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Effect of Starvation on Haematological and Serum Biochemical Changes in the Fresh Water Fish, *Notopterus Notopterus* (Pallas)

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Abstract: *The present study was carried out to find out the changes in the haematological and serum biochemical parameters induced by starvation in the fresh water fish, Notopterus notopterus. In the starved fish for fourteen days a significant reduction in the hemoglobin and hematocrite content has been noticed. Starvation is known to induce different responses on blood biochemical level, depending on how long the starvation lasts, as well as on the species-specific differences in the metabolism and its regulation. A significant decrease in protein, creatinine, triglycerides, enzymes like SGOT, SGPT and electrolyte sodium whereas glucose and BUN increased after starvation exposure to the fish, N. notopterus. The changes in the environmental factors such as deprivation of food cause stress to the fish which may bring disturbance in the blood parameters effecting the survival of fish.*

Keywords: *Haematology, Notopterus notopterus*

1. INTRODUCTION

Starvation is a condition in which many fish species may experience in the natural environments, as a part of their life cycle, both as a consequence of seasonal changes in water temperature or migration that may cause a lack of food or, to a greater extent food depletion. Several studies have previously investigated starvation in its multi-faced effects on morphological, biochemical (Hung *et al*., 1997; Vosyliene and Kazlauskiene, 1999) and haematological parameters (Mahajan and Dheer, 1983; Stepanowska *et al*., 2006). Most research focuses on metabolic changes in response to food deprivation (Guderley *et al*., 2003). However, effects of starvation on haematological and blood biochemical changes in Indian fishes are very less. Hence, in the present investigation effects of starvation on haematological and blood biochemical changes was observed in the locally available fresh water fish, *Notopterus notopterus.*

2. MATERIALS AND METHODS

Fresh water fish *Notopterus notopterus* (70-80g body weight) were brought from Bheema River around 40 km away from Gulbarga. Fish were acclimatized for laboratory conditions for 7 days before the beginning of the experiment as where fish were fed during this adaptation period. This supplementary aeration provided optimum dissolved oxygen. Stable temperature of 27±3 is an optimum temperature during the period of study. Fish were fed with earth worm and boiled egg pieces once daily from the day of arrival except experimental group which are kept in fasting aquaria. Daily change of water was around 10% that in the aquaria. The light provided for all aquaria period was the natural photoperiod, which was about 12 hours of light: 12 hours of dark/day during the days of study.

The fish; *N. notopterus* were exposed to starvation for a short period of 14 days. The control group was maintained simultaneously keeping under optimal environmental conditions and proper feeding. The response of hematological and blood biochemical parameters were studied after termination of exposure period.

The experimental data was analyzed statistically by adopting varied statistical methods. The student's t- test was carried out to know the levels of significance using the standard formula. The experimental data was analyzed statistically by adopting varied statistical methods using statistical software S.P.S.S 7.5

Blood Parameters : Blood samples collected from caudal blood vessels, blood was separated in two portions, one portion was mixed with anticoagulant another portion of sample was centrifuged without anticoagulant for serum separation.

Hemoglobin Concentration (Hb): Hemoglobin was measured using the standard cyanmethemoglobin method described by Baker and Silverton (1982). Hematocrite value was determined by standard Wintrobe method, and expressed in percentage. Blood sample were loaded in Wintrobe tubes and spun in a centrifuge at 3000 rpm for 5 min and measured.

Blood Biochemical Parameters:

Total Serum Proteins (TP) was measured by using the modified Biuret method, end point assay as described by Lawrence, (1986), **serum glucose** determined by (GOD-POD) Glucose oxidase – peroxidase, end point and assay method, **Blood urea nitrogen (BUN)was** determined by modified Berthelot method, **cholesterol was** determined by (CHOD-PAP) cholesterol oxidase - phenol aminophenazone method**, HDL** was determined by (CHOD-PAP) cholesterol oxidase - phenol aminophenazone method**,** LDL was determined by Friedewald's equation

$$\text{LDL} = \text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL cholesterol}$$

Triglycerides (TG) was determined by (GPO-PAP) glycerol-3-phosphate oxidase - phenol aminophenazone end point assay method. **Creatinine was** determined by modified Jaffe's method Kinetic test without deproteinisation according to the Jaffe's method. **Serum glutamate oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT)** activity was assayed following modified International Federation for Clinical Chemistry (IFCC) method using commercial kit. Serum Alkaline phosphatase activity was determined by kinetic assay (IFCC) method using commercial kit. **Sodium and Potassium: are** determined by colorimetric method. **Calcium** is determined by Modified Arsenazo method.

3. OBSERVATION

The exposure to starvation was for 14 days. No mortality was observed throughout the experiment in spite of food deprivation The hematological and serum biochemical parameters of control group and experimental (starvation) group is presented in the table-1. The hemoglobin and hematocrite were less in starved fish compared to feed ones. The serum glucose values were significantly higher ($t = 7.08$, $p < 0.001$) in the starved fish whereas the serum total protein values were significantly lower ($t = 3.43$, $p < 0.01$) in the starved fish than those measured in fed ones. The BUN values were significantly higher ($t = 14.14$, $p < 0.001$) in the starvated fish. The serum creatinine values were significantly lower ($t = 11.26$, $p < 0.001$) in the starved fish. The total cholesterol values were found to be increased ($t = 2.04$, $p < 0.05$) in the starved fish. The triglyceride values were found to be reduced. The high density lipoprotein were lower in the starved fish whereas the low density lipoprotein were found higher ($t = 0.15$, $p < 0.001$). The values of blood enzymes obtained in starved fish and control fish *N. notopterus* shows that the enzymes, SGOT and SGPT values were found lower in the starved fish than those measured in fed ones. The values of blood electrolyte, sodium was less in starved fish, *N. notopterus* whereas the potassium and calcium values were higher in starved fishes.

4. DISCUSSION

This study was undertaken to evaluate the response of fish to short term exposure to starvation causing stress which being assessed by determining different hematological and blood biochemical parameters. The knowledge of the hematological and blood biochemical strategies adopted by the fish *N. notopterus* to face different type of stress conditions may have important practical implications in farming this species, as it may help to prevent the probable damage to fish health resulting from these conditions. It is also well known that the homeostatic system enables fish to face the effect of environmental changes (temperature, salinity, oxygen, food availability), but the responses to environmental stressors can vary greatly according to the fish species, the developmental stage and the individual characteristics in addition to the type of stress and its duration (Pottinger *et al*., 1992).

Starvation affects the normal body metabolism and prolonged starvation may even cause death of animal (Joshi, 1973). A decline in various body constituents of fish, following experimental starvation have been reported by various authors considering starvation as a chronic stress condition, the response of fish to stress involves activation of the neuro – endocrine system, by releasing the stress

related hormones in the blood as a primary response followed by hematological and biochemical changes as a secondary response which includes growth inhibition, impaired reproduction and immune response in many fish species (1992; Reddy *et al*., 1995; Pascual *et al*., 2003).

In the starved fish, *N. notopterus* a significant reduction in the hemoglobin and hematocrite has been observed. However, conflicting results exists in the scientific literature concerning the effect of starvation on blood hemoglobin content and hematocrite value. Although the increase or decrease in hematocrite on exposure to starvation has been reported in earlier studies, recently Caruso *et al*., (2010) reported for the European eel (*Anguilla anguilla*) and found the reduction in the hemoglobin and hematocrite, hemoglobin found as 9.10 g/dl for fed once and 7.92g/dl for starved, and hematocrite was found 34.50 % for fed once and 28.17% for starved. Similar results of reduction were noticed for the fish, *N. notopterus* in the present investigation.

Starvation is known to induce different responses on blood glucose level, depending on how long the starvation lasts, as well as on the species-specific differences in the metabolism and its regulation. A significant decrease in glucose levels was observed in the sturgeon after fasting for a period of 10 weeks (Hung *et al*. 1997), and in carp after fasting for 6 weeks (Friedrich and Stepanowska, 2001). The present study shows that the concentration of serum glucose increased significantly during starvation, suggesting that fasted fish were able to maintain their value of glycaemia by enhancing gluconeogenesis. The good gluconeogenic capability in the starved fish, *N. notopterus* suggested their adaptive response to food shortage by means of the mobilization of non carbohydrate sources in order to preserve their glucose homeostasis. During the starvation period, enhanced gluconeogenesis rates have been observed in many species of teleosts (Foster and Moon, 1991). During fasting, glycogen breakdown in the liver (and Kidney) releases the glucose in to the blood. It is characterized by hyperglycemia. The result obtained in this study agrees with the observations of above authors.

The amount of protein, glucose and glycogen also decreased as the period of starvation increased (Letcher *et al*., 1996). Tripati and Verma, (2003) found decrease in rate of protein in the fresh water cat fish *Clarias batrachus* and suggested that the rate of protein synthesis were also could result from reduced protein synthesis capacity brought about by reduction in the concentration of ribosome's. In the present study a significant reduction in the serum total protein observed in the fish *N. notopterus*. Love (1980) demonstrated and suggested that, during prolonged starvation, the fish use protein as an energy source via, gluconeogenesis.

Liver function would likely result in a decrease of urea production as these pathways are energetically expensive. However the increasing urea content in plasma likely as an indicator of failing gill osmoregulatory capability (Wood *et al*., 2003). In the present study the elevated BUN indicate that failure gill osmoregulatory function due to starvation and given the link to osmoregulatory stress. Differences are difficult to estimate because of the limited studies on fishes. However, the levels of blood urea nitrogen (BUN) are related to the protein content (Asper *et al*., 1990). Plasma chemistry can demonstrate excessive protein metabolism caused by starvation (increases in blood urea nitrogen [BUN]), breakdowns in lipid metabolic regulation (free fatty adds and ketone bodies) and carbohydrate regulation (glucose), all of which are altered in pinnipeds that are fasting for extended periods or have begun to starve (Castelini and Rea, 1992). Elevated BUN levels in teleosts may serve as a clinical indication of respiratory and excretory compromise due to respiratory epithelial cell hypertrophy and hyperplasia as reported by Keith Nelson, (1999) in the goldfish, *Carassius auratus*. In the present study blood urea nitrogen found to be elevated and creatinine levels were not changed significantly after starvation in the fish *N. notopterus.*

The cholesterol is an important component of cell membranes and functions as a precursor of the synthesis of sexual hormones. Black and Skinner, (1986) did not found significant change in the cholesterol level during starvation in the serum of fish, rain bow trout. In the present study the fish *N. notopterus* was found to be same in the serum cholesterol levels in the starved fish as compared to control may be due to shorter period of exposure of starvation.

Triglycerides (TG) are the fat reserves stored in the body; triglycerides are normally transported in to the blood to provide energy and nutrients to cells. The decrease in TG ratio was observed in the fish, *N. notopterus* in the present study after starvation for two weeks. In the fish *Plecoglossus altivelis* decreased TG was seen and suggested that preferential decrease in phospholipids in the serum of

starved fish, as the serum is an important lipid carrier for fish. The decrease in the blood triglycerides level observed allows to presume that lipolysis during starvation and probably it was major source of energy (Hung *et al*., 1997) particularly during first two weeks of starvation. Friedrich and Stepanwska, (2001) reported the triglycerides levels 374 ± 95.7 mg/dl found for control and after two week 111.4 ± 25.7 found in the carp *cyprinus carpio*. In the present study the fish *N. notopterus* subjected to starved for two weeks and similar results were found in the fish under starvation. The HDL levels were decreased and LDL levels were increased in the starved fish as compared to control fish. A significant difference found in the HDL and LDL for the carp *cyprinus carpio* exposed to starvation was reported (Stepanwska *et al*., 2006).

The decrease in the blood serum protein level was accompanied by a significant ($p < 0.001$) reduction in SGOT and SGPT activity, which pointed out to a slowed-down rate of amino acid transformations via transamination indicates that the starvation causes damage either in the hepatopancreas or in the muscles, as their activity is evidenced in the carp, *cyprinus carpio*. (Friedrich and Stepanowska, 2000). Similarly a significant reduction in the enzymes SGOT and SGPT was found in the fish *N. notopterus*. Alkaline phosphatase activity was found to decline markedly during starvation in the cat fish, *H. fossilis reported* by Shaffi, (1979) and suggested that the decline in the activity of enzyme is attributed to some factors like a fall in rate of synthesis caused by lowered metabolic demand and electrolyte imbalance caused by tissue over hydration. In the present study the alkaline phosphatase activity was reduced in the fish *N. notopterus* during starvation. Blood electrolyte research has been focused mainly on physiology (osmoregulation), biochemistry (acid base regulation), metabolic diseases (renal and hepatic diseases) and toxicity in fishes, such as tilapia, carp, salmon, trout, (Harikishnan *et al*., 2003; Petri *et al*., 2006). The sodium levels were decreased during starvation and potassium and calcium level have remained unchanged during starvation in the fish *N. notopterus.*

5. CONCLUSIONS

Starvation induces different responses on blood biochemical level, depending on how long the starvation lasts, as well as on the species-specific differences in the metabolism and its regulation .The present study indicated that short term starvation affects hematology and blood biochemistry in the fresh water fish, *Notoptrus notopterus.*

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Table1. *Showing haematological, serum biochemical, enzymes and electrolyte concentration of the fish N. notopterus exposed to starvation.*

Sl. No.	Ex- group/ parameters	Control	Starvation
1	Haemoglobin (Hb) g/dl	8.70 ± 0.74	6.77 ± 1.35**
2	Haematocrite (Hct) %	21.5 ± 2.21	18.33 ±3.88**
3	Glucose	48.23 ±8.87	75.51 ± 3.20***
4	Protein	5.82 ± 0.79	3.99 ± 1.47 **
5	BUN	6.97 ± 0.59	39.16 ± 10.38***
6	Creatinine	2.44 ± 0.37	0.56 ± 0.26***
7	Cholesterol	253.5 ± 29.21	253.76 ± 74.67NS
8	Triglycerides	302.96 ± 65.09	275.65±149.41**
9	HDL	67.32 ± 18.45	20.35 ± 4.54***
10	LDL	41.54 ± 12.38	178.13 ± 48.28***
11	SGOT	15.65 ± 0.69	1.87 ± 1.21***
12	SGPT	16.94 ± 0.26	6.64 ± 3.48***
13	ALP	59.55 ± 6.64	58.89 ± 7.50NS
14	Sodium (Na)	74.13 ± 13.07	17.14 ± 3.26***
15	Potassium (K)	14.96 ± 1.59	16.61 ± 1.35NS
16	Calcium (Ca)	9.01 ± 0.68	9.32 ± 1.36NS

Each value is expressed as mean ± SD, N = 6.

*NS = Not significant, * = significant P = < 0.05, ** = significant P = < 0.01, *** = significant = P < 0.001*

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