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Effect of probiotics on the haematological parameters of Indian major carp (*Labeo rohita*)

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Abstract
The present study was carried out to evaluate the influence of dietary supplementation of probiotic bacteria (*Bacillus pumilus*) on haematological parameters of *Labeo rohita*. The Probiotic was isolated from the intestine of Indian major carp (*Labeo rohita*). The feeding trail was conducted for 60 days. The fish with similar body weight (5±1gm) were distributed randomly into three treatment groups, which fed a feed containing *Bacillus licheniformis*, *Bacillus cereus* and *Bacillus pumilus* in T1, T2, and T3. The control group (T4) was fed without probiotics for the same period. Blood samples were collected at the intervals 0, 15, 30, 45 and 60 days. The haematological parameters such as Total erythrocytes count (RBC), Haematocrit (Hct), Haemoglobin concentration (Hb), and Haematological indexes (MCV, MCH and MCHC) were examined. The *Bacillus pumilus* treated fish (T3) showed maximum percentage Total erythrocyte count, haemoglobin concentration and haematocrit concentrations than in other groups. The result suggests that *Bacillus pumilus* could be used effectively as a probiotics for the use in aquaculture.

Keywords: *Labeo rohita,* Probiotics and Haematological parameter.

1. Introduction
Rohu, *Labeo rohita* (Hamilton, 1822), a major candidate species for aquaculture in India as well as in other south-east Asian countries [1] contributed more than 0.95 million tones production during 2006 [2]. The Indian major carp *Labeo rohita* is a most important commercial fish in India with maximum market demand and acceptability as food by the consumers due to their taste and flesh. *Labeo rohita* contributes a major portion to the fresh water fish production in south India. Fish disease is widely distributed worldwide and is considered to be serious problems in aquaculture [3]. Aquaculture has made significant advances in recent years in the production of a wide range of aquatic organisms, both for human consumption and as ornamental species [4].
Bacterial infections are one of the most important causes of disease problems in Indian aquaculture especially in the production of cat fish [5]. *Aeromonas hydrophila* is the most common pathogen, and it can easily spread through accidental abrasions [6]. Among the common bacteria pathogens, *Staphylococcus xylosus*, *A. hydrophila* and *Streptococcus agalactiae* are known to seriously infect mortalities [7]. The blood parameters of fish provide accurate indications of any changes occurring in the organism as a result of injuries to organs or tissues related to infectious disease, similar to those of warm blooded animals [7, 8]. The use of probiotics in aquaculture is thus anticipated to be an excellent strategy for the prevention of infectious microbial diseases and to replace antibiotics and chemotherapeutic [9].
The term probiotics is defined as live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring an improved use of the feed or enhancing its nutritional value, by increasing the host response towards disease, or by improving the quality of its environment [10]. Probiotics in aquaculture have been shown to have several modes of action: competitive exclusion of pathogenic bacteria through the production of inhibitory compounds; improvement of water quality; enhancement of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes [10]. Because Bacillus bacteria secrete many exoenzymes [11], these bacteria have been widely used as putative probiotics. Thus, the use of probiotic in aquaculture has received some attention [12, 13, 14]. Some common

Bacterial strains are used as probiotic products such as *Lactobacillus acidophilus*, *L. bulgaricus*, *L. plantariu*, *Streptococcus lactis* and *Saccharomyces cerevisiae* [15]. Piraret *et al*. [16] found that number of mortality was significantly lower in probiotic supplemented fish than in control fish.

The knowledge of the haematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological changes in the fishes [17]. Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology [18, 19]. The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals [20]. So, the present study was designed to evaluate the effect of probiotic *Bacillus pumilus* On Haematological parameters Indian major carp *Labeo rohita*.

Materials and Methods

Experimental design

The study was carried out in the PG and Research Department of Zoology, Periyar E.V.R. College, Trichy (India). The experiment was conducted in laboratory condition for 60 days. Indian major carps were obtained from Raja private fish farm in Thanjavur district at Tamilnadu. The collected fish was transferred alive in polyethylene bags and brought to the laboratory and acclimated for two days feeding on mixed plankton. One hundred acclimated Indian major carp of similar size (average weight 5±1gm) were randomly distributed in plastic containers filled with unchlorinated water. Constant aeration was provided to each container using air compressor.

Experimental diets

The formulation of the experimental diet is given in feed diet was prepared containing similar ingredient composition (soya bean meal 25%, ground nut oil cake 25%, rice bran, 38%, wheat flour 10%,vitamin and mineral mixture 2%). Bacterial strain of *Bacillus cereus, Bacillus licheniformis* and *Bacillus pumilus* at three different levels (T_2), (T_3) and (T_4) X 10^7 CFU g-1 were mixed with feed supplements. The control diet (T1) was not Supplemented with bacterial cells.

Collection of blood sample

During the experimental period 15, 30, 45 and 60 days intervals, blood samples were collected randomly, Blood was drawn from both probiotic fed fishes and control fishes by cardiac puncture using 2ml syringes and gauge hypodermic needles. The point of insertion for heart puncture is ventral, midway between the anterior bases of the pectoral fins. The syringe is flushed with EDTA (Anticoagulant) about 150 to 200µl of anticoagulant were retained in the needle and then the blood was drawn to avoid coagulation. The collected blood was transferred in to eppendorfs of 1.5 ml capacity and stored in refrigerator for further analysis.

Haematological examination

Total Red blood cells counts were determined by using Improved Neubauer haemocytometer. [21], Haemoglobin (Hb) concentration was estimated by cyanmethemoglobin [22] and haematocrit value (Hct) was determined by micro haematocrit capillary tube [23]. Mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), and Mean cell volume (MCV) were calculated using the formulae mentioned by Dacie and Lewis [24].

MCHC (g/dl) = Hb / Hct x 100
MCH (pg) = Hb / RBC x 10
MCV (fl) = Hct / RBC x 10

Statistical analysis

The results are presented as means ± SD, difference between parameters were analyzed by one way analysis of variance (ANOVA) and Statistical assessment of result was carried out using SSPS software 16 version.

Results

The haematological parameter of *Labeo rohita* fed with different level of probiotic was shown in Tables (1, 2, 3, 4 and 5). The blood samples were collected at 0, 15, 30, 45 and 60 day's intervals during the experimental period. The Red blood cells count was significantly higher at (T3, X 10^7 CFU g^{-1}) for 60 days (4.48 ± 0.01) when compared to control (2.12 ± 0.11) and other treated groups. The maximum Hb% were recorded at T3 for 60 days (6.23 ± 0.03) and minimum in control group (5.08 ± 0.01). The Hct % were recorded the maximum value was observed in T3 for 60 days (35.44 ± 0.12) compared to control group (27.67 ± 0.01). The Red Cell Indices like MCV, MCH and MCHC values were calculated, minimum MCV values was observed in T3 for 60 days 137.34 ± 0.12 and maximum values was recorded in control group (201.25 ± 0.39) and MCH maximum values was recorded in control group (39.53 ± 0.78) and minimum in T3 (29.21 ± 0.38). MCHC maximum values was recorded in T3 (24.34 ± 0.16) and minimum in control group (19.21 ± 0.65).

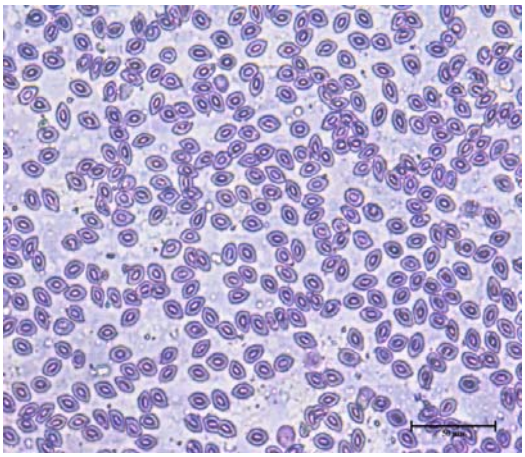

Fig 1: Structure of red blood cell

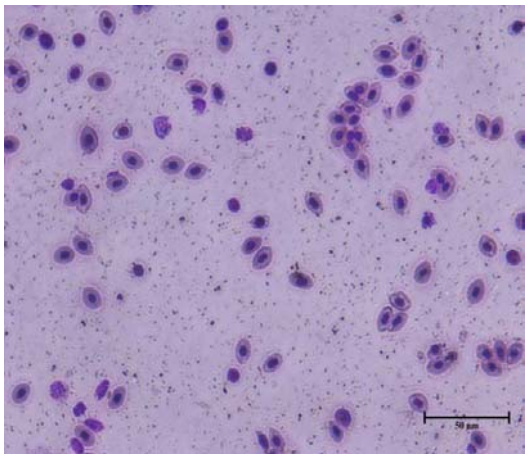

Fig 2: Different structure of haematological parameter

Table 1: Haematological parameters of *Labeo rohita* fed with diets of different levels (mean ± SD) of probiotics initial level of the experiment.

Haematological parameter	T_1	T_2	T_3	T_4
RBC ($x10^6$ µl^{-1})	2.16 ± 0.17	2.34 ± 0.16	2.24 ± 0.06	2.12 ± 0.11
Hb (g/dl)	5.18 ± 0.03	5.21 ± 0.04	5.24 ± 0.02	5.08 ± 0.01
Hct ($x10^3$ µl^{-1})	28.67 ± 0.01	28.46 ± 0.03	29.86 ± 0.02	27.67 ± 0.01
MCV (fl)	139.76 ± 0.16	138.37 ± 0.12	137.34 ± 0.12	144.27 ± 0.18
MCH (pg)	29.36 ± 0.46	29.33 ± 0.27	29.21 ± 0.38	29.26 ± 0.33
MCHC (g/dl)	19.63 ± 0.87	19.21 ± 0.54	19.27 ± 0.36	19.21 ± 0.65

Table 2: Haematological parameters of *Labeo rohita* fed with diets of different levels (mean ± SD) of probiotics 15 days of the experiment.

Haematological parameter	T_1	T_2	T_3	T_4
RBC ($x10^6$ µl^{-1})	2.56 ± 0.16	2.47 ± 0.14	2.87 ± 0.09	2.34 ± 0.11
Hb (g/dl)	5.37 ± 0.06	5.23 ± 0.02	5.45 ± 0.07	5.12 ± 0.04
Hct ($x10^3$ µl^{-1})	29.35 ± 0.01	28.37 ± 0.04	30.23 ± 0.06	28.16 ± 0.03
MCV (fl)	147.87 ± 0.17	140.24 ± 0.19	138.47 ± 0.22	156.48 ± 0.27
MCH (pg)	31.25 ± 0.67	30.86 ± 0.48	30.43 ± 0.35	32.45 ± 0.87
MCHC (g/dl)	19.96 ± 0.69	19.86 ± 0.64	20.35 ± 0.38	19.37 ± 0.76

Table 3: Haematological parameters of *Labeo rohita* fed with diets of different levels (mean ± SD) of probiotics 30 days of the experiment.

Haematological parameter	T_1	T_2	T_3	T_4
RBC ($x10^6$ µl^{-1})	2.89 ± 0.22	2.78 ± 0.35	3.12 ± 0.23	2.68 ± 0.19
Hb (g/dl)	5.67 ± 0.08	5.86 ± 0.04	5.82 ± 0.04	5.23 ± 0.03
Hct ($x10^3$ µl^{-1})	28.68 ± 0.03	30.86 ± 0.02	31.23 ± 0.05	28.35 ± 0.06
MCV (fl)	158.97 ± 0.21	147.86 ± 0.24	140.84 ± 0.25	167.25 ± 0.17
MCH (pg)	32.65 ± 0.56	31.75 ± 0.27	31.12 ± 0.35	34.25 ± 0.82
MCHC (g/dl)	19.87 ± 0.48	20.75 ± 0.57	21.98 ± 0.38	19.48 ± 0.29

Table 4: Haematological parameters of *Labeo rohita* fed with diets of different levels (mean ± SD) of probiotics 45 days of the experiment.

Haematological parameter	T_1	T_2	T_3	T_4
RBC ($x10^6$ µl^{-1})	3.86 ± 0.18	3.78 ± 0.15	4.15 ± 0.11	2.87 ± 0.08
Hb (g/dl)	5.67 ± 0.02	5.68 ± 0.7	5.91 ± 0.04	5.48 ± 0.08
Hct ($x10^3$ µl^{-1})	31.98 ± 0.03	31.07 ± 0.02	32.23 ± 0.05	29.67 ± 0.02
MCV (fl)	176.34 ± 0.28	167.97 ± 0.46	154.27 ± 0.34	188.35 ± 0.23
MCH (pg)	36.76 ± 0.37	34.78 ± 0.42	31.23 ± 0.38	36.35 ± 0.24
MCHC (g/dl)	22.46 ± 0.59	22.47 ± 0.63	22.95 ± 0.78	19.87 ± 0.67

Table 5: Haematological parameters of *Labeo rohita* fed with diets of different levels (mean ± SD) of probiotics 60 days of the experiment.

Haematological parameter	T_1	T_2	T_3	T_4
RBC ($x10^6$ µl^{-1})	4.12 ± 0.01	4.19 ± 0.03	4.48 ± 0.01	3.12 ± 0.02
Hb (g/dl)	5.98 ± 0.02	5.76 ± 0.03	6.23 ± 0.03	5.57 ± 0.04
Hct ($x10^3$ µl^{-1})	33.35 ± 0.04	32.35 ± 0.05	35.44 ± 0.12	31.86 ± 0.08
MCV (fl)	186.94 ± 0.38	179.47 ± 0.67	158..34 ± 0.46	201.25 ± 0.39
MCH (pg)	37.86 ± 0.67	36.85 ± 0.65	31.58 ± 0.49	39.53 ± 0.78
MCHC (g/dl)	23.46 ± 0.21	23.74 ± 0.26	24.34 ± 0.16	20.32 ± 0.17

RBC- Red blood cell count, Hb%- haemoglobin percentage, Hct- haematocrit value, MCV- mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration.
T1= *Bacillus cereus*, T2= *Bacillus licheniformis*, T3= *Bacillus pumilus* and T4= Control (Without probiotics).

Discussion

Haematology is an important factor that could be considered for the fish diet quality assessment. Ologhobo [25] reported that the most common blood variables consistently influenced by diet are the haematocrit (Ht) and haemoglobin (Hb) levels. Probiotics have been used in tilapia [26], which reported positive effects on haematological parameters. On the other hand, *O. niloticus* fed diet supplemented with *B. subtilis* [27] or supplemented with *Pediococcus acidilactici* [28] showed some variation (but not significant) in Hb and Ht contents among the control and fish that were fish groups fed diet enriched with probiotics. Fish fed the diet supplemented with probiotics showed the highest values of Hb, RBCs and WBCs [29]. Reported that both fish groups fed the diet supplemented with dead *Saccharomyces cerevisiae* yeast and both of live *B. subtilis* and *S. cerevisiae* showed significant ($P < 0.05$) increase in the Ht level when compared to fish fed the control diet [30]. Reported that, Hb concentration, in rainbow trout (*Oncorhynchus mykiss*) fed different levels of probiotic was significantly ($P < 0.05$) different from the control.

This study was planned to evaluate the effect of the probiotic on the blood parameters of the fish *Labeo rohita*. Concerning the effect of the laboratory isolated probiotic *Bacillus pumilus* on the health status and haematological parameters of *Labeo*

rohita, the results indicated a positive effect represented by significant increase in RBC s count, Hb%, HCT% and red cell indices like MCV, MCH and MCHC in the Tables (1, 2, 3, 4 and 5). These could be attributed to the fact that, the probiotics used increased the blood parameter values as a result of hematopoietic stimulation. These results supported the results of [31, 32, 33].

High proportion of *Bacillus pumilus* in the intestinal of experimental fish may shows that intestinal environment is suitable for the given probiotic to settle and grow and also lead into harbor a great number of microbial cells of host intestine. Increase in survival associated with Bacillus probiotic proportion in the gut flora is probably due to competitive exclusion of other bacteria. One of the identified bacteria, in T4 disappeared and the population of the other bacteria in probiotic treatments declined. It can strongly confirm the idea of out-competing the other bacteria by colonization of probiotic in intestine. On the other hand, survival in T3 was higher, so we cannot definitely conclude that the exclusion of other bacteria by the probiont results in improved survival. However, this effect should not be ignored. Because growth rate throughout the experiment was improved in T3, not in T4, it can be certainly suggested that the more probiotic cells in diets and host intestine necessarily does not result in the more improved growth and survival. Better growth, as observed in T3, may establish better health conditions in *Labeo rohita* and therefore increased haematological values [34, 35].

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