Effect of probiotic (Proteus) on hematological parameters of *Labeo rohita* (Hamilton, 1822) fingerlings

Praveen Kumar, Rohitash Yadav, BK Sharma, SK Sharma and Rajpal Yadav

Abstract

The study deals with the “Effects of Feed Probiotic (Proteus) on *Labeo rohita* (Hamilton) Fingerlings.” The trial conducted in triplicate for each treatment diet. During the procedure of 60 days, the fingerlings of rohu (*Labeo rohita*) were fed on four different probiotics (Proteus), mixed in the basal diet containing rice bran, groundnut cake, fish meal, and mineral mixture. The four different feed ingredients combined with conventional carp diet at 2g/kg, 3g/kg, 4 g/kg, and 5g/kg of feed was used. The control containing rice bran, groundnut cake, fish meal, and the mineral mixture also run separately. These experimental feeds were fed to fishes @ 4% of body weight per day. The growth performance of treatment fishes observed every fortnight, and accordingly, the fishes readjusted. During the experimental period, the water quality parameters were found suitable for fish growth. T₃ group of fishes showed a higher value of T.L.C. (35.114×10⁶/µl), T.E.C. (3.35×10⁶/µl) and Hb concentration (10.2g%) as compared to control and other treatments. The results indicated that T₃ was the best treatment that realized a significant (P = 0.05) increase in all growth parameters, hematological, and biochemical parameters. According to results, it can be achieved that the inclusion of the commercial probiotic (Proteus) @ 4g/kg in the diet of *Labeo rohita* is useful to get the best growth fish performance with aqua friendly effects on the environment.

Keywords: *Labeo rohita*, probiotic (Proteus), hematological parameters

1. Introduction

Aquaculture, the fastest-growing animal food-producing sector, is perceived as having the highest potential to meet the increasing demand for aquatic food [1]. In the World, production attained another all-time high of 158.0 million tons from which aquaculture contributes about 66.6 million tons. The aquaculture development worldwide is gaining status in the total fish supply chain, which has endured uninterrupted-Asia, producing more farm cultured fish than wild catch since 2008. Share of Asia's global aquaculture production has touched 89%. Aquaculture practices involved using high-quality feeds with high crude protein content, which should contain not only necessary nutrients but also complementary additives to keep organisms healthy and result in excellent growth performance. The aquaculture industry faces the challenges of inadequate supply, and the high cost of quality fish feeds. Probiotics were initially incorporated into the feed to increase the animal's growth and improve its health [2].

At present, probiotics have become an integral part of the aquaculture practices to gain high production. Probiotics the live microorganisms are beneficial to the host organism/animal. The presence of probiotics in feed nutrition, disease resistance, and other useful activities in fish has proven beyond doubt. Probiotics have numerous health benefits, improve in the immune system is one of the most commonly purported benefits of probiotics and their potency to stimulate systemic and local immunity. The term probiotic, initially given by Lilley and Stillwell in which coined from the green words "Pro" and "bios" means "for life", an abstract secreted by a microorganism, which stimulated the growth of another [3]. The exact approach of stroke of probiotics is yet to be established in any animal, including fish. Probiotic therapy helps to treat several gastrointestinal infections. In aquaculture for the treatment of may disease, Probiotics have become an alternative to antibiotics which commercially available as feed/water additives in pond water [4, 5, 6]. Probiotics may help in arouse appetite and improve nutritional value by producing vitamins, detoxifying compounds in the diet, and the breakdown of indigestible components.
These also help to keep the water clean. Supplementary feeds bear the 60% culture cost in aquaculture, quality of feed forms criterion in the yield and profit. Feed cost is a crucial operating expance that should receive attention from the aquaculturists. It is feasible to use cheap, readily available, and formulated diets, which are palatable and have high growth efficiency, protect the fish from stress and should be able to induce immunity in the fish. Therefore, in the present study, investigate the probiotic effects of Proteus through supplementary diet on the growth performance and hematological parameters of Labeo rohita (Hamilton) fingerlings.

2. Materials and Methods
The present study was carried out for sixty days with a view to investigate immunostimulatory effects of feed probiotic (Proteus) in Labeo rohita (Hamilton) fingerlings for assessing aqua cultural possibilities. For this purpose, laboratory studies were conducted in the College of Fisheries, Udaipur.

2.1 Experimental fish
Labeo rohita fingerlings were procured from aquaculture research and seed unit, DOR, MPUAT, Udaipur. Then after the fish fingerlings acclimatized for a week. Fish fingerlings were feeding the duration of the acclimatized period.

2.2 Experimental structure
The experimental structure was four treatments and one control, and all the treatments and control are triplicates. In each tank, per replicate ten Labeo rohita fingerlings. The experimental duration period 60 days and growth sampling fortnightly was done-the feeding was scheduled morning and evening, and the feeding rate 3% body weight.

2.3 Carp feed (with 24% protein) were formulated using groundnut cake, rice bran, fish meal, and mineral mixture and a commercially available probiotic (Proteus). Probiotics were purchased from Forever GENESIS Company at Nandanar Nagar, North Lallaguda, Secunderabad, Andhra Pradesh, India. The treatment tank (T0) considered as control, which was without probiotic, while groups T1, T2, T3, and T4 were included with probiotic and added at @ 2g/kg, 3g/kg, 4 g/kg and 5g/kg of feed levels respectively. The dry ingredients of the experimental diets (groundnut cake, rice bran, fish meal, and minerals mixture) were thoroughly mixed and boiled water added to give pellet form. The paste was then extruded through a commercial pelleting machine. The resulting spaghetti-like diet (2.0 mm diameter) were air-dried and stored in airtight containers for further use.

2.4 Hematological analysis
The hematological examination was carried out to estimate the effect of the diet on the fish to determine the growth, stress resistance, and specific and non-specific immunity of the fish.

2.4.1 Blood samples
Each fish was individually caught using a scoop net, weighed, and blood was collected from the caudal peduncle using a 2 ml syringe. Blood was collected in EDTA anti-coagulant vials. Blood parameters were analyzed through the standards protocol.

2.4.2 Estimation of Total Leukocyte Count (T.L.C.)
T.L.C. implies the total number of WBC in 1 µl of blood. Well, mixed-blood was drawn in WBC pipette up to 0.5 marks. Excess blood was wiped out. The WBC pipette fluid was sucked into the WBC pipette by 11 points. The material was mixed well by moving the pipette between the palms. The first 2-3 drops were rejected with a diluted pipette. Neubauer's counting chamber was charged by touching the tip of the pipette at the junction of the chamber and cover glass. The counting chamber was left for 2-3 minutes. Then focusing on a microscope on the central grid having 25 squares, all the cells in four corners and small central square were counted using high power objectives.

\[
TLC = \text{(No. of cells in four corner grids} \times 50) / \mu l
\]

2.4.3 Estimation of Total Erythrocyte Count (T.E.C.)
T.E.C. implies the total number of R.B.C. in 1 µl of blood. Well, mixed-blood was drawn in R.B.C. pipette up to 0.5 marks. Excess blood was wiped out. R.B.C.s diluting fluid was sucked up to 101 targets in R.B.C. pipette. The content was mixed well by rotating the pipette between the palms. The first 2-3 drops were discarded from the diluting pipette. Neubauer's counting chamber was charged by touching the tip of the pipette at the junction of the chamber and cover glass. The counting chamber was left for 2-3 minutes. All the cells in four corners and small central square, Focusing the microscope on the primary grid having 25 squares, were counted using high power objectives.

\[
TEC = \text{(No. of cells in 5 small squares} \times 10,000) / \mu l
\]

2.4.4 Hemoglobin estimation
A graduated measuring tube of Sahli's haemoglobinometer was filled with a few drops of N/10 HCl. Blood was collected in EDTA anti-coagulant vials. Blood was drawn in WBC pipette up to 0.5 marks. Excess blood was wiped out. Blood transferred into a haemoglobinometer. N/10 HCl added drop by drop, mixing with the help of stirring rod till the color matches with the standard. Results were taken at the lower meniscus in terms of gm%.

2.4 Statistical analysis
The experiment in the results mean, percent, standard error of the mean, coefficient of variation, and the critical difference was used to analyze the effect of probiotic (Proteus) on hematological parameters of Labeo rohita fingerlings.

3. Results and Discussions
3.1 Hematological parameters
The hematological parameters of Labeo rohita before and after feeding with different levels of probiotic (Proteus) are shown in Table 2.

Table 1: The details of the ingredients used for basal diets (g/100g).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredients</th>
<th>Control T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Groundnut Oil Cake (GOC)</td>
<td>18.4</td>
<td>18.4</td>
<td>18.4</td>
<td>18.4</td>
<td>18.1</td>
</tr>
<tr>
<td>2</td>
<td>Rice Bran</td>
<td>18.4</td>
<td>18.2</td>
<td>18.1</td>
<td>18.0</td>
<td>18.0</td>
</tr>
<tr>
<td>3</td>
<td>Fish Meal</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>Wheat Flour</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td>Tapioca Flour</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Mineral Mixer</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Probiotic (Proteus) (%)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

2.4 Hematological analysis
The hematological examination was carried out to estimate the effect of the diet on the fish to determine the growth,

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3.2 Total Erythrocyte Count (T.E.C.)

The observation of Total Erythrocyte Count in different treatment groups as depicted in Table 2. The highest T.E.C. level was found in T3 (diet with 4g/kg), significantly different from T4, T2, T1, and Control (P = 0.05, CD= 0.146). The values of means revealed that the best total erythrocyte count was recorded in T3 (3.35 x1012/µl), followed by T4 (3.25 x1012/µl), T2 (3.12 x1012/µl), T1 (2.98 x1012/µl) and minimum in T0 control (2.45 x1012/µl). Higher R.B.C. count is a sign of good health condition of fishes.

The highest Total Erythrocyte Count (T.E.C.) level was found in T1 (diet with 4g/kg), which was significantly different from T4, T2, T3, and T0 (control). The significant erythrocyte count was recorded in T3 (3.35 x1012/µl), followed by T4 (3.25 x1012/µl), T2 (3.12x 1012/µl), T1 (2.98 x1012/µl) and minimum in T0 control (2.45 x1012/µl). The above findings were observed higher R.B.C. count in poultry and mix feed; this can be due to the age of the fish cycle of sexual maturity and good health condition [10-6].

3.3 Total Leukocyte Count (T.L.C.)

The details of the total leukocyte count in different treatment groups of fishes have been depicted in Table 2. The highest T.L.C. level was found in T3 (diet with 4g/kg), which was significantly different from T4, T2, T1, and Control (P = 0.05, CD= 1.415). The means values of revealed that the best total leukocyte count was recorded in T3 (35.114 x 10³/µl), followed by T4 (32.112 x 10³/µl), T2 (29.9 x 10³/µl), T1 (25.3 x 10³/µl) and minimum in T0 control (22.240 x 10³/µl). Thus, treatment T3 showed the best T.L.C. level, which is significantly (P = 0.05, CD= 1.415) higher control T0, and all other treatments.

The highest Total Lymphocyte Count (T.L.C.) level was found in T1 (diet with 4g/kg), which was significantly different from T4, T2, T3, and T0 (control). The finest leukocyte count was recorded in T3 (35.114 x 10³/µl), followed by T4 (32.112 x 10³/µl), T2 (29.9 x 10³/µl), T1 (25.3 x 10³/µl) and minimum in T0 control (22.240 x 10³/µl). Sherwani observed WBC count highest in cow dung supplemented treatment; this may be due to an infection or due to stress, which can be due to metabolic disturbances [13]. Immune system of major carp revealed similar responses to unfavourable conditions and an increase in leukocyte cells of fish infected with the parasite in Europe [1]. It also reported comparable results while evaluation the potential of Bacillus subtilis on growth, hematology, and biochemical parameters in the Indian major carp Labeo rohita challenged with Aeromonas hydrophilia. The T.L.C., Hb content, total protein, and globulin content were significantly (P< 0.05) higher in control after two weeks of the experimental period [11].

3.4 Hemoglobin

Profile of hemoglobin obtained in different treatment groups has been presented in Table 2. Hemoglobin analysis of fish blood showed the highest Hb level in treatment T1 (diet with 4g/kg.). The mean values of treatment revealed that the best Hb level was recorded in T3 (10.2g%), followed by T2 (10.0g%), T4 (9.8g%), T1 (7.9g%) and minimum in T0 control (7.6g%). Treatment T4 showed the second-best Hb concentration level, which is significant (P = 0.05, CD= 0.452) high compared to control and other treatments.

Hemoglobin (Hb) analysis of fish blood showed the highest Hb level in treatment T1 (diet with 4g/kg.). The significant Hb level was recorded in T3 (10.2g%), followed by T4 (10.0g%), T2 (9.8g%), T1 (7.9g%), and minimum in T0 control (7.6g%). This that the hematological parameters of fish are affected by a range of factor which includes size, age, physiological status, environmental conditions and dietary regime (e.g., quality and quantity of food, nutritional ingredients, protein sources, vitamins, and probiotics) [10].

It is to state that besides growth performances and other related parameters obtained in the present study, in general, the experimental fish were received in good health without a sign of any stress or disease. Thus, the use of probiotics in the test fish certainly helped in importing immunostimulatory effects. Such effects have also been reported earlier, he assessed the development and immunostimulatory effects of the biomedicine biogen R on mono sex Nil tilapia, Oreochromis niloticus under different stocking densities [8]. The significant increase was observed in T4, i.e., Biogen at a level of 3 g/kg of diet at a stocking density rate of 30 fish/m³ of mono sex Nile tilapia (Oreochromis niloticus). It was regarded as the excellent treatment which significantly (P< 0.05) increased all growth performances function (final weight, A.W.G., A.D.G.), hematological parameters (hemoglobin, R.B.C. count, PCV, blood platelets and WBC count), plasma proteins (total proteins, albumin, globulin, and albumin/globulin ratio), improved F.C.R., blood indices (MCV, M.C.H. and MCHC), differential leucocytes, carcass composition and histometric examination of dorsal fish muscle.

Table 2: Initial and final observations of hematological parameters in Labeo rohita fingerlings:

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T.E.C. x10³/µl</th>
<th>T.L.C. x10³/µl</th>
<th>Hemoglobin (g%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Initial</td>
</tr>
<tr>
<td>Control</td>
<td>2.23±0.022</td>
<td>2.45±0.004</td>
<td>20.24±0.095</td>
</tr>
<tr>
<td>T1</td>
<td>2.73±0.016</td>
<td>2.98±0.003</td>
<td>23.30±0.041</td>
</tr>
<tr>
<td>T2</td>
<td>3.03±0.125</td>
<td>3.12±0.002</td>
<td>27.89±0.052</td>
</tr>
<tr>
<td>T3</td>
<td>3.15±0.041</td>
<td>3.35±0.034</td>
<td>33.10±0.017</td>
</tr>
<tr>
<td>T4</td>
<td>3.10±0.016</td>
<td>3.25±0.034</td>
<td>30.107±0.017</td>
</tr>
<tr>
<td>CD at 0.5%</td>
<td>0.140</td>
<td>0.146</td>
<td>1.323</td>
</tr>
<tr>
<td>CV%</td>
<td>2.70</td>
<td>2.64</td>
<td>2.70</td>
</tr>
</tbody>
</table>

Data expressed as Mean ± SEM
T.E.C = Total Erythrocyte count (10³/µl)
T.L.C = Total lymphocyte count (10³/µl)
HGB = Hemoglobin (g%)
Proteus probiotics on the physic-chemical parameters in the water tanks.

6. Acknowledgment
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7. References