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On the Immune Response of *Clarias batrachus* After Immunisation with 25% SRBC

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ABSTRACT

Effect of experimental immunization with 25% sheep red blood cells (SRBC) on the immune system of *Clarias batrachus* was observed. The impact on immune system was studied in terms of serum proteins of the fish. Total Serum Proteins, Albumin and Globulin were determined for the control group and the immunized group. The amount of globulin increased slightly after administering the antigen, thus decreasing the albumin globulin ratio. The results obtained are analysed and presented in this communication.

Keywords: *Clarias batrachus*, Albumin globulin ratio, Serum protein, SRBC.

INTRODUCTION

The fishes are the largest vertebrate class and constitute approximately 24600 species. (Nelson, 1994). All groups of lower vertebrates possess well developed immunological capacity to respond to soluble and particulate antigens (Smith *et al*., 1967; Hildemann, 1970) In addition to comparative investigations, fish also represent potential experimental model for vertebrate immunology as a whole. Extensive studies of various fish species show that the basic mechanism of acquired immunity in the fish and mammals are surprisingly similar (Faisal and Hetrick 1992). Foundation of immune system was laid in vertebrates in class Pisces with a prehistory in the agnathans. The study of immune system in a fish not only enlightens about the details of a system in a particular species but also throws light on the basic pattern responsible for subsequent development of immune system in other classes of vertebrates. After immunization, fish most often produce antibodies with specificity and measurable affinity for the immunization antigen. Determining the serum proteins in the fish contributes to the appraisal of the condition and the course of disease. This report is a part of the doctoral work of the first author. It deals with the kinetics of the immune response to SRBC immunization in terms of albumin globulin ratio.

MATERIALS AND METHODS

The live fishes for present work were obtained from the local fish markets and different water sources of Meerut and Muzaffarnagar region. Fishes were fed on different types of food, including commercially available pelleted food, minced goat liver etc. Injured, abnormally coloured, morbid or ecto-parasitized fishes were discarded for all the observations as they might have altered the results. After a long acclimatization period the fishes kept in tanks and glass aquaria were used for observational purpose.

Eight fishes were selected and divided into two groups, one of them was used as control and rest seven as experimental group. Fishes of experimental group were immunized with antigen (0.5ml of 25% SRBC). Fishes thus immunized were routinely anaesthetized and used for serum collection on day 1,5,10,15,20,25, and 30 days respectively.

For SRBC, sheep blood, collected from jugular vein, with the help of sterilised syringe, was added to same amount of Alsever's solution and then centrifuged. After discarding the supernatant the pellet was resuspended in Phosphate Buffer Saline. Fishes were immunized with 0.5 ml of 25% SRBC in PBS. For immunization purpose and for blood collection, later on, each fish was caught manually, taken out of the aquarium and anaesthetized as per Kumar *et al.,* (2005). Antigen was injected intraperitoneally

(Sinha and Chakravarty, 1997). For collection of blood for serum protein analysis, cardiac puncture method was used. The total proteins and amount of albumin in the serum was estimated following the Gornall's biuret method (Kulow 1967 as cited by Schaperclaus 1991). The globulin component is the difference between the total proteins and the albumin. The obtained results were used to get the A :G value. The same steps were repeated five times.

RESULTS AND DISCUSSION

Serum protein values of *Clarias batrachus* on different days after injecting 0.5ml of 25% SRBC intra-peritoneally are presented in Figure 1 and Table 1. The value of Total Serum Proteins ranged between 2.05-3.88, the value of globulin ranged from 0.71-2.01 and amount of albumin was recorded in the range of 1.32-1.91. These values of the serum protein were used to evaluate the albumin-globulin ratio for the fish which ranged between 0.71-1.92.

Table1. Serum Protein Values, Albumin-Globulin Ratio (A: G) of the fish *Clarias batrachus* recorded on different Days after injecting 0.5ml of 25% SRBC intra-peritoneally.

	Total serum protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	A: G
control	2.61±0.36	1.53±0.13	1.08±0.24	1.44±0.27
Day 1	2.59±0.34	1.52±0.17	1.07±0.18	1.42±0.13
Day 5	3.14±0.23	1.58±0.17	1.56±0.12	1.02±0.08
Day 10	3.63±0.30	1.69±0.24	1.94±0.06	0.86±0.11
Day 15	3.27±0.34	1.68±0.15	1.59±0.16	1.06±0.19
Day 20	2.78±0.22	1.49±0.16	1.29±0.10	1.15±0.12
Day 25	2.72±0.19	1.53±0.22	1.19±0.18	1.31±0.36
Day 30	2.64±0.15	1.56±0.15	1.08±0.14	1.45±0.30

Values are mean ± standard deviation; n=5

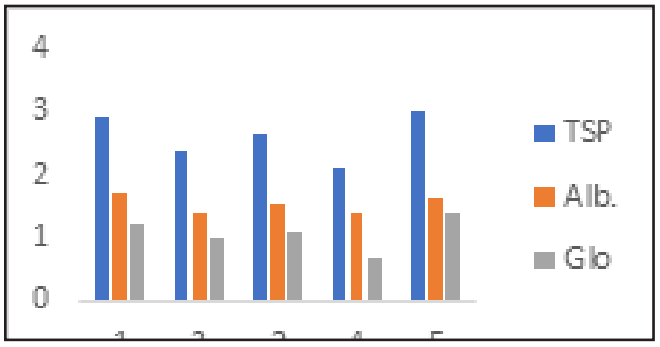

Fig.1a

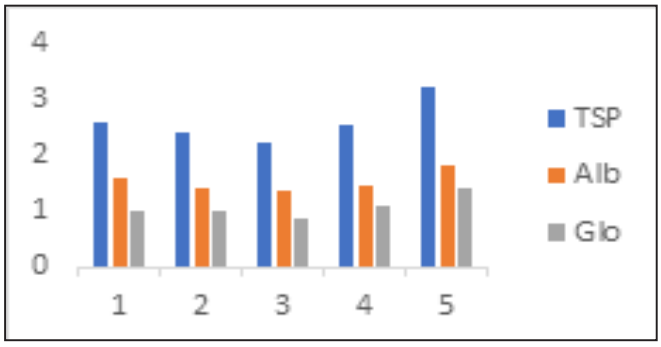

Fig. 1b

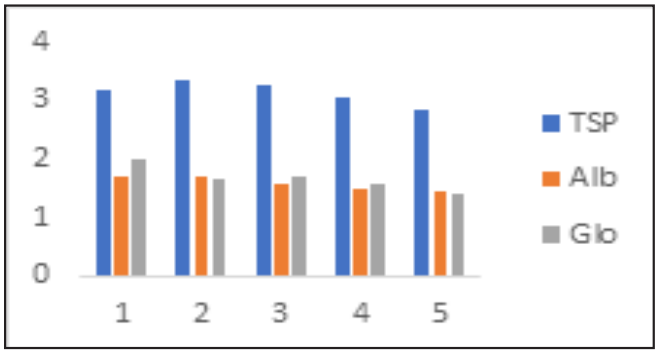

Fig. 1c

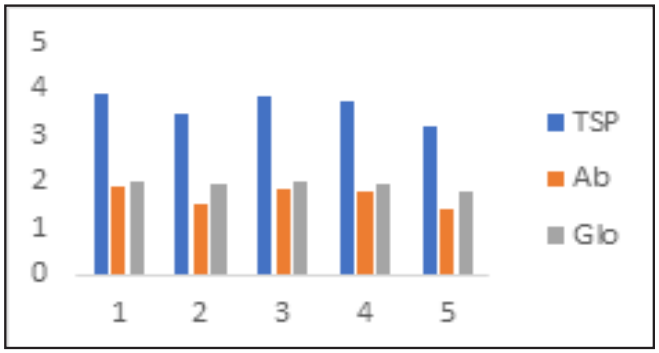

Fig. 1d

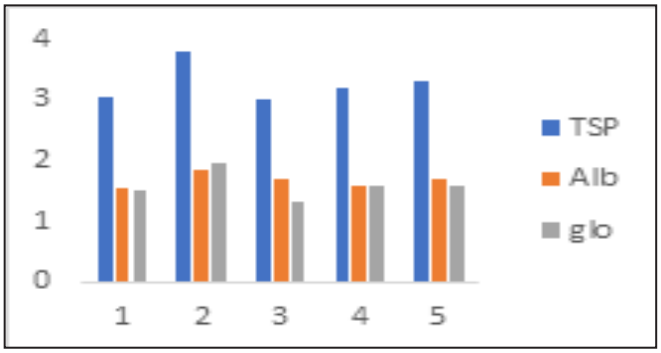

Fig. 1e

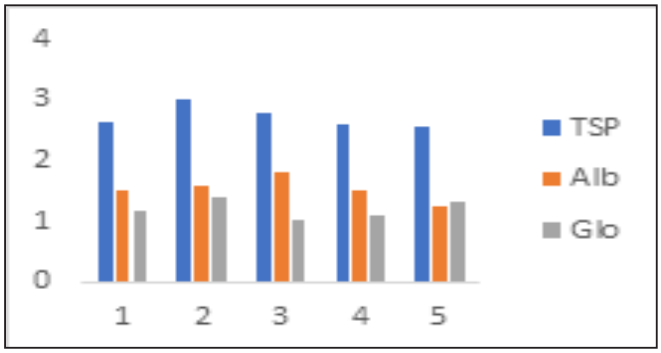

Fig. 1g

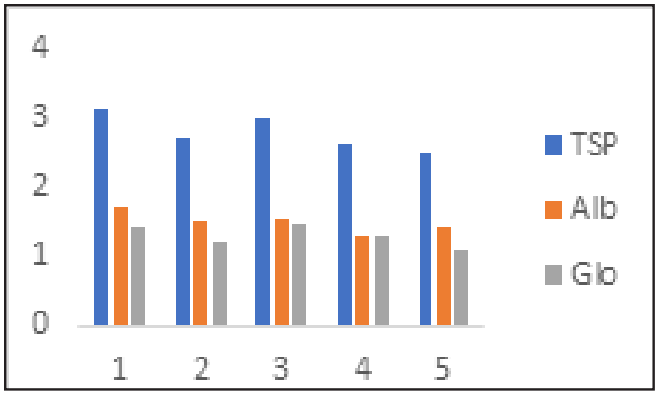

Fig. 1f

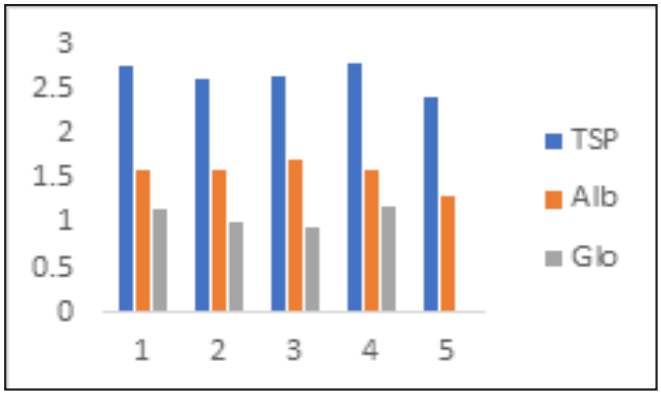

Fig. 1h

The values of the Serum Proteins of *Clarias batrachus* increased after injecting 0.5 ml of 25% SRBC intraperitoneally. The value of Total Serum Protein was recorded to decrease in the fish sacrificed on day 1 (2.59±0.34) as compared to the fish of the control group (2.61±0.36), the value was recorded to increase gradually in the fish sacrificed on day 5 (3.14±0.23) and day 10 (maximum 3.63±0.30) then the value was recorded to decrease gradually in the fish sacrificed on day 15 (3.27±0.34), day 20 (2.78±0.22),day 25 (2.72±0.19) and day 30 (2.64±0.25) reaching back approximately to the value in the fish of control group. The amount of globulin protein was recorded to decrease in the fish sacrificed on day 1 (1.07±0.18), as compared to the fish of control group (1.08±0.24) the value was recorded to increase gradually in the fish sacrificed on day 5 (1.56±0.12) and day 10 (maximum 1.94±0.06), then the value was recorded to decrease gradually in the fish sacrificed on day 15 (1.59±0.16), day 20 (1.29±0.10), day 25 (1.19±0.18) and day 30 (1.08±0.14) reaching back approximately to the value in the fish of control

The value of the Total Serum Proteins and the value of globulin proteins was shown to increase in the fish after the immunization. The value of the Total Serum Proteins was at maximum in the fish sacrificed on day 10 and the values of the globulin proteins was also maximum in the fishes sacrificed on the day 10. The value of albumin and globulin shows a definite trend in response to the immunization in the experimental fishes, it decreased as result of immunization, reached a minimum value in the fish sacrificed on day 10 and then again increased coming back near to the value in the fish of control group.

Fig. 1 Serum proteins of Clarias batrachus on different days after injecting 0.5 ml of 25% SRBC intra- peritoneally. 1.a- control, 1.b- day 1, 1.c- day 5, 1.d- day 10, 1.e- day 15, 1.f- day 20, 1.g- day 25 and 1.h- day 30.

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