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ORIGINAL ARTICLE

IMPACT OF LEAD AND INFLUENCE OF DIFFERENT FEEDS ON CARBOHYDRATE METABOLISM IN THE MUSCLE TISSUE OF FRESH WATER FISH, *OROECHROMIS MOSSAMBICUS* (PETERS)

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ABSTRACT

The present study was aimed to investigate the effect of lead and influence of different feeds on carbohydrate metabolism in fresh water fish, Oreochromis mossambicus for 15 and 30 days. The glycogen content decreased in the muscle tissue of lead exposed fish fed with feed 1 (Group 4) when compared with control fish fed with feed 1 (Group 1) showed a slight decrease in the glycogen in muscle tissue when compared with control fish fed with feed 2 (Group 2) and feed 3 (Group 3). Remarkable minimum glycogen content was found in the lead exposed fish fed with feed 2 (Group 5)than feed 3 (Group 6) when compared to corresponding control group 2 and 3. There was no noticeable changes in control fish fed with feed 2 (Group 2) and feed (Group 3) when compared to feed 1(Group 1) for 15 and 30 days of exposure periods. The decreased level of muscle glycogen content in group 4, 5 and 6 for both 15 and 3 days exposure periods was statistically significant.

Key words: Lead, Supplementary feeds, Glycogen, Muscle, *Oreochromis mossambicus*

1.INTRODUCTION

Environmental pollution is a global problemand is common to both developed and developing countries (Agarwal, 1940). The developing and progressive country like India is also facing the problem of air, water, land and noise pollution (Lodha, 1991). Heavy metal pollutants cause direct toxicity both to humans and other living beings, due to their presence beyond specified limits (Rai *et al*., 1998).

In the recent years, industrial development and agricultural process have resulted in the increased levels of toxic metals in the environment, although relatively high concentrations can also occur naturally (Lopez Alonso *et al*., 2002). Heavy metals have been recognized as strong biological poisons because of their present nature, toxicity, tendency to accumulate in organisms and undergo food chain amplification (Kamble and Muley, 2000: Dinodia *et al*, 2002); they also damaging the aquatic fauna including fish.

Most heavy metals released into the environment find their way into the aquatic phase as a result of direct input, atmospheric deposition and erosion due to rain water. Therefore, aquatic animals may be exposed to elevated levels of heavy metals due to their wide use for anthropogenic purpose (Mustafakalay and Canli, 2000). Heavy metal contamination has been reported in aquatic organisms (Rashed, 2001; Adham *et al*., 2002). These metals build up in the food chain and are responsible for chronic illness and death in aquatic organisms (Farkas *et al*., 2000).

The aquatic animals are susceptible to various pollutants like heavy metals, pesticides and industrial effluents, but they have to adjust to these new circumstances by changing their metabolic activities (More *el al*, 2003). Fish become one of the immediate targets of various pollutants as they are comparatively more susceptible to such pollutants (Prasad Nanda *et al*, 2000). Lead poisoning is the most important environmental health problem "(Committee on Environmental Health, 1993). Dietary constituents have been pointed out both as causative agents in oxidative stress and as protective agents in the antioxidant defense mechanisms against stress (Thomas, 1994). Studies on the elimination of metals from animal are most important from the point of view of human and animal health. Medicinal plants have been used in various traditional system, as they have immune potential against numerous diseases (Kottai Muthu *et al*., 2005).

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Plant products have been shown to have a good therapeutic potential as anti-inflammatory agent and promoters of wound healing due to the presence of active alkaloids terpenes and flavonoids (Porras - Reyes *et al*. 1993; Mensah *et al*, 2001).Many plant extracts and phytochemicals have been shown to have antioxidant free radical scavenging properties. (Larson, 1986; Bagal *et al*. 2003). Role of many plant extracts and Ayurvedic formulations in scavenging free radicals has been reported (Vani *et al*, 1997; Bangui *el al*, 2003). Experimental work on several plants has been carried out to evaluate their efficacy against chemically induced toxicity. Experimental studies conducted with the *Emblica officinalis* indicate the they have significant cytoprotective against radiation and heavy metal induced toxicities (Sharma, 1978). Juice and extract of the fruit of the plant *Emblica officinalis* are used as Rosayanas, which are claimed to have preventive, curative and health restorative functions (Satyavathi *et al*, 1976).

Lead is a common pollutant distributed in air, water and soil and even in living organisms. Lead is used in many industries like ceramic, plastics and chemical industries. Aquatic organisms are in great danger due to the pollution of heavy metals like lead. Lead present in water is known to be highly toxic to fishes. Lead is the most serious heavy metal contaminant of soil. Routes of exposure to lead are diverse, with the most common pathways being the intake of lead – containing air, food, water, soil or dust (Kyung – Hwa Beak *et al*., 2005).

Supplementary feeding in tilapia culture is an important one. Among the feeds employed Asia are rice bran, broken rice, oil cakes, flour, corn meal, kitchen refuse, rotten fruit coffee pulp and a variety of aquatic and terrestrial plants. Plants are a rich source of phytochemical. Vallarai leaves *(Centella asiatica)* are widely used in traditional medicines to treat wounds, leprosy, venous insufficiently and disorders of the central nervous system. In recent studies, Vallarai has been shown to improve antioxidant levels which inhibit lipid peroxidation. Freshwater fish,*Oreochromis mossamhicus*. showed retardation in growth under a sub-leihai gallium exposure (Lin and Hwong, 1998). The use of a biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish (Jee-Lee yang and Hong-Cheng Chen, 2003).

2.MATERIALS AND METHODS

Procurement and rearing of experimental fishes

Adult healthy fishes weighing 25-30 gm and 13-14cm length were collected from Annamalai Nagar, India and acclimatized to laboratory conditions for at least 2 weeks. The LC50 values were determined by following the method of Finney, (1971). Sub -lethal studies are helpful to assess the response of test organisms under augmented stress caused by metals. According to Konar, (1969) and Sprague, (1971). one tenth of 96 h LC_5o values represent the lower sub-lethal concentration. 96 h LC50 value of lead was found to be 20.03 ppm. Hence the 1/10 of LC50 value (2.06ppm) was selected for the period of 30 days. The fishes were divided into 2 batches of 10 each. The first batch was exposed to sub-lethal concentration of lead acetate (2.06ppm) for 15 and 30 days. The second group was maintained as control. In the present investigation, sub-lethal concentration of lead acetate was selected and no mortality of fish was recorded throughout the experiment. The dechlorinated water was changed daily for both control and lead acetate stressed fishes.

Group I (untreated control)	:	Kept on standard diet and clean water ad *libitum* and observed for 30 days.
Group II (Lead acetate treated)	:	2 ppm of sub lethal concentration for 30 days
Group III (Lead acetate and Amla fruit – feed 1	:	2 ppm of sub lethal concentration for 30 days and plant 1 treatment for 45 days
Group IV lead acetate and vallarai leaf – feed 2	:	2 ppm of sub lethal concentration for 30 days and plant treatment 2 for 45 days
Group V Amla fruit	:	2 ppm upto 30 days
Group VI vallarai leaf	:	2 ppm upto 30 days

Experimental design

At the end of 15 and 30 days, the tissue was taken out form the experimental and control groups for Carbohydrate estimation. The calorimetric micro method of Kemp and Kits Van Heijininger (1954) was employed for the quantitative estimation of glucose and glycogen. The mixture was cooled and made upto 5.0ml with deprotainizing solution. Once again and later centrifuged at 2,000 rpm for 10 minutes. The mixture was heated in a boiling water bath for 6.5 minutes and subsequently cooled and developed colour was measured in greating spectrophotometer (Cecil, Model CE 3313) against the reagent black (3.0 ml cone. Sulphuric acid at 520nm). The glycogen values were expressed as mg/g wet weight of tissue.

Statistical Analysis

The data obtained from the control and experimental parameters were subjected to determine the level of significance at exposure periods and metal concentrations by ANOVA (DMRT).

3.RESULTS

In the present investigation, lead exposed fish fed with feed 1 shows a decrease in the content of glycogen which compared to control fish fed with feed*l. The decreased level of glycogen**4*ver** content in group 4,5 & 6 for both 15 days and 30 days exposure periods was statistically significant (Table 1 & Figl). The present study, *Oreochromis mossambicus* exposed to sub-lethal concentration of lead (group2 fed with control feed 1) shows a significant decrease in the glycogen level of muscle tissue of 15 and 30 days of exposure periods (Table 1).

4.DISCUSSION

The biochemical parameters in fish are sensitive for detecting potential adverse effects (Almeida *el al*, 2002). Carbohydrate metabolism plays an important role in energy yielding process. Glycogen, being the chief source of energy for the fish, is the metabolite to be affected by an stress. Glucose, a reliable source

Table 1.Level of glycogen content in the muscle tissue of fresh water fish *Oreochromis mossambicus* exposed with lead

Groups	Exposure periods in days 15 days	30 days
Groups 1(Control+ Feed 1)	2.73±0.017	2.77±0.012
Groups 2 (Control+ Feed 2)	2.72±0.031	2.75±0.014
Groups 3 (Control+ Feed 3)	2.73±0.042	2.76±0.015
Groups 4 (Lead+ Feed 1) (%COC)	1.88±0.031 -31.13	1.69±0.014 -38.98
Groups 5 (Lead+ Feed 2) (%COC)	2.47±0.012 -9.19	2.56±0.025 6.90
Groups 6 (Lead+ Feed 3) (%COC)	1.97±0.013 29.13	2.05±0.010 -25.72

Mean± S.E. indicates the mean of six individual observations; Values expressed in mg/g wet wt. of tissue

of energy, is present in almost all tissues. Glycogen, the food reserve is utilized more to meet the extra demand of energy during stress conditions which leads to the decrement.

The present study showed that the decreased glycogen content was observed in the muscle tissue of fresh water fish exposed with different feeds when compared with control fish. Decreased glycogen synthesis is attributed to inhibition of enzyme glycogen synthesis (Stamp and Lesker, 1967). Alteration of carbohydrate metabolism is observed in *Tilapia mossambicus* exposed to arsenic toxicity (Sobha Rani, 2000) in *Labeo rohita* exposed to arsenic trioxide (Pazhanisamy, 2002) and in *Mystal guili* exposed to lead (Kasthuri and Chandran, 1997). Dietary intake of ascorbic acid to protect the body against toxic substances has been studied in Albino rats (Schlegal *et ah*, 1970 and Kamm *et ah*, 1973). Alteration in the level of glucose and lactic acid levels in blood, liver and muscle has been reported in the fish, *Heteropenustes fossilis* (Singhal, 1994). The present study has been undertaken to investigate the influence of different feeds (Vallarai leaves mixed feed *Centella asiatica* and *Emblicu officinalis* fruit mixed feed on the changes in the glycogen contents in the liver tissue of freshwater fish, *Oreochromiss mossambicus* for 15 and 30 days.

Several investigations have been made on the effect of heavy metals on the glycogen and glucose levels of fishes (Karuppasamy, 2000; Datil and Dhande, 2000). Vincent *et al*. (1995) have reported a decrease of carbohydrate content in liver, gill and intestine after exposure to chromium in Indian major carp *Catla catla*. They have further reported that increased glycogenosis in fish under the stress of heavy metals may be expressed as depleted levels of tissue carbohydrates. Shoba Rani (2000) has reported a significant depletion of glucose content in the liver, muscle and gill of *Tilapia mossambica*. exposed to arsenic. Sunita shailajan *et al*, (2005) have reported the significant variation in rat tissue glycogen after treatment with CCU impairment of liver metabolism. Since CCI4 treatment causes reduction in tissue glycogen levels. Reduced glycogen level has been reported in *Channa punctatus* exposed to monocrotophos (Samuel and Sastry, 1989), in freshwater crab *Barytelphusa guerhi* exposed to monocrotophos (Venkateswarlu and Sunitha, 1995).

Alkakhera *et al*., (2005) have reported the depletion of glycogen reserves of liver in the atrazine administered animal. Shakoori and All (1987) and Towry *et al*., (1985) have reported decreased glycogen content in the liver and muscle of fish treated with mercury. Decreased level of carbohydrate content in muscle, liver and gill tissues has been reported in larvivorous fish, *Gambusia affinis* exposed to different sub-lethal concentration of tannery effluent collected from a tannery in Chromepet on the Madras Chingalpet National Highway (Revathi *et al*, 2005).

Dietary intake of feed 2 mixed with Vallarai leaves and feed 3 mixed with *Emblica officinalis* fruit to lead exposed fish show a significant increase in tissue glycogen when compared with lead exposed fish fed with normal feed (feedl). The glycogen content are significantly increased in lead exposed fish fed with feed 2 than fish fed with feed 3 and it indicate that Vallarai mixed feed (feed 2) is more effective than Emblica fruit mixed feed (feed 3). Lead exposed fish fed with feed 2 brings back tissue glycogen values to near normal. In the control fish fed with feed 2 and feed 3 the level of tissue glycogen shows no significant changes when compared to control fish fed with basal feed (feedl). Muthulingam (2002) has reported oral administration of chloroform and ethyl acetate extract of *Astercantha longifolia* and also *Silymarin* to carbon tetra chloride treated rats shows significant increase in glycogen content in liver and kidney when compared to CCU alone treated rats.

Mohamed Salahy and Abd Allah Mohamoud, (2003) have reported hypoglycemia, hypolepidaemia, hypocholesteraemia, hypotriglyceridaemia, promotion of glycogenesis and lipogenesis in white muscle of carnivorous fish, *Chrysichthyl auratus* after oral administration of garlic Juice *{Allium sativum)*. A similar decreasing trend of glycogen has been reported in *(Tenopharyngodone idellus*, Tilak *et al*, 2000). Ascorbic acid alts as a protective agent against aldrin pollution in *Channa punctatus* in which mortality is reduced after addition of ascorbic acid in diet (Agrawal and Mahajah, 2000). An improvement in the level of total protein and glucose level near to normal has been reported in the blood of lead intoxicated wistar rat supplemented with Vitamin C and silymarin (Shalan *et al*, 2005). Thus feed 2 mixed with Vallarai leaves has more antioxidant properties than feed 3 (with *Emblica officinalis)* fruit that may minimize the deleterious effects generated by heavy metal lead thereby suggesting its use as a potent causative action.

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