RESEARCH ARTICLE

Protective effect of dietary supplementation of *Spirulina platensis* on improvement of growth parameters in mercuric chloride exposed fish, *Labeo rohita*

Shelke AD* and Wani GP

P.G. & Research Dept. of Zoology, B.P. Arts, Science & Commerce College, Chalisgaon, Dist. Jalgaon. State-Maharashtra, Pin 424101, Affiliated to North Maharashtra University, Jalgaon. MS, India
*Corresponding author E-mail Id: adshelke@gmail.com

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ABSTRACT

Metal contamination in freshwater bodies is a matter of serious concern from the human health point of view since many aquatic organisms, particularly fish, form an integral part of the human diet. *Labeo rohita* is one of the important cultured carp species in Asia. Among the freshwater fishes, carps are most affected to environmental contamination. The reduction of toxic elements in aquatic systems and organisms by acceptable methods is a need of the hour. The effect of spirulina supplementation on reduction of mercuric chloride toxicity based on food utilization was studied in a freshwater fish, *Labeo rohita*. The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days. The results showed that sublethal exposure of *Labeo rohita* fed with *spirulina* free diet (T1 groups) significantly reduced the food utilization parameters than those exposed to sublethal level of mercuric chloride and fed *Spirulina* supplementation diet (T2 - T5 groups).

Keywords: Protective effect, *Spirulina platensis*, Growth parameters, Mercuric chloride, *Labeo rohita.*

INTRODUCTION

Public health concern over mercury exposure to human beings due to consumption of contaminated fish has been a topic of political and medical debate. Pathological effects due to inorganic mercury in certain fishes have been reported. (Sastry and Gupta 1978, Naidu *et al*., 1983). In the aquatic environment inorganic mercury is converted to methyl mercury and is the predominant form of mercury reported in fishes caught from contaminated waters.

Spirulina is one of the most concentrated natural sources of nutrients for all animals. *Spirulina* contains protein (60-70%), essential amino acids and fatty acids, phycocyanin (14%), chlorophyll (1%) and carotenoid pigments (0.37%), vitamin B-12, and minerals that play important roles in animals in various ways (Venkataraman 1993).

Spirulina improves the intestinal flora in fish by breaking down indigestible feed components (Ramakrishnan et al., 2008). It stimulates the production of enzymes that transport fats in fish for growth instead of storage (Henrikson, 1994). β-carotene in spirulina firmly maintains the mucous membrane and thereby prevents the entry of toxic elements into the body (Henrikson, 1994). Chlorophyll in *Spirulina* acts as a cleansing and detoxifying factor against toxic substances (Henrikson, 1994). Researchers have reported the therapeutic effects of *Spirulina* as a growth promoter, probiotic, and booster of the immune system in animals including fishes (Venkataraman, 1993). So far, *Spirulina* is known for its nutritive value only; its role in alleviating metal toxicity in fishes and other cultivable organisms remains unexplored. In the present study, experiments were designed to investigate the impact of dietary *Spirulina* supplementation on the growth, and alleviation of mercuric chloride in carp, *Labeo rohita*. In the present investigations concentrated on damage induced by inorganic mercury in the Indian major carp *Labeo rohita* and protective role done by *Spirulina platensis.*

MATERIALS AND METHODS

The live major carp, *Labeo rohita* were obtained from a Girna river dams near Chalisgaon city. They were acclimatised in laboratory condition for more than two weeks. The temperature, PH, Salinity and dissolved oxygen of the water were found to be 27 ± 1Ċ, 7.55 ± 0.1, 0.76 ± 0.09% and 7.20 ± 0.12 ml/l respectively. During the acclimatisation, water was changed daily and fish were fed ad libitim with pelletised diet containing 35% protein. Acclimatized fish (1.30 ± 0.10g) were exposed to different concentrations (0, 0.03, 0.06, 0.09., 0.12, 0.15, 0.18 ppm) of mercuric chloride $HgCl_2$ obtained from Merk India Ltd. (Mumbai, India) and mortality was observed for 96 h. A static bioassay method was adopted for the determination of 96 h median lethal concentration. Probit analysis was followed for the calculation of 96 hours LC_{50} Control group of fish was maintained in mercury free freshwater.

Feed: In the present experiment, 35% protein diet was used as basal diet for *Spirulina patensis* supplementation. The intergradient of dried fish meal, ground oil cake, cod liver oil, egg yolk, tapioca flour, vitamins and mineral mixtures were used to prepare the 35% protein diet, with appropriate proportion by square method. In addition to the control diet, five diets (0, 2, 4, 6, 10 %) were prepared with different *Spirulina patensis* levels. The experimental diets were by adding the appropriate level of *Spirulina patensis* with chosen intergradient to boiled water, mixed well and steam cooked for 15-20 min. After moderate cooling, pellets (2mm) were prepared with operated pelletizer and dried in sunlight. After drying diets were separated stored in refrigerator. Active and healthy fish (1.30 ± 0.10 g) were chosen from the acclimatisation tank and starved for 24 h. prior to the commencement of experiment. The fish were divided in to six groups of 10 individuals each and were exposed to 0.12 ppm. (50% 96h LC_{50} value) of mercuric chloride for 21 days. Triplicates were maintained for each group.

Group-I: served as control and reared in mercuric chloride free freshwater and fed with *Spirulina patensis* free diet. Test animals belonging to 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} groups were exposed to 0.12 ppm of mercuric chloride.

Group-II: Individuals was fed with *Spirulina patensis* free diet, however 3^{rd} , 4^{th} , 5^{th} and 6^{th} groups were fed with 2, 4, 6, and 10% *Spirulina patensis* diets respectively. The experimental groups 1, 2, 3, 4, 5, and 6 are designated as C, E1, E2, E3, E4 and E5 respectively. The experiment was conducted in glass aquaria containing 100L water. The water was not changed during the experiment but was aerated for 14 h. to avoid depletion of oxygen. The hydrobiological parameters like dissolved oxygen, temperature, PH, salinity and hardness of water were estimated during non- aeration period. Two series of experiment were conducted in the present study.

Exp.I: Feeding and growth- During the experiment period, the chosen groups were fed with weighed quantities of experimental diets twice a day at 07:00 and 18:00 hrs. Unconsumed feed was removed after 1 h feeding and dried in hot air oven at 80Ċ for two days. Feed intake was estimated by subtracting the amount of unconsumed dry feed from the total dry weight of the offered feed. The feeding rate (mg/g live fish/day) was computed as the amount of feed consumed / (initial wet wt of the fish × no. days). Feed samples and unconsumed feed were weighted in an electric monopan balance to 1 mg accuracy. The duration of

the experiment was 7, 14, 21 days. The sacrifice method was adopted to estimate the growth of the experimental fish. Calculation of selected food utilisation parameters has been described in detail.

At the beginning the experiment, the total weight of the fish in each groups was weighed in an electric monopan balance. Five fish from the stock were sacrificed to estimate water content and determine the initial dry weight of the fish. All fish in each group were weighed at the end of the experiment and dry weight was calculated using the percent water content of fish sacrificed at the beginning of the experiment. Weight gain (growth) was calculated as the difference between initial and final dry fish weight. Growth rate (mg/g live fish/day) was calculated as growth / (initial weight of fish × no. of days). Gross conversion efficiency (%) was calculated as growth /feed intake × 100. Feed conversion ratio (FCR) was computed as the relation between feed intake and growth.

RESULTS AND DISCUSSION

Inorganic mercury salts are unable to cross tissue blood barriers and is eliminated at a faster rate than methyl mercury (Ulfvarson, 1966). Animals start accumulating mercury when the rate of uptake exceeds the rate of elimination. When inorganic mercury treatment was stopped, the elimination of accumulated mercury resulted in the decline of its residue level in liver and this brought about a corresponding histological recovery studied by (Paulose , 1988).

The accumulation of heavy metals in the tissues of fishes may cause various physiological defects and mortality (Torres *et al.,* 1987). Heavy metals accumulated in the tissues of aquatic animals may become toxic when accumulation reaches a substantially high level (Kalay and Canli, 2000). The pattern of bioaccumulation of metals in animals differs from metal to metal and organ to organ during their functional status. Most of the investigations pertaining to heavy metals contaminants in aquatic systems are dealt either with toxicity or with accumulation (Rushforth *et al.,* 1981; Khadiga *et al.,* 2002). Heavy metals have been shown to be concentrated in the liver of various fishes (Sorensen, 1991 and Rao *et al.,* 1998).

The Dietary ascorbic acid supplementation at a level of 2000 mg kg-1 diet resulted in decreased copper accumulation in the gills and liver of rainbow trout and also decreased copper levels in the gills, haepatopancreas, Kidney and intestine. These results demonstrated that dietary ascorbic acid decreased the toxicity of water borne copper accumulation in the tissues. It is likely that, dietary *spirulina* may also reduce the metal level in tissues (Lanno *et al.,* 1985) and protect *Labeo rohita* from mercuric chloride toxicity.

In the present work the feed intake was decreased in the sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet but it was significantly increased in the mercuric chloride with *Spirulina platensis* diet from 20.49 to 28.32 (g dry matter) and the consumption rate was increased from 32.38 to 41.90 (mg/g live fish/day) as the percent dose of *Spirulina platensis* increased.

Table 1: Effect of dietary supplementation of *Spirulina platensis* content on selected food utilization parameters in mercuric chloride exposed *Labeo rohita*.

Parameters	**Diet (*Spirulina* content)**					
	Control	**T_1 (0%)**	**T_2 (2%)**	**T_3 (4%)**	**T_4 (6%)**	**T_5 (10%)**
Food intake (g dry matter)	31.21 3.43	15.82 1.36	20.49 2.03	23.11 2.32	27.87 2.69	28.32 2.45
Consumption rate (mg/g live fish/day)	56.38 5.43	25.26 2.16	32.38 3.47	39.48 4.02	42.11 4.53	41.90 4.21
Weight gain (g wet wt.)	6.76 0.71	1.71 0.19	3.61 0.36	5.20 0.57	5.58 0.51	5.90 0.43
Weight gain (%)	26.06 2.17	3.61 0.36	6.89 0.65	18.96 1.75	20.60 0.55	19.53 1.53
Growth rate (mg/g live fish/day)	12.95 1.15	2.88 0.32	3.09 0.28	6.89 0.65	8.36 0.78	7.05 0.38
Gross conversion Efficiency (%)	23.72 2.38	6.00 0.60	11.00 1.29	20.49 2.03	19.95 1.83	18.56 1.39
Feed conversion ratio	5.28 0.51	5.92 1.73	9.81 0.61	7.05 0.38	5.10 0.43	5.09 0.48

The sublethal exposure of mercuric chloride fed *Spirulina platensis* free diet resulted in significant decrease in weight gain (%) but the weight gain were increased in the mercuric chloride with *Spirulina platensis* diet from 6.89% to 19.53 % and the growth rate was increased from 3.09 to 7.05 (mg/g live fish/day) as the percent dose of *Spirulina platensis* increased. Mercuric chloride exposed *Labeo rohita* fed *spirulina* supplemented diets might have eliminated the copper from the body tissues through feces, *Spirulina* reduced genotoxicity and oxidative stress of several antibiotics in mice (Premkumar, 2004) and lead (Pb) toxicity in rats (Upasani, 2003).The present study showed that feeding and growth parameters improved in mercuric chloride exposed fish fed *Spirulina*-supplemented diets. *Spirulina* reduced mercuric chloride accumulation in tissues and increased mercuric chloride elimination through feces, lessening the metal burden and its toxicity to fish. The reduced growth rate in fish given a sublethal level of mercuric chloride was probably due to the tissue burden of mercuric chloride which, in turn, could have caused a reduction in feed intake, an increase in metabolic cost, or poor food conversion efficiency. Growth reduction in copper-exposed *Salmo gairdneri* was partly due to increased metabolic costs and reduced food consumption (Lett *et al.,* 1976).

Supplementation of *Spirulina* in the diet, improved the food utilisation parameters in mercuric chloride exposed fish. The feed conversion ratio (FCR) value of fish belonging to T4 groups was low as (5.10) as compared to other groups other groups and close to the FCR value of control fish. (Table-1). It was due to the *Spirulina palatensis* which reduced the accumulation of mercuric chloride in tissues in elimination of accumulated metal through faces, lessening the metal burden and its toxicity on fish. Spirulina contains phycocynin (14%) cholorophyll (1%) and carotenoid (0.37%) pigments. (Henrikson, 1994) B-carotene of Spirulina maintains the mucous membrane firmely (Henrikson, 1994) and thereby entry of toxic elements in to the body is prevented. Chlorophyll of *Spirulina* acts as a cleasing and detoxifying phytonutrient against the toxic substances (Henrikson, 1994).

It indicates that *Spirulina* has the ability to eliminate and detoxify and accumulated mercuric chloride and it was proved by improvement of feeding and growth parametrers in sublethal exposure of *Labeo rohita* fed *Spirulina patensis* supplementation diets. Working on rainbow trout *Salmo gaiedheri*, (Lanno *et al.,*1985) found that high level of dietary ascorbic acid (10g Kg-1 diet) improved the body weight gain in copper exposed fish as compared to fish fed on low levels on ascorbic acid (0.9g kg-1 diet).

CONCLUSION

The present study shows that, the dietary supplementation of *Spirulina* reduced the metal toxicity in mercuric chloride exposed *Labeo rohita* and improved the food utilization parameters like feed intake, consumption rate, weight gain, growth rate and feed conversion ratio (FCR) value significantly as the percent dose of *Spirulina platensis* was increased in a short period of time.

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