Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(4): 1885-1888

Received: 15-05-2018
Accepted: 20-06-2018

Parishmita Handique
Dept of Aquaculture, CFSc, Raha, Nagaon, India

Hemanta Pokhrel
Dept. of Aquatic Animal Health Management, CFSc, Raha, Nagaon, India

Binod Kalita
Dept. of Aquatic Animal Health Management, CFSc, Raha, Nagaon, India

Kaustubh Bhagawati
Dept of Aquaculture, CFSc, Raha, Nagaon, India

Arnab Narayan Patowary
Dept of Fisheries Extension, Economics and Statistics. CFSc, Raha, Nagaon, India

Correspondence
Hemanta Pokhrel
Dept. of Aquatic Animal Health Management, CFSc, Raha, Nagaon, India

Effect of dietary selenium incorporated feed on haematological parameter like RBC, WBC and Haemoglobin content of *Cirrihinus mrigala*

Parishmita Handique, Hemanta Pokhrel, Binod Kalita, Kaustubh Bhagawati and Arnab Narayan Patowary

Abstract
The present experiment was conducted for 45 days to evaluate the effect of dietary selenium on haematological parameters. Experiment was conducted in glass aquariums of size 0.75 m X 0.45m X 0.45m. All treatments were carried out in triplicates. Four numbers of experimental selenium incorporated diets were prepared including control which are as follows Control (0 mg organic selenium/kg of diet), T_1 (2.5 mg organic selenium/kg of diet), T_2 (5.0 mg organic selenium/kg of diet) and T_3 (10 mg organic selenium/kg of diet). During the experiment, the water quality parameters (temperature, DO, pH, conductivity, hardness, ammonia, nitrate and nitrite) were found to be insignificant (P<0.05) among all the treatments. RBC's count in T_1 (4.90 million/mm3) was significantly (P<0.05) higher than Control and T_3. The value of WBC's count in T_1 (1.58 thousands/mm3) was significantly (P<0.05) higher from that of T_2 and T_3. Haemoglobin level in T_1 (6.41 g/dl) was also significantly (P<0.05) higher from T_3. (185.88%). The result of the present study indicated that the experimental diet containing 2.5 mg organic selenium/kg of diet appears to be suitable for improved haematological parameters compared to the other treatments.

Keywords: Inorganic selenium, Haematology, red blood cells, haemoglobin

1. Introduction
Aquaculture is the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants. It is the fastest growing and emerging food producing factor in the world. Farming implies some form of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators etc. There is tremendous growth in aquaculture since the past few years; the global production has increased from 70.3 million tons in 2013 to 73.8 million tons in 2014, which accounted for 44.1% of total fisheries production, compared with capture fisheries, aquaculture provided more fish interns of food supply and the inland finfish aquaculture accounted for 65% of the increase in fish production in period 2005-2014. Inland finfish culture in earthen ponds is by distant the largest contributor from aquaculture to food security and nutrition in the developing world [2].
Selenium is one of the traced mineral which has recently received a considerable amount of attention in animal nutrition. Selenium is an essential trace element for animals including fish, and has been considered as an excellent essential nutrient for aquaculture product enhancement [6]. It is an essential micronutrient in standard animal nutrition plan, even though it is required in trace amounts, which acts as an antioxidant, compensative in metabolism, immune system, growth increments as immune stimulant as well as in normal body functions of fish. Therefore, an attempt has been made in the present investigation to study the effects of selenium incorporated diet in *Cirrhinus mrigala* on different hematological parameters.

2. Material and Methods
2.1 Experimental location
The experiment was carried out in 150 liter capacity glass aquarium at the unit of the College of Fisheries, Assam Agricultural University, Raha, Nagaon. The recorded geographical location is 26˚13'52" N latitude and 92˚06'90" E longitudes (GPRS, etrex 30, Garmin).

2.2 Collection and acclimatization of experimental fish

Experimental fishes were collected from the fish farm, College of Fisheries, AAU, Raha. After collection, fishes were acclimatized for one week in cement cistern prior to actual experiment. Fishes were fed daily with a formulated diet consisting of rice polish and mustard oil cake @ 3% body weight twice in the morning and the evening.

2.3 Preparation of experimental diets

Four experimental diets (T_c, T_1, T2 and T_3) were prepared. Basal feed (T_c) were prepared as a control diet and other three diets (T_1, T_2 and T_3) was prepared with adding varying selenium level. Feed ingredients used for preparing the diets were mustard oil cake, rice polish, fish meal, wheat flour and vitamin and mineral.Ingredients were collected from the local market, Raha. Selenium level in T_1, $T_2$and T_3were 2.5, 5.0 and 10.0 mg/kg of diet.

2.3Blood parameter analysis

About 1 ml of blood was collected from the two fishes and pooled to obtain one replication. Likewise, eight fishes were selected from each treatment to obtain four replications. Analysis of the blood parameters was performed immediately.

2.4 Erythrocyte count (RBC)

The RBCs count was carried according [1]. A 1:20 dilution of blood was obtained using Dacies fluid. The diluted blood was mixed by tilting the sealed tube gently to avoid destroying of the cells. A cover slip was placed over an Improved Neubauer hemocytometer (Hi-media) which is a special type of slide designed to act as blood cell counting chamber. Diluted blood sample was drawn into a Pasteur pipette and tip of the pipette was touched to the edge of the cover slip. Capillary action drew the diluted blood into the chamber. The hemocytometer was placed under microscope and erythrocytes (RBC) were counted in five small squares at the center of the grid, a total area of 0.02 mm^3. The number of cells per cubic mm was calculated using the following formula –

No of cells per mm^3 = no of cells counted in 0.02 mm^3 X 50 (area counted) X 50 (dilution)

2.5 Leucocytes count (WBC)

Leucocyte numbers were determined by direct counting under the microscope using a Neubauer hemocytometer chamber. The procedure for WBCs count is almost same as that of RBCs count. Step 1 to 4 of RBC count was followed to count WBCs in fish blood. Leucocytes occurring in the four corner squares marked on the grid (a total area of 0.1 mm^3) was counted.The number of cells per cubic mm was calculated using the following formula –

No of cells per mm^3 = No of cells counted in 0.01 mm^3 X 10 (area counted) X 50 (dilution)

2.6 Haemoglobin determination:

Haemoglobin concentration in blood was determined by haemometer (Sahlis haemometer). Graduated measuring tube was filled up to the bottom graduation line (mark - 2) with n/10 hydrochloric acid.Fingertip or lobe of the ear was cleaned thoroughly with either or alcohol and a drop of blood were taken by blood lancet. 20 µl blood was sucked into the capillary pipette preciously up to the mark and blown into the measuring tube. Repeated suction and blowing was done to achieve a good mixture of the liquid. The mixture was dark brown and clear after one minute. Water added by means of water pipette and was mixed with the glass stirrer until the colour of the solution matches the colour of the test rods. Reading was taken by diffused day – light exactly three minutes after adding the blood to the hydrochloric acid.The instrument indicates the quantity of haemoglobin in grams of haemoglobin contained in 100 ml of the blood.

3. Result

3.1 RBC (red blood cell) (million/mm^3)

During the experimental period the range of RBCs was between 1.74 to 4.90 million/mm^3which is shown in Table 2 and Fig. 1.On the day of stocking, the RBC count was recorded 1.75, 1.77, 1.74 and 1.74 million/mm^3 in Control, T_1, T_2 and T_3 respectively. On the 15th day of sampling, RBC count was recorded to be 2.17, 3.42, 2.99 and 2.24 million/mm^3 in Control, T_1, T_2 and T_3 respectively. On the 30th day of sampling, RBC count was noted 2.50, 4.12, 3.61 and 2.85 million/mm^3 in Control, T_1, T_2 and T_3 respectively. On the 45th day of sampling, RBC count was found to be 2.95, 4.90, 4.34 and 3.15 million/mm^3 in Control, T_1, T_2 and T_3 respectively. At the end of the experiment (45th day sampling) the highest value (4.90 million/mm^3) was recorded in T_1 and the lowest value (2.95 million/mm^3) was observed in Control. RBCs count in T_1 was significantly (P<0.05) different from Control and T_3 (Table1).

3.2 WBC (white blood cell) (thousands/mm^3)

During the experimental period the WBC count was varied from 1.12 to 1.58 thousands/mm^3which is shown in the Table 2 and Fig. 2. On the day of stocking, the WBC countwas recorded 1.14, 1.14, 1.13 and 1.12 thousands/mm^3in Control, T_1, T_2 and T_3 respectively. On the 15th day of sampling, WBC countwas noted 1.21, 1.32, 1.20 and 1.18thousands/mm^3 in Control, T_1, T_2 and T_3 respectively. On the 30th day of sampling, WBC count was noted1.23, 1.40, 1.25 and 1.20thousands/mm^3 in Control, T_1, T_2 and T_3 respectively. On the 45th day of sampling, WBC countwas recorded 1.36, 1.58, 1.29 and 1.23thousands/mm^3 in Control, T_1, T_2 and T_3 respectively. At the end of the feeding (45th day of sampling) the highest value of WBCs (1.58 thousands/mm^3) was counted in T_1, while the lowest (1.23 thousands/mm^3) was counted in T_3. The value of WBCs count in T_1 was significantly (P<0.05) different from T_2 and T_3.

3.3 Haemoglobin (g/dl)

The range of hemoglobin level was varied from 3.25 to 6.41g/dl during the experimental period which is given in Table 2 and Fig. 3. On the day of stocking, the hemoglobin content was recorded 3.27, 3.30, 3.41 and 3.25 g/dlin Control, T_1, T_2 and T_3 respectively. On the 15th day of sampling, the hemoglobin content was noted 4.64, 4.82, 4.71 and 4.53 g/dl in Control, T_1, T_2 and T_3 respectively. On the 30th day of sampling, the hemoglobin content was noted 5.67, 5.91, 5.34 and 3.91g/dl in Control, T_1, T_2 and T_3 respectively. On the 45th day of sampling, the hemoglobin content was recorded 5.91, 6.41, 5.12 and 3.73 g/dl in Control, T_1, T_2 and T_3 respectively.At the end of the experiment (45thday of sampling) the highest hemoglobin level (6.41 g/dl) was observed in T_1, whereas the lowest (3.73 g/dl) was recorded in T_3. Hemoglobin level in T_1 was significantly (P<0.05) different from T_3 (Table 1)

Table 1: Mean variation in various blood parameters in *Cirrhinus mrigala*

Blood parameters	Treatment			
	Control	T_1	T_2	T_3
RBC (million/mm^3)	2.34±0.02^a	3.55±0.11^b	3.17±0.11^{ab}	2.49±0.17^a
WBC (thousands/mm^3)	1.25±0.02^{ab}	1.35±0.02^b	1.21±0.03^a	1.18±0.02^a
Haemoglobin (g/dl)	4.87±0.04^a	5.11±0.10^b	4.64±0.07^{ab}	3.86±0.04^a

*Values of each parameter are mean ±SE of triplicate determination

Table 2: Means and SEM of hematological parameters of *Cirrhinus mrigala* fed on selenium in corporated diet.

Parameters	T_c				T_1				T_2				T_3			
	0 days	15 days	30 days	45 days	0 days	15 days	30 days	45 days	0 days	15 days	30 days	45 days	0 days	15 days	30 days	45 days
RBC	1.75±0.02	2.17±0.12	2.50±0.02	2.95±0.21	1.77±0.04	3.42±0.09	4.12±0.15	4.90±0.21	1.76±0.04	2.99±0.23	3.61±0.11	4.34±0.14	1.74±0.02	2.24±0.02	2.85±0.18	3.15±0.13
WBC	1.14±0.02	1.21±0.02	1.23±0.03	1.26±0.02	1.14±0.02	1.32±0.02	1.40±0.02	1.58±0.05	1.13±0.02	1.20±0.03	1.25±0.03	1.29±0.03	1.12±0.01	1.18±0.02	1.20±0.02	1.23±0.03
HB %	3.27±0.03	4.64±0.05	5.67±0.05	5.91±0.05	3.30±0.23	4.82±0.03	5.91±0.07	6.41±0.08	3.41±0.08	4.71±0.05	5.34±0.07	5.12±0.09	3.25±0.03	4.53±0.03	3.91±0.06	3.73±0.06

*Values of each parameter are mean ± SE of triplicate determination

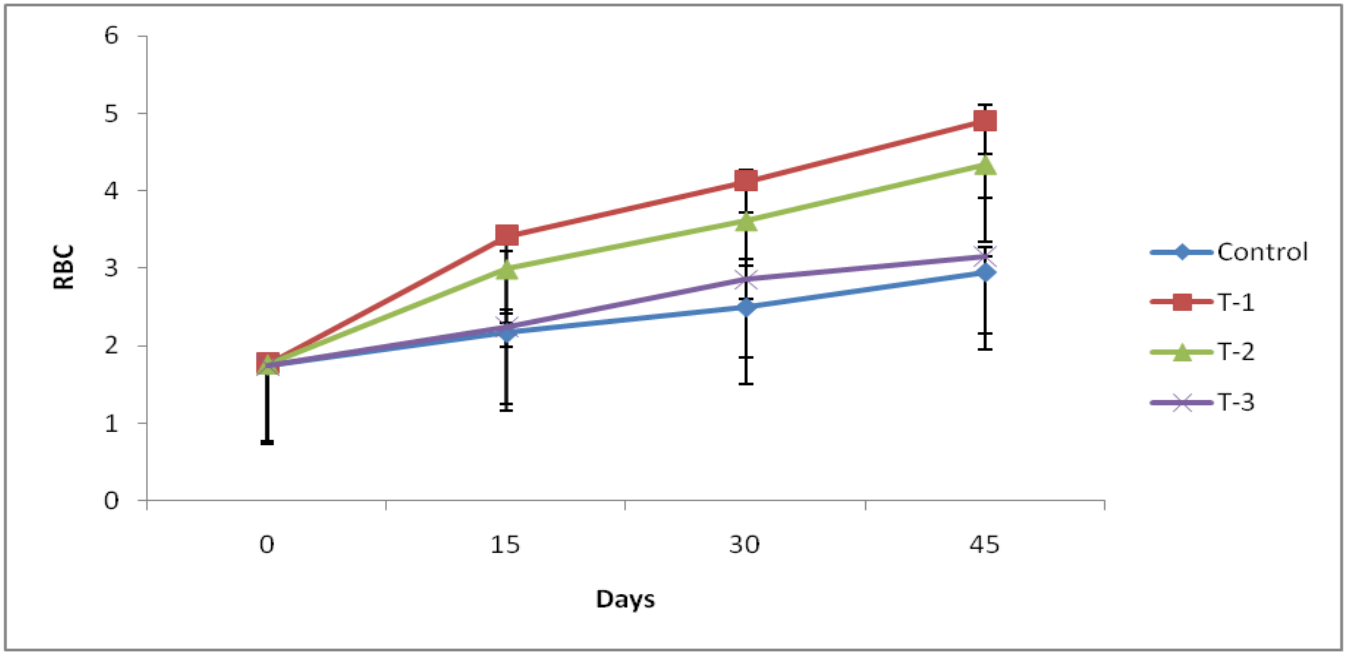

Fig 1: Fluctuation in RBCs count (million/mm^3) (mean ± SE) in different treatments during experiment

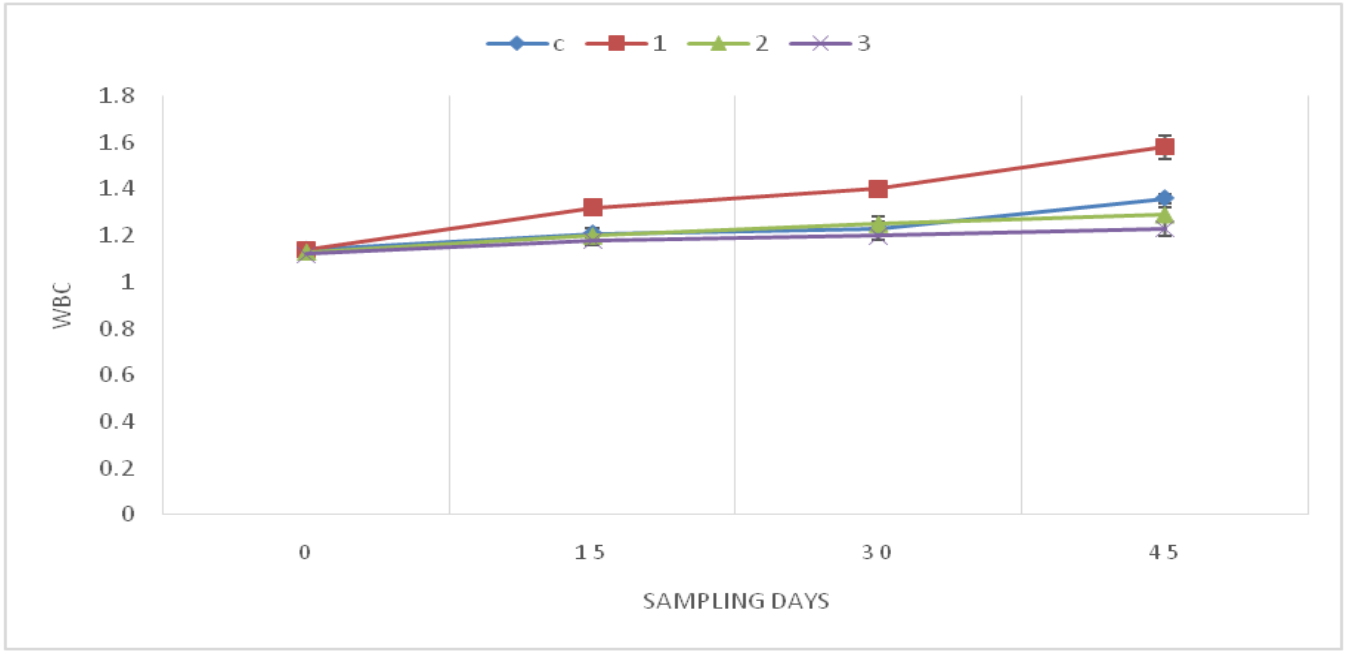

Fig 2: Fluctuation in WBCs count (thousands/mm^3) (mean ± SE) in different treatments during experiment

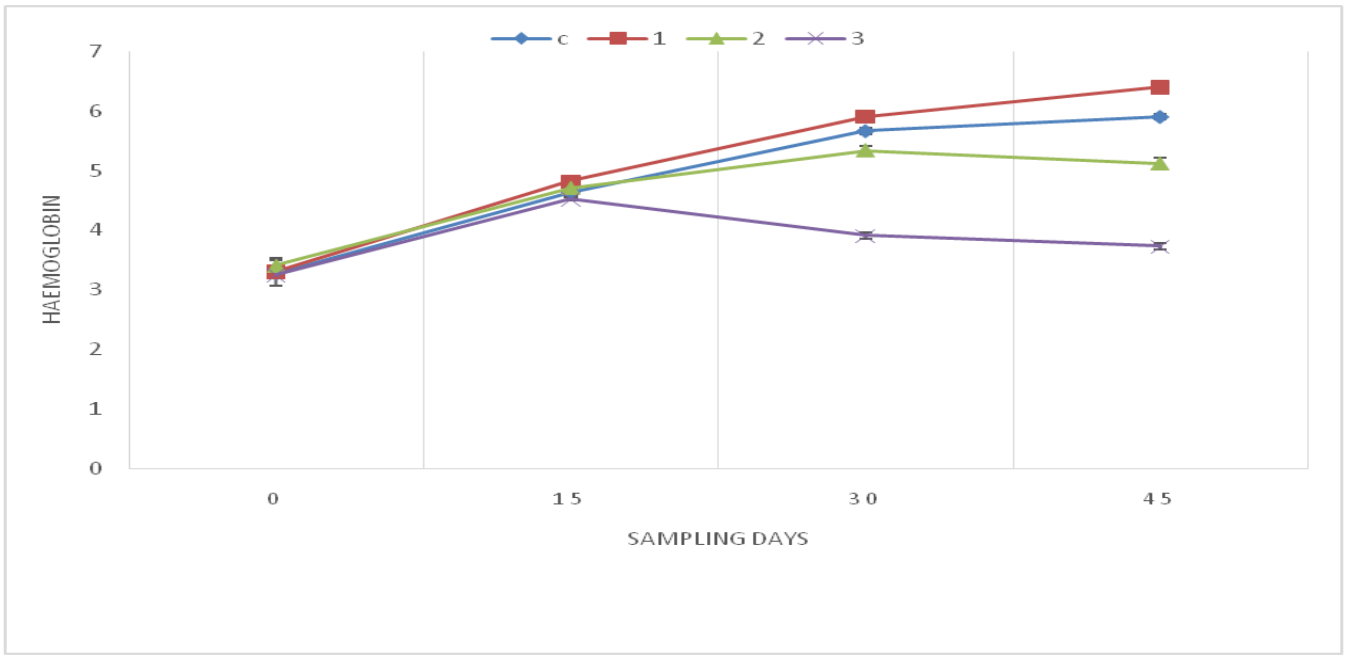

Fig 3: Fluctuation in hemoglobin level (g/dl) (mean ± SE) in different treatments during experiment

4. Discussion

In the present study, the highest value of RBC (4.90 million/mm^3), WBCs (1.58 thousands/mm^3) and hemoglobin content (6.41 g/dl) was recorded in T_1 (2.5 mg OS/kg diet),compared to the other treatments and lowest value of RBC 2.95 million/mm^{3,} WBC (1.23 thousands/mm$^{3)}$ and Haemoglobin (3.73 g/dl) was recorded in Control (0 mg OS/kg diet), T_3 (10 mg OS/kg diet) and T_3 (10 mg OS/kg diet)respectively. The values of the RBC's, WBC and hemoglobin in the present study are in agreement with [4], who revealed that the amount of RBC's, WBC and hemoglobin concentration was highest in case of 2 mg OS/kg of diet fed in tilapia. Moreover, [7] also mentioned the highest count of RBC's and hemoglobin in black seabream for 0.30 mg Se/kg diets and the lowest value in Control (0 mg OS/kg diet) group of fish.Declines in RBC, WBC's and hemoglobin content may be due to the stress, as a results of handling of fish during sampling or may be selenium toxicity at slightly higher dose in diet, reported by [5]. Findings were also at par with [3] in common carp (0.08, 0.16, 0.32 and 0.64 mg/kg diet). However, [7] revealed that hemoglobin content of black seabream fed with experimental diet containing selenium (0.21, 0.30, 0.52, 1.29 and 12.3 mg/kg diet) were not significantly (P>0.05) different among the treatments.Overall, this study showed that there is an effect of selenium on increasevalues in hematological parameter (RBC, WBC and Haemoglobin content).

5. Conclusion

The present study showed the effect of dietary selenium on haematological parameters (Hamilton, 1822). Addition of organic selenium in diet enhanced the haematological parameters in *Cirrhinus mrigala* which documented the important health status of the fish. The result of the present study indicated that the experimental diet containing 2.5 mg organic selenium/ kg of diet appears to be suitable for improved haematological parameters (red blood cell, white blood cell and hemoglobin).

6. Acknowledgement

The present work was performed through the project attachment with National Surveillance Programme Aquatic Animal Diseases funded by NFDB, Hyderabad. The authors here sincerely thanks to the authority of the project. The authors are also grateful to Dr. K.M Bujarbaruah, Vice chancellor of the University for kind Help for providing the facilities to carry out the research project.

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