STUDIES ON EFFECTS OF DIETARY AFLATOXIN ON HAEMATOLOGICAL PARAMETERS OF THE FISH CLARIAS BATRACHUS.

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ABSTRACT

Studies were conducted to determine the effects of different doses of aflatoxin contaminated feed on haematological parameters of the fish clarias batrachus. There was a significant decrease in RBC count, WBC count, hemoglobin content, PCV, MCH,MCV and MCHC with increasing contamination of aflatoxin in the feed of the fish.

Keywords: Aflatoxin, RBC count, WBC count, haemoglobin content.

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 deseases , decrease in water quality,increase of contamination and decrease of food quality which can effect 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). 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) and produce 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 immunosuppression, kidney disfunction , 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). The Asian cat fish popularly known as Mangur 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 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 (Khedkar *et. al.*, 2014) .Haematological parameters are the most important indicators of health status and toxic effects of a xenobiotic in various animals including fish.

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

Materials and methods

A total of 72 apparently healthy *Clarias batrachus* were obtained from private fish farm at Dholpur district of Rajasthan. 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 set 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).

WBC count was done by the method of Shah and Altindag (2005).

Differential Leucocyte Count(DLC) was carried out by the method of Lilli(1972) using Wright Giemsa stain for the preparation of blood film.

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

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

$$\text{MCV(fl)} = \frac{PCV}{RBC(million/L)\ X\ 100}$$

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

$$\text{MCHC(\%)} = \frac{Hemoglobin(g/dl)\ X\ 100}{PCV}$$

Statical analysis

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

Results and discussion

Feed	RBC(million/mm$^{3)}$	WBC(thousand/mm$^{3)}$	Hemoglobin(g/dl)
Feed I	1.98 ± 1.24	11.05 ± 0.36	7.10 ± 0.15
Feed II	1.86 ± 0.95	9.80 ± 0.20	6.07 ± 0.10
Feed II	1.75 ± 0.04	8.95 ± 0.13	5.11 ± 0.17
Feed IV	1.44 ± 0.02	5.89 ± 0.42	3.31 ± 0.12

Table 1 Showing effect of dietary aflatoxin on haematological parameters of Clarias batrachus.

Feed	Eosinophils (%)	Basophils (%)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
Feed I	1.33 ± 0.68	0.27 ± 0.18	22.00 ± 2.54	75.50 ± 2.84	0.88 ± 0.76
Feed II	1.60 ± 0.63	0.20 ± 0.45	24.33 ± 2.25	73.26 ± 2.18	0.60 ± 0.63
Feed II	1.45 ± 0.52	0.18 ± 0.40	32.54 ± 2.01	65.10 ± 2.70	0.72 ± 0.46
Feed IV	2.00 ± 0.25	0.28 ± 0.47	36.28 ± 1.80	60.57 ± 1.29	0.85 ± 0.50

Table II- Showing effect of dietary aflatoxin on Differential Leucocyte Count of Clarias batrachus.

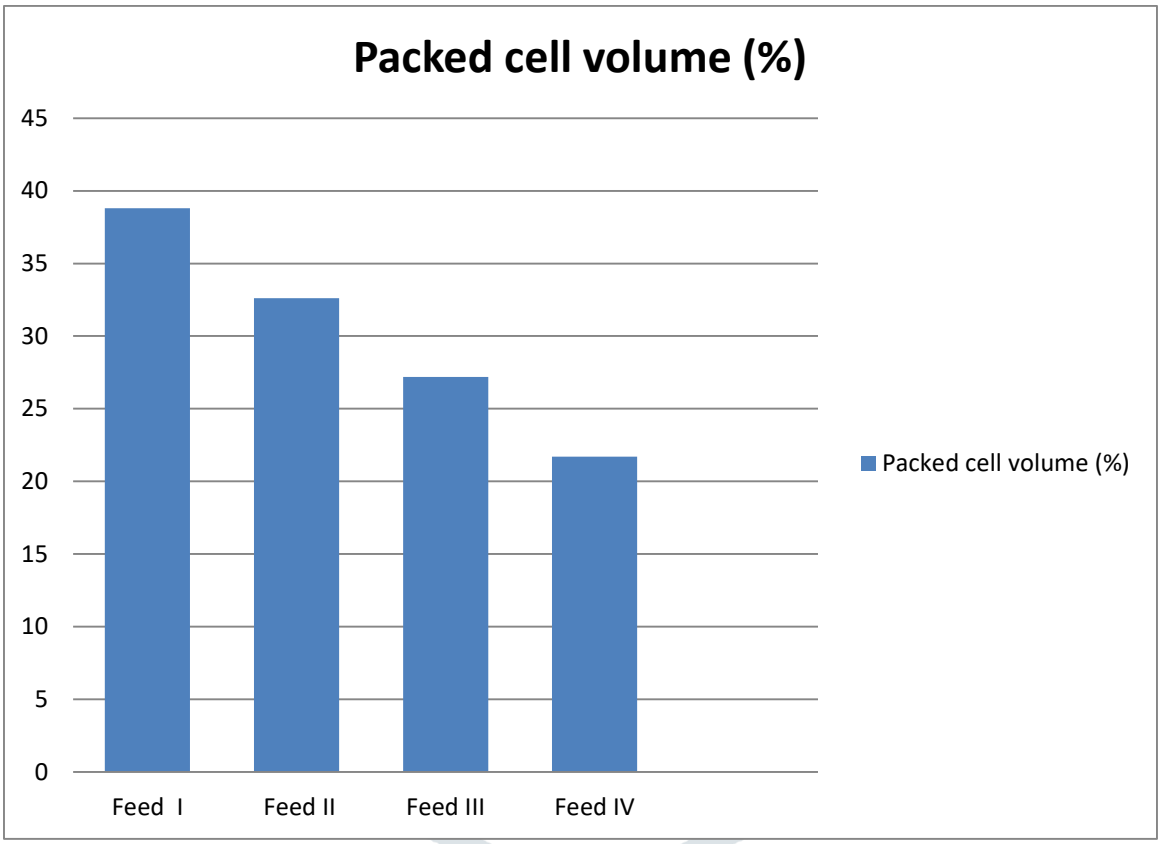

Fig. 1: Showing effect of aflatoxin on paked cell volume of the fish.

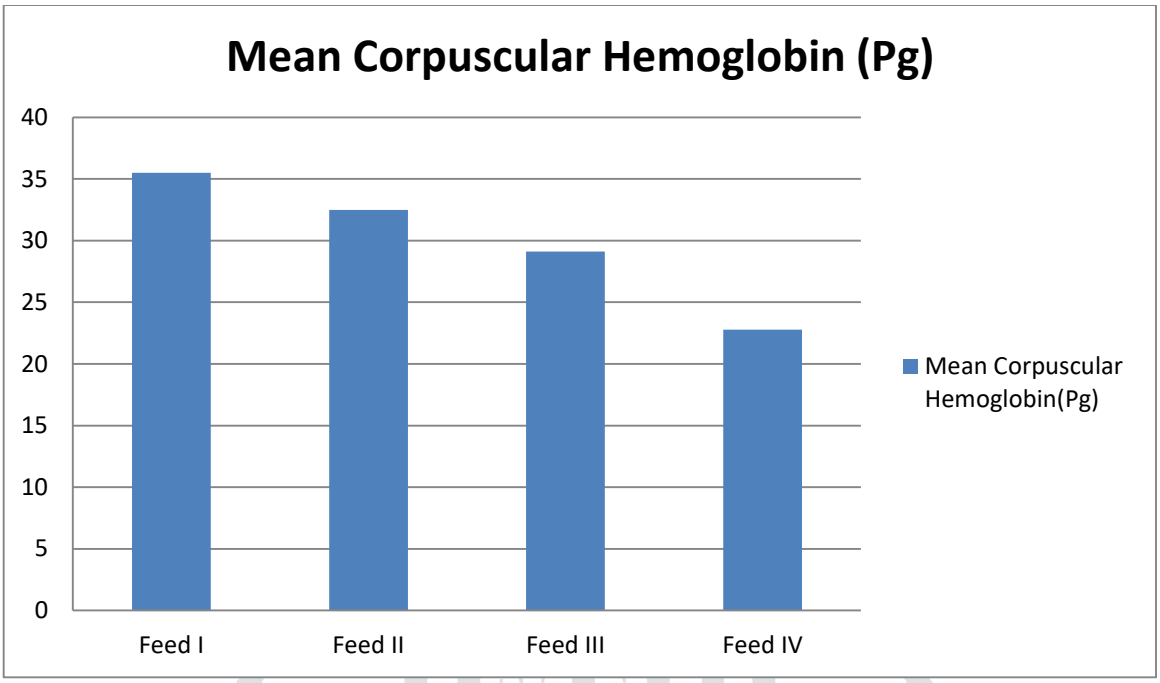

Fig. 2: Showing effect of aflatoxin Mean corpuscular haemoglobin of the fish.

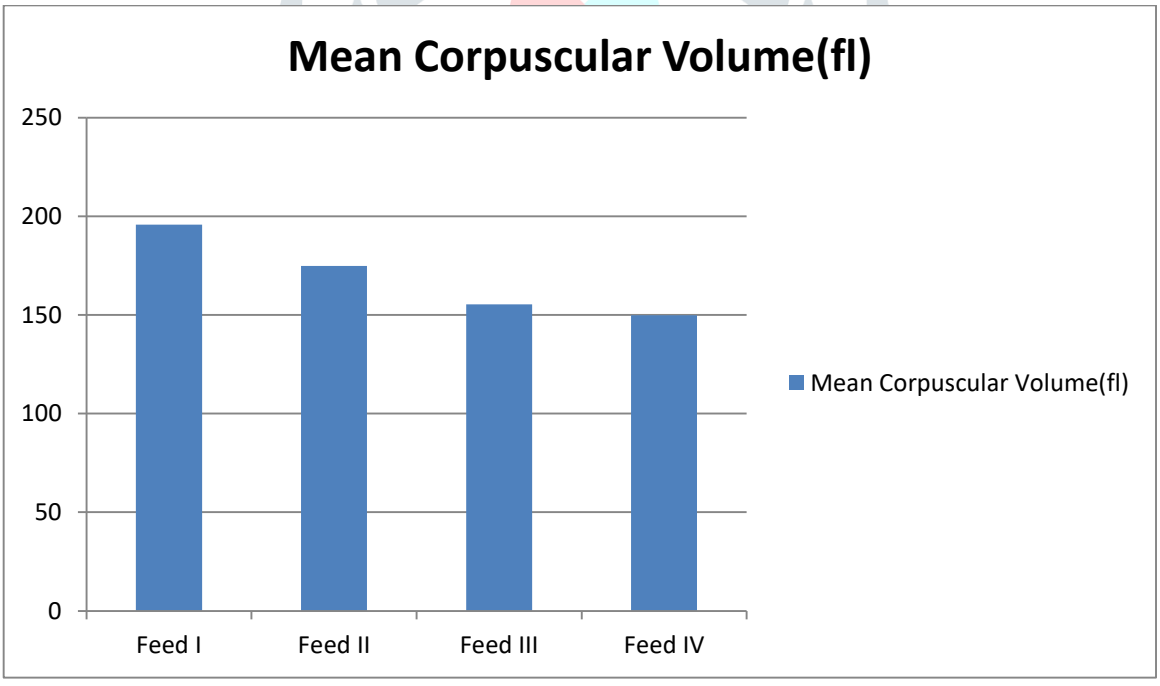

Fig. 3: Showing effect of aflatoxin on Mean corpuscular volume of the fish.

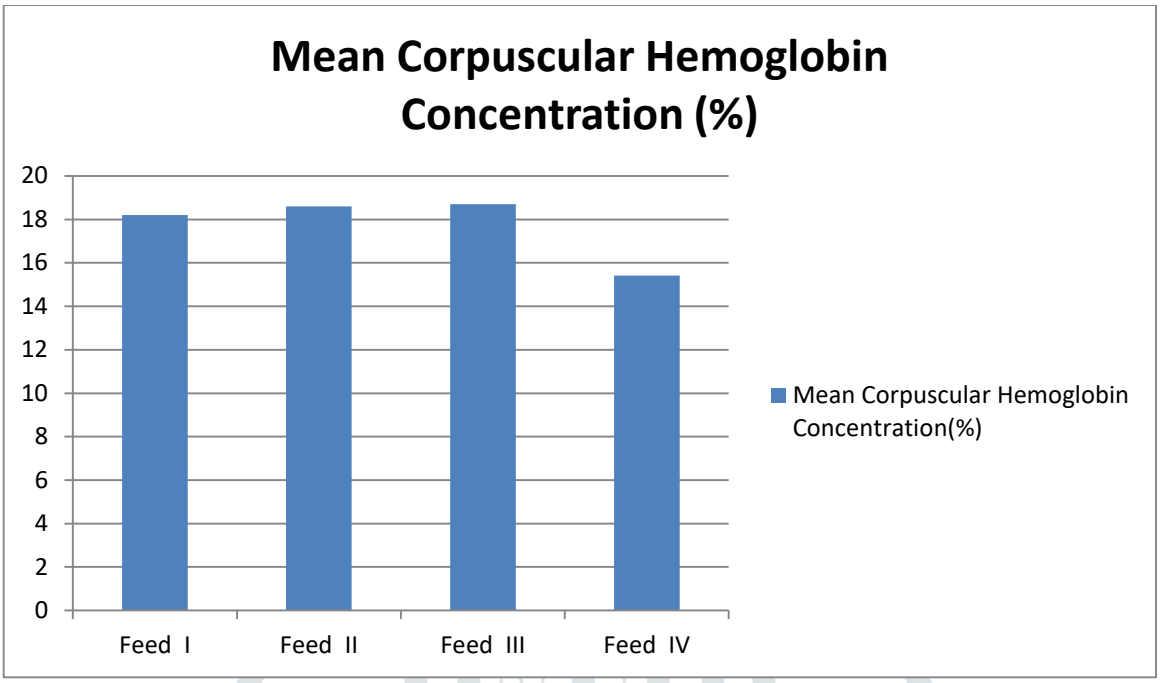

Fig. 4: Showing effect of aflatoxin Mean corpuscular hemoglobin concentration of the fish.

Hemoglobin content , RBC Count and RBC indices :

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).

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).It 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).

WBC Count and Differential Leucocyte count

The results of WBC Count and Differential Leucocyte Count are depicted in the table I and table II. In the present studies there was a significant decrease in WBC count in the experimental fishes as compared to control which agrees with those of Rizkalla (1997) and Hussain *et al*., (2000) in *Oreochromis niloticus* . Witeska (2003) reported that stress increases epinephrine and a simultaneous contraction in spleen resulting in decrease WBC count. Thus the decrease in WBC count in the present studies may be due to increased stress as a result of aflatoxin. In the present findings there was an increase in neutrophil percent. This

may be due to non specific immune response sets in various organs as a result of aflatoxin (Jantrarotai and Lovel, 1990) .In the present investigation there was a significant fall in lymphocyte which agrees with those of Sahoo *et. al.,* (2002) and Dimitri and Gobal (1996).It may be attributed to increased cortisol level due to stress induced by AFB_1 which in turn shortens the life span of lymphocytes, promote their apoptosis and reduce their proliferation in animals(Radhakishan,2010,Wytes *et. Al.,* 1998, Verberg 1999, Zaki *et. al.,* 2012, Espelid *et. al*., 1996). In the present investigation no significant changes were found in Monocyte and Basophils but Eosinophils showed a non significant increase.

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