

Effect of Aflatoxin Contaminated Feed On Energy Reserves of Fish *Labeo rohita* (Hamilton)

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ABSTRACT

Effect of aflatoxin contaminated feed on glycogen content of liver, blood glucose, total serum protein and blood urea of the fish *Labeo rohita* was studied. The result revealed that administration of aflatoxin decreased liver glycogen and total serum protein but blood level of glucose and urea increased.

Key words: Aflatoxin, *Labeo rohita*, Blood glucose

INTRODUCTION

In Teleost the level of glucose and protein is an indicator of stress in fishes. The major source of blood glucose is liver glycogen. The level of urea in blood indicates the degree of damage to kidney and utilization of protein in the body. Aflatoxin is the metabolic by product of molds *Aspergillus flavus* and *Aspergillus parasiticus* on a variety of food such as cotton seed, corn, wheat, milk, fish meal etc. It is a toxic compound, a potent immunosuppressive and carcinogenic agent and the cause of high mortality in livestock, (Reed and Kasali., 1987, Montessano *et al.*, 1997). Due to growth requirement of the molds aflatoxin poses a greater risk in warmer climate. Effects of aflatoxin on fishes and other animals have been reported by many workers. Nunez *et al.* (1991) reported hepatocellular adenoma and hepatocellular carcinoma in Rainbow trout when exposed to aflatoxin B₁. Caguan *et al.* (2004) reported loss of appetite, low survival percent and decreased mean total biomass in tilapia when fed with aflatoxin contaminated feed. Faisal *et al.* (2008) reported spermatotoxic effect of aflatoxin in male wistar rat. Aflatoxin causes elevated blood glucose level in aflatoxin treated Nile Tilapia (El-Boshy *et al.*, 2008). The extent of damage produced by aflatoxin

depends upon the species, the toxin concentration and time period of the exposure (Coulombe *et al.*, 1984; Centroducati *et al.*, 2009; Sepahdari *et al.*, 2010). In the present investigation effect of aflatoxin on glycogen content of liver, blood glucose level, total serum protein and blood urea level of *Labeo rohita* has been evaluated in order to explore the effect of toxin in the fish.

MATERIALS AND METHODS

The fish *Labeo rohita* was collected from river Sone near Ara. 72 fishes measuring about 10-20 cm and weighing about 30 – 50 gm were selected and kept in twelve aquaria measuring 3' x 2' x 1'. Six fishes were kept in each aquarium. Three aquaria containing six fishes each were kept as control and nine aquaria containing six fishes each were kept as experimental set.

Four feeds 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 aquaria 2A, 2B and 2C.
- Feed III contained 50% good feed and 50% moldy feed. Feed III 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 fourth set of fishes comprising three aquaria 4A, 4B and 4C.

Moldy feed were prepared in 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 10 ml of cultured *Aspergillus flavus*. The inoculation was made in a transfer chamber to avoid contamination. The mixed feed was then covered with a plastic sack. The infected feed was kept in a condition which is favourable for the growth of mold.

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

The quantitative estimation of glycogen content of liver was estimated according to a modified method of Kemp and Andrein (1954). Blood glucose was estimated by O. Toluidine method of Cooper and McDanile (1970). Quantitative estimation of total serum protein was done according to the method of Kinsley (1942) followed by Mehl (1945) and Weichselbaum (1946). Blood urea was quantitatively estimated according to the Phenol hypochlorite method using the Berthelot Reaction (Fawcett and Scott 1960; Chaney and Marbach 1962).

RESULT AND DISCUSSION

The glycogen content of liver was 31.9 ± 0.24 mg/g in control and that of experimental fish fed with feed IV (100 % moldy feed) was 22.0 ± 0.24 mg/g. The blood glucose level was observed as 71.5 ± 0.69 mg/100ml in control and 101.3 ± 1.49 mg/100ml in fishes given feed IV. Thus there was a significant ($p > 0.05$) and gradual decline in the content of liver glycogen and a simultaneous rise in the blood glucose observed in the experiment fish

when aflatoxin contamination increased in the food. Thus the present findings are in agreement with those of Nunez *et al.* (1991) in *Oreochromis mykiss*, El- Boshy *et al.* (2008) Cicik and Engin (2005) reported depletion of liver and muscle glycogen in *Cyprinus carpio* under the condition of stress, when it is utilized for detoxification process. Nunez *et al.* (1991) reported increased glycogen catabolism through glycogenolysis and pentose phosphate pathways having hepatocellular carcinoma and hepatocellular adenoma when exposed to aflatoxin. El- boshy *et al.* (2008) reported increased blood glucose level in aflatoxin treated *Oreochromis niloticus*.

Blood glucose level closely correlate to stress level in fish and represent the state of respiratory and nutritional disturbance (Hideaki., 2009; Porchse *et al.*, 2009; Mehmet and Sen., 2011). Zaki (2012) reported significant increase in cortisol level and significant decrease in insulin level in aflatoxin treated fish *Clarius lazera*. An increase in cortisol level increases glycogenolysis in liver. In the present investigation liver glycogen level is low and blood glucose level is high in fishes fed with aflatoxin contaminated feed which indicates that the increase in blood glucose level is probably due to increased liver glycogenolysis. Also the increase in blood glucose level may also be attributed to decrease in secretion of insulin as an effect of aflatoxin, which in turn reduces the process of glycogenesis resulting in rise of blood glucose level. In addition the rise in blood glucose many also be due to gluconeogenic breakdown of protein to form glucose for energy production to be utilized in detoxification process at the time of stress induced by aflatoxin.

Thus in the present investigation depletion of liver glycogen and increase in blood glucose might have resulted due to increased glycogenolysis, gluconeogenesis and decreased glycogenesis as an effect of dietary aflatoxin mediated by increased cortisol and decreased insulin level under the condition of stress.

In the present study there was a gradual fall in total serum protein and a simultaneous rise in the blood urea level with the increase in the aflatoxin contaminated feed in the food. The present findings are in agreement with Pepeljnjak *et al.* (2003)

Treatments	Feed I	Feed II	Feed III	Feed IV
Liverglycogen(mg/g)	31.9±0.24	30.5±0.23	25.7±0.25	22.0±0.24
BloodGlucose(mg/100ml)	71.5±0.69	77.4±0.64	87.2±1.08	101.3±1.49
Bloodurea(mg/100ml)	2.40±0.01	2.86±0.01	3.42±0.01	4.57±0.04
TotalSerum Protein(mg/100ml)	4.33±0.10	3.93±0.04	3.59±0.02	3.20±0.02

inrainbow trout,El-Boshy *et al.* (2008) and Shehata *et al.*(2009) in *O.niloticus*.Martinez *et al.* (2004) reported that fish under stress may mobilize protein to meet energy requirement needed to sustain increase physiological activities.

Since fishes 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 protein synthesis in *Onchorhynchus mykiss*.Aflatoxin causes loss of appetite resulted

in hypophagia and this might have also caused the decrease in total serum protein level.

The serum urea level showed an increase with increase in aflatoxin contaminated feed. A rise in Blood urea level indicates to abnormal kidney function(Newberne 1981). Similar findings were reported by Zaki *et al.* (2008, 2010) in aflatoxin treated fishes *Tilapia nilotica* and *Tilapia zilli* . Increase in urea level due to necrosis in kidney was reported by Mansfeld (1989), Pier(1987). Thus in the present studies aflatoxin might have caused necrosis in kidney resulting in increase in urea level.

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