Original Article

Ameliorative Potential of Vitamins on Haematological and Biochemical Profiles of *Clarias gariepinus* Fed Diets Contaminated with Fumonisin B$_1$

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ABSTRACT

This study examined ameliorative impact of antioxidants –vitamins C and E on blood profile of *Clarias gariepinus* fed diets contaminated with varied levels of fumonisin B$_1$ (FB$_1$). *Fusarium* cultured maize containing FB$_1$ was used to formulate diets containing approximately 2.5, 5.0, and 7.5 mg FB$_1$/kg constituting diets 2, 3, and 4, respectively and other nine diets constituting diets 5-13 with the same varied dietary FB$_1$ concentrations but supplemented with 0.6g of vitamin C or E or vitamins C and E. These 12 diets and a control diet were used in 12-week feeding trial. Significant decrease ($P<0.05$) in PCV, RBC, Hb and platelet values were observed in *C. gariepinus* fed diet containing 7.5 mg FB$_1$/kg compared to those on the control diet, but significantly increased ($P<0.05$) in those fed diets supplemented with vitamin C or E. The WBC counts of fish fed diets containing 5.0 and 7.5 mg FB$_1$/kg were significantly higher ($P<0.05$) than those fed the control diet. The serum total protein of fish fed diet containing 2.5 mg FB$_1$/kg supplemented with vitamins C and E was significantly higher ($P<0.05$) than of those fed other diets containing higher concentrations of FB$_1$ supplemented with vitamins. The serum enzymes of *C. gariepinus* decreased with increase in the concentrations of the mycotoxin. Supplementation of FB$_1$-contaminated diets of *C. gariepinus* with vitamins ameliorated the adverse effect of FB$_1$ on most blood parameters in the fish through mitigation of FB$_1$-evoked toxicity by free radicals.

Key words: Antioxidant, Blood, *Clarias gariepinus*, Fumonisin B$_1$, Mycotoxin, Vitamin

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 rich animal food protein

and generating employment, which is a means of poverty alleviation. However, the quality of the ingredients used in feeds of farmed fish has become a limiting factor for the activity because these feedstuffs are ideal substrates for the growth of fungi, which under favourable conditions may favour the synthesis of mycotoxins.

Mycotoxins are toxic secondary metabolites produced by fungi in many agricultural products (Reddy *et al.,* 2006). Fumonisin, a mycotoxin produced by *Fusarium verticillioides* (= *F. moniliforme*) and other *Fusarium* species that grow on maize worldwide, has been documented to cause various physiological responses in humans and animals.

The economic consequences of mycotoxin contamination are profound, and exposure of humans and livestock to mycotoxin-contaminated food is particularly a serious problem in the tropics (Reddy and Raghavender, 2008). Crops with large amounts of mycotoxins often have to be destroyed. Alternatively, contaminated crops are sometimes diverted into animal feeds. Giving contaminated feeds to susceptible animals poses a serious threat to the health and productivity of the animals and causes great economic losses (Griessler and Encarnação, 2009).

The effects of mycotoxins are generally associated with reduced growth and negative effect on the health status of fish and other animals (Mohamed *et al.*, 2017). The threat of mycotoxins to aquaculture industry is a major concern because mycotoxin contamination is persistent in fish flesh (Spring and Fegan, 2005; Santacroce *et al.,* 2008) and residues have been found in marketed fish products beyond acceptable levels (Santos *et al.,* 2010). Hence, mycotoxin contamination of feedstuffs is not only a serious threat to animal welfare and production but can also result in dramatic economic losses (Hooft *et al.,* 2010; Santos *et al.,* 2010; Iheshiulor *et al.,* 2011). There are several studies on the toxic effects of FB_1 in different fish species (Yildirim *et al.*, 2000; Nguyen *et al.*, 2003). Mycotoxins presence in fish feed and feeds of other animals has been a major threat causing disturbances in the immune systems (Voss *et al.,* 2007) and blood abnormalities (Ewuola and Egbunike, 2008; Ewuola *et al.,* 2008; Gbore and Egbunike, 2008, 2009; Gbore *et al.*, 2010).

The ingestion of numerous dietary components has measurable effects on blood constituents (Bhatti *et al.,* 2009). The monitoring of mycotoxins in biological fluids such as blood or urine is useful in the generation of information on exposure incidence at the individual level compared to dietary assessments (Warth *et al.,* 2013). Haematological parameter is a good indicator to determine the health of an organism (Joshi *et al.,* 2002). It also acts as pathological reflector of the whole body; hence, haematological parameters are important in diagnosing the functional status of exposed animals to toxicants (Joshi *et al.,* 2002). Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health state of fish. The assessments of blood biochemical parameters are important to evaluate the health of many vertebrates, including fish (Tavares-Dias and Moraes, 2007). Through biochemical constituents of the fish blood, the metabolic disturbances of fishes could easily be assessed (Jamalzadeh *et al.,* 2009). Alterations in biochemical processes can result in changes in the biochemical blood profile of organism (Firat *et al.*, 2011).

In a study conducted by (Barbosa *et al.,* 2013) to determine species of the fungal genera contamination from feed intended for farmed fish, it was shown that a high percentage of samples (98%) was contaminated with FB_1. The occurrence of this mycotoxin emphasizes the need for further research to better assess the risk to the health of farmed fish. The reflection of this hazardous toxicant shows a need to mitigate the effect of mycotoxins in

aquaculture in order to prevent its continuous effects on aquacultural species. Fumonisin has been reported to increase oxidative stress, which has been frequently proposed as a biochemical mechanism for fumonisin toxicity *in vitro* and *in vivo* (Stockmann-Juvala *et al.*, 2004; Hassan *et al.*, 2010; Abbès *et al.*, 2016).

Several studies have shown that the threats posed by mycotoxins on aquacultural species and other animals susceptible to these toxic metabolites can be mitigated with the use of antioxidants. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might have caused. The most widely used antioxidants in aquaculture and in animal care include vitamins C and E (Sies, 1997). This study therefore evaluated the effects of feeding varied levels of dietary FB_1 on blood of *Clarias gariepinus* and the ameliorative impact of vitamins C and E on the health of *C. gariepinus* fed diets contaminated with FB_1.

Materials and methods

Experimental animal and management

Experimental site: This experiment was carried out at the Fish Hatchery of the Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

Experimental design and procedure: A total of 780 fingerlings of *C. gariepinus* were obtained from the Hatchery Unit of the Department of Animal and Environmental Biology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The fish were used for the experiment, adopting a completely randomised design after acclimatization for two weeks.

Animal management: The fingerlings of the same cohort were sorted to similar average weight of ± 13.08 g and divided into 39 tanks which contained 60 litres of water with average depth of 31.7cm having 13 treatments in triplicates. Each tank was stocked with 20 fingerlings.

Fumonisin extraction, experimental diet formulation and preparation

Fumonisin extraction: A toxigenic strain of *F. verticillioides* (MRC 826) was obtained from the Plant Pathology Laboratory of the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The fungus isolate was grown on acidified potato dextrose agar (PDA) under a controlled environment in an incubator for a period of 7 days. After the incubation period, the mycelia were then washed into a suspension with sterile distilled water and this was used to inoculate autoclaved maize grits in polyethylene bags. One-millilitre suspension of conidia was inoculated into 1000 mL of the medium and incubated at 25 °C for 15 days to produce FB_1. The procedure of extraction was repeated to collect the necessary amount of FB_1. The FB_1 content of the extract from the *Fusarium*-cultured maize grits was determined using fumonisin quantitative test kits (The ROSA WET Fumonisin Quantitative Test (FUMQ-WET) Charm Sciences, Inc., Lawrence, MA, USA) and HLPC. A stock solution of FB_1 was prepared in distilled water (5 mg/mL^{-1}) and kept refrigerated at 4 °C. This stock solution was further diluted with distilled water for uniform admixing during the formulation and preparation of the experimental diets.

Experimental diet formulation and preparation: Equal parts of crushed commercial pellets for Clariid (crude protein content of 45%), corn gluten and an adequate amount of distilled water (with or without FB_1) were mixed to obtain a dough. A household machine was modified to form 2 mm pellets from the dough to make three diets containing approximately 2.5, 5.0, and 7.5 mg FB_1/kg constituting diets 2, 3, and 4, respectively and the other nine diets constituting Diets 5-13 with the same varied dietary FB_1 concentrations but supplemented with 0.6 g of vitamin C or E or vitamins C and E (*i.e.,* 2.5 mg FB_1/kg, 5.0 mg FB_1/kg, 7.5 mg FB_1/kg, 2.5 mg FB_1/kg + Vit C, 5.0 mg FB_1/kg + Vit C, 7.5 mg FB_1/kg + Vit C, 2.5 mg FB_1/kg + Vit E, 5.0 mg FB_1/kg + Vit E, 7.5 mg FB_1/kg + Vit E, 2.5 mg FB_1/kg + Vit C & E, 5.0 mg FB_1/kg + Vit C & E and 7.5 mg FB_1/kg + Vit C & E). Diet 1 that contained non-*Fusarium* cultured maize meal served as the control diet. After drying, the feed was stored in a refrigerator.

The fish were fed the respective experimental diets twice daily at 7:00 and 15:00 hours at 3% body weight for 12 weeks. The fish were weighed every two weeks and feed weight was adjusted accordingly throughout the period of the experiment. Leftovers and faeces in each tank were siphoned daily. The water in the tanks was also changed with pre-conditioned deep well water every week. Fingerlings in each tank were observed daily in case of any behavioural and morphological changes, wounds, lesions and general well-being.

Blood collection and analysis

At the end of the feeding experiment, the fish were randomly selected from each treatment tank and blood samples of the fish were collected through the caudal vein using plastic syringe, fitted with 21-gauge hypodermal needles. Each blood sample was collected in duplicate, some into heparinized (50 IU per mL of blood) bottles which were used for haematological analysis and the other were collected into non-heparinized EDTA bottles used for serum biochemical analysis. The blood samples collected were stored in pre-iced collecting bottles and later put in refrigerator for further handling and analyses within 30 minutes of their collection.

The haematological analyses of blood samples were carried out at the University Health Centre of Adekunle Ajasin University, Akungba-Akoko, Nigeria. Evaluation of the haemogram involves the determination of the total erythrocyte count (RBC), total white blood cell count (WBC), haematocrit (PCV), haemoglobin (Hb) concentration and platelet using Mindray® Auto Haematology Analyser (3000 plus).

The serum biochemical parameters, including serum free cholesterol, total proteins, albumins, glucose, triglycerides and urea were determined by enzymatic colorimetric methods using commercial test kits (Quimica Clinica Applicada, S.A.), while the serum enzymes –alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), were quantified using test kits (Randox Laboratories Ltd, UK). All determinations were carried out in duplicate.

Statistical analysis

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

Results

The haematological values of *C. gariepinus* fed diets contaminated with varied levels of FB_1 with or without vitamin are as shown in Table 1. The PCV of the fish decreased with increase in the concentrations of the mycotoxin, with the PCV of those fed diet containing the highest FB_1 concentration being significantly lower (P<0.05) than those fed the control diet. The PCV values of the fish fed diets containing FB_1 supplemented with vitamins also decreased with increase in the toxin contents. However, the PCV of fish fed diets supplemented with vitamin E (Diets 8, 9, and 10) were significantly lower (P<0.05) than the control but statistically the same with those fed the diet containing the highest concentration of FB_1 (Diet 4).

Table 1. Haematological parameters of *C. gariepinus* fed diets contaminated with varied levels of FB_1 with or without vitamins (Mean ± SD)

	Parameter				
Treatment	**PCV* (%)**	**WBC** (10^9/L)**	**RBC*** (10^9/L))**	**Haemoglob in (g/L)**	**Platelets (10^9/L)**
Diet 1 (Control)	34.0±2.0[a]	87.0±2.0[b]	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]	101.0±18.0[ab]	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]	113.5±4.5[a]	2.03±0.43[b]	73.5±1.5[c]	35.0±0.0[a]
Diet 4 (7.5mg FB_1)	26.5±6.5[b]	116.0±12.0[a]	1.68±0.05[c]	92.0±18.0[b]	23.0±3.0[b]
Diet 5 (2.5mg FB_1 + Vit C)	32.5±1.5[ab]	106.5±0.5[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]	113.5±2.5[a]	2.51±0.07[a]	108.0±0.0[a]	18.0±4.0[bc]
Diet 7 (7.5mg FB_1 + Vit C)	28.5±1.5[ab]	117.0±3.0[a]	2.31±0.00[ab]	97.0±3.0[ab]	21.0±2.0[bc]
Diet 8 (2.5mg FB_1 + Vit E)	27.0±0.0[b]	96.5±3.5[c]	2.60±0.07[a]	93.5±0.5[ab]	13.0±1.0[d]
Diet 9 (5.0mg FB_1 + Vit E)	25.0±1.0[b]	98.0±4.0[bc]	2.15±0.04[b]	88.0±4.0[bc]	14.0±4.0[cd]
Diet 10 (7.5mg FB_1 + Vit E)	26.0±1.0[b]	100.5±2.5[abc]	2.05±0.15[bc]	88.0±5.0[bc]	15.5±1.5[cd]
Diet 11 (2.5mg FB_1 + Vit C&E)	32.0± 2.0[ab]	106.0 ± 6.0[a]	2.57±0.20[a]	109.0 ±5.0[a]	18.5±7.5[bc]
Diet 12 (5.0mg FB_1 + Vit C&E)	29.0 ±2.0[ab]	108.5±6.0[a]	2.33±0.13[ab]	98.5 ± 5.5[a]	16.0±16.0[c]
Diet 13 (7.5mg FB_1 + Vit C&E)	28.5 ±1.5[ab]	113.5±4.5[a]	2.23±0.08[ab]	98.0 ±2.0[a]	15.5±0.5[cd]

[a b c d]:- 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

The WBC counts of fish fed the experimental diets with or without vitamin generally increased with increase in FB_1 concentrations. The WBC counts of fish fed diets containing 5.0 and 7.5 mg FB_1/kg were significantly higher (P<0.05) than those fed the control diet. The WBC of fish fed diets containing lower concentrations of FB_1 supplemented with vitamin E (Diets 8 and 9) had the lowest WBC values.

The RBC values of *C. gariepinus* fed diets contaminated with varied levels of FB_1 with or without vitamin decreased with increase in FB_1. The fish fed diets containing higher concentrations of FB_1 of 5.0 and 7.5 mg/kg (Diets 3 and 4) had RBC values that were significantly lower (P<0.05) than those fed the control diet (Diet 1). The RBC of the fish fed diets supplemented with vitamin C or E decreased with increase in the FB_1 concentrations. Similarly, the Hb concentrations in fish fed Diets 3 and 4, containing higher FB_1 without

vitamin supplementation, were statistically lower (P<0.05) than in those fed diets containing same concentration of FB_1 but supplemented with vitamins C and E (Diets 12 and 13) and those fed diets containing lower concentrations of FB_1 supplemented with vitamin C. The platelet values of the fish fed diets containing varied concentrations of FB_1 increased with increase in the mycotoxin concentrations but decreased with supplementation with vitamins C and E.

Table 2 shows the serum proteins of *C. gariepinus* fed diets contaminated with varied concentrations of FB_1 with or without vitamin. The values of the serum proteins decreased with increase in FB_1 concentrations. The serum total protein value of fish fed diet containing 7.5 mg FB_1/kg (Diet 4) was significantly lower (P<0.05) than of those fed the control diet and diets containing lower concentrations of FB_1 (Diets 1, 2 and 3). The serum total protein of fish fed diet containing 2.5 mg FB_1/kg supplemented with vitamins C and E (Diet 11), which was statistically similar to the serum protein values of those fish fed the control diet and Diet 2, was, however, significantly higher (P<0.05) than of those fed other diets containing higher concentrations of FB_1 supplemented with vitamins C and E and other diets supplemented with vitamin C or E.

Table 2. Serum proteins of *C. gariepinus* fed diets contaminated with varied levels of FB_1 with or without vitamin (Mean ± SD)

Treatment	Protein (g/L)	Albumin (g/L)	Globulin (g/L)	Albumin/Globulin
	Parameter			
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]	15.70± 0.70[cd]	11.30±3.70[cd]	0.72±0.20[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]
Diet 8 (2.5 mg FB_1/kg + Vit E)	26.00±6.00[b]	15.20±0.80[cd]	10.80±6.80[cd]	2.08±1.69[a]
Diet 9 (5.0 mg FB_1/kg + Vit E)	27.00±3.00[b]	12.70±2.70[d]	14.30±5.70[bcd]	1.06±0.66[a]
Diet 10 (7.5mg FB_1/kg + Vit E)	24.00±2.00[b]	14.30±1.90[d]	9.70±3.90[cd]	1.73±0.97[a]
Diet 11 (2.5mg FB_1 + Vit C&E)	47.00± 2.50[a]	20.0 ± 1.80[bc]	27.00±17.20[a]	0.87 ± 0.33[b]
Diet 12 (5.0mg FB_1 + Vit C&E)	38.00± 0.00[ab]	12.3 ± 0.90[c]	25.70 ± 0.90[a]	0.48 ± 0.05[b]
Diet 13 (7.5mg FB_1 + Vit C&E)	34.00 ± 2.00[ab]	13.6 ± 2.80[c]	20.40 ± 4.80[a]	0.72 ± 0.31[b]

[a b c d]:- Means within the same column with different superscripts differ significantly (P< 0.05)

The serum albumin and albumin-globulin ratios of the fish did not follow any particular trend. However, the serum globulin of the fish fed diets containing lower concentration of 2.5 mg of the

mycotoxin per kg supplemented with vitamin C or vitamins C and E (Diets 5 and 12) were significantly higher (P<0.05) than the serum globulin of fish fed diets containing higher concentrations of FB_1 and those supplemented with either vitamin C, E or vitamins C and E.

Table 3 shows the serum biochemical values of *C. gariepinus* fed diets contaminated with varied levels of FB_1 with or without vitamin. The glucose levels of the fish decreased significantly (P<0.05) with increase in the concentrations of FB_1 in the diets. The serum glucose levels of fish fed diets containing varied concentrations of FB_1 were significantly lower (P<0.05) than the glucose level of those fed the control diet. The glucose level of fish fed diet containing 2.5 mg FB_1/kg supplemented with vitamin E was significantly higher (P<0.05) than of those fed diets containing 2.5 mg FB_1/kg supplemented with either vitamin C or vitamins C and E.

Table 3. Serum biochemical parameters of *C. gariepinus* fed diets contaminated with varied levels of FB_1 with or without vitamin (Mean ± SD)

Treatment	Parameter			
	Glucose (mg/L)	Cholesterol (mg/L)	Urea (mg/L)	Triglyceride (mg/L)
Diet 1 (Control)	129.00± 1.00[a]	144.00 ± 12.00[ab]	3.43 ± 0.31[a]	120.00±36.00[a]
Diet 2 (2.5 mg FB_1/kg)	77.00±1.20[b]	171.00±19.00[a]	3.61±0.21[a]	121.00± 9.00[a]
Diet 3 (5.0 mg FB_1/kg)	65.50±5.50[c]	158.50±11.50[ab]	2.59±1.14[ab]	121.00±19.00[a]
Diet 4 (7.5mg FB_1/kg)	66.50±5.50[c]	138.00±12.00[b]	1.80±0.60[b]	114.00±18.00[a]
Diet 5 (2.5 mg FB_1/kg + Vit C)	70.00 ±1.00[bc]	153.00±21.00[ab]	3.69 ± 0.04[a]	111.00 ± 3.00[a]
Diet 6 (5.0 mg FB_1/kg + Vit C)	66.00±1.00[c]	154.00±20.00[ab]	2.54±0.89[ab]	88.00 ± 8.00[a]
Diet 7 (7.5mg FB_1/kg + Vit C)	65.00±1.00[c]	140.00±3.00[b]	2.64±0.83[ab]	96.00 ± 8.00[a]
Diet 8 (2.5 mg FB_1/kg + Vit E)	77.50±3.50[b]	140.00±5.00[bc]	2.74±0.80[ab]	130.50±10.50[a]
Diet 9 (5.0 mg FB_1/kg + Vit E)	70.50±1.50[bcd]	121.00±11.00[c]	2.63±1.01[ab]	109.00±9.50[a]
Diet 10 (7.5mg FB_1/kg + Vit E)	68.00±3.00[bcd]	168.00±14.00[a]	1.69±0.04[b]	122.50±8.50[a]
Diet 11 (2.5mg FB_1 + Vit C&E)	65.50 ± 1.50[c]	160.00 ±16.00[abc]	3.10 ± 0.06[ab]	135.00 ± 9.00[a]
Diet 12 (5.0mg FB_1 + Vit C&E)	65.50 ± 5.50[c]	131.00 ± 23.00[c]	2.10 ± 0.27[c]	107.00±35.00[ab]
Diet 13 (7.5mg FB_1 + Vit C&E)	61.50 ± 1.50[c]	164.00 ± 14.00[ab]	1.73 ± 0.13[c]	77.00 ± 41.00[b]

[a b c d]:- Means within the same column with different superscripts differ significantly (P< 0.05)

The serum cholesterol level of fish fed diet containing the highest concentration of FB_1 without vitamin supplementation (Diet 4) was significantly lower (P<0.05) than of those fed the control or other diets containing lower concentration of FB_1 (Diets 2 and 3). Similarly, the serum cholesterol level of those fish fed diets containing 7.5 mg FB_1/kg supplemented with vitamin C was lower (P<0.05) than of those fed diet containing lower concentrations of FB_1 supplemented with vitamin E. The serum urea levels in the experimental fish decreased significantly (P<0.05) with increase in the concentrations of FB_1. The serum triglyceride tended to decrease with increase in the FB_1 concentrations across the treatments.

The levels of serum enzymes of *C. gariepinus* fed diets contaminated with varied levels of FB_1 with or without vitamin are as shown in Table 4. The results show that the serum AST levels generally increased with increase in the FB_1 concentrations. Fish fed diet containing the highest concentration of 7.5 mg FB_1/kg without supplementation (Diet 4) or with vitamin E supplementation (Diet 10) recorded significantly highest (P<0.05) serum AST level than those fed the control diet, which was not significantly different (P>0.05) from the AST levels of fish fed the other diets.

Table 4. Serum enzymes of *C. gariepinus* fed diets contaminated with varied levels of FB_1 with or without vitamin (Mean ± SD)

Treatment	Parameter		
	Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)	Alkaline phosphatase (U/L)
Diet 1 (Control)	152.50± 12.50[c]	93.00 ± 37.00[b]	70.50± 13.50[d]
Diet 2 (2.5 mg FB_1/kg)	150.00 ± 10.00[c]	181.00 ± 30.00[ab]	99.00±3.00[abc]
Diet 3 (5.0 mg FB_1/kg)	220.00±70.00[b]	167.00±37.00[ab]	105.50±18.50[ab]
Diet 4 (7.5mg FB_1/kg)	220.00±20.00[b]	225.00±23.00[a]	115.00±5.00[a]
Diet 5 (2.5 mg FB_1/kg + Vit C)	155.00±10.00[c]	154.00±47.00[ab]	73.00 ±9.00[d]
Diet 6 (5.0 mg FB_1/kg + Vit C)	270.00± 20.00[ab]	167.50±22.50[ab]	84.50±17.50[bcd]
Diet 7 (7.5mg FB_1/kg + Vit C)	287.50±7.50[a]	193.50±7.50[ab]	78.00± 5.00[cd]
Diet 8 (2.5 mg FB_1/kg + Vit E)	155.00±5.00[c]	154.00±20.00[ab]	96.00±49.00
Diet 9 (5.0 mg FB_1/kg + Vit E)	295.00±5.00[a]	138.50±2.50[ab]	96.00±24.00
Diet 10 (7.5mg FB_1/kg + Vit E)	285.00±5.00[a]	200.00±40.00[a]	92.50±9.50
Diet 11 (2.5mg FB_1 + Vit C&E)	240.00± 25.00[ab]	148.00±24.00[ab]	70.00 ± 11.00[c]
Diet 12 (5.0mg FB_1 + Vit C&E)	295.00± 5.00[a]	196.00±10.00[ab]	80.50 ± 13.50[bc]
Diet 13 (7.5mg FB_1 + Vit C&E)	300.00± 10.00[a]	199.00± 65.00[ab]	119.00 ± 7.00[a]

[a b c d]:- Means within the same column with different superscripts differ significantly (P< 0.05)

The serum ALT levels in fish fed diets containing 5.0 and 7.5 mg/FB_1 without vitamin supplementation were significantly higher (P<0.05) than those fed the control diet and Diet 2, containing 2.5 mg FB_1/kg. However, the ALT levels in fish fed diet containing 5.0 mg FB_1/kg but supplemented with vitamin C or E were statistically lower (P<0.05) than in those fed diets containing higher concentration of FB_1.

The serum ALP of the fish fed the experimental diets containing varied concentrations of FB_1 without vitamin supplementation increased significantly (P<0.05) with increase in the FB_1 concentrations. However, the ALP level of fish fed diets containing 2.5 mg FB_1/kg supplemented

with vitamin C or vitamins C and E were generally statistically lower (P<0.05) than of those fed diets containing higher concentrations of FB_1 supplemented with the antioxidants.

Discussion

Haematological and serum biochemical indices are becoming increasingly important diagnostic tools in fish medicine. 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). Several studies have reported nutritional and pathological signs of mycotoxin poisoning in fish and shrimp species. Although the detailed importance of fumonisins as toxic agents in fish remain obscure, adverse effects of fumonisin-contaminated diets have been reported in catfish and tilapia (Gbore *et al.*, 2010; Barbosa *et al.,* 2013). In this study, the significant decrease in PCV, RBC, Hb concentrations and platelet values of *C. gariepinus* fed Diet 4 (containing 7.5 mg FB_1/kg diet) shows that *C. gariepinus* exposed to high concentrations of FB_1 might have suffered significantly from the red blood cell synthesis (erythropoiesis), concentration of erythrocytes, and anaemia. In Nile tilapia (*Oreochromis niloticus*) fed dietary FB_1 for 8 weeks, Tuan *et al.* (2003) reported that haematocrit values were reduced in fish fed 150 mg FB_1/kg. Also, in young carps exposed to FB_1-contaminated feed (0.5 - 50 mg FB_1/kg body weight) by Pepeljnjak *et al.* (2003), the results showed alterations of haematological and biochemical parameters indicating liver and kidney damage. Gbore *et al.* (2010) reported significant alterations in haematological parameters in *C. gariepinus* fingerlings exposed to *Fusarium*-contaminated diets containing about 5.0 or more mg FB_1/kg for six weeks.

In contrast, the mean values for PCV and RBC of *C. gariepinus* fed Diet 7 also containing 7.5 mg FB_1/kg but supplemented with vitamin C were higher than for those fed Diet 4 and similar to the control diet showed that the antioxidant was efficient in reducing the effects of FB_1 on the PCV and erythrocyte counts of the fish. Also*,* the Hb of *C. gariepinus* fed diets containing FB_1 supplemented with vitamin C (Diets 5 and 6) or vitamins C and E (Diets 11, 12 and 13) which were higher than for those fed Diets 3 and 4 and similar to the control diet showed that the antioxidants were efficient in reducing the effects of FB_1 on the Hb concentrations of the fish. Vitamin C is a significant antioxidant with several cellular functions. It is involved in a number of metabolic processes in the human body, including those that are important for the optimal functioning of the oxygen energy system (Femi-Oloye *et al.*, 2019). In addition, it is an important free radical scavenger in extracellular fluids, trapping radicals and protecting bio-membranes from peroxide damage (Salah *et al.*, 2010; Adikwu and Deo, 2013; Jaiswal *et al.*, 2015, 2017).

Vitamin C is chemically adept of reacting with most of the physiologically vital radicals and oxidants and acts as an established hydro-soluble antioxidant (Eboh, 2014). Being an antioxidant, it defends the body from the detrimental effects of free radicals and contaminants. Furthermore, vitamin C helps vitamin E to return to its active form in the cell membrane. Because of this regeneration process, the combination of vitamins C and E provides better antioxidant protection than vitamin C or vitamin E alone. Indeed, combination of vitamins C and E was shown to have protective effects in many diseases associated with enhanced oxidative stress (Hfaiedh *et al.*, 2012). Vitamin C plays an important role in protection against different harmful oxidation reactions that involve molecular oxygen. Its properties as a reducing agent and its reaction with oxygen-derived free radicals seem to be its most important biological functions.

According to (Douglass and Jane, 2000), leucocytes (i.e., 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 showed significant changes in WBC counts of *C. gariepinus* exposed to feed contaminated with FB_1 at 5.0 and 7.5 mg/kg, which were significantly higher than those fed diets containing 2.5 mg FB_1/kg and the control diet revealed that the fish on these diets might have suffered leucocytosis. This may have also resulted from the excitation of the defence mechanism of the fish to counter the effect of the toxicant. The WBC counts of fish fed diets containing lower concentrations of FB_1 supplemented with vitamin E (Diets 8 and 9) recording the lowest WBC values indicated the role vitamin E might play in immune response in the face of intoxication. Most mycotoxins have been reported (Balogh *et al.*, 2007; Pál *et al.*, 2009) to provoke oxygen free radical formation. Several studies have shown that the threats posed by mycotoxins on aquacultural species and other animals susceptible to these toxic metabolites can be mitigated with the use of antioxidants. Antioxidants are reported to be potentially efficacious as they are able to act as superoxide anion scavengers (Citil *et al.*, 2005; Dvorska *et al.*, 2007). Vitamin E is recognized as an essential nutrient for all species of animals, including fish, and it is generally ingested along with fat-containing foods (Zingg, 2007). It is a fat-soluble antioxidant that acts as an antioxidant primarily by scavenging active free radicals by stopping the production of reactive oxygen species formed when fat undergoes oxidation (Riccioni *et al.,* 2007). Vitamin E has been reported to be one of the most important nutrients influencing the fish immune system (Ispir *et al.*, 2011). All these properties may have contributed to the ability of vitamin E in mitigating FB_1-evoked toxicity in the fish.

An estimation of the total quantity of serum proteins may be utilized as an evaluation of the nutritive state of an animal (Gbore and Egbunike, 2009). The total protein in serum can be used as an indicator of fish innate immune responses (Sahoo and Mukherjee, 2002; Ai *et al.*, 2004; Lin and Shiau, 2005). Therefore, in this present study, a decrease in the serum protein levels following increase in the concentrations of FB_1 as observed in this study, could be attributed to their damage by singlet oxygen, often due to oxidation of essential amino acids as reported by Okuda *et al.* (2000).

According to Mommsen *et al.* (1999), glucose concentration may vary greatly depending on the physiological status of the animal. Plasma glucose levels can increase, decrease, or keep constant under high plasma cortisol. The findings in this study showed decline in serum glucose and triglycerides of *C. gariepinus* with increase in FB_1 concentrations in the diets with or without vitamin. This result showed that vitamin C or E does not have influence on the serum glucose and triglycerides.

Harvey and Ferrier (2011) stated that aminotransferases are normally intracellular enzymes, with low levels found in the plasma representing the release of cellular contents during normal cell turnover. Alanine aminotransferase and AST are non-plasma specific enzymes that are localized in tissues cells of liver, heart, gills, kidneys, muscles and other organs, and their presence in the blood (plasma) may give specific information about organ dysfunctions (Gabriel and George, 2005). In this study, the higher levels of serum ALT and AST of *C. gariepinus* exposed to varied concentrations of FB_1 compared to those on control diet are an indication that the animals might have suffered several hepatic cell damages which caused the leakage of the enzymes into the extracellular space. A significant decrease in serum ALP of *C. gariepinus* fed Diets 5, 6, and 7 as observed in this study were similar to those fed the control diet, indicating the protective roles of supplemental vitamin C on serum enzymes alteration by FB_1. The presence of vitamin C diminished the adverse effect of FB_1 on most enzymatic activities in the

fish, as reported for AFB$_1$ in rabbits by (Yousef *et al.*, 2003). Also, vitamins C, E, and C+E partially prevented increase in liver enzymes and biochemical parameters that were induced by FB$_1$ in the fish, as reported for AFB$_1$ in rabbits (Karakilcik *et al.*, 2004).

Vitamin E is the most important lipophilic antioxidant and is residing mainly in the cell membranes, and thus helps to maintain membrane stability (Sun *et al.*, 2012). It is a powerful chain-breaking antioxidant that inhibits reactive oxygen species induced generation of lipid peroxyl radicals, thereby protecting cells from oxidative damage of cellular proteins (Shrivastava, 2012). While vitamin C, the most abundant hydrophilic antioxidant in the body, acts primarily in cellular fluid, particularly in combating free radical and protecting biomembranes from peroxidative damage induced by pollution (Hassan and Jassim, 2010; Skŕivan *et al.*, 2012).

Conclusions

The findings of this study showed how varied levels of FB$_1$ impaired the haematological and serum biochemical parameters of *C. gariepinus*. However, this study was able to demonstrate the ameliorative impact of vitamins C and E on the negative impacts of FB$_1$ on blood parameters of *C. gariepinus*. This impact was clearly noticed on the RBC, PCV, Hb, and ALP in the fish. The results showed that supplementation with vitamins C or E and/or both ameliorated deficits in haematological and biochemical changes induced by FB$_1$ administration in the fish, partly due to their manifested antioxidant properties.

Acknowledgements

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