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(3): 700-706
© 2020 JEZS
Received: 13-03-2020
Accepted: 15-04-2020

Tasok Leya
(1) School of Fisheries, Centurion University of Technology and Management, R-Sitapur, Gajapati, Odisha, India
(2) ICAR-Central Institute of Fisheries Education, Mumbai, Maharashtra, India

Ram Prakash Raman
ICAR-Central Institute of Fisheries Education, Mumbai, Maharashtra, India

Prem Prakash Srivastava
ICAR-Central Institute of Fisheries Education, Mumbai, Maharashtra, India

Corresponding Author:
Tasok Leya
(1) School of Fisheries, Centurion University of Technology and Management, R-Sitapur, Gajapati, Odisha, India
(2) ICAR-Central Institute of Fisheries Education, Mumbai, Maharashtra, India

Effect of curcumin supplemented diet (CSD) on haematological parameters of *Cirrhinus mrigala* against *Edwardiella tarda* infection

Tasok Leya, Ram Prakash Raman and Prem Prakash Srivastava

Abstract
The aim of the current study was to evaluate the effect of CSD on haematological parameters of *Cirrhinus mrigala* fingerlings fed on four CSD viz: T1 (0.25%), T2 (0.5%), T3 (1.0%), T4 (1.5%), and a control group (0% curcumin level), and the experimental was carried for 45 days. The sampling was done by taking blood on 0, 15, 30, and 45 days interval from different treatment groups. After 45 days of the experimental period, fifteen fishes from each replicate were challenged with virulent *Edwardsiella tarda*. Mortality was recorded up to 14 days post challenged. The experimental results showed that there was a significant (p>0.05) difference in all treatment group in all haematological parameters in compared to control group. However, among treatment groups there was insignificant differences was observed in haematological parameters. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) showed no significant (p>0.05) difference among the treatment group. Total erythrocyte count (TEC) and haemoglobin (Hb) was, also not significantly difference in all treatment group. However, total leukocyte count (TLC) was significantly (p>0.05) different among the treatment groups. The high values were observed in T4 followed by T3, T2, and T1, respectively. Dietary supplement curcumin at 1.5% (T4) showed significantly (p<0.05) higher percentage survival (80%) against *Edwardsiella tarda* challenged than control. Thus, the study has concluded that curcumin supplement diet fed to fish showed no any significant difference among treatments but it improves the immunity of the fish because of an increase in TLC.

Keywords: Curcumin, *Haematology*, *Libero rohita*, *Edwardsiella tarda*

1. Introduction
Haematology is a branch of science concerned with the study of physiology and pathology of the cellular elements of blood [1, 2]. Haematological parameters in teleost constitute of haemoglobin concentration (Hb), haematocrit (Hct), Erythrocytes (Red Blood Cell) and Leukocytes (White Blood Cell), platelet (PLT), Packed cell volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) [3-5]. All these haem parameters can be altered by intrinsic and external element affecting the appearance of blood cells and its quantitative [5, 6].
Haematological indices affect physiological and cellular elements status, provide a significant indication on well-being of the fish health [7] and are used as important diagnostic tools to assess the health status of fish [2, 8].
The Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial herbal plant of the ginger family, Zingiberaceae [9]. It is native to southeast India, and well grow at the temperatures between 20 °C and 30 °C (68 °F and 86 °F) [10]. The active turmeric compound such as curcumin is believed to have a wide range of biological effects including antibacterial, antifungal, anti-inflammatory, antioxidant, antitumor and immunostimulant activities [11, 12].
Edwardsiella tarda is one of the most important disease-causing pathogens [13] which caused high mortality in freshwater, brackish water, and marine fish population [14]. The infected fish developed extensive skin lesions, affecting internal organs (liver, kidney, spleen), and epidermal tissues [12, 15]. This bacterium also a zoonotic in nature, causing gastroenteritis and generalized infections in human and animal [16].
Therefore, the objective of the present study was to evaluate the effect of curcumin supplemented diet on the haematological parameters and percentage survival against *Edwardsiella tarda* in *Cirrhinus mrigala*.

2. Methods

2.1 Experimental animals

The experiment was conducted for a period of 45 days in Aquatic Animal Health Management Laboratory of the Central Institute of Fisheries Education (CIFE), Mumbai. Animals used for experimental purpose were advanced fingerlings of Indian major carp (*Cirrhinus mrigala)* with an average weight of 10.5 ± 1.4gm. The fishes were procured from Namdev Fish Farm, near Titwala Ganesh Mandir, Mumbai, Maharashtra. The fishes were transported in 21 L polythene packing with sufficient oxygen (15 fish /pack). On reaching the wet laboratory, the fish were treated with Potassium permanganate ($KMnO_4$) @ 2ppm for 30 second, and carefully transferred to a big circular cement tank (1000L capacity) and were left undisturbed for whole night with sufficient aeration. To check any kind of bacterial infection they were given antibiotic using Oxytetracycline @ 15 ppm (APHA) for first three days. The stock was acclimatized under aerated conditions for a period of 20 days and was fed with commercial floating fish diet (FISHER GOLD: crude protein 32 %, carbohydrate-34%, crude fat-5%, fibre-5% and moisture-10%) at the rate of 3% body weight. The water quality parameters were monitored regularly and maintained at an optimum level throughout the research period [17].

2.2 Experimental design and sampling

The experimental animals were maintained in rectangular plastic tubs (80 × 57 × 42 cm, 150 L capacity) covered with perforated lids and the water used for rearing was drawn from bore well. The tubs were initially washed and filled with potassium permanganate solution @ 4ppm that was left overnight. They were flashed out the following day and tubs were thoroughly washed with clean water. The total volume of the water in each tube was maintained at 100L throughout the experimental period. The aeration was provided round the clock. The aeration pipe in each tub was provided with an air stone and a regulator valve to control the air pressure uniformly in all the tubs. Two hundred and seventy (270) fingerlings of *Cirrhinus mrigala* were randomly distributed into five distinct experimental groups. Each group was having three replicates following a completely randomized design (CRD), fifteen fish (18) were stocked in each tub. Fishes were sampled on 0^{th} day (before feeding trial), 15^{th}, 30^{th} and 45^{th} day for haematological study. Nine fishes were taken for blood from each treatment, respectively.

2.3 Herbal extract and diet preparation

The curcumin was procured from Multi Enterprise Pvt Ltd., Mumbai, India. The experimental diet was prepared as per the previous study [12].

2.4 Bacteria

The *E. tarda* (ATCC 15947) used in the present study was obtained from ATCC, USA. The *E. tarda* were revived using brain heart infusion broth (BHI Broth). Briefly, the broth was incubated for 18–20 h at 37 °C, culture was then streaked on sterile Salmonella-Shigella agar (SS-agar) plate and incubated at 28 °C. The single black colony was picked up with a sterile loop and inoculated in BHI broth at 28 °C for 20 h [12].

2.5 Collection of blood

Six fish from each treatment group were selected randomly and anaesthetized with clove oil @50 µl/L of water before taking blood from fish. Blood was taken from Vena caudal is using a medical syringe which was previously rinsed with 2.7% EDTA solution. Blood collected was then transferred immediately to test tube containing thin layer of EDTA powder (as an anticoagulant) and shaken well in order to prevent haemolysis of blood.

2.6 Total erythrocyte count (TEC)

Blood (20µl) mixed with 3980 µl of RBC diluting fluid in a clean test tube. The mixture was shaken well to suspend the cells uniformly in the solution. A small drop of this mixture was charged to Neubauer's counting chamber of haemocytometer then counted in five groups of squares. All the cells lying inside the five small squares were counted under high power (40X) of compound light microscope. The following formula is used to calculate the number of RBC per mm^3 of the blood sample:

$$\text{Number of RBC/mm}^3 = \frac{\text{N x dilution}}{\text{Area counted x Depth of fluid}}$$

$= N \times 200 / 0.2 \times 0.1$

$= N \times 10000$

Where, N is the total number of red blood cells counted in 5 squares of the haemocytometer. 0.2 is the area counted and 0.1 is depth of fluid.

2.7 Total Leukocyte Count (TLC)

In TLC count, 20 µl of blood was mixed with 3980 µl of WBC diluting fluid in a clean glass vial. The mixture was shaken well to suspend the cells uniformly in the solution. Care was taken that here were no air bubbles trapped. The numbers of cells were counted in four big squares under high power (40X) magnification of compound light microscope. The number of WBC per mm^3 of the blood sample was calculated using the following formula:

Number of WBC/mm^3 = N × dilution / Area counted × depth

$= N \times 200 / 4 \times 0.1$

$= N \times 500$

Where, N denotes the total number of white blood cells counted in 4 squares of the haemocytometer.

2.8 Packed cell volume (PVC) / Haematocrit value

PCV was determined by drawing non-dotted blood by capillary action into micro haematocrit tubes. One end of the tube was sealed with synthetic sealant. The sealed tubes were centrifuged in a micro haematocrit centrifuge for 5 mins at 10,500 rpm. The PCV measured using micro haematocrit reader and expressed as percentage.

29 Haemoglobin content (Hb)

The haemoglobin level of blood was analysed following the Cyanmethemoglobin method using Drabkins Fluid (Qualigens). Blood (20µl) was mixed with 5ml of Drabkin's working solution. The absorbance was measured using a spectrophotometer at wavelength of 540 nm. The final concentration was calculated by comparing with the standard cyanmethemoglobin (Qualigens Diagnostics). The haemoglobin concentration was then calculated by using the following formula:

$$\text{Haemoglobin content (\%)} = \frac{\text{OD (T) x 251 x 60}}{\text{OD (S) x 1000}}$$

Where, OD (T) = Absorbance of test
OD (S) = Absorbance of standard

2.10 MCV, MCH and MCHC
The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were measured according to the following equation
MCV (fl) = Hct (%) ×10 / RBC (million/mm^3)
MCH (pg) =Hb (gm/dl) ×10 /RCB (million/mm^3)
MCHC (%) = Hb (gm/dl) × 100 / Hct (%)

2.11 Challenge study
After days 45 days, 5 fishes from each replicate (n=15) were selected for the challenge test. The fishes were injected intraperitoneal (IP) with the virulent strain of *E. tarda* suspended in 200 µL (5.4 x 10^5 CFU^{-1}). The challenged fishes were kept under observation for 14 days and subsequently, percentage survival was estimated as descripted in previous study [12].

2.12 Percentage survival
Survival at the end of 14 days post infection was calculated using the following formula

$$\text{Survival (\%)} = \frac{\text{Total number of fish survived}}{\text{Total number of fish injected}} \text{ x } 100$$

2.13 Statistical Analysis
The data were statistically analysed by using statistical package SPSS version 16 in which data were subjected to one-way ANOVA and Duncan's multiple range tests was used to determine the significant differences between the means. Student t-test was also used to determine the significant difference among the mean of pre- and post-challenge condition. Comparisons were made at the 5% probability level.

3. Results
3.1 Diet preparation
Experimental diet and water quality parameters analysis was described in our previous study [12].

3.2 Collection of blood
Blood was taken from vena caudal using a 1 mL tuberculin syringe (Fig.1), and blood collected was then transferred immediately to test tube containing thin layer of EDTA powder and shaken well in order to prevent haemolysis of blood.

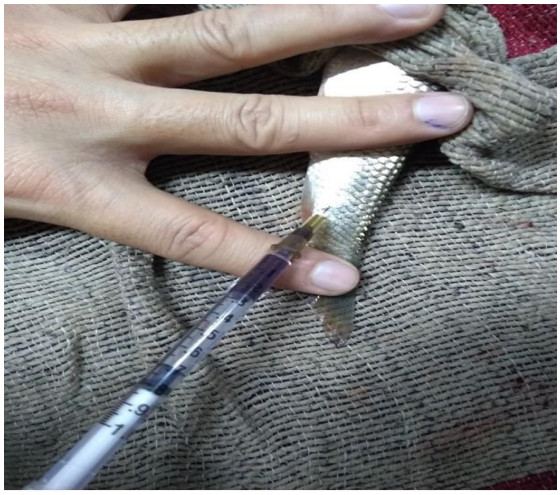

Fig 1: Blood collecting from caudal vein

3.3 Total Erythrocyte Count (TEC)
The effect of curcumin enriched diet on total erythrocyte count of the different treatment groups are presented in Fig.2. There was no significant difference noticed among the different treatment groups on 0-day sampling. However, on 15th day significant (p<0.05) difference was noticed in different treatment groups in to control group. Similarly, slight increasing trend of TEC was observed in subsequent days of sampling day.

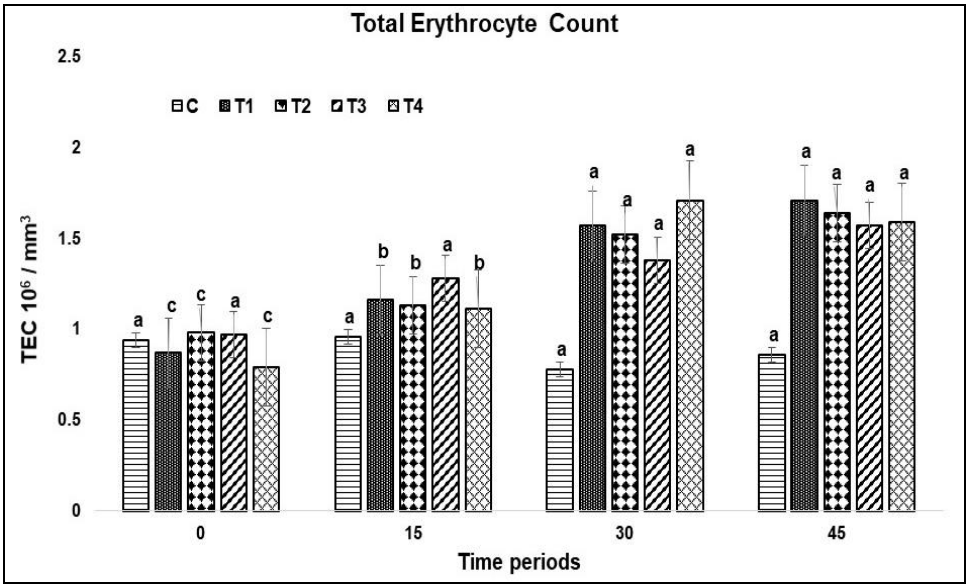

Fig 2: Total Erythrocyte Count (TEC 10^6 / mm^3) of *C. mrigala* in different treatment groups fed with diet containing various levels of Curcumin.

3.4 Total Leukocyte Count (TLC)

The effect of curcumin enriched diet on total leukocyte count of the different treatment groups are given in Fig.3. There was no significant difference ($p < 0.05$) among the various treatment groups on 0 day. However, on 15th and 30th day, the significant ($p<0.05$) difference was noticed in treatment fed with curcumin enriched diet compared to control, and significant difference was also observed in subsequent 45th days also. The highest TLC level was observed in T4 treatment group and lowest in control group on 15th, 30th, and 45th day, respectively.

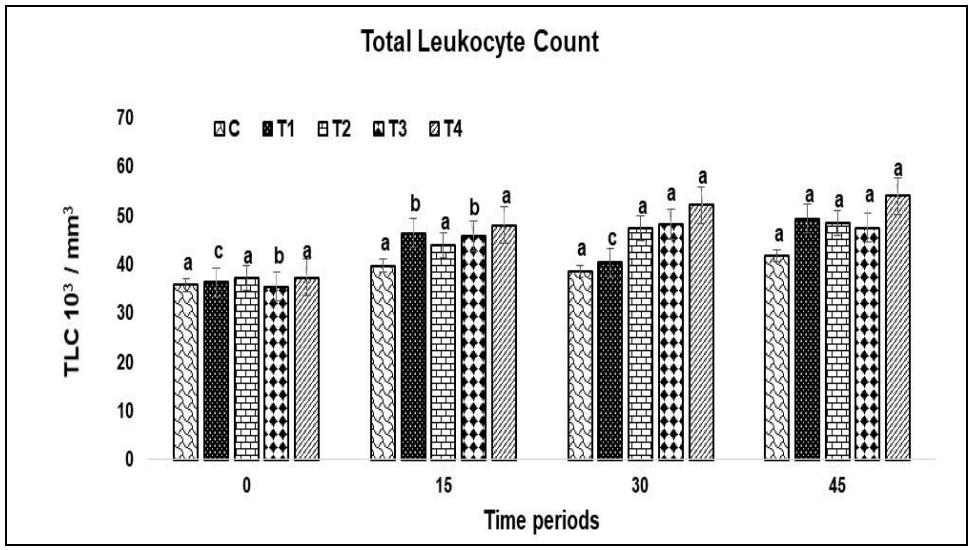

Fig 3: Total Leukocyte Count (TEC 10^3 / mm^3) of *C. mrigala* in different treatment groups fed with diet containing various levels of Curcumin.

3.5 Packed Cell Volume (PCV)

The effect of curcumin enriched diet on PCV content in the different treatment groups are presented in Fig.4. As TEC same trend was observed in PCV also. However, significant difference ($p<0.05$) was noticed on 30th and 45th days. The highest value was observed in T4 (24.33±1.23) and lowest value in control group (17.79±1.22) on 30th day. On 45th day sampling the highest PVC value was observed in T4 (34.98±1.17) and lowest in T5 (26.39±1.28).

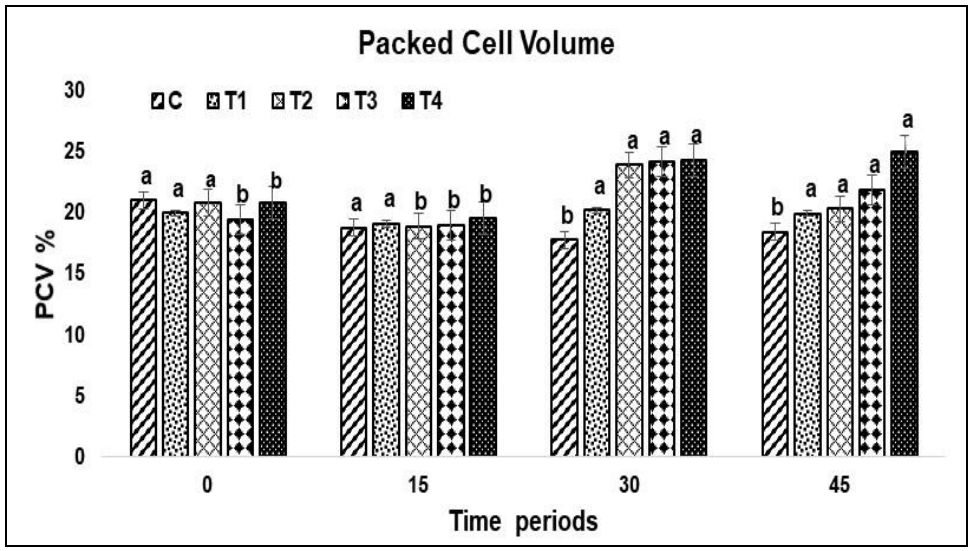

Fig 4: Packed cell volume (PCV %), of *C. mrigala* in different treatment groups fed with diet containing various levels of Curcumin.

3.6 Haemoglobin content (Hb)

The effect of curcumin enriched diet on haemoglobin content in different treatment groups are presented in Fig. 5. No significant difference was noticed between control and the treatment groups on 0-day sampling. However, there was significant difference ($p < 0.05$) found between control and treatment groups on 15th day sampling and subsequent sampling days. The highest value being observed was in T5 (6.27±0.09) and lowest in control group (3.53±0.12). The highest value was recorded in T4 (9.50±0.06) and lowest in control group (6.40±0.06) on 45th day of sampling.

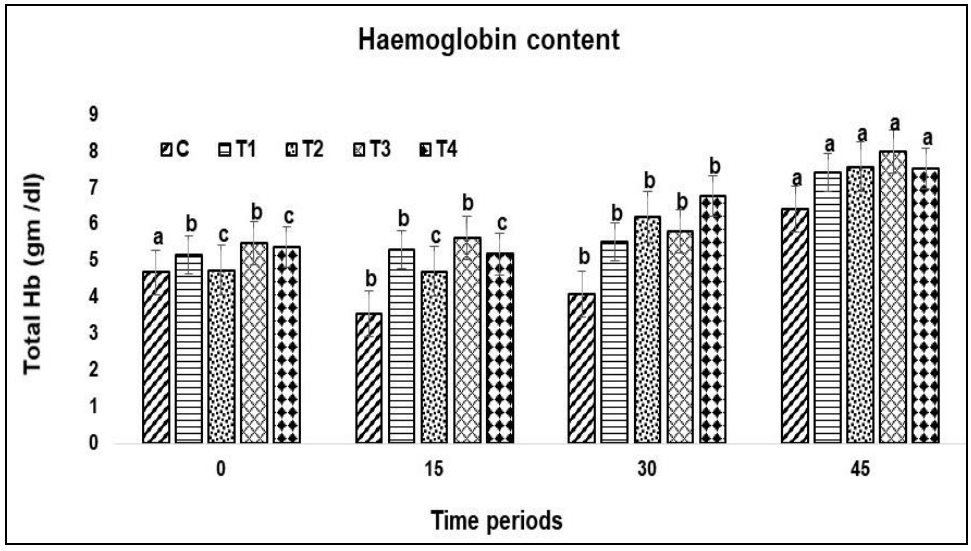

Fig 5: Haemoglobin content (Total Hb) (gm / dl) of *C. mrigala* in different treatment groups fed with diet containing various levels of Curcumin.

3.7 Mean Corpuscular Volume (MCV)

Effect of curcumin on MCV value of *C. mrigala* is represented in table 1. MCV value of experimental groups were not differed significantly ($p<0.05$) with control on 0 day and 15th day sampling. On day 30th, the value of MCV was observed significantly ($p<0.05$) differed between control and treatment groups and highest value being observed in control group and lowest in T4 group. Similarly, on day 45th, the MCH value was noticed highest in control group and lowest in T2 treatment.

Table 1: Mean Corpuscular Volume (MCV) (in fl), of *C. mrigala* in different treatment groups fed with diet containing various levels of Curcumin

Treatment	Time periods			
	0th day	15th day	30th day	45th day
Control	141.67^{a}±0.88	157.33^{a}±1.45	183.02^{a}±0.58	186.01^{a}±0.58
T1	144.67^{a}±1.45	154.01^{a}±0.58	170.33^{b}±0.88	182.33^{b}±2.60
T2	142.67^{a}±2.33	155.33^{a}±1.20	167.01^{c}±1.15	171.33^{c}±088
T3	139.01^{a}±1.73	153.01^{a}±1.53	180.01^{a}±0.58	183.67^{b}±1.45
T4	140.67^{a}±2.40	154.67^{a}±1.45	166.67^{c}±0.88	172.03^{c}±1.73

The means with no superscript letter in common per factor indicates significant difference. If the effects were significant, ANOVA was followed by Duncan multiple range test. $p<0.05$. Values are presented as mean ± SE.

3.8 Mean Corpuscular Haemoglobin (MCH)

Effect of curcumin on MCH value of *C. mrigala* is represented in table 2. MCH value of experimental groups were not differed significantly ($p<0.05$) in all treatment group compare to control group. The highest value among the experimental groups was found in control group on 30th and 45th days.

Table 2: Mean corpuscular haemoglobin (MCH) (in pg), of *C. mrigala* in different treatment groups fed with diet containing various levels of Curcumin

Treatment	Time periods			
	0th day	15th day	30th day	45th day
Control	41.47^{b}±0.91	45.56^{a}±0.58	44.42^{abc}±0.36	47.78^{a}±0.58
T1	42.51^{ab}±1.25	44.27^{b}±0.62	43.62^{ab}±0.64	46.76^{b}±0.29
T2	44.47^{a}±0.34	41.09^{b}±0.62	42.17^{c}±1.18	46.79^{b}±0.44
T3	41.49^{b}±0.69	41.20^{b}±1.20	43.05^{bc}±0.79	45.62^{b}±0.46
T4	44.70^{a}±0.46	45.54^{ab}±0.73	42.71^{a}±0.53	46.85^{b}±0.58

The means with no superscript letter in common per factor indicates significant difference. If the effects were significant, ANOVA was followed by Duncan multiple range test. $p<0.05$. Values are presented as mean ± SE.

Mean Corpuscular Haemoglobin Concentration (MCHC)

Effect of curcumin on MCHC value of *C. mrigala* is represented in table 3. MCHC value of experimental groups were also not differed significantly ($P<0.05$) in control and treatment group on 0th, 15th and 30th days, respectively. But there was a significantly ($p<0.05$) differed in between control and treatment groups on 45th. The highest value being observed on 45th day was in T4 (27.05^{a}±3.62) and lowest in T1 (23.86^{b}±0.09).

Table 3: Mean Corpuscular Haemoglobin Concentration (MCHC) (in %), of *C. mrigala* in different treatment groups fed with diet containing various levels of Curcumin.

Treatment	Time periods			
	0th day	15th day	30th day	45th day
Control	29.59^{b}±0.55	26.93^{a}±0.33	26.42^{a}±0.61	24.61^{b}±0.60
T1	29.47^{b}±0.42	26.86^{cd}±0.40	25.6^{ab}±0.56	23.86^{b}±0.09
T2	28.97^{b}±0.73	25.62^{d}±0.63	24.17^{b}±0.37	26.78^{a}±0.35
T3	29.33^{b}±0.79	26.62^{bc}±0.64	24.66^{ab}±0.26	24.16^{b}±0.19
T4	28.64^{b}±0.35	26.98c±0.67	25.90^{ab}±0.44	27.05^{a}±3.62

The means with no superscript letter in common per factor indicates significant difference. If the effects were significant, ANOVA was followed by Duncan multiple range test. $p<0.05$. Values are presented as mean ± SE.

3.10 Challenge and percentage survival

After 45 days of feeding trial, fish were challenged with *E. tarda* at the rate of 200 µL (5.4 x 10^5 CFU^{-1}/fish) through intraperitoneal injection. The mortality and morbidity of challenged fish were observed till 14 days post-challenge. Significantly higher percentage survival was recorded in T4 (80%), followed by T3 group (73.33%), and T2, T1 (66.66 %), respectively (Fig. 6).

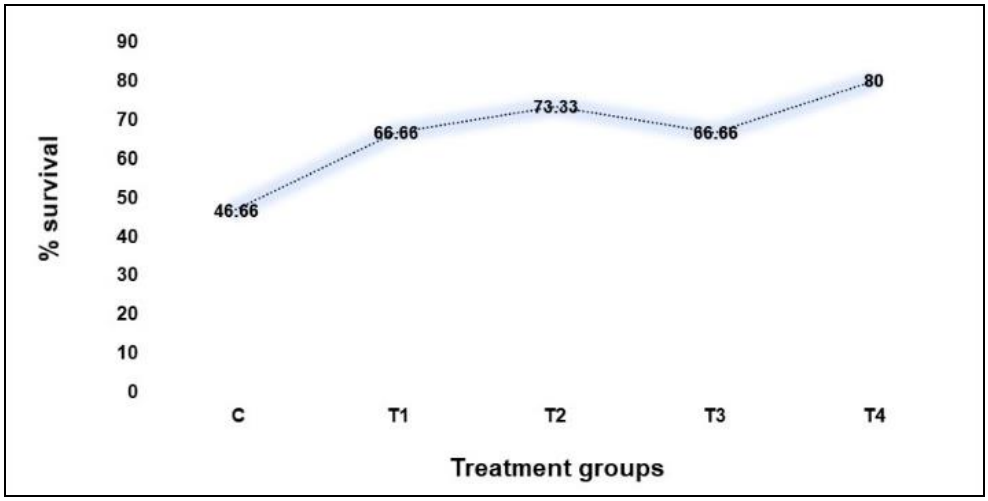

Fig 6: Percentage survival of different treatment groups after challenged with *E. tarda* (5.4 x 10^5 CFU $-^1$/ fish).

4. Discussion

Haematological parameters in fish including Erythrocytes (RBC), Leukocytes (WBC), Haematocrit (Hct) / Packed cell volume (PCV), Haemoglobin concentration (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) [3-5].These haem parameters can be influenced by internal and external factors, affecting the quality and the quantitative values of cells [6]. The any changes in blood parameters is considered as a prognostic or diagnostic which gives primary warning signal of the abnormalities in homeostatic and defence system of fish [6].

The fish erythrocytes are usually elliptical or ovoid, but species have varied in erythrocytes dimensions such as length 10– 20 mm and 6–10 mm in width [2, 18]. The primary function of RBCs is the carrier of oxygen by the high concentrations of the respiratory pigment Haemoglobin (Hb) and pH regulation [18]. Fish fed with probiotic-supplemented diets increased RBCs levels of fish compared to control fish, reported previously [19]. However, our finding was not agreed with this report, where we observed that there was not significantly increased in RBC number. But our result was agreement with the finding of Bbole *et al*. [20] where, they reported that, there was a decrease in the erythrocytes count with increase of Moringa above 10% inclusion rate in feed.

Fish leukocytes role is to involved in defence system against the invading antigens. In our study, the leukocytes results showed significantly different in treatment groups, compared to control group, which was supported by others studies [21, 22]. The high values of erythrocytes may be an indicator that the fish feeding on supplemented curcumin diet have high immune response and will have effective against pathogens [23].

Haematocrit (hct) or Packed cell volume (PCV) is the measure of blood capacity to carry oxygen [24]. The higher the PVC value, the higher the blood's moving ability of oxygen [2]. In our finding the PCV was observed no significant ($p>0.05$) difference in all treatment groups on 0th and 15th days. However, on 30th and 45th days, significant ($p>0.05$) difference was recorded and highest PCV was showed in T3 followed by T4, T2, and T1 respectively. The Hb, MCV, MCH, and MCHC were also showed similar trends as like PCV value.

5. Conclusion

The present study concluded that curcumin supplement diet did not show much significance differences in all treatment group in terms of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total erythrocytes. However, there was significant differences observed in treatment group in compared to control group in total leukocyte numbers which may help in protection against *Edwardsiella tarda* infection.

6. Acknowledgement

The authors are grateful to Director/Vice-Chancellor, ICAR-Central Institutes of Fisheries Education, Mumbai, India for

providing facilities for carrying out this experiment. We would also like to thanks to Dean, Centurion University of Technology and Management, Paralakhemundi, Odisha, India, for encourage and support to write this manuscript.

7. References

1. Bolliger AP, Everds N. Haematology of Laboratory rodents. Edited by Weiss D.J, Wardrop K.J. Schalms Veterinary Hematology, 6th Edition. Ames, IA: Wiley Blackwell. 2010:852-862.
2. Michael SE, Abarike ED, Cai J. A Review on the Probiotic Effects on Haematological Parameters in Fish. Journal of FisheriesSciences.com. 2019; 13(3):025-031.
3. Grant KR. Fish hematology and associated disorders. Veterinary Clinics: Exotic Animal practice. 2015; 18:83-103.
4. Neelima P, Sunitha K, Gopala Rao N, Chandra Sekhara Rao J. Haematological alterations in Cyprinus carpio as biomarker of Cypermethrin toxicity. International Journal of Current Research. 2015; 7(8):18864-18870.
5. Burgos-Aceves MA, Lionetti L, Faggio C. Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. Science of the Total Environment. 2019; 670:1170-1183.
6. Clauss TM, Dove ADM, Arnold JE. Hematologic disorder of fish. Veterinary Clinics of North America: Exotic Animal Practice. 2008; 11:445–462.
7. Harikrishnan R, Balasundaram C, Heo MS. Lactobacillus sakei BK19 enriched diet enhances the immunity status and disease resistance to streptococcosis infection in kelp grouper, *Epinephelus bruneus*. Fish & Shellfish Immunology. 2010; 29(6):1037-1043.
8. Pradhan SC, Patra AK, Sarkar B, Pal A. Seasonal changes in hematological parameters of *Catla catla*. Comparative Clinical Pathology. 2012; 21(6):1473-1481.
9. Chan EWC, Lim YY, Wong SK, Lim KK, Tan SP, Lianto FS *et al*. Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. Food Chemistry. 2009*;* 113(1):166-172.
10. Prasad S, Aggarwal BB, Benzie IFF, Wachtel-Galor S. Turmeric, the Golden Spice: From Traditional Medicine to Modern Medicine. 2011, 1-10.
11. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. Advances in Experimental and Medical Biology. 2007*;* 595(1):1-75.
12. Leya T, Raman RP, Srivastava PP, Kumar K, Ahmad I, Gora AH *et al*. Effects of curcumin supplemented diet on growth and non-specific immune parameters of *Cirrhinus mrigala* against *Edwardsiella tarda* infection. International Journal of Current Microbiology and Applied Sciences. 2017; 6(9):1230-1243.
13. Mohanty BR, Sahoo PK. Edwardsiellosis in fish: a brief review. Journal of Biosciences. 2007; 32:1331-1344.
14. Ewing WH, McWhorter AC, Escobar MR, Lubin AH. Edwardsiella, a new genus of Enterobacteriaceae based on a new species; International Bulletin of Bacteriological Nomenclature and Taxonomy. 1965; 15:33-38.
15. Nucci C, Silveira da WD, Silva da SC, Nakazato G, Bando SY, Ribeiro MA *et al*. Microbiological comparative study of isolates of *Edwardsiella tarda* isolated in different countries from fish and humans. Veterinary Microbiology. 2002; 89:29-39.
16. Plumb JA. *Edwardsiella septicaemias*. In Fish Disaeses and Disorders (Eds Woo, P.T.K. and Bruno, D.W.), CAB International, UK.1999; 3:479-521.
17. APHA. Standard Methods for the Examination of Water and Wastewater, 20th edition, American Public Health Association, Washington, D.C.1988; 3:32
18. Farrell AP. Cellular Composition of the Blood. Encyclopedia of Fish Physiology: From Genome to Environment. 2011; 2:984-991.
19. Azarin H, Aramli MS, Imanpour MR, Rajabpour M. Effect of a Probiotic Containing Bacillus licheniformis and Bacillus subtilis and Ferroin Solution on Growth Performance, Body Composition and Haematological Parameters in Kutum (*Rutilus frisii kutum*) Fry. Probiotics and Antimicrobial proteins. 2015; 7(1):31-37.
20. Bbole I, Mumba C, Mupenda N, Kefi AS. Analysis of growth performance and haematological parameters of *Oreochromis niloticus* fed on a varying diet of *Moringa oleifera* Lam. leaf meal as an additive protein source. International Journal of Fisheries and Aquaculture. 2016; 8(11):105-111,
21. Ozovehe BN, Nzeh GC. Effects of varying levels of Moringa oleifera leaf meal diet on growth performance, hematological indices and biochemical enzymes of African catfish *Clarias gariepinus* (Burchell 1822). Elixir Aquaculture. 2013; 57A:14459-14466.
22. Ncha OS, Michael PB, Nnabuchi UO, Alex E. Effect of diets with Moringa leaf meal on growth, carcass composition and haematology of *Clarias gariepinus*. International Journal of Fisheries and Aquatic Studies. 2015; 3(2):397-401.
23. Hrubec TC, Cardinale JL, Smith SA. Hematology and Plasma Chemistry Reference Intervals for Cultured Tilapia (Oreochromis Hybrid). Veterinary Clinical Pathology. 2000; 29(1).
24. Gallaugher PE. The role of haematocrit in oxygen transport and swimming in salmonid fishes. PhD Thesis, August, Simon Fraser University, UK. 1994, 129