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Effect of dietary *Spirulina platensis* on blood physiology of common carp (*Cyprinus carpio*var. *communis*)

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Abstract
The present study aimed to assess the effect of dietary *Spirulina platensis* on some haematological parameters of common carp, *Cyprinus carpio* var. *communis*. The trail was conducted for a period of 90 days using 150 fingerlings ofcommon carp with a mean weight of (50g ± 5). Fishes were kept in plastic tubs (80 litre capacity) for a period of 10 days for acclimatization. During this period, the fishes were fed on basal diet. Five experimental diets were used and replacement of fish meal with *Spirulina platensis* was done @ 0% (T0), 2.5% (T1), 5% (T2), 7.5% (T3) and 10% (T4).The highest mean±S.D value for haemoglobin (mg/dl) was found in treatment group T3 (6.4 ± 0.96) and the lowest mean±S.D for haemoglobin (mg/dl) was observed in control group T0 (4.2 ± 0.57).The highest mean±S.D value for packed cell volume (PCV%) was found in treatment group T3 (19.20 ± 2.89) and the lowest mean±S.D value for packed cell volume (PCV%) was found in control group T0 (12.60 ± 1.71). The highest mean±S.D value for total erythrocyte count ($10^6/mm^3$) was found in treatment group T3 (3.60 ±0.46) and the lowest mean±S.D value for total erythrocyte count was observed in control group T0 (3.22 ± 0.19).

Keywords: Common carp, *Spirulina*, haematology, fish feed.

1. Introduction
Intimate contact of aquatic vertebrate such as fish with the environment makes them major target for toxicants and susceptible to pollutants. Thus, fish could be used as a warning system to indicate contaminants in natural water (Prashanth and Neelgund, 2007) [17]. Haematology is one of the valuable tools to evaluate health and welfare in many animal species. Haematology can be considered an essential index to the general health status in a number of fish species. This results from the close association of the circulatory system with the external environment (Casillas and Smith, 1977) [5]. Nutrition is crucial to the reproductive performance of all vertebrates, including fish. Successful reproduction requires adequate resources in order to sustain high-energy demands for the production of gametes, and reproductive behaviours. In fishes, studies have shown that decreased food availability or starvation causes gonad regression, decrease in spawning and a decrease in the number of eggs produced by females whereas increased food availability promotes growth and larger body sizes, causes earlier maturation and higher fecundity in some species. *Spirulina* is one such nutritional supplement. The major polymeric component in *Spirulina platensis* is a branched polysaccharide, structurally similar to glycogen. High molecular weight polysaccharides with antiviral and immuno modulating activities have been isolated from *Spirulina*. Their applications are mainly to provide nutrition. The present study was therefore undertaken to analyse the effect of dietary *Spirulina platensis* on various haematological variables, such as haemoglobin (Hb), packed cell volume (PCV) and Total erythrocyte count (TEC) in common carp (*Cyprinus carpio* var. *communis*. Common carp makes up more than half of the total fish caught in Kashmir's lacustrine waters (almost 85%). Common carp is regarded as the best economic species for culture practices in Kashmir because of its fast growth, tasty flesh, hardy nature and adaptability to cold waters and ease with which it can be bred in confined waters.

2. Material and Method
The experimental set up consisted of 15 plastic circular tubs (80L capacity) covered with net. The tubs were initially washed and filled with potassium permanganate solution (4mgL-1) and left overnight.

The tubs were flushed out the next day and were thoroughly washed with cleanwater. The experimental design consisted of 4 treatments and a control. All the treatments and control were replicated thrice (R1, R2, R3) following a CRD (Completely Randomized Design).

2.1 Haematological studies

For estimation of various haematological parameters, 2 ml blood was collected by heart puncture using a 3 ml syringe. Before the blood collection, fishes were anesthetized using clove oil 40 mg/l (Javahery *et al*., 2012) [11].

2.2 Haemoglobin content (mg/dl)

Haemoglobin is a reasonable index of the red cell population and was estimated by acid haematin method (Sahli, 1962) [20] with the help of a haemometer. The 0.1 N Hydrochloric acid was taken up to mark 20 in the graduated tube, and a drop (0.1 ml) of blood was added and allowed to stand for 5 minutes until it changed to dark brown colour. The solution was diluted by adding distilled water drop by drop (each time mixing the solution with a stirring rod) until it matched the standard colour. The readings were then taken from the scale on the graduated tube and the haemoglobin concentration was expressed in grams per dl.

2.3 Packed cell volume (PCV) (%) (Wintrobes method)

Packed cell volume was determined by Wintrobes method. The blood was drawn into the Wintrobes tube up to 0 mark. The blood-filled tubes were centrifuged for thirty minutes at the rate of 3000 rpm.

2.4 Total Erythrocyte Count (10^6/mm^3)

Enumeration of formed elements (blood cells) is a quantitative measure of the population of blood cells in circulation. The counting of cells can be done manually with the help of a microscope after diluting the blood and making a special type of wet mount. The technique is popularly known as haemocytometry and added by Neubauer grid, on the haemocytometer which show cell counting areas for the estimation.

The Haemocytometer (neubauer counting chamber) has a ruled area of 9 sq. mm consisting of a central heavy ruled area of 1 sq. mm and four ruled areas of the same size in the four corners. The central area is divided into 25 squares which are subdivided into 16 small squares. For counting total RBC's, the ruled areas at 5 centres were counted, and total RBC's were measured as number/cubic millimetre. For counting white blood corpuscles (WBC's), corner small squares i.e. 16 x 4 = 64 sq.mm were counted and number of cells were reported as number/cubic millimetre. Anticoagulated, non-haemolysed blood was drawn into the RBC pipette up to 0.5, and then RBC dilution solution (Hayem's solution) was drawn up to 101 mark. The pipette was shaked for one minute and immediately after shaking, the Neubauer counting chamber was charged with a mixture free from bubbles and the ruled areas were covered with a cover glass. The ruled counting area was observed under the microscope, and the number of RBC's were counted in fine small squares of the counting area under high 40 x objective (HPF), the number of erythrocytes was calculated by using the following formula:

$$TEC(/MM^3) = \frac{\text{No of cells} \times \text{dilution factor} \times \text{depth}}{\text{Total number of small squares}}$$

3. Statistical analysis

The results obtained were analysed with the help of appropriate statistical tools using SPSS and Microsoft Excel.

4. Results

4.1 Haemoglobin (mg/dl)

The highest mean value for haemoglobin (mg/dl) was found in treatment group T3 (6.4 ± 0.96 mg/dl) and the lowest mean value for haemoglobin (mg/dl) was observed in control group T0 (4.2 ± 0.57mg/dl). A significant difference (p<0.05) in haemoglobin was observed between different treatments.

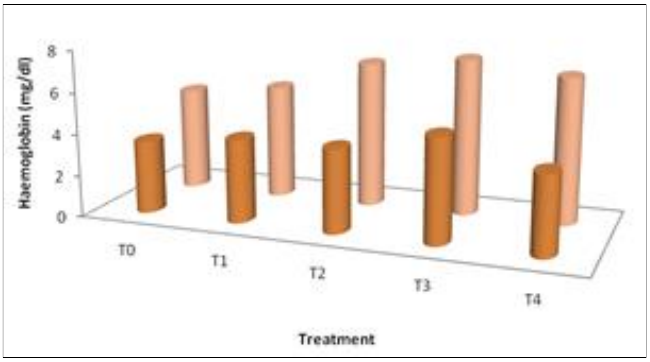

Fig 1: Haemoglobin (g/dl)

4.2 Packed cell volume (%)

The highest mean±S.D value for packed cell volume (PCV%) was found in treatment group T3 (19.20 ± 2.89) and the lowest mean±S.D value for packed cell volume (PCV%) was found in control group T0 (12.60 ± 1.71). Packed cell volume (PCV %) between the treatments varied significantly (p<0.05).

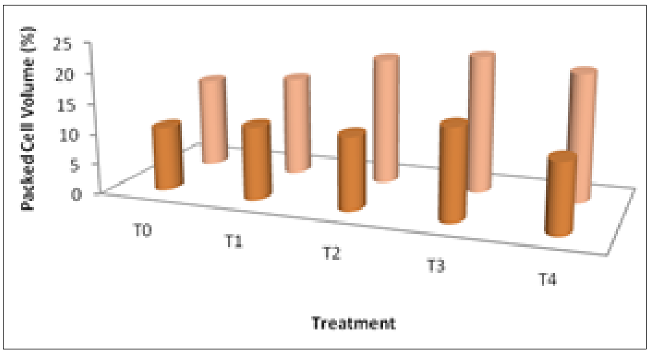

Table 2: Packed Cell Volume (%)

4.3 Total erythrocyte count (10^6/mm^3)

The highest mean value for total erythrocyte count was found in treatment group T3 (3.60 ±0.46) and the lowest mean value for total erythrocyte count was observed in control group T0 (3.22 ± 0.19).Treatment T3 varied significantly from the control group (T0) and other treatments (T4, T2 and T1) having a mean total erythrocyte count of 3.30 ± 0.20, 3.46 ± 0.20 and 3.26 ± 0.27 respectively. No significant difference (p<0.05) in total erythrocyte count was observed between different treatments.

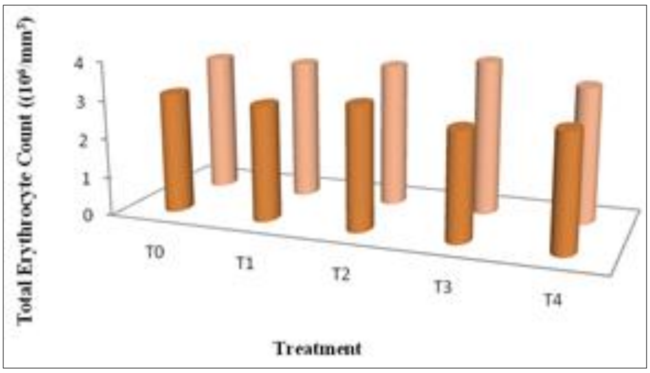

Table 3: Total Erythrocyte Count ($10^6/mm^3$)

4. Discussion

Vertebrate haemoglobin is a globular protein with quaternary structure composed of 4 globin chains (2 alpha and 2 beta) and a prosthetic group. Having myoglobin as an ancestor, haemoglobin acquired the capacity to respond to chemical stimuli that modulate its function according to tissue requirements for oxygen. Fish are generally subjected to spatial and temporal O_2 variations and have developed anatomical, physiological and biochemical strategies to adapt to the changing environmental gas availability. Structurally, most fish haemoglobins are tetrameric; however, those from some species such as lamprey and hagfish dissociate, monomeric when oxygenated and oligomeric when deoxygenated (De souza and Bonilla-Rodriguez, 2007) [6]. The main function of haemoglobin is to transport oxygen from the gas exchange organs to peripheral tissues. In the present study, treatment group (T3) exhibited the highest mean haemoglobin value while the lowest mean value for haemoglobin was observed in control group (T0). The possible reason for this could be that the dietary *Spirulina* facilitates oxygen transportation capacity consequently improving the wellbeing of the fish. The results of the present study are in line with the findings of Mohammadiazarm *et al*., 2021 [16] in Oscar fish (*Astronotus ocellatus*).Yaganesh *et al*., 2015 [26] and Arabi *et al*., 2016 [3] also reported that the haemoglobin levels increased significantly in the groups supplemented with *Spirulina platensis* in rainbow trout and common carp respectively.

The highest mean value for PCV was found in treatment group T3 and the lowest mean value for PCV was found in control group T0. *Spirulina* is known to contain phycocyanin which may have stimulated the erythropoietic hormone production for hematopoiesis. Our results are in line with the findings of Moe (2011) [15] who reported that *Spirulina platensis* incorporated diets fed to *Oreochromis niloticus* improved haematological parameters reflecting higher weight gain due to health improvement. Moreover, the haematological parameters were improved in *Cirrhinus mrigala* fed with *Spirulina* supplemented diets (James *et al*., 2009) [10].The results of the present study are also in line with the findings of (Hussain *et al*., 2021) [9] in *Cyprinus carpio* fingerlings. Zeinab *et al*., 2015 [27] reported an increase in packed cell volume in nile tilapia (*Oreochromis niloticus*) fed with *Spirulina* supplemented diets and attributed this increase in packed cell volume to the fact that probiotic used i,e. *Spirulina* increased the blood parameter values as a result of haematopoetic stimulation (Sarma *et al*., 2003) [21].

Erythrocytes are the most numerous (98–99%) blood cells in fish (Fange 1994) [7]. Fish erythrocytes (similarly as in other vertebrates with exception of mammals) are ellipsoidal and contain nucleus. Lay and Baldwin (1999) [13] found an inverse relationship between erythrocyte size and aerobic swimming ability in teleost fishes. They explain that a higher ratio of surface area to volume in smaller cells results in a shorter diffusion distance and allows faster oxygen transfer. The number of erythrocytes (RBC) differs among species. According to Soldatov (2005) [24], there are species with a very low erythrocyte count ($0.5–1.5 \times 10^6$ /mm^3) and with an extremely high RBC ($3.0–4.2 \times 10^6$ /mm^3), and the differences result mainly from locomotor activity. Erythrocyte count in fish is strongly dependent on environmental conditions (mainly on temperature and dissolved oxygen level). Therefore, seasonal changes in erythrocyte numbers take place, and this makes establishing of hematologic reference values difficult (Luskova 1998) [14]. For example, erythrocyte counts in healthy *Cyprinus carpio* may range from 0.79 to 2.90×10^6 /mm^3 (Witeska 2013) [25], while in *Oncorhynchus mykiss* from 0.28 to 1.34×10^6 /mm^3 (Rehulka *et al*., 2004) [19]. In the present study, the highest mean value for total erythrocyte count (TEC) was found in treatment group (T3) and the lowest mean value total erythrocyte count (TEC) was found in control group (T0). Higher total erythrocyte count in fish fed *Spirulina* diets may be related to the presence of iron elements in *Spirulina*. Kapoor and Mehta, 1992 [12] reported that *Spirulina* contains a substantial amount of iron and that it had significant effects on erythropoiesis increasing RBC counts and hemoglobin concentrations. According to (Henrikson, 1994) [8], *Spirulina* has 14% phycocyanin and it stimulates the erythropoietin hormone production for hematopoesis and that the increase in RBC count is a response to tolerate stress or on the other hand is a measure to maintain general health (Sivagurunathan *et al.*, 2012) [23]. Another point of view is that RBC is regarded as an indicator of oxidative stress because it is one of the major production site of free radicals (Babak *et al*., 2012) [4]. Consumption of *Spirulina platensis* which is known as a natural antioxidant, can prevent RBC degeneration.(Qureshi and Ali, 1996 [18]; Savidov, 2004) [22] Reported that the fish fed on diets containing 5.0 - 10.0 g *Spirulina*/kg diet exhibited higher RBCs than the fish fed the control diet. The results of the present study are in line with the findings of Abdel-Tawwab *et al*., 2008 1] who also reported the highest RBC count in *Oreochromis niloticus* when fed on diets containing 5.0 - 10.0 g *Spirulina* /kg diet. Andrews *et al*. (2011) [2] also reported that the erythrocyte count was significantly higher in fish fed on diets containing *Spirulina* supplementation compared to the control group. In a study of Abdel-Tawwab and Ahmad (2009) [1], fish fed on diet containing 5.0 g *Spirulina* kg_1 diet exhibited higher RBC count than the fish fed control diet. These results indicate an improvement in fish health when fed *Spirulina*-enriched diets.

5. Conclusions

Use of plant products as protein sources in fish feeds shows considerable application potential for aquaculture worldwide. *Spirulina* is known to increase the chance of blood regeneration and has immunostimulatory effects on the immune system. It is therefore concluded that the use of *Spirulina platensis* in the diet of Common carp (*Cyprinus carpio* var. *communis*) @ 7.5% improved haematological parameters, thereby maintaining good health of fish.

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