Effect of Probiotic Supplementation on Growth of Carp Fingerlings

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Bonika Pant
Krishi Vigyan Kendra

Vibha Lohani
Govind Ballabh Pant University of Agriculture and Technology

Ashutosh Mishra
Govind Ballabh Pant University of Agriculture and Technology

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SHORT COMMUNICATION

Effect of Probiotic Supplementation on Growth of Carp Fingerlings

Bonika Pant[1] · Vibha Lohani[1] · Ashutosh Mishra[1] · M. D. Trakroo[1] · Hema Tewari[1]

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Abstract A probiotic product (PLANKTO PLUS) consisting adequate colony-forming units of beneficial bacteria was experimented for 90 days to evaluate the effect of growth of carp fingerlings. The selected probiotic was applied regularly @ 2 ml/tank (water volume 4 m^3) to the carp fingerlings kept in fibreglass-reinforced plastic tanks under five different treatments, i.e. control (without probiotics), once in a day, weekly, fortnightly and monthly with two replicates. Tanks were stocked with *Hypophthalmichthys molitrix, Labeo rohita* and *Cirrhinus mrigala* @ 10 fingerlings/m^3 in the ratio of 4:3:3, and water quality parameters were monitored regularly. The use of probiotic resulted in good conclusion by bringing feed cost down, improving water quality, increasing plankton concentration, quick breakdown of unused feed and checking algal bloom. The best result was obtained in the tanks with 'weekly' dose in the form of diverse assemblage of zooplankton, good condition factor and highest specific growth rate of the stocked fishes.

Keywords Probiotic · Algal blooms · Zooplankton · Condition factor · Specific growth rate

Aquaculture has evolved as the fastest growing food-producing sector and developed as an important component in food security [1]. It has become an important economic activity in many countries. In large-scale production facilities, where aquatic animals are exposed to stressful conditions, problems related to diseases and deterioration of environmental conditions often occur and result in serious economic losses [2]. The contribution of probiotics is quite considerable compared to other chemical additives used in aquaculture as there are many environmental problems. Etymologically, the term "probiotic" was originated from the Latin word "pro" which means "for" and the Greek word "bios" which means "life". The Food and Agriculture Organization [3] defined probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host. An antonym of antibiotic, probiotics, involves in multiplying few good/useful microbes to compete with the harmful ones, thus suppressing their growth. These include certain bacteria and yeasts that are not harmful on continued use for a long time [4]. Moriarty [5] suggested that the definition of a probiotic in aquaculture should include the addition of live naturally occurring bacteria to tanks and ponds in which animals live. Improved water quality has especially been associated with probiotics. Aquaculture probiotics have a very important role to play in the degradation of organic matter, thereby significantly reducing the sludge and slime formation. General selection criteria are mainly determined by bio-safety (non-pathogenic) considerations, methods of production and processing, method of administration of the probiotic and the location in the body where the microorganisms are expected to be active [6]. Probiotic can be established well in static or low water exchange systems. They are effective if applied as soon as the water medium is sterilized before contamination with other microbes [7]. The role of probiotics in health management of fish is also studied by several workers [8–12]. The probiotics prepared with microorganisms have important roles in pond culture, particularly with respect to productivity, the nutrition of the

✉ Ashutosh Mishra
ashutosh36in@yahoo.co.in

[1] College of Fisheries, G.B. Pant University of Agriculture and Technology, U.S. Nagar, Pantnagar, Uttarakhand 263145, India

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cultured animals, disease control, water quality, and environmental impact of the effluent [13].

Many pharmaceutical companies have introduced commercial preparations of probiotics into the market as feed/food supplement in various commercial names. PLANKTO PLUS (Bovian Healthcare Ltd.) is a unique multi-strain probiotic blend, which consists of adequate colony-forming units of *Bacillus* sp., *Lactobacillus* sp.:, *Rhodococcus* sp.; *Rhodobacte*r sp.; nitrifying and denitrifying bacteria in liquid form for better water miscibility and dispersion in the pond. The main objective of the present investigation was to check the performance of selected probiotic for zooplankton enhancement, protein breakdown and absorption, inhibiting the growth of harmful bacteria and competing with them, complete water quality improvement and stabilizing algal growth.

Materials and Methods

The present investigation was conducted in ten circular FRP tanks of diameter 2.5 and 1.0 m depth (water volume 4 m^3) for 90 days (from March to May 2014) kept under re-circulatory complex of the College of Fisheries, Pantnagar, in Tarai belt of Shivalik range of Himalayan foot hills located at 29°N latitude, 79.3°E longitude and at altitude of 243.8 m above mean sea level. The climatic condition of Pantnagar is humid, sub-tropical and is characterised by very hot and dry summer and extremely cold conditions. The tanks were equipped with an inlet, outlet, proper aeration, drainage, etc., facilities. Two replica of each tank were created and named as T_1–T_1', T_2–T_2', T_3–T_3', T_4–T_4' and T_5–T_5' for control, once in the experimental period, weekly, fortnightly and monthly dose, respectively. An inoculation of pond water having plankton population was introduced to all the tanks. Measured quantities of the probiotic (PLANKTO PLUS) @ 2 ml/tank/dose as per its recommendation (1–2 l/acre) were added to the specific water tanks after their proper preparation. Several studies have shown that a single treatment with probiotic culture is not enough and that the organism(s) must be added on a more continuous basis [14]. After 3 days of probiotic application, the stocking of 40 fish fingerlings per tank (@ 10 fingerlings/m^3) was made. The weight of stocked fingerlings varied according to species, i.e. Rohu (*Labeo rohita,*) 11–20 g, Mrigal (*Cirrhinus mrigala)* 32–41 g and Silver Carp (*Hypophthalmichthys molitrix)* 20–30 g. Supplementary feeding with 28% protein in the form of pellets made up of soybean oil cake (20%), mustard oil cake (20%), fish meal (19%), rice bran (20%), maize flour (20%),vitamin and mineral mixture (1%) was done @ 5% body weight of the fingerlings twice daily in the morning and in evening. Aeration was done regularly to maintain optimum dissolved oxygen (5–7 mg/l) concentration in all the experimental tanks. At the end of experiment, specific growth rate was calculated as follows [15].

$$\text{Specific Growth Rate (SGR)} = \frac{\text{In log (final weight)} - \text{In log(initial weight)}}{\text{Time (days)}} \times 100$$

Assessment of water quality parameters, qualitative and quantitative estimation of plankton population was performed on weekly basis following standard methods [16]. Regular check-up and monitoring for any abnormality, reduction in dissolved oxygen, fluctuation in physical and chemical parameters, presence of toxicant, algal bloom, improper feed breakdown of artificial feed and excessive sludge formation was done.

Results

To observe the effect of the selected aquaprobiotic (PLANKTO PLUS) on water quality of different treatment trials [Control (T_1), once in the experimental period (T_2), weekly (T_3), fortnightly (T_4) and monthly dose (T_5)], the physico-chemical parameters of water were analysed weekly (Tables 1(a)–(e)). The physico-chemical characteristics of water in T_1, T_2, T_3, T_4 and T_5 throughout the experimental period were water temperature 20.1–22.67 °C; average turbidity from 1.30 to 1.75 NTU, average pH ranged from 7.75 to 7.85; average dissolved oxygen varied from 6.65 to 6.90 mg/l; average value of free CO_2 varied from 0.0 to 1.93 mg/l; total alkalinity varied between 168.21 and 171.46 mg/l; average nitrite ranged between 0.01 and 0.04 mg/l and average chloride varied from 9.79 to 12.50 mg/l.

To observe the growth of plankton, the qualitative (Table 1(f)) and quantitative (Table 1(g)) estimation was made weekly. A total of 12 species of phytoplankton belonging to Chlorophyceae (5 species), Bacillariophyceae (5 species), Cyanophyceae (1) and Euglenophyceae (1) were recorded from control tanks. A total of 13 species of phytoplankton belonging to Chlorophyceae (6 species), Bacillariophyceae (6 species) and Euglenophyceae (1) were recorded from treatment tanks. Initially, the algal bloom was noticed in some experimental tanks (monthly, fortnightly, one dose and controlled tanks), but after 2–4 weeks, these were not seen in probiotic-treated tanks. There were no cyanobacterial algae in the treatment tanks, but its presence in controlled tanks revealed that the probiotic supplementation checked the growth of harmful algae. A total of 7 species of zooplankton belonging to Rotifera (2), Copepoda (3) and Cladocera (2) were recorded from control and treatment tanks.

Table 1 (a) Weekly variations in water quality parameters of controlled tank (T_1) during the experimental period. (b) Weekly variations in water quality parameters of once during the experiment dose tank (T_2) during the experimental period. (c) Weekly variations in water quality parameters of weekly dose (T_3) during the experimental period. (d) Weekly variations in water quality parameters of fortnightly dose (T_4) during the experimental period. (e) Weekly variations in water quality parameters of weekly dose (T_5) during the experimental period. (f) The qualitative analysis of plankton population in treatment tanks. (g) Quantitative analysis of plankton in different tanks (Nos./l)

(a)

Week	Temp (°C)	Turbidity (NTU)	pH	DO (mg/l)	Free CO_2 (mg/l)	Total alkalinity (mg/l)	Nitrite (mg/l)	Chloride (mg/l)
1	20.05	1.25	7.8	6.31	1.95	175	0.01	5
2	20.85	1.33	7.6	6.5	1.6	177.5	0.01	5
3	21.65	1.36	7.5	6.65	1.35	166.5	0.02	5
4	23.0	1.4	7.65	6.65	1.25	162.5	0.02	10
5	22.25	1.45	8	7	1.95	161.5	0.03	10
6	23.0	1.25	7.9	7.05	2.45	167	0.03	10
7	23.2	1.5	7.85	6.8	2.45	168.5	0.04	12.5
8	23.3	1.4	8	6.75	2.15	172	0.02	12.5
9	22.95	1.45	7.85	7	2.45	173	0.02	15
10	23.3	1.4	7.6	6.65	1.95	172.5	0.05	10
11	23.85	1.55	7.85	6.65	1.65	155	0.06	12.5
12	24.05	1.65	7.85	6.85	2	167.5	0.01	10
Mean	22.62	1.42	7.79	6.74	1.93	168.21	0.03	9.79
SD	1.16	0.11	0.16	0.21	0.39	6.09	0.02	3.14

(b)

Week	Temp (°C)	Turbidity (NTU)	pH	DO (mg/l)	Free CO_2 (mg/l)	Total alkalinity (mg/l)	Nitrite (mg/l)	Chloride (mg/l)
1	20.2	1	7.6	6.75	1.8	176	0.01	5
2	20.5	1.35	7.85	6.55	1.6	172	0.02	5
3	21.25	1.45	7.85	6.45	1.55	173	0.01	10
4	22.55	1.25	8	6.6	1.75	172.5	0.02	10
5	23.2	1.45	7.75	6.85	1.35	168.5	0.03	15
6	23.15	1.55	7.85	7.05	2.05	166	0.02	15
7	23.3	1.5	8	7.05	2.15	171	0.01	10
8	23.05	1.45	7.85	6.75	2.2	168.5	0.03	10
9	23.25	1.65	8	7	2	169	0.055	5
10	23.75	1.45	7.6	7.2	2.1	170	0.02	15
11	23.7	1.65	7.6	6.85	1.95	176	0.03	12.5
12	24.65	1.75	8	7.05	1.95	175	0.03	10
Mean	22.71	1.46	7.83	6.85	1.87	171.46	0.02	10.21
SD	1.36	0.20	0.16	0.23	0.26	3.21	0.01	3.76

(c)

Week	Temp (°C)	Turbidity (NTU)	pH	DO (mg/l)	Free CO_2 (mg/l)	Total alkalinity (mg/l)	Nitrite (mg/l)	Chloride (mg/l)
1	20.1	1.3	7.85	6.65	1.6	168.5	0.01	5
2	20.1	1.45	7.6	6.45	1.75	169.5	0.01	5
3	21.5	2	7.6	6.85	2	171.5	0.02	10
4	22.3	1.9	7.85	7	2.1	169.5	0.02	10
5	23.05	2.2	8	7.15	1.95	173	0.03	10
6	23.35	2.3	7.6	6.95	1.95	177	0.03	15
7	23.15	2.45	7.85	7.3	2.15	173	0.04	10
8	23.5	2.6	8.25	7	2.35	175.5	0.02	10
9	23.85	2.95	7.6	6.85	2.35	176.5	0.02	15
10	24	3.05	7.85	6.8	2.2	172.5	0.05	12.5
11	23.95	3.55	7.85	7	2.2	170	0.06	20
12	24.45	3.6	8	6.85	2	172	0.06	15
Mean	20.1	1.3	7.85	6.65	1.6	168.5	0.01	11.45
SD	20.1	1.45	7.6	6.45	1.75	169.5	0.01	4.32

Table 1 continued

(d)

Week	Temp (°C)	Turbidity (NTU)	pH	DO (mg/l)	Free CO_2 (mg/l)	Total alkalinity (mg/l)	Nitrite (mg/l)	Chloride (mg/l)
1	20.05	1.29	7.6	6.5	1.75	166.5	0.02	5
2	20.85	1.36	7.5	6.65	2	162.5	0.02	5
3	21.65	1.4	7.65	6.65	2.1	161.5	0.03	10
4	23	1.45	8	7	1.95	167	0.03	12.5
5	22.25	1.25	7.9	7	1.95	169.5	0.04	12.5
6	23	1.5	7.85	7.15	1.75	171.5	0.02	15
7	23.2	1.45	7.85	6.95	1.35	169.5	0.01	10
8	23.3	1.55	8	7.3	2.05	173	0.02	15
9	23.05	1.5	7.6	7.05	2	168.5	0.03	12.5
10	23.25	1.45	7.85	6.75	1.9	169	0.02	17.5
11	23.75	1.65	7.6	7	1.8	170	0.03	15
12	23.7	1.55	7.6	6.85	1.85	170	0.03	12.5
Mean	22.59	1.45	7.75	6.90	1.87	168.21	0.03	11.88
SD	1.16	0.11	0.18	0.23	0.20	3.39	0.01	3.86

(e)

Week	Temp (°C)	Turbidity (NTU)	pH	DO (mg/l)	Free CO_2 (mg/l)	Total alkalinity (mg/l)	Nitrite (mg/l)	Chloride (mg/l)
1	20.5	1.4	7.6	6.5	1.35	161.5	0.03	5
2	20.2	1.45	7.5	6.65	1.25	167	0.04	10
3	21.65	1.25	7.65	6.65	1.35	169.5	0.02	5
4	23	1.5	8	7	1.6	171.5	0.02	5
5	22.25	2	7.9	7	1.75	168.5	0.03	10
6	23	1.9	7.85	6.65	2	169.5	0.03	12.5
7	23.3	2.2	7.85	6.45	1.95	171.5	0.05	12.5
8	23.05	2.05	7.6	6.85	1.75	169.5	0.06	15
9	23.25	1.8	7.85	6.6	1.35	173	0.06	20
10	23.85	1.85	7.85	6.85	1.7	168.5	0.05	20
11	24	1.95	7.85	6.8	2.05	169	0.06	15
12	23.95	1.7	7.85	6.55	1.35	170	0.06	20
Mean	22.67	1.75	7.78	6.71	1.62	169.08	0.04	12.50
SD	1.28	0.30	0.15	0.18	0.29	2.87	0.02	5.74

(f)

Plankton	Control tanks	Treatment tanks
Phytoplankton	*Cholorophyceae—*	*Cholorophyceae—*
	Chlorella sp., *Cosmarium* sp.	*Chlorella* sp., *Cosmarium* sp.
	Spirogyra sp. *Pediastrum* sp.	*Spirogyra* sp. *Pediastrum* sp.
	Scenedesmus sp.	*Chlamydomonas* sp.
		Scenedesmus sp.
	Bacillariophyceae—	*Bacillariophyceae—*
	Synedra sp.	*Synedra* sp.
	Navicula sp.	*Navicula* sp.
	Nitzschia sp., *Melosira* sp.	*Nitzschia* sp.
	Tabellaria sp.	*Melosira* sp.
		Tabellaria sp.
		Cymbella sp.
	Euglenophyceae—Euglena sp.	*Euglenophyceae—Euglena* sp.
	Cyanophyceae—Oscillatoria sp.	

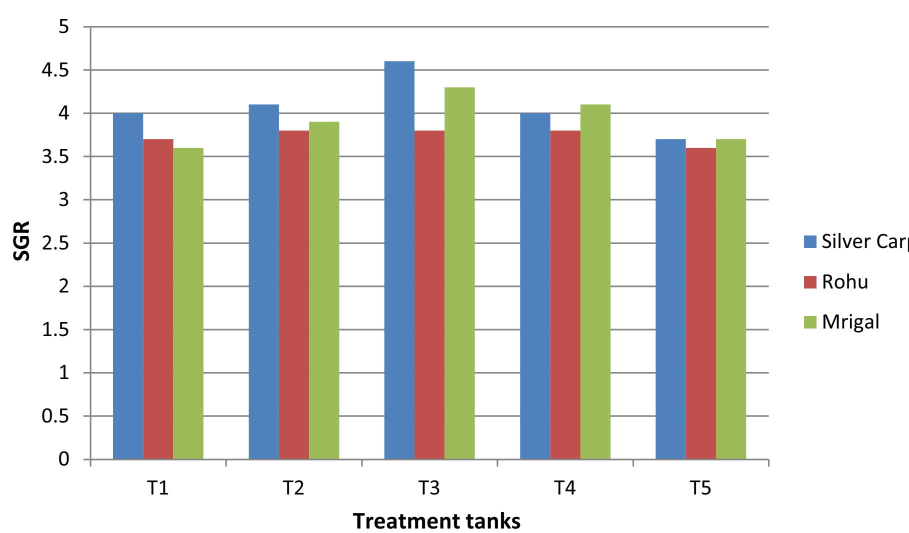
The fish growth was estimated in the form of SGR. The highest value of SGR was recorded from weekly dose tank for all the experimental fishes, i.e. Silver Carp (4.6), Rohu (3.8) and Mrigal (4.3) which shows that this dose of the probiotics (2 ml/m^3 weekly) is most suitable for maximum sustainable growth of fishes (Fig. 1) with Silver Carp growing the fastest. At 5% level of significance using ANOVA, the average specific growth rate of fishes was found significantly different.

The water temperature was almost same in all the tanks and it increased gradually with rise in air temperature. The probiotic regulated the murkiness of water and out of the treated tanks least turbid were the tanks with less algae and plankton due to the gap between two doses. The most turbid was weekly dose tank where the turbidity was high due to plankton growth stimulated by the probiotic. The pH of water in all the tanks was normal and in productive range. pH values above 7 are most suitable and responsible for uniform mixing of nutrients in water. The weekly and fortnightly dosed tanks showed good trends of dissolved oxygen as compared to other ones. All the tanks were equipped with the facility of aeration, and DO level was also maintained when needed. This resulted in better functioning and growth of fishes. The level of free CO_2 in all the tanks was optimum and thus probiotic helped in improving the water quality. The optimum range of alkalinity for aquaculture is > 100 mg/l [17] and the use of the probiotic helped in maintaining the higher values of carbonate and bicarbonate ions in the water.

The physico-chemical parameters and nutrient content of water play a significant role in the distributional patterns and species composition of primary organisms [18]. The moderate temperature (20.1–22.67 °C) is favourable for good growth of plankton. Similarly, the pH values (7.75–7.85) are also suitable for a high rate of biological production. The range of alkalinity in the experimental tanks was found in optimum range (168.21–171.46 mg/l).

When bacteria are added into the water, they could decompose the excreta of cultivable aquatic species, viz. fish and shrimp, remaining food materials, remains of the plankton and other organic materials to CO_2, nitrate and phosphate. These inorganic substances provide the nutrition for the growth of microalgae, while the bacteria grow rapidly and become the dominant group in the water, inhibiting the growth of the pathogenic microorganisms. The photosynthesis of the micro algae provide sufficient dissolved oxygen for oxidation and decomposition of the organic materials and for the respiration of the microbes and cultured animals. This kind of cycle may improve the nutrient cycle, and it can create a balance between bacteria and microalgae, and maintaining a good water quality environment for the cultured animals [19]. The probiotics brought feed cost down, improve water quality, healthy plankton production, proper feed degradation, reduction in malodour, breakdown of unwanted nutrients and checked algal bloom [19]. Probiotic brought feed cost down by providing natural food, viz. phytoplankton and zooplankton for planktivorous fishes (Silver Carp and Rohu) and detritus for bottom dwelling fish (Mrigal) [17]. Probiotic helps in rapid fish growth by improving water quality parameters [20]. In the present investigation, unused feed and other organic matters were degraded very quickly in the probiotic-treated tanks and less sludge formation was seen there comparison to the tanks without treated with the probiotics. Although for first 2–4 weeks an initial algal bloom was noticed in monthly, fortnightly and one-dose tank, yet soon it was stabilized by the probiotic and was not seen further. It is a matter of fact that none of the fishes showed any sign of pathogen and disease, as checking the

Fig. 1 Specific growth rate (SGR) of experimented fishes in various treatment tanks



growth of the pathogenic organism was one of the effects of probiotic.

To conclude, it can be stated that using the probiotic weekly (T_3) showed the best results with maximum effects and benefits including maximum growth of experimented fishes (Silver Carp, Rohu and Mrigal), i.e. highest specific growth rate and better condition factor followed by fortnightly (T_4), monthly (T_5) and once (T_2) tanks and this is supported by [21]. Probiotic treatment based practices of aquaculture would be very effective and sustainable without causing any harmful effect to the environment. Also it will increase the yield of cultured fish species and income of fish farmers throughout the globe.

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