g on 60th day and 8.5±0.1g at the end of the experimental period than the control fish as 1.63±0.05g on15th day, 2.43±0.05g on 30th day, 3.13±0.05g on 45th day, 4.43±0.05g on 60th day and 5.13±0.05g at the end of the experimental period. Increased weight was recorded in both control fish and probiotic fed fish, but weight increase was found to be more prominent (8.5 ± 0.1g) in the fish fed with isolated probiotic bacteria on 75th day of the experimental period than the control group (5.13±0.05g) (Tab 1 & Fig.1).

Table 1: Weight of the *C.carpio* fed with isolated gut probiotics *Bacillus licheniformis* after different experimental period.

Experimental Period (Days)	**Weight(cm)**	
	Control Tank	**Experimental Tank**
15	1.63±0.05	2.13±0.05
30	2.43±0.05	3.43±0.05
45	3.13±0.05	4.23±0.05
60	4.43±0.05	6.83±0.05
75	5.13±0.05	8.5±0.1

Each value is expressed as Mean ± SD

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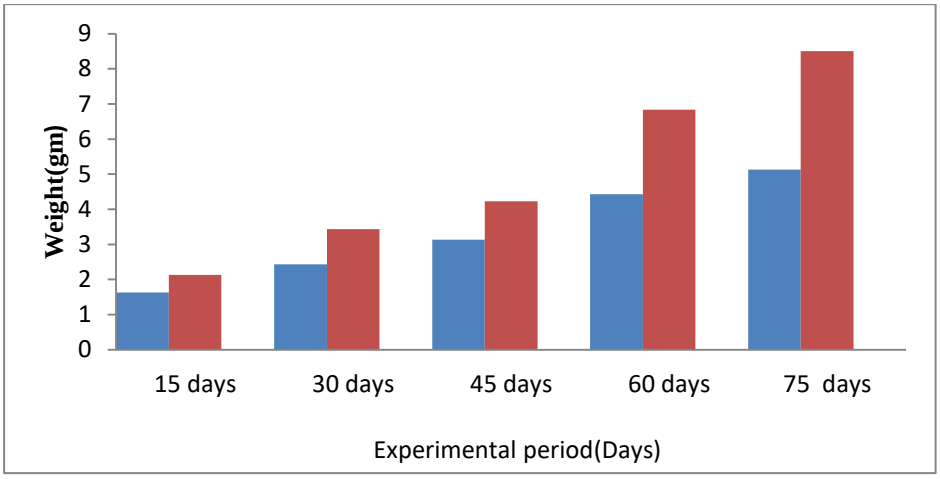
Fig 1: Weight of the *C.carpio* fed with isolated gut probiotics *Bacillus licheniformis* after different experimental period.



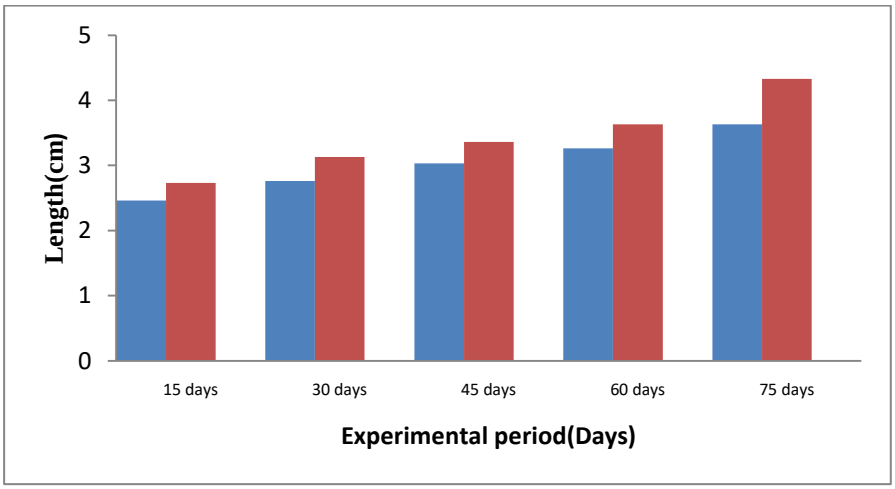
Increased length of the fish was recorded in the both control and experimental fish (Table 2 & Fig.2). The length of the control fish was recorded as 2.46±0.05cm on 15th day, 2.76±0.05cm on 30th day, 3.03±0.05cm on 45th day, 3.26±0.05cm on 60th day and 3.63±0.05 cm on 75th day. The length of the probiotic fish was observed as 2.73±0.05cm, 3.13±0.05cm, 3.36±0.05cm, and 3.63±0.05cm and 4.33±0.05 cm on 15th, 30th, 45th, 60th and 75th day of the experimental period. Significantly increased length was recorded in probiotic fed fish than the control fish but length increase was found to be more prominent in the fish fed with isolated probiotic bacteria than the control fish on 75th day of the experimental period (Fig.2).

Table 2: Length of the *C.carpio* fed with isolated gut probiotics *Bacillus licheniformis* after different experimental period.

Experimental Period (Days)	Length(cm)	
	Control Tank	Experimental Tank
15	2.46±0.05	2.73±0.05
30	2.76±0.05	3.13±0.05
45	3.03±0.05	3.36±0.05
60	3.26±0.05	3.63±0.05
75	3.63±0.05	4.33±0.05

Each value is expressed as Mean ± SD

Fig 2: Length of the *C.carpio* fed with isolated gut probiotics *Bacillus licheniformis* after different experimental period.



4. DISCUSSION

Probiotics are the live microbial feed supplement which helps to enhance the growth of aquatic animals by improving the host intestinal microbial balance [13]. In aquaculture, growth rate is an important factor because it reflects the production yield. The application of probiotics in diets for an aquatic animal is suggested as a preventive measure of diseases [14] and increases the growth rate (length and weight) and may increase of immune response to allow better survival [15]. Microorganisms present in the intestinal tract are generally seemed to be those from the environment or the diet [16]. Weight gain is an important parameter to evaluate types of diet and protein value in it and probiotics have been found to improve growth, protein efficiency ratio and FCR in *Labeo rohita* [17]. The higher growth efficiency reported in Koi carp was higher in feed containing 1ml of *Lactobacillus* spp [18]. Probiotics may stimulate appetite and improve nutrition by the production of vitamins detoxification of compounds in the diet and by break down of indigestible components [19].

In the present study, weight and length was significantly increased in the probiotic fed fish group than the control group. This weight gain was more prominent in fish fed with isolated gut probiotic *Bacillus licheniformis* at the end of the experimental period. Increased weight and length of the fish may be due to the ability of probiotic bacteria to adhere to the intestinal mucosa of *Cyprinus carpio* producing wide range of relevant digestive enzymes, which have the ability to denature the indigestible components in the diets, the ability to detoxify the potentially harmful components of feed and the ability to produce a lot of essential vitamin B.complexs [20], which results in high food utilization and an increase in digestibility of diet which may result in better growth of the experimental animal. Similar increase in the weight gain and SGR were reported in *Macrobrachium rosenbergii* fed with diet containing *B. licheniformis* [21]. The present study was agreement with the findings of [22] who reported significant increase in weight gain in *Labeo rohita* fed diet supplemented with *Bacillus sp*.

5. CONCLUSION

Isolated gut probiotic bacteria *Bacillus licheniformis* produces more digestive enzymes and essential vitamins for high food utilization and digestibility which results in better growth in the experimental fish. Hence, it is concluded that *B. licheniformis* increased the growth performance of *Cyprinus carpio*.

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Conflicts of interest:

The authors declare that there is no conflict of interest regarding the publication of this research article.

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