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ALTERATIONS IN THE BIOCHEMICAL PROFILES AND METABOLIC ENZYMES IN TISSUES OF *LABEO ROHITA*

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ABSTRACT
The present study is aimed on the estimation of the biochemical composition and assay of metabolic enzymes in liver, kidney and brain tissues of *L. rohita* reared in the freshwater lakes of Bangalore. The study was conducted for a period of 15 months in three sites- Hebbal fish farm (control site), Vengaiah lake (Lake A) which is being used for recreation purpose and Yellamallappa chetty lake (Lake B) which receives sewage and industrial pollutants and variations in these organic constituents was recorded. The study revealed a considerable reduction in the levels of proteins and glycogen whereas the levels of cholesterol showed a significant increase in the tissues of test fish sampled from lake (B) whereas fish tissues obtained from the lake (A) did not show significant variation when compared to those of control. Metabolic enzymes such as succinic-, malic- and lactic dehydrogenases also showed a considerable variation in the tissues of test fish sampled from lake (B) when compared to that of control to meet the energy demands in hypoxic condition in polluted lake water.

INTRODUCTION

Fish is an indirect target to pollutants, pathogens and xenobiotic substances present in these water bodies. These substances cause stress which in turn affects the metabolism and biochemical profiles of fish.

Fish are excellent subjects for the study of various effects of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants. They are sensitive to contamination and the pollutants may damage some physiological and biochemical processes when they enter the organs of fishes (Tulasi *et al*., 1992). Changes in quality of water, interactions between individuals, high fish stocking density (Flos *et al*., 1990, Mazur and Iwamma, 1993) may bring about a wide range of physiological changes in fish. The altered physiological adaptations are however variable and flexible in fish in response to water quality of a large variety of aquatic habitats. Being at a higher level of food chain, they accumulate a significant amount of pollutants and this accumulation depends on the intake and elimination from the body (Karadede *et al*., 2004).

Cell inclusions such as glycogen, proteins and cholesterol are the energy reserves available for a cell for its activities. Adequate energy reserves are required by organisms to mediate the effects of stress (Lee *et al*., 1983). Any disturbance in the external environment is first reflected in the biochemical composition of cells. Changes in these components indicate a change in the metabolic activities due to an external stress. Dehydrogenases play an important role in dehydrogenation to release energy for the normal function of biological systems under various situations (Giesy and Weiner, 1977). They are associated with cellular metabolic activity and changes in their activity may be due to the imbalance or due to the intracellular action of the pollutants. Succinic and malic dehydrogenases are important enzymes involved in Krebs cycle whereas lactic dehydrogenase plays an important role in carbohydrate metabolism and converts lactate to pyruvate. Changes in the activities of these metabolic enzymes indicate changes in the oxidative metabolism of the animal.

In the present investigation, profiles of biochemical constituents and metabolic enzymes of liver, kidney and brain tissues from *L.rohita* reared from unmanaged (lakes) and managed (fish farm) water bodies have been studied.

MATERIALS AND METHODS

Water samples were collected from the three sites - Hebbal fish farm (control site), Vengaiah lake (A- receiving domestic sewage) and Yellamallappa chetty lake (B - receiving industrial effluents from an adjacent pharmaceutical factory). The samples were collected during the morning hours at about 09.00 to 10.00 AM once every month for about 15 months. The Physico-chemical parameters such as temperature, pH, BOD, COD, DO, TDS, phosphates, sulphates, nitrates, nitrites and alkalinity were estimated by standard methods (APHA *et al.,* 2005). Test fish was sampled with the help of dragnet and brought to the lake bank alive from the three water bodies simultaneously along with water samples. They were anaesthetized using MS222 and dissected at the site itself. After dissecting, the fish tissues such as liver, kidney and brain

were carefully removed and transferred to a suitable media for estimation of biochemical constituents and assay of dehydrogenases.

Proteins were estimated by Lowry's (1951) method using bovine serum albumen (BSA) as a standard. Glycogen content was estimated by Anthrone reagent (Seifer *et al.,* 1950) using standard glucose solutions. Cholesterol was estimated by Zlatki's (1953) method using standard cholesterol solution in $FeCl_3$- acetic acid reagent. Succinic-, mailc- and lactic dehydrogenases were estimated by continuous spectrophotometric rate determination method (Bergmayer, 1974).

Each assay was replicated six times and the values are expressed as mean ±SE. The results were then statistically analyzed with the help of ANOVA and students "t" test was applied to find out significance at 5%, 1%.and 0.1% level.

RESULTS

The physico-chemical characteristics of control Hebbal fish farm, lake A and lake B were analyzed and the data is represented in Table 1.

In the present investigation there was a marked decrease in the protein and glycogen content and an increase in the cholesterol content in all the three tissues of the fish collected from lake B. The variations in the level of protein, glycogen and cholesterol in liver, kidney and brain of freshwater fish *L. rohita* sampled from the fish farm (control), lake (A) and lake (B) is presented in Table 2.

In the present study the protein content showed high level in liver (37.8 ± 3.31) when compared to those of kidney (23.67 ± 1.63) and brain (12.5 ± 0.49) in the control fish. A significant reduction of 38.57%, 46.47% and 36.8% respectively of protein content was recorded in the tissues of fish collected from lake (B) when compared to those of control. Maximum reduction in percentage of protein content was recorded in kidney (46.47 %) which was followed by liver (38.57%) and brain (36.8%). But protein content in fish sampled from lake A (33.67 ± 2.07 to 10.66 ± 0.56) did not show much variation when compared to those of control.

Glycogen content in the control fish was recorded highest in liver (5.62 ± 0.22), followed by kidney (3.28 ± 0.39) and brain (1.85 ± 0.05). Fish sampled from lake B showed significantly less glycogen content when compared to those

Table 1: Physico-chemical parameters of water of Fish farm (Control), Vengaiah lake (A) and Yellamallappa chetty lake (B)

S. No	Parameters	Control (farm)	Lake A	Lake B
1	Temperature (C°)	21	21	22
2	pH Value	7.07	7.09	7.22
3	Sulphates as SO_4, mg/L	72	128	253
4	Total Dissolved solids (TDS), mg/L	380	523	793
5	Total Suspended solids(TSS), mg/L	90	162	287
6	Nitrates as NO_3, (mg/L)	2.88	3.17	5.2
7	Chemical oxygen demand (C.O.D)	80	153	377
8	Biological oxygen demand (B.O.D)	14	21	97
9	Dissolved oxygen(D.O)	3.9	3.8	1.7
10	Nitrites as NO_2 (mg/L)	3.6	4.2	3.2
11	Total Phosphorus	1.30	2.08	3.98
12	Color	4.2	4.8	7.3
13	Odor	UOB	UOB	Fishy
14	Conductivity mmho /cm	483	654	988
15	Acidity as CaCO3	27	31	76
16	Turbidity, NTU	7.2	24	33
17	Total Alkalinity as $CaCO_3$, mg/L	180	221	470
18	Cadmium as mg/L	0.001	0.04	0.184
19	Copper as mg/L	0.013	0.03	0.39
20	Iron as mg/L	0.04	0.14	3.92

UOB - Unobjectionable

Table 2: Levels of protein, glycogen and cholesterol in the different tissues *viz.,* liver, kidney and brain of *Labeo rohita* from Fish farm (control), Vengaiah lake (A) and Yellamallappa chetty lake (B)

Tissue	Biochemical constituent	Control	Lake A	Lake B
Liver	Protein	37.8 ± 3.31	33.67 ± 2.07 (-10.92%)	23.22 ± 1.28^c (-38.57%)
	Glycogen	5.62 ± 0.22	5.08 ± 0.12 (-9.6%)	2.38 ± 0.33 ^{c} (-57.65%)
	Cholesterol	2.4 ± 0.31	2.6 ± 0.21 (+8.3%)	4.2± 0.13 (+75.00%)
Kidney	Protein	23.67 ± 1.63	20.25 ± 0.44 (-14.44%)	12.67 ± 1.03^b (-46.47%)
	Glycogen	3.28 ± 0.39	2.88 ± 0.08 (-12.19%)	1.35 ± 0.14^c (-58.84%)
	Cholesterol	1.8 ± 0.09	1.9 ± 0.09 (+5.55%)	3.4 ± 0.14 (+88.00%)
Brain	Protein	12.5 ± 0.49	10.66 ± 0.56 (-14.72%)	7.9 ± 0.52(-36.8%)
	Glycogen	1.85 ± 0.05	1.65 ± 0.12 (-10.81%)	1.13 ± 0.14^c (-38.91%)
	Cholesterol	1.7 ± 0.06	1.9 ± 0.09 (+11.76%)	2.4 ± 0.22(+41.17%)

Values expressed mg/g wet weight of tissues,Values are expressed as mean ± S.E; sample size (n) = 6,Values given in parenthesis are % change over control,(-) indicates % decrease over control and (+) indicates % increase over control,Super scripts **a, b** and **c** indicate significance at 5%, 1% and 0.1% levels respectively.No super scripts – indicating no significance

of control ones in the order as liver > kidney > brain(2.38 ± 0.33, 1.35 ± 0.14 and 1.13 ± 0.14, respectively). An insignificant reduction of glycogen was recorded in liver (9.6%) followed by brain (10.81%) and then kidney (12.19%) from the fish of lake A.

Cholesterol content showed a marked increase in all the tissues in the fish from lake B. The control fish collected from the farm showed highest cholesterol content in liver (2.4 ± 0.31) when compared to those of kidney (1.8 ± 0.09) and brain (1.7 ± 0.06). The fish sampled from lake B showed a remarkable increase of percentage in kidney cholesterol content (88.00%), followed by liver (75.00%) and brain (41.17%), whereas fishes from lake A showed a mild increase in kidney cholesterol content (5.55%,), in liver (8.3%) and brain (11.76%) when compared to those of control ones.

Changes in the activity levels of metabolic enzymes such as Succinic, malic and lactic dehydrogenase indicate a change in the redox metabolism of the fish. The variations in the activity level of these enzymes in liver, kidney and brain of freshwater fish *L. rohita* sampled from the fish farm (control), lake (A) and lake (B) is presented in Table 2.

Succinic dehydrogenase activity in the control fish was recorded highest in liver (48.07 ± 0.54), followed by brain (42.63 ± 0.51) and kidney (27.12 ± 0.50). Fish sampled from lake B showed significantly less activity of succinic dehydrogenase when compared to those of control ones in the order as brain > liver > kidney (34.33 ± 0.96 , 29.48 ± 1.24 and 16.48 ± 0.5 respectively). An insignificant reduction of the enzyme activity was recorded in kidney (12.42%) followed by liver (8.40%) and then brain (7.17%) from the fish of lake A.

The activity of malic dehydrogenase showed high level in liver (52.0 ± 1.41) when compared to those of brain (43.58 ± 1.11) and kidney (30.17 ± 0.98) in the control fish. A significant reduction of 30.44%, 16.02% and 36.65% respectively of the activity level of this enzyme was recorded in the tissues of fish collected from lake (B) when compared to those of control. Maximum reduction in percentage of malic dehydrogenase activity was recorded in kidney (36.65%) which was followed by liver (30.44%) and brain (16.02%). But the enzyme activity in fish sampled from lake A (46.5 ± 1.22 to 27.5 ± 1.05) did not show much variation when compared to those of control.

Lactic dehydrogenase activity showed a significant increase in all the tissues in the fish from lake B. The control fish collected from the farm showed highest lactic dehydrogenase activity in liver (323.5 ± 3.94) when compared to those of brain (301.5 ± 4.59) and kidney (251.33 ± 3.56). The fish sampled from lake B showed a remarkable increase of percentage in the enzyme activity in liver (42.40%), followed by brain (39.19%) and kidney (33.62%) whereas fishes from lake A showed a mild increase in Lactic dehydrogenase activity in liver (8.08%), in kidney (10.28%) and brain (14.2%) when compared to those of control ones.

DISCUSSION

The protein, cholesterol and glycogen levels and its correlation with dehydrogenases were studied in the tissues (liver, kidney and brain) of *L.rohita* sampled from fish-farm (Control) and sewage and industrially polluted lakes (A and B) of Bangalore (Table 2 and 3).

Hymavathi (2001) reported a decrease in the biochemical profile (total proteins, carbohydrates and lipids) of *Channa orientalis* from a habitat polluted by slaughterhouse wastes when compared to an unpolluted habitat of Mudasarlova stream of Visakhapatnam. This is in agreement with the present investigations on *L.rohita*. In the present study depletion in the total proteins was observed in all the tissues of *L.rohita* sampled from the two lakes A and B (sewage and industrially polluted respectively) when compared to control fish sampled from fish farm (Table 2). The variation in protein distribution suggests a gradual difference in metabolic calibers of various tissues and it is a physiological strategy adopted by the animal to adjust itself to the changing metabolic systems. Liver is the seat for the synthesis of various proteins and is the regulating centre of protein metabolism. The decreased trend of the protein content as observed in the present study in the fish tissues from lake B (brain > liver > kidney) over the control could be due to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, as also reported by Somnath, (1991) in tissues of fish *L. rohita* exposed to acute and sublethal concentration of tannic acid and by Anita *et al*. (2010), in liver tissue *L. rohita* exposed to sublethal and lethal concentrations of fenvalerate. Reduction in the protein content in kidney and brain also suggests disturbances in physiological activity as protein undergoes hydrolysis and oxidation through TCA cycle to meet the increased demand for energy. Glycogen is the primary and immediate source of

Table 3: Activity levels of Succinic- (SDH), Malic – (MDH) and Lactic dehydrogenases (LDH) in the different tissues *viz.,* liver, kidney and brain of *Labeo rohita* from Fish farm (control), Vengaiah lake (A) and Yellamallappa chetty lake (B)

Tissue	Enzyme activity	Control	Lake A	Lake B
Liver	SDH	48.07 ± 0.54	44.03 ± 0.65(-8.40%)	29.48 ± 1.24^{c}(-38.67%)
	MDH	52.0 ± 1.41	46.5 ±1.22 (-10.5%)	36.17 ± 1.33^{c} (-30.44%)
	LDH	323.5 ± 3.94	349.66 ± 5.20 (+8.08%)	460.67 ± 7.15^{c} (+42.40%)
Kidney	SDH	27.12 ± 0.50	23.75 ± 0.44 (-12.42%)	16.48 ± 0.5^{c} (-39.23%)
	MDH	30.17 ± 0.98	27.5 ± 1.05 (-8.85%)	19.11 ± 0.54^{c} (-36.65%)
	LDH	251.33 ± 3.56	277.17 ± 3.06 (+10.28%)	335.83 ± 6.55 (+33.62%)
Brain	SDH	42.63 ± 0.51	39.57 ± 1.02 (-7.17%)	34.33 ± 0.96 (-19.46%)
	MDH	43.58 ± 1.11	40.33 ± 1.97 (-7.45%)	36.60 ± 0.52 (-16.02%)
	LDH	301.5 ± 4.59	344.5 ± 4.68 (+14.2%)	419.68 ± 2.94^{c} (+39.19%)

Values expressed as U/ml enzyme, Values are expressed as mean ± S.E; sample size (n) = 6, Values given in parenthesis are % change over control, (-) indicates % decrease over control and (+) indicates % increase over control, Super scripts a, b and c indicate significance at 5%, 1% and 0.1% levels respectively. No super scripts – indicating no significance.

energy for all animals. Glycogen content show maximum levels in the liver tissue when compared to kidney and brain of control fish, as it is the chief organ in carbohydrate metabolism. Liver being a detoxifying organ, it reacts with toxic substances causing glycogen depletion. In the present study, significant reduction in glycogen content was observed in all the tissues of the fish sampled from lake B. Stress stimulates mobilization of glycogen in the liver of fish, causing rapid glycogenolysis and inhibition of glycogenesis through activation of glycogen phosphorylase and depression of transferase as reported by Jha and Pandey, (1989) and Jha and Jha, (1995 a, b). Since glycogen reserves in the liver tissue of fish under stress are used as an emergency energy supply, changes in the glycogen levels in liver tissue of fish sampled from lake B which is polluted with the effluent discharge from industry. Depletion of glycogen content was also observed in kidney and brain tissue indicating the health status of fish population. According to Lehninger, (1983) brain is metabolically active and lacks the inherent potential to store glycogen and is dependent on blood glucose for all its metabolic activities therefore lower glycogen content is generally observed. According to Panigrahi and Mishra (1980) accumulation of toxins in brain may cause disintegration of nerve cells, clotting of blood and reduced oxygen transport to brain which may further decrease the glycogen contents. The present study also shows a significant decrease in the glycogen content in the following trend B >L> K of fish sampled from lake B and L>B>K when compared to those of control fish. Dezwaan and Zandee (1972) reported that glycogen depletion is more prevalent under hypoxic conditions and a situation similar to hypoxia or anoxia might be occurring in the tissues of fish. This is in corroboration with the present data of depletion in glycogen content in all the tissues of fish from lake B in which the dissolved oxygen content is of the minimum as 1.7mg/l (Table 1 and 2). In the present study, the three tissues (liver, kidney and brain) showed a marked increase in cholesterol content in the fish sampled from lake B. The control fish collected from the farm showed highest cholesterol content in liver when compared to those of kidney and brain. A significant increase in percentage of cholesterol content observed in the fish tissues sampled from lake B when compared to lake A and control was in the trend of K>L>B (Table 2). Further it is known that cholesterogenesis increases with the interference of xenobiotic material and then with the feed back mechanism. When pollutants exert a metabolic stress on liver, the excretory mechanism is disturbed leading to an increase in cholesterol content. Sabine, (1977) also suggested that the cholesterol synthesis is maintained at a below maximum level in liver due to continuous absorption of cholesterol from the digestive tract and also due to the negative feedback effect. Meenakumari *et al*. (2010) reported that heavy metals inhibit steroidogenesis which in turn results in the increase in liver cholesterol. According to Kruzynski, (1979) and Brauffaldi (1989) lipid deposits exert protective effects by removing and inactivating organic chemicals from the metabolism or even actively sequestering them, thus improving toxicity tolerance and resistance. Goks*yr, *et al*. (1994) reported an increase in lipid contents and its accumulation in juvenile Atlantic cod (*Gadus morhua*) caged in a polluted fjord, may be due to the increased bioconcentration of lipophilic toxicants which can be due to the effect of aromatic and chlorinated hydrocarbons. The significant increase in cholesterol content in tissues such as kidney and brain can be attributed to the impairments in cell membrane organization due to the toxic effect of pollutants.

Succinic dehydrogenase (SDH) and malic dehydrogenase (MDH) are the primary enzymes in the oxidative catabolism of sugars (Lehninger *et al*., 1993). SDH is effectively used as a marker of mitochondrial abundance and its activity to identify any possible physiological disturbance in fish. The activity of Krebs cycle enzymes such as SDH and MDH showed a significant decrease whereas lactic dehydrogenase, a glycolytic enzyme, showed a marked increase in all the tissues of the fish sampled from polluted lake B when compared to control and those from lake A. Depletion was observed in DO levels in the water analysis of lake B (Table 1). These hypoxic conditions in the water due to the presence of pollutants may, by disrupting the oxygen binding capacity of respiratory pigment, diminish the activity of SDH. Thus there is a direct correlation between depletion in dissolved oxygen level in lake B and reduction of SDH and MDH activity (Table 1 and 3). The results also prove that pollutants cause hypoxia in tissues and suppression of respiratory enzymes with simultaneous stimulation of glycolytic enzymes. Decrease in SDH activity indicates a generally inhibited mitochondrial oxidation of succinate that may lead to drop in energy production. This might be the reason for the decreased SDH activity observed in all the tissues of the fish from lake B in the present study. SDH and MDH activity was recorded highest in liver followed by brain and kidney in the control fish. Fish sampled from lake B showed significantly less SDH and MDH activity when compared to those of control ones in the order as brain > liver > kidney (Table 3). These results are in corroboration with the reports by Radhakrishnaiah (1992), Reddy (1998), Rajamannar (2000) and Suneetha (2012) that there is gradual decrease in SDH activity in tissues like gill, liver, muscle and brain of fish over a period of time in adult and fingerlings of *L.rohita* exposed artificially to lethal and sub lethal concentrations of heavy metals and pesticides. When oxygen becomes restricted in the surrounding environment, fish respond to it by an increase in anaerobic metabolism. Lactate dehydrogenase (LDH) is widely distributed in metabolically active tissues and catalyses the reversible oxidation-reduction reaction involving lactate, pyruate, NAD and NADH. It converts lactate to pyruvate anaerobically to release energy. LDH activity showed a significant increase in all the tissues in the fish from lake B when compared to control in the present study. The control fish collected from the farm showed highest LDH activity in liver when compared to those of brain and kidney (Table 3). The fish sampled from lake B showed a remarkable increase of percentage in the enzyme activity in the order K>B>L whereas fishes from lake A showed a mild increase in the activity of this enzyme (Table 1 and 2). Szegletes *et al*. (1995) and Simon *et al*. (1983) have reported that the increased LDH activity indicate metabolic changes in the stressed fish due to the hypoxic conditions where the catabolism of glycogen and glucose shifts towards the formation of lactate, which can be lethal for the fish. Increased levels of LDH in all the tissues in fishes from polluted lakes in this investigation indicate that it favors anaerobic respiration to meet the energy demands when aerobic

oxidation is lowered.

On the basis of above investigation it may be concluded that significant decrease in the biochemical constituents such as protein and glycogen in the tissues reflects to the exposure of the fish to hypoxic condition as a result of pollutants present in the lake water. This reduction in the constituents may be attributed to glycogenolysis and blocking of protein synthesis which interrupts the amino acid synthesis causing hypoxic condition. These hypoxic conditions in the water also have direct correlation with the reduction of SDH and MDH activity in the test fish *L.rohita*.

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