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Haematological changes in *Cyprinus carpio* subjected to transportation stress

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ABSTRACT

The stress response of *Cyprinus carpio* subjected to transportation with or without oxygen was evaluated by investigating the haematological parameters of the fish. A significant increase in Total Erythrocyte Count (TEC), Haemoglobin (Hb), Haematocrit (Hct) and Total Leucocyte Count (TLC) but a fall in the levels of Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) was observed immediately after transport of fish without oxygen, whereas Mean Corpuscular Haemoglobin Concentration (MCHC) appears not to be affected. On the other hand when fish were transported with oxygen, the only noticeable change was found in TLC. All elevated parameters returned back to their control values within 14 days. Results show that transport is a considerable stressor for fish and it takes some time to come out of this stress.

Key words: Haematological parameters, oxygen, transport, stress.

INTRODUCTION

In aquaculture, transporting fish from one location to another is a widespread practice, particularly in rural areas of a developing country like India, often representing the only means of supplying fry or juveniles to small-scale aquaculturists (Taylor & Ross, 1988). Transportation which usually involves moving large number of fish in a small amount of water is a traumatic procedure that exposes fish to several adverse stimuli evoking hormonal, metabolic as well as haematological dysfunction in fish (Robertson *et al.*, 1987).

Transport of fish though an inevitable procedure, elicits stress leading to decreased fish performance (Pickering, 1981), which may often be caused by low oxygen levels, temperature differences between storage pond and tank water, or poor transport water quality due to inadequate water exchange that cause accumulation of carbon dioxide and ammonia (Erikson *et al.*, 1997). These conditions produce unwanted variations in qualitative and quantitative blood parameters besides other changes.

Haematological measures happen to be useful indicators of sublethal environmental stress in fish (Bridges *et al.*, 1976; Warner & Williams, 1977 and Folmar, 1993) and can serve as valuable physiological indices that may offer critical feedback on transportation stress in aquaculture (Gbore *et al.*, 2006). Most often, fish after transport arrive at the site in poor conditions and if not properly managed can lead to poor performance afterwards (Akinrotimi, 2007).

Presently, therefore an endeavor has been made to assess the haematological responses of *Cyprinus carpio*, when transported with or without oxygen.

MATERIAL AND METHODS

Fish were obtained from National fish seed farm, Kathua and were transported in non-aerated ordinary containers as well as in oxygen packed bags to Jammu and the time taken for transport was 3 hours. In the laboratory, they were immediately distributed in plastic tubs of 50 liter capacity. 6 fish were sampled in the fish farm before transport and those samples were used as a control group for transport study. 6 fish were immediately sampled at arrival to the laboratory so as to measure

the immediate stress response to transport. 6 fish were used for each one of the sampling times i.e. 7, 14 & 21 days after transport. Total erythrocyte count (TEC) and Total leucocyte count (TLC) were calculated with the help of an Improved Neubauer Haemocytometer (Shaw, 1930). Haemoglobin (Hb) was calculated with the help of Sahli's Haemoglobinometer (Dethloff et al., 1999). Packed cell volume (PCV) was measured using a wintrobe tube by centrifuging the blood in it (Wintrobe, 1967). MCV, MCH &MCHC were calculated by their respective formulas. The results were statistically analysed with the help of Student's t-test.

RESULTS

Transporting fish from site of collection to laboratory, covering a distance of 80 km, does exert changes in the haematological parameters (Table 1). Immediately after transport without oxygen, as evident from table 2, an increase in TEC and its observed values (Hb & Hct) but a reduction in calculated values of MCV & MCH was observed. MCHC yet another calculated index of TEC was not found to be affected by transportation stress.

The data on haematological parameters of fish transported without oxygen when compared with that obtained after transport of fish in oxygen packed bags further reveals that much variation could not be recorded in the red blood cell count and its related parameters (i.e. Hb, Hct, MCH & MCHC) except for MCV wherein though a slight increase of 4.5% was recorded but that too was not statistically significant ($p>0.05$).

Changes in white blood cell system however were similar in both type of transportation conditions i.e. with and without oxygen and as clearly evident from table 2, the total leucocyte count was observed to increase immediately after transportation. Since the experiment was carried out for a period of 21 days post transportation, so the haematological parameters were monitored weekly i.e. on day 7, 14 & 21 also. Analysis of results (Tables, Figs) reveal that all haematological

Table 1: Alterations in haematological profile of *Cyprinus carpio* exposed to transport (without oxygen) strees

Haematological Indices	Pre transport	Post transport	7 days	14 days	21 days
TEC ($\times10^6$ cells/mm^3)	2.60±0.72	3.40±0.14	2.90±0.06	2.60±0.19	2.59±.03
Hb (g/l)	6.2±0.52	7.1±0.23	6.6±0.26	6.0±0.36	6.4±0.40
Hct/PCV (%)	6.2±0.52	7.1±0.23	6.6±0.26	6.0±0.36	6.4±0.40
MCV (fl)	119.2±2.90	102.9±6.53	113.7±9.93	115.3±18.03	123.5±9.13
MCH (pg)	23.8±2.60	20.8±0.15	22.7±1.38	23.0±2.89	24.7±1.80
MCHC (g/l	20.0±2.30	20.2±1.20	20.0±1.98	20.0±0.88	20.0±1.20
TLC ($\times10^3$ cells/mm^3)	18.6±0.100	19.5±0.20	18.2. ±0.26	18.7±0.26	18.7±0.96

Table 2: Alterations in haematological profile of *Cyprinus carpio* exposed to transport (with oxygen) stress

Haematological Indices	Pre transport	Post transport	7 days	14 days	21 days
TEC ($\times10^6$ cells/mm^3)	2.61±0.03	2.58±0.07	2.63±0.06	2.62±0.09	2.64±0.03
Hb (g/l)	6.2±0.20	6.3±0.17	6.3±0.30	6.2±0.26	6.1±0.43
Hct/PCV (%)	30±2.64	31±1.73	30±1.73	30±0.00	30±1.00
MCV (fl)	114.9±11.51	120.1±9.58	114.0±8.36	114.5±3.90	113.6±2.62
MCH (pg)	23.7±0.60	24.4±1.25	23.9±0.78	23.6±0.69	23.1±1.81
MCHC (g/l)	20.6±2.10	20.3±0.57	21.0±1.58	20.6±0.85	20.3±1.65
TLC ($\times10^3$ cells/mm^3)	18.5±0.52	19.8±0.17	18.3±0.45	18.6±0.15	18.4±0.20

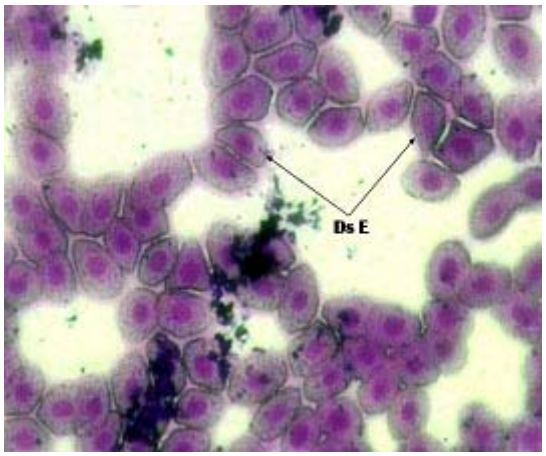

Fig. 1: Alterations in haematological profile of *Cyprinus carpio* exposed to transport (without oxygen) stress

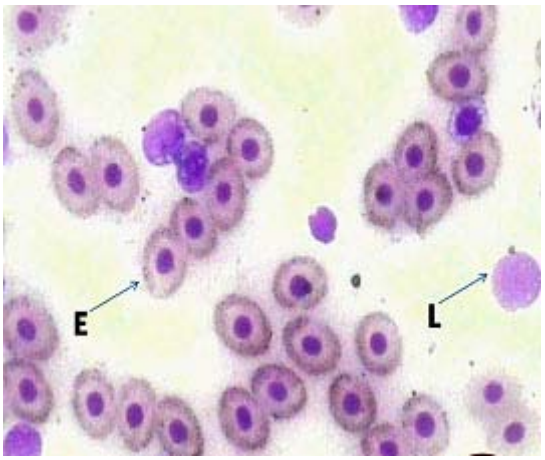

Fig. 2: Alterations in haematological profile of *Cyprinus carpio* exposed to transport (with oxygen) stress

parameters which had increased immediately after transport, declined on day 7th, to attain a level equivalent to control on day 14 which was subsequently maintained up to day 21.

DISCUSSION

The presently observed post transportational increase in the red blood cell numbers indicates stress related polycythemia (increase in the number of cells particularly RBCs) in *Cyprinus carpio*. That polycythemia has occurred is evident from blood smear (Fig 1-2) which very clearly illustrates increased number of erythrocytes compared to control

There was a decline in dissolved oxygen (DO) content of water from 5mg/l at the pond (from where fish were captured in Kathua) to 3.7mg/l when brought to laboratory. Thus this means that the gradually declining oxygen level by posing stressful condition might have resulted in an increase in RBC count as also of Hb and Hct simply to meet the high energy demands of the fish caused by rapid swimming during transportation procedure. Puczkow who as early as 1962 stated that oxygen deficiency triggers a contraction of spleen and results in release of erythrocyte reserves into blood stream, may plausibly be the reason of polycythemic condition witnessed in *C. carpio* after transportation.

Besides the quantitative changes, erythrocytes also witnessed changes in their quality because a large chunk of erythrocytes exhibit distorted shape (Fig. 1-2). Distorted shape of RBC, present authors feels, by (1) reducing the surface area of erythrocytes reduces their oxygen carrying capacity which in turn may ultimately result in an imbalance in the respiratory physiology of fish, (2) disfiguring RBCs affects their Hb content. While due to reduced volume of cells, Hb content is also reduced and hence results in low oxygen capacities.

The only observed effect of transportation on TEC in fish transported in oxygen packed bags was slight increase in MCV which present author feels may seemingly be because of endosmosis exhibited by red blood cells under the conditions of stress of transportation. Stress related swelling of erythrocytes due to osmotic imbalance is already on record (Nikinmaa & Huestis, 1984).

Other important component of blood which reacts to stressful condition is TLC which has been observed to exhibit an appreciable increase immediately after transportation. Increase in TLC it may be categorically mentioned appears to be a protective response of the fish under the conditions of stress caused by transportation. This stress seemingly appears to cause an alarming reaction in *C. carpio* resulting thereby in stimulation of leucopoetic organs which increase lymphocyte

count. Gbore *et al.* (2006) in fingerlings of *Tilapia zilli*, Akinrotimi *et al*.(2007) in *S. melanotheron* too held release of lymphocytes from lymphoid tissue to be responsible for increase in TLC.

To conclude, it can be stated that the return of elevated haematological parameters to control levels after 14 days and subsequent stabilization is suggestive of the fact that the fish comes out of the stress of transportation in fourteen days. This should be taken into consideration by aquaculturists/fish farmers and any further activity on fish should be performed after taking cognigence of such reference period which in case of *C. carpio* of present studies has been found to be 14 days. This time period it may be added may tend to differ for different fishes from different ecosystems.

On the basis of results obtained in *C. carpio* following transportational stress and review of literature (Svobodova *et al*., 1999, Dobsikova *et al*., 2006, Gbore *et al*., 2006, Akinrotimi *et al*., 2007), present author is of viewpoint that in order to limit losses and damage to fish, following points may be taken care of:

- Fish should be sensitized/acclimatized to handling prior to transportation.
- As far as possible, identical water temperature and DO content should be maintained both in transporting vessels and those where they are to be kept after transportation and that should in turn be in the range of temperature and DO content of pond from where fish are to be caught.

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