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Original Research Article

Effect of Probiotic (*Lactobacillus acidophilus*) on Haematological parameters of *Catla catla* (Hamilton)

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A B S T R A C T

The present study was carried out to evaluate the influence of dietary supplementation of probiotic bacteria (*Lactobacillus acidophilus*) on growth performance, hematological parameters of *Catla catla*. The Probiotic was isolated from the intestine of common carp. The feeding trail was conducted for 60 days, to determine the effect of dietary probiotic on growth and health status of fish. The fish with similar body weight (25±1gm) were distributed randomly into five treatment groups, which fed a feed containing *Lactobacillus acidophilus* in four concentrations viz., 1.0 (T1), 1.5 (T2), 2.0 (T3) and 2.5 (T4) X 10^7 CFU g^{-1} feed. The control group (T5) was fed without *Lactobacillus acidophilus* for the same period. Blood samples were collected at the intervals 15, 30, 45, 60 days. The hematological parameters such as Total erythrocytes count (RBC), Total leucocytes count (WBC), Hematocrit (Hct), Hemoglobin concentration (Hb), and Hematological indexes (MCHC, MCH and MCV) were examined. The *Lactobacillus acidophilus* treated fish (T_3, 2.0 X 10^7 CFU g^{-1} feed) showed maximum percentage hemoglobin contents than in other groups. The result suggests that *Lactobacillus acidophilus*. Could be used effectively as a probiotics for the use in aquaculture.

Keywords

Lactobacillus acidophilus, Probiotics, Hematological parameters

Introduction

The Indian major carp *Catla calta* 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. *Catla catla* contributes a major portion to the fresh water fish production in south India.

Fish disease is most common problems in aquaculture, bacterial infections are one of the most important cause of disease problems in Indian aquaculture (Kesarcodi *et al*., 2008; Sahoo *et al*., 2011) .Prevention and control of diseases have led during recent decades to substantial increases in the use of veterinary medicines include vaccines and antibiotics or chemotherapeutics, but they cannot be used alone as a universal

disease control measures in aquaculture. Although the excessive use of broad spectrum antibiotic in aquaculture has led to development of antibiotic resistance among pathogenic bacteria (Villami *et al* .,2002; Sakai *et al.,* 2001). This concern has also been raised in aquaculture industry and has led to suggestions for other disease controls including non-specific immuo stimulants, use of non pathogenic bacterial probiotics such as Lactic acid bacteria (LAB) (Ringo and Gatesoupe 1998; Kim and Austin 2006). 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 (Joseluis Balcozar *et al.,* 2006).

The term Probiotics is defined as "Live microbial feed supplements which when administered in adequate amount beneficially affect the host by improving its microbial balance (FAO/WHO 2005). Lactic acid bacteria (LAB) have been used as probiotics due to their properties of anti bacterial activity against pathogens (Byun *et al.,* 1997; Garrga *et al.,* 1998).

The use of probiotic as feed supplements has attracted considerable attention by feed manufactures as mean of improving livestock performance. Most of the studies concerned with the effect of probiotics on cultured aquatic animals have emphasized a reduction in mortality increased survival (Change and Liu, 2002), improved resistance against disease (villamil *et al*., 2003); enhance the ability to adhere and colonize the gut (vine *et al.,* 2004;Abo-State 2009); improved the ability to antagonize other organism (Burgents *et al*., 2004;Li *et al*., 2004; Brunt and Austin,2005).

The knowledge of the hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological changes in the fishes (Satheeshkumar *et al.,* 2011). Normal ranges for various blood parameters in fish have been established by different investigators in fish physiology and pathology (Rambhaskar and Srinivasa Rao 1986; Xiaoyun *et al*., 2009). The analysis of blood indices has proven to be a valuable approach for analyzing the health status of farmed animals (Bahmani *et al*., 2001). So, the present study was designed to evaluate the effect of probiotic *Lactobacillus acidophilus* On growth performance and hematological parameters Indian major carp *Catla catla.*

Materials and Methods

Fish sampling

The investigated 10 individual's fingerlings of common carp (*Cyprinus carpio*) were collected at regular intervals from the National fish seed farm B.R. Project, Karnataka.

Isolation of Lactic acid bacteria

Healthy fishes were selected for the isolation of lactobacilli; fishes were brought to laboratory alive and sacrificed. The ventral surface was sterilized using 70% ethanol and aseptically dissected to remove the intestines. The intestines were opened by a longitudinal incision and thoroughly flushed with sterilized normal saline solution (NSS) to remove the feed materials, dirt and other impurities. Excess moisture was blotted with filter paper and the intestines were weighed, macerated with sterile glass rod and homogenized in sterile NSS (1:10: wt: vol) using a vortex mixer. These samples were serially diluted in NSS and aseptically plated by the spread plate technique on MRS media (Hi media, India)

(Gohs *et al.*, 2007). The inoculated agar plates were incubated at 30-40 c for 5-7 days. MRS agar was used for enumeration and cultivation of LAB (De man et al, 1960). Well isolated colonies with typical characteristics namely pure white, small (2-3 mm diameter) with entire margins were picked from each plates for further identification.

Identification of the lactic acid bacteria

The cultures were identified according to their morphological, cultural, and physiological, biochemical characteristics based on gram reaction, motility, spore formation, catalase and oxidase activity, nitrate reduction, hydrogen sulfide production. Casein and urea hydrolysis, gelatin liquefaction and IMVIC test were done. Phonotypical identification of Lactic acid bacteria were done by using carbohydrate fermentation test kit.

Experimental diets

The formulation of the experimental diet is given in Table 1. 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%). Soya bean meal was used as sources of protein, ground nut oil cake was used as lipid sources, wheat and rice bran were used as carbohydrate source. Bacterial strain of *Lactobacillus acidophilus* at five different levels (1.0 (T_1), 1.5 (T_2), 2.0 (T_3) and 2.5 (T_4) X 10^7 CFU g^{-1} were mixed with feed supplements. The control diet (T_0) was not Supplemented with bacterial cells.

Experimental design

The experiment was conducted in laboratory condition for 60 days. Common carps were obtained from National fish seed farm B.R. Project, Karnataka. 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 common carp of similar size (average weight 25±1gm) were randomly distributed in plastic containers filled with unchlorinated water. Constant aeration was provided to each container using air compressor.

Collection of blood sample

During the experimental period 15, 30, 45, 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 insertation 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 eppendrofs of 1.5 ml capacity and stored in refrigerator for further analysis.

Hematological examination

Total RBCs count and WBCs count were determined by using Improved Neubauer hemocytometer. (Hesser, 1960), Hemoglobin (Hb) concentration was estimated by cyanmethemoglobin (Blaxhall & Daisley, 1973) and hemocrite value (Hct) was determined by micro hematocrite capillary tube (Wintrobe, 1967). Differential leukocyte count was done by using giemas's staining method (Abdul wahid shah *et al.*, 2009). Mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and Mean cell volume (MCV) were calculated using the formulae mentioned by

Dacie and Lewis (2001).

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 significance was tested at $p< 0.05$ and $p< 0.001$ level. Statistical assessment of result was carried out using SSPS software.

Results and Discussion

Isolation of lactic acid bacteria

The LAB was isolated from fish intestine. After isolation the isolated organism was identified up a genes level based on their morphological, cultural, physiological and biochemical characteristics (Sharpe *et al*., 1979). After Incubation on MRS agar for 24to72 hr, isolate formed round, creamy white colony, grown at 30 to 400C, the optimum pH was 5.5-6.5. The isolate was tested for biochemical and other physiological characteristics. Their distinguishing feature is shown in (Table II). The isolate was hetero fermentative *Lactobacillus acidophilus* with negative patter of H2S formation, nitrate reduction, catalase activity and urease activity. Fermentation test was shown in (Table III).

Haematological parameters

The haematological parameters of catla catla fed with different level of probiotic was shown in tables (IV, V VI VII VIII). The blood samples were cooolected at 0,15,30,45 and 60 days intervals during the experimental period. The RBC's count was significantly higher at (T_3, 2.0 X 10^7 CFU g^{-1}) for 45 days (6.34±0.016) when compared to control (2.25 ± 0.11) and other treated groups. The maximum Hb% were recorded at T_3 for 45 days (8.18±0.03) and minimum in control T_0 (4.1±0.01). The Hct % were recorded the maximum value was observed in T_3 for 45 days (25.55±0.12) compared to control group (23.70±0.01). The Red Cell Indices like MCV,MCH and MCHC values were calculated, minimum MCV values wea observed in T_3 for 45 days 4.30±0.12 and maximum values was recorded in control group (9.31±0.39). maximum MCH values was recorded in control group(18.77±0.78)and minimum in T_3 (12.21 ±0.38). Maximum MCHC values was recorded in T_3(29.97±0.16 and minimum in control group (17.72±0.65)

Fish culture is increasing to compensate the shortage of animal protein all over the world. Fish under intensive culture conditions will be badly affected and often fall prey to different microbial pathogens that have been treated with chemotherapeutic substances of which antibiotics were intensively used. These curative substances produce the problem of bacterial drug fastness on one hand and the public health hazards on the other hand (Robertson *et al*., 2000) . These awaited drawbacks enforced the fish pathologists to seek for other alternatives; the use of natural immune stimulants in fish culture for the prevention of diseases is a promising new development and could solve the problems of massive antibiotic use. Natural immune stimulants are biocompatible, biodegradable and safe for both the environment and human health. Moreover, they possess an added nutritional value (Jessus *et al.*, 2002). The parallel use of biological products namely the probiotic is recently the goal of the disease bio control strategy in aquaculture as they improve the fish health and modify the fish associated microbial community (Gibson and Roberfroid, 1995).

This study was planned to evaluate the effect of the probiotic on the blood parameters of the fish *Catla catla*. Concerning the effect of the laboratory isolated probiotic lactobacillus acidophilus on the health status and hematological parameters of *Catla catla*, 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 (IV,V,VI,VII,VIII). These could be attributed to the fact that, the probiotics used increased the blood parameter values as a result of hemopiotic stimulation. These results supported the results of (Sarma *et al*.,2003; Manohar *et al*.,2005; and Rajesh Kumar *et al*.,2006).

Table.I List of isolated of Diatoms

Table I. Ingredient composition (g kg^{-1} dry weight) of the experimental diet

Ingredient	Composition
Soya bean meal	25%
Ground nut oil cake	25%
Rice bran	38%
Wheat flour	10%
Vitamin and mineral mixture	2%

Table II. Morphological, cultural and physiological characteristics of the Isolated organism

Test	Result
Growth temperature	$30\text{-}40^0$ C
Colony color	pure white
Colony size	small (2-3mm)
Colony margin	entire
Gram stain	positive
Shape	rod
Motility test	negative
Catalase test	negative
Oxidase test	negative
Indole test	negative
Citrate utilization	negative
Nitrate reduction	negative
Gelatin liquefaction	negative
H_2S production	negative
Methyl red test	negative
Voges –proskaur test	negative
Casein hydrolysis	negative

Table.III Biochemical characteristics of the tested isolate by utilization of carbohydrate sources

Carbohydrate Source	Reactions
Arabinose	+
Cellobiose	+
D- Fructose	+
Galactose	+
Lactose	+
Maltose	+
Mannitol	+
Mannose	+
Melibiose	+
Rafffinose	+
Rhamnose	-
Ribose	+
Salicin	+
Srobitol	+
Sucrose	+
Terhaldose	+

Symbols: + Positive, - Negative

Table.IV Hematological parameters of *Catla catla* fed with diets of different levels of probiotics 0 days of the experiment

Hematological Parameters	T_0	T_1	T_2	T_3	T_4
RBC (X10⁶/µl)	2.25±0.11	2.96 ±0.07	3.05 ±0.10	3.11± 0.02	3.06 ±0.09
Hb% (g/dl)	4.77± 0.01	4.1± 0.09	4.19 ±0.06	4.36 ±0.05	4.39± 0.26
Hct (%)	23.7 ±0.01	23.16± 0.42	23.58± 0.06	23.54± 0.09	23.41± 0.06
MCV (fl)	9.31±0.39	7.94± 0.19	7.74 0±.24	7.56 ±0.06	7.64 ± 0.22
MCH (pg)	18.77± 0.78	13.88± 0.62	13.75 ± 0.57	14.01± 0.07	14.38± 1.25
MCHC (g/dl)	20.12 ±0.05	17.72 ±0.65	17.76± 0.28	18.52 ±0.24	18.75 ± 1.12

Table.V Hematological parameters of *Catla catla* fed with diets of different levels of probiotics 15 days of the experiment

Hematological Parameters	T_0	T_1	T_2	T_3	T_4
RBC (X10⁶/µl)	2.84 ±0.98	3.2± 0.03	3.88 ±0.06	3.59± 0.14	3.36 ±0.21
Hb% (g/dl)	5.13± 0.02	4.99± 0.07	5.02 ±0.07	4.9± 0.28	4.8± 0.25
Hct (%)	23.92 ±0.06	23.80± 0.44	24.14± 0.25	24.49 ± 0.16	23.81± 0.33
MCV (fl)	8.42± 0.30	6.88± 0.30	7.01 0±.08	6.81±0.28	7.09 ±0.33
MCH (pg)	18.06 ±0.67	14.47± 1.16	13.66 ±1.48	13.84 ±1.31	14.37± 1.58
MCHC (g/dl)	21.44± 0.03	20.98± 0.69	20.85± 0.49	20.25± 0.49	20.20 ±1.13

Table.VI Hematological parameters of *Catla catla* fed with diets of different levels of probiotics 30 days of the experiment

Hematological Parameters	T_0	T_1	T_2	T_3	T_4
RBC ($X10^6$/µl)	3.2± 0.03	3.88± 0.6	3.96± 0.01	4.4 ±0.15	3.98 ±0.01
Hb% (g/dl)	5.27± 0.02	5.75± 0.03	5.96 ±0.01	6.10 ±0.08	5.52 ±0.37
Hct (%)	24.23± 0.05	24.52 ± 0.06	24.72± 0.13	25.55 ± 0.12	24.17 ± 0.42
MCV (fl	7.57 ±0.87	6.31 ±0.08	6.23 0.01	5.80 ±0.17	5.08 ±0.25
MCH (pg)	16.48± 0.12	14.81± 0.21	15.04± 0.04	13.83 ±0.44	13.92± 1.22
MCHC (g/dl)	21.77 ±1.32	23.45 ± 0.08	24.09± 0.14	32.87± 0.15	21.59 ±0.37

Table.VII Hematological parameters of *Catla catla* fed with diets of different levels of probiotics 45 days of the experiment

Hematological Parameters	T_0	T_1	T_2	T_3	T_4
RBC ($X10^6$/µl)	3.49± 0.04	4.28± 0.02	4.82± 0.08	6.34± 0.16	4.82± 0.04
Hb% (g/dl)	5.58± 0.03	6.30 ±0.11	6.69± 0.04	8.18± 0.03	6.56 ±0.11
Hct (%)	24.72 ±0.08	25.07 ±0.04	25.55± 0.05	27.29 ± 0.13	26.10 ±0.85
MCV (fl)	7.08± 0.08	5.85 ±0.03	5.23± 0.08	4.3± 0.12	5.31± 0.28
MCH (pg)	16.00± 0.15	14.72± 0.31	13.68± 0.40	12.91 ±0.38	13.6± 0.4
MCHC (g/dl)	22.59 ±1.32	25.14± 0.14	26.39± 0.41	29.97 ±0.16	26.26± 0.88

Table.VIII Hematological parameters of *Catla catla* fed with diets of different levels of probiotics 60 days of the experiment

Hematological Parameters	T_0	T_1	T_2	T_3	T_4
RBC ($X10^6$/µl)	3.64± 0.04	4.24 ±0.03	4.74± 0.10	5.78± 0.07	4.75± 0.03
Hb% (g/dl)	5.72± 0.01	6.18± 0.12	6.61 ±0.06	7.09± 0.07	6.62± 0.23
Hct (%)	24.83 ±0.09	24.94± 0.01	25.04± 0.08	25.38 ± 0.35	24.79 ± 0.53
MCV (fl)	6.8 ±0.06	5.9 ±0.04	2.27± 0.10	4.38 ±0.09	5.21 0±.14
MCH (pg)	15.8± 0.2	14.58± 0.40	13.98± 0.37	12.26± 0.12	13.45 ±0.20
MCHC (g/dl)	23.01 ±0.12	24.78± 0.46	25.44± 0.95	29.01±0.70	26.02± 0.83

RBC –Red blood cell count**,**Hb%- haemoglobin percentage, Hct –haematocrit value ,MCV- mean corpuscular volume, MCH- mean corpuscular hemoglobine, MCHC- mean corpuscular hemoglobin concentration .

High proportion of *Lactobacillus acidophilus* 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 Lactobacillus

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 T_3, not in T_4, 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 T_3, may establish better health conditions in *Catla catla* and therefore increased hematological values. (Rosvitz *et al.,* 1998; T.Bagheri *et al*., 2008).

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