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# Impact of Experimental Immunisation on Leucocyte Count of *Clarias batrachus*

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**ABSTRACT**

Fresh water catfish *Clarias batrachus* was immunised with cellular antigen and the immune response of the fish was observed. The impact on immune system was studied in terms of leucocytes: TLC and DLC were determined for the experimentally immunized group and compared with the control group. The number of lymphocytes and TLC increased slightly after administering the antigen. The results obtained are analysed and presented in this communication.

**Keywords**: Immune system, leucocytes, lymphocytes, TLC, *Clarias batrachus*

## INTRODUCTION

The fishes besides being the largest vertebrate class, have greater species diversity than any other vertebrate groups and are found in almost every aquatic environment on earth. The evolution of fishes and the tetrapods diverged from each other about 300 million years ago and it is natural that fishes should be the subject of investigation of the evolution of lymphoid tissues and the development of immune system (Press *et al.*, 1998).

Fish and their immune system also represent an important scientific tool in the monitoring of environmental quality. Fish occupy a variety of ecological niches in the aquatic environment and so changes in the immune parameters of fish have the potential to be the sensitive gauge of environmental deterioration (Wester *et al.*, 1994). In all vertebrates, immune responses are mediated by leucocytes therefore an understanding of immunology must be based upon an understanding of leucocyte function. This study is a part of the doctoral work of the first author. The basic structure of the fish leucocyte is described in short along with the impact of SRBC immunisation on the number of leucocytes.

## MATERIALS AND METHODS

The study was conducted on the fishes obtained from the different water sources of Meerut and Muzaffarnagar region. Fishes were then maintained in suitable tanks and glass aquaria and fed upon different types of food such as pelleted food, minced goat liver etc. The fishes were acclimatized for at least three weeks in order to bring them to their normal state before using them for observational purpose.

For the experimental setup the fishes were divided into two groups. One was used as control which was not immunized and second group consisted of seven fishes which were immunized. The seven fishes which were immunized with a cellular antigen constitute the experimental group. The antigen in the present experiment was SRBC. The immunized fishes were routinely anaesthetized and used for blood collection after every five days for one month. The data was collected on day 1, 5, 10, 15, 20, 25 and day 30. This set up was repeated five times and then the mean of the five observations was taken as the final value.

For preparation of the antigen, blood was collected from jugular vein of the sheep, with the help of sterilised syringe. To the sheep blood, same amount of Alsever's solution was added. It was shaken and then centrifuged at 2000 rpm at room temperature for five minutes. The pellet obtained after discarding the supernatant was resuspended in Phosphate Buffer Saline. It was then again spun at 2000 rpm at room temperature for ten minutes

and then suspended in Phosphate Buffer Saline. Fishes of the experimental group were immunized with 0.5 ml of 25% SRBC in Phosphate Buffer Saline. For immunization purpose and for blood collection, each fish was caught manually, taken out of the aquarium and anaesthetized using clove oil, at a concentration of 50 mg/l, also reported by Woody *et al.*, 2002. Antigen was injected intraperitoneally. For the preparation of blood smear Brauer (1968) method was followed. These smears were used to record the values for DLC. In the interpretation of the differential blood counts of fish, the correct identification of different cells of fish blood is of particular importance and requires basic knowledge of morphology of blood cells and practical experience. For the classification of different cell types, the shape and size as reported by Ellis (1977) was considered during observations. The total leucocyte count was obtained by the method Of Shaw as described by Schaperclaus ( 1991), using Neubauer counting chamber.

## RESULT AND DISCUSSION

Total Leucocyte Count of *Clarias batrachus* on different days after injecting 0.5% ml of 25% SRBC intraperitoneally was recorded and it was observed that the value of TLC during this experiment ranged in between 2.21-3.42 (average value 2.74). The reference value for the TLC was 2.59. The value of TLC was recorded to decrease in fish sacrificed on day 1 (2.54 ± 0.29) as compared to the value of TLC in the fish of control group (2.59 ± 0.10), the value increased gradually in the fish sacrificed on Day 5 (2.64 ± 0.16), Day 10 (2.84 ± 0.08), Day 15 (2.87 ± 0.18) and Day 20, reaching its maximum in the fish sacrificed on Day 20 (2.92 ± 0.22) and then was recorded to decrease gradually in fish sacrificed on Day 25 (2.78 ± 0.32) and Day 30 (2.71 ± 0.13).

Differential Leukocyte Count of *Clarias batrachus* on different days was recorded. The number of neutrophils was recorded to increase in the fish sacrificed on Day 1 (29.2 ± 7.2), as compared to the fish of the control group (28.0 ± 8.0), the number was lower in the fish sacrificed on Day 5 (22.2 ± 5.8) which was recorded to decrease gradually in the fish sacrificed on Day 10 (20.2 ± 3.7), Day 15 (20.2 ± 4.97) and Day 20 (minimum value 19.0 ± 2.12), the value was recorded to increase in the fish on Day 25 (22.2 ± 4.32) and then in the fish sacrificed on the Day 30 (25.6 ± 3.29).

The number of lymphocytes was recorded to decrease in the fish sacrificed on Day 1 (67.8 ± 8.1), as compared to the fish of control group (68.6 ± 8.4), then the number was recorded to increase gradually in the fish sacrificed on Day 5 (74.4 ± 4.62), Day 10 (76.6 ± 4.7), Day 15 (78.2 ± 4.49) and Day 20 (maximum 79.2 ± 2.59) the number was again recorded to decrease in the fish sacrificed on Day 25 (75.6 ± 4.72) and Day 30 (71.6 ± 3.36).

The number of Eosinophils was same in the fish of control group (1.4 ± 0.5) and the fish sacrificed on Day 1 (1.4 ± 1.1), it was recorded to decrease in the fish sacrificed on Day 5 (1.2 ± 0.84) and was same in the fish sacrificed on Day 10, again a decreased number was recorded in the fish sacrificed on Day15 (0.8 ± 0.84), the number increased in the fish sacrificed on Day 20 (1.0 ±0.71), which was recorded to decrease in the fish sacrificed on Day 25 (0.6 ± 0.89), and again increase in the fish sacrificed on Day 30 (1.2 ± 1.30).

The number of Monocytes was recorded to decrease in the fish sacrificed on Day 1 (1.0 ± 0.7) as compared to the fish of the control group (2.0 ± 1.0), the number was noticed to increase in the fish sacrificed on Day 5 (maximum 2.2 ± 1.48), which slightly decreased in the fish sacrificed on Day 10 (2.0 ± 1.6) and Day 15(minimum 0.8 ± 0.84), was same as on day 15 in the fish sacrificed on Day 20. The number was recorded to increase in the fish sacrificed on Day 25 (1.6 ± 0.89) and was the same in the fish sacrificed on Day 30.

**Table1. Mean Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC) of the fish *Clarias batrachus* recorded on different Days after injecting 0.5ml of 25% SRBC intra-peritoneally.**

| | TLC ($x10^4$ $\mu l^{-1}$) | Differential Leucocyte Count (DLC) | | | |
|---|---|---|---|---|---|
| | | Neutrophils % | Lymphocytes% | Eosinophils% | Monocytes% |
| Control | 2.59 ±0.01 | 28.0 ±8.0 | 68.6 ±8.0 | 1.4 ±0.5 | 2.0 ±1.0 |
| Day 1 | 2.54 ±0.29 | 29.8 ±7.2 | 67.8 ±8.1 | 1.4 ±1.1 | 1.0 ±0.7 |
| Day 5 | 2.64 ±0.16 | 22.2 ±5.81 | 74.4 ±4.62 | 1.2 ±0.84 | 2.2 ±1.48 |
| Day 10 | 2.84 ±0.08 | 20.2 ±3.7 | 76.6 ±4.7 | 1.2 ±0.8 | 2.0 ±1.6 |
| Day 15 | 2.87 ±0.18 | 20.2 ±4.97 | 78.2 ±4.49 | 0.8 ±0.84 | 0.8 ±0.84 |
| Day 20 | 2.92 ±0.22 | 19.0 ±2.12 | 79.2 ±2.59 | 1.0 ±0.71 | 0.8 ±0.84 |
| Day 25 | 2.78 ±0.32 | 22.2 ±4.32 | 75.6 ±4.72 | 0.6 ±0.89 | 1.6 ±0.89 |
| Day 30 | 2.71 ±0.13 | 25.6 ±3.29 | 71.6 ±3.36 | 1.2 ±1.30 | 1.6 ±0.89 |

*Values are mean ± standard deviation; n=5*

The blood smears show erythrocytes, which are easily differentiated from the leukocytes, thrombocytes and the various types of leukocytes such as neutrophils, lymphocytes, monocytes, and sometimes eosinophils. Erythrocytes are oval and disk-shaped cells with a centrally located compact nucleus. Thrombocytes are nucleated cells that are smaller than erythrocytes and vary in shape.

Lymphocytes, are usually the most commonly observed leucocyte type present in the blood of fishes accounting for as much as 85% of total leucocyte population (Rowley *et al*., 1988). They are smaller than erythrocytes, with a centrally located round or oval nucleus and scanty cytoplasm. Lymphocytes of several fish species have been shown to carry Ig in their surface membranes that can be induced to form caps. This phenomenon may constitute a triggering mechanism whereby lymphocytes are induced to transform into antibody producing cells or their precursors (Ellis, 1977). In the experimental fish *Clarius batrachus* the number of lymphocytes directly appears to be affected by the selected antigen, the number of the lymphocytes increased after immunization, reached a maximum value in the fish sacrificed on Day 20.

Monocytes have round or indented, eccentric nuclei and deeply basophilic cytoplasm with clear punctate cytoplasmic vacuoles. They are characterized by a prominent eccentric, kidney shaped to bilobed nucleus with a granular cytoplasm (Ellis, 1976). The cytoplasm contains numerous profiles of RER, an active golgi complex and centrioles usually located in the nuclear cleft and golgi derived vesicles (Rowley *et al*., 1988). Monocytes are participants in acute inflammatory responses of fish in which they actively phagocytose invaders (Finn and Neilson, 1971, MacArthur *et al*., 1984, MacArthur and Fletcher, 1985). No definite trend was noted on the number of monocytes in *Clarias batrachus* after immunisation with the selected antigen.

There is a great variation in the bony fishes in both the abundance and staining reactions of the granulocytes suggesting that different populations of granulocytes have arisen several times during the divergent evolution of this group (Rowley *et al*., 1988).

Neutrophils are round or oval shaped cells and usually larger than Erythrocytes, with an eccentric kidney shaped or two to three lobed nucleus and granular cytoplasm. Neutrophil is the most commonly encountered granulocyte in gold fish *Carassius auratus* with a diameter of 10 micrometer; having an eccentric nucleus with partially lobate appearance (Weinreb, 1963). Neutrophils are characterized by granules, some of them with a central electron dense or electron lucent crystalline rod, which may have a fibrillar appearance (Cenini, 1984). Plaice neutrophils appeared not to be phagocytic towards carbon particles (Ellis, 1976). While neutrophils in rainbow trout migrated to an inflammatory site caused by bacteria, they did not take part in phagocytic activity (Klontz, 1972). Migration and phagocytosis by neutrophils during experimentally induced bacterial inflammation in rainbow trout was reported by Finn and Nielson (1971). Chemotaxis has not been demonstrated convincingly for fish neutrophils. However rapid migration of neutrophils from blood vessels in the neighbourhood of an infected or otherwise injured site strongly suggest that a chemotactic stimulus is involved (Hines and Spira, 1973). No definite trend was noted on the number of neutrophils on different day intervals after immunization with the selected antigen.

Reports of the presence of basophils in the blood of fish vary with some authors reporting them (Watson *et al.,* 1963, Weinreb, 1963, Garavini and Martelli, 1981) and others claiming them to be absent in the fish blood (Javaid and Akhtar, 1977 and Barber and Westermann, 1978). Watson *et al*., 1963, claimed that basophils were fragile and often disrupted in smear preparations with dispersion of the granules. During our experiment we could not find basophils in *Clarias batrachus.*

The entire literature concerning eosinophils in teleost fish is contradictory; there being claims of their presence (Conroy, 1972) and absence (Blaxhall and Daisley, 1973). Eosinophils have round, often eccentric nuclei and lightly basophilic cytoplasm with eosinophilic granules. Eosinophils in fish are identified by the presence of large cytoplasmic granules. The nucleus tends to the pyknotic and the cytoplasm packed with large eosinophilic granules. The only direct evidence of a functional role for eosinophils in fish is the observation of phagocytic activity. Watson et al. 1963 and Jakowska and Nigrelli, 1953, reported phagocytosis of bacteria by eosinophils in the goldfish *Carassius auratus* and guppy respectively. Ainsworth (1992) stated that they are not practically phagocytic but are important in parasitic infections. No definite trend was noted on the number of eosinophils in *Clarias batrachus* after immunization with the selected antigen during the course of this experiment.

## REFERENCES

Ainsworth, A.J. (1992). Fish granulocytes: Morphology distribution and function. *Annu. Rev.Fish Dis.* **2.** 123-148.

Barber, D.L. and Westermann, J.E.M. (1978). Occurrence of the periodic acid -schiff positive granular leucocytes (PAS-GL) in some fishes and its significance. *J. Fish Biol.* **12.** 35-43.

Blaxhall, P.C. and Daisley, K.W. (1973) Routine

haematological methods for use with fish blood. *J. Fish Biol.* **5.** 771-782.

Brauer, A. (1968). Laboratory directions for histological techniques. Burgess publishing company. Minneapolis 15-Minnesota.

Cenini, P. (1984). The ultrastructure of leucocytes in carp (*Cyprinus carpio*). *J. Zool.* **204**. 509-520.

Conroy, D.A. (1972) Studies on the haematology of the Atlantic Salmon (*Salmo salar* L). In '*Diseases of Fish '*(ed. L.E. Mawdesley-Thomas), *Symp. Zool. Soc. Lond.* No. 30. London: Academic Press. pp. 101-127.

Ellis, A.E. (1976). Leucocytes and related cells in the plaice, *Pleuronectes platessa*. *J. Fish Biol.* **8**. 143-156.

Ellis, A.E. (1977). The leucocytes of fish: A review. *J. Fish Biol.* **11**. 453-491

Finn. J.P. and Nielsen, N.O. (1971) The inflammatory response of rainbow trout. *J. Fish Biol.* **3**. 463-478.

Garavini, C. and Martelli, P. (1981). Alkaline phosphatase and peroxidase in gold fish (*Carassius auratus*) leukocytes. *Basic Appl. Histochem.* **25**. 133-139.

Jakowska, S. and Nigrelli, R.F. (1953). Localised responses in fish to experimental inflammation caused by pathogenic bacteria. *Anat. Rec.* **177**. 526.

Javaid, M.Y. and Akhtar, N. (1977). Haematology of fishes in Pakistan. II. Studies on fourteen species of teleosts. *Biologia*. **23**. 79-90.

Klontz, G. W. (1972). Haematological techniques and the immune response in rainbow trout. In '*Diseases of Fish'* (ed. L.E. Mawdesley-Thomas) *Symp. Zool. Soc. Lond.* No. 30. London: Academic Press. pp. 89-99.

MacArthur, J.I. and Fletcher, T.C. (1985). Phagocytosis in fish. In '*Fish Immunology'.* (eds. M.J. Manning and M.F. Tatner), Academic Press, Orlando. pp. 29-46.

MacArthur, J.I.; Fletcher, T.C.; Pirie, B.J.S.; Davidson, R.J.I. and Thomson, A.W. (1984). Peritoneal inflammatory cells in plaice, *Pleuronectes platessa* L.: effects of stress and endotoxin. *J. Fish Biol.* **25** .69-81.

Press, C.M.; Jorgensen, T.; Miller, N.W.; Ellis, A.E.; Robertson, B.; Hordvik, I.; Charlemagne, J.; Pilstrom, L.; Lie, O.; Grimholt, V. *et al.* (1998). Immunology of fishes. In "*Handbook of Vertebrate immunology".* (eds. P.P. Pastoret.; P. Griebel.; H. Bazin. And A. Govaerts), Academic Press Inc., San Diego. 3-62.

Rowley, A.F.; Hunt, T.C.; Page, M. and Mainwaring, G. (1988). Fish. '*In Vertebrate Blood Cells'* (eds. A.F. Rowley and N.A. Ratcliffe), Cambridge University Press, Cambridge, UK. pp. 19-127.

Schaperclaus, W. (1991). Haematological and serological techniques. In "*Fish diseases."* (eds. W. Schaperclaus, H. Kulow and K. Schrekenbach), Amerind Publishing Co. Pvt. Ltd., New Delhi. Vol.1. 71-107.

Watson, L.J.; Shechmeister, I.L. and Jackson, L.L. (1963). The haematology of goldfish (*Carassius auratus*). *Cytologia.* **28**. 188-230.

Weinreb, E.L. (1963). Studies on the fine structure of teleost blood cells. I. Peripheral blood. *Anat. Rec.* **147**. 219-238.

Woody, C.A.; Nelson, J. and Ramstad, K. (2002). Clove oil as an anaesthetic for adult sockeye salmon: field trials. *J. Fish Biol.* **60** 340-347.