

# EFFECT OF AFLATOXIN ON TOTAL SERUM PROTEIN AND LIVER GLYCOGEN OF LABEO CALBASU

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**\*Corresponding author****ABSTRACT**

In the present investigation the effect of aflatoxin contaminated feed on liver glycogen, total serum protein, serum albumin and serum globulin has been studied on *Labeo calbasu* collected from river Chambal. A marked decrease in liver glycogen, total serum protein and a simultaneous increase in albumin globulin ratio were noted.

**INTRODUCTION**

Aflatoxin is a toxic compound produced as a secondary metabolite by fungi *Aspergillus flavus* and *Aspergillus parasiticus* on a variety of food products such as cotton seed, corn, wheat, milk, fish meal etc. It is an established potent carcinogenic immunosuppressive agent. Till now 18 different types of aflatoxin has been identified. Among them aflatoxin B1 is the most potent one and is found in maximum quantity in a culture as well as in food products. Due to the growth requirement of the fungi, aflatoxin poses a greater risk in warmer climate aflatoxicosis is the poisoning that results from ingestion of aflatoxin in contaminated food. Aflatoxicosis in fish is reported by many workers. Aflatoxin causes hepato cellular adenoma and hepato cellular carcinoma in fishes (Nunez et al., 1991) Gumbmen et al. (2004) reported decreased liver glycogen and elevated serum albumin globulin ratio in pig. Aflatoxin also produces hypoglycemia in fishes (Hussain et al., 2000; El-Boshy et al., 2008). The extent of damage produced by Aflatoxin depends upon the toxin concentration and time period of the exposure (Sephadari et al., 2010; Centroducat, 2009). Aflatoxicosis seems to be species specific in fishes. Toxic effect of aflatoxin seems to be species specific. Coulombe (1984) reported greater aflatoxin sensitivity in rainbow trout than in Coho salmon. Over the years plant based commercial diet has increasingly been used as fish feed. This has considerably increased the risk of aflatoxin contamination in fish feed.

*Labeo calbasu* is a widely distributed fresh water major Indian carp of family cyprinidae. It is a bottom dweller fish and is commercially important for his high quality flesh.

In the present work total serum protein and liver glycogen were estimated in order to explore the effect of aflatoxin on the physiology of the fish *Labeo calbasu*.

**MATERIALS AND METHODS**

The fish *Labeo calbasu* where collected from river Chambal near Dholpur. 48 fishes majoring 10-12 cm. and weighing 30-50g. were selected and kept in twelve aquaria is majoring  $2^1 \times 1^1 \times 1^1$ . Four fishes were kept in each aquarium. Three aquaria containing four fishes each were kept as control and nine aquaria containing four fishes each were kept as experimental set.

**Four treatments were employed as follows:**

- Feed I or good feed contained 0% moldy feed or unmixed feed. Feed I were given to control.
- Feed II contained 10% moldy feed and 90% good feed. Feed II were given to first set of experimental fishes comprising three aquaria 2A, 2B and 2C.
- Feed III contained 50% moldy feed and 50% good feed. Feed II were given to second set of experimental fishes comprising three aquaria 3A, 3B and 3C.
- Feed IV contained 100% moldy feed. Feed IV were given to third set of experimental fishes comprising three aquaria 4A, 4B and 4C.

Moldy feed were prepared in the laboratory. The commercial fish feed was first sprinkled with small amount of tap water to make the feed moist and then infected with cultured *Aspergillus flavus* by mixing it with 10 mL. of cultured *Aspergillus flavus*

obtained from department of Botany Veer Kunwar Singh University Arrah, Bihar. The inoculation was made in a transfer chamber to avoid contamination. The mixed feed was then covered with a plastic sack. The infected feeds were kept in a condition which is favourable for the growth of mold.

Required amount of moldy feed and good feed were weighed carefully for each treatment and then mixed thoroughly. The fish were fed a day after and daily thereafter two times a day at 8.00 AM. And at 6.00 PM. at a feeding rate of 4% of the body weight.

Blood sample was collected by inserting the syringe directly into the heart.

Total Serum protein were estimated by the method of Kingsley (1942) followed by Mehl (1945) and Weichselbaum (1946). The quantitative estimation of glycogen from liver was done according to a modified method of Kemp and Andrienne (1954).

## RESULTS AND DISCUSSION

### Liver glycogen

There is a gradual decline in the content of liver glycogen observed in the experimental fish. The content of liver glycogen in control was 31.07 + 2.12 mg/g and that of experimental fish fed with 100 percent moldy feed (feed IV) was 23.22 + 1.34 mg/g. (Table 1)

**Table 1: Showing effect of aflatoxin contaminated feed on serum protein and liver glycogen in *Labeo calbasu***

Treatment	Liver glycogen (mg/g)	Total serum protein (g/100 mL)	Serum albumin (g/100 mL)	Serum globulin(g/100 mL)
Feed I	31.07 + 2.12	4.11 + 0.51	2.98 + 0.39	1.13 + 0.29
Feed II	30.05 + 1.28	3.88 + 0.42	2.81 + 0.27	1.07 + 0.28
Feed III	26.92 + 3.12	3.52 + 0.26	2.68 + 0.42	0.84 + 0.14
Feed IV	23.22 + 1.34	3.12 + 0.34	2.54 + 0.15	0.58 + 0.12

The liver glycogen content gradually decrease with the increase in the percentage of moldy feed in the food of the fishes.

So the present findings are in agreement with that of Nunez et al. (1991) in rainbow trout (*Oncorhynchus mykiss*). El-Boshy et al. (2008) reported elevated serum glucose level in aflatoxin treated Nile tilapia (*Oreochromis niloticus*). Carbohydrates are stored as glycogen in liver and muscle of fish and liver glycogen is the major source of blood glucose as a result of glycongenesis. So in the present findings decrease in liver glycogen is due to increase in glycogenolysis. It is reported that induction of xenobiotics in the environment increases stress on the fishes. Cicik and Engin (2005) reported depletion of liver and muscle glycogen in *Cyprinus carpio* under the stress condition when it is utilized for detoxification purpose. Similar findings have been reported by Sarvanan et al. (2010) in *Labeo rohita*. Carbohydrates are the primary and immediate source of energy (Tiwary and Singh 2006). Kohli et al. (1975) reported xenobiotics induced hypoxia and increased energy demand in animals. In the present study, aflatoxin in the feed probably have created a condition of stress and a simultaneous increase in the energy demand in the fish which resulted in increase in utilization of carbohydrate to meet the energy demand. Nunez et al. (1991) reported increased

glycogen catabolism through glycolysis and pentose phosphate cycle in the liver of rainbow trout having hepato cellular adenoma and hepato cellular carcinoma when exposed to aflatoxin.

Aflatoxin causes loss of appetite (Cheeke and Shull, 1985). Thus the depletion of liver glycogen in the present study might have resulted from increase in glycogenolysis in liver to meet the energy demand under the condition of stress for detoxification purpose, by the hepatocyte necrosis and also due to loss of appetite.

### Serum protein

In the present study the maximum total serum protein was found in control and then a gradual decline was observed reaching its minimum in fish treated with hundred percent moldy feed. The present findings are in agreement with those of Hussain et al. (2000), in *O. niloticus*, Pepelnjak et al. (2003) in rainbow trout and Shehata et al. (2009) in *O. niloticus*. El-Boshy et al. (2008) reported elevation of blood urea in aflatoxin treated *O. niloticus*. An increase in utilization of protein results in increased concentration of blood urea. Martinez et al. (2004) reported that fish under stress may mobilize protein to meet energy requirement needed to sustain increased physiological activities.

Since fish have a very little amount of carbohydrate (Rao, 1999), the next alternative source of energy is protein to meet the increased energy demand under the condition of stress.

Buhler et al. (2000) reported that exposure to mycotoxin decreases the protein synthesis in *Oncorhynchus mykiss*. Thus in the present study decrease in the level of total serum protein were probably due to its increased utilization for the production of energy in the condition of stress or due to decrease in protein synthesis caused by the aflatoxin. Serum albumin and serum globulin level were also low in the treated fish group as compared to that of control group but the decline in serum globulin level is more as compared to that of serum albumin (Table 1).

Aflatoxin is a potent immuno suppressive agent in fish and antibodies are globulin in nature in the present studies decrease in serum globulin is probably due to immuno suppressive effect of aflatoxin which resulted in decrease in synthesis of globulin.

## REFERENCES

- Buhler, D. R., Miranda, C. L., Henderson, M. C., Yang, Y. H., Lee, S. J. and Wang Buhler, J. L. 2000. Effects of 17 beta estradiol and testosterone on hepatic mRNA \ protein levels and catalytic activities of CYP2M1 , CYP2K1 and CYP3A27 in rainbow trout (*Oncorhynchus mykiss*). *Toxico. Appl. Pharmacol.* **168**(2): 91-101.  
 Centroducat, G., Shantacroce, M. P., Lestingi, A., Casalino, E.,

- Crescenzo, G. 2009.** Characterization of cellular damage induced by aflatoxin B1 in Sea bream (*Sparus aurata* L. 1758.) hepatocytes. *Ital. J. Anim. Sci.* **8(Suppl. 2):** 848-850.
- Cheeke, P. K. and Shull, L. R. 1985.** Natural Toxicant in feeds and poisonous plants. Abi. Publishing company. ING. West Port. Connecticut.
- Coulombe, R. A., Jr, Bailey, G. S. and Nixon, J. E. 1984.** Comparative activation of aflatoxin B1 to mutagens by isolated hepatocytes from rainbow trout (*Salmo gaudneri*) and coho salmon (*Oncorhynchus kisutch*) carcinogenesis. **5:** 29-33.
- El-Boshy, M. E., A.M.M.EL- Ashram and Nadia A. Abdel-Ghany. 2008.** Effect of dietary beta-A, 3, glucagon on immunomodulation on diseased oreochromis niloticus experimentally infected with Aflatoxin B1. 8<sup>th</sup> International symposium on tilapia in aquaculture.
- Hussain, S. Y., Mekkawy, I. A., Moktar, Z. Z. and Mubarak, M. 2000.** Protective effect of Nigella Sativa seed against aflatoxicosis in oreochromis niloticus. Mycotoxin conference, Mycotoxin and environmental Poland. Bydgoszcz. 25-27. pp. 109-130.
- Kemp, A. and Andrienne, J. M. K. V. H. 1954.** A colorimetric micro method for the determination of glycogen in tissue. *Biochem. J.* **56:** 646-648.
- Kingsley 1942.** A colori metric method of blood analysis in " Hawk's physiological chemistry" Oser. B.L. (Ed.) Tata Mcgraw-Hill. New Delhi.
- Kohli, K. K., Sharma, S. C. Bhatia, S. C. and Venkita Subramonian, T. A. 1975.** Biochemical effects of chlorinated insecticides DDT and dieldrin. *J. Science and Industrial Research.* **34:** 462.
- Martinez, C.B.R., Nagae, M. Y., zaia, C. T. B. V. and Zaia, D. A. M. 2004.** Morphological and physiological acute effect of lead in the neotropical fish prochilodus lineatus. *Braz. J. Biol.* **64:** 797-807.
- Mehl, O. 1945.** A. colorimetric method of blood analysis . In "Hawks physiological chemistry". Oser. B.L. (Ed.) Tata Mc Graw-Hill New Delhi.
- Nunez, J. D. Hendriks and Duimishra, J. R. 1991.** Ultra structure of hepato cellular neoplasm in aflatoxin B1 (AFB1) – initiated rainbow trout (*oncorhynchus mykiss*). *Toxicol. Pathol.* **19(I):** 11-21.
- Pepeljnjak, S., Petrinec, Z., Kovack, S. and Segvic, M. 2003.** Screeningtoxicity study in young carp (*cyprinus carpio* L.) on feed amended with fumonisins B1. *Mycopathologia.* **156(2):** 139- 45.
- Rao, S. K.R.S. 1999.** Pesticide impact on fish metabolism . Discovery Publishing House. New Delhi, India. pp. 66-70.
- Sarvanan, M., Kumar, D. V., Malarvizhi, A. and Ramesh, M. 2010.** Biosafety of Azadirachta indica (A. Juss) leaves extracts on certain biochemical parameters of Labeo rohita. *J. Biopesticide.* **3(1 Special Issue)** 227-231.
- Sephdari, A., Ebrahimzadeh Mosavi, H.A., Sharif pour, I., Khosravi, A., Motallebi, A. A., Mohseni, M., Kakoolaki, S., Pourali, H. R. and Hallajian, A. 2010.** Effect of different dietary level of AFB1 on survival rat and growth factor of Beluga (*Huso huso*). *I. J.Fish. Sci.* **9(1):** 141-150.
- Shehata, S. A., EL. Melegy. Kh. M., Ebrahim, M. S. 2009.** Toxicity reduction of Aflatoxin B1 by vitamin –C in fish. *J. Arabian Aquaculture Society.* **4(2):** 73-82.
- Tiware, S. and Singh, A. 2006.** Biochemical stress response in fresh water fish channa punctatus induced by aqueous extracts of euphorbia tirucalli plant. *Chemosphere.* **64:** 36-42.
- Weichselbaum, C. 1946.** An improved procedure of protein estimation by Biuret reagent method. *J. Clin. Pathol.* **7:** 40-43.

