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Studies on effects of dietary aflatoxin on biochemical and haematological parameters of the fish *Labeo rohita*.

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ABSTRACT

Studies were conducted to determine the effects of different doses of aflatoxin contaminated feed on hematological parameters of the fish Labeo rohita. There was a significant decrease in RBC count, hemoglobin content, PCV, MCH, MCV and MCHC and a simultaneous increase in SGPT, SGOT, Serum Bilirubin and Serum Cholesterol with increasing contamination of aflatoxin in the feed of the fish.

Keywords: Aflatoxin, RBC count, MCV, MCH, PCV MCHC, SGPT SGOT Hemoglobin content.

INTRODUCTION

The aquaculture has shown a rapid rise in the past years (Jana .H 2016, subasinghe *et. al.,* 2009). However extensive fish farming is also associated with risk of spread of infectious diseases, decrease in water quality, increase of contamination and decrease of food quality which can effects the fish health(Nomoto. K. 2005). One of the risks which are associated with aquaculture and fish farming is aflatoxicosis in fish as a result of exposure to aflatoxin (Santacroce *et. al.*,2008). Exposure is mainly associated with contaminate feed used for fish farming.Aflatoxins are compounds predominantly produced by two molds *Aspergillus flavus* and *Aspergillus parasiticus* (Oliviera *et. al.*, 2013). These molds grow on improperly stored food(Cheeke and Shull 1985, Ellis *et.al.,* 2000) thus have access to fish feed also (Evalyn *et.al.*,2018). These molds aproduce four types of aflatoxin B_1,B_2,G_1.G_2(Kurtzman *et.al.*, 1987, Kosalec and Pipeljnjak 2005).Among them aflatoxin B_1 is the most fatal and found in maximum quantity in the culture (Yu,2012).It shows resistance to both heating and freezing which enable it to remain in food chain for indefinite period of time and also reach human beings Zaki *et. al.*, 2011) and the toxic effects depend upon the species, dose of the toxin as well as the time of exposure (Columbe *et. al.*, 1984, Ngethe *et. al.*,1993, Centoducati 1993). The principal target organ is liver and time exposure to aflatoxin adversely effects

growth, increase mortality, causes immunosuppressive effects, kidney dysfunction, hepatocellular sarcoma and hepatocellular carcinoma (Nunez *et. al*., 1991, Joner 2000, Caguan *et. al*., 2004, Sepahdari *et. al*.,2010, Zaki *et. al*., 2012, Selim *et. al*., 2013, Mehfouz *et. al.*, 2015, Nunez *et al*.,2019). Biochemical and hematological parameters are the most important indicators of health status and toxic effects of a xenobiotics in various animals including fish.

The objective of the present investigation was to explore the effect of aflatoxin contaminated feed on biochemical and haematological parameters of the fish.

MATERIALS AND METHODS

A total of 72 apparently healthy *Labeo rohita* were obtained from private fish farm at Arrah. The length of fishes was about 10 to 20 cm and the weight was about 35 to 55 grams. The fishes were kept in twelve aquaria measuring 2^1X 1^1 X1^1 .Six fishes were kept in each aquarium. Three aquaria were kept as control and nine aquaria were divided into three sets. Each set consisted of three aquaria and kept as experimental sets.

Preparation of feed

Four types of feeds were prepared for the fishes on the basis of percentage of contaminated feed present in them 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 moldy 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 moldy feed. Feed II were given to fishes of second set of Aquaria comprising 2A 2B and 2C.

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

Feed IV was made of 100 percent moldy feed. Feed IV was given to fishes of fourth sets of aquaria comprising 4A 4B and 4C.

Moldy feed was prepared in the laboratory. The commercial fish feed was procured from market was first sprinkled with small amount of water to make the feed moist and the 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 growth of the mould. Required amount of moldy 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.

RBC count was carried out by the method of Blaxhall and Daisley(1973).

Hemoglobin was estimated by Cyanmethemoglobin method (Blaxhall and Daisley,1973;Dacie and Lewis, 1991).

Serum Cholesterol was estimated by the method of Zak (1957)

Serum AST/SGOT was estimated by the method of karmen (1955)

Serum ALT/SGPT was estimated by the method of Wroblewski and La Due (1956)

Serum Billirubin was estimated by the method of Evelyne and Malloy (1937)

Packed cell Volume (PCV) was determined by the microhematocrit technique of Blaxhall and Daisley (1973).

The following RBC indices were calculated according to the method of Dacie and Lewis (1975).

$$\text{MCV(fl)} = \frac{\text{PVC}}{\text{RBC (million/L)}} \text{ X100}$$

$$\text{MCH(pg)} = \frac{\text{Hemoglobin (g/L)}}{\text{RBC (million/L)}}$$

$$\text{MCHC(\%)} = \frac{\text{Hemoglobin (g/L)}}{\text{PCV}} \text{ X100}$$

Statical analysis

Biostatical analysis of the parameters was carried out by the method of analysis of variance (ANOVA).

RESULT AND DISCUSSION

SGPT (SERUM ALT) AND SGOT (SERUM AST)

In the present investigation the level of SGPT and SGOT was significantly high($p>0.05$) in fishes fed with feed II, III and feed IV or aflatoxin containing feed as compared to the fish group given feed I which contained no aflatoxinThus the present findings are in agreement with those of Adel *et al.* (2008), Nogaim *et al.* (2011) and Zaki *et al.(*2008,2011).

Liver is the premium site of aflatoxin metabolism where it causes maximum damage.SGPT(ALT) and SGOT(AST) are enzymes located in liver and leaks out into general circulation when liver cells are damaged raising their level in blood(Schmidt and Schmidt, 1973).Nunez and Duimishra (1991) reported hepatocelluler adenoma and hepatocelluler carcinoma in rainbow trout when exposed to aflatoxin Susan *et al.* (2010) reported hepatocyte necrosis and autolysis and a simultaneous rise in level of SGPT and SGOT in *Labeo rohita* when treated with fenvalerate a synthetic pyrethroid .Thus in the present investigation, rise in the levels of SGPT and SGOT in these fish group might be due to hepatocyte necrosis and autolysis as a result of exposure to aflatoxin.

The degree of damage of liver cells are directly proportional to the levels of SGPT and SGOT and it is evident from the present findings that the increase in percentage of moldy feed or the amount of aflatoxin increases the extent of damage to liver of the experimental fishes.

Serum bilirubin

AS evident from the Table there was a significant rise($p>0.05$) in serum bilirubin level in the fishes fed with aflatoxin contaminated feed as compared to control. The rise in the level of serum bilirubin was in proportion to the increase in the amount of aflatoxin contaminated feed in the food of the fishes .Thus the present finding are in agreement 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,Caguan *et. al.,*2004). Hepatocyte degeneration causes the release of bilirubin in general circulation and a simultaneous rise in its level in blood. Increase in level of serum bilirubin also indicates an increased break down in RBC and hemoglobin. In the present finding there was a decrease in RBC count and hemoglobin percentage in the fishes fed with aflatoxin contaminated feed. Thus in the present studies the rise in serum bilirubin was probably due to hepatocyte degeneration and destruction and also due to break down of RBC and hemoglobin as a result of aflatoxin.

Serum cholesterol

Serum Cholesterol level showed a gradual and significant rise with the rise in the content of aflaxin with feed (Table-1). So the present findings are in agreement with those of Zaki *et al*.(2008).Cholesterol acts an antioxidant and as a free radical scavenger. Thus the increase in serum cholesterol was probably due to increase in its synthesis as a result of aflatoxin.

Hemoglobin percent ,rbc count and rbc indices

In the present study there was an steady and significant decrease in the hemoglobin percent with increase in the percentage of moldy feed in the experimental fishes . (Table).Similar findings were reported by Zaki *et al*. (2011) in aflatoxin treated cat fish *Clarias lazera*.

In the present study there was a significant effect of aflatoxin on RBC Count, WBC count, hemoglobin content as well as other RBC parameters such as packed cell volume (PCV) Mean Corpuscular haemoglobin (MCH) Mean Corpuscular Volume (MCV) and mean corpuscular hemoglobin concentration (MCHC).

Table 1: Showing effects of aflatoxin on biochemical and Hematological parameters of Labeo rohita

FEED	Feed I	Feed II	Feed III	Feed IV	Total Mean ± SE
SGPT(IU/L)	18.2± 0.19	19.9 ± 0.19	22.4 ± 0.21	26.2 ± 0.51	21.6 ± 1.54
SGOT(IU/L)	82.0 ± 0.65	85.7 ± 0.57	100.7 ± 1.21	125.6 ± 1.04	98.5 ± 8.57
Serum Bilirubin (mg/dl)	0.58 ± 0.01	0.68 ± 0.07	0.90 ± 0.01	1.18 ± 0.07	0.83 ± 0.11
Serum Cholesterol (mg/dl)	151.1 ± 0.45	165.8 ± 1.52	185.9 ± 0.54	202.9 ± 1.62	176.4 ± 9.8
Hemoglobin(gm/dl)	8.05 ± 0.02	7.06 ± 0.02	6.25 ± 0.03	4.50 ± 0.12	6.46 ± 0.65
RBC (million/µl)	1.76 ± 0.07	1.66 ± 0.07	1.56 ± 0.01	1.24 ± 0.02	1.55 ± 0.09

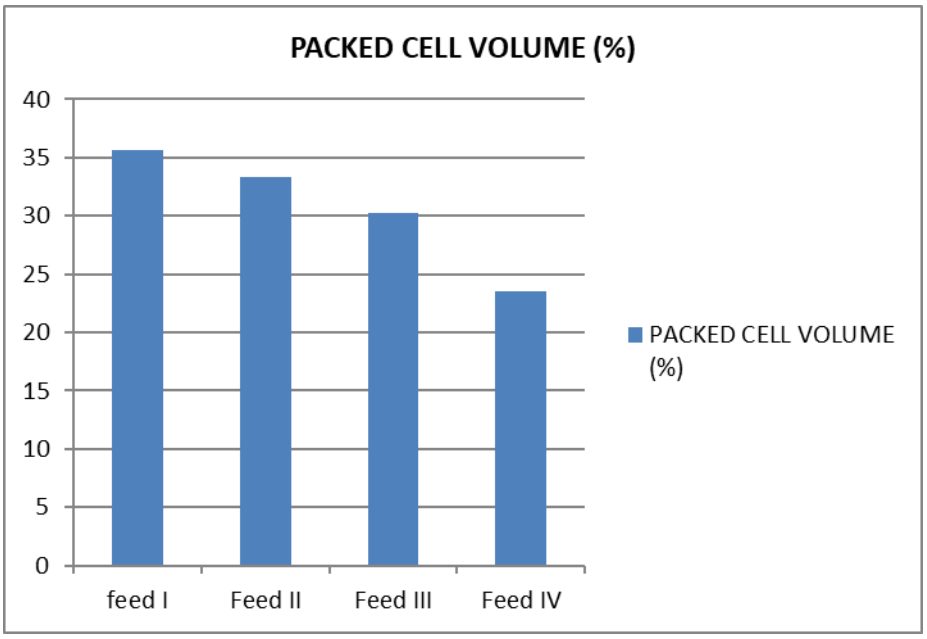

Fig.1 showing effect of Aflatoxin on packed cell volume (PCV) in the fish

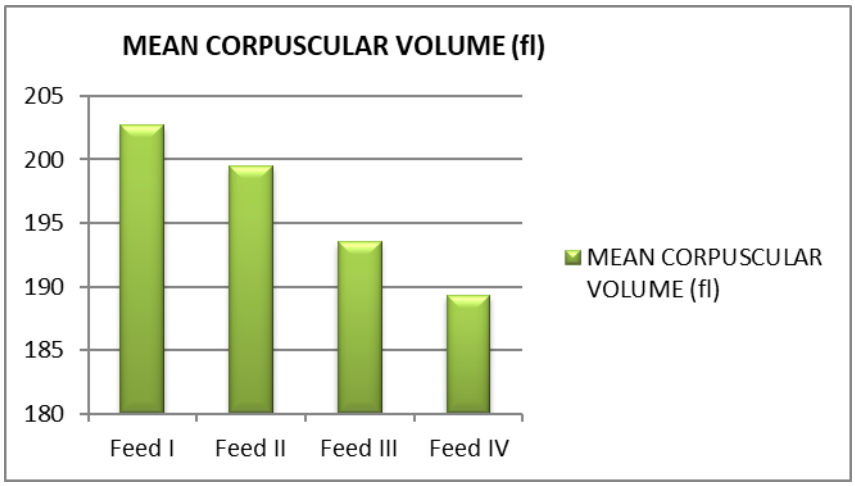

Fig.2 showing effect of Aflatoxin on Mean Corpuscular Volume (MCV) in the fish

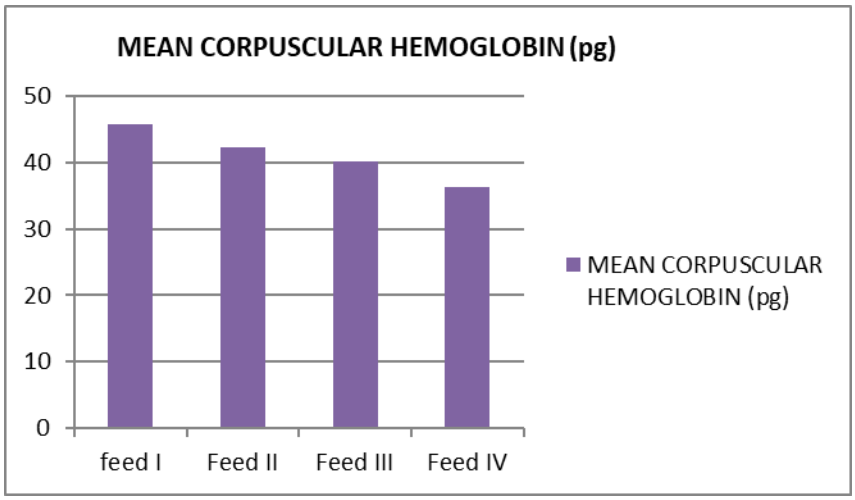

Fig.3 showing effect of Aflatoxin on Mean Corpuscular Hemoglobin (MCH) in the fish

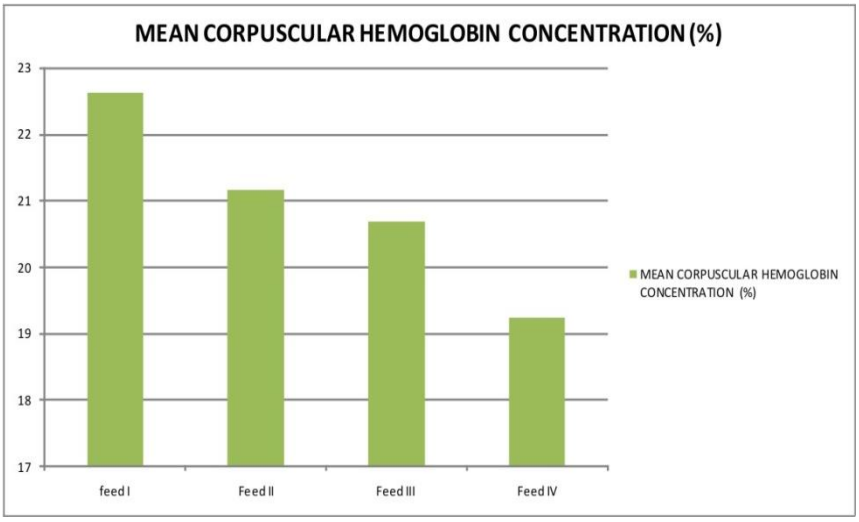

Fig.4 showing effect of Aflatoxin on Mean Corpuscular Hemoglobin Concentration (MCHC) in the fish

The results of RBC Count, haemoglobin content as well as RBC Indices like PCV, MCV, MCH MCHC are shown in the table I and fig. 1-5. These haematological parameters showed a significant ($p>0.05$) decrease in experimental fish as compared to control . The present findings agree with those of Nurcan *et. al.,* (2012), Selim *et. al., (*2013) and Mahfouz *et. al.,*(2015) and Palaniswamy (2018).

The decrease in these parameters indicates anaemia. AFB_1 causes hepatocellular carcinoma and anaemia is one of the symptoms of HCC (Murrary – Lyon 1983). The decrease in RBC count, hemoglobin percent, MCV and MCHC indicate anaemia. It may be due to inhibition of those enzymes which are responsible for synthesis of heme (ATSDR 2005). Serum protein level decreases in fish groups which were fed with aflatoxin contaminated food indicating decreased protein synthesis (Ruby *et. al*., 2014). So the decline in hemoglobin percent in the present investigation may also be attributed to decrease in synthesis of globin fraction of hemoglobin.Thus decrease in hemoglobin and RBC count may be due to many factors such as decreased protein synthesisIt may also be occurred due to hemopoitic, hepatic and osmoregulatory disfunction as a result of aflatoxin (Jantrarotai and Lovel 1990, Pepeljnjak *et. al*.,2003, Caguan 2004, Centoducati *et. al.,* 2009 Sepahdari *et. al*., 2010, Deng 2010, Selim *et. al*., 2014, Mahfouz and Sherif 2015).The decrease in these parameters may also be due to increased destruction of RBC in spleen and hemopoitic organs as a result of exposure to aflatoxin (Verma and Raval 1989, Jenkins and Smith2003).

CONCLUSION

The present study brought about information on the effect of dietary aflatoxin on L. rohita. It was found that aflatoxin present in feed causes liver damage and destruction of RBCs in the fish. Decrease in serum cholesterol was due to the aflatoxin in the feed. It would be pertinent to investigate the residual aflatoxin in the flesh of the fish which is being used as food thus may have implication on human beings as well.

Conflict of Interest

The author declares that there is no conflict of interest.

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