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RESEARCH ARTICLE

EFFECTS OF STRESSORS ON HEMATOLOGICAL PARAMETERS IN THE FRESH WATER FISH, *Cyprinus carpio*

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ABSTRACT

Fish cultures are especially at risk to the adverse effects of stress. Blood chemistry and hematological measurements can provide valuable physiological indices that may offer critical feedback on different stressors. Blood samples were collected from the finger lings of *Cyprinus carpio* after subjected to stressors and parameters such as Hemoglobin, RBC, and WBC and activated neutrophils were estimated for 0,6,24 and 48 hours. Fishes were treated with Stresroak immersion in four concentration ie ,10, 20, 30 and 40mg/l. In all the concentrations, Stressroak can effectively suppresses the stress in relation to hematological parameters. Among the dose tested 20mg/l of stressroak performed better results compared with other and is recommended as an effective dose in fish culture.

INTRODUCTION

Stress is an unavoidable component in intensive aquaculture .Fish culture in intensive system and artificial farms cause the fish exposed to different stressful factors that there are not similar of the situation in natural position. Fish are exposed to stressors in nature as well as in artificial conditions such as in aquaculture or in the laboratory. The response of the fish to such stressors involves all levels of organization from the cell to the individual organism (Barton and Iwama, 1991) to the structure of the population. In response to stressors a fish will undergo a serious of biochemical and physiological changes in an attempt to compensate for the challenges imposed upon it and thereby cope up with the stress. The initial response represents the perception of an altered state and initiates a neuroendocrine response that forms a part of the generalized stress response in fish (Gamperl *et al*., 1994). The secondary stress response comprises the various biochemical and physiological effects associated with stress and mediated to a large extent by the above stress hormone. The stress hormone activates a number of metabolic pathways that result in alteration in blood chemistry (Oyagbemi *et al*., 2008). Hematology is often used to detect physiological changes following different stress conditions by measuring specific physiological and biochemical alterations in the blood of fish exposed to short periods of sublethal stressors may provide a sensitive method for predicting the effects of chronic exposure and survival reproduction. It has been illustrated that the use of hematological variable as indictor of stress.

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Stresroak is a scientifically proven botanical with adaptogenic, immmunomodulatory, free radical scavenging and antioxidant rejuvenating actions (Shukla and Srivastava, 1999).Its active ingredients are derived from extract of plants such as *Phyllatus emblica, Ocimum sanctum, Withania, somnifera, Mangefira indica and Shilajit* extract. It would be of interest to examine the changes related on stress related syndromes in common carp an economically important, edible, culturable and sensitive fish to slight environmental changes. Hence the aim of the present study was thus to elucidate how stress affects on hematological parameters and evaluate the effect of administration of stresrock in common carp, *Cyprinus carpio.*

MATERIALS AND METHODS

The fish common carp, *Cyprinus carpio*, were obtained from Aliyar fish farm at pollachi, Coimbatore, Tamil Nadu. Fish were acclimatized to the laboratory condition for 15days in fiber tanks of 150 ltr capacity. Then experimental fish weighting 5-8 gms were used in the study. Water was changed for everyday to avoid stress due to ammonia accumulation. The experimental animals were fed *ad libitum* with a balanced fish diet prepared in our laboratory. The water temperature was not controlled since it showed only a minor daily fluctuation ($28.5\pm1^{o+}C$). Three stresses for selected for the present study thermal stress (39ºC for 20 min) (Jasper, 1981), salinity stress (8ppt for 30 min) (Pillai *et al*., 2003) and crowding stress (10 fish were kept in 5 liter of water for 1hr) (Yin *et al* .,1995) were applied to the fishes. Unstressed fishes were taken as the control and care was taken and it is free from stress. Blood samples for collected immediately after

stress was given (0,6,24,and 48 hours) and the samples were tested for hematological parameters such as Haemoglobin, RBC and WBC count, Activated neutrophil number were estimated by classical methods. Five groups of fish were maintained for testing the efficacy of Stressroak as a stress reliever. Four groups are fishes such as T1, T2, T3, and T4 were exposed to the Stressroak immersion in different concentration 10, 20, 30 and 40 mg/l respectively for 24 hours. To taken as the control for the Stressroak was not applied after 24 hours of Stressroak exposure and the fishes were again subjected to stresses and the blood samples were tested for hematological stress response at 0,6,24 and 48 hours. The data were expressed as means (SD). The test of significance between groups was determined by one factor ANOVA and the groups were compared.

RESULTS AND DISCUSSION

Stress in fish can cause immunosuppression result in a an increased susceptibility to disease, therefore understanding the physiology of stress and its careful management of the environment in intensive aquaculture is needed .In the present study some haematological parameters were investigate in the fish, *Cyprinus carpio* exposed to different stressors and an attempt was made to relieve the stress using herbal stress reliever stresroak. Result shows that the RBC, WBC count, observed in the RBC and WBC count in the stressed fish. In 0 hours of exposure the WBC count was decreased with a minimum level of 2.15, 2.56 and 2.59 (10^4 cells / mm^3) for crowding, thermal and salinity stressors respectively. The decreasing number of erythrocytes has been investigated in the crowding and salinity stressed fishes. The value reaches up to 1.0 and 1.1 (10^6 cells / mm^3) in 24 hours of crowding and salinity stressed fishes respectively.

The decreased RBC results in hypochronic anemia which attributed to the deficiency of iron and its decreasing utilization for hemoglobin synthesis. In the present study, the thermal stress exposure RBC count was increasing up to 8.1(10^6 cells / mm^3) in 48 hours. Study reported that stress results in an increased number of erythrocytes (Jobling,1994) has been correlated with the effect of released catecholamine on the spleen (Val and Val, 1995) as a consequence of spleen concentration immature erythrocytes released in tom the circulation. Activated neutrophil number was also decreased in the fishes exposed salinity and crowding stress, a minimum value of 9.9 in 24 hours of crowding stress exposure and 8.9 in 0 hours of salinity stress exposure was observed. Stress hormones induce a various biochemical and physiological effects associated with stress activated a number of metabolic pathway that result in alteration in blood chemistry and haemotology.

Table.1.Changes in the level of haematological parameters in the blood of *Cyprinus carpio* exposed to different stresses at different durations

Parameters	Time after Exposure (Hours) (Mean±S.D)	Treatment groups														
		Crowding stress					Thermal stress					Salinity stress				
		Control	T_1	T_2	T_3	T_4	Control	T_1	T_2	T_3	T_4	Control	T_1	T_2	T_3	T_4
Hemoglobin	0	1.1 ±0.36	1.2 ±0.18	7.2 ±0.20	4.1 ±1.82	1.9 ±0.18	2.2 ±.0.18	5.2 ±.0.17	7.5 ±.0.29	1.9 ±.0.18	5.2 ±.0.18	7.58 ±0.04	11.0 ±0.82	9.38 ±0.36	8.27 ±0.23	8.69 ±0.19
	6	4.2 ±0.18	2.2 ±0.27	6.6 ±0.31	3.2 ±0.18	5.2 ±0.18	3.9 ±.0.12	17.4 ±.0.17	7.2 ±.0.18	13.2 ±.0.18	5.4 ±.0.18	6.92 ±0.03	10.1 ±0.76	8.49 ±0.18	8.5 ±0.15	8.2 ±0.55
	24	5.1 ±0.18	3.0 ±0.17	6.1 ±.17	3.1 ±0.18	12 ±0.18	6.2 ±.0.18	9.9 ±.0.18	8.3 ±.0.20	11.6 ±.0.18	4.2 ±.2.36	7.9 ±0.01	11.1 ±0.62	9.9 ±0.18	9.3 ±0.32	8.3 ±0.66
	48	3.7 ±0.18	5.1 ±0.17	8.2 ±0.18	3.3 ±0.18	10.6 ±0.18	4.0 ±.0.18	9.7 ±.0.17	12 ±.2.16	11 ±.0.18	5.9 ±.0.18	8.5 ±0.02	11.8 ±0.45	9.7 ±0.22	9.6 ±0.17	8.0 ±0.89
WBC	0	3.1 ±0.01	6.2 ±0.05	5.1 ±0.02	4.2 ±0.02	3.2 ±0.01	2.5 ±.0.18	3.7 ±.0.02	4.7 ±.0.18	4.8 ±.0.18	5.6 ±.0.18	2.5 ±0.01	6.6 ±0.22	5.0 ±0.00	4.6 ±0.23	3.8 ±0.19
	6	2.1 ±0.01	2.1 ±0.01	4.2 ±0.02	4.2 ±0.02	3.95 ±0.02	2.9 ±.0.18	2.7 ±.0.03	4.1 ±.0.18	5.0 ±.0.46	6.0 ±.0.18	2.6 ±0.02	7.3 ±0.77	5.0 ±0.18	4.9 ±0.09	3.8 ±0.18
	24	2.7 ±.0182	5.7 ±.0.02	4.7 ±.0.01	4.2 ±.0.01	4.1 ±.0.04	3.5 ±.0.18	4.3 ±.0.01	3.8 ±.0.18	4.9 ±.0.01	5.7 ±.0.14	3.5 ±.0.05	7.0 ±.0.76	5.3 ±.0.20	5.4 ±.0.09	4.2 ±.0.40
	48	4.4 ±.0.01	7.2 ±.0.02	4.3 ±.0.02	4.5 ±.0.02	4.2 ±.0.02	3.9 ±.0.18	4.1 ±.0.03	4.2 ±.0.51	4.5 ±.0.01	7.5 ±.0.18	4.0 ±.0.01	7.9 ±.2.9	5.8 ±.0.15	5.7 ±.0.32	4.2 ±.0.24
RBC	0	5.2 ±.0.02	4.2 ±.0.02	4.9 ±.0.08	5.4 ±.0.08	4.6 ±.0.18	5.9 ±0.18	2.1 ±0.20	3.7 ±0.18	5.9 ±0.29	3.4 ±0.18	1.11 ±.0.01	2.3 ±.0.10	2.0 ±.0.06	1.9 ±.0.01	1.6 ±.0.01
	6	1.6 ±.0.25	6.4 ±.0.20	6.6 ±.0.38	5.7 ±.0.18	5.8 ±.0.18	4.2 ±0.18	2.9 ±0.20	2.8 ±0.43	4.0 ±0.29	3.1 ±0.18	1.2 ±.0.02	2.3 ±.0.00	2.1 ±.0.04	1.8 ±.0.07	1.7 ±.0.10
	2	1.0 ±.0.81	3.6 ±.0.18	4.4 ±.0.46	4.8 ±.0.18	4.8 ±.0.18	4.9 ±0.18	3.9 ±0.38	3.8 ±0.28	4.1 ±0.18	3.2 ±0.18	1.1 ±.0.02	2.5 ±.0.02	2.0 ±.0.03	1.8 ±.0.11	1.9 ±.0.03
	48	1.9 ±.0.25	3.7 ±.0.18	4.2 ±.0.18	4.2 ±.0.18	4.1 ±.0.18	8.1 ±0.29	3.0 ±0.18	2.8 ±0.23	3.6 ±0.18	2.8 ±0.18	1.5 ±.0.02	2.6 ±0.03	2.1 ±.0.01	1.9 ±.0.03	1.8 ±.0.01
Activate neutrophils	0	3.17 ±.0.64	31.1 ±.0.47	17.2 ±.0.18	17.2 ±.0.18	15.1 ±.0.18	55.3 ±0.18	18 ±2.58	16 ±2.16	20 ±0.18	14 ±2.16	8.9 ±.0.01	28.8 ±.0.95	17.3 ±.17.3	15.3 ±.0.12	11.2 ±.0.16
	6	17.0 ±.0.21	36.2 ±.0.22	19.0 ±.0.91	19.0 ±.0.18	14.1 ±.0.18	26.7 ±0.18	24 ±0.81	18 ±0.18	13 ±0.18	15 ±2.44	9.1 ±.0.01	27.4 ±.0.77	18.3 ±.0.68	17.4 ±.1.05	11.2 ±.0.14
	24	9.9 ±.0.18	27.0 ±0..44	20.0 ±.0.12	22.1 ±.0.18	18.4 ±.0.18	28.8 ±0.12	16 ±0.18	21 ±2.16	19 ±2.38	16 ±2.16	11.2 ±.0.02	28.6 ±.0.76	19.6 ±.1.56	18.7 ±.1.24	15.2 ±.0.26
	48	19.1 ±.0.25	19.1 ±.0.75	16.2 ±.0.18	15.2 ±.0.18	18.1 ±.0.18	26.4 ±0.18	15 ±2.44	25 ±0.18	24 ±0.81	13 ±0.18	11.5 ±.0.02	30.5 ±.2.90	22.3 ±.1.31	19.3 ±.0.71	20.3 ±.0.26

Hemoglobin level and activated neutrophils number showed a decreased trend in the fish expose to different stress except in the case of RBC in thermal stress (Table 1). All the three stress can reduce the hemoglobin level with a minimum value of 1.1g/dl in 0 hours of exposure to crowding stress .The concentration of hemoglobin in fish blood vary greatly in the fish exposed to different stress may be due to the stressors caused the anemic condition in fish. A decreased trend is Haemotological parameters are related to response of the animal to changing environmental conditions. In the present study, the RBC count and Hemoglobin content and activated netrophils showed a decreased trend in the fish exposed to different stressors. Studies reported that stress result in an increased number of erythrocytes (Polycythemic) (Jobling, 1994). The increase in the number of circulating erythrocytes has been correlated with the effect of released catecholamine

on the spleen (Val and Val, 1995) as a consequence of spleen concentration, immature erythrocytes are also released into the circulation. Stressroak when given through immersion and is effective in all concentration in reducing stress in terms of RBC count.

The concentration of Hemoglobin in the blood of fish very greatly, the stressors caused an anemic condition. The decrease in number of RBC and other hematological parameters indicates acute haemolytic anemia and decreased physiological function of haemopolitic system which are considered to the most sensitive indicators of the stress conditions observed by Solati and Falahatkar (2007). In the present study similar result were obtained when the effect of different stresses were compared. Fish subjected to stress had the lowest RBC, WBC and neutrophil numbers but stressrock when given through immersions is effective in all concentrations. The concentrations of Hb in the blood of fish very greatly. Values ranging from 4-5 g/dl upto 20-21 g/dl are very common. In the present study it has been observed that the stressroak is efficient in relieving stress at a concentration of 20mg/l both through immersion and through feed. Blood Hb concentration decreased to about 2gm/d although not healthy can be tolerated if the fish are not forced to swim actively lower values become life threatening. From the present study it is concluded that the application of stresroak as a stress reliever, fish showed a positive effect in all the parameters tested and is illustrated in the Table 1.Among the four concentration of the stresroak 20mg/l can effectively to relieve the stress in relation to haematological parameters. The increase in hemoglobin, total RBC and WBC count and activated neutrophils concentration obtained especially for the therapeutic dose, though not statistically significant; show that Stresroak probably enhances erythropoietin. It was clearly established in this study that this effect of stresroak was dose-dependent within the range of concentration of stresroak used. Immuno-stimulation is one of the major reasons for which stresroak remains a valuable tool in the hands of its users. (Deka *et al*., 2004).The herbo-mineral constituents of stresroak include *Shilajit* species, *Phyllatus emblica*, *Ocimum sanctum*, *Mangifera indica* and *Withania somnifera*. Each of these plants has been reported singly potent enough to boost immunity.

These findings show that as much as the administration of stresroak can be of immense benefit in stress management. From the present study, it is concluded that the stresroak is a potent stress reliever in the field of stress management in the aquaculture and also we recommended for increasing efficiency in rearing should avoid of stressors possibly that it needs to right management on rearing practice and this action caused to increasing in production, fish welfare restoration and economic efficiency.

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