Journal of Entomology and Zoology Studies
Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(5): 103-106

Received: 04-07-2020
Accepted: 15-08-2020

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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. T_3 group of fishes showed a higher value of T.L.C. (35.114×10^3/µl), T.E.C. (3.35×10^6/µl) and Hb concentration (10.2g%) as compared to control and other treatments. The results indicated that T_4 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 [4]. 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 [5].

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" [7]. 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 [9, 3, 13, 14] 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 expanse that should receive attention from the aqua culturists. 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 Experimental diet

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 (T_0) considered as control, which was without probiotic, while groups T_1, T_2, T_3, and T_4 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 pelletizing machine. The resulting spaghetti-like diet (2.0 mm diameter) were air-dried and stored in airtight containers for further use.

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

S. No.	Ingredients	Control	T_1	T_2	T_3	T_4
1	Groundnut Oil Cake (GOC)	18.4	18.4	18.4	18.4	18.1
2	Rice Bran	18.4	18.2	18.1	18.0	18.0
3	Fish Meal	45	45	45	45	45
4	Wheat Flour	12	12	12	12	12
5	Tapioca Flour	5	5	5	5	5
6	Mineral Mixer	1	1	1	1	1
7	Probiotic (Proteus) (in%)	_	0.2	0.3	0.4	0.5

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 pedal 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 = (No. of cells in four corner grids × 50) / µl.

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 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 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 = (No. of cells in 5 small squares × 10,000) / µl.

2.4.4 Hemoglobin estimation

A graduated measuring tube of Sahli's haemoglobinometer was filled with a few drops of N/10 HCl. Well, mixed-blood was drawn up to 20 µl marks in the pipette provided with Sahli's haemoglobinometer. 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.

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 T_3 (diet with 4g/kg), significantly different from T_4, T_2, T_1, and Control (P = 0.05, CD= 0.146). The values of means revealed that the best total erythrocyte count was recorded in T_3 (3.35 x10^6/µl), followed by T_4 (3.25x 10^6/µl), T_2 (3.12 x 10^6µl), T_1 (2.98 x 10^6µl) and minimum in T_0 control (2.45 x 10^6µl). 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 T_3 (diet with 4g/kg), which was significantly different from T_4, T_2, T_1, and T_0 (control). The significant erythrocyte count was recorded in T_3 (3.35 x10^6/µl), followed by T_4 (3.25x 10^6/µl), T_2 (3.12x 10^6µl), T_1 (2.98 x 10^6µl) and minimum in T_0 control (2.45 x 10^6µl). 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 [2, 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 T_3 (diet with 4g/kg), significantly different from T_4, T_2, T_1, and Control (P = 0.05, CD= 1.415). The means values of treatment revealed that the best total leukocyte count was recorded in T_3 (35.114 x 10^3/µl), followed by T_4 (32.112 x10^3/µl), T_2 (29.9 x 10^3/µl), T_1 (25.3 x 10^3/µl) and minimum in T_0 control (22.240 x 10^3/µl). Thus, treatment T_3 showed the best T.L.C. level, which is significantly (P = 0.05, CD= 1.415) higher control T_0, and all other treatments.

The highest Total Lymphocyte Count (T.L.C.) level was found in T_3 (diet with 4g/kg.), which was significantly different from T_4, T_2, T_1, and T_0 (control). The finest leukocyte count was recorded in T_3 (35.114 x 10^3/µl), followed by T_4 (32.112 x10^3/µl), T_2 (29.9 x 10^3/µl), T_1 (25.3 x 10^3/µl) and minimum in T_0 control (22.240x 10^3/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 [12]**.** 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 than 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 T_3 (diet with 4g/kg.). The mean values of treatment revealed that the best Hb level was recorded in T_3 (10.2g%), followed by T_4 (10.0g%), T_2 (9.8g%), T_1 (7.9g%) and minimum in T_0 control (7.6g%). Treatment T_4 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 T_3 (diet with 4g/kg.). The significant Hb level was recorded in T_3 (10.2g%), followed by T_4 (10.0g%), T_2 (9.8g%), T_1 (7.9g%), and minimum in T_0 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 T_4, i.e., Biogen at a level of 3 g/kg of diet at a stocking density rate of 30 fish/m^3 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., S.G.R.), 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:

Treatment	T.E.C. ×10^6 /µl		T.L.C. ×10^3 /µl		Hemoglobin (g%)	
	Initial	Final	Initial	Final	Initial	Final
Control	2.23±0.022	2.45±0.004	20.243±0.095	22.240±0.040	7.40±0.294	7.6±0.0514
T_1	2.73±0.016	2.98±0.003	23.300±0.041	25.3±0.39	7.70±0.216	7.9±0.520
T_2	3.03±0.125	3.12±0.402	27.897±0.052	29.9±0.394	9.60±0.245	9.8±0.032
T_3	3.15±0.041	3.35±0.034	33.107±0.017	35.114±0.013	10.01±0.164	10.2±0.0244
T_4	3.10±0.016	3.25±0.034	30.107±0.017	32.112±0.013	9.82±0.304	10.0±0.0244
CD at 0.05%	0.140	0.146	1.323	1.415	0.443	0.452
CV%	2.70	2.64	2.70	2.69	2.73	2.73

Data expressed as Mean ± SEM
TEC = Total Erythrocyte count (10^6 /µl)
T.L.C = Total lymphocyte count (10^3 /µl)
HGB = Hemoglobin (g%)

5. Conclusions

Results depict that the inclusion of the commercial probiotic (Proteus) @ 4g/kg in the diet of *Labeo rohita* was useful to get the best growth fish performance with aqua friendly effects on the environment. It is probiotic (Proteus), not affect the haematological parameters. There was no side effect of

Proteus probiotics on the physic-chemical parameters in the water tanks.

6. Acknowledgment
The author records their sincere thanks to Dean and HoD, Department of Aquaculture, College of Fisheries, MPUAT, Udaipur for their encouragement and for extending facilities for conducting this research.

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