AMELIORATIVE POTENTIAL OF AN ANTIOXIDANT ON BLOOD PARAMETERS OF *CLARIAS GARIEPINUS* FED DIETS CONTAMINATED WITH FUMONISIN B_1

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Abstract

This study was conducted to evaluate the ameliorative potential of an antioxidant, vitamin C, against the impact of diets contaminated with varied levels of fumonisin B_1 (FB_1), a mycotoxin produced by Fusarium verticillioides, on haematology and serum biochemical constituents of Clarias gariepinus in a 12-week feeding experiment. Extracted FB_1 were added to six diets at the rate of approximately 2.5, 5.0, and 7.5 mg FB_1/kg constituting diets 2, 3, and 4 respectively and the other three diets constituting diets 5, 6, and 7 containing the same varied dietary concentrations of FB_1 with 0.6g of vitamin C i.e., 2.5mg FB_1/kg + 0.6g vitamin C, 5.0mg FB_1/kg + 0.6g vitamin C and 7.5mg FB_1/kg + 0.6g vitamin C, respectively. Diet 1, which served as the control, had no FB_1. Generally, ssignificant ($P<0.05$) decrease in PCV, RBC, and Hb values were observed in C. gariepinus fed diet 4 compared to those on the control diet, but significantly ($P<0.05$) increased in those fed diets supplemented with vitamin C (diets 5, 6 and 7). The WBC counts of the fish increased with increase in the concentrations of FB1 in the diets. The total serum protein values of the fish fed diets 4, 6 and 7 containing ≥ 5.0 mg FB_1/kg with or without the antioxidant were lower compared to those on other diets. The study revealed the mitigating effect of vitamin C against the negative impact of fumonisin B_1 on blood parameters of C. gariepinus and the potentials of the antioxidant to ameliorate the impact of the toxin on blood parameters of the fish decreased with increase in the dietary FB_1 concentrations.

Key words: Antioxidant, Blood, *Clarias gariepinus*, Fumonisin B_1, Mycotoxin, Vitamin C.

Introduction

Fish plays a major role in human diets and serves as rich source of animal protein. In Nigeria, fish contributes immensely to the national economy by providing high animal food protein and generating employment, which is a means of poverty alleviation (Solarin, 1992). The quality of the products used in feeds for farmed fish has become a limiting factor in aquaculture because these feeds are ideal substrates for the growth of fungi, which, under favourable conditions, may favour the synthesis of mycotoxins.

Mycotoxins are toxic secondary metabolite produced by fungi in many agricultural products (Reddy *et al.*, 2006). The threat of mycotoxin to aquaculture industry is a major concern because mycotoxin contamination is persistent in fish flesh (Santacroce *et al.*, 2008) and residues have been found in marketed fish products beyond acceptable levels (Santos *et al.*, 2010).

Haematological parameter is a good indicator to determine the health of an organism (Joshi *et al.*, 2002). Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health state of fish. Serum biochemical parameters are also valuable tools for monitoring the health of fish.

Considerable research has been directed at finding methods to prevent the negative impact of the mycotoxin. Most of the mycotoxins have been reported (Balogh et al., 2007; Pál et al., 2009) to provoke oxygen free radical formation. For this reason, the addition of natural and synthetic antioxidants to feeds contaminated with mycotoxins has been reported to be potentially efficacious as they are able to act as superoxide anion scavengers (Citil et al., 2005; Dvorska et al., 2007). However, there are only few data about the positive effect of antioxidants on some important mycotoxins, including fumonisin B_1.

The objective of this study was to evaluate the ameliorative potential of an antioxidant, vitamin C, on blood parameters of *Clarias gariepinus* fed diets contaminated with varied levels of fumonisin B_1.

Materials and Methods

Four hundred and twenty (420) fingerlings of *Clarias gariepinus* obtained from the Department of Animal and Environmental Biology Fish Hatchery, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria were used for the experiment. The fingerlings of the same cohort were sorted to similar average weight of ± 13.08g and they were acclimatized for 2 weeks prior to the

commencement of the experiment. After 2 weeks the fingerlings were divided into 21 tanks which contained 60 liters of water with average depth of 31.7cm having seven treatments in all and three replicates per each treatment. Each tank was stocked with 20 fingerlings fed at 3% body weight daily at 8:00 and 16:00 hours for 12 weeks.

A toxigenic strain of *Fusarium verticillioides* (MRC 826) obtained from the Plant Pathology Laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State Nigeria was used to produce fumonisin B_1 (FB_1) as previously described (Adu and Gbore, 2015).

The extracted FB_1 were added to a commercial fish feed (Durantee®) to prepare three diets at the rate of 2.5, 5.0, and 7.5 mg FB_1/kg constituting diets 2, 3, and 4 respectively and the other three diets constituting diets 5, 6, and 7 with the same dietary fumonisin B_1 but supplemented with 0.6g of vitamin C. Diet 1 contained no FB_1 and served as the control diet. The fish were fed twice daily, at 8.00 hours and 16.00hours at 3 % body weight throughout the 12-week experimental period.

At the end of the feeding trial, fish samples were selected from each treatment tank and blood samples were collected through the caudal vein using a hypodermic needle into 2 sets of sample bottles. One set contained EDTA which were used for haematological analysis and the other set without EDTA were used for serum biochemical analysis. Evaluation of haematological parameters were determined using Mindray® Autohaematology Analyser (3000 plus) while the biochemical parameters (total serum protein, albumin, globulin and albumin/globulin ratio) were determined as earlier described by Gbore et al. (2006). All determinations were carried out in duplicates for each sample.

Data obtained were subjected to one-way analysis of variance (ANOVA) using Statistical Analysis System software. The significant treatment means were compared at ($P<0.05$) probability level using Duncan's multiple range test (DMRT) of the same software.

Results and Discussion

The haematological values of *Clarias gariepinus* fed diets contaminated with varied levels of fumonisin B_1 (FB_1) with or without vitamin C are as shown in Table 1. The PCV level of *C.gariepinus* fed the control diet (diet 1) was significantly ($P< 0.05$) higher than those fed other diets, with fish fed diet 4 having the least value which was also statistically lower ($P> 0.05$) than the PCV values of those fed diet 6. The erythrocyte (RBC) count of *C. gariepinus* fed the control diet and diets 5 and 6 were significantly ($P<0.05$) higher than those of the fish on other diets but statistically similar to those of fish fed diet 2. The highest mean haemoglobin values were observed in fish on diets 5 and 6, with the least value recorded in fish fed diet 3. Fish on control diet had the least platelet value compared with those on diets 3 and 4 but not statistically different ($P>0.05$) from those on diets 2, 5, 6, and 7.

Table 2 shows the serum proteins of *C. gariepinus* fed diets contaminated with varied levels of fumonisin B_1 with or without vitamin C. The fish fed diet 4 had the highest albumin/globulin ratio but invariably had the least globulin value. Meanwhile, the albumin content of *C. gariepinus* fed diet 2 was significantly ($P<0.05$) higher compared to those on diet 5 which had the least albumin content with the same level of dietary FB_1 but supplemented with 0.6g of vitamin C.

Haematological and serum biochemical indices are becoming increasingly important diagnostic tools in fish biology. Haematological indices are a reflection of the effects of dietary treatments on animals in terms of the type, quality and amounts of feed ingested and nutrients available to an animal to meet its physiological and metabolic requirements (Gbore and Akele, 2010). In this present study, the reduction in PCV, RBC, and Hb concentrations of *C. gariepinus* fed diets 3 and 4 (containing 5.0 and 7.5 mg FB_1/kg diet. respectively), was as a result of higher concentration of FB_1 in these diets. This result shows that *C. gariepinus* exposed to high concentrations of FB_1 might have suffered significantly from the synthesis (erythropoiesis), concentration of erythrocytes, and anaemia. This agrees with the results of Mousa and Khattab (2003) that reported decrease in RBC and PCV valuesin the blood of African catfish after Ochratoxin intoxication. In contrast, the mean values for PCV, RBC, and haemoglobin (Hb) of *C. gariepinus* fed the control diet and diets 5, 6 and 7 containing FB_1 supplemented with the antioxidant were generally higher than those fed diets containing FB_1 without vitamin C showed that the antioxidant was efficient in reducing the effects of FB_1 on these important blood parameters.

According to Douglass and Jane (2010), leucocytes (WBC) are the defensive cells of the body; their levels have implications for immune responses and the ability of the animal to fight infections. The findings in this study indicated that the animals that were fed diets 3 and 4 might have suffered leucocytosis. According to Coles (1986), leucocytosis may result from intoxication including those produced by metabolic

disturbance. This might have also resulted from the excitation of the defense mechanism of the fish to counter the effect of the toxicant.

An estimation of the total quantity of serum proteins may be utilized as an estimation of the nutritive state of an animal. According to Gbore and Egbunike (2009), the nutritive state may be dependent not only on proper and adequate intake of protein building materials in the diet, but may also be a reflection of the nutritive state existing within the animal body, reflecting alterations in metabolism. The total protein in serum can be used as an indicator of fish innate immune responses (Lin and Shiau, 2005) and the maintenance of osmotic balance between the circulating blood and tissue fluid (Harper *et al.*, 1977). Therefore, in this present study the significantly ($P<0.05$) decrease in serum protein and globulin ratio valuesin fish fed diets 4 and 6 with increased FB_1 concentrations within 12 weeks revealed a dietary FB_1 concentration-dependent response to the toxin.

The result of this present study indicated the toxin as a protein metabolism inhibitor as reported for sphingolipid synthesis (Riley and Norred, 1996). The concentration of FB_1 were able to suppress the effect of vitamin C supplements in fish fed diets 6 thereby impairing the serum protein constituents of the animals. Since *C. gariepinus* were fed with iso-nitrogenous diets which contained varied levels of FB_1, the result revealed the roles which dietary FB_1 could play in serum protein alterations, as previously observed in rabbits (Ewuola and Egbunike, 2008), growing swine (Gbore and Egbunike, 2009), and fingerlings (Gbore *et al.*, 2010) fed different concentrations of dietary FB_1. The results from this study suggested that FB_1 could lead to disorder in protein biosynthesis in animal body systems as well.

Conclusion

The findings of this study have shown how varied levels of FB_1 impaired the haematological and serum biochemical parameters of *C. garepinus*. However, this study was able to demonstrate the ameliorative impact of vitamin C on the effect of FB_1 on blood parameters of *C. gariepinus*. The strategy of using food additives, such as antioxidants, to protect livestock from the mycotoxin may also provide effective and economical new approaches to protecting human populations.

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Table 1: Haematological parameters of *C. gariepinus* fed diets contaminated with FB_1 with or without vitamin C.

Parameter	PCV* (%)	WBC** (10^9/L)	RBC*** (10^9/L))	Haemoglobin (g/L)	Platelet (10^9/L)
Diet 1 (Control)	34.0 ± 2.0^{a}	113.5 ± 4.5^{a}	2.53 ± 0.09^{a}	105.5 ± 1.5^{ab}	16.5 ± 3.5^{c}
Diet 2 (2.5 mg FB_1)	30.5 ± 4.5^{ab}	116.0 ± 12.0^{a}	2.37 ± 0.26^{ab}	105.5 ± 12.5^{ab}	18.0 ± 2.0^{bc}
Diet 3 (5.0 mg FB_1)	29.0 ± 2.0^{ab}	87.0 ± 2.0^{b}	1.68 ± 0.05^{c}	73.5 ± 1.5^{c}	35.0 ± 0.0^{a}
Diet 4 (7.5mg FB_1)	26.5 ± 6.5^{b}	101.0 ± 18.0^{ab}	2.03 ± 0.43^{b}	92.0 ± 18.0^{b}	23.0 ± 3.0^{b}
Diet 5 (2.5mg FB_1 + Vit C)	32.5 ± 1.5^{ab}	113.5 ± 2.5^{a}	2.52 ± 0.07^{a}	109.0 ± 3.0^{a}	19.5 ± 2.5^{bc}
Diet 6 (5.0mg FB_1 + Vit C)	33.0 ± 1.0^{a}	117.0 ± 3.0^{a}	2.51 ± 0.07^{a}	108.0 ± 0.0^{ab}	18.0 ± 4.0^{bc}
Diet 7 (7.5mg FB_1 + Vit C)	28.5 ± 1.5^{ab}	106.5 ± 0.5^{a}	2.31 ± 0.00^{ab}	97.0 ± 3.0^{ab}	21.0 ± 2.0^{bc}

^{abcd}:- Means within the same column with different superscripts differ significantly ($P< 0.05$).
*PCV: Packed cell volume; **WBC: White blood cell; ***RBC: Red blood cell.

Table 2: Serum proteins of *C. gariepinus* fed diets contaminated with FB_1 with or without vitamin C.

Parameter	Protein (g/L)	Albumin (g/L)	Globulin (g/L)	Albumin/Globulin
Diet 1 (Control)	48.00 ± 0.00^{a}	20.30 ± 0.70^{bc}	27.70 ± 0.70^{a}	0.73 ± 0.04^{a}
Diet 2 (2.5 mg FB_1/kg)	47.00 ± 1.70^{a}	29.15 ± 2.75^{a}	17.85 ± 11.25^{abc}	2.13 ± 1.25^{a}
Diet 3 (5.0 mg FB_1/kg)	38.00 ± 1.00^{ab}	14.00 ± 2.40^{cd}	24.00 ± 5.60^{ab}	0.58 ± 0.05^{a}
Diet 4 (7.5mg FB_1/kg)	27.00 ± 3.00^{b}	22.70 ± 0.70^{ab}	4.30 ± 3.70^{c}	15.68 ± 2.02^{a}
Diet 5 (2.5 mg FB_1/kg + Vit C)	37.00 ± 1.70^{ab}	9.50 ± 3.70^{d}	27.50 ± 13.30^{a}	0.36 ± 0.04^{a}
Diet 6 (5.0 mg FB_1/kg + Vit C)	25.00 ± 1.00^{b}	14.80 ± 2.20^{cd}	10.20 ± 8.20^{bc}	4.29 ± 0.54^{a}
Diet 7 (7.5mg FB_1/kg + Vit C)	27.00 ± 3.00^{b}	11.50 ± 1.70^{d}	15.50 ± 4.70^{abc}	0.82 ± 0.37^{a}

^{abcd}:- Means within the same column with different superscripts differ significantly ($P< 0.05$).