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Effect of Probiotics on the Haematological Parameters of Indian Magur (*Clarius batrachus* L.)

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ABSTRACT
Haematological profile of an animal is the reflection of its immunological status. Fishes are prone to infections by several pathogens which influence the immunity of the affected individuals. The effect of three probiotics on four haematological parameters viz., level of haemoglobin, total erythrocyte count, total leucocytes count hematocrit/packed cell volume of Indian magur (*Clarius batrachus* L.) was studied over a period of eight weeks. The level counts of all these four parameters decreased significantly in the blood of fish administered with pathogenic organisms as compared to the control; the fish administered with pathogenic organisms died after three weeks. The level counts of hematological parameters showed significant increase in the blood of fish treated with probiotics alone versus control and also in fish administered with pathogenic organisms versus probiotics. A comparison among the three probiotics revealed that, probiotic 1 was more effective than probiotic 3 and probiotic 2; the effect of latter stood at the lowest level.

Key words: Probiotics, *Clarius batrachus*, bacteria, haematological parameters

INTRODUCTION
Probiotics are commonly defined as mono- or mixed cultures of live microbes that, when applied to animal or human, generate a beneficial effect on health of the host. These beneficial effects include disease treatment and prevention as well as improvement of digestion and absorption in the host (Yazid *et al.*, 1999; Chang and Chen, 2003; El-Naggar, 2004; Ghafoor *et al.*, 2005; Igbasan *et al.*, 2005; Kabir *et al.*, 2005; Trachoo and Boudreaux, 2006; Anukam, 2007; Anukam and Koyama, 2007; Raj *et al.*, 2008; Capcarova *et al.*, 2008; Fazeli *et al.*, 2008; Hung *et al.*, 2008; Patel *et al.*, 2008; Radfar and Farhoomand, 2008; Soundarapandian and Sankar, 2008; Vamanu *et al.*, 2008; Vijayabaskar and Somasundaram, 2008; Abdelhamid *et al.*, 2009; Al-Otaibi, 2009; Raja *et al.*, 2009; Vali, 2009; Nikfar *et al.*, 2010; Vamanu and Vamanu, 2010; Agouz and Anwer, 2011; Bansal *et al.*, 2011; Yesillik *et al.*, 2011). Improved resistance against infectious diseases can be achieved by the use of probiotics (Havenaar *et al.*, 1992). Probiotics were used in crustacean (Anderson and Klontz, 1965), shrimps (*Penaeus monodon, Macrobrachium rosenbergii*) (Ajitha *et al.*, 2004) freshwater eggs, hatchlings and larvae of *Scophthalmus maximus* (Calo-Mata *et al.*, 2008); juveniles of goldfis (*Carassius auratus*) (Ahilan *et al.*, 2004; Ringo *et al.*, 1997, 1995), gilthead sea bream (*Sparus aurata*) for their health benefits (Chabrillon *et al.*, 2006). Hematological parameters of fishes are used as indicators of their physiological state (Bansal *et al.*, 1979). The total erythrocyte count (Irianto and Austin, 2002;

Ranzani-Paiva *et al.*, 2004), total leucocyte count fed with *Saccharomyces cerviase* (Selvaraj *et al.*, 2005), haemoglobin, mean corpuscular volume, haemoglobin concentration and mean corpuscular haemoglobin concentrations, total protein, albumin, globulin, albumin-globulin ratio, alkaline phosphatase activity, alanine and aspartate aminotransferase activities, creatinine, sodium, cortisol, insulin and glucose were reported to increase in *Labeo rohita* provided with *Bacillus subtilis* as probiotics (Das *et al.*, 2006; Kumar *et al.*, 2006, 2008; Nayak *et al.*, 2007), *Catla catla* (Jha *et al.*, 2007), *Tilapia nilotica* (Ramadan *et al.*, 1994) and *Cyprinus carpio* (Palikova *et al.*, 2004; Olafsen, 2001) when fed with probiotics.

Indian magur (*Clarius batrachus* L.) is a new introduction in the freshwater ponds of Haryana (India). This fish is being used selectively for pisciculture. However, the fish has been reported to suffer from hemorrhagic septicemia and EUS (Epizootic Ulcerative Syndrome). Use of antibiotics in aquaculture has raised many questions about the development of resistance in the pathogenic organisms and persistence of the drugs in the food chain. Therefore, use of probiotics seems to make an alternative means of disease control in culture fishery. With this aim the present study was undertaken.

MATERIALS AND METHODS

This study was carried out in the Department of Zoology and Aquaculture, CCS Haryana Agricultural University, Hisar (India). Ten catfishes of three months age,were kept in each 30 L flat bottom cubical tubs filled with a well aerated and dechlorinated tap water. Fresh tap water was also stored in big rectangular tanks for 24 h and was well aerated in order to dechlorinate it. Each tub was cleaned on alternate days by siphoning catfish's fecal matter and food remains and 70% of its water was then refilled to ensure clean water in the tubs. Three commercial probiotics were and used to control the infections caused by pathogenic bacteria. Probiotics 1 contained only single bacterium named lactic acid bacteria (*Lactobacillus sporogenes*) while Probiotics 2 only single fungus-the yeast named *Saccharomyces boulardii* and Probiotics 3 contained a mixture of many bacteria viz., *Nitromonas, Rhodococcus, Bacilus megaterium, Lecheni formis, Desulphovibrio sulphuricum, Psuedomonas, Chromatium, Chlorobium, Thiobacillus, Thioxidants, Thiobacilus ferroxidant, Methylomonas metyhanica, Glucon acetobactor, Azospirillum, Trichoderma, Shizophyllum commune* and *Sclertium gluconicum*). The following treatments were used:

- **Control:** In this treatment, 200 μL of Physiological Buffer Saline (PBS) was given into the intraperitoneal cavity of each acclimated catfish
- **Control+bacterium 1 (*Aeromonas hydrophila*):** Here, 200 μL bacterial suspension in PBS with 5×10^{11} cfu mL^{-1} of bacteria was inoculated into intraperitoneal cavity of each catfish
- **Control+bacterium 2 (*Micrococcus* sp.):** Here, 200 μL bacterial suspension in PBS with 5×10^{11} cfu mL^{-1} of bacteria was inoculated into intraperitoneal cavity of each catfishes
- **Control+probiotic 1 (*Lactobacillus sporogenes*):** Here, 0.1 g of probiotic l dissolved in PBS, with 5×10^{11} cfu mL^{-1} of bacteria, was injected into the intraperitoneal cavity of each catfish
- **Control+probiotic 2 (*Saccharomyces boulardii*):** Here, 0.1 g of probiotic 2 dissolved in PBS, with 5×10^{11} cfu mL^{-1} of bacteria, was injected into the intraperitoneal cavity of each catfish
- **Control+probiotic 3 (mixture of many bacteria):** Here, 0.1 g of probiotic 3 dissolved in PBS, with 5×10^{11} cfu mL^{-1} of bacteria, was injected into the intraperitoneal cavity of each catfish

- **Control+bacteria 1+probiotic 1:** Here, 0.1 g of probiotic 1 dissolved in PBS; along with 250 μL of bacterial suspension with 5×10^{11} cfu mL^{-1} of bacteria, was injected into the intraperitoneal cavity of each catfish
- **Control+bacteria 1+probiotic 2:** Here, 0.1 g of probiotic 2 dissolved in PBS; along with 250 μL of bacterial suspension with 5×10^{11} cfu mL^{-1} of bacteria, was injected into the intraperitoneal cavity of each catfish
- **Control+bacteria 1+probiotic 3:** Here, 0.1 g of probiotic 3 dissolved in PBS; along with 250 μL of bacterial suspension with 5×10^{11} cfu mL^{-1} of bacteria, was injected into the intraperitoneal cavity of each catfish
- **Control+bacteria 2+probiotic 1:** Here, 0.1 g of probiotic 1 dissolved in PBS; along with 250 μL of bacterial suspension with 5×10^{11} cfu mL^{-1} of bacteria, was injected into the intraperitoneal cavity of each catfish
- **Control+bacteria 2+probiotic 2:** Here, 0.1 g of probiotic 2 dissolved in PBS; along with 250 μL of bacterial suspension with 5×10^{11} cfu mL^{-1} of bacteria, was injected into the intraperitoneal cavity of each catfish
- **Control+bacteria 2+probiotic 3:** Here, 0.1 g of probiotic 3 dissolved in PBS; along with 250 μL of bacterial suspension with 5×10^{11} cfu mL^{-1} of bacteria, was injected into the intraperitoneal cavity of each catfish

Collection of blood from the magur (*C. batrachus*) under different treatments: Blood samples of treated fish were taken at weekly intervals after the initiation of treatments. Sampling was also done at the same time from the control group. Blood was drawn from the caudal peduncle region using a sterile syringe of 2 mL rinsed with 2.7% Ethylene Dimethyl Tetra Amine (EDTA) solution used as an anticoagulant to prevent the blood cells from lysis and clotting. Blood was collected in small glass vials after drying the vials in hot air oven.

Estimation of hemoglobin, total erythrocyte count, total leukocyte count and hemotocrit (packed cell volume) in the blood of magur (*C. batrachus*) under different treatments: The hemoglobin contents of blood were analyzed following the Cyanmethemoglobin methods using Darbkin's Fluid (Anderson and Klontz, 1965). Twenty micro litre of blood was mixed with 5 mL Darbkin's working solution. The absorbance was measured using a spectrophotometer at wavelength of 540 nm. Hemoglobin contents were expressed as g dL^{-1}.

For the estimation of total erythrocyte count the blood was diluted to 1:200, with RBC counting pipette. The mixture was shaken well to suspend the cells uniformLy in the solution. Then the cells were counted using a haematocytometer as follows (Anderson and Klontz, 1965):

$$N=\frac{\text{No. of RBC mm}^{-3}}{1000}$$

where, N is total number of red blood cells counted in 5 squares of the haematocytometer slide and 10,000 is the dilution factor.

To estimate the total number of leukocyte, blood was diluted 1:20 with WBC diluting fluids using WBC counting pipette. The mixture was shaken well to suspend the cells uniformly in the solution. Then the cells were counted using a haematocytometer as follows:

$$N=\frac{\text{No. of WBC mm}^{-3}}{50}$$

where, N is total number of white blood cells counted in 4 squares of the haematocytometer slide and 50 is dilution factor.

To determine the volume of packed cells volume in the blood, a heparinised capillary tube was filled with blood up to mark 100 and sealed with plasticize sealant and then the capillary tube was centrifuged for 5 min at 12,000 rpm for 30 min in a microhaematocrit centrifuge. The reading of packed cells volume in percent was recorded i.e., mass of erythrocytes settled down in tube.

Statistical analysis: The obtained results were analyzed statistically using Completely Randomized Design (CRD) to evaluate differences among different treatments means at 0.05 significant levels (Snedecor and Cochan, 1989).

RESULTS

The results of *in vivo* tests revealing the effect of probiotics on the hematological parameters of Indian magur (*C. batrachus*) over a period of eight weeks are presented in tabulated from. Blood contains mobile cells viz., red blood cell (erythrocytes), white blood cells (leucocytes) and platelets. These are made in bone marrow-the spongy tissues filled the centre of bones of fishes, bone marrow in skull, sternum, ribs, vertebral column and pelvic bones. Each type of cells play an important role in the body's normal functioning.

Level of hemoglobin in the blood of Indian magur (*C. batrachus*) under different treatments: The hemoglobin level of normal catfish (T1) remained in the range of 7.32±0.17 to 7.80±0.01 g 100 mL. However, in catfishes inoculated with pathogenic bacterium, *A. hydrophila* (T2) and *Micrococcus* sp. (T3), respectively; the level of hemoglobin fell drastically and remained in the range of 5.76±0.02 to 7.63±0.01 and 5.62±0.03 to 7.35±0.06 g 100 mL, respectively. The decrease was more in T2 (1.83 g 100 mL) than T3 (1.73 g 100 mL); indicating that *A. hydrophila* was more pathogenic than *Micrococcus* sp. and caused anemic conditions in catfishes. The hemoglobin level increased in the range of 6.56±0.0 to 7.47±0.02, 6.91±0.01 to 7.60±0.01 and 6.88±0.08 to 7.81±0.02 g 100 mL in catfishes inoculated with *Micrococcus* sp.+probiotic 1 (T10), *Micrococcus* sp.+probiotic 2 (T11) and *Micrococcus* sp.+probiotic 3 (T12) treatments, respectively. The increase in the levels of hemoglobin in *Micrococcus* sp.+probiotic 1 (T10) (1.11 g 100 mL), *Micrococcus* sp.+probiotic 2 (T11), (0.69 g 100 mL) and *Micrococcus* sp. +probiotic 3 (T12), (0.93 g 100 mL) indicated that probiotic 1 was more antagonistic and stimulated higher hemoglobin production than probiotic 3 followed by probiotic 2. On the other hand, the catfishes given the treatment of probiotics only i.e., probiotic 1 (T4), probiotic 2 (T5) and probiotic 3 (T6) showed maximal value of hemoglobin level as compared to all other treatments including control. The hemoglobin level was in the range of 4.79±0.07 to 6.41±0.10, 6.78±0.08 to 7.75±0.05 and 5.30±0.00 to 6.68±0.03 g 100 mL in catfishes administrated with probiotic 1, probiotic 2 and probiotic 3. The increase in the levels of hemoglobin in T4, T5 and T6 were 1.62, 0.97 and 1.38, respectively. These results revealed that probiotic 1 gives better results in increasing the hemoglobin level of catfishes and stimulated more production of hemoglobin as compared to probiotic 3 and probiotic 1 (Table 1).

Table 1: Effects of probiotics on the hemoglobin levels of Indian magur (*C. batrachus*) under *in vivo* induced pathogenicity over a period of eight weeks

	Hemoglobin (g 100 mL) level over a period of 8 weeks							
Treatments	1	2	3	4	5	6	7	8
Normal diet (T1)	7.59±0.00	7.32±0.17	7.63±0.00	7.69±0.00	7.75±0.01	7.79±0.01	7.79±0.01	7.80±0.01
A. hydrophila (T2)	7.63±0.01	6.83±0.02	6.13±0.02	5.76±0.02	-	-	-	-
Micrococcus sp. (T3)	7.35±0.06	6.87±0.03	6.23±0.23	6.05±0.23	5.62±0.03	-	-	-
Probiotic 1(T4)	4.79±0.07	4.91±0.08	4.93±0.05	4.95±0.07	5.23±0.17	5.34±0.07	5.97±0.09	6.41±0.10
Probiotic 2(T5)	6.78±0.08	6.89±0.02	6.92±0.07		7.32±0.07	7.45±0.06	7.70±0.04	7.75±0.05
Probiotic 3(T6)	5.30±0.00	5.55±0.05	5.79±0.05	5.58±0.07	5.94±0.02	7.16±0.03	6.40±0.02	6.68±0.03
A. hydrophila+probiotic 1 (T7)	6.97±0.06	7.29±0.05	7.43±0.00	7.49±0.03	7.62±0.04	7.82±0.04	7.90±0.02	7.96±0.04
A. hydrophila+probiotic 2 (T8)	7.03±0.07	7.27±0.05	7.49±0.04	7.56±0.12	7.62±0.04	7.66±0.03	7.77±0.02	7.79±0.03
A. hydrophila+probiotic 3 (T9)	6.99±0.07	7.46±0.09	7.34±0.10	7.40±0.07	7.47±0.07	7.71±0.02	7.77±0.08	7.85±0.04
Micrococcus sp.+probiotic 1 (T10)	6.56±0.08	6.69±0.07	6.88±0.03	6.90±0.09	6.97±0.04	7.23±0.05	7.34±0.05	7.47±0.02
Micrococcus sp.+probiotic 2 (T11)	6.91±0.01	6.98±0.01	7.05±0.04	7.28±0.01	7.39±0.01	7.47±0.01	7.51±0.01	7.60±0.01
Micrococcus sp.+probiotic 3 (T12)	6.88±0.08	7.28±0.04	7.41±0.11	7.51±0.04	7.57±0.07	7.81±0.02	7.67±0.11	7.76±0.08
CD value ($p\leq 0.01$)	0.27	0.15	0.26	0.26	0.15	0.15	0.17	0.17

Values are as Mean±SD. N = 30 (10 catfishes×3 replications), -: Catfishes died after four and five week

Level of total erythrocyte count in the blood of Indian magur (*C. batrachus*) under different treatments: The erythrocyte count of normal catfish remained in the range of 1.80±0.01 to 1.89±0.01. However, in catfishes inoculated with pathogenic bacterium, *A. hydrophila* (T2) and *Micrococcus* sp. (T3), respectively; the counts of erythrocyte fell drastically and remained in the range of 1.73±0.02 to 1.81±0.00 and 1.75±0.00 to 1.82±0.01, respectively. The counts of erythrocyte fell drastically seemed to be due to the haemolysis caused by pathogenic bacteria. The decrease was more in the *A. hydrophila* 0.08 than *Micrococcus* sp. 0.07; indicated that *A. hydrophila* was more pathogenic than the *Micrococcus* sp. bacteria and caused anemic condition. The erythrocyte count increased and remained in the range of 1.76±0.01 to 1.87±0.00, 1.84±0.00 to 1.92±0.01 and 1.76±0.01 to 1.86±0.00 in the catfish inoculated with *A. hydrophila*+probiotic 1 (T7), *A. hydrophila*+probiotic 2 (T8) and *A. hydrophila*+probiotic 3 (T9), respectively. The increase in the counts of eryrthrocyte in T7 (0.11), T8 (0.08) and T9 (0.10) indicated that probiotic 1 was more antagonistic and stimulate higher production of eryrthrocyte than probiotic 3 followed by probiotic 2. The erythrocyte count increased and remained in the range of 1.76±0.01 to 1.90±0.00, 1.80±0.00 to 1.86±0.00 and 1.78±0.01 to 1.91±0.01 in the catfish inoculated with *Micrococcus* sp.+probiotic 1 (T10), *Micrococcus* sp.+probiotic 2 (T11) and *Micrococcus* sp.+probiotic 3 (T12), respectively. The increase in the counts of erythrocyte in T10 (0.14), T8 (0.06) and T9 (0.13) indicated that probiotic 1 was more antagonistic and stimulate higher production of erythrocyte than probiotic 3 followed by probiotic 2. On the other hand, the catfishes given the treatment of probiotics in treatments probiotic 1 (T4), probiotic 2 (T5) and probiotic 3 (T6) showed maximal value of erythrocyte counts as compared to all other treatments including control. The erythrocyte counts were remained in the range of 1.53±0.01 to 1.76±0.00, 1.72±0.00 to 1.87±0.01 and 1.60±0.01 to 1.78±0.00 in catfishes administrated with probiotic 1, probiotic 2 and probiotic 3. The increase in the counts of erythrocyte in T4, T5 and T6 were 1.62, 0.97 and 1.38; respectively. These results revealed that probiotic 1 gives better results in increasing the erythrocyte counts of catfishes and stimulated more production of erythrocytes or increased the rate of erythropoesis as compared to probiotic 3 and probiotic 1 and reduced the chances of anemia in the catfishes (Table 2).

Table 2: Effects of probiotics on the total erythrocyte counts of Indian magur (*C. batrachus*) under *in vivo* induced pathogenicity over a period of eight weeks

	Total erythrocyte counts (10^6 cells mL^{-1}) level over a period of 8 weeks							
Treatments	1	2	3	4	5	6	7	8
Normal diet (T1)	1.80± 0.01	1.81±0.01	1.83±0.01	1.84±0.00	1.85±0.00	1.87±0.00	1.89±0.00	1.89±0.01
A. hydrophila (T2)	1.80±0.00	1.77±0.01	1.75±0.01	1.74±0.02	-	-	-	-
Micrococcus sp. (T3)	1.82±0.01	1.78±0.01	1.76±0.01	1.74±0.00	1.72±0.00	-	-	-
Probiotic 1 (T4)	1.60±0.01	1.63±0.01	1.63±0.01	1.64±0.01	1.64±0.01	1.67±0.01	1.76±0.00	1.70±0.01
Probiotic 2 (T5)	1.72±0.00	1.75±0.01	1.79±0.01	1.81±0.01	1.84±0.00	1.85±0.01	1.85±0.01	1.87±0.01
Probiotic 3 (T6)	1.62±0.01	1.60±0.01	1.63±0.01	1.68±0.01	1.70±0.01	1.74±0.01	1.69±0.01	1.78±0.00
A. hydrophila+Probiotic 1 (T7)	1.76±0.01	1.74±0.00	1.78±0.03	1.83±0.00	1.85±0.00	1.87±0.00	1.89±0.01	1.87±0.00
A. hydrophila+Probiotic 2 (T8)	1.84±0.00	1.82±0.01	1.87±0.00	1.88±0.01	1.89±0.00	1.89±0.02	1.92±0.01	1.91±0.01
A. hydrophila+Probiotic 3 (T9)	1.76±0.01	1.76±0.01	1.74±0.01	1.77±0.02	1.80±0.01	1.85±0.01	1.86±0.01	1.86±0.00
Micrococcus sp.+Probiotic 1 (T10)	1.76±0.01	1.81±0.01	1.84±0.01	1.85±0.00	1.87±0.01	1.87±0.00	1.89±0.01	1.90±0.00
Micrococcus sp.+Probiotic 2 (T11)	1.80±0.01	1.81±0.02	1.80±0.01	1.80±0.01	1.82±0.00	1.86±0.00	1.85±0.00	1.84±0.00
Micrococcus sp.+ Probiotic 3 (T12)	1.78±0.01	1.80±0.01	1.81±0.01	1.85±0.00	1.87±0.01	1.90±0.01	1.91±0.01	1.90±0.01
CD value ($p \le 0.01$)	0.03	0.03	0.03	0.02	0.02	0.03	0.02	0.02

Values are as Mean±SD; N = 30 (10 catfishes×3 replications), -: Catfishes died after four and five week

Level of total leukocyte count (TLC in 10^3 cells mL^{-1}) in the blood of Indian magur (*C. batrachus*) under different treatments: The leukocyte counts of normal diet (T1) fed catfish remained in the range of 6.61±0.03 to 7.68±0.06. However, in the catfishes inoculated with, *A. hydrophila* (T2) and *Micrococcus* sp. (T3); leukocytes counts increased and remained in the range of 6.53±0.12 to 7.72±0.05 and 6.67±0.03 to 7.81±0.09, respectively. The counts of leukocyte increased steeply seemed to be due to the increased production simulated by *A. hydrophila* and *Micrococcus* sp. as well as stimulation of immune system of catfishes and increased phagocytes. The increase was more in the T2 (1.19) than T3 (1.14); indicated that *A. hydrophila* was more pathogenic than the *Micrococcus* sp. bacteria. The leukocyte count increased and remained in the range of 5.70±0.03 to 6.48±0.11, 6.69±0.11 to 7.69±0.08 and 6.65±0.06 to 7.46±0.03 in catfish inoculated with the *A. hydrophila*+probiotic 1 (T7), *A. hydrophila*+probiotic 2 (T8) and *A. hydrophila*+probiotic 3 (T9), respectively and the increase in the counts of leukocytes in T7, T8 and T9 were 0.78, 1.00 and 0.81, respectively. The erythrocyte count increased and remained in the range of 6.34±0.06 to 7.35±0.04, 6.22±0.11 to 7.11±0.10 and 6.89±0.09 to 7.80±0.03 in catfish inoculated with *Micrococcus* sp.+probiotic 1 (T10), *Micrococcus* sp.+probiotic 2 (T11) and *Micrococcus* sp.+probiotic 3 (T12), respectively and the increase in the counts of leukocytes in T10, T11 and T12 were 1.01, 0.89 and 0.91, respectively. On the other hand, the catfishes given probiotics in treatments probiotic 1 (T4), probiotic 2 (T5) and probiotic 3 (T6) showed maximal value of leukocyte counts as compared to all other treatments including control. The counts were remained in the range of 6.40±0.05 to 7.72±0.05, 5.20±0.04 to 6.46±0.15 and 6.59±0.08 to 7.80±0.01 in catfishes administrated with probiotic 1, 2 and 3, respectively. The decrease in the counts of leukocyte in T4, T5 and T6 were 0.87, 0.63 and 0.81, respectively. These results revealed that probiotic 1 gives better results in decreasing the leukocyte counts of catfishes and stimulated more less production of leukocyte or decreased the rate of leucopoiesis as compared to probiotic 3 and probiotic 1 (Table 3).

Table 3: An effect of probiotics on the total leucocytes counts of Indian magur (*C. batrachus*) under *in vivo* induced pathogenicity over a period of eight weeks

	Total leucocytes counts (10^3 cells/mL) level over a period of 8 weeks							
Treatments	1	2	3	4	5	6	7	8
Normal diet (T1)	6.61±0.03	6.85±0.13	7.11±0.08	7.37±0.15	7.54±0.06	7.58±0.03	7.68±0.13	7.68±0.06
A. hydrophila (T2)	6.53±0.12	6.57±0.07	7.28±0.09	7.72±0.05	-	-	-	-
Micrococcus sp. (T3)	6.67±0.03	6.78±0.04	6.74±0.01	6.68±0.04	7.81±0.09	-	-	-
Probiotic 1 (T4)	6.40±0.05	6.38±0.09	6.36±0.06	6.30±0.04	6.18±0.03	6.06±0.04	5.82±0.05	5.53±0.04
Probiotic 2 (T5)	6.46±0.04	6.42±0.04	6.39±0.07	6.35±0.07	6.23±0.06	6.08±0.10	5.86±0.10	5.83±0.15
Probiotic 3 (T6)	6.59±0.08	6.51±0.10	6.43±0.00	6.38±0.07	6.26±0.12	6.12±0.06	6.01±0.02	5.88±0.01
A. hydrophila+Probiotic 1 (T7)	5.70±0.03	5.34±0.04	5.81±0.01	6.15±0.05	6.37±0.07	6.49±0.03	6.48±0.11	6.44±0.10
A. hydrophila+Probiotic 2 (T8)	6.69±0.11	6.86±0.03	6.85±0.09	7.04±0.07	7.19±0.15	7.36±0.04	7.48±0.07	7.69±0.08
A. hydrophila+Probiotic 3 (T9)	6.65±0.06	6.86±0.03	7.12±0.05	7.26±0.02	7.22±0.05	7.30±0.01	7.46±0.03	7.46±0.03
Micrococcus sp.+Probiotic 1 (T10)	6.44±0.12	6.34±0.06	6.58±0.14	6.63±0.11	6.75±0.04	6.97±0.05	7.26±0.12	7.35±0.04
Micrococcus sp.+Probiotic 2 (T11)	6.22±0.11	6.32±0.05	6.50±0.03	6.58±0.03	6.65±0.05	6.84±0.05	7.11±0.10	6.93±0.00
Micrococcus sp.+Probiotic 3 (T12)	7.13±0.03	6.89±0.09	7.35±0.06	7.41±0.01	7.52±0.08	7.63±0.05	7.71±0.04	7.80±0.03
CD value ($p \le 0.01$)	0.22	0.22	0.21	0.21	0.27	0.20	0.24	0.23

Values are as Mean±SD; N = 30 (10 catfishes×3 replications), -: Catfishes died after four and five week

Level of Packed Cell Volume (PCV) in the blood of Indian magur (*C. batrachus*) under different treatments: The packed cell volume of normal diet (T1) catfishes remained in the range of 38.29±0.99 to 48.60±0.67. However, in catfishes inoculated with *A. hydrophila* (T2) and *Micrococcus* sp. (T3), respectively; the packed cell volume fell drastically and remained in the range of 28.79±0.96 to 36.51±2.34 and 24.79±0.22 to 31.64±0.64, respectively. The drastic fall in packed cell volume seemed to be due to the haemolysis due to *A. hydrophila* and *Micrococcus* sp. The decrease was more in T2 (7.72) than T3 (6.85); indicated that *A. hydrophila* was more pathogenic than the *Micrococcus* sp. bacteria caused anemic situation. The packed cell volume increased and remained in the range of 22.79±0.61 to 36.52±0.50, 34.83±1.35 to 45.32±0.29 and 32.77±0.17 to 44.86±1.12 in the catfish inoculated with *A. hydrophila*+probiotic 1 (T7), *A. hydrophila*+probiotic 2 (T8) and probiotic 3 (T9), respectively. The increase in the levels of packed cell volume in T7 (13.73), T8 (10.49) and T9 (12.09) indicated that probiotic 1was more antagonistic and stimulated increased production blood cells than probiotic 3 followed by probiotic 2. The packed cell volume increased and remained in the range of 40.55±1.00 to 54.92±1.27, 34.81±0.46 to 46.71±1.53 and 32.76±0.46 to 43.68±1.45 in the catfish inoculated with *Micrococcus* sp.+probiotic 1 (T10), *Micrococcus* sp.+probiotic 2 (T11) and probiotic 3 (T12), respectively. The increase in the volume of packed cells in T10 (14.37), T11 (11.90) and T12 (10.92) indicated that probiotic 1was more antagonistic and stimulated increased production blood cells than probiotic 3 followed by probiotic 2. On the other hand, the catfishes given probiotics in treatments probiotic 1 (T4), probiotic 2 (T5) and probiotic 3 (T6) showed maximal value of packed cell volume as compared to all other treatments including control. The packed cell volume were remained in the range of 33.25±0.23 to 53.70±1.21, 35.02±0.27 to 51.55±0.39 and 41.70±0.75 to 59.98±0.88 in catfishes administrated with probiotic 1, probiotic 2 and probiotic 3, respectively. The increase in the volumes of packed cells in T4, T5 and T6 were 20.45, 16.53 and 18.28, respectively. These results revealed that probiotic 1 gives better results in increasing the packed cell volumes of the blood of catfishes and stimulated more production of erythrocytes and leukocyte as compared to probiotic 3 and probiotic 1 (Table 4).

Table 4: Effects of probiotics on the packed cell volume of Indian magur (*C. batrachus*) under *in vivo* induced pathogenicity over a period of eight weeks

	Packed cell volume level over a period of 8 weeks							
Treatments	1	2	3	4	5	6	7	8
Normal diet (T1)	38.29±0.99	39.15±1.21	40.13±0.86	40.87±0.93	42.43±0.55	43.96±0.38	45.88±0.66	48.60±0.67
A. hydrophila (T2)	36.51±2.34	34.02±1.04	3.36±2.45	28.79±0.96	-	-	-	-
Micrococcus sp.(T3)	31.64±0.60	31.63±0.59	26.83±0.19	25.69±0.40	24.79±0.22	-	-	-
Probiotic 1 (T4)	33.25±0.23	33.99±0.54	35.49±0.74	39.25±1.99	44.74±1.01	47.24±1.79	51.35±0.65	53.70±1.21
Probiotic 2 (T5)	35.02±0.27	35.02±0.30	43.29±0.39	45.54±0.98	47.72±0.66	48.26±0.23	49.62±0.37	51.55±0.39
Probiotic 3 (T6)	41.70±0.75	44.04±0.90	46.53±0.33	49.79±0.39	52.51±1.32	56.65±0.50	58.49±0.35	59.98±0.88
A. hydrophila+Probiotic 1 (T7)	29.10±0.32	25.00±1.21	22.79±0.61	24.26±0.39	29.10±0.39	32.10±0.50	34.19±1.08	36.52±0.50
A. hydrophila+Probiotic 2 (T8)	34.83±1.35	34.83±1.36	40.99±0.57	39.31±0.97	42.58±0.28	41.84±0.75	45.27±0.26	45.32±0.29
A. hydrophila+Probiotic 3 (T9)	32.77±0.17	33.88±0.59	36.16±0.28	38.29±1.05	41.39±0.63	44.86±1.12	42.64±0.62	42.08±0.37
Micrococcus sp.+probiotic 1 (T10)	40.55±1.00	42.19±0.97	44.13±1.10	45.13±0.90	47.07±0.99	48.77±0.85	51.35±0.87	54.92±1.27
Micrococcus sp.+probiotic 2 (T11)	34.83±0.47	34.81±0.46	38.00±1.31	38.60±0.95	40.11±0.35	42.26±0.51	46.71±1.53	43.28±0.48
Micrococcus sp.+probiotic 3 (T12)	32.76±0.46	33.51±0.34	34.36±0.48	37.49±2.11	39.41±0.37	41.39±0.54	43.55±1.05	43.68±1.45
CD value ($p \leq 0.01$)	2.80	2.81	2.87	3.33	2.16	2.60	2.31	2.83

Values are as Mean±SD; N = 30 (10 catfishes×3 replications), -: Catfishes died after four and five week

DISCUSSION

Dead probiotic cells were used to control disease in *Onchorhynchus mykiss* (Walbaum) and observed higher number of leucocytes, erythrocytes and macrophages (Irianto and Austin, 2002). Scientists from different parts of the world were found the development of disease resistance due to the use of probiotics *Bacillus* sp. (Brunt *et al.*, 2007; Rengpipat *et al.*, 2008, 2000; Siwicki *et al.*, 2003). As probiont and also reported an increase in the level of selected hematological parameters in the blood Red Blood Cell Count (RBC), Haematocrit (Ht), hemoglobin (Hb), various leukocyte counts, the total leukocyte level and Mean Corpuscular Volume (MCV), mean hemoglobin concentration (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC). The studies on hematological parameters in the three species of Indian major carps viz., catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigal*) were carried out for 21 days by subjecting fingerlings to acidic (pH 5.5, 6.5) and alkaline (pH 8.0, 8.5 and 9.0) waters and control groups were maintained at neutral pH. A change in water pH either to acidic or alkaline conditions exerted stress in fish characterized by increased size of erythrocytes, production of immature erythrocytes and reductions in the total erythrocyte counts, hemoglobin and serum protein content. There were also increases in total leukocyte counts and blood glucose. However, the degree of these responses varied among the three species. Rohu was found to be least affected followed by mrigal to the stress of altered water pH, while catla was the most vulnerable to pH changes (Nayak *et al.*, 2007). Significantly higher respiratory burst activity, hemoglobin content, total erythrocyte count, blood serum, total leukocyte count, serum globulin content, serum lysozymes activity and relative survival percentage in *C. catla* juveniles were observed when fed with omega-3 fatty acid (3%) in the diet.

CONCLUSION

In conclusion, the results of present study revealed that probiotic had a positive effect on haemoglobin level which increased approximately to 24% in its value (Table 1), erythrocytes count which increased approximately to 10% in its value (Table 2), leucocyte count which decreased approximately to 8% in its value (Table 3) and packed cell volume increased approximately to 20% in its value (Table 4). This clearly indicated that there was positive response in the values of haematological parameters of fishes.

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