

Ameliorating effects of Vitamin C on haematological parameters of the fish *Clarias Batrachus* exposed to dietary Aflatoxin B1

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ABSTRACT

Aflatoxins are produced mainly by moulds *Aspergillus flavus* and *Aspergillus parasiticus*. They cause toxicity called aflatoxicosis in animals and human beings which is of great concern in aquaculture also. Aflatoxin B1 is the most potent of all the known aflatoxins which mediates its effects by producing reactive oxygen species (ROS) and by irreversible damage to DNA and proteins leading to genotoxicity and cytotoxicity. Vitamin C is a potent antioxidant and reduces reactivity of ROS by donating electrons to them. Previous investigations suggest its role in alleviating aflatoxicosis in Nile tilapia. Present studies were conducted to determine the reduction in the negative effects of aflatoxin B1 on haematological parameters of fish *Clarias batrachus* by dietary supplementation of vitamin C. In the present investigation 60 fish were divided into four groups. Each group comprised fifteen fish. Four types of feed were prepared on the basis of presence of aflatoxin B1 and vitamin C. Feed I consists of a basal diet given to the first group of fish or control. Feed II consisted of aflatoxin B1 contaminated feed given to second group of fish. In feed III and IV 300 mg/kg and 600 mg/kg vitamin C was added to aflatoxin B1 contaminated feed. The objective of the present investigation was to explore the role of Vitamin C in reduction of adverse effects of aflatoxin on plasma biochemical parameters and liver glycogen of the fish *Clarias batrachus*. The group of fish that was given the feed II showed a significant increase in serum level of ALT, AST, bilirubin, blood urea, glucose and albumin globulin ratio but significant decrease was observed in total serum proteins and liver glycogen as a result of dietary aflatoxin. Supplementation of Vitamin C in the feed significantly decreased the adverse effects of aflatoxin and the parameters showed significant improvement. The group of fish fed with 600 mg/kg vitamin C showed best results and were nearly similar to those of the control. The present investigation revealed that feed containing 600 mg/kg vitamin C diminishes the effect of aflatoxin B1 in the fish reducing the health risk in humans consuming the fish as aflatoxins has the potential to accumulate and stay in the tissues of animals for long period of time.

Keywords: Aflatoxin, Vitamin C, RBC, Haemoglobin, WBC, *Clarias batrachus*

INTRODUCTION

Fisheries and aquaculture are one of the fastest growing industries in the world (Tacon et al., 2020) and provides livelihood for more than 10 % of world population (Anonymous, 2020). Fish flesh is considered as a rich source of protein, essential amino acids, omega 3 fatty acids and vitamins

(Jayasankar, 2018). The national per capita consumption of fish is about 11 kg which makes fish an important component of domestic food security in India (Jayasankar *et al.*, 2015). To meet the requirement aquaculture production in India has been increasing over the years contributing significantly in national economy and employment opportunity particularly in the economically less developed rural area (Kumar *et al.*, 2015). However fish farming is also associated with the risk of infectious diseases, contamination and decrease of food quality that can adversely affects the fish health (Nomoto, 2005). One of the risks associated with aquaculture is contamination of aflatoxin in the feed (Conversano *et al.*, 2008). Aflatoxins are mainly produced by two moulds *Aspergillus flavus* and *Aspergillus parasiticus* (Oliviera *et al.*, 2013) which can grow on a variety of improperly stored food (Cheeke *et al.*, 1985, Pitt *et al.*, 1997, Ellis, 2000) and produce mainly four types of aflatoxins, B1, B2, G1 and G2 (Kurtzman *et al.*, 1987, Kosalac and Pipeljnjak 2005). Among them, aflatoxin B1 is the most fatal and found in maximum quantity in culture (Yu 2012). It shows resistance to both heating and freezing which enables it to remain in the food chain for an indefinite period of time (Zaki *et al.*, 2011). The toxic effects of aflatoxin B1 are attributed to its ability to increase production of reactive oxygen species (ROS) which harms both human and animals through oxidative stress. The toxicity depends upon the species, dose of the aflatoxin as well as the time of exposure (Coulombe *et al.*, 1984, Ngethe *et al.*, 1993, Centoducati *et al.*, 2009). The principal target organ is liver and long-time exposure to aflatoxin retards growth and increase mortality due to immunosuppression, kidney and liver dysfunctions (Caguan *et al.*, 2004, Sephadari, *et al.*, 2010, Zaki *et al.*, 2012, Mahfouz *et al.*, 2015). Longtime exposure to aflatoxin B1 causes hepatocellular sarcoma and hepatocellular carcinoma in rainbow trout *O. mykiss* (Nunez *et al.*, 1991). Dietary administration of aflatoxin B1 decreased growth performance and haematological parameters but increased serum ALT, AST and creatinine in *Oreochromis niloticus* (Selim *et al.*, 2014). In catfish *Rhamdia quelen*, low level of aflatoxin B1 decreased.

Total Leucocyte Count but TLC increased when the fish is exposed to a higher level of the toxin (Anatar *et al.*, 2020). Aflatoxin B1 residues were detected in the tissues after exposure to the mycotoxin in seabass *Dissentracus labrax* (El Sayed *et al.*, 2009) and

Oreochromis niloticus (Deng *et al.*, 2020). This may lead to health risk in human also due to consumption of contaminated fish. (Murjani *et al.*, 2003). Maximum accumulation of aflatoxin B1 was reported in tissues of African catfish *Clarias gariepinus* when compared with Carp *Cyprinus carpio*, Tilapia *Oreochromis niloticus* and Canga *Heterotis niloticus* (Tacadong *et al.*, 2020). Previous investigations suggested that aflatoxins are mutagenic, hepatotoxic and hepatocarcinogenic in both animals and humans. Even small amount of the toxin causes oxidative stress leads to dysfunction of cellular components such as enzymes, nucleic acid, proteins and membranes (Meki, *et al.*, 2004, Rastogy, *et al.*, 2001). Vitamin C is recognised for its antioxidant and immune boosting properties (Chambial, *et al.*, 2013) and considered as an important component of the diet for growth and other biological functions. It increases protein synthesis (Lovel, 1972). Dietary administration of vitamin C improved the growth performance in Asian catfish *Clarias batrachus* (Pal and Chakrabarti, 2012). Positive effects of vitamin C on growth performance, plasma proteins and haematological parameters have been reported in *Catla catla* (Reddy, 2018). Japanese sea bass *Lateolabrax japonicus* (Ai *et al.*, 2004) and starry flounder *Platichthys stellatus* (Young *et al.*, 2020).

Vitamin C Improved immunity through enhanced phagocytosis, lysozyme activities, increased antibody level and respiratory burst activity of phagocytes in Indian major carp *Labeo rohita* exposed to aflatoxin B1 (Nayak, *et al.*, 2007, Sahoo *et al.*, 2003). Dietary supplementation of vitamin C improved the growth performance, improved blood level of liver function enzymes and total serum protein in Nile tilapia *Oreochromis niloticus* exposed to aflatoxin B1 (Shehata *et al.*, 2009). Vitamins C also inhibits *Aspergillus* growth and aflatoxin gene expression (Maryam, *et al.*, 2018). Asian catfish *Clarias batrachus* popularly known as Mangur is found throughout Asia and Africa. It is considered as an important fish due to its taste and excellent nutritional profile (Rui *et al.*, 2007). In many parts of India, it is frequently prescribed to lactating pregnant, anaemic and malnourished individuals (Debnath 2011). However, the fish showed a drastic decline from their natural habitat in India during the last few years. The objective of the present investigation was to explore the role of Vitamin C in mitigation of adverse effects of aflatoxin on haematological parameters of the fish *Clarias batrachus*.

MATERIALS AND METHODS

Experimental Design

A total of 60 apparently healthy *Clarias batrachus* were obtained from a private fish farm at Dholpur. The length of the fish was about 10 to 20 cm, and the weight was about 40 to 50 grams. The fishes were kept in twelve aquariums measuring 2' × 1' × 1'. Five 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 were kept as experimental sets.

Preparation of feed

Four types of feeds were prepared for the fishes on the basis of the presence of aflatoxin or vitamin C in the feed, and they were distinguished as Feed I, Feed II, Feed III, and Feed IV. Feed I (good feed) contained 100% good feed and no mouldy feed or vitamin C. Feed I was given to the control or fishes of the first set of aquaria comprising 1A, 1B, and 1C. Feed II consisted of 100% mouldy feed without vitamin C. Feed II was given to fishes of the second set of aquaria comprising 2A, 2B, and 2C. Feed III consisted of 100% mouldy feed mixed with vitamin C at a concentration of 300 mg vitamin C per kg of feed. Feed III was given to fishes of the third set of aquaria comprising 3A, 3B, and 3C. Feed IV consisted of 100% mouldy feed mixed with vitamin C at a concentration of 600 mg vitamin C per kg of feed. Feed IV was given to fishes of the fourth set of aquaria comprising 4A, 4B, and 4C.

Mouldy feed was prepared in the laboratory. The commercial fish feed procured from the market was first sprinkled with a small amount of 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. For the preparation of feed III and feed IV, 300 mg and 600 mg of vitamin C per kg of feed were added to feed III and feed IV, respectively. The infected feed was kept in conditions favourable for the growth of the mould. The feeding started from the second day, two times a day, at a feeding rate of 4% of the body weight.

Estimation of parameters

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

- Haemoglobin 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 microhematocrit technique of Blaxhall and Daisley (1973).

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

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

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

$$MCHC (\%) = Hemoglobin(g/dl) \times 100 / PCV$$

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) of Guenther (1964). The data were presented as mean ± standard deviation (SD). A probability value (p) of less than 0.05 was considered statistically significant. Besides, graphs were drawn on the basis of data obtained as mean ± SEM.

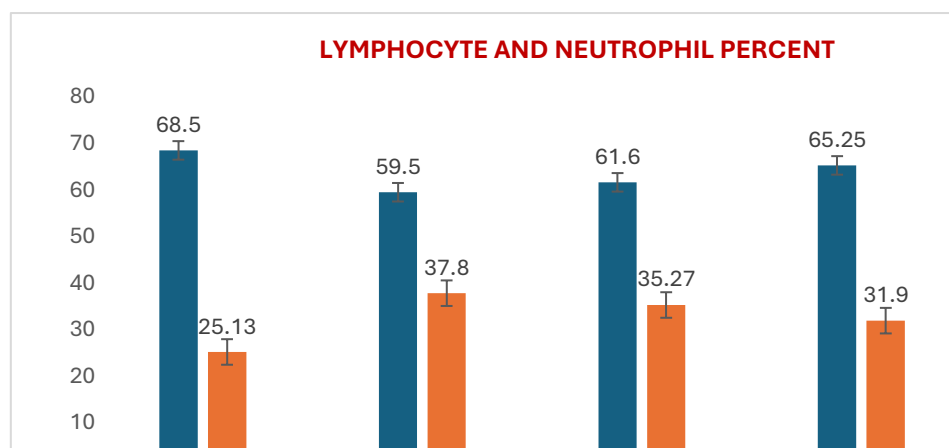
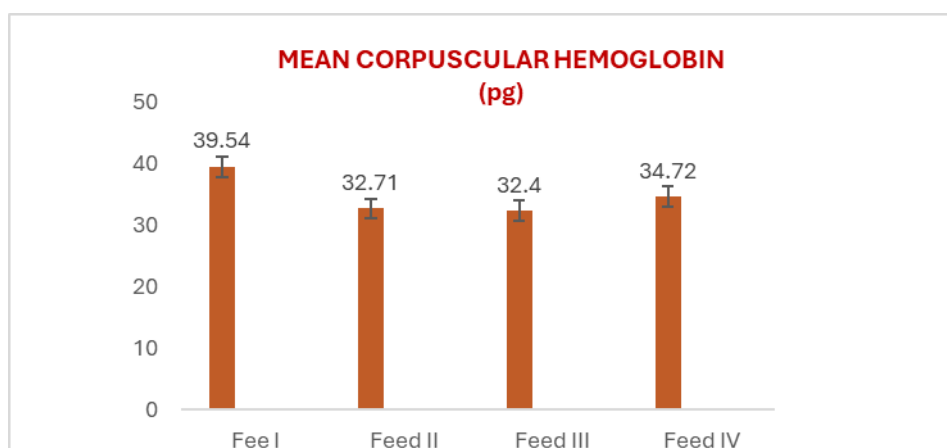
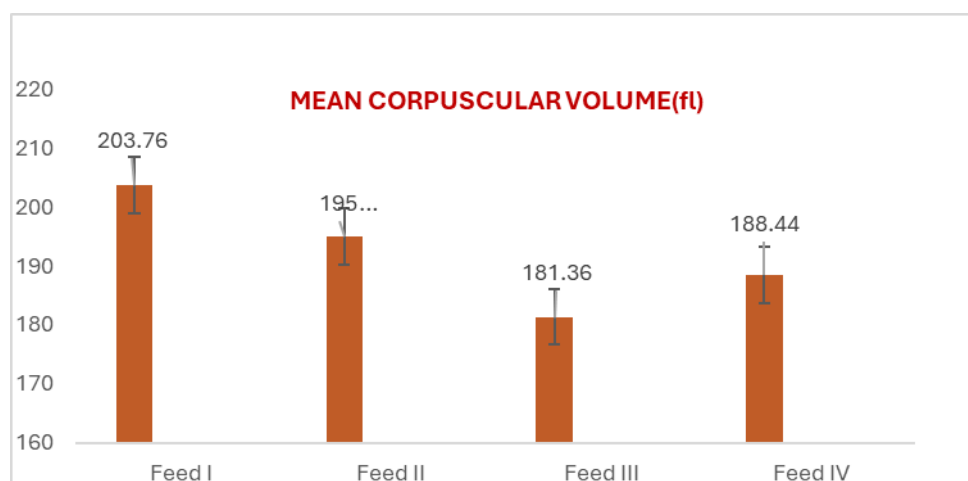
RESULT AND DISCUSSION

Reduction of the growth of toxigenic fungi and elimination of the effects of toxins produced by these fungi has been a priority in agriculture and aquaculture. There are several studies investigating this domain. Bluma et al. reported that clove oil reduces the production of aflatoxin. Kim et al. (2010) suggested that freeze-dried green onion inhibits the growth of *A. parasiticus*. Maryam et al. (2018) found that vitamin C inhibits the growth of *A. parasiticus* and reduces the expression of genes responsible for the production of aflatoxin.

In the present investigation, Table I shows the impacts of vitamin C supplements in the feed of fish exposed to aflatoxin B1. In the present study, it was observed that RBC count, haemoglobin concentration, and packed cell volume were significantly lower in fish exposed to aflatoxin B1 alone (*p* > 0.05) as compared to the control, but a significant reduction in the negative effects of aflatoxin was observed in groups supplemented with vitamin C.

Table I- Showing effects of aflatoxin B1 and Vitamin supplementation on haematological parameters

Feed	I	II	III	IV
Hemoglobin(g/dl)	7.76 $\pm$ 0.13	4.38 $\pm$ 0.05	5.29 $\pm$ 0.03	6.25 $\pm$ 0.02
RBC (million/mm ³)	1.96 $\pm$ 0.06	1.33 $\pm$ 0.07	1.63 $\pm$ 0.04	1.79 $\pm$ 0.03
TLC(Thousand/mm ³)	11.21 $\pm$ 0.05	6.37 $\pm$ 0.02	8.56 $\pm$ 0.03	10.39 $\pm$ 0.37
PCV	39.96 $\pm$ 0.02	26.13 $\pm$ 0.17	29.58 $\pm$ 0.08	33.38 $\pm$ 0.04

**Fig 1: Effects of Aflatoxin B1 and Vitamin C supplementation on lymphocyte % and neutrophil****Fig 2: Effects of Aflatoxin B1 and Vitamin C supplementation on Mean corpuscular haemoglobin (MCH)****Fig 3: Effects of Aflatoxin B1 and Vitamin C supplementation on Mean Corpuscular Volume (MCV)**

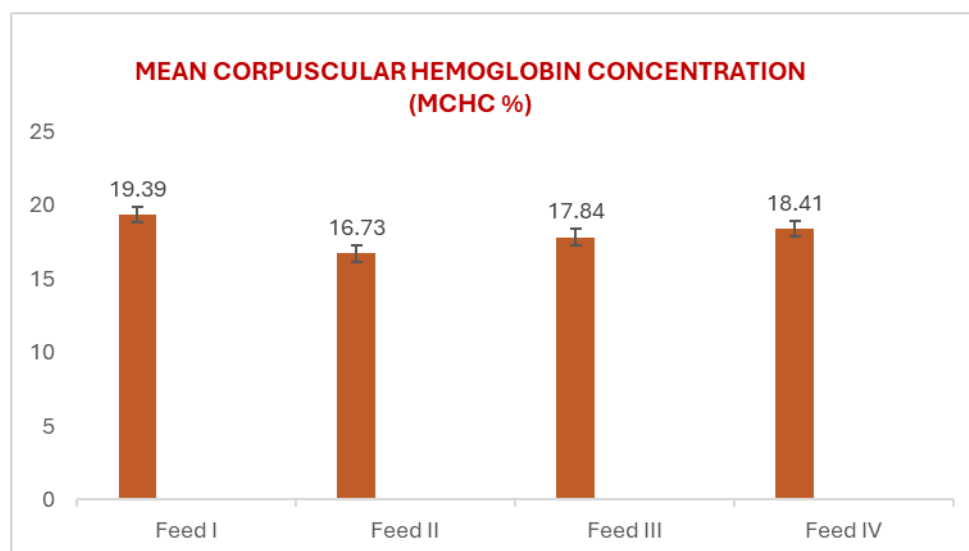


Fig 4- Effects of AflatoxinB1 and Vitamin C supplementation on MCHC %

Haematological indices are crucial for assessing the health and physiological status of fish. In the present study, there was a significant effect of aflatoxin on RBC count, WBC count, haemoglobin content, as well as other haematological indices such as packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC). The results of RBC count, haemoglobin content, as well as RBC indices like PCV, MCV, MCH, and MCHC are shown in Table I and Figs. 1-4. These haematological parameters showed a significant ($p < 0.05$) decrease in the fish exposed to AFB1 alone as compared to the control and those groups supplemented with vitamin C. The present findings agree with Nurcan et al. (2012), Selim et al. (2013), Mahfouz et al. (2015), Palaniswamy (2018), and Olugbenga et al. (2025). The decrease in these parameters indicates anaemia, which is consistent with the known haematotoxic effects of AFB1 (Oloruntola, 2024). AFB1 causes hepatocellular carcinoma, and anaemia is one of the symptoms of HCC (Murray-Lyon, 1983). The decrease in RBC count, MCV, and MCHC may be due to inhibition of those enzymes which are responsible for the synthesis of heme (ATSDR, 2005). It may also occur due to haemopoietic, hepatic, and osmoregulatory dysfunction as a result of aflatoxin (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 RBCs in the spleen and haemopoietic organs as a result of exposure to aflatoxin (Verma and Raval, 1989; Jenkins and Smith, 2003). The absence of any significant difference in these parameters between the control and vitamin C-supplemented groups

suggests a potential ameliorative effect of the antioxidant vitamin C (Cotoraci et al., 2021; Olugbenga et al., 2025).

The results of WBC count, neutrophil, and lymphocyte count are depicted in Table I. In the present study, there was a significant decrease in WBC count in the experimental fishes as compared to the control, which agrees with those of Rizkalla (1997) and Hussain et al. (2000) in *Oreochromis niloticus*. Witeska (2003) reported that stress increases epinephrine and causes a simultaneous contraction in the spleen, resulting in decreased WBC count. Thus, the decrease in WBC count in the present study may be due to increased stress as a result of aflatoxin. In the present findings, there was an increase in neutrophil percentage. This may be due to a non-specific immune response set in various organs as a result of aflatoxin (Jantrarotai and Lovel, 1990). In the present investigation, there was a significant fall in lymphocytes, which agrees with those of Sahoo et al. (2002) and Dimitri and Gopal (1996). It may be attributed to increased cortisol levels due to stress induced by AFB1, which in turn shortens the lifespan of lymphocytes, promotes their apoptosis, and reduces their proliferation in animals (Radhakrishnan, 2010; Zaki et al., 2012). Conversely, a higher leucocyte count, lymphocyte percentage, and increased neutrophil percentage in groups supplemented with vitamin C indicate a protective effect of vitamin C in fishes exposed to aflatoxin B1. These findings indicate the potential of vitamin C to ameliorate mycotoxin-induced oxidative stress and its negative haematological consequences (Alayash, 2022; Tzounakas et al., 2022).

CONCLUSION

In the present study, it was found that 600 mg of vitamin C per kg of formulated feed significantly mitigated the adverse effects of aflatoxin contamination in the fish *Clarias batrachus*. Vitamin C supplementation significantly ameliorated the haematological indices of the fish exposed to dietary aflatoxin. Therefore, vitamin C may be supplemented in the fish feed used for aquaculture to achieve better results.

Conflict of interest

The authors declare no competing interests.

Data Availability Statement: Not applicable.

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