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Effect of Probiotic *Enterococcus gallinarum* N3 Supplemented Feed on Growth Performance of Freshwater Fish *Cyprinus carpio*

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ABSTRACT

Background: Probiotic is the best solution (Produce antimicrobial compounds, inhibit colonization of pathogenic microorganism, increase digestive ability and confer better health to the host) as an alternative to antibiotics against various microbial infection in freshwater and marine water. The freshwater fish *Cyprinus carpio* was selected for this research work (due to high growth rate, high protein content, ability to survive under different climatic conditions and its availability throughout the year) to observe the effect of probiotic *Enterococcus gallinarum* N3 supplemented feed on its growth.

Methods: In this present research work Prepared three different concentration of probiotic *Enterococcus gallinarum* N3 (0.1%. 0.5% and 1.0%) along with basal diet containing other ingredients (fish meal, groundnut oil cake, wheat bran, starch and Vitamin mineral premix). Initially 14 days acclimatized freshwater fish *Cyprinus carpio* to laboratory condition selected randomly for four treatment groups as Control (C) and probiotic feed treated T1, T2 and T3 group. The experiment was carried out for 42 days with continuous aeration, natural photoperiod of 16 hrs light and 8 hrs dark, maintain temperature constant and fishes fed diet twice daily. The feed ingredients and experimental diets analysed for proximate composition and measured Weight gain, Specific growth rate (SGR) and Feed conversion efficiency (FCE).

Result: The proximate composition of the feed was determined as percentage dry matter in which 36.21% protein, 6.8% moisture and 7.8% of lipid. The probiotic feed experiment was conducted for 42 days and entire group of fishes was survived. The effect of probiotic feed on growth performance of *Cyprinus carpio* was measured as initial weight, final weight, weight gain, specific growth rate and feed conversion efficiency. The growth changes were observed before and after treatment with 0.1%, 0.5% and 1.0% of probiotic *E. gallinarum* N3 fed to *Cyprinus carpio.* The final weight (g) and final length (cm) was measured in treatment T1 (3.107 g; 3.32 cm), T2 (3.258 g; 3.40 cm) and T3 (3.413 g; 3.52 cm) respectively as compared with Control group C (2.255 g; 2.98 cm). The percentage weight gain obtained in group T1 (37.78%), T2 (44.45%) and (51.55%) respectively as compared with Control group C (29.54%). Maximum specific growth rate was observed in treatments T1 (0.94 % day^{-1}), T2 (0.99% day^{-1}) and T3 (1.02% day^{-1}) as compared with control group C (0.56% day^{-1}). The results showed better growth performance and feed utilization in all the three treated groups T1, T2 and T3. The best growth performance (final weight, final length, weight gain, specific growth rate and feed conversion efficiency) and feed utilization (moisture, protein and lipid) was observed best in T3 (1 g kg^{-1}) group of fishes as compared to control C group. There was significant difference (P>0.05) in final weight, final length, weight gain, specific growth rate and feed conversion efficiency.

Key words: *Cyprinus carpio*, *Enterococccus gallinarum*, Feed conversion efficiency, Freshwater fish, Probiotic, Specific growth rate.

INTRODUCTION

Aquaculture is one of the fastest growing food producing sector accounted for 46 percent of the total production and 52 percent of fish for human consumption. The Global fish1 production is estimated to have reached about 179 million tonnes in 2018. Aquaculture includes mainly study of fish, shellfish and crustaceans which provides high protein content for human being. Common carp *Cyprinus carpio* contributes 7.7% as major species produced in world aquaculture (FAO, 2020). Different types of pathogenic microorganisms including bacteria, fungi, viruses, parasites responsible for causing various diseases in freshwater as well as in marine environment (Axelrod and Untergasser, 1989). Aquaculture production is severely affected by the various disease-causing microorganisms as bacteria (*Aeromonas, Klebsiella pneumoniae, Proteus mirabilis, Plesiomonas shigelloides, Acinetobacter baumannii, Morganella morganii, Serratia marcescens, Vibrio parahemolyticus, Edwardsiella tarda, Pseudomonas*

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aeruginosa and *Streptococcus*. (Wang *et al*., 2008, Sugita *et al*., 1996; Defoirdt *et al*., 2011; Sahoo *et al*., 2020).

To overcome this problem used various antimicrobials and inhibitory compounds but there is development of resistance to these compounds by microorganisms. Probiotics are defined as "live microorganisms which when consumed in sufficient amounts, affect beneficially the health of the host." Probiotic microorganism properties are to reduce the concentration of pathogenic microorganisms producing bacteriocin, some dietary enzymes which play role in digestion and absorption of nutrients (Amylase, Phytase, Lipase, Protease), enhanced epithelial barrier function, increases ability to adhesion to intestinal mucosal membrane, restrict adhesion of pathogens, colonization of beneficial probiotic organisms, competitive exclusion of pathogenic microorganisms and production of anti-microbial substances besides modulating immunity (Bermudez-Brito *et al.,* 2012). Fifty-four isolates of lactic acid bacteria *Lactococcus garvieae*, *Pediococcus acidilactici* and *Enterococcus faecium*) isolated from the intestines of the common carp *Cyprinus carpio* and freshwater prawn (*Macrobrachium rosenbergii*) in Nakorn-Pathom province, Thailand (Cai *et al.,* 1999). Enterococci groups of lactic acid bacteria used as starter culture in various food fermentation due to their enzymatic activity and proteolytic activity, production of bacteriocins as probiotics with number of beneficial properties as stimulate immunity, anti-inflammatory activity, hypocholesterolemic effect and contribute for prevention/ treatment of some diseases (Braiek *et al.,* 2018). There is an isolation of various strains of *Enterococcus faecalis, Enterococcus casseliflavus, E. faecium.*

Some enterococcal strains used as safe and effective feed supplements in various probiotic preparations (*E. faecium* M74 and *E. faecium* SF-68), treatment and prevention of certain animal and human diseases (alleviates irritable-bowl syndrome, antibiotic induced diarrhea, prevents different functional and chronic intestinal diseases, antimutagenic, anticarcinogenic effect). *E. mundtii* ST4SA and *E. durans* KLDS 6.0930 considered probiotic candidate which lowers human serum cholesterol levels (Chaiecka-Wierzchowska *et al.,* 2016; Higuita Huycke, 2014, Ghosh *et al.,* 2013; Liu *et al*., 2016). The objectives of the research work is to prepare probiotic feed and observe the effect of probiotic supplemented feed on growth performance of freshwater fish *Cyprinus carpio* for 42 days.

MATERIALS AND METHODS

This experiment was conducted in the year 2011-2012 at School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra.

Preparation of the probiotic bacteria

The probiotic bacteria *Enterococcus gallinarum* N3 used in this study was grown in DeMan Rogosa and Sharpe (MRS) broth for 24 h at 30°C and checked routinely for purity. The preparation of probiotic bacteria was carried out by inoculating *Enterococcus gallinarum* N3 in MRS broth and incubated at 30°C for 48 h and then centrifuged at 3500 × g for 30 min. After centrifugation bacteria were washed twice with sterile saline and the final suspension concentration was adjusted to 10^8 cells/ ml of saline (Totewad and Gyananath, 2018). Bacterial cell numbers were estimated by serial dilutions method being plated in triplicate on MRS agar plates and counted after 48 h of incubation at 30°C. The saline containing the fresh cells of probiotic *Enterococcus gallinarum* N3 was added to the laboratory prepared feed to give an initial number of 10^5 cells/g of wet weight of diet and were harvested and maintained at 4°C (Wang, 2007). Aliquots of cells were kept in a sterilized container at 4°C. Three different concentrations of probiotic *Enterococcus gallinarum* N3 (0.1 g, 0.5 g and 1 g) were used to define feed formulations.

Preparation of experimental diet

The dietary dry feed ingredients fish meal. Ground nut oil cake, wheat bran, Vitamin and mineral mix selected for the growth study of freshwater fish Cyprinus carpio were finely powdered and sieved (Pore size < 400 µm) separately. The feed ingredients (Fish meal, groundnut oil cake, wheat bran and cod liver oil) as basal ingredients were mixed thoroughly with lukewarm water to make dough using 2% starch as a binder (Ghosh *et al*., 2005) and formulated the basic diet to contain 30.14% crude protein. Then vitamin- mineral mixture was added to the diets before pelletization.

The probiotic bacterial suspension of *Enterococcus gallinarum* N3 was added to the basal diet to conduct three treatments as basal diet without probiotic bacteria (T1) as control, basal diet containing 0.1 g of *Enterococcus gallinarum* N3 (T2), basal diet containing 0.5 g of *Enterococcus gallinarum* N3 (T3) and basal diet containing 1.0 g of *Enterococcus gallinarum* N3 (T4). The prepared feed mixture was rapidly squeezed through a hand pelletizer with 1 mm diameter mesh size. The pellets obtained were sundried till no moisture is observed. The pellets were transferred and packed in air-tight plastic bags and stored in refrigerator at 4°C and this preparation was repeated every two weeks. The ingredients were used for the preparation of basal diet were given in the Table 1 and Table 2.

Fish feeding and culture system

In this present research work freshwater fish common carp *Cyprinus carpio* was selected on the basis of high growth rate, high nutritive value, ability to survive under different climatic conditions, resist common diseases and attack of parasites and available throughout the year. The fingerlings of common carp *Cyprinus carpio* 0.793±0.010 g was selected for the growth study and maintained in plastic tubs of 23 L capacity and size (46 × 26 cm). The *Cyprinus carpio* were acclimatized to the laboratory conditions in plastic tubs for 14 days and fed ad libitum with commercially available diet (Gold Tokyo). The *Cyprinus carpio* were selected randomly and assigned to the four groups of 5 fish in each tub with three replicates. The fishes were randomly stocked

at a rate of 5 fishes per 15 L of plastic tub. Each plastic tub was supplied with fully aerated tap water. The fishes were fed diet twice daily at the rate of 5% body weight regularly at the visible satiation per day (9.00 A.M. and 5.30 P.M) for 42 days. The fish fed was readjusted regularly every 2 weeks.

Each group of fishes was maintained at natural photoperiod (16 h light and 8 h dark) and with continuous aeration by aerator (Aquarium air pump RS-180, RS Electricals). Water was changed daily by siphoning. Static water system was used and water temperature remained constant 28±1°C during experimental period. Temperature measured daily and dissolved oxygen (DO), total ammonium, nitrite and pH were measure weekly. The level of dissolved oxygen was maintained above 6 mg/lit by an air pump.

Sampling and analytical methods

The fishes of each plastic tubs were counted and length and weight of the fishes were measured at 7 days, 14 days, 21 days, 28 days, 35 days and 42 days respectively. The feed ingredients were analysed for proximate composition (Anonymous, 1990) as follows:

At the end of feeding trial, growth and survival performance were calculated by using following formulae so as to evaluate the efficacy of feeds prepared. The fishes of each tub were counted and weighed at the end of the experiment. The growth parameters and feed utilization were calculated as follows:

Weight gain= Final weight W2 - Initial Weight W1

$$\text{Specific growth rate} = \frac{100\ (\ln W2 - W1)}{\text{Number of days}}$$

$$\text{Feed conversion efficiency} = \frac{\text{Weight gain}}{\text{Dry feed given}} \times 100$$

Statistical analysis

One way ANOVA was used to evaluate the effect of the bacterial supplementation. Mean separations were determined at the 5% probability level. All the statistical analysis were performed using the StatistiXL program.

RESULTS AND DISCUSSION

The final length and weight changes in all the treatment groups have shown statistically significant growth changes. The values obtained for T1 group was found to be 3.3±0.05 cm and 3.10±0.05 g. Similarly, the values obtained for T2 and T3 group were found to be 3.4±0.0 cm and 3.23±0.03 g and 3.5±0.0 cm and 3.41±0.04 g respectively given in the Table 3.

A comparison of the results was obtained for percent weight gain following treatment with (T1) 0.1 g/kg, (T2) 0.5 g/kg and (T3) 1.0 g/kg of probiotic *E. gallinarum* N3 was examined and the percent weight gain was found to be 37.78%, 44.45% and 51.56% respectively (Table 4).

The specific growth rate was also calculated, and the value of the control (C) group was found to be 0.56±0.08% day. The specific growth rate values of the three groups (T1, T2 and T3 groups) values were found to be 0.94±0.06% /day, 0.99±0.11%/day and 1.02±0.08% /day respectively (Table 5).

Similarly, the feed conversion efficiency (FCE) was calculated shows the comparison of FCE between C and T1, T2 and T3 groups was found to be 57.29±0.13 and the comparative value for T1, T2 and T3 groups were found to be 70.60±0.13, 70. 44±0.12 and 72.76±0.13 respectively. In the present investigation effect of *Enterococcus gallinarum* N3 supplemented feed on the growth performance and feed utilization of freshwater *Cyprinus carpio* was studied. The experiment was carried out for 42 days. The fishes were divided into four groups as control (C) and treated (T1, T2 and T3). The basal diet without addition of *Enterococcus gallinarum* N3 was used as control (C). The results suggest that probiotic *Enterococcus gallinarum* N3 may primed growth rate and thus improve nutrition by the production of vitamins. The improvement in live body weight in probiotic treated groups of fish is mainly due to the beneficial bacteria such as *Enterococcus gallinarum* N3 in the intestinal tract which can compete with the undesirable organisms for nutrients.

Bogut *et al.* (1998) confirmed that the commercial *Streptococcus faecium* improved the growth and feed efficiency of Israeli carp and observed the effects of supplementary Israeli carp feeds with different additives including antibiotics, yeast (*S. cerevisiae*) and bacteria (*S. faecium*) and observed better growth response with probiotic supplemented diets but obtained the best growth with a bacterium. Zhou *et al.* (2010) studied the effect of probiotic on immunostimulatory effect in Tilapia (*Oreochromis niloticus*). All the probiotic supplemented diets

Table 1: Formulations of probiotic *Enterococcus* gallinarum N3 supplemented experimental diet.

Ingredients	Treatment			
	C	T1	T2	T3
Fish meal	30.14 g	30.14 g	30.14 g	30.14 g
Groundnut oil cake	30.14 g	30.14 g	30.14 g	30.14 g
Wheat bran	36.72 g	36.72 g	36.72 g	36.72 g
Cod liver oil	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Starch	2.0 g	2.0 g	2.0 g	2.0 g
Vitamin and mineral mix (Supradyn)	1.0 g	1.0 g	1.0 g	1.0 g
Probiotic (*Enterococcus gallinarum* N3)	0.0 g	0.1 g	0.5 g	1.0 g

Table 2: Vitamins and mineral premix per kilogram feed.

Vitamin mineral premix contained the following vitamins per kilogram feed	Quantity
Vitamin A I.P (as acetate)	10000 I.U.
Vitamin D3 I.P (Cholecalciferol I.P)	1000 I.U
Vitamin B1 I.P. (Thiamine mononitrate)	10 mg
Vitamin B2 I.P (Riboflavin)	10 mg
Vitamin B6 I.P (Pyridoxine hydrochloride)	3 mg
Vitamin B12 I.P (Cyanocobalamin I.P)	15 mcg
Nicotinamide I.P (Niacinamide)	100 mg
Calcium D Pantothenate I.P (D-Pantothenic acid Calcium salt)	16. 30 mg
Vitamin C I.P (Ascorbic acid)	150 mg
Vitamin E I.P. (α- Tocopherol acetate I.P)	25 mg
Biotin U.S.P	0.25 g
Mineral mix contained the following minerals as mg per Kg feed	
Tribasic calcium phosphate	129 mg
Magnesium oxide	60 mg
Dried ferrous sulphate I.P.	32.04 mg
Manganese as Sulphate monohydrate B.P.	2.03 mg
Total Phosphorus in the preparation	25.80 mg
Copper sulphate pentahydrate B.P.	3.39 mg
Zinc sulphate I.P.	2.20 mg
Sodium molybdate dihydrate B.P.	0.25 mg
Sodium borate B.P.	0.85 mg

Table 3: Effect of probiotic *E. gallinarum* N3 supplemented feed on body length and weight changes of *Cyprinus carpio.*

Parameter	Days	Treatment			
		C	T1	T2	T3
Weight of fish (g)	0	0.763±0.03	0.799±0.04	0.807±0.03	0.805±0.05
	7	0.936±0.02	1.004±0.04	1.057±0.03	1.079±0.03
	14	1.132±0.03	1.280±0.03	1.366±0.03	1.411±0.04
	21	1.369±0.02	1.652±0.02	1.754±0.03	1.828±0.03
	28	1.462±0.03	2.083±0.03	2.217±0.03	2.323±0.02
	35	1.820±0.04	2.574±0.03	2.723±0.03	2.856±0.03
	42	2.255±0.05	3.107±0.05	3.258±0.03	3.413±0.04
Length of fish (cm)	0	2.34±0.05	2.32±0.00	2.38±0.05	2.36±0.05
	7	2.40±0.02	2.51±0.01	2.53±0.02	2.56±0.02
	14	2.52±0.02	2.66±0.03	2.70±0.03	2.79±0.03
	21	2.64±0.03	2.80±0.02	2.87±0.02	2.92±0.03
	28	2.76±0.02	2.98±0.02	3.04±0.02	3.11±0.02
	35	2.86±0.02	3.13±0.02	3.23±0.03	3.34±0.03
	42	2.98±0.02	3.32±0.02	3.40±0.02	3.52±0.03

Table 4: Effect of probiotic *E. gallinarum* N3 on per cent weight gain in *Cyprinus carpio.*

Concentration of probiotic *E. gallinarum* N3	Weight gain (%)
C	29.54
T1	37.78
T2	44.45
T3	51.55

suggesting that the addition of probiotics mitigated the effects of the stress factors. It can be extended from the findings that the improvement in fish growth and feed resulted in growth higher than that of the control diets utilization observed with probiotics supplemented diets may be linked to improved nutrient digestibility, improved intestinal microbial balance, reduced pathogenic flora which accelerated food absorption could be result of the ability of enzymes capable of converting certain components of the diet into more digestible nutrients for the hosts. The improvement in the live body weight in probiotic treated groups of fish is mainly due to the beneficial bacteria such as *E. gallinarum* N3 in the intestinal tract which compete with the undesirable organisms in the current research work.

Table 5: Effect of probiotic *E. gallinarum* N3 supplemented food on the growth performances of freshwater fish *Cyprinus carpio* by One Way ANOVA method.

Parameters	Treatment			
Group/treatment	C	T1	T2	T3
Initial weight (IW) g	0.76±0.03a	0.79±0.04a	0.80±0.03a	0.80±0.05a
Final weight (FW) g	2.25±0.05a	3.10±0.05b	3.25±0.03bc	3.41±0.04d
Initial length (IL) cm	2.30±0.05a	2.30±0.00a	2.4±0.05a	2.4±0.05a
Final length (FL) cm	3.0±0.05a	3.3±0.05b	3.4±0.00c	3.5±0.00dc
Weight gain (WG) g	1.49±0.14a	2.30±0.11b	2.53±0.12cb	2.60±0.12db
Specific growth rate (SGR)	0.56±0.08a	0.94±0.06b	0.99±0.11cb	1.02±0.08db
Feed conversion efficiency (FCE)	57.29±0.13a	70.6±0.13b	70.44±0.12cb	72.76±0.13d

Means in the same row with different letters were significant with different values (P<0.05).

The better feed conversion efficiency value observed with probiotic food supplemented diet suggested that addition of probiotics improved feed utilization of common carp. Similar results had been reported for probiotics use in diets *Nile tilapia* (*Oreochromis niloticus*) (Lara-Flores *et al.*, 2003). It is clear from the study that positive effect of probiotics isolated from the intestine of freshwater fish *Cyprinus carpio*. It is documented that probiotic bacterium have been effective in inhibiting a wide range of fish pathogens.

CONCLUSION

The effect of *Enterococcus gallinarum* N3 was examined as feed supplement under *in vivo* conditions for understanding the growth in the freshwater fish *Cyprinus carpio*. The results indicate that at the end of the 42 days after treatment with probiotic *Enterococcus gallinarum* N3 in experimental animals, there was significant growth in terms of length and weight as compared to control group. At 1% concentration of probiotic *Enterococcus gallinarum* N3 noticeable changes were observed in groups of fishes. The parameters that were selected to indicate growth changes of the *Cyprinus carpio* include final length, final weight, per cent weight gain, specific growth rate, feed conversion efficiency and feed conversion ratio. It can be inferred that the probiotic *Enterococcus gallinarum* N3 can be proposed as feed supplement for improvement in growth rates of freshwater and may be beneficial in marine fishes.

Conflict of interest

We have no conflicts of interest to disclose.

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