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EFFECT OF PROBIOTICS ON GROWTH PERFORMANCE OF COMMON CARP *CYPRINUS CARPIO VAR COMMUNIS*

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ABSTRACT

The present study deals with the isolation and identification of intestinal bacteria from Common carp *Cyprinus carpio.* and its effect on growth performance. Six experimental feeds such as FI control (without probiotics), F2 (1ml of *Proteus spp.*), F3 (2ml of *Proteus spp.*), F4 (3ml *Proteus spp.*), F5 (4 ml of *Proteus spp.*), and F6 (1ml of yeast). Where prepared by using fish meal, Ground nut oil cake, wheat flour and tapioca. Fish were fed with the feeds at a rate of 4% of live body weight. And reared for a period of 30 days. Growth performance such as condition factor, FC, FCE, FCR, Growth, PG, RGR, Assimilation, Metabolism, GGE, and NGE, were calculated, after 30 days. Where ANOVA was studied FC, Growth, GGE and NGE. The results showed that growth parameters such as Feed Consumption, Feed Conversion Efficiency, Feed Conversion Ratio, Growth, Percentage Growth, Relative Growth Rate, Assimilation, Metabolism, Gross Growth Efficiency, Net Growth Efficiency was higher in F5 containing 4 ml of *Proteus spp*. and lower in F1 control (without probiotics).

KEYWORDS: Probiotics, Isolation, Identification, *Cyprinus carpio, Proteus spp.* Growth Performance.

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INTRODUCTION

In India, providing sustainable livelihood opportunities and food security for ever increasing rural population is perhaps the greatest national challenge, where aquaculture plays a pivotal role in meeting this challenge. we should assume aquaculture as the most appropriate farm activities and as a tool for poverty reduction, food security and overall rural development. So blue revolution can be a solution to feed this ever increasing population. Although aquaculture in India has shown a rapid progress during past few years but some major problems are hindering the progress path and disease being one of them. Hence for successful aquaculture we must ensure quality feed, good environment and disease free seeds. In recent years the feed probiotics intestinal floras enhance the probiotic nature of the fish which helps in the nutritional benefits in the fish. The normal Micro flora in the intestinal tract of the fish includes microbes such as *Pseudomonas*, *Aeromonas, Enterobacteriaceae, Micrococcus, Bacteroides* etc. In aquatic animals the intestinal micro flora has been reported to aid in the digestion of algal cells the production of amino acids and the secretion of inhibitory substances that prevent colonization by bacterial pathogens. Fong & Mann (1980), Rimmer & Wiebe (1987). The study related to the isolation, identification of intestinal bacteria in common carp and its effect on growth performance was totally wanting. Hence the present study was carried out.

MATERIALS AND METHODS

Experimental design

Isolation and identification of bacteria

The experimental fish Common carp *(Cyprinus carpio)* were purchased from Pandiyan fish farm Dindigul, Tamil Nadu, India. and transported to the laboratory in polythene bags filled with oxygenated water. The fish were acclimated for a period of ten days with trainee feed. The sample collected from the intestinal content of Common carp *(Cyprinus carpio)* was serially diluted, 10^{-5} dilution was plated on nutrient agar medium for the isolation of bacteria and incubated at 37^0C for 24 hrs. After incubation the colonies were enumerated and the predominant colony were selected and identified based on the morphological, microscopic and biochemical characteristics. Bacterial enumeration per 1 ml of sample was determined by plate count method Cappuccino and Sherman (1998). After determining the microbial load in the sample the sample was confirmed as a probiotic organism in MRS agar medium Hennan *et al* (2002).

Feed preparation

Different ingredients used in the feed preparation (Fish meal, Groundnut oilcake, Wheat flour and Tapioca) were tested for its protein content Lowry *et al* (1951). After knowing their protein contents, feeds were prepared according to square method Ali, (1980) with varying concentration of feeds F1 (control diet without probiotics), F2 (1ml of *Proteus spp.* /100g feed), F3 (2 ml of *Proteus spp.*/100g feed), F4 (3ml of *Proteus spp.* / 100g feed), F5 (4ml of *Proteus spp.*/100g feed), F6 (1ml of yeast / 100g feed), The experimental feeds were prepared separately using known quantities of ingredients to maintain required protein level. The selected ingredients (Fish meal, Ground nut oil cake, Wheat flour, Tapioca) were powdered and sieved to get fine particles of uniform size. Then the ingredients were weighed according to the formulation and added sufficient quantity of distilled water and finally made into dough. The dough was autoclaved for 15 minutes. To the cooled dough probiotics were added and stored in air – tight containers. Song Zeng – Fu *et al* (2006). The ingredients used in the experimental feeds were given in Table.2. Triplicates were maintained for each experimental diet (Control diet without probiotics). The experimental feeds fed twice a day (10 am and 5 pm) at a rate of 4 % of its body weight per day Haniffa *et al* (1987) and water was changed once in three days. Experimental duration is about 30 days. After 30 days feed utilization parameters were calculated.

Statistical Analysis

The experimental results are presented in the form of tables using Microsoft Excel (Version 2007). Mean and Standard deviation were calculated with the help of the same tool, the feed utilization parameters were calculated Statistical analysis dietary treatments were compared by One-way ANOVA method was used for the analysis using DMRT (Version 2005). The data was input manually and computed. The output results obtained from the software indicate whether the difference is between the treatments and days. (CD) Critical difference, (CV) Critical variance, (Prob.) Probability, (NS) Non significant. were also obtained.

RESULTS AND DISCUSSION

One bacterial strain was isolated from Common carp intestinal content and identified as *Proteus spp* the result was supported by Rajan and Akilandeswari (2008). The organism of our interest was confirmed for its probiotic nature with the MRS agar medium and by biochemical tests. Table 1,2 and 3. Verschuere et al., (2000) defined Probiotics as "a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment." the probiotic organism The condition factor (K) of the fish was calculated according to the weight and length of the fish on 30^{th} day. Addition of intestinal bacteria in the feed showed increase of condition factor in all the experimental feeds reared for a period of 30 days. The condition factor was best in F5 (3.56±0.26) containing 4 ml of *Proteus spp.* when compared to other feeds (F1, 0.93±0.26; F2, 1.16±0.61; F3, 1.06±0.40; F4, 1.33±0.53; F6, 0.93±0.38). The feed utilization parameters were presented in Table 4. Growth parameters like Feed consumption (FC), feed conversion efficiency (FCE), feed conversion ratio (FCR) was higher in F5 contain 4 ml of *Proteus spp.* and lower in F1 control (without probiotics). Similar result observed by Marzouk et al., (2008). The Specific growth rate (SGR), Feed conversion ratio (FCR) and Protein efficiency (PER) higher in Group II and lower in Control group without probiotics. Growth (G), percentage growth (PG), and Relative growth rate of *Proteus spp.* were significantly ($P<0.05$) higher in F5 and lower in F1 (Control). Similar results were observed by Arthl Manju *et al.,* 2011. The mean values of SGR (Specific growth rate) were significantly different ($P<0.05$) among different groups. The highest SGR was observed in F4 (3.46± 0.01 % / day). Assimilation (A), Metabolism (M), Gross growth efficiency (GGE), and Net growth efficiency (NGE) were higher in F5 containing 4 ml of *Proteus spp.* and lower in F1 (without probiotics). Rajan and Revathi (2011) reported that the assimilation and metabolism were higher in F4 and lower in F3. Chandra and Rajan, (2009) reported that the gross and net growth efficiency of koi carp was higher in feed II containing 1 ml of *Lactobacillus spp*. Abd El-halim *et al.,* (1989) founded that the addition of living yeast in diet improve the performance of *Oreochromis niloticus.* Analytical variance of Growth performance of Conmen carp given Table 5.

Table 1
The Intestinal microbial load per ml of organism

S.No	Sample (Intestine of Gold fish)	Number of colonies	CFU/ml of the sample
1.	$10^{-5\,(O)}$	101	$100X10^5$
2.	$10^{-5\,(R)}$	98	

Table 2
Biochemical tests

S.No	Name of Tests	Observation
1	Gram staining	Negative
2	Indole Test	Positive
3	Methyl Red Test	Positive
4	Voges – Proskauer test	Negative
5	Citrate test	Negative
6	Urease	Positive
7	Catalase test	Positive
8	Starch Test	Negative
9	Glucose Utilization test	Positive
10	Gelatin hydrolysis	Positive
11	Growth in MRS agar medium	Positive
12	Identification result	*Proteus spp.*

Table 3
Composition of different ingredients in experimental feeds (g/100gm)

S.No	INGREDIENTS	EXPERIMENTAL FEEDS					
		Feed I (control)	Feed II	Feed III	Feed IV	Feed V	Feed VI
1	Fishmeal	33.75	33.75	33.75	37.50	33.75	33.75
2	Groundnut oil cake	33.75	33.75	33.75	33.75	33.75	33.75
3	Wheat flour	11.25	11.25	11.25	11.25	11.25	11.25
4	Topioca	11.25	11.25	11.25	11.25	11.25	11.25
5	Fish oil	2	2	2	2	2	2
6	Sunflower oil	2	2	2	2	2	2
7	Suppelvite mix	4	4	4	4	4	4
8	Sodium chloride	1	1	1	1	1	1
9	Sodium benzoate	1	1	1	1	1	1
10	Microbes (10^6 Cells)		1ml	2ml	3ml	4ml	1ml Yeast

Table 4
Feed utilization and growth parameters of Common carp Cyprinus carpio in relation to different concentration of Proteus. spp. (cells). Each value is the average (± SD) performance of 5 individuals in triplicates reared for 30 days

S.NO	PARAMETERS	EXPERIMENTAL FEEDS					
		FEED I (Control)	FEED II (1 ml)	FEED III (2ml)	FEED IV (3ml)	FEED V (4ml)	FEED VI (1mlYeast)
1	Feed Consumption(FC) (g/g live wt/30 days)	1.10 ± 0.03[a]	1.12 ± 0.12[b]	1.15 ± 0.06[c]	1.15 ± 0.09[d]	2.20 ± 0.08[e]	1.17 ± 0.07[f]
2	Feed Conversion Efficiency (FCE)	0.55 ± 0.29	1.37 ± 0.99	2.51 ± 0.46	3.12 ± 0.30	4.42 ± 0.42	3.57 ± 0.91
3	Feed Conversion Ratio (FCR)	2.59 ± 0.95	4.51 ± 1.20	5.41 ± 0.88	5.56 ± 0.43	7.14 ± 0.24	6.48 ± 0.42
4	Growth (G) (g/g live wt/ 30 days)	0.11± 0.04[a]	0.48 ± 0.59[b]	0.38 ± 0.22[c]	0.41 ± 0.23[d]	0.59 ± 0.53[e]	0.44 ± 0.33[f]
5	Percentage Growth (PG) (%)	16.11 ± 9.53	17.10 ± 7.0	37.66 ± 6.16	37.62 ± 15.17	51.12 ± 22.30	41.66 ± 23.28
6	Relative Growth Rate (RGR)	0.32 ± 0.20	0.39 ± 0.22	0.47 ± 0.13	0.53 ± 0.12	0.61 ± 0.42	0.48 ± 0.24
7	Assimilation (A)	0.60± 0.07	0.65± 0.20	0.72 ± 0.47	0.76 ± 0.32	0.88 ± 0.31	0.63 ± 0.28
8	Metabolism (M)	0.48 ± 0.08	0.63 ± 0.04	0.61 ± 0.19	0.74 ± 0.26	0.82 ± 1.21	0.60± 0.19
9	Gross growth Efficiency (GGE) (%)	15.22 ±0.02[a]	16.71 ± 0.07[b]	18.31 ± 0.23[c]	17.97 ± 0.31[d]	19.36 ±0.25[e]	17.81 ± 0.26[f]
10	Net growth Efficiency (NGE) (%)	12.11 ±0.06[a]	14.16 ± 0.03[b]	13.15 ± 0.04[c]	15.13 ± 0.05[d]	17.14 ± 0.02[e]	14.76 ± 0.04[f]

Table 5
Analytical variance of Growth performance of Conmen carp

S.No	Growth parameters	SD	CD (.05)	CD (.01)	CV	Prob
1	Feed consumption	0.0614	0.1367	0.1945	43.08	0.824 (NS)
2	Growth	0.2502	0.5574	0.7929	75.96	0.548 (NS)
3	Gross Growth Efficiency	3.9752	8.8573	12.5991	31.34	0.438 (NS)
4	Net Growth Efficiency	3.6882	8.2179	11.6896	29.94	0.603 (NS)

(SD) Standard deviation, (CD) Critical differences, (CV) Critical variance, (Prob.) Probability, (NS) Non significant.

CONCLUSION

The present study concludes that the identified bacterial strain from the intestine of common carp (*Cyprinus carpio)* was *Proteus spp.* The growth parameters like Feed Consumption, Feed Conversion Efficiency, Feed Conversion Ratio, Growth, Percentage Growth, Relative Growth Rate, Assimilation, Metabolism, Gross Growth Efficiency, and Net Growth Efficiency were higher in F5 and lower in F1 (control Without probiotis). Presence of this organism in the intestinal flora of the fish enhance the probiotic nature and helps in the nutritional benefits to the fish.

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