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Effect of Dietary Probiotic (*Lactobacillus acidophilus*) on the hematological parameters of *Labeo rohita*

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Abstract

The present study was conducted to examine the influence of nutritional supplementation of probiotic (*Lactobacillus acidophilus*) on hematological parameters of *Labeo rohita*. The Probiotic in the form of *Lactobacillus acidophilus* was purchased from market. The feeding trials were conducted for 60 days, to determine the effect of dietary probiotic of blood parameters of fish. The fish with similar body weight (25±1gm) were distributed randomly into six treatment groups, which were fed with a feed containing *Lactobacillus acidophilus* in five concentrations viz., 0.5 (T1), 1.0 (T2), 1.5 (T3), 2.0 (T4), and 2.5 (T5) X 10^7 CFU g-per feed along with control (T0). The control group (T0) was fed without *Lactobacillus acidophilus* for the same period. Samples of blood were collected continuously after the intervals 0, 15, 30, 45, 60 days. The hematological parameters such as Total Erythrocytes Count (RBC), Haematocrit value (Hct), Hemoglobin concentration (Hb), and Hematological indices (MCHC, MCH and MCV) were calculated. The *Lactobacillus acidophilus* treated fish (T5, 2.5 X 10^7 CFU g-per feed) showed maximum percentage of hemoglobin contents. The result suggests that *Lactobacillus acidophilus* as a probiotic could be utilized efficiently in aquaculture.

Keywords: Feed, *Lactobacillus acidophilus*, Probiotics, Hematology, *Labeo rohita*

Introduction

Aquaculture is the quickest growing food manufacturing sector in the world that has grown tremendously in last few decades and has made significant advances in recent years in the production of a wide range of aquatic organisms, for human consumption as well as ornamental species (Javaid, 1990; Mishra ,2001; Irianto and Austin, 2002; Kesarcodi, 2008 and Sahu, 2008). It also provides employment, food and nutrition to millions of people in the world with an all-time high fish production of 80 million tons in 2016 (FAO, 2018). In India, aquaculture contributes over 95% of the total aquaculture production, in which carps have a maximum contribution.

The three Indian major carps (IMCs), namely catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) contribute as much as 87% of the total fresh water aquaculture production (with highest contribution of rohu, which contributed 3% of total inland fin fish production with 1843 thousand tons during 2016 in comparison to 1133 thousand tons during 2010. (Department of Fisheries,2018).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 (Khan *et al.,* 2004 and Giri, 2014). It 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. (Verschuere, 2000 and Sahoo *et al.*, 2011). Among bacterial infections *Aeromonas hydrophila, Staphylococcus xylosus etc.* are some of the most important causes of disease problems in Indian aquaculture. (Kalleswaran *et al.*, 2011).To treat these diseases, overuse of antibiotics and therapeutics are causing negative impacts like development of antimicrobial and drug resistance, accumulation of chemical residues in tissue.(Thrall *et al.*, 2004).

Probiotics are “live microbes” used in aquaculture that helps the host by several modes of action: developing microbial balance; improvement of water quality; enrichment of immune response of host species and enhancement of nutrition of host species through the production of supplemental digestive enzymes. (Ringo, 1998; Cruce, 2001; Yanbo, 2006; Suzer, 2008; Essa, 2010; Faramarzi *et al.*, 2011; Allameh *et al.*, 2017; Das *et al.*, 2020 and Chaudhary *et al.*, 2021). Common Bacterial strains used as probiotic products are *Lactobacillus acidophilus, L. bulgaricus, L. plantarium, Streptococcus lactis* and *Saccharomyces cerevisiae.* Piraret *et al.*, (2006) found that number of mortality was significantly lower in probiotic supplemented fish than in control fish.

Lactobacillus, as a main group of probiotics, is employed by several workers in terms of animal nutrition, to enhance the growth performance of fishes (Cruce, 2001; Bagheri, 2008 and Rahman *et al.*, 2021).

The knowledge of the hematological parameters is an important tool that can be used as an effective and sensitive index to monitor physiological changes in the fishes (Kumar *et al.*, 2011). Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology (Rambhaskar and Rao, 1986; Xiaoyun *et al*., 2009 and Dutta and Ghosh, 2021).

The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals. Many researchers reported that the hemato-biochemical quantity is known as the most significant physiological indicators of fish health, stress, and welfare. Keeping this view in mind, the present study was conducted to examine the influence of *Lactobacillus acidophilus* as dietary supplement on the hematological parameters of *Labeo rohita*. The results of this study will be very helpful in understanding the blood responses of *L. rohita* towards probiotics, and thereby will enhance our knowledge for the healthy use of probiotics to flourish the aquaculture industry.

Materials and Methods

For the present study, *Labeo rohita* were procured from a pond Sagar Taal(28°03’01.8’’N 79°07’55.5’’E), Badaun, U.P. The collected alive fishes were then transported to the PG Department of Zoology, Bareilly College Bareilly, U.P (28°21’21.7’’N 79°25’35.6’’E), in polyethylene bags and acclimatized by keeping in aquaria and providing basal diet for ten days. The fishes were provided with powdered basal feed (soya bean meal 25%, groundnut oil cake 25%, rice bran 38%, wheat flour 10%, vitamins and mineral mixture 2%), crushed in mortar and

pestle, twice daily during acclimatization. Afterwards, the length-weight measurements of all fishes were done. *L. rohita* with similar body weight, were then distributed in 6 glass aquaria (n= 10 fishes/aquarium) containing 75 litres water. Constant aeration was provided to each aquarium using air compressor. In the control (T_0) group, the fishes were fed with normal basal feed for next 60 days. While in second group, the fishes were fed with basal food + *L. acidophilus*, in different concentrations of 0.5% (T1), 1.0% (T2), 1.5% (T3), 2.0% (T4) and 2.5% (T5) respectively for 60 days. Thus, for *L. acidophilus* probiotic experiment, five aquaria were used, while single aquarium was employed as control.

During experimental period, bacterial strain of *Lactobacillus acidophilus* at five different levels 0.5 (T_1), 1.0(T_2), 1.5(T_3), 2.0 (T_4) and 2.5 (T_5) X 10^7 CFU g-1 were mixed with basal diet. The control diet (T_0) was fed with basal diet and was not supplemented with bacterial cells.

Collection of blood sample

During the experiments, after the interval of every 15 days, *i.e.*, 0, 15, 30, 45 and 60 days intervals, blood samples were collected randomly, Blood was drawn from both probiotic fed fishes and control fishes from caudal peduncle using 2 ml syringes and gauge hypodermic needles. The syringe was flushed with EDTA (anticoagulant) in such a way that about 150 to 200 µl of EDTA was retained in the needle and then the blood was drawn to avoid coagulation. The collected blood samples were transferred separately to K3E Hemo Tubes (MB Lab consumables India) of 2 ml capacity and stored in refrigerator for further analysis.

Hematological examination

Total Red blood cells (RBC) counts were determined by using Improved Neubauer haemocytometer, Haemoglobin (Hb) concentration was estimated by cyanomethemoglobin and Haematocrit value (Hct) was determined by filling blood in micro haematocrit capillary tubes and their centrifugation in micro-haematocrit rotor (REMI) @ 6000 rpm for 15 minutes. Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH), and Mean Cell Volume (MCV) were calculated by adopting the methods of Dacie and Lewis (1991).

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

The mean values of multiple observations were calculated to determine the better results of hematological parameters *in L. rohita* of control group and of experimental group that were fed with bacterial probiotic *L.acidophilus* together with basal diet. The observations were recorded in triplicates and their mean values are presented in table 1-6.

Results

The hematological parameters of *Labeo rohita* fed with basal diet and different levels of probiotic were shown in Tables 1-6. The blood samples were collected at 0, 15, 30, 45 and 60 days intervals during the experimental period. The Red Blood Cells count was found higher at probiotic concentration of 2.5X 10^7 CFU g_1 for 60 days (T5) when compared to control (T0) and other treated groups. The maximum value of Hb% was recorded in T5 and minimum in control group (T0). The maximum findings of Hct % were also recorded in T5 for 60 days experimental period. The Red Cell Indices like MCV, MCH and MCHC values were determind by calculation, minimum MCV value was observed in T-4 and maximum value was recorded in probiotic treated group T5. The highest MCH was recorded in the group T5 and minimum inT4 whereas MCHC maximum value was recorded in T2 and minimum in control group (Fig.,1).

Table-1 Control group (T0)

Control Group		Days			
Blood Parameters	0 day	15	30	45	60
RBCs(×10$^{6)}$	1.73	1.81	1.87	1.95	2.03
Hb	5.63	5.86	6.09	6.34	6.59
Hct	27.87	28.98	30.14	31.34	32.59
MCHC	20.21	20.22	20.22	20.22	20.22
MCH	32.49	32.49	32.53	32.49	32.46
MCV	160.77	160.73	160.89	160.71	160.55

Table-2 Experimental group (T1)

Lactobacillus (0.5)					
Experimental Group		Days			
Blood Parameters	0 day	15	30	45	60
RBCs(×10$^{6)}$	1.73	1.82	1.91	2.06	2.11
Hb	5.63	5.92	6.21	6.52	6.84
Hct	27.86	29.26	30.72	32.25	33.87
MCHC	20.22	19.99	20.08	20.21	20.21
MCH	32.49	32.48	32.48	32.47	32.47
MCV	160.77	160.81	160.85	160.75	160.77

Table-3 Experimental group (T2)

Lactobacillus (1.0)					
Experimental Group		Days			
Blood Parameters	0 day	15	30	45	60
R.B.C	1.73	1.84	1.95	2.06	2.18
Hb	5.63	5.97	6.33	6.72	7.12
Hct	27.87	29.53	31.3	33.17	35.16
MCHC	20.22	20.22	20.23	20.23	20.24
MCH	32.49	32.49	32.53	32.53	32.54
MCV	160.77	160.77	160.79	160.79	160.82

Table-4 Experimental group (T3)

Lactobacillus (1.5)					
Experimental Group		**Days**			
Blood Parameters	**0**	**15**	**30**	**45**	**60**
R.B.C	1.73	1.85	1.98	2.12	2.27
Hb	5.63	6.03	6.45	6.9	7.38
Hct	27.86	29.82	31.89	34.13	36.52
MCHC	20.22	20.22	20.21	20.22	20.22
MCH	32.49	32.52	32.49	32.54	32.52
MCV	160.78	160.87	160.83	160.99	160.85

Table-5 Experimental group (T4)

Lactobacillus (2.0)					
Experimental Group		Days			
Blood Parameters	0 day	15	30	45	60
R.B.C	1.73	1.87	2.03	2.19	2.36
Hb	5.63	6.08	6.56	7.09	7.65
Hct	27.87	30.09	32.51	35.11	37.91
MCHC	20.22	20.19	20.19	20.19	20.19
MCH	32.48	32.39	32.32	32.32	32.34
MCV	160.78	160.38	160.12	160.05	160.19

Table-6 Experimental group (T5)

Lactobacillus (2.5)					
Experimental Group		Days			
Blood Parameters	0 day	15	30	45	60
R.B.C	1.73	1.88	2.06	2.24	2.44
Hb	5.63	6.14	6.69	7.28	7.94
Hct	27.87	30.37	33.11	36.08	39.34
MCHC	20.22	20.21	20.21	20.19	20.18
MCH	32.49	32.55	32.52	32.47	32.49
MCV	160.77	161.02	160.98	160.85	161.02

Discussion

Hematology is an important factor that could be considered for the fish diet quality assessment. Ologhobo (1992) reported that the most common blood variables consistently influenced by diet are the haematocrit (Hct) and haemoglobin (Hb) levels. Abd El-Rhman *et al.,* (2009) observed positive effects on haematological parameters by using probiotics in tilapia, On the other hand, *O. niloticus* fed diet supplemented with *B. subtilis* or supplemented with *Pediococcus acidilactici* (Soltan and El-Laithy ,2008). Ferguson *et al.,* (2010) showed some inconsequential variation in Hb and Hct contents among the control and fish groups that were fed with probiotics enriched diet. Fish fed with the diet supplemented with probiotics showed the highest values of Hb, RBCs and WBCs. Marzouk *et al.,* (2008) reported that both fish groups fed the diet supplemented with dead *Saccharomyces cerevisiae* yeast and both of live *B. subtilis* and *S. cerevisiae* showed increasement in the Hct level when compared to fish fed the control diet. Firouzbakhsh *et al.,* (2012), reported that, Hb concentration, in rainbow trout (*Oncorhynchus mykiss*) fed different levels of probiotic was significant.

This study was planned to determine the effect of the bacterial probiotic *L. acidophilus* on the blood parameters of the fish *L. rohita*. Concerning the effects of the probiotic *Bacillus acidophilus* on the health status and haematological parameters of *L. rohita.* The results indicated a positive effect represented by significant increase in RBCs count, Hb%, Hct and Red Cell indices like MCV, MCH and MCHC values. These could be attributed to the fact that, the use of probiotics increases the blood parameter values as a result of hematopoietic stimulation. These results are in agreement of the observations of Manohar *et al.,* (2005), that supported the results of high proportion of *Bacillus acidophilus* in the intestinal part of experimental fish. This may be attributed to 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.

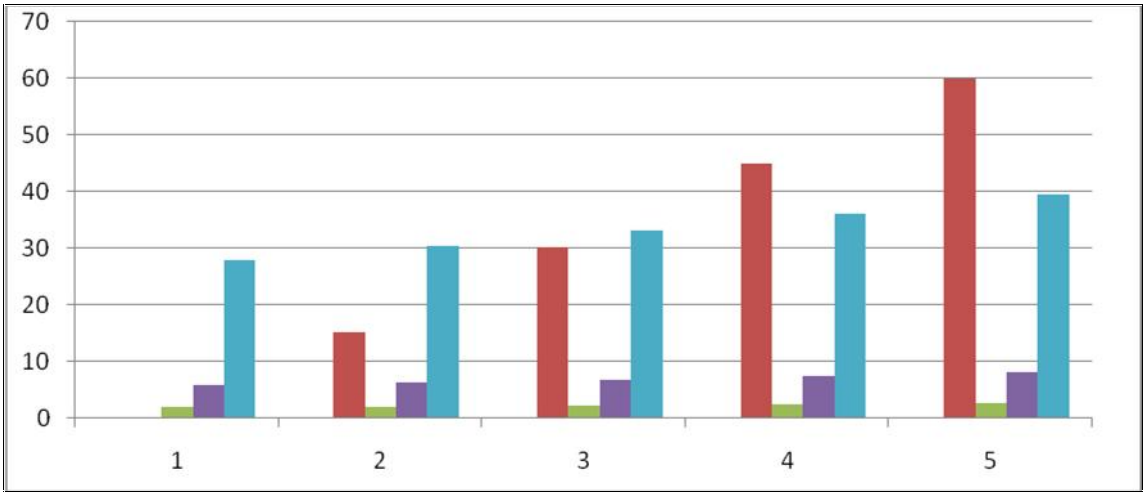

Fig., 1. Graph plotted to represent increased levels of blood parameters as per increased concentration of probiotic provided the during experimental days.

However, this effect should not be ignored. Because growth rate throughout the experimental period was improved in T5, it can be 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 T5, may establish better health conditions in *Labeo rohita.*

Conclusion

From the present study, it can be determined that the adding of probiotic *L. acidophilus* with formulated diet enhanced fish growth, mitigates and effects of stress factors in freshwater fish *L. rohita*. The *Labeo rohita* fish used in the existing research have been of high quality in stimulating the hemopoietic organ and improved the haematological parameters. As *L. acidophilus* produced the excellent outcomes, therefore, it is the possible option for better growth and feed consumption in intensive culture of freshwater fishes.

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