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STUDIES ON EFFECTS OF DIETARY AFLATOXIN ON CERTAIN BIOCHEMICAL PARAMETERS OF THE FISH *CLARIAS BATRACHUS*

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ABSTRACT

Studies were conducted to determine the effects of different doses of aflatoxin B1 contaminated feed on total serum protein, blood glucose, SGPT, SGOT and serum bilirubin. There was a significant decrease in total serum protein but blood glucose, serum bilirubin, SGPT and SGOT showed a gradual rise with the increase in contamination of aflatoxin in the feeds.

INTRODUCTION

Aquaculture in the world has shown a rapid rise in the past year. However rising in fish farming is also associated with risk for spread of infectious diseases. Several factors such as decrease in water quality, increase in contamination and decrease in food quality can affect the fish health (Nomoto.k, 2005). One of the potential threat to fish farming is contamination of commercial fish feed with aflatoxin producing molds, *Aspergillus flavus* and *Aspergillus parasiticus* which can rapidly grow on improperly stored food (Cheeke and Shull, 1985). Aflatoxins are group of compounds secreted by these fungi as metabolic by products (Handan and Guleray, 2005). Among four major type of aflatoxin B_1, B_2, G_1 and G_2 produced by *A. flavus* and *A. parasiticus* (Kurtzman *et al.*, 1987, Kosalec and Pipeljnjak, 2005), aflatoxin B_1 is the most potent and found in maximum quantity in culture(Yu, 2012) . It is a potential immunosuppressive and carcinogenic agent and cause of high mortality in animals and in some cases of human beings (Reed and Casali, 1987, Montessano *et al*., 1995, Bbosa *et al*., 2013).

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The principal target organ is the liver where it causes infiltration of lipids into hepatocytes leading to necrosis and cell death (Joner 2000).The fatal characteristics of this toxin is attributed to its resistance to both heating and freezing which enable it to remain in food chain for indefinite period and reach human beings also (Zaki *et al.*,2011). Aflatoxicosis results when fish feed upon aflatoxin contaminated feeds (Ashley, 1970). The toxic effect of aflatoxin in fishes depends upon the species, doses of the toxin as well as the exposure time (Coulombe *et al.*, 1984, Ngethe *et al.*, 1993, Centoducati *et al.*, 2009). Chronic exposure to aflatoxin B_1 in fishes results in poor growth, increased mortality, hepatocyte necrosis, hepatocellular sarcoma, hepatocellular carcinoma and immuno suppression (Nunez *et al.,* 1991; Caguan *et al.*, 2004; Sepahdari *et al.,* 2010; Zaki *et al.*, 2012; Selim *et al.,* 2013). The Asian cat fish popularly known as Magur is an important fish owing to its taste and excellent nutritional profile (Rui *et al*., 2007). It is frequently prescribed among the lactating and pregnant women and for anaemic and malnutrition individuals (Debnath *et al.,* 2011). But the fish is showing drastic decline from their natural habitats in india during the last few years due to various environmental factors (Khedkar *et al.,* 2014). Liver function enzymes such as ALT (SGPT), AST (SGOT), serum bilirubin, total serum protein and blood glucose are the

indicators of effects of a toxic compound in various animals including fish .The objective of the present study was to explore the effects of aflatoxin contaminated feeds on *Clarias batrachus*.

MATERIALS AND METHODS

A total 72 apparently healthy *Clarias batrachus* were obtained from private fish farm at Dholpur district of Rajasthan. The length of the fish was about 10 to 20 cm and the weight was about 35 to 55 grams. The fishes were kept in twelve aquaria measuring 2^1 X 1^1 X 1^1. Six fishes were kept in each aquarium. Three aquaria were kept as control and nine aquaria were divided into three sets of three aquaria each and kept as experimental sets.

Preparation of Feed

Four types of feeds were prepared for the fishes depend upon the percentage of contaminated feed and they were distinguished as Feed I, Feed II, Feed III and Feed IV.

Feed I or good feed contained 100 percent good feed and no mouldy feed. Feed I were given to control or fishes of first set of aquaria comprising IA, IB and IC.

Feed II consisted of 90 percent good feed and 10 percent mouldy feed. Feed II were given to second set of aquaria comprising 2A, 2B and 2C.

Feed III contained 50 percent mouldy feed and 50 percent good feed. Feed III were given to fishes of third set of aquaria comprising of 3A, 3B and 3C.

Feed IV contained 100 percent mouldy feed. Feed IV was given to fishes of fourth set of aquaria comprising 4A, 4B and 4C.

Mouldy feed was prepared in laboratory. The commercial fish feed procured from market was first sprinkled with small amount of tap water to make the feed moist and then mixed with cultured *Aspergillus flavus* procured from ICAR New Delhi. The inoculation was made in a transfer chamber to avoid contamination.

The mixed feed was then covered with a plastic sac. The infected feed was kept in a condition which is favourable for the growth of mould. Required amount of mouldy feed and good feed were weighed carefully for each treatment and then mixed thoroughly. The feeding was started from the second day two times a day at a feeding rate of 4% of the body weight.

- Quantitative estimations of SGOT (ALT) and SGPT (AST) were carried out by the methods of Karmen (1955) and Wroblewsky & La Due (1956) respectively.
- Quantitative estimation of serum bilirubin was done by the method of Evelyne and Malloy (1937).
- Quantitative estimation of blood glucose was made by O- Toluidine method of Cooper and McDanile (1970).
- Quantitative analysis of total serum protein was carried out by the method of Kingsley (1942) followed by Mehl (1945) and Weichselbaum (1946).

Statistical analysis: Statistical analysis of biochemical parameters was carried out by the method of Analysis of Variance (ANOVA.)

RESULTS AND DISCUSSION

Blood glucose: There was a significant ($p>0.05$) and gradual rise in the level of blood glucose with increase in contamination of aflatoxin in the feeds of the fishes. The level of blood glucose was 69.9 $\pm$ 2.22 in control whereas that of fishes fed with feed IV was 95.5 $\pm$4.11 (table 1).

Total serum Protein

Total serum protein showed a steady decline with the increase in aflatoxin contamination in the feed. The maximum total serum protein 4.84 $\pm$0.18 g/100ml was found in control whereas the minimum 3.25 $\pm$ 0.08 g/100ml was found in the set of experimental fishes which were fed with Feed IV.

SGPT (ALT), SGOT (AST) and Serum Bilirubin

Serum levels of Alanine transaminase (ALT), Aspartate transaminase (AST) and bilirubin also revealed significant ($p > 0.05$) and gradual rise with increase in the contamination of aflatoxin in the fish feed.

Table 1. Effect of dietary aflatoxin on Biochemical parameters of *Clarias batrachus*

Feed	Total serum protein (g/100ml)	Blood Glucose (mg/100ml)	Serum bilirubin (mg/100 ml)	SGOT (IU/L)	SGPT(IU/L)
Feed I	4.84 $\pm$ 0.18	69.9 $\pm$ 2.22	0.65 $\pm$ 0.03	84.8 $\pm$ 2.44	19.3 $\pm$ 0.80
Feed II	4.19 $\pm$ 0.21	79.3 $\pm$ 1.13	0.77 $\pm$ 0.04	89.6 $\pm$ 3.41	20.8 $\pm$ 0.80
Feed III	3.57 $\pm$ 0.15	86.2 $\pm$ 1.73	0.97 $\pm$ 0.05	103.9 $\pm$ 3.45	23.5 $\pm$ 0.76
Feed IV	3.25 $\pm$ 0.08	95.5 $\pm$ 4.11	1.28 $\pm$ 0.03	128.4 $\pm$ 2.0	27.7 $\pm$ 0.86
Total mean $\pm$SD	3.96 $\pm$ 0.70	82.7 $\pm$ 10.79	0.83 $\pm$ 0.28	101.6 $\pm$ 19.5	22.8 $\pm$ 3.6

Table 2. Coefficient of correlation and coefficient of variation % in the biochemical parameters in *Clarias batrachus* exposed to dietary aflatoxin

Parameters	Coefficient of variation %	Coefficient of correlation
Blood glucose	13	(+) 0.10
Total Serum Protein	17.6	(-) 0.69
Serum bilirubin	33	(+) 0.36
SGPT	16	(+) 0.14
SGOT	19.2	(+) 0.16

The minimum level of ALT, AST and bilirubin in the serum 19.3 ± 0.8 IU/L, 84.8 ±2.44 IU/L and 0.65±0.03 mg/100ml respectively were found in the control the maximum level of these parameters 27.7 ±0.86 IU/L, 128.4 ±2.0 IU/L and 1.28 ± 0.03 mg/100ml respectively were observed in fishes fed with feed IV or hundred percent moldy feed (Table 1).

DISCUSSION

Blood glucose

There was a gradual and significant (p>0.05) rise in the level of blood glucose with increase in the contamination of aflatoxin in the feed. The result agrees with the findings of EL-Boshy *et al*. (2008) in *O. niloticus*. Hyperglysemia in fishes are a part of stress response (Winkaler *et al.*, 2007, Mousa *et al.,* 2007). Glucose is the immediate source of energy and increase under the condition of stress to meet the increased energy demand as a result of hypoxia induced due to stress (Tiwary *et al.*, 2006, Sarvanan *et al.,* 2010, Coban *et al.*, 2011). Zaki *et al*. (2011) reported increased cortisol and decreased insulin levels *in Clarias lazera* when exposed to aflatoxin. Fatmi *et al*. (2011) reported depletion of liver glycogen in *Labeo calbasu* when treated with aflatoxin contaminated feed. Thus in the present investigation increase in blood glucose level may be attributed to increased glycogenolysis, gluconeogenesis and decreased glycogenesis as a result of alteration in the levels of insulin and cortisol as well as the increased demand of energy formation under the condition of stress induced by aflatoxin.

Total serum protein

There was a gradual and significant (p>0.05) decrease in the total serum protein as the contamination of aflatoxin increased in the feed of the fish. The present investigation is in accordance with those of Pepeljnjak *et al.* (2003), EL- Boshy *et al*. (2008), Shehata *et al*. (2009); Ruby *et al*. (2014) and Hassan *et al*. (2014). Aflatoxin decreases protein synthesis at both transcription and translation level (Buhler *et al.,* 2000, Joner *et al.,* 2000, Bbosa *et al.,* 2013). Fishes have very little amount of carbohydrate (Rao 1999) and may utilize protein as alternative source of energy to meet the increased energy demand under the condition of stress (Martinez *et al.,* 2004). Thus in the present studies decrease in total serum protein may be due to decrease in its synthesis as well as increased utilization for energy formation through gluconeogenic pathways in the condition of toxic stress as a result of aflatoxin.

SGPT (ALT), SGOT (AST) and bilirubin

There was a significant (p >0.05) increase in serum alanine transaminase (ALT) and aspartate transaminase (AST) in experimental group as compared to control. The present findings are in agreement with those of Kheir eldin *et al.* (2008), Centoducati *et al.* (2009), Selim *et al.* (2013) and Mahfouz *et al*. (2015). Previous findings suggested hepatocyte necrosis (Caguan *et al.,* 2004; Lewis *et al.,* 2005; Kenawy *et al.,* 2009) and hepatocelluler lipid deposition (Zycowski *et al.,* 2013) in fishes exposed to aflatoxin. Any increase in these enzymes in serum is an indicator of cellular damage (Palanivelu *et al.,* 2005). Varior and Philips (2012) reported that aflatoxin B_1 significantly alters the stability of lysosomal membrane and thereby alters the permeability of hepatocytes resulting in high levels of ALT and AST in the serum. Thus in the present findings increase in serum ALT and AST in experimental fishes are probably due to hepatocyte degeneration, altered hepatic function as a result of exposure to aflatoxin. Serum bilirubin also showed a significant (p>0.05) rise in experimental fishes as compared to control. The rise was in proportion to the increase in aflatoxin contamination in the feed. The present findings are in accordance with those of Rizvi *et al. (*2000) in broiler chicken. The rise in the level of serum bilirubin is an indicator of abnormal liver function. Sepahdari *et al.* (2010) reported hepatocyte degeneration in Beluga (*Huso huso*) when exposed to aflatoxin. Caguan *et al.* (2004) reported yellowing of the body of aflatoxin treated Nile tilapia indicating an increased serum bilirubin level. Administration of aflatoxin increases the size of RBC and thus enhances its break down during passage through reticuloendothelial cells (Verma *et al*., 1989). Thus the increase in serum bilirubin may also be due to enhanced break down of RBC as a result of aflatoxin.

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