



## Effect of feed supplemented exogenous bacteria, *Lactobacillus sporogenes* on the growth and body composition of *Cirrhinus mrigala* fingerlings

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**Abstract:** Probiotics are known to improve the growth rate of aquatic organisms. Little experimentation has focused on supplementation of live colony forming units of *L.sporogenes* in *Cirrhinus mrigala* to access the growth performance. A feeding trial was conducted for 50 days to study the effects of *Lactobacillus sporogenes* on growth and body composition of *Cirrhinus mrigala* (Hamilton-Buchnan, 1822) fingerlings. Fishes were fed with five experimental feeds containing  $3.31 \times 10^5$  colony forming units (CFU)/100g<sup>-1</sup> of *L. sporogenes* in diet D<sub>2</sub>,  $1.65 \times 10^6$  CFU/100g<sup>-1</sup> in diet D<sub>3</sub>,  $3.31 \times 10^6$  CFU/100g<sup>-1</sup> in diet D<sub>4</sub> and  $4.96 \times 10^6$  CFU/100g<sup>-1</sup> in diet D<sub>5</sub>. Control diet D<sub>1</sub> was kept devoid of *L. sporogenes*. The diets prepared were containing 0.0% (D<sub>1</sub>), 0.1% (D<sub>2</sub>), 0.5% (D<sub>3</sub>), 1.0% (D<sub>4</sub>) and 1.5% (D<sub>5</sub>) of live cells of *L. sporogenes* CFU/100gm<sup>-1</sup> of feed. Increasing trend of weight gain, specific growth rate (SGR), protein efficiency ratio (PER) improved food conversion ratio (FCR) was observed in treatments fed with probiotic added feeds than control feed. Best growth performance was found in the treatment fed with D<sub>4</sub> of *L. sporogenes* containing  $3.31 \times 10^6$  CFU 100g<sup>-1</sup> of feed. At increased level  $4.96 \times 10^6$  CFU 100g<sup>-1</sup> of *L. sporogenes* inclusion the growth performance was dropped. Tissue protein content was also observed to be higher in animals fed with D<sub>4</sub> feed. The lipid content was significantly ( $P < 0.05$ ) higher in fingerlings fed with control feed (D<sub>1</sub>). In short, *L. sporogenes* addition to a level of  $3.31 \times 10^6$  CFU 100g<sup>-1</sup> in feed improved growth performance of *C. mrigala* fingerlings. Therefore, it is evident that  $3.31 \times 10^6$  CFU 100g<sup>-1</sup> of feed of *L. sporogenes* can be considered as suitable concentration for attaining good growth in *C. mrigala* fingerlings.

**Keywords:** Bacteria, *C. mrigala*, Growth performance, *L. sporogenes*, Probiotics

### INTRODUCTION

Aquaculture is currently the fastest growing animal production sector in the world, expanding at an average annual rate of 8-11% since 1984 (Pike and Barlow, 2002; FAO, 2006). In fish farming, nutrition is critical because feed represents 60% - 71% of the production costs (De Silva, 1985), thus fish nutrition investigations are mainly directed towards reducing feed cost by manipulating the feed formulation. To reduce the cost and to improve the performance a number of feed additives inclusive of micro-organisms have been tested in aquaculture, some of the most utilized growth promoting additives includes hormones, antibiotics, ionophores and some salt (Klaenhammer and Kullen, 1999). During last decade, use of chemical drugs especially antibiotics have become limited due to development of resistant strains of certain pathogens (Lara-Flores *et al.*, 2003; Nihan *et al.*, 2013). Presence of residues of certain antibiotics in aquaculture products may also cause intestinal disorders and problems to human health (WHO, 2006). Probiotics have been identified as live microorganisms colonized in the

intestine and/or animal originated microbial supplements could effected fish health by improving the intestinal microbial balance (Wang and Wang, 2008; Dimitroglou *et al.*, 2011). As an alternative to growth promoting antibiotics probiotics have been used in feed supplementations. Probiotics improves the growth rate in aquatic animals without causing any residual effect to human health unlike antibiotics. *Lactobacillus* species are one among the most commonly used microbes for nutritional and enzymatic contribution to the digestion process in animals (Suzer *et al.*, 2008; Ganguly *et al.*, 2010). Gatesoupe (1999) mentioned that indigenous beneficial bacterial strains isolation and multiplication for further use in aquaculture is desirable. However, isolation of indigenous species -specific microorganism from fish and its subsequent multiplication to use under farm conditions is difficult (Prasad *et al.*, 2013). Little experimentation has focused directly on supplementation of non indigenous probiotic microbes in freshwater fishes. In present study 50 days feeding trial was conducted to evaluate the effect of exogenous *L. sporogenes* live colony forming units of on growth and body composition of

*C. mrigala* fingerlings. It was observed that supplementation of *L. sporogenes* improves the growth performance of *C. mrigala* fingerlings and the counts of colony forming units in feed decreased with the increasing feed storage duration. Since probiotics are known to effectively improve growth performance of host organisms, in the present work an attempt has been made to identify an optimal concentration of *L. sporogenes* for attaining good growth and their concentration of biochemical constituents in *C. mrigala* fingerlings through prepared feed.

## MATERIALS AND METHODS

### Experimental rearing of *C. mrigala* fingerlings:

Induced bred *C. mrigala* fingerlings were obtained from Central Institute of Freshwater Aquaculture (CIFA), Orissa, India. The fingerlings were acclimatized for twelve days. During acclimatization period they were fed with control diet and supplied with round the clock aeration from a compressed air pump. After acclimatization *C. mrigala* fingerlings (average weight  $2.54 \pm 0.038$ g) were introduced into fifteen uniform size rectangular containers.

Fifteen fishes of uniform size were kept in each treatment container following a completely randomized design (CRD). Fishes were fed with five experimental feeds containing  $3.31 \times 10^5$  colony forming units (CFU)/ $100\text{g}^{-1}$  of *L. sporogenes* in diet D<sub>2</sub>,  $1.65 \times 10^6$  CFU/ $100\text{g}^{-1}$  in diet D<sub>3</sub>,  $3.31 \times 10^6$  CFU/ $100\text{g}^{-1}$  in diet D<sub>4</sub> and  $4.96 \times 10^6$  CFU/ $100\text{g}^{-1}$  in diet D<sub>5</sub>. Control diet D<sub>1</sub> was kept devoid of *L. sporogenes*. Three replicates were used for each feeding scheme. The total volume of water in each container was maintained at 30 liters throughout the experimental period. Aeration was continuously provided to all containers. The containers were cleaned daily and 25% of water was replaced on alternate days with mature bore well water. Water temperature, pH, dissolved oxygen, free carbon-dioxide, carbonate hardness were recorded once a week following methods suggested by APHA (1995).

**Preparation of feed:** The compositions of experimental diets are given in table 1. Feed ingredients were finely powdered and thoroughly mixed with water to make dough. The dough was then steam cooked for twenty minutes in autoclave. The vitamin and mineral premix was added homogeneously after cooling the dough. The bacterial cultures harvested, washed were also added to the dough before pelleting. The bacterial culture supplement was prepared in the following way; One gram of lyophilized *L. sporogenes* was suspended in a liter of De man Rogosa and Sharpe (MRS) broth (Hi-Media, Mumbai, India) and kept for incubation. After 24 hours, the culture was centrifuged at 5000 rpm in a refrigerated centrifuge (Remi, Mumbai, India) to obtain a pellet. The pellet was then washed twice with normal saline (0.85%) and weighed. Required portions of the pellets were added to the feeds at different levels of bacteria. A portion (0.1g) of the feed pellet

was then suspended in 10ml of sterile normal saline (0.85%). To find out the number of colony forming units of *L. sporogenes* present per gram of feed, 0.1ml of this serially diluted sample were pelleted on MRS agar following spread plate technique. The number of live bacteria added to each category of feed was calculated from the enumerated sample as per gram of feed. The required amounts by weight of *L. sporogenes* were mixed in four different diets replacing the equal amount of rice bran. The dough prepared was pressed in a hand pelletizer to get feed of 1.5 mm diameter. The feeds were dried in a hot air oven at 45°C overnight and were stored at 4°C in air tight containers until used. The pellets were crumbled before being dispensed. The curdling test was done on every 15<sup>th</sup> days by addition of *L. sporogenes* containing feeds in sterilized milk as suggested by Hugenholtz (1993).

**Biochemical analysis:** Proximate compositions of the fingerlings of *C. mrigala* (Caracass) and the feeds were analyzed for their proximate composition following standard methods (AOAC, 1995) in Nutrition and Biochemistry Laboratory of CIFA. Feed ingredients, experimental diets samples and fish carcass were analyzed for proximate composition according to AOAC (1995) procedures as follows: Moisture was determined by oven drying at 105°C for 24 hours; crude protein (Nitrogen  $\times 6.25$ ) by micro Kjeldahl digestion and distillation after acid digestion; lipid was determined by extracting the residue with 40-60°C petroleum ether as a solvent for 7-8 hours in a Soxhlet apparatus; and ash was determined by ignition at 550°C in a Muffle furnace to constant weight. Total carbohydrate was calculated by subtracting the value of protein, fat, moisture and ash from the total weight and expressed in percentage as described by Halver (1976). The values were expressed as percentage of the sample analyzed. The proximate analysis of the carcasses was done before the initiation and after termination of the experiment.

**Screening of native bacteria for presence of lactic acid bacteria:** To establish whether the *L. sporogenes* bacteria are autochthonous micro-flora of *C. mrigala*, intestine samples were weighed and homogenized in sterile saline and plated both on MRS agar and on nonselective agar (Soybean casein digest agar). The representative colonies were biochemically analyzed to find if any isolate resembled with the lactic acid bacteria.

**Growth study:** Fish performance in terms of weight gain (%), SGR, FCR, protein efficiency ratio (PER) and apparent net protein utilization (ANPU, %) was calculated using the following formulae:

Weight gain (%) = [(Final body weight – Initial body weight)]  $\times 100$ ; SGR = [(ln Wt - ln Wi) / T]  $\times 100$  ; where Wt is the final weight of fish at the time of termination of experiment, Wi is initial weight of fish at the time of start of experiment, and T is the culture period in days; FCR = Feed consumed/Weight gain,

PER = Weight gain/Protein intake; ANPU % = (Net increase in

carcass protein / Protein consumed) × 100.

**Statistical analysis:** The data was statistically analyzed using statistical software (SPSS version 11.0). One way analysis of variance (ANOVA) and Duncan's multiple range test (Duncan, 1955) were applied to compare means between two treatments.

## RESULTS AND DISCUSSION

**Biochemical composition of feeds:** The biochemical compositions of experimental diets are presented in table 2. Protein content of the diets was varying between 31.31.60±0.04 to 31.62±0.04 percent. Lipid

content varied between 5.63±0.03 to 5.66±0.01. The diets prepared were statistically similar to their protein, lipid and carbohydrate content.

**Viability of *L. sporogenes* in feed:** The *Lactobacillus*, *Bacillus* and yeast micro-organisms have been used as probiotics, in crustacean and fish culture (Gomezgil, 1995; Rengpipat *et al.*, 2000; Hong *et al.*, 2005; Pooramini *et al.*, 2009; Boonthai *et al.*) Uma (1995), Gabriel, *et al.*, (2002) Venkat, *et al* (2004) Seenivasan, *et al*, (2012) have also reported effectiveness of commercially available probiotics to improve growth performance of different crustaceans and fin fishes. The, degree of survivability of probiotic bacteria in the experimental feeds is an important criterion to assess their effect on growth performance of host. In the present study all experimental feeds, when inoculated in sterilized skim milk broth were able to curdled milk within 24-48 hours indicated the presence of *L. sporogenes* in feed which was also confirmed using microscopic examinations. Milk curdling test indicate all the test diet containing *L. sporogenes* were viable till 30<sup>th</sup> day. Curd formation by Lactic Acid Bacillus (LAB) results in formation of plain curd with sweet aroma and the curd formation by other bacteria is characterized by bad odour, presence of flakes and with different consistencies (Molimard and Spinnler, 1996). It was observed that the curding of skimmed milk agar occurred till 30<sup>th</sup> day, the colony forming

**Table 1.** Composition of diet ingredients (% dry weight).

Ingredients	Amount %
Ground nut oil cake	40
Wheat flour	16
Soybean oil cake	15
Rice bran	12.5
Fish meal	10
Fish oil	4.5
*Vit-mineral mix	1.0
Carboxymethylcellulose	1.0

\*Vitamins and minerals mixture added in the experimental diets.

**Table 2.** Proximate composition (mean±SD) of diet on % dry weight basis.

Proximate composition (%)	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>
Crude protein	31.62±0.04	31.61±0.02	31.6 ±0.03	31.61± 0.02	31.61 ± 0.02
Crude lipid	5.65 ± 0.50	5.66 ± 0.01	5.65 ± 0.01	5.66 ± 0.02	5.63 ± 0.03
Carbohydrate	48.25±0.31	48.37 ± 0.24	48.29±0.52	48.31±0.66	48.29 ± 0.57
Ash	14.37±0.21	14.23±0.25	14.27±0.30	14.28± 0.17	14.30 ± 0.10
Energy (kcal/100g)	371.94±0.72	371.98±0.45	371.90±0.85	371.91±0.04	371.83±0.14

**Table 3.** *L. sporogenes* counts in different diet after 15 days interval.

Days	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>
CFU/100g ±SD	0.0	3.31×10 <sup>5</sup>	1.65×10 <sup>6</sup>	3.31×10 <sup>6</sup>	4.96×10 <sup>6</sup>
15 (%survival)	0.0	67.01 ±1.21	67.67 ±3.87	67.51 ±3.41	67.56 ±3.11
30 (%survival)	0.0	46.90 ±2.18	46.77 ±3.72	46.96 ±4.40	46.75 ±1.97

**Table 4.** Proximate composition of *C. mrigala* (% dry matter basis) tissues fed with *L. sporogenes* supplemented diets and control diets.

Proximate composition(%)	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>
Moisture	76.34±1.62	77.29±0.64	77.54±1.10	77.54±0.58	77.62±0.46
CP	57.77 <sup>a</sup> ±0.03	57.78 <sup>a</sup> ±0.05	57.87 <sup>ab</sup> ±0.06	58.00 <sup>b</sup> ±0.12	57.79 <sup>a</sup> ±0.08
EE	6.60 <sup>c</sup> ±0.42	6.38 <sup>c</sup> ±0.66	5.82 <sup>b</sup> ±0.41	5.26 <sup>a</sup> ±0.14	5.17 <sup>a</sup> ±0.03
Ash	16.55±0.26	16.47±0.20	17.57±0.26	16.50±0.32	16.54±0.29
TC	19.06 <sup>a</sup> ±0.65	19.35 <sup>a</sup> ±0.18	19.80 <sup>ab</sup> ±0.14	20.36 <sup>b</sup> ±0.41	20.48 <sup>b</sup> ±0.34
OM	83.44±0.26	83.52±0.20	83.42±0.26	83.50±0.32	84.45±0.30
Energy (Kcal/100g)	366.79 <sup>c</sup> ±1.31	366.01 <sup>bc</sup> ±1.12	362.81 <sup>ab</sup> ±1.22	360.30 <sup>a</sup> ±1.22	359.11 <sup>a</sup> ±1.11

\*Values with identical superscript letters in the same row are not significantly different (p<0.05), OM - Organic matter = 100 -Ash, CP- Crude protein =(N%×6.25), EE- Ether extract, TC=Total carbohydrate

**Table 5.** Growth performance of *C. mrigala* fingerlings fed experimental diets for 50 days (mean±SD) supplemented with *L. sporogenes* supplemented diets.

Performance	D <sub>1</sub>	D <sub>2</sub>	D <sub>3</sub>	D <sub>4</sub>	D <sub>5</sub>
Initial weight (g)	2.56 ±0.04	2.55 ±0.05	2.53 ±0.01	2.52 ±0.02	2.51 ±0.04
Final weight (g)	5.57 <sup>a</sup> ±0.04	5.78 <sup>b</sup> ±0.02	5.90 <sup>c</sup> ±0.02	5.89 <sup>c</sup> ±0.01	5.59 <sup>a</sup> ±0.02
Live weight gain (%)	117.58 <sup>a</sup> ±2.09	119.50 <sup>ab</sup> ±1.39	133.34 <sup>c</sup> ±1.58	133.89 <sup>c</sup> ±3.07	122.44 <sup>b</sup> ±2.80
SGR (% per day)	1.55 <sup>a</sup> ±0.02	1.63 <sup>b</sup> ±0.04	1.69 <sup>c</sup> ±0.01	1.70 <sup>c</sup> ±0.02	1.60 <sup>ab</sup> ±0.02
FCR	2.36 <sup>b</sup> ±0.04	2.30 <sup>b</sup> ±0.03	2.12 <sup>a</sup> ±0.01	2.11 <sup>a</sup> ±0.05	2.31 <sup>b</sup> ±0.04
PER	1.33 <sup>a</sup> ±0.02	1.37 <sup>a</sup> ±0.02	1.49 <sup>b</sup> ±0.01	1.50 <sup>b</sup> ±0.03	1.37 <sup>a</sup> ±0.02
ANPU	29.84 <sup>a</sup> ±1.94	29.85 <sup>a</sup> ±1.89	34.38 <sup>ab</sup> ±2.69	40.19 <sup>b</sup> ±5.72	30.95 ±3.61
Survival (%)	100	100	100	100	100

\*Values with identical superscript letters in the same row are significantly different (p<0.05)

units were of *L. sporogenes* in the feeds reduced. On the day of preparation (0<sup>th</sup> day), the viable counts of *L. sporogenes* were 3.31×10<sup>5</sup>, 1.65×10<sup>6</sup>, 3.31×10<sup>6</sup>, 4.96×10<sup>6</sup> CFU/100g<sup>-1</sup> in D<sub>2</sub>, D<sub>3</sub>, D<sub>4</sub> and D<sub>5</sub> feeds, respectively. The D<sub>1</sub> (Control feed) did not curdle the media and when plated did not have *L. sporogenes*. The percent counts of *L. sporogenes* present after 15 and 30 days of feed preparation were are presented in table 3.

In the present study the CFU of *L. sporogenes* decreased up to 76.96 percent on 30<sup>th</sup> day of feed preparation, which shows that CFU in the feeds reduces as the storage life of feed increases. Similar to our observations Gabriel *et al.* (2002) also observed reduced counts of agglomerated *S. cerevisiae* in the fine sphere pelleted shrimp feed were slightly higher (1.6×10<sup>10</sup> CFU/g and 1.3×10<sup>10</sup> cells/ g) than the large spheres (8×10<sup>9</sup> CFU/g and 8.2×10<sup>9</sup> cells/g) pelleted shrimp feeds. Gabriel *et al.* (2002) mentioned that the level of reduction may vary according to the procedure applied to prepare the feed pellet, shear force and temperature and moisture level used for drying. Venkat *et al.* (2004) reported survival of *L. plantarum* in feed till 60 days but they have not reported the change in number of added probiotics. Similarly, Prasad *et al.* (2013) also reported reduced counts of *S. cerevisiae* by 34.44% by 30<sup>th</sup> day in pelleted feed and best growth performance were observed in *M. rosenbergii* post larvae at CFU concentration of 65.83×10<sup>3</sup> CFU/100g of feed.

**Water quality parameters:** During the experiment water quality parameters like water temperature ranged 29.0-31<sup>o</sup>C, dissolved oxygen level was 5.3.0-6.8 mg/l and total alkalinity as mg CaCO<sub>3</sub> l<sup>-1</sup> ranged between 126-128, total hardness as mg CaCO<sub>3</sub> l<sup>-1</sup> ranged from 110-116. Ammonia (NH<sub>3</sub>) level ranged 0.22-0.37 mg/l, while nitrate-N ranged from 0.19-0.27 mg/l. Nitrite-N and PO<sub>4</sub> were observed in the optimum range of 0.01-0.03 mg/l and 0.45-0.68 mg/l, respectively. There was no significant change observed in the water quality in the test or control diet rearing tanks. Similar to our observations Ventak *et al.*, (2004) also reported significant higher growth rate, feed efficiency ratio, protein efficiency ratio were recorded in *M. rosenbergii*

post larvae when bioencapsulated *L. sporogenes* were fed through *Artemia*.

**Gut screening for *L. sporogenes*:** Before start of feeding trial intestinal bacterial isolates were subjected to series of biochemical tests comprising of gram staining, catalase, indole, motility, Methyl Red Voges-Proskauer (MRVP), citrate utilization, oxidase, and glucose, mannitol, sucrose, lactose utilization were performed to find out if *L. sporogenes* are indigenous micro-flora of *C. mrigala* fingerlings. The colony characteristics and biochemical tests performed showed that *L. sporogenes* is not present in the indigenous micro-flora of *C. mrigala* fingerlings. In our study dominant genera identified were *Aeromonas* spp., *Pseudomonas* spp. and *Streptococcus* spp., *Enterobacter* spp., *Bacillus* spp. Similar kind of micro-flora in aquatic organisms have been also reported by Sivakami *et al.* (1996) in Indian major carps and Al-Harbi and Uddin *et al.* (2005) in *Oreochromis niloticus*. The higher prevalence of *A. hydrophila*, *Pseudomonas* *Streptococcus* and *Enterobacter* species in the study period suggests that these bacteria may be common bacterial species in the ponds. The bacterial flora of the water may vary with the feeding rates (Tucker, 1985) and with the variation in nutrient load (Buras *et al.* 1987) in the pond. The bacterial composition may also vary from species to species and from one environment to another environment. (Nayak,2010).

**Carcass composition and growth performance:** Biochemical compositions of the fingerlings are presented in table 4. Significantly higher (p<0.05) carcass protein was

recorded in fingerlings fed with D<sub>3</sub> and D<sub>4</sub> feed. Lowest protein was recorded in fingerlings fed with control feed D<sub>1</sub> (57.77%). Lowest lipid content was observed in *C. mrigala* fingerlings fed with D<sub>4</sub> feed (5.26%) and significantly higher (p<0.05) lipid content (6.60%) was found in treatment fed with D<sub>1</sub> control feed. Moisture content was minimum (76.34%) in treatment fed with D<sub>1</sub> feed and maximum in treatment fed with D<sub>5</sub> (77.62%) feed. No specific trend and significant difference was observed for ash and organic matter content among treatments.

Son *et al.*,(2009) also reported that significant increase

in percent weight gain (PWG) of 404.6%, and feed efficiency (FE) of 1.26 respectively of grouper *Epinephelus coioides* when fed a diet containing *L. plantarum* at the levels  $10^8$  CFU  $\text{kg}^{-1}$ . Parthasarathy and Ravi (2011) reported best growth rate, feed utilization and survival rate in *Catla catla* when fed with commercial fish feed containing  $10^7$  CFU of *L. plantarum* per gram of feed. In the present study we also observed 12.39 % higher live weight gain percent with  $D_4$  fed diets containing  $3.31 \times 10^6$  CFU/100g of feed than control feed. The better growth performance by  $D_4$  diet fed fingerlings may be because of better role played by *Lactobacillus* spp. as beneficial flora in the gastrointestinal tract among others include: production of antimicrobial substances; enhancement of the immune response and improve resistance of fish against bacterial pathogens; increase of availability of nutrients and use of some non-digestible carbohydrates (Dimitroglou *et al.*, 2011).

In the present study we observed improved growth of *C. mrigala* fingerlings as results of feeding the live colony forming units of *L. sporogenes* through prepared feed. Highest weight gain percent, SGR, PER, and ANPU and lowest FCR were obtained in fingerlings fed with  $D_4$  feed (Table 5). However, no significant difference ( $P < 0.05$ ) in weight gain percentage, SGR, PER, and ANPU and FCR were recorded between fingerlings reared with  $D_4$  and  $D_3$  feed. Treatments fed with  $D_2$  and  $D_5$  feeds also resulted in better performance than those fed with  $D_1$  (control) feed. Fingerlings reared with highest inclusion level ( $D_5$ ) of *L. sporogenes* resulted in reduced performance as compared with the other test feeds. 100 % survival was obtained in all the treatments including control. The beneficial effects of probiotics in aquatic organisms have been documented by Charlton and Bergot (1984) in common carp (*Cyprinus carpio*), Gatesoupe (1991) in turbot (*Scophthalmus*) larvae, Gomezgil (1995) in shrimp (*P. monodon*) larvae and Seenivasan *et al.* (2012) in freshwater prawn (*M. rosenbergii*). Probiotics are used aquaculture to manipulate the microbial population of the aquatic environment and to mitigate or eliminate pathogenic microorganisms, thereby leading to better growth and survival of the cultured species (Irianto and Austin, 2002).

In control group percent per day weight gain was lower than other treatment groups. Best percent live weight gain were obtained in the fingerlings reared with  $3.31 \times 10^6$  CFU/100g<sup>-1</sup> of probiotics added feed gives an impression that the probiotic effect is best achieved at this level. Which is also analyzed by drawing parallel changes with other parameters like SGR, FCR, PER and ANPU (Table 5). The PER and ANPU results indicate that supplementing feeds with probiotics significantly improves the protein utilization capacity of *C. mrigala* fingerlings. Ramakrishnan *et al.* (2008) observed improved survival, growth, feed conversion ratio, protein efficiency ratio in common

carp (*Cyprinus carpio*) fed with probiotics and spirulina. Improved protein digestibility upon addition of *L. acidophilus* have also been reported by Lara-Flores *et al.* (2003) in Nile tilapia. Similar results of improved growth and survival of *Penaeus indicus* larvae were observed when *Lactobacillus plantarum* were added in rearing medium by Uma *et al.* (1999). Venkat *et al.* (2004) also observed improved growth and feed conversion efficiency when bio-encapsulated *L. sporogenes* were fed to *M. rosenbergii* through *Artemia*. Douillet and Langdon (1994) used bacteria strain CA2 at  $1 \times 10^5$  CFU/ml to increase growth of *Crassostrea gigas* larvae. Similar observations of enhanced growth in Pacific oysters (*C. gigas*) have been reported by Douillet and Langdon (1994). Subhash and Lipton (2007) also reported that *L. acidophilus* supplemented diets showed significantly higher survival ( $78.7 \pm 8.1$  and  $85.7 \pm 2.9\%$ , respectively) than the control groups ( $60.7 \pm 1.2\%$ ) in *P. margaritifera*. Best PER and ANPU obtained at 1% probiotic inclusion level contributes to optimizing protein use for growth, a significant quality protein being the most expensive feed nutrient. Reduced weight gain, SGR, PER were observed when the inclusion level of *L. sporogenes* colony forming units was raised to 1.5%. The reduced growth at higher inclusion level of *L. sporogenes* may be because of increased catabolic effect and reduced digestion efficiency in *C. mrigala* fingerlings. Ghosh *et al.* (2004) also reported reduced live weight gain percentage in Rohu (*Labeo rohita*) fingerlings when *L. acidophilus* was fed at higher level through pelleted feed. Similar observations have been documented by Uma *et al.* (1999) in *P. indicus*. Better efficiency of protein uptake may be due to better digestion and assimilation of the nutrients in the gut by the supplemented micro-flora possibly by virtue of extra cellular enzymes that play an important role in the digestion process as observed in turbot larvae (Munilla *et al.*, 1990). No mortality was observed in any of the experimental group this may be due to the favorable range of environmental parameters in all the treatments. This also indicates that *L. sporogenes* when fed through feed do not have adverse impact on survival of *C. mrigala*. The possible reason for the overall growth response in *C. mrigala* may be because of increased digestibility due to presence of *L. sporogenes*, which ultimately lead to increased absorption as reported by Douillet and Langdon, (1993) and Uma *et al.*, (1999) in the oyster, *Crassostrea gigas* and the shrimp, *P. indicus* respectively. Improved feed utilization by probiotic supplementation have extensively been reported in aquaculture species (Suralikar and Sahu, 2001; Lara-Flores *et al.*, 2003; Ziaei-Nejad *et al.*, 2006; Shinde *et al.*, 2008). Increased growth may also be attributed due to vitamins as lactic acid bacteria are reportedly produced B-complex vitamins in the gut of

aquaculture animals (Goldin and Gorbach, 1992; Mondal *et al.*, 2003; Abraham *et al.*, 2007).

## Conclusion

The present study concluded that commercially available non-indigenous *L. sporogenes* can be used as feed additives for *C.mrigala* fingerlings. When viable culture of *L.sporogenes* are added it feed their viability must be regularly monitored as increased duration of feed storage results in reduced counts of added CFU of probiotics. *C.mrigala* fingerlings showed best growth performance in terms of increased live weight gain (133.89±34%), specific growth rate (1.70±0.02%), apparent net protein utilization (40.19±5.72%), protein efficiency ratio (1.50±0.03) and decreased food conversion ratio (2.11±0.05) at inclusion level of  $3.31 \times 10^6$  CFU/100g<sup>-1</sup>. Probiotics improve the growth rate of fish and therefore can be used as feed additives. Further studies are required to elucidate their commercial application in aquaculture.

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