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Influence of *Spirulina* and Probiotic Bacteria (*Lactobacillus sporogenes*) on Gill and Brain tissues of Zinc toxicated *Cyprinus carpio*

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**1. Introduction**

Aquaculture has seen a worldwide expansion over the past 20 years and it seems that growth is set to continue (Naylor et al., 2000). World total demand for fish and fishery products is projected to expand by almost 50 million tonnes to 183 million tonnes by 2015, and it is expected that out of this increase, 73% will come from aquaculture, accounting for 39% of global fish production (FAO, 2004). Heavy metals are persistent contaminants in the environment that come to the forefront of serious health hazards in humans and animals. Such metals are absorbed from polluted water through gills, skin and digestive tract of fish by bio-concentration and bio-magnification.

Zinc is one of the major important heavy metal which gets accumulated in the tissues of the fresh water fish. Aquatic pollutions are frequently decimated in zinc-polluted waters (Solbe and Flook 1975; Everall et al., 1989).

Zinc and its compounds induce testicular sarcomas in mammals. Excess zinc is teratogenic to fish embryos. A probiotic is a live microbial feed supplement, which beneficially affects the host by improving its intestinal microbial balance (Fuller, 1987). *Lactobacillus sporogenes* is universally occurring beneficial bacteria. *Spirulina* is symbiotic, multicellular and filamentous blue-green microalgae with symbiotic bacteria that fix nitrogen from air enabling detoxification. The *Cyprinus carpio* is a widespread freshwater fish most closely related to the common goldfish (*Carassius auratus*), with which it is capable of interbreeding.

Hence, a study has been undertaken in the tissues of gills and brains which were biochemically analysed for content of protein, carbohydrates, lipids, enzymes, acid and alkaline phosphatases and transaminases to evaluate the toxicity of zinc, detoxication ability of *Spirulina* and healthy maintenance by *L. sporogenes*. 

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**Abstract**

The present study concluded the biochemistry of gill and brain tissues in the zinc treated *Cyprinus carpio* were heavily affected. *Spirulina* and the probiotic bacteria *Lactobacillus sporogenes* increased more of protein, carbohydrate and lipids in the tissues. The enzymes (GOT, GPT, ACP and ALP) were slightly modified in gills and brain tissues compared to control groups. The *spirulina* alleviates and lessens the zinc metal burden and its toxicity in *C. carpio*. *L. sporogenes* along with *Spirulina* maintain the health of aquatic organism in farm culture. Hence it can be utilized in large scale healthy production of fresh water fishes.
2. Materials and Methods

2.1 Collection and Acclimatization
The common carp *Cyprinus carpio* of the average body weight 3.5 g were collected from the Tamil Nadu Fisheries Development Corporation Ltd, Aliyar nagar, Coimbatore, Tamil Nadu, India and were transported in oxygenated plastic bags to the laboratory. The collected fingerlings were maintained in large cement tanks, containing bore well water from college campus having the temperature 27 ± 1°C, pH 7.2 to 7.5 and dissolved Oxygen 1.97 ml/lt. During acclimation the fish were fed with control feed prepared in the laboratory.

2.2 LC$_{50}$ value of zinc
The test metal compound zinc tetraoxosulphate IV heptahydrate, (ZnSO$_4$, 7H$_2$O) was prepared by dissolving 43.97 g of British Drug Houses (BDH) reagent equivalent to 1 g of zinc in 1000 ml distilled water at concentration of 1000 mg/l. Likewise different concentrations (4.54, 5.11, 5.68, 6.25, 6.82 mg/litre) of zinc was prepared and mixed in the glass tank containing 20 litres of water for 10 fishes. The mortality of fishes was noted at the end of 24, 48, 72, 96, 120,148 hours for different concentration of zinc solution. Using the mortality rate for different concentration, the LC$_{50}$ value of zinc for different hours was calculated by employing Probit analysis of Finney (1978) as described by Busvine. For the present investigation 0.005 mg/l of zinc was chosen as the sub-lethal concentration.

40% protein rich artificial feed was prepared (Pearson square method) to fetch the control fish and 10 gm of *Spirulina* added with one kg of control feed and served as experimental feed, given to the experimental fish.

2.3 Probiotic Treatment
*Lactobacillus sporogenes* of trade name Sporlac procured from local pharmacy. 1 gm of sporlac powder contains 150 million spores of *Lactobacillus sporogenes*. For the present experiment 1 gm of sporlac powder was mixed in 40 l of water

2.4 Experimental setup
Group A: Control fishes fed with control feed and treated with zinc
Group B: Fishes fed with experimental feed. Group C: Fishes fed with control feed and treated with *Lactobacillus sporogenes*
Group D: Fishes fed with control feed and treated with zinc
Group E: Fishes fed with control feed, treated with *Lactobacillus sporogenes* and Spirulina

2.5 Objective of Research
To find the lethal concentration (LC$_{50}$) of the heavy metal zinc to the fish *C. carpio*. To study the toxic effects of zinc on the tissue biochemistry and understand the effects of *L. sporogenes* and Spirulina to recure the toxic effect of zinc on the tissues of *C. carpio*.

3. Results and Discussion
The brain tissues show a very little change in the amount of protein in different groups, compared to the control A group (Fig.1). Group B and C show insignificant increase and group D shows insignificant decrease. Group E, F and G show insignificant decrease than the control A and zinc toxicated D groups. Free amino acids in group D fishes increased compared to other groups. The effect of zinc is lesser in gill tissues since the reduction of protein content is not significant when compared to the control fishes. The group B and C fishes show a little increase in amount over the control fishes. Group E, F and G fishes show reduced amount of protein, even less than group D fishes, showing the insignificant effect of the feed additives, on zinc toxicated gill tissues.

Insignificant and significant increase of carbohydrates (Fig. 3) in brain tissues of groups B and C over group A have been noted in 10 days and 20 days of experimented fishes. In 30 days treatment, the increase is significant. 10 days treatment of zinc in group D has reduced the level significantly. 20 and 30 days treatment have increased the level in group D fishes. Among groups E, F and G
fishes, *spirulina* in group E can rectify the defect for 30 days of treatment and *Lactobacillus* (Group F) has reduced the defect significantly for 30 days of treatment. The group B has increased the level insignificantly for 10 days of *Spirulina* supplementation. But 20 and 30 days of supplementation reduced the level significantly. Group C fishes have increased

**Fig 1:** Protein (mg/g) present in 10, 20, 30 days of gill and brain tissues of *C. carpio*

**Fig 2:** Free amino acids (mg %) present in 10, 20, 30 days of gill and brain tissues of *C. carpio*

**Fig 3:** Carbohydrate (mg/g) present in 10, 20, 30 days of gill and brain tissues of *C. carpio*

**Fig 4:** Lipid (mg/g) present in 10, 20, 30 days of gill and brain tissues of *C. carpio*
Fig 5: GOT (AST) (µM oxalo acetate formed/mg protein/h) present in 10, 20, 30 days of gill and brain tissues of C. carpio

Fig 6: GPT (ALT) (µM pyruvate formed/mg protein/h) present in 10, 20, 30 days of gill and brain tissues of C. carpio

Fig 7: ACP (mg/g) present in 10, 20, 30 days of gill and brain tissues of C. carpio

Fig 8: ALP (mg/g) present in 10, 20, 30 days of gill and brain tissues of C. carpio

the amount significantly for 10 days and 30 days of L. sporogenes treatment. Group D fishes increased significantly for all periods of treatment. The effect of zinc can be recurred in
group F and G even for 10 to 20 days of supplementation. 30 days of supplementation reduces the carbohydrates more significantly. The amount of lipid is more in brain when compared to all other tissues (Fig. 3). The amount has been raised significantly in group B and C for 10 days and insignificantly for 20 days and 30 days. Group D has lost the amount significantly in 10 days and 30 days but the lost is insignificant in 20 days. Supplementation cannot bring the amount to normal level even after 30 days in E, F and G groups.

The amount has been raised significantly in group B and C for 10 days and insignificantly for 20 days and 30 days. Group D has lost the amount significantly in 10 days and 30 days but the lost is insignificant in 20 days. Supplementation cannot bring the amount to normal level even after 30 days in E, F and G groups. The lipid content is comparatively less in gill tissues (Fig 5). Groups B and C tissues have raised the amount significantly for 10 days, insignificantly for 20 days and 30 days of period. Group D has lost the lipid significantly for 10 and 20 days and insignificantly for 30 days. Group E, F and G are not able to compensate the effect even for 30 days of supplementation.

Brain is the most affected tissue. Here the GOT level is tripled in group D fishes due to toxicity. Feed additives are not able to reduce the GOT level in all groups. Gills are containing more of GOT when supplemented with feed additives in group B, C, D, E, F and G. Zinc toxicity in group D also doubled the GOT level in gills (Fig 5). The amount of ACP in gills, the amount is increased in all groups for all days. In brain, it is increased in groups B and C and reduced in all other groups. The amount of ALP is high in brain tissues of control fish compared to other tissues. In gills, the ALP is reduced in B, C and F and increased in D, E and G tissues (Fig. 7).

The dietary protein spirulina in the feed has increased the amount of protein in both the tissues of group B fishes. This is in accordance with the work of Mohsen Abdel et al. (2008) who has pointed that the Spirulina supplementation increase the protein content of the whole – fish body Oreochromis niloticus significantly. In group C fishes, the increase in proteins are due to the active feeding of the group. Several studies have demonstrated the modes of probiotic actions in effect in the aquatic environment. Bairagi et al. (2002) have determined that some probiotic bacteria produce digestive enzymes, thus facilitating feed utilization and digestion. The group D fishes have reduced the protein content in both the tissues due to the metal toxicity. Proteins are highly sensitive to heavy metal poisoning (Jacobs et al., 1977). In group E, F and G fishes which are fed with supplementary feed and treated with L. sporogenes the protein contents has increased in the zinc toxicated fish D. Because in group E where spirulina is supplemented, it reduces the zinc accumulation in tissues and increases its elimination or it lessens the metal burden and its toxicity to fish.

The amounts of free amino acids in both the tissues, in different groups of fish have been increased. The increase in amount of free amino acids in group D is presumed to be an indication of acute effect of lethal concentration of zinc on those tissues. De Smet and Blust (2007) have reported that proteolysis is intended to increase the role of protein in the energy production during cadmium stress in Cyprinus carpio. In Catla catla, Sobha et al. (2007) have observed the increased aminoacids in the fish exposed to sub-lethal concentration of cadmium chlorides.

In the present investigation, the carbohydrate has been increased in all experimental groups. Zinc is known for its inhibition of glycolysis, Kreb’s cycle, electron transport chain and glutamate release. The two glycolytic enzymes that might be inhibited by increased zinc concentration are glyceraldehydes-3 phosphate dehydrogenase (GAPDH) and phosphofructokinase. Zinc also inhibits α-ketoglutarate dehydrogenase complex (KGDHC) and lipoamide dehydrogenase (LADH) subunits of the tricarboxylic acid cycle (Strydom et al., 2006). Zinc is also known for its inhibition of the electron transport chain between cytochrome b and c1 (Dineley et al., 2003). The glucose-6 phosphate dehydrogenase of the pentose-phosphate pathway is also inhibited by the influence of zinc (Naab et al., 2001). In group E and F fishes, the zinc toxicity is being alleviated by spirulina and L. sporogenes and by both tissues, in group G fishes, the amount of carbohydrates have been reduced when compared to group D fishes.

The lipid levels increased in group B and C when compared to the group A in both the tissues experimented in C. carpio for the present work. The increase in amount can be attributed to the feed additive spirulina which provides protein which is converted into stored...
lipids in the group B fishes and the healthy environment is provided to group C fishes by *L. sporogenes*. In the case of group D fishes, the amount is less compared to the control fishes. This is due to the toxicity of zinc. Similar results are reported by earlier investigators such as Levensgue et al. (2002); Dubale and Punita Shah, (1981) in the yellow perch *Perca flavescens* exposed to metal and on *Channa punctatus* and *Anguilla anguilla* exposed to cadmium.

The two transaminases are enhanced in all types of groups and it is more in zinc intoxicated fishes. The increased GOT correlates in general well with the severity of liver cellular damage (Hochachka and Somero, 1973; Kristoffersson et al., 1974). But there are evidence in gill tissues also. Due to the inhibitory effect of metals such as cadmium and copper, there has been alteration in these enzyme activities in the marine fish *Mugil sehnai* (Abou El-Naga et al., 2005). The increased transaminase enzyme activities in fish treated with high ammonia concentrations caused tissue damage such as gill cells (Nemedk et al., 1981)

The metal toxicant has enhanced the level of ALP and ACP in all the tissues of zinc intoxicated fishes. Stress induced the secretion of enzymes whereas in Spirulina fed fishes the level of ACP and ALP are below normal and the same is the result in *L. sporogenes* treated fishes. The present study also states that dietary supplementation and probiotic treatment improve food utilization and phosphatase activity in zinc contaminated *C. carpio* even within a short period of experimental treatment.

**Conclusion**

We can understand by this experiment that sub-lethal exposure to zinc proved to be toxic to *Cyprinus carpio* and induced many deleterious effects in the gill and brain tissues and affect the biochemical parameters of protein, free aminoacids, carbohydrates, lipids and enzymes such as GOT (AST), GPT (ALT), ACP and ALP. The heavy metal decreased the protein, free aminoacids and lipid content but increased the carbohydrates of the tissues. The feed additive *spirulina* create positive effects on the tissue biochemistry of the fish. The probiotic *Lactobacillus sporogenes* also has provided a healthy exterior and interior environment to the fish and improving the biochemical aspects of the fish.

**References**


