Impact of probiotic supplementation on water quality and behaviour parameters in *Cyprinus carpio*

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Abstract

The present study was carried out to study the effects of probiotic formulation product, Vapflex Aquafloc Pro on water quality in *Cyprinus carpio* fish aquaculture system. The observations were taken for a duration of four weeks during which the parameters of ammonia concentration, pH, temperature, total dissolved oxygen and behaviour were considered. All the parameters were greatly controlled in the probiotic treated group as compared to control, indicating beneficial effects of probiotics and efficient recycling of organic waste. The formulation of the product with a suitable carbon source could prove to be very efficient supplement for aquaculture systems.

Keywords: probiotics, ammonia concentration, pH, total dissolved oxygen, behaviour

1. Introduction

Probiotics are a vital component of the functional foods that have been studied widely in recent years for the wide range of functions [1,2]. The functions of probiotics are not limited to humans, they exhibit several functions in animals as well. They are used in poultry for better body weight increase and the feed conversion ratio. Apart from this the probiotics were also observed to control the pathogenic microorganisms in poultry [3]. The probiotic microorganisms have been known to enhance swine production. The probiotic *Lactobacillus plantarum* JDFM LP11 promotes beneficial microbial flora that helped in the development of intestine in weaned piglets [4]. The probiotics have recently been evaluated for their number of benefits in aquaculture. Antibiotics that are given with feed to aquatic animals, kill beneficial bacteria as well. Scientists have searched for suitable alternatives such as probiotics to be supplemented with diet, as these microorganisms have several functions and enhance healthy microflora in aquatic ecosystem [5]. The present study was aimed to study the effects of probiotics in common carp *Cyprinus carpio* since it plays a very important role in aquaculture production systems [6].

2. Materials and Methods

2.1 Study design

*Common carp* (10 ± 2 g) were purchased from a local commercial fish farm and transferred to aquarium located at R&D Biotech Lab of Bioprocessing Unit, Tropilite Foods Pvt. Ltd., in polyethylene bags. Ten fishes were randomly distributed into each aquarium (2 aquariums of 35 L each), which were filled with unchlorinated water. The tanks were labelled as control and treated. Both the tanks were maintained at a photoperiod of 12 h light/12 h dark during the period of trial. The feed was given to fishes twice per day for a period of four weeks and it was 2% of the total fish biomass. Vapflex Aquafloc Pro, a multistrain probiotic formulation was selected for the study. Each 100 g of the product contains probiotics (1200 Billion spores), consisting of *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus licheniformis* and *Saccharomyces cerevisiae*. The product also contained Maltodextrin, Citric acid, Yucca Schidigera, Digestive Enzymes and other active ingredients. The probiotic was supplemented as per manufacturer’s protocol. The required quantity of probiotic (dosage: 300g/10,000 litre of water) was calculated for fish tank containing 30 litres of water.
The calculated amount was weighed and dissolved in 100 ml of water with 10% jaggery (carbon source), mixed thoroughly and spread all over the tank \[7,8\]. The probiotic starter was added along with carbon source every week.

2.2 Analysis of Water Quality Parameters

2.2.1 pH: The sampling of water was done every week for pH measurement from both control and treated tank. The pH was measured using Systronics \(\mu\) pH System 361 electrode.

2.2.2 Total dissolved oxygen: The total dissolved oxygen was measured using oxygen probe of Hamilton (Oxyferm FDA 225) PN 237452.

2.2.3 Total ammonia content: The ammonia content was measured using the method mentioned in FSSAI manual \[9\].

2.2.4 Temperature: The temperature was recorded using mercury thermometer.

2.3 Behaviour: Some behavioral changes were observed in fishes given probiotic treatment \[10,11\]. Since we are a part of a corporate organization, we do not have an ethical committee to seek approval for the study, however, the experiments were carried out ethically to the best of our knowledge. Results are expressed as mean \(\pm\) SE of six set of observations. Statistical analyses were performed using Sigma Stat Statistical software version 2.0. All the statistical analyses were performed using one-way analysis of variance with post hoc Bonferroni’s multiple comparison test applied across the treatment groups. Significance was based on P value < 0.05.

3. Results and Discussion

In the present study, the major parameters being affected by probiotic supplementation were ammonia concentration and pH. These parameters play an important role in determining water quality \[12\]. From the experiments it was observed that the pH of water and ammonia concentration was well regulated in the probiotic supplemented group. During the first week the concentration of ammonia in the probiotic supplemented group was 25% less as compared to control. In the second week the decrease in ammonia levels was 30.84% in the probiotic supplemented group. During the third and fourth week, the decrease was 66.66% and 100% respectively as compared to control, thereby indicating efficient recycling of nitrogenous waste in the probiotic containing aquaculture system (Table 1).

### Table 1: Effect of probiotic supplementation on ammonia concentration in fish tank

| Group     | Exposure period in Weeks - (mg/L) |
|-----------|-----------------------------------|
| Control   | I      | II       | III      | IV       |
|           | 0.150 \(\pm\) 0.01 | 0.140 \(\pm\) 0.01 | 0.170 \(\pm\) 0.01 | 0.20 \(\pm\) 0.01 |
| Probiotic | 0.120 \(\pm\) 0.01*** | 0.107 \(\pm\) 0.01*** | 0.102 \(\pm\) 0.01*** | 0.10 \(\pm\) 0.01*** |

\(P<0.05, \ P<0.01, \ **P<0.0001\) and \(P>0.05\) when compared with respective control.

Similarly, the pH was also controlled in the probiotic treated group. The pH was 2.9% less as compared to control during the first week, followed by a decrease of 6.59%, 3.89% and 7% during the second, third and fourth week respectively (Table 2).

### Table 2: Effect of probiotics on pH of water in fish tank

| Group     | Exposure period in Weeks |
|-----------|--------------------------|
| Control   | I      | II       | III      | IV       |
|           | 8.08 \(\pm\) 0.09       | 7.92 \(\pm\) 0.02   | 8.01 \(\pm\) 0.01   | 7.89 \(\pm\) 0.01   |
| Probiotic | 7.85 \(\pm\) 0.06*       | 7.43 \(\pm\) 0.05**  | 7.71 \(\pm\) 0.01   | 7.37 \(\pm\) 0.02   |

\(P<0.05, \ P<0.01, \ **P<0.0001\) and \(P>0.05\) when compared with respective control.
cancer prevention, cholesterol reduction, Vitamin A synthesis, the ability of probiotics in enhancing the gastrointestinal microbiota of fishes is also mentioned [19]. In our study, the temperature was maintained in the range that is optimum for the growth and survival of Cyprinus carpio [20] (Table 3).

Table 3: Temperature of fish tank water during trial period

| Group         | I  | II | III | IV  |
|---------------|----|----|-----|-----|
| Control       | 27.03 ± 0.09 | 25.97 ± 0.03 | 25.0 ± 0.03 | 22.07 ± 0.03 |
| Probiotic     | 26.90 ± 0.06* | 25.93 ± 0.07* | 24.97 ± 0.03* | 21.67 ± 0.33* |

*P<0.05, *P<0.01, **P<0.0001 and *P>0.05 when compared with respective control.

The levels of dissolved oxygen were closer to control values, indicating increased oxygen demand due to increased numbers of probiotic microorganisms in the fish tank (Table 4).

Table 4: Effect of probiotic supplementation on total dissolved oxygen

| Group         | I         | II         | III        | IV         |
|---------------|-----------|------------|------------|------------|
| Control       | 125 ± 0.58% | 121.33 ± 0.88% | 133.33 ± 0.88% | 135 ± 0.58% |
| Probiotic     | 120.67 ± 0.88%* | 118 ± 0.58%* | 130.00 ± 0.58%* | 130 ± 0.58%** |

*P<0.05, *P<0.01, **P<0.0001 and *P>0.05 when compared with respective control.

In a study conducted by Reddy and Naik, it was observed that there were increased dissolved oxygen reduction rates in fishes receiving combination of probiotics with a carbon source i.e. molasses, wheat flour and rice flour, as compared to control [21]. Combination of our probiotic formulation with carbon sources other than jaggery could probably increase the dissolved oxygen concentration in aquaculture water. A remarkable difference was also observed in behavior of fishes given probiotics treatment. They were active in consuming feed and also swam freely whereas the control fishes were confined to a corner of the tank most of the time (Table 5).

Table 5: Comparison of behaviour in fishes given probiotic supplementation with control during duration of the study

|                   | Rate of swimming | Corner Behaviour |
|-------------------|------------------|-----------------|
| Control           | ++               | +++             |
| Probiotic         | +++              | +               |

(-) None, (+) Mild, (++) Moderate, (+++) Strong

Similar findings have been reported in a study where a combination of Lactobacillus rhamnosus CECT8361 and Bifidobacterium longum CECT7347 altered the swimming pattern in zebrafish model. Here the bottom dwelling behaviour of zebrafish was greatly reduced, indicating lower levels of anxiety due to probiotic consumption [22].

4. Conclusion
The present study demonstrates the ability of probiotic formulation Vapflex Aquafloc Pro in improving quality of water in fish tank, thereby making them an essential supplement for aquaculture systems. The property of probiotics to improve behavioral aspects of aquatic animals further makes them a beneficial dietary supplement.

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