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Effect of Probiotic Dietary on Growth Performances and Feed Utilization of *Cyprinus carpio* Fingerlings

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Abstract:
The present study was carried out to evaluate the influence of dietary supplementation of probiotic bacteria on the growth performance and biochemical composition of *Cyprinus carpio* fingerlings. The probiotic was isolated from the intestine of *C. carpio* and *Carassius auratus*. In the present study, *Micrococcus luteus, Micrococcus lylae, Micrococcus varians,* and *Micrococcus roseus* were used as probiotic in the pelletized feed. The experiment was conducted for 35 days to determine the effect of dietary probiotic on the growth of *C. carpio* fingerlings. They were maintained in different feeding regimes such as pelletized feed, commercial feed, commercial probiotic feed, mixed probiotic feed and plankton. The growth was assessed by morphometric measurements, percentage of weight gain, specific growth rate, and feed conversion ratio. At the end of the experiment, *C. carpio* fingerlings shows highest biomass (1.06 ± 0.02 g), percentage of weight gain (97.47 ± 0.41%) and specific growth rate (2.92 ± 0.66%) in fingerlings fed with mixed probiotic pelletized feed, while, feed conversion ratio (1.83 ± 0.10) was the lowest value when compared to other feeding regimes. Mixtures of probiotic bacteria has enhanced the growth of *C. carpio* fingerlings. From this study, suggested that mixed cultures of probiotics are effective for the rearing of *C. carpio* fingerlings for sustainable aquaculture.

Keywords: Probiotics, Gut microbiota, Aquaculture, Growth parameters, *C. carpio*.

INTRODUCTION

Aquaculture has become an important economic activity in many countries. In large scale production, aquatic animals are exposed to stressful conditions and having diseases which results in serious economic losses. Bacterial diseases are responsible for severe economic losses and high mortality in the aquaculture industries (FAO, 2020). Usage of antibiotics and chemicals has been increasing in aquaculture, which increases the selective pressure exerted on the microbial world and encourages the natural emergence of bacterial resistance(Manyi-Loh et al., 2018). Probiotics are microbiota both as a tool for nutritional management of specific gut-related diseases and as a source of new microbes for future probiotic bacteriotherapy applications (Wang et al., 2018). Probiotics are live bacteria which confers health benefits to host when administered via feed or to the rearing water (Merrifield et al., 2010). The use of probiotics in aquaculture is well adapted to compete with pathogens for nutrients and preventing the adhesion of pathogens to the gut wall (Gobi et al., 2017).

Probiotic organisms must meet resistance to the different environments of stomach, capacity of colonization in the host and production of antimicrobial substances against to pathogenic bacteria. For developing a new, safe and effective probiotics should not be harmful to the host and it should be

accepted by the host (Pringsulaka et al., 2015). The species normally used as probiotics in animal nutrition are usually non-pathogenic microflora, such as lactic-acid bacteria (*Bifidobacterium, Lactobacillus, Lactococcus, Streptococcus* and *Enterococcus*) and yeast (*Saccharomyces* sp.) (Zorriehzahra et al., 2016). Probiotics incorporated dietary supplements showed enhanced growth performance, survival and feeding effectiveness in fin and shellfishes (Huynh et al., 2017). The present study is highlighted the effect of gut microflora on feed efficacy and growth of *C. carpio* fingerlings.

MATERIALS AND METHODS

Isolation of gut associated bacteria

C. carpio and *C. auratus* were procured from Thambi fish farm, Chennai. They were washed in running tap water and aseptically eviscerated. Gut samples were washed with sterile saline to remove extraneous matter. Gut tissues were homogenised and transferred into 1% peptone broth containing 0.5% NaCl and were kept for 24 h. The enriched broth media were serially diluted and plated in nutrient agar and incubated at 31-32 ^{o}C for 2-3 days (Ghosh et al., 2014).

Characterization of isolated microorganisms

The isolated microorganisms were characterized by biochemical methods described in Bergey's manual of determinative bacteriology (Buchanan and Gibbons, 1974).

Mass culture of isolated bacteria

The *M. luteus, M. lylae, M. varians* and *M. roseus* bacteria were mass cultured in nutrient broth for 5 days. After the incubation period, the culture was centrifuged. Pellet was resuspended in the cryoprotectant solution (15% sucrose solution). The resultant cryoprotected bacterial cells were freeze dried using lyophilizer (Dharmaraj and Kandasamy, 2010). The lyophilised form of bacteria was used as probiotic in the study.

Feed preparation

The feed was prepared with ingredients shown in the table 1. The ingredients were mixed according to the formulation and water was added to obtain smooth dough. The dough thus prepared was steam cooked for 30 min and it was allowed to cool. Lyophilized form of probiotics strains *M. luteus, M. lylae, M. varians* and *M. roseus* of each 0.5 g were added to it and extruded through a pelletizer. The pellets were dried and stored in airtight container (Wang, 2007).

Table 1: List of Ingredients for Pelletized feed

Ingredients	Amount (g/kg-1)
Fish meal	33.84
Groundnut oil cake	25
Soya bean meal	24
Corn flour	4
Tapioca flour	5.10
Egg albumin	5.06
Cord liver oil	2ml
Vitamin B-complex	1
Probiotics (*Micrococcus lylae, Micrococcus luteus, Vibrio cincinatiensis, Micrococcus roseus*)	2.0 gm

Experimental setup

C. carpio fingerlings (0.3-0.35 g) were introduced into the aquaculture tank and they were maintained in triplicate with aeration. They were fed with different feed in *adlibitum*. The experiment was conducted in 35 days, every 5 days of interval, measures the length, weight and feed utilization. Changes of water in alternate days and siphoning out the fishes faeces and uneaten feed regularly.

The fishes were divided into following groups,
Group 1: fed with control feed (i.e., without probiotic)
Group 2: fed with commercially available feed.
Group 3: fed with commercially available probiotic feed,
Group 4: feed with mixed cultures of probiotics (*Micrococcus lylae, Micrococcus luteus, Micrococcus varians, Micrococcus roseus)*,
Group 5: fed with plankton (*Thermocyclops decipiens*)

Growth parameters
The growth parameters, and feed conversion ratio was calculated according to (Dash et al., 2014).
%Weight gain (WG) = [(Final weight (g)-Initial weight(g)) / initial weight (g)]*100
Specific growth rate (SGR) =100 (lnW2-lnW1)T^{-1}
Where W1 and W2 are initial and final weights and T is the number of days of feeding.
Feed conversion ratio (FCR) =Total feed given / Total weight gain

Proximate analysis
The proximate composition of feed and *C. carpio* fingerlings fed with different feed were analyzed according to the AOAC standard method (AOAC, 1990).

Statistical analysis
The experimental data were subjected to calculated mean and standard deviation. The significance of differences was determined by ANOVA followed by Duncan Multiple Range's test using SPSS 21.0ver for windows.

RESULTS AND DISCUSSION

Isolation and characterization of bacteria from gut of fish
In the present study, the bacterial species, *Serratia liquefaciens, Aeromonas veronii, Micrococcus luteus, Micrococcus lylae, Aeromonas schubertii, Vibrio cincinatiensis, Micrococcus varians, Micrococcus roseus* were present in the gastrointestinal tract of *C. auratus* and *C. carpio* (Table 2). The isolates were further subcultured to get pure colonies. Biochemical characteristics of these bacterial isolates depicted in table 3.

Table 2: Isolation of bacteria from *Carssius auratus* and *Cyprinus carpio*

S. No.	Name of the Bacteria	*Carassius auratus*	*Cyprinus carpio*
1	*Aeromonas veronii*	$5x10^5$cfu / ml	-
2	*Aeromonas schubertii*	-	$600x10^5$cfu / ml
3	*Micrococcus luteus*	$1x10^3$cfu / ml	$2x10^3$cfu / ml
4	*Micrococcus lylae*	$1x10^3$cfu / ml	$4x10^3$cfu / ml
6	*Micrococcus roseus*	-	$1x10^3$cfu / ml
5	*Micrococcus varians*	-	$1x10^3$cfu / ml
7	*Serratia liquefaciens*	$1x10^5$cfu / ml	-
8	*Vibrio cincinatiensis*	-	$4x10^5$cfu / ml

Table 3: Biochemical characterization of bacteria

Test	Organism							
	A. veronii	*A. schubertii*	*M. luteus*	*M. lylae*	*M. roseus*	*M. varians*	*V. cincinatiensis*	*S. liquefaciens*
Gram stain	–ve	-ve	+ve	–ve	–ve	-ve	+ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve
Motility	Motile	Motile	Non-motile	Motile	Motile	Motile	Non-motile	motile

Citrate	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
Growth in 6.5% NaCl	+ve	-ve	+ve	+ve	+ve	NA	+ve	+ve
Voges-Prausker	+ve	-ve	NA	+ve	+ve	+ve	NA	+ve
Gelatine	NA	NA	NA	NA	NA	+ve	NA	+ve
Indole	Native	Native	NA	Native	Native	+ve	NA	NA
MR-test	+ve	+ve	+ve	+ve	+ve	NA	+ve	NA
VP- test	+ve	+ve	Resistant	-ve	-ve	NA	Resistant	NA
H2S	-ve	-ve	NA	-ve	-ve	-ve	NA	NA
Esculin hydrolysis	-ve	-ve	-ve	-ve	-ve	NA	-ve	NA
Coagulase	-ve	-ve	-ve	-ve	-ve	NA	-ve	NA
Clumping factor	NA	NA	-ve	NA	NA	NA	-ve	NA
Urea	NA	NA	-ve	NA	NA	-ve	-ve	NA
Growth in Mac	NA	NA	Resistant	NA	NA	NA	Resistant	NA
Arginine dihydrolase	NA	NA	NA	NA	NA	NA	NA	NA
Growth in Nacl	NA	NA	-ve	NA	NA	-ve	-ve	NA
Glucose	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Lactose	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve
Sucrose	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Mannitol	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Sorbitol	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Arabinose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Rafinose	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

+ve – Positive, -ve – Negative, NA – Not applicable

Proximate composition of different feed

The result of the proximate composition of different feed is depicted in table 4. The Nitrogen free extract (NFE) (19.85 ± 0.43%) and crude protein (37.25 ± 0.75%) were high in the probioitic feed (mixed) compared to control feed (NFE 16.24 ± 0.33% and crude protein 24.67 ± 0.37%) (Table 4).

Table 4: Proximate Composition of feed

Type of feed	Moisture (%)	NFE (%)	Crude Protein (%)	Crude lipid (%)	Ash (%)
Control	9.21 ± 0.34	16.24 ± 0.33	24.67 ± 0.37	6.55 ± 0.67	3.51 ± 0.35
Commercial Control	7.52 ± 0.25	16.17 ± 0.22	23.58 ± 0.36	6.57 ± 0.57	3.61 ± 0.31
Commercial Probiotic	7.15 ± 0.21	17.26 ± 0.24	34.13 ± 0.82	4.57 ± 0.22	2.13 ± 0.54
Probiotic Feed (Mixed)	8.35 ± 0.41	19.85 ± 0.43	37.25 ± 0.75	3.67±0.45	2.34 ± 0.42
Plankton	70.50 ± 1.56	10.34 ± 0.22	36.86^{e} ± 0.21	4.13 ± 0.31	2.75 ± 0.75

The values are represents as Mean ± SD

Effect of different feeds on growth performance and feed utilization of *Cyprinus carpio* fingerlings

Probiotics are used in aquaculture to increase the growth and survival of fishes. Probiotics provides benefits to the host by increasing appetite and digestibility nature of fish. In the present study, *M. luteus, M. roseus, M. varians* and *M. lylae* were used as probiotics to study the growth and feed utilization of *C. carpio* fingerlings. Adorian *et al.,* 2018 reported that more than two probiotic bacteria (*Bacillus licheniformis* and *Bacillus subtilis*) enhanced the growth of fishes and impedance to the pathogenic bacteria.

The *C. carpio* fingerlings fed with mixed pelletized probiotic feed showed the increases in length (3.29 – 4.69 g) and weight (0.39 – 1.06 g) of the fish (Figure 1 and 2) which indicates that gradual increase in body mass and length of fish. Krishnaveni et al., 2013 have reported that the mixed form two probiotic bacteria (*L. Plantarum and B.megatarium)* as a growth promoter in *Catla catla* fingerlings. The combination of probiotics with spirulina feed to fishes enhance the growth as well as biochemical profile. Dietary mixture of yeast *Groenewaldozyma salmanticensis* and bacterium *Gluconacetobacter liquefaciens* enhanced the growth, survival and immune response in the *Paralichthys olivaceus*(Rhee et al., 2020).

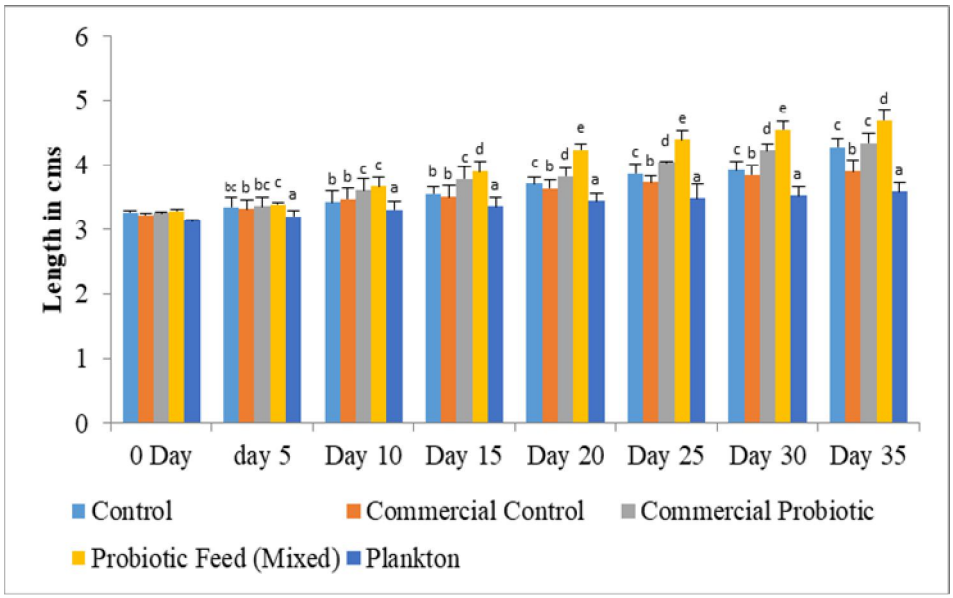

Figure 1: Length of *C. carpio* fingerlings fed with different feed
The values are represents as Mean ± SD
Different superscripts on the mean bar of the same day shows significantly different at P < 0.05 level

Similarly, in the present study, it was proved that combination of probiotic fed fingerlings was significantly increased the growth parameters, followed by fed with commercial probiotic feeding regime compared to other groups. Hence, these result indicates that probiotic containing feed enhanced the growth and survival of fish. And also it was evident that mixed probiotic feed increased the growth of fishes. The growth improvement of fishes, sword tail (*Xiphophorus helleri, X. maculatus*) and guppy, (*Poecilia reticulate, P. sphenops*) was significant increases in growth and survival in fed the supplemented with *Bacillus subtilis* and *Streptomyces* after 50 days of administration (Dharmaraj and Dhevendaran, 2010; Ghosh et al., 2008). This shows that combination of two different probiotic shows significant increases in growth.

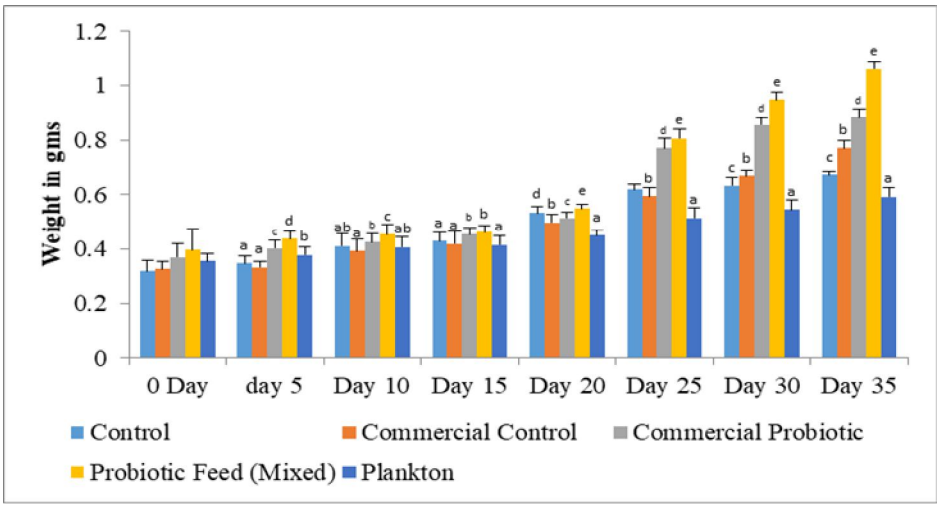

Figure 2: Weight of *C. carpio* fingerlings fed with different feed
The values are represents as Mean ± SD
Different superscripts on the mean bar of the same day shows significantly different at P < 0.05 level

The percentage of weight gain was recorded highest (97.47 ± 0.41%) in mixed pelletized probiotic feed, whereas in plankton feeding regime was 55.48 ± 0.23% (Figure 3). ANOVA for percentage of weight gain for fishes fed with different feed regimes showed that during 15th day and 35th day was significantly increase (P < 0.05) while on 5th day and 10th day was nosignificant in weight gain percentage (P > 0.05) (Table 4). (Umadevi and Krishnaveni, 2013) reported that *M. luteus* possess antibacterial activity and also enhanced the growth of fish was higher weight gain 81.6 ± 0.13% compared to commercial probiotic (52.36 ± 0.03%).

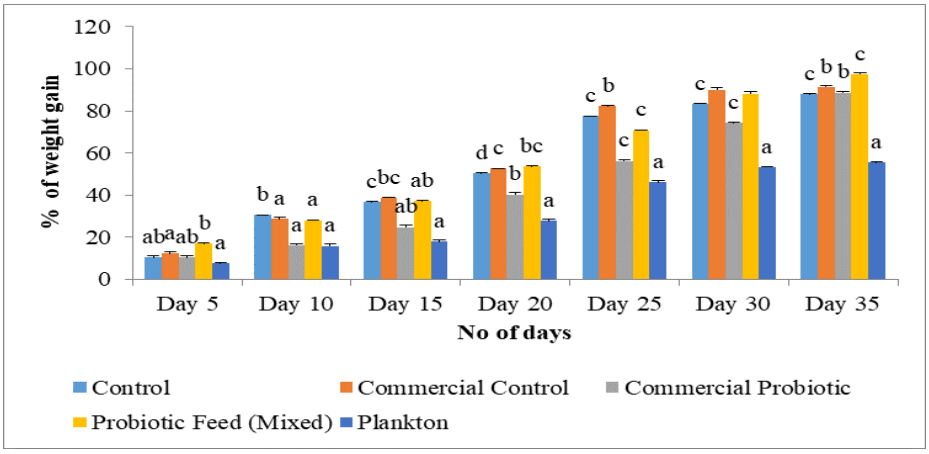

Figure 3: Percentage of weight gain of *C. carpio* fingerlings fed with different feed
The values are represents as Mean ± SD
Different superscripts on the mean bar of the same day shows significantly different at P < 0.05 level

The specific growth rate of fishes fed with different feed showed that higher SGR in mixed probiotic feed (2.91 ± 0.66%), whereas commercial probiotic feed showed lower SGR of 2.83 ± 0.54% (Figure 4). ANOVA for specific growth rate for fishes fed with different feed shows that on 10th day to the 35th day was significant difference (P < 0.05), while, on 5th day was nosignificant difference (P > 0.05) between the feeding regimes (Table 4). (El-Rahman et al., 2009) has reported that *O. niloticus* SGR was higher (1.47 ± 0.73%) in fed with diet containing mixed bacteria (equal amounts of *Pseudomonas sp* and *M. luteus*). In the present study, the highest value SGR was recorded in fishes fed with pelletized

probiotic feed. (Bairagi et al., 2002) reported that probiotics like *Bacillus subtilis* and *B. circulans* were supplemented in the diets of Rohu (*Labeo rohita*) fingerlings, the final body weight and SGR significantly increased than fed only formulated diets. The combination of probiotic shows high SGR compared to commercial feed.

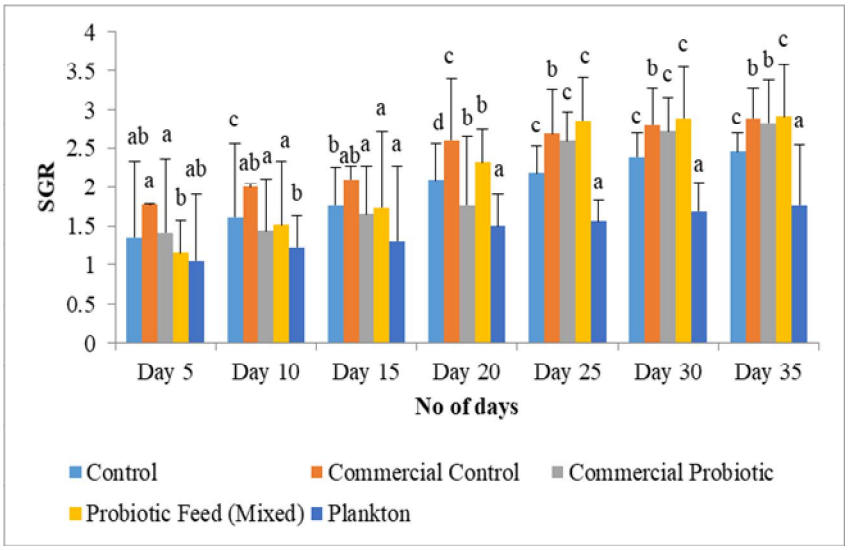

Figure 4: Specific Growth Rate of *C. carpio* fingerlings fed with different feed
The values are represents as Mean ± SD
Different superscripts on the mean bar of the same day shows significantly different at P < 0.05 level

Feed utilization and feed conversion ratio is the most important growth parameters. The mixed probiotic feed shows a lower value of FCR (1.82 ± 0.29) and *C. carpio* fingerlings enhanced their growth and survival, whereas it was fishes fed with commercial probiotic feed has higher FCR value of 1.89 ± 0.29 (Figure 5). Therefore using pelletized probiotic feed could be used for larval rearing of fishes. Anova for feed conversion ratio for fishes fed with different feed showed nosignificant difference (P > 0.05) during 5th day to 20th day while from 25th day to 35th day significant increased the FCR value (P < 0.05) (Table 4).

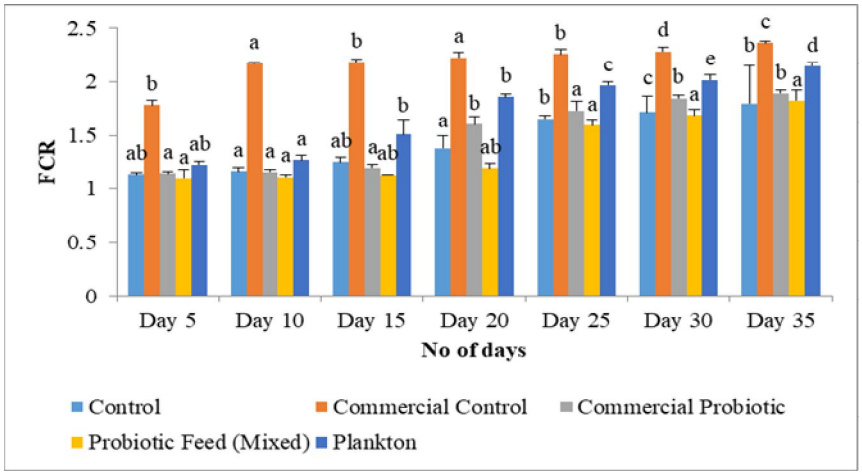

Figure 5: Feed Conversion Ratio of *C. carpio* fingerlings fed with different feed
The values are represents as Mean ± SD
Different superscripts on the mean bar of the same day shows significantly different at P < 0.05 level

Effect of different feeds on survival performance of *C. carpio* fingerlings

Survival percentage of *C. carpio* fingerlings was high (92.2 ± 1.90%) in probiotic pelletized feed (Mixed) and low (67.73 ± 1.96%) in plankton fed *C. carpio* fingerlings. The survival rate of *C. carpio* fingerlings fed with probiotic pelletizied feed (Mixed) showed a significant highest survival percentage (P<0.05) compared with commercial feed (Figure 6). Similarly, effect of probiotic *Pediococcus acidilacti* fed on *Rutilus kutum* fingerlings showed an significant increase in survival rate compared to the control group(Valipour et al., 2018).

Proximate composition of fish

At end of the experiments, different feeding regime fishes were subjected to analyzed the biochemical constituents. The *C. carpio* fingerlings, the protein (14.90 ± 0.43%) value was significantly increased in mixture of probiotic bacteria feeding regimes when comparted to other feeding regimes. (Parthasarathy and Ravi, 2011) has reported that changes of fish protein and carbohydrate level could be related to their synthesis and deposition in fish muscles. The lipid content was higher (5.86 ± 1.62%) in fishes fed with plankton feed and lower (3.69 ± 0.56%) in fishes fed with pelletized feed (Table 5). Comparing our result with Chemical composition of Rainbow trout after feeding with probiotic feed showed the protein content of 14.53% (Bairagi *et al*., 2002) which was low content compared to our study fed with pelletized mixed probiotic feed.

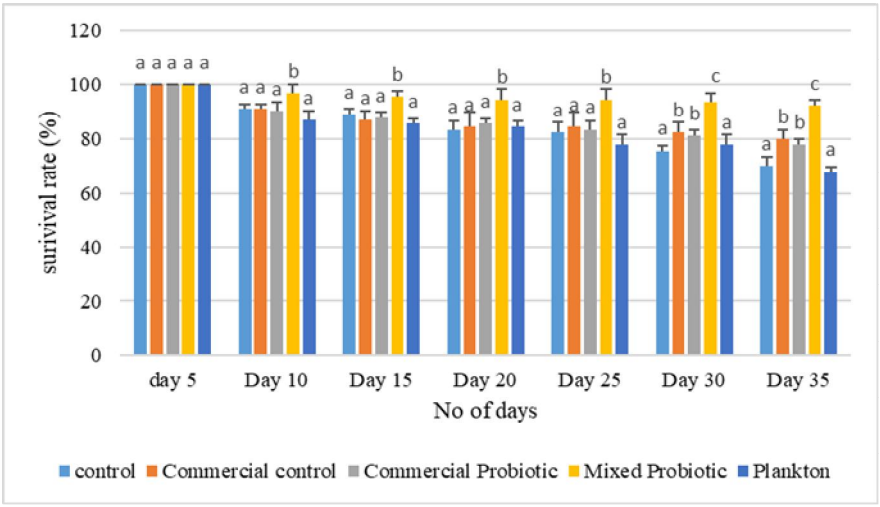

Figure 6: Survival rate (%) of *C. carpio* fingerlings in different feeding regimes

The values are represents as Mean ± SD

Different superscripts on the mean bar of the same day shows significantly different at P < 0.05 level

Table 5: Proximate composition of *C. carpio* fingerlings fed with different feed

Type of feed	Moisture (%)	Nitrogen free extract (%)	Crude Protein (%)	Crude lipid (%)	Ash (%)
Control	11.05 ± 1.08^{a}	31.61 ± 0.33^{c}	13.15 ± 0.26^{c}	3.69 ± 0.56^{a}	2.4 ± 0.35^{a}
Commercial Control	11.46 ± 1.25^{b}	32.77 ± 1.15^{e}	11.72 ± 1.15^{b}	3.86 ± 0.35^{b}	2.6 ± 0.41^{b}
Commercial Probiotic	12.47 ± 1.21^{c}	32.92 ± 1.04^{a}	10.46 ± 1.04^{a}	3.92 ± 0.60^{a}	2.75^{c} ± 0.56^{c}
Probiotic Feed (Mixed)	12.07 ± 1.22^{d}	30.20 ± 0.43^{d}	14.09 ± 0.43^{c}	3.46 ± 0.53^{c}	2.83 ± 0.52^{d}
Plankton	65.61 ± 1.34^{e}	26.94 ± 0.35^{e}	11.94 ± 0.35^{a}	5.86 ± 1.62^{d}	2.96 ± 0.33^{de}

The values are represents as Mean ± SD

Different superscripts in the same column shows significantly different at P < 0.05 level

CONCLUSION

In the present investigation, it was found that combination of probiotic bacteria results good effect in growth and biochemical profile. The biochemical analyses often provide vital information about health status of fishes. From this study it is evident that the microbial flora in the gut of fish varies greatly depending on the surrounding environment. The effect of these microbial strains adversely affects the growth of the fish. The major study area in aquaculture research is to analyse the disease causing bacteria and evaluation for treatment. Recently, most of the researchers are focused in the gut microflora to asses their activitiy against the pathogens and served as a probiotic for sustainable aquaculture The fishes fed with probiotics will antagonise the disease causing microorganisms in gut of fish. The mixed probiotic(*M. lylae, M. luteus, M. varians, M. roseus*) have potential feed for and suggested that combination of these probiotic bacterias are recognized as safe, healthy feed and ecofriendly approach for sutainable aquaculture.

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Conflict of Interest

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

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