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Calorific changes in liver, ovary and muscle of hill stream fish *Garra mullya* (sykes) due to cadmium toxicity

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Abstract: Acclimatized specimens of *Garra mullya* were treated with sub lethal concentration of Cadmium chloride and different tissue (liver, muscle and ovary) were analysed for biochemical component i.e. fat, protein and carbohydrate apart from moisture and ash content during preparatory phase of reproduction (April-June) and calorific values in Kcal of liver, ovary and muscle were estimated. The data revealed that the net energy changes between April to June indicate a different energy profile in all the three tissues as well as different net energy change. In control group mobilization of energy is 38.5%, 8.2% and 18.6% in ovary, liver and muscle respectively while treated group showed overall reduction of 7.8%. The reduction was maximum (30.3%) in liver. The paper deals with the impact of cadmium toxicity on energy profile of fish tissue.

Key Words : Cadmium toxicity, calorific changes, liver, ovary, muscle.

Introduction

Cadmium is one of the non-essential heavy metals having high toxic impact on organisms whether terrestrial or aquatic. The whole of Jharkhand area is predominantly occupied by mining and industrial activities hence contamination of soil and water by heavy metals is a common thing for the region. According to Klein (1972) coal washing, quarrying, oil and grease processing, electroplating etc. are the major source of heavy metal pollution resulting into adverse toxic impact. Weathering of rock is a potential natural source of heavy metal import in water courses. As heavy metals get accumulated over period of time at different trophic levels of a food chain in fairly high concentration coming from very low concentrations in water and sediment, the need of study of their toxicological impact on organism is underlined. The mechanism of cadmium toxicity is not fully understood (Nilsson, 1970; Rosenthal and Sperling, 1974). However according to Nilsson (1970) and Sax & Sax (1974) the mechanism of cadmium toxicity is probably due to high affinity of cadmium with sulphydryl and hydroxyl groups and ligands containing nitrogen. Therefore, the binding with such groups in chemical system make the control functions of the organisms vulnerable to cadmium even at low concentrations. It is possible, therefore, that the cadmium acts at cellular level to inhibit the enzyme system and other related biochemical processes of synthesis and breakdown. Keeping this idea in background the present project was taken to estimate the impact of cadmium toxicity on energy profile and energy loss of fish tissue based on biochemical parameters particularly during phase of gonadal maturation (March to June) when vitellogenesis takes place.

Materials and Methods

Mature specimens of Garra mullya were collected from the Subarnrekha river at Getalsud, 30 km west of Ranchi town (23^0 20' latitude and 85^0 30' longitude) with the help of local fishermen. Mature specimens of similar weight (40 ± 3 g) and length (15.5 ± 1.00 cm) the fishes (7 in number) were kept in aquaria for a week in normal tap water for acclimatisation. The 96 hour LC_{50} value of cadmium chloride was determined by Probit analysis (Finney, 1971; Fisher and Yates, 1974), as 8.6 mg/l. The water used in the experiment had the physico-chemical characteristics as given in table-1.

Table 1: Physico-chemical characteristics of aquaria-water during cadmium treatment.

Parameters	Range of variation (March -June)	Mean	Mean at 95% CL
Temp ^{0}C	23.3-26.3	24.975	24.975 ± 1.594
pH	7.2-7.5	7.325	7.325 ± 0.203
Total Hardness (ppm)	61.0-72.0	67.000	67.000 ± 6.462
Total alkalinity (ppm)	105.0-116.0	110.000	110.000 ± 6.841
Dissolved oxygen (ppm)	6.3-7.5	6.875	6.875 ± 0.779

Experimental plan : Ovary of Garra mullya at the collection site enters the preparatory phases in February and spawning being by mid-July (Khan and Mehrotra, 1991). Ovary remains in the maturing phase during April and eggs become ripe and ready for spawning by June end. The fishes were kept in Cd-containing water from beginning of March to June end. As the LC_{50} value was 8.6 mg/l for cadmium chloride, sublethal dose was considered to be safe (Khan *et al.*, 1992 a) at 4.00 mg/l for producing effect during prolonged period. Experiment was started on 1st March, 1998 and continued till June 30th, 1998. About eighty specimens were used each for control and Cd-treatment. Fishes were lodged in four aquaria of similar size, each for control and treated, as keeping them in one aquarium would have caused crowding condition. All the aquaria had sufficient aeration as well as light, and water was changed weekly. Specimens were fed on *Hydrilla* leaves and some bryophytes and lichens sticked to small stone chips. Average mortality during the experiment was around 5.0%.

Tissue Analysis : After every 30 days, beginning from March 1, the liver, ovary and muscle of both control and treated fish were dissected out and weighed. Gonosomatic index (GSI i.e. ovary wt/100 g body wt) and hepatosomatic index (HSI i.e., liver wt/100 g body wt) were determined for each fish. About ten to fifteen fishes were sacrificed each month. Blood was collected by heart puncture. Ovary, liver and muscle samples were kept in fish saline (5.5 g NaCl, 0.14 g KCl and 0.12 g $CaCl_2$ per litre distilled water) until processed for estimation of various biochemical constituents. Extraction of protein in tissues and in blood serum was done by the method of Munro and Fleck (1966) as modified by Abalain *et al.* (1980) and estimated by the method of Lowry *et al.* (1951) taking bovine serum albumin as standard. Fat content was determined by using the method of Floch *et al.* (1957). Carbohydrate was estimated by the methods of Eliassen and Vahl (1982). Energy Content: Energy content of tissue was calculated taking calorific values of 9.5, 5.7 and 4.0 KCal g^{-1} for fat, protein and carbohydrate respectively (Kleiber, 1975).

Results

The results of the tissue analysis for biochemical profile in both control and treated specimen have been presented in fig 1 to 3. Protein contents in liver in control tissue in the month of April, May and June were recorded to be 28.40 ± 0.38, 25.90 ± 0.31 and 21.90 ± 28% respectively while in the same months in treated specimen 18.10 ± 0.28, 19.00 ± 0.35 and 16.35 ± 0.52% (fig-1). The difference between control and treated when analysed was found statistically significant ($P<0.001$). The protein content values revealed a trend of a continuous increase from April to June in tissue of ovary in control samples being 23.50 ± 54, 25.64 ± 0.52 and 27.80 ± 0.44 percent while in treated samples a significant decrease ($P<0.001$) was noticed. The muscle protein, lesser in comparison to those of ovary and liver showed a very narrow range of reduction after treatment i.e. by 0.32%, 0.65% and 37% in the three months but statistically significant ($P<0.001$).

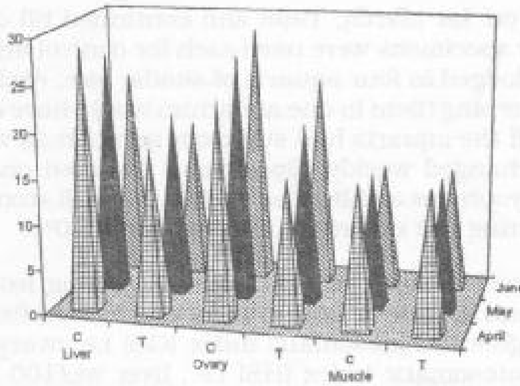

Fig. 1: Variation in protein content in liver, ovary and muscle during three months in control and treated condition. (C= Control, T= Treated)

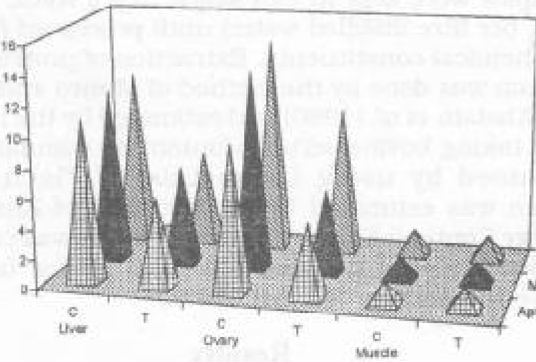

Fig. 2: Variation in cholesterol content in liver, ovary and muscle during three months in control and treated condition. (C= Control, T= Treated)

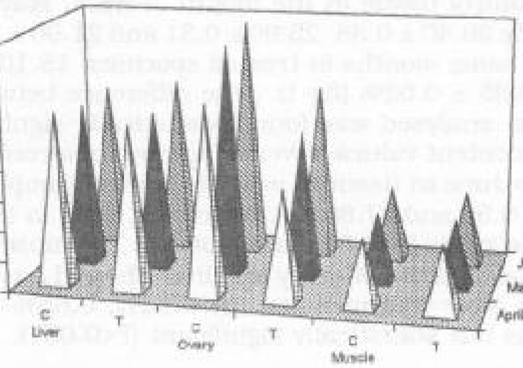

Fig. 3: Variation in glycogen content in liver, ovary and muscle during three months in control and treated condition. (C= Control, T= Treated)

The glycogen content was significantly reduced (P<0.001) in all the three types of tissue taken for experiment. In liver tissue initially the glycogen value (mg per gram tissue) was found to be 5.70 ± 0.38, 5.30 ± 0.26 and 5.40 ± 0.21 which showed a decrease of 5.62, 1.89 and 5.56% respectively (fig-2). The treated ovarian tissue showed a reduction of glycogen content by 39.28%, 42.85%, 26.62%.The glycogen content in ovary of control fish was 5.60 ± 0.25, 6.30 ± 0.15 and 7.70 ± 0.15 in corresponding months. A marginal decrease by 0.90%, 2.2% and 2.1% was recorded in glycogen content of muscle of treated specimen over control.

Cholesterol content exhibited highly significant (P<0.001) decrease in all the three months in the three tissues of Cd-exposed specimen. There was not a high fluctuation in cholesterol content of liver in the three months (10.90 ± 0.22 in April, 11.60 ± 0.26 in May and 11.70 ± 0.25 in June) with respect to other parameters (fig-3). The cholesterol content showed a fairly high level of decline by 45.87%, 43.96%, 46.78% in Cd treated specimen of *Garra mullya*. In ovary the reduction in cholesterol content due to Cd toxicity was higher than that of liver as it revealed a reduction by 50.00%, 57.84% and 58.01% in the three months. The value of cholesterol as mg per gram tissue of ovary was a bit higher in ovary during the period of experiment being 10.90 ± 0.40, 10.20 ± 0.20, 13.10 ± 0.60 respectively. The cholesterol content of muscles was fairly low in comparison to liver and ovary. In the month of April, May and June it was 1.50 ± 0.12, 1.40 ± 0.15 and 1.70 ± 0.25 which due to Cd treatment decreased marginally by 3.33%, 3.20% and 4.70%.

The gonadosomatic index and hepatosomatic index reveal a pronounced impact of cadmium treatment. The decrease in GSI value was more significant in April and May (P<0.001) than in June (P<0.02). Similarly HSI exhibited more significant decrease in May and June (P<0.01) and April (P< 0.05). The values of GSI and HSI for *Garra mullya* in treated and control condition have been presented in table- 2.

Table 2: Gonadosomatic index (GSI) and hepatosomatic index (HSI) of *Garra mullya* under control and Cd-exposed condition (n=7 i.e. 7 replicates).

	GSI		HSI	
	Control	Treated	Control	Treated
April	6.60 ± 0.60	2.40 ± 0.75***	2.50 ± 0.34	1.00 ± 0.30**
May	10.50 ± 0.66	5.70 ± 0.80***	2.70 ± 0.36	1.30 ± 0.15***
June	11.40 ± 0.90	8.10 ± 0.60*	2.10 ± 0.17	1.30 ± 0.25***

Significance : * = P< 0.02; ** = P< 0.05; *** = P< 0.001

Table- 3 provides the total energy change of the three tissues (liver, ovary and muscle) during the 4 months along with percent changes between March and June based on changes in protein, fat and carbohydrate content. Month wise data indicate that while liver and ovary were affected by Cadmium chloride, the muscle remained almost unaffected. However, the net energy changes between April and June indicate a different energy profile. In control group the mobilization of energy in ovary is maximum (38.5%) between the period of experiment. But liver shows lesser mobilization (8.2%) and muscle exhibited depletion (18.6%) in terms of calorific content. However, the energy profile obtained from the experiment reveal that the Cd causes significant fall in the calorific content of all the three tissues. In liver of Cd-treated fish the reduction of energy was 30.3 per cent between April and June. Though mobilisation by ovary was also evident in Cd-treated fish, it recorded 22.0 per cent fall in energy. When compared to control it accounted for net loss of 16.5 per cent in ovary. Similarly, depletion in energy content of muscle was evident both in control (18.6%) and Cd-treated (13.1%) specimens which indicated depletion in the muscle of Cd-treated by 5.6 per cent in comparison to control. As far as the total energy content of liver, ovary and muscle are concerned, there was a net gain of 15.5 per cent in control group between April and June while the Cd-treated specimens recorded a net loss of energy by 7.8 per cent during the same period when the total loss of energy by Cd-exposed fish was compared with control it accounted for a net loss of 23.3 per cent between April and June.

Table 3: Changes in the calorific profile of the liver, ovary and muscle of control and Cd-treated fish (between March and June).

		March	April	May	June	Difference (between March and June)	Percent gain/ loss (between March and June)	
Liver	C	1.982	2.237	2.335	2.161	+0.179	+8.2%	
	T	1.686	1.229	1.253	1.175	-0.511	-30.3%	-38.5%*
Ovary	C	1.381	1.628	2.088	2.247	+0.866	+38.5%	
	T	1.248	1.246	1.536	1.589	+0.351	+22.0%	-16.5%*
Muscle	C	1.159	1.121	1.061	0.944	-0.215	-18.6%	
	T	1.149	1.099	1.102	0.998	-0.151	-13.1%	-5.6%*
Total	C	4.522	5.022	5.484	5.352	+0.830	+15.5%	
(L+O+M)	T	4.083	3.574	3.891	3.762	-0.321	-7.8%	-23.3%*

L = Liver; O = Ovary; M = Muscle; (+) = Gain, (-) = Loss; C = Control; T = Cd-treated.

* Percent change in treated with respect to control.

Discussion

Since a sublethal concentration mimics the actual situation of polluted and stressed condition a concentration of 4 mg/CdCl L^{-1} (sublethal) against LC_{50} value 8.6 mg Cd/L was selected for the experiment and a duration of 30 days was opted to have an idea of impact of toxicity (Khan *et al.*, 1992 a). Deposition of yolk protein in the ovary is reflected as the increased gonadosomatic index and hepatosomatic index (Emmersen and Emmersen 1976; Quinitio *et al.*, 1989). A similar trend of increase in GSI and HSI was observed during the present study in the control group of specimens of *Garra mullya* [Table- 2] while a sharp and significant decrease was noticed in these values in Cd treated specimen probably due to histological damages done to experimental specimen (*Garra mullya*) by Cd toxicity. Wani and Latey (1982) have also reported a significant fall in GSI along with disappearance of mature oocytes and HSI in Cd-treated specimens of *Garra mullya*.

In Cd-treated specimens all the parameters exhibited significant decrease in both ovary and liver except liver glycogen which remained unaltered, suggesting the possibility that Cd-toxicity either blocks hormone action or the complex enzyme systems responsible for vitellogenesis and steroidogenesis (Idler and Bitners, 1960; Upadhyaya *et al.*,1985). An elevated level of serum glucose and depletion of liver glycogen among Cd-treated *Clarias batrachus* and Tilapia mossambica have been reported (Banerjee *et al.*, 1978). The retention of glycogen level in the Cd-treated specimens as has been observed during the present study may, however, be attributed to the stress condition under which liver has a tendency to retain glycogen (Love, 1970).

There is great paucity of information pertaining to the variation of calorific content in the tissues of fish maintained in heavy metal containing water. However, the biochemical constituents like protein, fat and carbohydrate, producing calorific energy have been found to decrease on exposure to heavy metals in several species of fish viz. blue gill (Hiltibran, 1971); fathered minnow, Pimephales promelas (Banerjee *et al.*, 1978); *Clarias batrachus* and *Tilapia mossumbica* (Banerjee *et al.*, 1978); flag fish, *Jordanella floridae* (Spehar *et al.*, 1978) and *Garra mullya* (Khan *et al.*, 1992 a, b). The energy data of the present study in Table-3 indicate that cadmium toxicity significantly reduced the energy content in liver, ovary and muscle. While in liver, the energy content was reduced by 38.5%, in ovary the reduction was 16.1% between April and June. The stored nutrient like protein, fat and carbohydrate in liver and body muscle are transferred to gonad and subsequently utilized as source of energy for metabolism as well as growth and maturation of gonad (Diana and Mackay, 1979; Blay Jr. and Eyeson, 1982; Strange and Pelton, 1987). In the present study maximum energy mobilisation was noticed in ovary of both control as well as treated groups. On the other hand energy depletion was also noticed from liver as well as muscle as the time of experiment advanced. Cadmium however, was found to greatly influence the mobilisation-deposition process in all three tissues. Energy profile of control group indicates heavy depletion in muscle (18.36%) between April and June. However, the liver of control group mobilised lesser percentage (8.2%) of energy indicating that liver too might have contributed substantially in ovarian calorific value where there was a gain of 38.5 per cent between the same period. However, the energy profile of Cd-treated specimens revealed that the depletion of energy content in muscle in this group was lesser (5.5%) with respect to control. Similarly, the liver of Cd-treated group recorded sharp decrease in the calorific content (38.5%) with respect to control between April and June which indicates that cadmium either caused large depletion from liver or inhibited energy mobilisation. However, latter possibility seems to be more likely as is evident from the month wise biochemical analysis of liver wet weight. Reduced percentage depletion of energy from muscle and reduced mobilisation in liver of Cd-treated specimens is reflected well by a significant fall by 16.5 per cent in the energy content of ovary of treated fish. Data of total energy content of liver, ovary and muscle exhibited a gain of 15.5 per cent in control group between April and June where as in Cd-treated specimens there was a net loss of 7.8 per cent energy between the same period. Hence, it may be concluded that cadmium chloride inhibit energy mobilisation through depletion of fat, protein and carbohydrate contents in liver, ovary and muscle of *Garra mullya* which accounted for nearly 23.3 per cent over all loss of total energy in the tissues in question with respect to control group.

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Corrigendum

*The paper entitled "Hydrobiological Characteristics of the Gulf of Kachch Saurashtra region by V.W. Lande appeared in the J.Ecophysiol. Occup. Hlth. Vol. **1** No. 1 & 2 on page No. 179-185 had few spelling errors. A corrected Copy of the same may be obtained from the author directly.*