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Growth Performance and Serum Lipids Profile of *Clarias gariepinus* Catfish Following Experimental Dietary Exposure to Fumonisin B_1

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Abstract

Fumonisin B_1 (FB_1) is produced by fungus of the genus *Fusarium* (*Fusarium verticiloides* and *F. proliferatum*), and occurs predominantly in maize. The consumption of feed contaminated with FB_1 has been reported to cause deleterious effects in some fish species. This study was designed to determine the effects of dietary FB_1 on growth and lipids profile of *Clarias gariepinus*. 450 juvenile catfish were stocked into 5 groups of tanks consisting of 3 tanks per group and fed one of five diets amended with FB_1 (0.0 mg; 10.0 mg; 20.0 mg; 40.0 mg and 80.0 mg FB_1/kg) for 56 days. At time point's day 7, 14, 28 and 56, five fish were sampled from each tank weighted, length measured and bled for of lipids profile determinations. Results show that there was a significant reduction ($P < 0.05$), in the mean body length of the fish fed diets amended with various amounts of FB_1 compared with those fed control diet; also, there was a significant reduction ($P < 0.05$) in the weight gain of fishes fed diets amended with FB_1 compared with the control. The specific growth rate and the feed conversion ratio at 56 days shows fish fed 0.0 mg FB_1/kg had the highest specific growth rate (0.39 ± 0.14%/day) and the lowest feed conversion ratio (0.59 ± 0.01) whereas, fish fed 80.0 mg FB_1/kg had the least specific growth rate (0.07% ± 0.01%/day) and the highest feed conversion ratio (1.95 ± 0.11). Dietary FB_1 caused significant increases ($P < 0.05$) in serum cholesterol, HDL-C; LDL-C; triglycerides and the sphinganine-sphingosine ratio. Dietary FB_1 at an inclusion rate ≥ 20 mg FB_1/kg of diet produced significant reduction in weight gain and hyperlipidemia

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marked by hypercholesterolemia, increased blood high-density lipid cholesterol, increased blood low-density lipid cholesterol, elevated blood triglycerides and elevated sphinganine-sphingosine ratio.

Keywords

***Clarias gariepinus*, Fumonisin B_1, Lipids Profile, Growth Performance, Cholesterol, Catfish**

1. Introduction

Fumonisin B_1 (FB_1) is a mycotoxin produced by fungus of the genus *Fusarium* (*Fusarium verticiloides* and *F. proliferatum*), and occurs predominantly in maize and in products made from maize [1]. FB_1 inhibits sphinganine N-acyltransferase (ceramide synthase), the enzyme that catalyzes the production of sphingolipids. The reduction of sphingolipids by FB_1 alters the control of cellular growth and cell-cell interactions with a resultant elevation of sphingoid bases [2]. The accumulation of the sphingoid bases and their metabolites alters phosphatidic acid phosphatase and monoacylglycerol acyltransferase activity with a concomitant interruption of phospholipids and fatty acid biosynthesis [2] [3]. Phospholipids and fatty acids have been shown to play an important role in the structure and function of biological membranes [4].

Whereas the consumption of feed and/or feed ingredients contaminated with FB_1 has been reported to cause deleterious effects such as diminution of weight gain, reduction of haematological parameters and immunosuppression, in some fish species [5] [6], other fish species notably the warm water catfishes are reported not to be sensitive to dietary fumonisin B_1 [7] [8]. The Clariid fish *Clarias gariepinus* is the largest group of cultured fish species in Nigeria [9]. These fish species are preferred because not only they command a good commercial value [10], but also they are generally considered to be hardy and easy to culture [10] [11]. The culture of clariid fishes involves the utilization of feeds containing up to 30% maize as a source of energy [12], thereby inadvertently imposing the risk of the introduction of FB_1 in the diets formulated for feeding the fishes [13] [14]. Aside from the report of [15], on the effects of fumonisin B_1 on growth and haematology of *Clarias gariepinus* catfish fingerlings, there is a paucity of information on the effects of dietary FB_1 on the lipids profile of clariid catfishes; this study therefore, was designed to determine the effects of dietary fumonisin B1 on growth and the lipids profile of *Clarias gariepinus* catfish following dietary FB_1 exposure.

2. Materials and Methods

Research grade FB_1 used for the study was purchased from Sigma Aldrich (St Loius, M.O USA). Purity was ascertained by HPLC-fluorescence detection after derivatization with *o*-phthaldiadehyde (OPA, Sigma) to be greater than 98%. Other chemicals and reagents used for the study were purchased commercially at the highest degree of purity available.

2.1. Preparation of the FB_1 Stock Solution

Fumonisin B_1 stock solution was prepared by dissolving 1 gm fumonisin B_1 (Sigma chemicals St Loius, USA) with 1000 µl of acetonitrile-water (Vol.:Vol.) resulting in a 1 µL:1 mg solution of FB_1.

2.2. Preparation of the Basal Diets

The basal diet was formulated according to [16], with slight adjustments, using the following ingredients (fish meal 19%, Soybean cake 37%, maize 32.25%, palm oil 1.0%, fish oil 6.0%, Starch binder 2.0%, vitamin/mineral premix 0.5%, Bone meal 1.0%, salt 0.25%) to meet the nutritional requirements of juvenile Clariid fishes. Formulated diets were then subjected to proximate analysis to ascertain their nutritional status (**Table 1**).

Preparation of the Experimental FB_1 Diets

From the FB_1 stock solution, the volume of solution needed to produce the experimental diets for the various FB_1 inclusions were pipetted into 1000 ml beakers into which had been placed 200 ml warm distilled water. Af-

ter careful stirring, weighted portions of the starch binders were added, followed by the addition of the weighed portions of the basal diets and finally, pelletizing using a bench extruder. The finished feeds were oven dried at 65˚C for 5 hours, allowed to cool to room temperature and packed in cellophane bags, labelled (control diet, D0 = 0.0 mg FB_1/kg; diet D1 = 10.0 mg FB_1/kg; diet D2 = 20.0 mg FB_1/kg; diet D3 = 40.0 mg FB_1/kg and diet D4 80.0 mg FB_1/kg) and there after stored at 4˚C until used.

2.3. Experimental Fish

450 juvenile fish were procured from a commercial hatchery, acclimatized for 15 days. The fishes were then weighted (151.64 ± 2.11 g), total length measured (27.00 ± 1.39 cm) and then randomly distributed into fifteen 1000 L capacity tanks which were divided into five groups consisting of three tanks each labelled A1, A2, A3 (for the group A tanks); B1, B2 and B3 (for the group B tanks); C1, C2, C3 (for the group C tanks); D1, D2, D3 (for the group D tanks and E1, E2 and E3 (for the group E tanks) respectively at a stock density of 30 fish per tank. Stocked fish were then fed diets D0 (Control, 0.0 mg FB_1/kg); D1 (10.0 mg FB_1/kg); D2 (20.0 mg FB_1/kg); D3 (40.0 mg FB_1/kg) and diet D4 (80.0 mg FB_1/kg) for 56 days. At time points 7, 14, 28, and 56 days, using a hand held net, 5 fish were randomly selected from each tank, weighed and total length measured. At these same time intervals, blood for serum lipids determinations were obtained from the fish by caudal veni-puncture (using a 23 G needle fitted on a 5 ml syringe).

2.3.1. Determination of Growth Performance

The effects of dietary fumonisin B_1 on growth performance was determined by the evaluation of the Specific Growth Rate (SGR) and the Feed Conversion Ratio (FCR) after 56 days of feeding. The specific growth rate was determined according to [17], using the formulae $SGR = 100 \times [(\ln W_2 - \ln W_1)/t_2 - t_1]$. Were, W_2 is weight attained after period of feeding on diet; W_1 is weight at commencement of the feeding experiment; $t_2 - t_1$ is the time period (in days) of feeding on the experimental diets. The feed conversion ratio was calculated according to [18] using the formulae FCR = Amount of feed eaten (dry weight) (g)/fish weight gain (g).

2.3.2. Collection of Blood Sample for Determination of Serum Lipid Profiles

Blood samples were collected from both the control and the treatment groups. The aspirated blood were then dispensed into clean glass tubes and allowed to clot and then spurn with a bench centrifuge at 3000 RPM for 5 minutes. Serum were separated from the clotted blood using a clean syringe fitted with a clean needle and dispensed into clean ependorph tubes and stored at 0˚C until used.

1) Determination of serum lipid profile

Total serum cholesterol was estimated using automatic serum chemistry auto analyser and kit (AUTOPAK supplied by Beyer Diagnostics India) by the enzymatic (Cholesterol Esterase, Cholesterol Oxidase and Peroxidase method of [19]). Results obtained were expressed as $mg \cdot dL^{-1}$ of serum.

2) Determination of serum triglycerides

Serum Triglycerides estimation was carried out with the use of the automatic serum analyser and kit (AUTOPAK, Bayer Diagnostics India) by the enzymatic Lipoprotein lipase, Glycerol kinase, Glycerol-3-Phosphate Oxidase method of [20]. Results are expressed as $mg \cdot dL^{-1}$ of serum.

3) Determination of serum high density lipid (HDL)

The high density lipid in the serum was estimated using the automatic serum analyser and kits (AUTOPAK, Bayer Diagnostics India) by the phosphotungstate method. Results obtained were expressed as $mg \cdot dL^{-1}$ of serum.

Very Low Density Lipoprotein-Cholesterol (VLVD) in serum was estimated using the Friedewald formula [21].

$$\text{VLVD-Chol} = \text{Serum triglycerides}/5$$

The results obtained are expressed as $mg \cdot dL^{-1}$ of serum.

4) Determination of serum low density lipid

The serum Low Density Lipoproteins was estimated mathematically as the difference between the serum total cholesterol and the sum of the Very Low Density Lipid cholesterol and the High Density Lipid cholesterol according to the Friedwald formula [21].

$$\text{LDL-Chol}\ \left(\text{mg/dL serum}\right) = \text{Total Chol} - \left(\text{VLDL-Chol} + \text{HDL-Chol}\right).$$

2.3.3. Quantification of Biomarkers of FB_1 Exposure

The quantification of serum sphinganine (Sa) and sphingosine (So) in serum was done according to the method of [22]. Briefly, 500 µl of serum was de-proteinized with 2 ml of methanol (CH_3OH), and then centrifuged for 10 minutes at 1200 g and 100C. A 1.9 ml aliquot of the supernatant was collected and mixed with an equal volume of water (1.9 ml) and 1.2 ml NH_4OH 0.35 M, and was then extracted with 4 ml of $CHCL_3$. After thorough mixing, the phases were separated by centrifugation at 1200 g and 10˚C for 10 minutes. Then a 3 ml aliquot of the chloroformic phase was transferred to a silica gel mini column to eliminate the interferents.

The 3 ml chloroformic extracts was cleaned by passing it through polypropylene columns containing 5 g of anhydrous Na_2SO_4 crystals packed on top of 0.2 g of silica gel 60 (15 - 40 µm). The mini columns were preconditioned with 3 ml $CHCL_3$ (at a flow rate of less than 2 ml per minute), the chloroformic extracts were eluted and the column washed with 1 ml $CHCL_3$, discarding the eluate. The sphingoid bases (Sa and So) were eluted with 4 ml of $CHCL_3$:CH_3OH:NH_4OH (50:50:2) solution, thus evaporating the solvent from this eluate. The residues obtained was then dissolved with 1 ml of a 0.125 M KOH solution in CH_3OH:$CHCL_3$ (4:1, vol.:vol.), and were later incubated at 37˚C for 90 minutes. Then 1 ml of $CHCL_3$ was added to each sample, and the organic phase was washed with alkaline water (0.6 ml of NH_4OH 0.35 M in 250 ml of bi-distilled water). The chloroformic phases were recovered by centrifugation for 10 min at 1200 g and 10˚C; before being dried.

The dried residues obtained from the sera were then dissolved with 250 µL of CH_3OH:H_2O (9:1). They were then derivatized with 50 µL of *O*-pthaldialdehide (OPA) reagent, and were analysed by reverse-phase HPLC. The OPA was prepared by adding 10 ml 3% Boric acid (pH adjusted to 10.5 with KOH); to a solution prepared with 5 ml OPA dissolved in 100 µL CH_3OH and 5 µL of 2-mercaptoethanol. The Sa and So levels were then quantified by a Hewlett Packard 1100 HPLC equipped with fluorescence detector set at 335 nm and 440 nm wave lengths for excitation and emission respectively. The Sa and So levels were calculated by extrapolating the peak areas obtained for experimental samples, in a calibration curve constructed by injection of commercial standards of Sa and So (44.4; 177.7 and 355.3 ng/ml).

3. Statistical Evaluation

Results were expressed as the mean ± Standard Deviation of the mean of each group (n = 30), and analysed by a one way analysis of variance (ANOVA). Variant means were then separated by the Turkey-Kramer Post hoc test (SPSS version 20). Differences were considered significant at $P < 0.05$.

4. Results

4.1. Effects on Growth Performance

The nutrient composition and proximate analyses of the control and fumonisin B_1 amended diets as well as the FB_1 contents of the diets are as presented in **Table 1**. It shows the presence of fumonisin B_1 did not affect the percentage crude protein, metabolizable energy and digestible energy levels of the diets.

Table 2 depicts the growth performance of juvenile *Clarias gariepinus* catfish fed varied amounts of FB_1. It shows there were no significant difference ($P > 0.05$) in both the length and weight of the fishes fed the control and experimental diets at the start of the experiments. **Table 2** also show that after 56 days of dietary FB_1 exposures, there were significant difference ($P < 0.05$), in the mean body length of the fishes fed the control diets when compared with those of fishes fed the diets amended with various amounts of FB_1. **Table 2** shows the fishes fed the control diets had the highest gain in body length (47.63 ± 1.09 cm representing a 69.50% ± 1.20% gain in body length), while fishes fed the diets containing 40 mg FB_1/kg had the least gain in body length (34.02 ± 0.48 cm which represent 17.31% ± 0.48% gain in body length). Although, there was a significant difference ($P < 0.05$) in the mean final body length in a comparison of fishes fed the control diets and the experimental diets; Turkey post hoc evaluation reveals, there were no variation ($P > 0.05$) in the mean final length of fishes fed the diets amended with the various amounts of FB_1.

One way analysis of variance (ANOVA) reveals there was a significant difference ($P < 0.05$) in the percentage gain in total length in a comparison of fishes fed the control fish and those fed the diets amended with fumonisin B1. Turkey post hoc reveals there were significant differences ($P < 0.05$) in the percentage gain in

Table 1. Nutrient composition, fumonisin B_1 content and proximate analysis of formulated diets.

	FUMONISIN B_1 AMENDED DIETS				
Parameter	D0	D1	D2	D3	D4
Fish meal	19.00	19.00	19.00	19.00	19.00
SoyBean cake	37.00	37.00	37.00	37.00	37.00
Maize	32.25	32.23	32.23	32.25	32.25
Palm oil	1.00	1.00	1.00	1.00	1.00
Fish oil	6.00	6.00	6.00	6.00	6.00
Vit/min Premix	0.50	0.50	0.50	0.50	0.50
Bone meal	1.00	1.00	1.00	1.00	1.00
Salt (NaCl)	0.23	0.25	0.25	0.25	0.25
Fumonisin B1	0.00	10.00	20.00	40.00	80.00
Starch Binder	2.00	2.00	2.00	2.00	2.00
Crude Protein	40.01	40.00	40.04	40.01	40.02
Gross Energy	19.77	20.00	20.01	19.86	20.00
Digestible Energy	12.00	12.01	11.98	12.01	12.10

D0 = 0.0 mg FB_1/kg; D1 = 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4 = 80.0 mg FB_1/kg.

Table 2. Growth performance of *Clarias gariepinus* juveniles fed varied levels of fumonisin B_1 (FB_1) fifty six days.

	FUMONISIN B_1 AMENDED DIETS				
Parameter	D0	D1	D2	D3	D4
Mean initial body length (cm)	28.10 ± 0.10^a	27.00 ± 1.39^a	28.60 ± 1.41^a	29.00 ± 0.93^a	27.70 ± 0.66^a
Mean final body length (cm)	47.63 ± 1.09^a	35.24 ± 0.88^b	34.87 ± 0.41^b	34.02 ± 0.48^b	36.38 ± 1.75^b
Mean gain in length (%)	69.50 ± 1.20^a	30.52 ± 0.33^b	21.92 ± 0.09^c	17.31 ± 0.48^d	31.33 ± 0.93^b
Mean initial weight (g)	153.09 ± 1.37^a	151.64 ± 2.11^a	152.07 ± 1.33^a	152.61 ± 0.93^a	151.78 ± 1.27^a
Mean final weight (g)	190.01 ± 2.03^a	168.14 ± 0.01^b	166.30 ± 0.12^b	164.21 ± 0.81^c	163.41 ± 0.14^c
Mean gain in weight (%)	24.12 ± 1.31^a	10.88 ± 1.27^b	8.56 ± 0.01^c	7.60 ± 0.07^d	7.66 ± 0.12^d
SGR (%/day)	0.39 ± 0.14^a	0.19 ± 0.08^b	0.16 ± 0.02^b	0.13 ± 0.01^b	0.07 ± 0.01^c
FCR	0.59 ± 0.01^a	0.98 ± 0.01^b	1.01 ± 0.00^b	1.24 ± 0.01^c	1.95 ± 0.11^c

Rows with different superscript are significantly different ($P < 0.05$). D0 = 0.0 mg FB_1/kg; D1 = 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4 = 80.0 mg FB_1/kg.

length amongst the fishes fed the diets amended with fumonisin B1. One way analysis of variance (ANOVA) of the data obtained for the mean final weight gain shows there was a significant difference ($P < 0.05$) in the comparison of the weight gain of fishes fed the control diets and those fed the diets amended with fumonisin B1; Turkey post hoc test shows there are no variations ($P > 0.05$) in the weight gain of fishes fed the diets amended with 10.0 mg FB_1/kg and 20.0 mg FB_1/kg; these were however significantly different ($P < 0.05$) from those of fishes fed diets containing 40.0 mg FB_1/kg and 80.0 mg FB_1/kg. Also, there was no significant variation ($P > 0.05$) in the mean gain in weight of fishes fed diets containing 40.0 and 80.0 mg FB_1/kg (**Table 2**).

Evaluation of the data obtained for the specific growth rate (SGR) and the feed conversion ratio (FCR) at 56 days of dietary exposure to fumonisin B1 shows fishes fed the control feed (0.0 mg FB_1/kg) had the highest specific growth rate (0.39% ± 0.14%/day) and the lowest feed conversion ratio (0.59 ± 0.01) whereas, fishes fed

80.0 mg FB_1/kg had the least specific growth rate (0.07% ± 0.01%/day) and the highest feed conversion ratio (1.95 ± 0.11). One way analysis of variance (ANOVA) reveals significant variation ($P < 0.05$) in the SGR of fishes fed the control diets compared with fish fed the diets containing various amounts of fumonisin B_1. Also, Turkey post hoc shows whereas there were no significant difference ($P > 0.05$) in the SGR of fishes fed diets containing 10.0, 20.0 and 40 mg FB_1/kg, there was a significant difference ($P < 0.05$) in the SGR of fishes fed diets amended with fumonisin $B_1 \leq 40.0$ mg FB_1/kg of the diet compared with fishfed 80.0 mg FB_1/kg of the diet (**Table 2**).

4.2. Effects of FB_1 on Serum Lipids

The results obtained for the effects of dietary exposure to fumonisin B_1 on the serum total cholesterol are as shown in **Table 3**. It shows at 7 days of feeding on the diet, serum cholesterol increased from 92.20 ± 1.00 mg·dL^{-1} in fish fed diet D0(the control diet) to 107.29 ± 2.02 mg·dL^{-1} in fish fed diet D4 (80.0 mg FB_1/kg).One way ANOVA shows significant variations ($P < 0.05$) in the serum cholesterol levels of the fish fed the control diet compared with those fed diets amended with FB_1; further, Turkey post hoc showed whereas the serum cholesterol level of fish fed diet D2 (20.0 mg FB_1/kg) and D3 (40.0 mg FB_1/kg) are not significantly different ($P > 0.05$) they were however, significantly different from those of fish fed diet D1 (10.0 mg FB_1/kg) and diet D4 (80.0 mg FB_1/kg). At 14 day dietary exposure to fumonisin B_1, the serum cholesterol level ranged from 94.89 ± 3.48 mg·dL^{-1} in fish fed diet DF1 to 129.19 ± 3.41 mg·dL^{-1} in fish fed diet D4. There was no difference ($P > 0.05$) in the serum cholesterol concentration of fish fed diet D0 and the fish fed diet D1, D3 and D4; the serum cholesterol of fish fed D2 however, differed significantly ($P < 0.05$) from those of fish fed the other diets. At 28 days of fumonisin B1 exposure, the serum cholesterol levels ranged from 95.41 ± 1.11 mg·dL^{-1} (in fish fed the control diet) to 130.72 ± 2.40 mg·dL^{-1} in fish fed diet D4 (80.0 mg FB_1/kg). Turkey post hoc showed that except for fish fed diet D2, the serum cholesterol levels were not significantly different ($P > 0.05$) in the fish fed the diets amended with FB_1. At day 56 of the feeding trial, the serum cholesterol level of fish fed diet D2 (20.0 mg FB_1/kg) was not significantly different ($P > 0.05$) from the serum cholesterol of fish fed diet D3 (40.0 mg FB_1/kg) but were however, significantly different ($P < 0.05$) from those of fish fed the control diet (0.0 mg FB_1/kg), diet D1 (10.0 mg FB_1/kg) and diet D4 (80.0 mg FB_1/kg).

Table 4 shows the serum high density lipid cholesterol (HDL-C) concentration of juvenile *Clarias gariepinus*

Table 3. Serum total cholesterol concentration (mg·dL^{-1}) of juvenile *Clarias gariepinus* fed diets amended with varied amounts of fumonisin B_1 for fifty six days.

	FUMONISIN B_1 AMENDED DIETS				
Days feeding	D0	D1	D2	D3	D4
7	92.20 ± 1.00^a	98.07 ± 0.13^b	101.24 ± 1.09^c	103.31 ± 1.07^c	107.29 ± 2.02^d
14	95.19 ± 1.31^a	94.89 ± 3.48^a	112.81 ± 1.09^b	128.99 ± 1.78^c	129.19 ± 3.41^c
28	95.41 ± 1.11^a	96.69 ± 1.09^a	113.78 ± 1.06^b	128.43 ± 1.12^c	130.77 ± 2.40^c
56	93.56 ± 0.66^a	94.27 ± 1.96^a	135.44 ± 2.37^b	135.99 ± 1.08^b	155.41 ± 6.13^c

Rows with different superscript are significantly different ($P < 0.05$). D0 = 0.0 mg FB_1/kg; D1 = 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4 = 80.0 mg FB_1/kg.

Table 4. Serum high density lipid cholesterol concentration (mg·dL^{-1}) of juvenile *Clarias gariepinus* fed diets amended with varied amounts of fumonisin B_1 for fifty six days.

Days feeding	**D0**	**D1**	**D2**	**D3**	**D4**
7	10.01 ± 1.21^a	12.07 ± 1.00^b	10.23 ± 1.37^a	10.24 ± 1.41^a	10.19 ± 1.67^a
14	7.91 ± 0.83^a	9.37 ± 0.30^{bc}	9.81 ± 1.00^c	8.99 ± 1.00^b	9.10 ± 0.41^b
28	7.79 ± 0.77^a	10.94 ± 0.38^b	10.17 ± 1.21^c	9.29 ± 1.47^d	9.96 ± 0.41^{cd}
56	7.03 ± 0.01^a	9.48 ± 1.87^b	9.99 ± 0.67^b	9.71 ± 0.99^b	10.00 ± 0.01^b

Rows with different superscript are significantly different ($P < 0.05$). D0 = 0.0 mg FB_1/kg; D1 = 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4 = 80.0 mg FB_1/kg.

exposed to varied amounts of dietary FB_1 for 56 days. It shows although there were marginal increases in the serum HDL-C levels in fish exposed to dietary FB_1, except for fish fed diet containing 10.0 mg FB_1/kg, at 7 days of feeding, the serum HDL-C level of the control fish was not significantly different ($P > 0.05$) from those of the fish fed the diets amended with varied amounts of FB_1. At 14 days and as was at day 28 and 56 post dietary exposure to FB_1, the serum HDL-C levels in the exposed fish was significantly increased ($P < 0.05$) compared with those of the control fish; Turkey post hoc evaluation revealed the variation in the serum HDL-C of the fish fed the diets amended with FB_1 at days 14, 28 and 56 to be significant ($P < 0.05$).

Results obtained from the dietary exposure of juvenile *Clarias gariepinus* catfish to FB_1 on serum low density lipid cholesterol (LDL-C) are shown in **Table 5**. It shows at 7 days, except for fishes fed diets D4 (80.0 mg FB_1/kg), there was reduction of the LDL-C from 70.63 ± 0.98 mg·dL^{-1} (in fish fed 0.0 mg FB_1/kg) to 63.80 ± 0.84 mg·dL^{-1} (in fish fed diet containing 10.0 mg FB_1/kg). **Table 5**, also shows that at 7 days of feeding, there was no significant difference ($P > 0.05$) in the serum concentration of the LDL-C in fish fed diets containing between 10.0 and 40.0 mg FB_1/kg these values where however significantly ($P < 0.05$) different when compared with those of fish fed the control diet (0.0 mg FB_1/Kg) and diet D4 (80.0 mg FB_1/kg). At 56 days of dietary exposure, the serum LDL-C ranged from 64.59 ± 0.37 mg·dL^{-1} (in fish fed 10.0 mg FB_1/kg) to 118.99 ± 0.64 mg·dL^{-1} (in fish fed diet containing 80.0 mg FB_1/kg). ANOVA shows there was significant difference ($P < 0.05$) in the LDL cholesterol of fish exposed to dietary FB_1 for 56 days compared with the control; Turkey post hoc further reveals except for fish fed diets with FB_1 inclusion rates ≥ 40 mg at 28 days of feeding, there were no significant difference in the serum LDL cholesterol concentration of fish fed the diet amended with the varied amounts of FB_1 for 56 days.

Dietary exposure to FB_1 caused significant increases in the serum triglyceride concentrations of the exposed fish (**Table 6**). At seven days of the feeding trial, the serum triglyceride levels ranged between 82.79 ± 0.34 mg·dL^{-1} to 134.90 ± 0.72 mg·dL^{-1}. One way analysis of variance (ANOVA) showed there were significant variations ($P < 0.05$) in the serum triglycerides concentrations obtained for the fish fed the diets amended with FB_1 when compared with those of fish fed the control diets (**Table 6**). Further, Turkey-Kramer post hoc evaluation showed significant ($P < 0.05$) variation in the serum triglyceride concentration for the fish fed the diets amended with varied amounts of FB_1. At day 56, the serum triglyceride levels of the fish fed the control diet

Table 5. Serum low density lipid cholesterol concentration (mg·dL^{-1}) of juvenile *Clarias gariepinus* fed diets amended with varied amounts of fumonisin B_1 for fifty six days.

	FUMONISIN B_1 AMENDED DIETS				
Days feeding	D0	D1	D2	D3	D4
7	70.63 ± 0.98^a	63.80 ± 0.84^b	64.49 ± 0.31^b	66.09 ± 1.00^b	70.56 ± 1.02^a
14	71.24 ± 0.32^a	64.02 ± 0.19^b	77.45 ± 0.56^b	93.84 ± 0.75^b	93.81 ± 1.01^b
28	71.85 ± 0.17^a	62.87 ± 0.45^b	77.69 ± 0.92^b	93.61 ± 0.17^c	94.93 ± 1.76^c
56	**70.31 ± 0.64^a**	**64.59 ± 0.37^b**	**99.76 ± 0.55^b**	**100.19 ± 0.04^b**	**118.99 ± 0.64^b**

Rows with different superscript are significantly different ($P < 0.05$). D0 = 0.0 mg FB_1/kg; D1 = 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4 = 80.0 mg FB_1/kg.

Table 6. Serum Triglycerides concentration (mg·dL^{-1}) of juvenile *Clarias gariepinus* fed diets amended with varied amounts of fumonisin B_1 for fifty six days.

	FUMONISIN B_1 AMENDED DIETS				
Days feeding	D0	D1	D2	D3	D4
7	82.79 ± 0.34^a	111.02 ± 0.49^b	132.61 ± 0.39^c	134.90 ± 0.72^d	132.72 ± 0.33^c
14	80.22 ± 0.81^a	107.51 ± 0.54^b	128.13 ± 0.97^c	130.81 ± 0.19^d	131.40 ± 0.21^e
28	78.84 ± 0.37^a	114.42 ± 0.76^b	129.59 ± 0.83^c	127.67 ± 0.26^d	129.42 ± 0.37^c
56	81.17 ± 0.71^a	100.99 ± 0.28^b	128.44 ± 0.51^c	130.44 ± 0.90^d	132.08 ± 0.54^e

Rows with different superscript are significantly different ($P < 0.05$). D0 = 0.0 mg FB_1/kg; D1 = 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4 = 80.0 mg FB_1/kg.

differed significantly ($P < 0.05$) compared to values obtained for fish fed the diets amended with FB_1.

Table 7 shows the results obtained for the serum sphinganine (Sa) and serum sphingosine (So) following dietary exposure of juvenile *Clarias gariepinus* catfish to diets amended amended with varied amounts of FB_1 for 56 days. At 7 days of dietary exposure, the sphiganine-sphingosine ratio of the fish fed the control diet was significantly different ($P < 0.05$) from those of fish fed the diets amended with FB_1. At this time point, the Sa-So ratio ranged from 0.45 ± 0.01 (in fish fed the control diet) to 1.71 ± 0.03 (in fish fed diet D4). Further, Turkey-Kramer post hoc analysis shows that whereas the Sa-So ratio varied significantly in a comparison of fishes fed diets amended with 10.0 and 20.0 mg FB_1/kg, the Sa:So values obtained for fish fed diets greater than 20 mg FB_1/kg were not significantly ($P > 0.05$) different from one another. At day 14 and 56, the Sa-So ratio of fed the control diets were significantly different from those of fish fed the diets amended with FB_1. Turkey-Kramer post hoc further shows that the Sa-So ratio of fish fed the FB_1 amended diets differed significantly ($P < 0.05$) from one another.

Results for the assessment of the fish culture water quality parameters are within the range prescribed for fresh water fishes and are as follows: the pH of the culture water ranges between 7.0 and 7.4; alkalinity ranged from 0.55 mmol/L to 0.70 mmol/L; the nitrates concentration of the fish culture water ranged from 0.014 mg/L to 0.048 mg/L while the nitrites concentration in the fish culture water ranged from 5.70 mg/l to 10.40 mg/L. Also, the dissolved oxygen concentration of the fish culture water ranged between 5.17 mg/L and 7.05 mg/L while the temperature of the culture water ranged from 27.5˚C to 30.8˚C throughout the duration of the experiment [23] [24].

5. Discussion

5.1. Effects on Growth Performance

In the present study, dietary FB_1 significantly reduced growth performance parameters measured; similar effects have been reported in other fish species (*Ictalurus puntatus*, [25], *Oreochromis niloticus*, [26] [27]). The mean body length was reduced in the treated fish in a nonspecific manner as the FB_1 contents of the diets increased; except for fish fed 80.0 mg FB_1/kg, the percentage length gain decreased along the gradient of FB_1 inclusion. The mean weight gain and the percentage weight gain decreased along the gradient of concentration of FB_1 inclusion in the diets. Previous studies of the toxicity of FB_1 in other fish species have shown that age, weight and the duration of exposure modulates the susceptibility to FB_1. [7], reported dosages of up to 20 mg FB_1/kg reduced weight gain in 1.2 g Channel catfish (*Ictalurus punctatus*) compared with the controls while the thresh hold for toxicity in larger fish (6.1 g) was higher (40.0 mg FB_1/kg); the same authors further noted that, 2 years old channel catfish exposed to FB_1 for 98 days gained less weight only at concentrations above 80.0 mg FB_1/kg. In Nile Tilapia (*Oreocrhomis niloticus*), dietary exposure of up to 40 mg FB_1/kg or higher was reported to reduce weight gain only after 56 days of dietary FB_1 exposure [26]. The loss in growth performance induced by dietary exposure to FB_1 in *Clarias gariepinus* is expected as FB_1 has been demonstrated to interfere with cellular growth and cell-cell interactions [4]. The feed conversion ratio (FCR) of the fish exposed to FB_1 was significantly increased when compared to fish fed the control feed, indicating these groups of fish needed to consume more feed to produce the same weight gain as the control fish similar to the findings of [27] in broiler chicks.

Table 7. Serum sphinganine-sphingonine ratio of juvenile *Clarias gariepinus* fed diets amended with varied amounts of fumonisin B_1 for fifty six days.

	FUMONISIN B_1 AMENDED DIETS				
Days feeding	D0	D1	D2	D3	D4
7	0.45 ± 0.01^a	1.57 ± 0.12^b	1.70 ± 0.19^c	1.68 ± 0.01^c	1.71 ± 0.03^c
14	0.39 ± 0.01^a	1.66 ± 0.03^b	1.60 ± 0.09^c	1.70 ± 0.03^d	1.66 ± 0.01^b
28	0.41 ± 0.02^a	1.87 ± 0.01^b	2.03 ± 0.01^c	2.12 ± 0.01^d	2.16 ± 0.13^d
56	0.41 ± 0.01^a	1.79 ± 0.02^b	1.68 ± 0.11^c	1.69 ± 0.02^c	1.88 ± 0.12^d

Rows with different superscript are significantly different ($P < 0.05$). D0 = 0.0 mg FB_1/kg; D1 = 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4 = 80.0 mg FB_1/kg.

5.2. Effects on Lipids Profile

Dietary fumonisin B1 exposure caused a significant increase in the sphinganine-sphingosine (Sa:So) ratio starting from day 7 of the feeding trial. In addition to the nutritional parameters commonly employed as markers of toxicity in FB1 exposures; the increase in the Sa-So ratio has also been used as toxicity indicator in diverse experimental animal models [28]. The increased serum Sa-So ratio in the exposed *Clarias gariepinus* catfish juveniles was expected as FB_1 is structurally similar to sphinganine and sphingosine [29]-[31] and therefore competitively inhibits the enzyme ceramide synthase resulting in the accumulation of free sphinganine and to a lesser extent, sphingosine leading to an increase in the Sa-So ratio in exposed fish.

Serum total cholesterol increased significantly throughout the duration of the dietary exposure. The increase in serum total cholesterol occurred along the gradient of concentration of the FB_1 content of the diets as well as on the duration of dietary FB_1 exposure with the highest increase (66.11%) occurring in fish fed diet containing 80.0 mg FB_1/kg at 56 days of dietary exposure. The increase in serum cholesterol in the juvenile *Clarias gariepinus* was an expected result as hypercholesterolemia has been reported to be an early finding in a number of exposed animals [13] [27], including fishes [5]. Hypercholesterolemia in FB_1toxicosis has been attributed to a disruption of Cytochrome P450 enzymes activity [4] and may also be a result of hepatic dysfunction initiated by the hepatonecrosis caused by the ingested FB_1 in the diets [32].

High density lipoproteins are responsible for the reverse transport of cholesterol from the peripheral cells to the liver where cholesterol is transformed to bile acids which are excreted into the biliary tract. Elevated HDL-cholesterol concentrations particularly in conjunction with an elevated triglycerides increases cardiovascular risk [33]. LDL-C transports cholesterol to the arteries where they can be deposited on the arterial proteoglycans leading to the formation of plaques which have been shown to narrow the arterial lumen [4] predisposing to such diverse pathophysiologies as cardiovascular diseases (CVD) and atherosclerosis. The increase of LDL cholesterol in this study may be indicative of an increased risk of cardiovascular disease in the dietary FB_1 exposed catfish.

When compared with the fish fed the control diet, the serum triglycerides concentration of fish fed diets amended with FB_1 increased in a nonspecific manner. Fish under condition of stress have been shown to mobilize triglycerides and protein to fulfill an increased demand for energy not only to cope with the detrimental condition imposed by the toxicant, but also, to meet the energy required to sustain increased physical activity, biotransformation and the excretion of the noxious substance [34] [35]. Results obtained from this study shows that FB_1 imposed a nonspecific stress on the *Clarias gariepinus* juvenile catfishes which caused an increase in the serum concentration of triglycerides.

6. Conclusion

In conclusion, the study revealed that dietary exposure to fumonisin B_1 at an inclusion rate greater than or equal to 20 mg FB_1/kg of diet produced significant loss in weight gain and hyperlipidemia marked by hypercholesterolemia, increased blood high density lipid cholesterol, increased blood low density lipid cholesterol, elevated blood triglycerides and elevated sphinganine-sphingosine ratio. It is therefore recommended that for increased profitability in *Clarias gariepinus* catfish culture, dietary fumonisin B1 content should not exceed 20.0 mg FB_1/kg.

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Additional Data

Valuable data collected but not shown in the report are presented in **Tables A1-A3**.

Table A1 shows data for serum very low density cholesterol concentration it shows there was an increase in serum very low density cholesterol of fish fed the diets amended with FB_1 compared with the fish fed the control diets.

Table A2 and **Table A3** show values obtained for serum sphiganine and serum sphingosine respectively. **Table A2** and **Table A3** show there was an increase in both serum sphinganine and the serum sphingisin concentrations throughout the duration of the dietary exposure; although, the increase to a large extent occurred more with the serum sphinganine concentration than with the serum sphingosine concentration.

Table A1. Serum very low density lipids cholesterol concentration ($mg \cdot dL^{-1}$) levels of juvenile *Clarias gariepinus* fed diets amended with varied amounts of fumonisin B_1 for fifty six days.

	FUMONISIN B_1 AMENDED DIETS				
Days feeding	D0	D1	D2	D3	D4
7	16.56 ± 1.00^a	22.20 ± 1.87^b	26.52 ± 0.94^c	26.98 ± 1.32^c	26.54 ± 1.65^c
14	16.04 ± 0.93^a	21.50 ± 1.90^b	25.63 ± 1.27^c	26.16 ± 1.69^c	26.28 ± 2.02^c
28	15.77 ± 0.86^a	22.88 ± 1.34^b	25.92 ± 1.08^c	25.53 ± 1.91^c	25.88 ± 1.47^c
56	16.23 ± 0.05^a	20.20 ± 1.19^b	25.69 ± 1.39^c	26.09 ± 0.43^c	26.42 ± 1.85^c

D0 = 0.0 mg FB_1/kg; D1= 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4= 80.0 mg FB_1/kg.

Table A2. Serum sphinganine concentration ($ng \cdot mL^{-1}$) of juvenile *Clarias gariepinus* fed diets amended with varied amounts of fumonisin B_1 for fifty six (56) days.

	FUMONISIN B_1 AMENDED DIETS				
Days feeding	D0	D1	D2	D3	D4
7	29.57 ± 0.71	116.71 ± 2.09	123.97 ± 0.41	125.70 ± 1.76	130.43 ± 0.01
14	26.34 ± 1.57	119.71 ± 1.36	123.91 ± 1.27	123.40 ± 1.91	129.90 ± 1.41
28	27.31 ± 0.02	126.08 ± 6.11	139.23 ± 4.37	144.00 ± 1.36	148.09 ± 1.19
56	25.62 ± 1.30	123.49 ± 2.37	129.01 ± 1.24	130.11 ± 1.37	152.60 ± 0.13

D0 = 0.0 mg FB_1/kg; D1 = 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4 = 80.0 mg FB_1/kg.

Table A3. Serum sphingosine concentration ($ng \cdot mL^{-1}$) of juvenile *Clarias gariepinus* fed diets amended with varied amounts of fumonisin B_1 for fifty six (56) days.

	FUMONISIN B_1 AMENDED DIETS				
Days feeding	D0	D1	D2	D3	D4
7	65.93 ± 0.02	74.48 ± 0.13	73.11 ± 0.25	74.63 ± 0.71	76.11 ± 0.04
14	67.47 ± 0.19	70.13 ± 0.97	78.31 ± 0.12	72.71 ± 0.37	78.49 ± 0.65
28	64.41 ± 1.02	67.61 ± 0.01	68.53 ± 0.03	67.94 ± 1.53	69.51 ± 0.01
56	63.79 ± 0.14	69.34 ± 1.09	77.03 ± 1.22	81.29 ± 1.89	83.45 ± 1.46

D0 = 0.0 mg FB_1/kg; D1= 10.0 mg FB_1/kg; D2 = 20.0 mg FB_1/kg; D3 = 40.0 mg FB_1/kg; D4 = 80.0 mg FB_1/kg.