International Journal of Fisheries and Aquatic Studies 2015; 3(2): 287-294


**ISSN: 2347-5129**
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 3(2): 287-294

www.fisheriesjournal.com
Received: 24-08-2015
Accepted: 26-09-2015

**Southamani C**
Assistant Professor, P.G and Research Department of Zoology, Govt, Arts College for Men, Krishnagiri – 635001, Tamilnadu.

**Shanthi G**
Assistant Professor, P.G and Research Department of Zoology, Govt, Arts College for Men, Krishnagiri – 635001, Tamilnadu.

**Dr. Manivelu Deivasigamani**
Assistant Professor, P.G and Research Department of Zoology, Govt, Arts College for Men, Krishnagiri – 635001, Tamilnadu.

**Corresponding Author:**
**Dr.Manivelu Deivasigamani**
Assistant Professor, Department of Zoology, Govt, Arts College for Men, Krishnagiri – 635001, Tamilnadu.

# Hematological response in three Indian major carps in relation to supplementary feeding

**Southamani C, Shanthi G, Manivelu Deivasigamani**

**Abstract**
The present study was carried out on the fish growth and hematological analysis depends on the water quality and supplementary feeding in aquaculture. The cement rectangular tank was taken for proper acclimatization and complete study of fish in this assessment period. Thus the objectives of this study, to assess the influence of supplementary feeding methods in different combinations on Physico-chemical characteristic of tank water and haematological parameters like RBC, TLC, Platelets, Hct, Hb, PCV, MCH. MCHC, MCV of Catla *catla, Labeo rohita* and *Cirrhinus mrigala*. To compare the hematological changes of *Catla catla, Labeo rohita* and *Cirrhinus mrigala*, under the influence of supplementary feeding in various combinations was treated in cement tank. Each tank was stocked with *Labeo rohita, Catla catla* and *Cirrhinus mrigala* in the ratio of 20:20:20, respectively. In this experiment, all the tanks received the same quantity of fishes, but the different type of supplementary feeds given to all the treatments and results suggests supplementary feeds could be effective for aquaculture.

**Keywords:** Indian major carps, Physico chemical Parameters, Hematological Analysis, Supplementary feed.

## Introduction

Aquaculture practices involved using high quality feeds with high crude protein content which should contain not only necessary nutrients but also complementary additives to keep organisms healthy and result in good growth performance. Aquaculture industry is faced with the challenges of inadequate supply and high cost of quality fish feeds. Protein is a very important macronutrient in fish feeding. Fish meal is the widest protein source which is used in aquaculture feeds because of its high protein content, good protein quality and balanced amino acid profile (Ariyawansa, 2000) [7]. The Indian major carps are most important commercial fish in India with maximum market demand and acceptability as food by the consumers due to their taste and flesh. Carps contribute a major portion to the fresh water fish production in south India. Their flesh contains a high amount of calcium, phosphorus and proteins and low amount of cholesterol.

However, fish meal supplies witnessed significant fluctuations in supplies and thus in prices during the last decade. This encouraged the search for fish meal alternatives from plant and animal sources. Plant materials suffer from low digestibility, high fiber content and antinutritional factors which limit their use effectively in aquaculture feeds (Lall *et al.,* 2005; Atanasoff, 2014) [22, 8].

Water quality in fish pond is affected by the interaction of several physicochemical components i.e., pH, alkalinity and hardness to exhibit profound effects on pond productivity, availability of oxygen, the level of stress and ultimately on fish health. The Physico-chemical characteristics of both soil and water are not static, but are dynamic and change with the introduction of fish species, provision of supplementary feeds, manures and fertilizers and other inputs. Both the soil and water quality parameters of pond ecosystem undergo complex changes due to all these factors, as a sequence disrupting the aquatic life in pond (Ali *et al*., 2006) [5].

Hematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness towards the anthropological pollution of natural freshwater resources in the tropics. Such studies have generally been used as an effective and sensitive index to monitor physiological and pathological changes in fishes. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management disease investigations (Mulcahy, 1975) [26]. Sampath *et al.,* 1993) [28] observed that haematological studies in fish, lies in the possibility that the blood will reveal anomaly within the body of the fish long before there is any outward manifestation of symptoms of disease or effects of unfavorable environmental factors.

However fishes can adapt themselves according to the environmental conditions viz; pond, tanks and aquarium by changing their physiological activities up to optimum range but in extreme change in water quality i.e. dissolved oxygen level, free carbon dioxide level and hardness, pH, total dissolved solids, can change the hematological parameters than normal values or control. Qualitative and quantitative variations in haematological parameters including the red blood cell (RBC), dependent parameters i.e. haemoglobin (Hb), Packed cell volume (PCV), mean cell volume (MCV), Mean cell hemoglobin concentration (MCHC), erythrocyte sediment rate(ESR) are significant findings for the determination of anemia, polycythemia, inflammation and infection while total White blood cell numbers (WBC) and differential leukocyte count viz, neutrophils, lymphocyte, eosinophils and monocyte are significant findings for the determination in pathological condition viz. leucocytosis and leucopenia (Eroh *et al*., 2003, Abdul Wahid Shah *et al.,* 2009) [16, 2].

The aim of this study is to investigate the hematological parameters of Indian major carps such as *Catla catla*, *Labeo rohita* (F. Hamilton, 1822) [17] and *Cirrhinus mrigala* (Bloch, 1795) [11] in relation with water quality and feeding methods, so that fish farmers can provide better water quality and food for the culture of Indian major carps and reduce the mortality rate and achieve the maximum yield in culture practice. For this study the Indian major carps are the most important commercial fishes with a maximum market demand under the laboratory condition.

## Materials and Methods

### Animal collection and maintenance

The study was carried out in the Post Graduate and Research Department of Zoology, Government Arts College for Men, Krishnagiri, South India. The experiment was conducted in laboratory condition for 7 Weeks. Freshwater Indian major carp (Catla catla, Labeo rohita and Cirrhinus mrigala) fingerlings were collected from the Government Fish farm in K.R.P Dam in Krishnagiri District, Tamilnadu. This Dam is constructed by damming river across the Ponnaiyar near Periyamuthur village about 10 kms from Krishnagiri town. They were brought to the laboratory in the polythene bags filled with aerated water. They were maintained in a rectangular cement tank washed with Potassium permanganate to make the walls free from fungal attack. The chlorine free groundwater was used with aerators fixed in the entire tank and the pH of the water was 7.4 - 7.8. The fingerlings were acclimatized for 10 days and fed with oil cake. One hundred acclimatized carps of similar size (average weight 10g±1gm) were randomly distributed in plastic containers filled with unchlorinated water. Constant aeration was provided using aerator. The tank water was changed in to three times in a week, after consumptions of food. Twenty healthy fishes were selected in each variety (Table 1) and different feed conditions; it was transferred from the stock to small cement tank and was divided into three groups. It consisted of the fishes in tap water which served as control.

**Table 1:** Classification of the experimental Fishes

| **Binomial name / Taxonomy** | ***Catla catla* (F. Hamilton 1822)** | ***Labeo rohita* (F. Hamilton, 1822)** | ***Cirrhinus mrigala* (Bloch, 1795)** |
|---|---|---|---|
| Kingdom | Animalia | Animalia | Animalia |
| Phylum | Chordata | Chordata | Chordata |
| Class | Actinopterygii | Actinopterygii | Actinopterygii |
| Order | Cypriniformes | Cypriniformes | Cypriniformes |
| Family | Cyprinidae | Cyprinidae | Cyprinidae |
| Genus | *Catla* | *Labeo* | *Cirrhinus* |
| Species | *C. catla* | *L. rohita* | *C. mrigala* |

### Diet Preparation

The formulation of the experimental diet was felt to be representative of commercial diets. The supplementary feed prepared by us and their ingredients are shown in Table 2. In the above formulation scheme, oil cake was used as a fat substitute, Chicken waste is used as protein substitute, Corn powder and Soybeans used as carbohydrate and protein substitutes, similarly rice bran was used as carbohydrate and protein sources whereas Tapioca powder was used as binder. In addition vitamins and minerals were added as minor elements. The ingredients were mixed thoroughly and ground well with the help of mixer. Required quantity of water was added to get through mixing of the constituents. The supplementary diet was steam pelletized using a laboratory scale; Pellets were sifted to remove any fine particles, cooled to room temperature in a fan ventilated chamber, and stored in a 28ºC freezer until required for feeding.

**Table 2:** Composition of Experimental feeds.

| **Control** | Oil cake |
|---|---|
| **Treatment - I (T1)**<br>(Artificial feed / Supplementary feed) | Rice Bran – 120 gm<br>Oil cake – 800 gm<br>Soya beans – 200 gm<br>Corn powder – 200 gm<br>Tapioca powder – 80 gm<br>Chicken waste – 60 gm<br>Vit. B Complex – 15 tabs |
| **Treatment – II (T2)** | Rice Bran |
| **Treatment – III (T3)**<br>(Commercial feed) | Readymade feed -<br>Gold mohur foods and feeds Pvt. Ltd,<br>Sanathnagar, Mumbai-79 |

### Water criteria

The chlorine free Groundwater was used in the growth studies of experiments. The condition of the experimental water was maintained with constant characteristics throughout the experiment period.

### I. Limnological examination

### Collection of water samples

In this study, water samples were collected on fortnightly. But their average was calculated on the end of the experiment. The following Physico-chemical parameters of the tank water were estimated.

### Analysis of Physical factors

The Physical analysis of experimental water such as Temperature, Colour, Appearance (by vision), Odour (by smell), Turbidity (NTU), TDS and Electrical conductivity were determined on fortnightly by the methods of American Public Health Association (APHA, 1998) [1].

**Analysis of chemical factors**

The other chemical analysis of experimental water viz., pH, Total alkalinity, Total hardness, carbonates, bicarbonates, calcium, magnesium, nitrate, chloride and sulphate were determined at the end of experiment by following methods of American Public Health Association (APHA, 1998) [1].

**II) Hematological examination**

**Collection of blood sample**

Blood from fish was collected from the cardiac region by puncturing the heart using a plastic disposable syringe fitted with a 26-gauge needle, the syringe is flushed with EDTA (Anticoagulant) about 150 to 200µl of anticoagulant and then the blood was drawn to avoid coagulation. The plastic vials are immediately returned to the ice box. The collected blood was transferred in to eppendorfs of 1.5 ml capacity and stored in refrigerator for further analysis.

**Analysis of fish blood**

Total RBCs and WBCs counts were determined by using Improved Neubauer hemocytometer. (Hesser, 1960) [18], Hemoglobin (Hb) concentration was estimated by cyanmethemoglobin (Blaxhall & Daisley, 1973) [10] and hematocrit value (Hct) was determined by micro hematocrite capillary tube (Wintrobe, 1967) [35]. Differential leukocyte count was done by using giemas staining method (Abdul Wahid Shah *et al.,* 2009) [2]. Mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), and Mean cell volume (MCV) were calculated using the formulae mentioned below by Dacie and Lewis (2001) [12] method.

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

**Statistical Analysis**

The data thus obtained was subjected to statistical analysis. The variation for various parameters and the significance and their interaction among then different treatments for the various blood parameters were tested by using mean.

**Results and Discussion**

**1. Limnological Studies**

The Physico-chemical parameters of water which were analyzed during the course of study include temperature, pH, dissolved oxygen, total alkalinity, carbonates, bicarbonates, total hardness, calcium, magnesium and total dissolved solids was estimated on fortnightly and their averages were calculated on final of the experiment. These parameters are interlinked and estimate the water quality by influencing the biological productivity of the fish tank water (Table - 3)

**Table 3:** Physico Chemical parameters in fish tank waters.

| Physico Chemical parameters | Control | Treatment I | Treatment II | Treatment III |
|---|---|---|---|---|
| Appearance (by vision) | Clear | Clear | Clear | Clear |
| Colour (pt.co.scale) | Colourless | Colourless | Colourless | Colourless |
| Odour (by smell) | None | None | None | None |
| Turbidity (NTU) | 1.1 | 0.8 | 0.8 | 0.9 |
| Total dissolved solids (mg/l) | 1298 | 1224 | 1227 | 1230 |
| EC (mic mho/cm) | 1765 | 1748 | 1753 | 1757 |
| pH | 7.63 | 7.07 | 7.52 | 7.33 |
| Alkalinity (mg/l) | 468 | 456 | 464 | 470 |
| Total Hardness ($CaCo_3$ mg/l) | 454 | 448 | 460 | 455 |
| Calcium (Ca mg/l) | 121 | 112 | 116 | 114 |
| Magnesium (Mg mg/l) | 43 | 41 | 41 | 41 |
| Nitrate ($NO_3$ mg/ l) | 3 | 1 | 1 | 1 |
| Chloride (Cl mg/l) | 233 | 230 | 230 | 230 |
| Sulphate ($SO_4$ mg/l) | 78 | 70 | 74 | 75 |

**2. Haematological Studies**

Hematological studies on *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under the influence of supplementary feeds in various combinations under the three different treatments. The blood parameter was significantly increased in experimental groups compared to control group.

Blood of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* being similar to any other vertebrate, comprises of plasma and cellular components. Plasma consists of 97% water, dissolved salts, electrolytes and hormones. The cellular components include erythrocytes and leucocytes. These formed elements of normal values from the basis of the diagnosis for health status of human beings and fish was the same. The normal values for all these parameters were initially measured from the fish. The blood parameters of Hb to be varying in the range of 8.5-11.6g/dl, while the RBC varied in the range of 1.5 million - 1.94 million Cells/cu mm. The PCV values varied in the range of 28.3-38.6%. MCV values varied in the range of 155.6-226 $\mu m^3$, MCH in the range of 43.8-75µg and MCHC varying in the range of 25-39.9%.

**Hematological parameters of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala.***

**Hemoglobin (g/dl) - Hb**

The normal range of Hemoglobin (Hb) for the fish was 8.5-11.6 (Table 4, 5, 6). The Hb value in all the three treatment groups of three fish species (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) was found to be lower than the control groups. The Hb value of *Catla catla* was shown as 9.5, 8.3, 8.7 and 7.7 g/dl in Control, T1, T2 and T3 experiments respectively. The maximum value of Hb as 9.5 g/dl in Control and minimum value was 7.7 g/dl noted in T3. The Hb value of *Labeo rohita* in Contol, T1, T2 and T3 were showed as 9.0, 8.5, 7.9 and 8.8 g/dl. The minimum value was 7.9 g/dl in T2. This species attained the best value of Hb 9.0g/dl in Control. For *Cirrhinus mrigala* the values of Hb were recorded as 8.6, 8.8, 7.8 and 7.4g/dl in Control T1, T2 and T3 respectively. The minimum value 7.4g/dl was remained in T3. The maximum value of Hb was shown as 8.8 in T1.

The highest survival percentage was observed for the *Catla catla* 9.5g/dl in Control (Rice bran) as compared to the *Labeo* *rohita* 9.0g/dl in Control (Rice bran) and *Cirrhinus mrigala* 8.8g/dl in T1 (Artificial feed). The lowest value Hb was recorded for the *Catla catla* 7.7g/dl in T3 (Commercial feed) as compared to the *Labeo rohita* 7.9g/dl in T2 (Oil cake) and *Cirrhinus mrigala* 7.4g/dl in T3 (Commercial feed). The overall range of Hb was compared with all treatments were little changes.

**Table 4:** Hematological characteristics of *Catla catla.*

| Blood parameters | Control | Treatment I | Treatment II | Treatment III |
|---|---|---|---|---|
| Hb (gm/dL $^{-1}$) | 9.5 | 8.3 | 8.7 | 7.7 |
| RBC (×$10^6$mm³) | 1.18 | 1.25 | 1.1 | 1.35 |
| PCV (%) | 21.0 | 22.3 | 20.0 | 21.8 |
| TC (×$10^3$ mm³) | 18.5 | 16 | 21 | 16 |
| MCV (%) | 68 | 71 | 70 | 74 |
| MCH ( pg) | 41 | 39 | 38 | 44 |
| MCHC (%) | 47 | 55 | 44 | 61 |
| Platelets (lakhs /cu mm) | 0.95 | 0.7 | 0.9 | 0.72 |

**RBC count ($10^6$ mm³)**

The RBC counts of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under the influence of supplementary feeds in various combinations fewer than three different treatments. The normal range of RBC count for the fish were 1.5-1.94 ($10^6$mm³) shown in Table - 4, 5, 6. The RBC value of *Catla catla* in Contol, T1, T2 and T3 values were 1.18, 1.25, 1.1 and 1.35 ($10^6$mm³) shown in the minimum value of RBC as 1.1 ($10^6$mm³) in T2 treated with Oil cake. Maximum value 1.35 ($10^6$mm³) of RBC was noted in T3 feed with Commercial feed. The RBC value of *Labeo rohita* in Contol, T1, T2 and T3 were shown as 1.20, 1.28, 1.30 and 1.33($10^6$mm³). The minimum value was 1.20 ($10^6$mm³) in control treated with Rice bran. This species attained the highest value of RBC was 1.33 ($10^6$mm³) in T3 feed with Commercial feed. For *Cirrhinus mrigala* the values of RBC were recorded as 1.32, 0.80, 1.38 and 0.89 ($10^6$mm³) in Control, T1, T2 and T3 respectively. The maximum value of RBC was shown as 1.38 ($10^6$mm³) in T2 receiving Oil cake. The minimum value of RBC of *Cirrhinus mrigala* was 0.80 ($10^6$mm³) in T1 experiment which treated with artificial feed.

The highest RBC count was observed for the *Catla catla* 1.35 ($10^6$mm³) in T3 (Commercial feed) as compared to the *Labeo rohita* 1.33 ($10^6$mm³) in T3 (Commercial feed) and *Cirrhinus mrigala* 1.38 ($10^6$mm³) in T2 (Oil cake). The lowest value RBC was recorded for the *Catla catla* 1.1 ($10^6$mm³) in T2 (Oil cake). As compared to the *Labeo rohita* 1.20 ($10^6$mm³) in Control (Rice bran) and *Cirrhinus mrigala* 0.80 ($10^6$mm³) in T1 (Artificial feed).

**Table 5:** Hematological characteristics of *Labeo rohita*

| Blood parameters | Control | Treatment I | Treatment II | Treatment III |
|---|---|---|---|---|
| Hb (gm/dL $^{-1}$) | 9.0 | 8.5 | 7.9 | 8.8 |
| RBC( ×$10^6$mm³) | 1.20 | 1.28 | 1.3 | 1.33 |
| PCV % | 23.9 | 28.7 | 24.0 | 25.1 |
| TC (×$10^3$ mm³) | 14.1 | 13.7 | 14.6 | 13.9 |
| MCV% | 95 | 146.5 | 80 | 103.2 |
| MCH (pg ) | 44 | 58.5 | 40 | 49.2 |
| MCHC% | 57 | 40 | 55 | 52 |
| Platelets (lakhs /cu mm) | 0.9 | 0.61 | 0.85 | 0.85 |

**Packed Cell Volume (%) - PCV**

The PCV of three cultured fish species viz., *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under the influence of supplementary feeds in various combinations designated as Control, T1, T2 and T3 respectively. The normal range of PCV for the fish was 28.3-38.6% (Table – 4,5,6). The PCV value of *Catla catla* in 21.0, 22.3, 20.0 and 21.8% in Control, T1, T2 and T3 showed the minimum value of PCV as 20.0% in, T2. Maximum value of PCV 21.8% was noted in T3. The PCV value of *Labeo rohita* in Control, T1, T2 and T3 were shown as 23.9, 28.7, 24.0 and 25.1%. The minimum value was 23.9% in Control. This species attained the best value of PCV was 28.7% in T1.

For *Cirrhinus mrigala* the values of PCV were recorded as 25.0, 23.7, 27.0 and22.3% in Control T1, T2 and T3 respectively. The minimum value was 22.3% remained in T3. The maximum value of PCV was shown as 27.0% in T2. The highest PCV was observed for the *Catla catla* in 22.3% in T1 (Artificial feed) as compared to the *Labeo rohita* 28.7% in T1 (Artificial feed) and *Cirrhinus mrigala* 27.0% in T1 (Artificial feed). The lowest value PCV was recorded for the *Catla catla* 20.0% in T2 (Oil cake) as compared to the *Labeo rohita* 23.9 in Control (Rice bran) and *Cirrhinus mrigala* 22.3%in T3 (Commercial feed). The overall ranges of PCV values when compared with all treatments were occurred little changes.

**Table 6:** Haematological characteristics of *Cirrhinus mrigala*

| Blood parameters | Control | Treatment I | Treatment II | Treatment III |
|---|---|---|---|---|
| Hb (gm/dL$^{-1}$ ) | 8.6 | 8.8 | 7.8 | 7.4 |
| RBC ( ×$10^6$mm³) | 1.32 | 0.80 | 1.38 | 0.89 |
| PCV % | 25.0 | 23.7 | 27.0 | 22.3 |
| TLC (×$10^3$ mm³) | 11.4 | 12.4 | 12.8 | 8.0 |
| MCV% | 128 | 124.7 | 126 | 138.9 |
| MCH (pg) | 40 | 60 | 42 | 49.4 |
| MCHC% | 34 | 48.6 | 33.5 | 35.5 |
| Platelets (lakhs /cu mm) | 1.77 | 0.97 | 1.96 | 1.12 |

**Total Leucocyte Count ($10^3$ mm³)**

The Total Leucocyte Counts of three cultured fish species of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under the influence of supplementary feeds in various combinations designated as Control, T1, T2 and T3 respectively (Table - 4,5,6). The total leucocyte counts of *Catla catla* in Control, T1, T2 and T3 values were recorded as 18.5, 16.0, 21.0and 16.0 ($10^3$ mm³) and showed the minimum value of total count as 16.0 ($10^3$ mm³) in Control and T3 treated with Rice bran and Commercial feed. Maximum value of total count in 21.0 ($10^3$ mm³) was total leucocyte count noted in T2 feed with Oil cake.

The total leucocyte count value of *Labeo rohita* in Control, T1, T2 and T3 were shown as 14.1, 13.7, 14.6 and13.9 ($10^3$ mm³). The minimum value of T1 was recorded as 13.7 ($10^3$ mm³) treated with artificial feed. This species attained the highest value of total leucocyte count in 14.6 ($10^3$ mm³) in T2 feed with Oil cake.

For *Cirrhinus mrigala* the values of total leucocyte count were recorded as 11.4, 12.4, 12.8 and 8.0 ($10^3$ mm³) in Control, T1, T2 and T3 respectively. The maximum value of total leucocyte count was recorded as 12.8 ($10^3$ mm³) in T2 receiving Oil cake. The minimum value of T3 was recorded as 8.0 ($10^3$ mm³) which treated with Commercial feed.

The highest total leucocyte count was observed for the *Catla catla* 21.0 ($10^3$ mm³) in T2 treated with Oil cake as compared to the *Labeo rohita* 14.6 ($10^3$ $mm^3$) in T2 feed with Oil cake and *Cirrhinus mrigala* 12.8 ($10^3$ $mm^3$) in T2 (Oil cake). The lowest value total leucocyte count was recorded for the *Catla catla* 16.0($10^3$ $mm^3$) in Control and T3 treated with Rice bran and Commercial feed, as compared to the *Labeo rohita* 13.7 ($10^3$ $mm^3$) in T1 feed with Artificial feed and *Cirrhinus mrigala* 8.0 ($10^3$ $mm^3$) T3 which treated was Commercial feed.

**Platelets (Lakhs/Cu. mm)**

The platelet counts of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under the influence of supplementary feeds in various combinations under three different treatments. (Table – 4,5,6).The platelets counts value of *Catla catla* in Contol,T1, T2 and T3 values were0.95, 0.70, 0.90 and 0.72 (Lakhs/cu mm) showed the minimum value of platelets count as 0.70 (Lakhs/cu mm) in T2 treated with Oil cake. Maximum value of platelets count in 0.95(Lakhs/cu mm) of was platelets count noted in Control feed with Rice bran.

The platelet count value of *Labeo rohita* in Control, T1, T2 and T3 were recorded as 0.90, 0.61, 0.85 and 0.85 (Lakhs/cu mm). The minimum value was 0.61 (Lakhs/cu mm) in T1 treated with Artificial feed. The *Labeo rohita* was attained the highest value of platelet count (0.90 Lakhs/cu mm) in Control treated with Rice bran. For *Cirrhinus mrigala* the values of platelet count were recorded as 1.77, 0.97, 1.96 and 1.12 (Lakhs/cu mm) in Control, T1, T2 and T3 respectively. The maximum value of platelet count was showed as 1.96 (Lakhs/cu mm) in T2 receiving Oil cake. The minimum value 0.97 (Lakhs/cu mm) was remained in T1 which treated with Artificial feed.

The highest platelet count was observed for the *Catla catla* 0.95 (Lakhs/cu mm) in Control feed with Rice bran as compared to the *Labeo rohita* 0.90 (Lakhs/cu mm) in Control feed with Rice bran and *Cirrhinus mrigala* 1.96 (Lakhs/cu mm) T2 receiving Oil cake. The lowest value platelets count was recorded for the *Catla catla* 0.70 (Lakhs/cu mm) in T2 treated with Oil cake, as comparison of *Labeo rohita* (0.61 Lakhs/cu mm) and *Cirrhinus mrigala* (0.97 Lakhs/cu mm) in T1 which treated with artificial feed was low values.

**Erythrocyte Indices**

Mean Corpuscular Hemoglobin (MCH) i.e., average Hb content of single RBC, Mean Corpuscular Hemoglobin Concentration (MCHC) i.e., average Hb concentration in 100 ml of haematocrit and Mean Corpuscular Volume (MCV) i.e., size/state of RBCs constitute red blood indices. (Table – 4,5,6)

**i) Mean Corpuscular Volume (MCV) (μm3)**

The MCV of three cultured fish species viz., *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under the influence of supplementary feeds in various combinations designated as Control, T1, T2 and T3 respectively. The normal range of MCV for the fish was 155.6-226 μm3 The MCV value of *Catla catla* is 68.0, 71.0, 70.0 and 74.0 μm3 in Control, T1, T2 and T3 showed the minimum value of MCV as 68.0 μm3 in Control. Maximum value of MCV in 74 μm3 received in T3.

The MCV value of *Labeo rohita* in Control, T1, T2 and T3 were recorded as 95, 146.5, 80 and 103.2 μm3. The minimum value was 80 μm3 in T2. This species attained the best value of MCV was 146.5 μm3 in T1.

For *Cirrhinus mrigala* the values of MCV were recorded as 128, 124.7, 126 and138.9 μm3 in Control T1, T2 and T3 respectively. The minimum value was 124.7 μm3 remained in T1. The maximum value of MCV was showed as 138.9 μm3 in T3.

The highest MCV was observed for the *Catla catla* in 74.0 in T3 (commercial feed) as compared to the *Labeo rohita* 146.5 μm3 in T1 (Artificial feed) and *Cirrhinus mrigala* 138.9 μm3 in T3 (Commercial feed). The lowest value MCV was recorded for the *Catla catla* 68.0 μm3 in Control (Rice bran) as compared to the *Labeo rohita* 80.0 μm3 in T2 (Oil cake) and *Cirrhinus mrigala* 124.7 μm3 in T1 (Artificial feed). The overall ranges of MCV values when compared with all treatments were little changes.

**ii) Mean Corpuscular Hemoglobin (MCH) (pg)**

The Total counts of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under the influence of supplementary feeds in various combinations fewer than three different treatments. The normal range of MCH for the fish was 43.8-75.

The MCH value of *Catla catla* in Control, T1, T2 and T3 values were 41.0, 39.0, 38.0 and 44 pg showed the minimum value of MCH is 38.0 pg in T2 treated with Oil cake. Maximum value of MCH in 44 pg of was MCH noted in T3 feed with Commercial feed. The MCH value of *Labeo rohita* in Control, T1, T2 and T3 were observed as 44.0, 58.5, 40.0 and 49.2 pg. The minimum value was 40.0 pg in T2 treated with Oil cake. This species attained the highest value of MCH were 49.2 pg in T3 feed with Commercial feed.

For *Cirrhinus mrigala* the values of total MCH were recorded as 40.0, 60.0, 42.0 and 49.4 pg in Control, T1, T2 and T3 respectively. The maximum value of MCH was shown as 60 pg in T3 fed with Commercial feed. The minimum value of 40.0 pg remained in Control which treated with Rice bran. The highest MCH was observed for the *Catla catla* 44.0pg in T3 feed with Commercial feed as compared to the *Labeo rohita* 49.2 pg in T3 feed with Commercial feed and *Cirrhinus mrigala* 60.0 pg in T3 treated with Commercial feed. The lowest value MCH was recorded for the *Catla catla* 38.0 pg in T2 treated with Oil cake, as compared to the *Labeo rohita* 40.0 in T2 treated with Oil cake and *Cirrhinus mrigala* 40.0 pg Control which treated was Rice bran. Overall MCH increased when compared different treatment in T3 treated with Commercial feed.

**iii) Mean Corpuscular Hemoglobin Concentration (MCHC) (%)**

The MCHC of three cultured fish species viz., *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under the influence of supplementary feeds in various combinations designated as Control, T1, T2 and T3 respectively. The normal range of MCHC for the fish was 25-39.9.

The MCHC value of *Catla catla* was 47.0, 55.0, 44.0 and 54.0% in Control, T1, T2 and T3 showed the minimum value of MCHC as 44.0 % in T2. Maximum value of MCHC was 55.0 received in T1. The MCHC value of *Labeo rohita* in Control, T1, T2 and T3 were observed as 57.0, 40.0, 55.0 and 49.2 %. The minimum value was 40.0% in T1. This species attained the best value of MCHC was 57.0 in Control.

For *Cirrhinus mrigala* the values of MCHC were recorded as 34.0, 48.6, 33.5 and 35.5 % in Control T1, T2 and T3 respectively. The minimum value was 33.5% remained in T2. The maximum value of MCHC was shown as 48.6% in T1.

The highest MCHC was observed for the *Catla catla* in 55.0% in T1 (Artificial feed) as compared to the *Labeo rohita* 57.0 % in Control (Rice bran) and *Cirrhinus mrigala* 48.6 % in T1 (Artificial feed). The lowest value MCHC was recorded for the *Catla catla* 44.0% in T2 (Oil cake) as compared to the *Labeo rohita* 40.0 % in T1 (Artificial feed) and *Cirrhinus mrigala* 33.5 % in T2 (Oil cake).

The degree of ecosystem contamination of water can be estimated by the analysis of hematological changes. Blood is the most important fluid in the body and its composition often reflects the total physiological condition of an organism. Hematological parameters are nowadays not only used for clinical diagnosis of physiology but also help in addressing the effects of toxic substances on the fish (Wendlaar, 1997) [33]. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the hematological parameters (Van Vuren, 1986) [32]. Qualitative commutations in blood cell components can consequence in contamination (Landis. M 2004) [23].

Hemoglobin serves to transport oxygen from gills to different tissues of the fish in the form of oxyhaemoglobin and carbon dioxide from tissue to the gills in the form of carboxyhaemoglobin and its concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible. The Hb value in all the three treatment groups of fish was found to be significantly lower than the control groups which may be attributed to the fact that the oxygen carrying capacity of the fish was affected by the different treatment in water. The decreases in Hemoglobin concentration represents that the fish's power to supply adequate oxygen to the tissues is limited considerably and this will result in decline of physical stir. Similar results were reported in Common carp (*Cyprinus carpio*) exposed to diazinon (Ahmed 2011) [4].

The present study clearly indicated that the RBC count changes significantly on supplementary feeds. Mostly, the reduction in RBC counts in fish may imply the anemic condition of the fish under stress situations (Li *et al*., 2011) [24]. This represents a considerable reduction in the total amount of Hb carrying RBC, leading consequently to reduction in oxygen transport capacity of blood. This is caused by pathogens attacking haemopoietic tissues (kidney and spleen). It seems that erythropoiesis has been accelerated to avoid hemorrhage state leading to lower production of erythrocytes (Svoboda *et al.,* 2001 [32], Modesto and Martinez, 2010 [25], Rao, 2010 [27] and Ahmed, 2011) [4]. It is documented that under stress conditions, fish become hyperactive perhaps to get out of the stressful medium and would require a decreased amount of oxygen to meet their energy requirement is low.

Furthermore, amplitude of toxicants in the gill area may injury the structure of gill resulting hemolysis and toxicant induced damaged osmoregulation may lead to a diminution in RBC counts (Kavitha *et al*., 2010; Saravanan *et al*., 2011) [20, 29]. It clearly showed that the fish not only responded to the toxicant by increasing the quantity of RBCs, but also by changing the quality of these cells (Katalay and Parlak, 2004) [19].

PCV decreased on toxic water (20-28.7%). Elevated numbers of red blood cells and hemoglobin levels seem to be an indication of the increase in newly formed immature red blood cell population and shortening of the life span of mature red blood cells. Ergonul (2012) [15] also came up with more or less similar results. Hence the decrease in PCV points towards the fish suffering from a physiological disorder due to water or feeds. Total count of cells (TC) is the defense cells of the body which provide protection to the organism against environmental as well as anthropogenic stress. Total count of cells per cubic millimeter (TC) is a diagnostic feature of many diseases. The tremendous increase in TC count in the present study simply indicates the stress condition of the fish caused by environment condition which might have produced gill damage and fish becomes restless.

**Erythrocyte indices**

MCV gives an indication of the status or size of RBCs (Alwan *et al*, 2009) [6]. MCV value was significantly lower physiological changes in fish treated with different supplementary feeds while MCHC significantly decreased against the same concentration of feeding capacity. The increases in MCHC of the blood conform to the erythrocyte count and their production in the disorder. A decrease in MCV indicates that the erythrocytes have shrunk, either due to anemia, stress or impaired water balance or a large concentration of immature erythrocytes that have been released from the reduced Hb in tissue (Kumar *et al.,* 1999) [21]. MCH during low and high concentrations might be caused by the above said reason Further, since MCV and EC are inversely related to each other, therefore in the present study, decrease in MCV can also be attributed to elevation in EC values observed in the fish. In the later phases the high percentage of unripe red blood cells in the circulation might be the cause for MCV decrease in low concentrations. The significant expansion of MCHC value might be resulted from spherocytosis as mentioned by Sobecka (2001) [30].

The MCHC is a good indicator of red blood cell swelling or shrinkage (Wepener *et al*., 1992) [34]. The increase in the MCHC values in the exposed fish is thus probably an indication of shrinking of the red blood cells and/or an decrease in hemoglobin synthesis. The decreased Hb content may also be attributed to decreased hemorrhage or anemia and hemoglobin synthesis which, in turn, explains a decreased MCHC. Concentrations of circulating blood cell types are important parameters for use in detecting and evaluating the sublethal effects of toxicants on fish (Dick and Dixon, 1985) [13]. The evidence to date strongly suggests that the lymphocytes of fish probably play a role similar to lymphocytes in higher vertebrates because they are immune competent cells (Ellis, 1981) [14]. Decrease in the number of lymphocytes was found in the fish pathological changes which can be the result of activation of the immune system in the presence of contaminant, which in turn may be an adaptive response of the organism resulting in a more effective immune defense (Barreto-Medeiros *et al*., 2005) [9].

Decrease in the number of platelets for normal values (0.7 to 1.96). These affecting of fish blood capillary or spleen and bone marrow. Certain hematological parameters like RBC count and TC in the control groups of fish slightly deviated from their normal range values which are purely attributable to the stress caused to the fish under laboratory conditions. On the whole the present study revealed that water parameters and feed brings about considerable changes in hematological studies of *Catla catla, Labeo rohita* and *Cirrhinus mrigala* under laboratory conditions.

**Conclusion**

In conclusion, the result of present study showed that physico chemical parameters and supplementary feeds improved in hematological parameters of fingerlings Indian common carp (*Catla catla, Labeo rohita, Cirrhinus mrigala*) that this can associate with improve health statues and physiological response. Hematological analysis indicated that common carp (*Catla catla, Labeo rohita, Cirrhinus mrigala*) were more stressed as the level of artificial feed in the diet increased The hematological parameters like Hb, Hct, RBC, Platelets and TLC counts and other hematological indices like MCV, MCH and MCHC, could be delicate to sure types of contaminants because of its close relation with the exterior environment and often used to discover the physiological status of animals (Van Vuren 1986; Adhikari *et al*.,2004) [32, 3].

The total leucocyte count, red cell count and platelet count are quantitative evaluations of blood constituents. Hemoglobin (Hb) and hematocrit (HCT) are indicators of the oxygen-conveying valence of blood. This study revealed that the reversal effect of Common carps (*Catla catla, Labeo rohita and Cirrhinus mrigala*) supplementary feed on some hematological parameters which were observed to have decreased of all blood parameters. We also observed that the blood parameters of erythrocyte indices were also reduced the levels of their normal state and indicating that it is the concentration dependent.

**Summary**

The fish growth and yield depend on the water quality and supplementary feeding in aquaculture practices. The cement rectangular tank was taken for proper acclimatization and growth of fish for study period. Thus the objectives of this study, to assess the influence of supplementary feeding methods in different combinations on Physico-chemical characteristic of tank water and haematological parameters of Catla *catla, Labeo rohita* and *Cirrhinus mrigala*. To compare the hematological changes of *Catla catla, Labeo rohita* and *Cirrhinus mrigala*, under the influence of supplementary feeding in various combinations was treated in cement tank. Each tank was stocked with *Labeo rohita, Catla catla* and *Cirrhinus mrigala* in the ratio of 20:20:20, respectively. In this experiment, all the tanks received the same quantity of fishes, but the different type of supplementary feeds given to all the treatments.

The findings of the present study were summarized as follows:

1. The physicochemical characteristics of tap water remained within the favorable limits for fish culture. Similarly pH, Total dissolved solids, Alkalinity, Hardness and Minerals (Calcium, Magnesium, Sulphate etc.,) also close relationship in fish growth.
2. Fish production was highest in artificial feed compared with other feeds. Among these three major carps *Labeo rohita* and *Cirrhinus mrigala* were highest growth response in the same and high quantity of crude protein with low cost level.
3. The Physiological changes induced by toxicity were apparent in hematology of *Catla catla, Labeo rohita* and *Cirrhinus mrigala.*
4. These changes suggest that the treated fish were faced with a serious metabolic crisis. The elevated values of RBC count, hemoglobin concentration, hematocrit, PCV, MCV, MCH and MCHC values in the fish are indicative of stress mediated production of RBC and hemoglobin by the fish.
5. This resulted in tremendous increase in TLC count of the fish due to the activation of the immune system.
6. The use of water and feed in agricultural fields should be controlled to prevent in cultural system in contamination by leaching into the aquatic environments. In this way water and feed could be protected from these kinds of purpose.

**References**


1. APHA. Standard Methods for the Examination of Water and Wastewater (20th Ed.). American Public Health Association, the American Water Works Association and the Water Environment Federation 1998, 1220.
2. Abdul Wahid Shah, Muni praveen, Sajad hussain Mir, Sarwar SG, Yousuf AR. Imapact of Helminth Parasitism on fish Haematology of Anchar Lake, Kashmir. pak. jourl of nutrition 2009; 8(1):42-45.
3. Adhikari S, Sarkar B, Chatterjee A, Mahapatra CT, Ayyappan S. Effects of Cypermethrin and Carbofuran on Haematological Parameters and Prediction of their Recovery in a Freshwater Teleost, Labeo rohita (Hamilton), Ecotoxicology and Environmental Safety 2004; 58:220-226.
4. Ahmed Z. Acute toxicity and haematological changes in common carp (*Cyprinus carpio*) caused by diazinon exposure. African Journal of Biotechnology. 2011; 10(63):13852-13859.
5. Ali A, Bhatti MN, Khan N, Rehman MH. Role of soil and water chemistry in aquaculture. Proceedings of International conference on Solving problems of Freshwater Fish Farming in Pakistan November 27-28, UVAS 2006, 139-141.
6. Alwan SF, Hadi AA, Shokr AE. Alterations in haematological parameter of fresh water fish. Tilapia Zilli exposed to Aluminium. Journal of Science and Its Applications. 2009; 3(1):12-19.
7. Ariyawansa S. The evaluation of functional properties of fish meal, The United Nations Univ. Fish. Training Prog, Final project, 2000, 25.
8. Atanasoff AP. Replacement of fish meal by ribotricin in diets of carp Cyprinus carpio, Mac. Vet. Rev 2014; 37:55-59.
9. Barreto-Medeiros JM, Feitoza EG, Magalhaes K, Da Silva RR, Manhaes-de-Castro FM, Manhaes-de-Castro R *et al.* The expression of an intraspecific aggressive reaction in the face of a stressor agent alters the immune response in rats. Braz. J Biol. 2005; 65:203-209.
10. Blaxhall PC, Daisley KW. Routine haematological methods for use with fish blood. Journal of fish biology. 1973; 5:771-781.
11. Bloch M E. Naturgeschichte der ausländischen Fische. Schlesinger, Berlin. 1795, 91, 92.
12. Dacie JV, Lewis SM. Practical haematology, 6th edn. ELBS and Churchill, Livingston comp clinpathol, 2001.
13. Dick PT, Dixon DG. Changes in circulating blood cell levels of rainbow trout, Salmogairdneri Richardson, following acute and chronic exposure to copper. f. Fish Biol 1995; 26:475-484.
14. Ellis KE. Stress and the modulation of defense mechanisms in fish. In Stress and Fish (Edited by Pickering A. D.) 1981, 147-169.
15. Ergonul MB, Atasagun S, Kocaturk K. Alterations in the hematological and biochemical parameters and plasma ion concentrations of common carp, (*Cyprinus carpio* L., 1758) after short term exposure to sub-lethal concentrations of lead. Kafkas Univ Vet Fak Derg 2012; 18(2):297-302.
16. Eroh PO, Adonye, Canice C, Okhamafe Angustine O. Response of trypanosome brucei brucei– induced anaemia to a commercial herbal prepration. African journal of Biotechnology. 2003, 2(9).
17. Hamilton-Buchanan F. An Account of the Fishes of River Ganges and its Branches. George Ramsay and Co., London, 1822; 6:405-39.
18. Hesser EF. Methods for routine fish hematology. progve. fish cult 1960; 22:164-171.

19. Katalay S, Parlak H. Effects of pollution on haematological parameters of black goby (Gobiusniger L., 1758) in foca and aliagabays. E.U. Journal of fisheries and Aquatic sciences. 2004; 21(1-2):113-117.
20. Kavitha C, Malarvizhi A, Kumaran SS, Ramesh M. Toxicological Effects of Arsenate Exposure on Hematological, Biochemical and Liver Transaminases Activity in an Indian Major Carp, Catla Catla, Food and Chemical Toxicology 2010; 48:2848-2854.
21. Kumar S, Lata S, Gopal K. Deltamethrin Induced Physiological Changes in Freshwater Cat Fish Heteropneustes fossilis. Bull. Environ. Contam. Toxicol 1999; 62:254-258.
22. Lall SP, Anderson S. Amino acid nutrition of salmonids: Dietary requirements and bioavailability, in: Montero D, Basurco B, Nengas I, Alexis M, Izquierdo M. (Eds.), Mediterranean fish nutrition. Zaragoza: CIHEAM, Cahiers Options Mediterraneennes, 2005; 63:73- 90.
23. Landis WG, Yu MH. Introduction to Environmental Toxicology, Crc Press 2004, 509.
24. Li Z, Velisek H, Zlabek J, Grabic V, Machova RJ, Kolarova J. *et al.* Chronic Toxicity of Verapamil on Juvenile Rainbow Trout (Oncorhynchus Mykiss): Effects on Morphological Indices, Hematological Parameters and Antioxidant Responses, Journal of Hazardous Materials 2011; 185:870-880.
25. Modesto KA, Martinez CBR. Effects of roundup transorb on fish: Hematology, antioxidant defenses and acetylcholinesterase activity. Chemosphere 2010; 81:81-787.
26. Mulcahy MF. Fish blood changes associated with diseases. A haematological study of Pike lymphoma and salmon ulcerative dermal necrosis in fish. pathology of fish 1975, 925-944.
27. Rao DS. Carbaryl induced changes in the haematological, serum biochemical and immunological responses of common carp, Cyprinus carpio, (L.) with special emphasis on herbal extracts as immunomodulators. Ph. D. Thesis, Andhra University, India. 2010, 235.
28. Sampath K, Velamnia SK, James R. Haematological changes and their recovery in Oreochromis mossambicus as a function of exposure period and sublethal levels of Elalus. Acta Hydriobiology 1993; 35:73-83.
29. Saravanan M, Karthika S, Malarvizhi A, Ramesh M. Ecotoxicological Impacts of Clofibric Acid and Diclofenac in Common Carp (Cyprinus carpio) Fingerlings: Hematological, biochemical, Ionoregulatory and Enzymological Responses, Journal of Hazardous Materials. 2011; 195:188-194.
30. Sobecka E. Changes in the Iron Leveling the Organs and Tissues of Wells Catfish, Silurus glanis L. Caused by Nickel, Acta. Ichthyol. Piscat 2001; 31(2):127-143.
31. Svoboda M, Luskova V, Drastichova Zlabek V. The effect of diazinon on haematological indices of Common Carp (Cyprinus carpio L.). Acta vet. BRNO, 2001; 70:457-465.
32. Van Vuren JHJ. The Effects of Toxicants on the Hematology of Labeo Umbratus (TeleosteiCyprinidae), Comparative Biochemistry and Physiology Part C: Comparative Pharmacology 1986; 83:155-159.
33. Wendelaar BSE. The stress response in fish. Physiological Reviews. 1997; 77:591-625.
34. Wepener V, Van Vuren JHJ, Du Preez HH. Effect of manganese and iron at neutral and acidic pH on the hematology of the banded tilapia (Tilupiuspurrmunii). Bull. Environ. Cotam. Toxicol 1992; 49:613-619.
35. Wintrobe M. Clinical hematology, 4th edition. Lea and febiger, Philadelphia, 1967.