

Effects of *Telfairia occidentalis* and *Ipomoea batatas* on Biochemical and Antioxidant Assay of African Catfish (*Clarias gariepinus*)

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How to cite

Tomori, B., Adeyemo, O., Ola-Davies, O., Alarape, S. (2023). Effects of *Telfairia occidentalis* and *Ipomoea batatas* on Biochemical and Antioxidant Assay of African Catfish (*Clarias gariepinus*). *Aquaculture Studies*, 23(SI), AQUAST1116. <http://doi.org/10.4194/AQUAST1116>

Article History

Received 05 September 2022

Accepted 29 November 2022

First Online 18 January 2023

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Keywords

Telfairia occidentalis

Ipomoea batatas

Clarias gariepinus

Biochemical assay

Oxidative stress

Antioxidant assay

Abstract

This toxicological study was carried out to determine possible adverse effects of *Telfairia occidentalis* and *Ipomoea batatas* leaves in *Clarias gariepinus*. Ninety-six *Clarias gariepinus* growers (431.25±6.13 g) randomly distributed into four feeding groups were fed with herbal leaf diet containing 20 g/kg of *T. occidentalis* (group A), 100 g/kg of *I. batatas* (group B), mixture of 10 g/kg of *T. occidentalis* and 50 g/kg *I. batatas* (group C) and basal diet (group D) which served as the control group. After a 40-day feeding period, blood samples were collected for serum biochemical assay and organs were harvested for antioxidant and tissue biochemical assay. A significant decrease in creatinine was observed in the fish in group C when compared with those in group D. Also, there was no significant difference in the liver superoxide dismutase activity across all feeding groups but no significant increase in the intestinal superoxide dismutase activity of the fish in group C when compared to those in group A. There were no significant differences in the values of liver enzyme markers and lipid profile across all feeding groups. However, a significant increase in the level of intestinal malondialdehyde was observed in the fish in group B when compared with those in groups A and D. This study revealed that oral administration of a combination of *T. occidentalis* (10 g/kg) and *I. batatas* (50 g/kg) leaves enhanced antioxidant enzymes and is recommended for protection against oxidative stress in *Clarias gariepinus*.

Introduction

A number of factors have been shown to contribute to physiological changes in fish such as stress or immunosuppression thereby increasing their level of susceptibility to diseases. Some predisposing factors are overcrowding, high or sudden changes in temperature, poor water quality and poor nutritional status (Cabello, 2006). Veterinary drugs including antimicrobials are administered regularly as additives in fish food or sometimes in baths and injections and are used as prophylactics, therapeutics or growth promoters (Rico *et al.*, 2013, Saka, Adeyemo and Odeseye, 2017). The use of these antibiotics which is a popular practice has

been abused thereby increasing the risk of developing antibiotic resistance in both humans and animals (Yin *et al.*, 2008, Alarape and Adeyemo, 2017).

Medicinal plants have been reported to display more biological activity than single compound because of their richness in secondary metabolites such as essential oils, saponins, phenolics, tannins, alkaloids, polypeptides and polysaccharides (Hoseinifar *et al.*, 2020). Herbal medicines have outstanding features such as richness in protein, amino acids, lipids, vitamins and some unknown components that could promote growth and strengthen the metabolism of aquatic animals (Yousefi *et al.*, 2020). Medicinal plants can directly promote antibody production, participate in the specific

immune response and promote the production of cytokines that mediate specific/non-specific immunity, including interleukin, interferon and tumour necrosis factor. The main target organs of medicinal plants which are the thymus, spleen and kidneys, promote the maturation and development of animal immune organs. (Hoseinifar *et al.*, 2020). They also possess secondary metabolites which play a major role in stress mediation, antioxidant and immunopotentiality by modulating recognition, binding, catalytic activity and turnover of proteins and DNA (Chong *et al.*, 2020).

Telfairia occidentalis also known as fluted pumpkin is cultivated in various parts of southern Nigeria and the darkish green leafy vegetable is used as food and herbal medicine. The leaf of *Telfairia occidentalis* is low in crude fibre but a rich source of protein, oil, folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamins A, C and K (Ajibade *et al.*, 2006). *T. occidentalis*' leaf contains phytoconstituents such as phenol, flavonoid, tannins, saponins and alkaloids (Usunobun and Egharevba, 2014). *Ipomoea batatas* (sweet potato) is a popular staple food of the tropical and subtropical areas. The leaves are sometimes consumed as an alternative to other leafy vegetables but mostly harvested for its sweet-tasting tubers (Flores *et al.*, 2015). The leaves of sweet potato have been reported to be rich in potent antioxidants and vitamin C, which helps fight free radicals, prevent premature aging and disease, thereby boosting immune system and preventing infections and diseases (Islam, 2014).

Telfairia occidentalis has been proven to improve growth, feed utilisation and haematological parameters in African catfish fingerlings (Dada, 2015) while *Ipomoea batatas* has been reported to improve feed conversion ratio and feed efficiency ratio in *Clarias gariepinus* fingerlings (Oludayo, 2010) but the toxicological effects of these two medicinal plants in *Clarias gariepinus* are yet to be confirmed. This study was therefore aimed at investigating the toxicological effects of these plants in *Clarias gariepinus* when administered singly and in combination using biochemical tests and antioxidant assay as indices. The data and information generated from this study will guide the use of *Telfairia occidentalis* and *Ipomoea batatas* in fish culture to achieve sustainable, economical, and safe fish production.

Materials and Methods

Collection and Identification of Plant Materials

Fresh mature leaves of *Ipomoea batatas* and *Telfairia occidentalis* were collected from Olorunda area of Lagelu West Local Council Development Area in Ibadan, Oyo State and authentication of the leaves was carried out in the Department of Botany, University of Ibadan after which herbarium numbers were assigned; [*Ipomoea batatas* (UIH-22686) and *Telfairia occidentalis* (UIH-22687)].

Animal Use and Care Protocol

Ethical approval was obtained from the University of Ibadan Animal Care Use and Research Ethics Committee (UI-ACUREC) after which the research was assigned UI-ACUREC/18/0002 before the experiment commenced.

Herbal Feed Preparation

Herbal feed preparation was done according to Dada, (2015). Four diets were prepared to contain the standard nutritional requirement for *C. gariepinus* grower fish based on a known basal formulation by FAO, (2011). The leaves of *T. occidentalis* and *I. batatas* were air-dried, ground, and incorporated into experimental fish feed as follows: three diets were prepared to contain *Telfairia occidentalis* (20 g/kg inclusion level) and *Ipomoea batatas* (100 g/kg inclusion) leaves singly, and in combination (at 10 g/kg of *Telfairia occidentalis* and 50 g/kg of *Ipomoea batatas*). The fourth diet was the basal diet and also served as the control. The four diets were separately pelletized, air-dried at ambient temperature, packed and stored in tightly sealed bags until they are to be used in the feeding experiments. The inclusion level of the medicinal plants was based on the effective inclusion levels without adverse effects from previous studies (Oludayo, 2010, Dada, 2015).

Experimental Procedure

Ninety-six grow-out (five months old) *Clarias gariepinus* (431.25±6.13 g) of both sexes were purchased from a commercial fish farm in Ibadan and retained in the experimental laboratory of the Department of Fisheries and Aquaculture, University of Ibadan. They were fed with basal diet for the period of eight days acclimatization. They were randomly distributed into four feeding groups (A, B, C and D) of 8 fish per group and in triplicates (24 fish per experimental group). Group A was fed with feed containing 20 g/kg inclusion of *T. occidentalis* leaves, Group B with feed containing 100 g/kg inclusion of *I. batatas* leaves, Group C with feed containing 10 g/kg inclusion of *T. occidentalis* and 50 g/kg of *I. batatas* leaves while Group D was fed with basal diet (the proximate composition of experimental and basal diets is presented in Table 1). All experimental fish groups were kept in concrete ponds of 1 × 1.3 m per triplicate and fed at 3% of fish's body weight twice daily for forty days. The experiment was static-renewal whereby water was changed at 72 hourly throughout the duration of the forty days. During the exposure period, the following conditions were maintained: water temperature (24.08±0.06°C), Dissolved Oxygen (5.08±0.04 Mg/L), (pH 7.2±0.03), Ammonia (0.04±8.33E-04 Mg/L), Ammonium (6.81±0.1 Mg/L), Nitrate (144.16±1.63 Mg/L), Nitrite (0.22±0.00 Mg/L).

At the end of the experiment, blood samples were collected from each fish through the caudal vein using 21-gauge needle and syringe and decanted into EDTA sample bottles for serum biochemical assay. Fish were euthanized and intestine and liver were collected for biochemical and antioxidant assay.

Serum Biochemical Assay

Test kits obtained from Randox® Laboratories, UK were used to determine the concentrations of alanine and aspartate transaminases (ALT and AST), alkaline phosphatase (ALP), cholesterol, Triglycerides, creatinine, Low- and High-Density Lipoproteins (LDL and HDL), total and conjugated bilirubin and total proteins. Serum assay for biochemical indices was determined colourimetrically according to the methods described by Okoye *et al.*, 2016.

Preparation of Tissues for Biochemical Assays

After dissection of twelve fish, liver and intestine tissues were carefully removed and rinsed in 1.15% KCl. The organs were weighed to the nearest mg. The tissues were homogenized in aqueous potassium phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 10,000 *g* for 20 min to obtain the supernatant fraction stored at 4°C till use (Oyagbemi *et al.*, 2017).

Determination of Protein Concentration in Liver and Intestinal Tissues

Protein concentration was determined by the method of Lubran, (1978). Briefly, 150 µl of the supernatants from liver and intestinal tissues were added to 150µl of the biuret reagent. The reaction mixture was incubated at room temperature for 30 min. The mixture was thereafter read with spectrophotometer at 540 nm using distilled water as blank. The final value for total protein was deduced from total protein standard curve.

Hydrogen Peroxide Generation in Liver and Intestinal Tissues

Hydrogen peroxide (H₂O₂) generation was determined as described by Wolff, (1994). A reaction mixture containing 2.5 ml of 100 µL potassium phosphate buffer (pH 7.4), 50 µL of ammonium ferrous sulphate (AFS), 20 µL of sorbitol, 20 µL of xylenol Orange (XO), 10 µL of sulfuric acid (H₂SO₄) was added to 10 µL of each sample. The mixture was vortexed thoroughly until it formed a light pink colour. The reaction mixture was subsequently incubated at room temperature for 30 min. The absorbance was measured at 560 nm using distilled water as blank. The hydrogen peroxide generated was deduced from hydrogen peroxide standard curve.

Table 1. Proximate composition of experimental (*Telfairia occidentalis* and *Ipomoea batatas*) and basal diets.

Nutrient (%DM)	Diet A	Diet B	Diet C	Diet D
Moisture	9.7	9.7	10.5	10.5
Crude Protein	32.7	33.8	34.4	35.6
Crude Lipid	9.46	9.2	10	8
Crude Fibre	4.9	6.6	4.8	6
Ash	8	8	8	9
Carbohydrate	30.9	32.7	32.3	30.9

Diet A (basal diet + 20 g/kg of *Telfairia occidentalis*), Diet B (basal diet + 100 g/kg of *Ipomoea batatas*), Diet C (basal diet + 10 g/kg of *Telfairia occidentalis* + 50 g/kg of *Ipomoea batatas*) and Diet D (basal diet).

Table 2. Effect of *Telfairia occidentalis* and *Ipomoea batatas* on the blood biochemistry profile of *Clarias gariepinus*.

INDICES	GROUP A	GROUP B	GROUP C	GROUP D
Na ⁺ (mm/L)	137.44±1.29	140±0	138.22±1.02	138.67± 0.41
K ⁺ (mm/L)	3.75±0.12	3.66±0.09	3.77±0.09	3.91±0.03
Cl ⁻ (mm/L)	105.56±1.30	106.11±1.39	106.11±1.39	105.56±1.30
HCO ₃ ⁻ (mm/L)	23.33±0.62	23.44±0.63	22.44±0.67	22.56±0.44
Urea (mg/dl)	33.22±1.86	31±1.84	28±2.25	35.67±1.86
Creatinine (mg/dl)	0.73±0.03	0.71±0.03	0.62±0.04 ^a	0.79±0.04 ^a
Total Protein (g/L)	6.94±0.09	6.9±0.07	6.84±0.07	6.96±0.08
Albumin (g/L)	3.86±0.08	3.83±0.09	3.87±0.08	3.94±0.07
Globulin (g/L)	3.09±0.05	3.07±0.04	3.07±0.04	3.01±0.02
Total Bil (µmol/L)	0.47±0.06	0.47±0.06	0.47±0.06	0.44±0.04
Conj. Bil (µmol/L)	0.22±0.03	0.18±0.02	0.18±0.02	0.20±0.02

Values are presented as mean ± standard error of mean (n=36). Group A (20 g/kg of *Telfairia occidentalis*), Group B (100 g/kg of *Ipomoea batatas*), Group C (10 g/kg of *Telfairia occidentalis* + 50 g/kg of *Ipomoea batatas*) and Group D (Control). ^aIndicates significant difference at P<0.05.

Measurement of Lipid Peroxidation in Liver and Intestinal Tissues

Lipid peroxidation was determined using malondialdehyde (MDA) as index in micromole per gram tissue with a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$, using the method of Varshney and Kale (1990). 100 μL of each sample was added to 400 μL of Tris-KCl, 0.5 ml of 30% Trichloroacetic acid (TCA) and 125 μL of 0.75% Thiobarbituric acid (TBA) prepared in 0.2 M Hydrochloric acid (HCl). The reaction mixture was incubated in a water bath at 80°C for 45 mins, cooled on ice and centrifuged at 4000 rpm for 15 min. Absorbance was measured against a blank of distilled water at 532 nm.

Measurement of Liver and Intestinal Reduced Glutathione (GSH) Concentration

The concentration of GSH in the samples was determined at 412 nm using the method described by Buetler (1963). 0.1 ml of each sample was first deproteinized with 0.1 ml of 4% sulfosalicylic acid and the resulting solution was centrifuged at 4000 rpm for 5 min. To 20 μL of the resulting supernatant, 180 μL of Ellman's reagent (containing 0.04 g of 5,5'-dithio-bis-(2-nitrobenzic acid (DTNB) in 100 ml of 0.1 M phosphate buffer, pH 7.4) was added. The absorbance was then read at 412 nm against distilled water as blank.

Determination of Liver and Intestinal Glutathione S-transferase (GST) Activity

Glutathione-S-transferase (GST) activity was estimated by the method of Habig *et al.*, (1984) using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. The method involves the production of a complex formed from the enzymatic conjugation of reduced glutathione with the aromatic substrate, 1-chloro-2, 4 nitrobenzene. The complex formed has a characteristic absorption at 340 nm.

Determination of Liver and Intestinal Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase (GPx) activity was measured according to the method of Buetler *et al.* (1963). Reaction mixtures for each sample were prepared to contain 0.5 ml of potassium phosphate buffer (pH 7.4), 0.1 ml of Sodium azide, 0.2 ml of GSH solution, 0.1 ml of H_2O_2 and 0.5 ml of the sample. The mixture was then made up to 2 ml with distilled water. All mixtures were incubated in a water bath at 37°C for 5 min after which 0.5 ml of Trichloroacetic acid (TCA) was added and mixture centrifuged at 4000 rpm for 5 min. 1 ml of the supernatant from each mixture was taken and added to 2 ml of K_2PHO_4 and 1 ml of Ellman's reagent. Absorbance was read at 412 nm using distilled water as blank.

Determination of Liver and Intestinal Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was determined by measuring the inhibition of the auto-oxidation of epinephrine at pH 7.2 at 30°C as described by Misra and Fridovich (1972) with slight modifications introduced by Oyagbemi *et al.*, (2015). 100 mg of epinephrine was dissolved in 100 ml distilled water and acidified with 0.5 ml concentrated hydrochloric acid. 0.01 ml of sample was added to 2.5 ml of 0.05 M carbonate buffer (pH10.2) followed by the addition of 0.3 ml of 0.3 mM epinephrine. The increase in absorbance at 480 nm was monitored every 30 s for 150 s.

Determination of Liver and Intestinal Nitric Oxide Level

Nitric oxide level was determined by the method described by Olaleye *et al.*, (2007). 100 μL of Griess reagent was added to 100 μL of supernatant followed by incubation at room temperature for 20 mins. Absorbance was read at 540 nm.

Statistical Analyses

Statistical analyses was performed using SPSS Version 22 for windows (SPSS Inc., chicago) software. Quantitative data were analysed using descriptive and inferential statistics. Data were subjected to Analysis of variance (ANOVA) followed by Tukey test to determine the significant differences at 5% probability level. Results were expressed as mean \pm standard error of mean.

Results and Discussion

Results

Blood Biochemistry Profile

There were no significant differences ($P > 0.05$) in the values of sodium, potassium, chloride, bicarbonate and urea across the three feeding groups when compared with the control group (Table 2). Although, there was a decrease in the creatinine mean values in all groups but there was only a significant decrease ($P < 0.05$) in the group (C) of fish fed with a mixture of 10 g/kg of *T. occidentalis* and 50 g/kg of *I. batatas* when compared with the control group (D).

Body Weight and Organ weight

Table 3 summarizes the effect of *T. occidentalis* and *I. batatas* on the body weight and the organ weight of *C. gariepinus* fed with leaves of 20 g/kg of *T. occidentalis* (group A), 100 g/kg of *I. batatas* (group B), mixture of 10 g/kg of *T. occidentalis* and 50 g/kg of *I. batatas* (group C) and Control (group D). There were

no significant differences ($P>0.05$) in the body weight and organ weight across all feeding groups. There were significant differences ($P<0.05$) in the values of feed conversion rate, hepatosomatic index and viscerosomatic index across the different feeding groups but no significant difference in the specific growth rate across all groups.

Antioxidant Enzyme Assay

There were no significant differences in the activities of glutathione and glutathione-S-transferase in the liver and intestine across all groups fed with the three experimental diets when compared with the control group (Tables 4 and 5). The Control (group D) showed a significantly higher value in glutathione peroxidase compared to three experimental groups. Also, there was no significant difference in the liver superoxide dismutase activity across all feeding groups. However, there was a significant increase in the intestinal superoxide dismutase activity of group C fish

fed with a combination of 10 g/kg *Telfairia occidentalis* and 50 g/kg *Ipomoea batatas* when compared with group A fed with 20 g/kg *Telfairia occidentalis* (Table 5).

Biochemical Enzyme Profile

There were no significant differences ($P>0.05$) in the levels of Alanine aminoTransferase (ALT), Alkaline Phosphatase (ALP), Gamma Glutamyl-Transferase (GGT) and Aspartate AminoTransferase (AST) when experimental groups of *Clarias gariepinus* fed with leaves of 20 g/kg of *T. occidentalis*, 100 g/kg of *I. batatas*, a mixture of 10 g/kg of *T. occidentalis* and 50 g/kg of *I. batatas* were compared to those in the control group (Figure 1).

Lipid Profile

There were no significant differences ($P>0.05$) in the levels of triglyceride, total cholesterol, high density lipoprotein and low density lipoprotein when

Table 3. Effect of *Telfairia occidentalis* and *Ipomoea batatas* on the growth parameters of *Clarias gariepinus*.

Parameters	Group A	Group B	Group C	Group D
Initial Mean Body weight (g)	430±6.32	436±8.72	444±9.80	415±11.62
Final Mean Body weight (g)	560±12.65	562±9.70	594±29.26	540±18.71
Total Mean Liver weight (g)	0.40±0.04	0.37±0.04	0.30±0.10	0.30±0.10
Liver Mean weight used (g)	0.24±0.04	0.15±0.03	0.16±0.05	0.18±0.04
Intestine weight (g)	0.32±0.02	0.30±0.03	0.29±0.03	0.24±0.02
Duodenum weight used (g)	0.11±0.01	0.11±0.01	0.16±0.05	0.07±0.01
Feed Conversion Rate	8.00±0.27	7.21±0.14	6.83±0.46	8.37±0.51
Specific Growth Rate (%)	2.66±0.009	2.68±0.008	2.704±0.019	2.664±0.015
Hepatosomatic Index	0.07±0.004	0.06±0.005	0.05±0.008	0.06±0.007
Viscerosomatic Index	0.06±0.002	0.05±0.001	0.05±0.01	0.041±0.001

Values are presented as mean ± standard error of mean. Group A (20 g/kg of *Telfairia occidentalis*), Group B (100 g/kg of *Ipomoea batatas*), Group C (a mixture of 10 g/kg of *Telfairia occidentalis* and 50 g/kg of *Ipomoea batatas*) and Group D (Control).

Table 4. Effect of *Telfairia occidentalis* and *Ipomoea batatas* on the Liver antioxidant and antioxidant enzymes in *Clarias gariepinus*.

PARAMETERS	GROUP A	GROUP B	GROUP C	GROUP D
SOD	33.21±1.46	30.03±1.14	32.64±1.20	35.52±1.49
GST	0.28±0.01	0.25±0.03	0.20±0.03	0.21±0.02
GPX	34.56±1.31*	29.50±1.76*	34.51±1.80*	47.34±0.90*
GSH	132±2.01	130.17±1.62	127.23±2.47	132.39±1.84

Values are presented as mean ± standard error of mean (n=12). Group A (20 g/kg of *T. occidentalis*), Group B (100 g/kg of *I. batatas*), Group C (10 g/kg of *T. occidentalis* + 50 g/kg of *I. batatas*) and Group D (Control). *Indicates significant difference at $P<0.05$. SOD (Superoxide dismutase; units/mg protein), GST (Glutathione-S-transferase; mmole-1-chloro-2,4-dinitrobenzene-GSH complex formed/min/mg protein); GPX (Glutathione peroxidase; units/mg protein), GSH (Glutathione; complex formed/min/mg protein).

Table 5. Effect of *Telfairia occidentalis* and *Ipomoea batatas* on the Intestinal antioxidant and antioxidant enzymes in *Clarias gariepinus*.

PARAMETERS	GROUP A	GROUP B	GROUP C	GROUP D
SOD	34.18±1.00 ^a	37.01±1.54	42.23±1.36 ^a	36.79±1.03
GST	0.27±0.04	0.22±0.04	0.29±0.03	0.27±0.02
GPX	35.86±4.28	32.48±1.76	39.30±1.12	36.69±1.66
GSH	132±2.01	130.17±1.62	127.23±2.47	132.39±1.84

Values are presented as mean ± standard error of mean (n=12). Group A (20 g/kg of *T. occidentalis*), Group B (100 g/kg of *I. batatas*), Group C (10 g/kg of *T. occidentalis* + 50 g/kg of *I. batatas*) and Group D (Control). ^aIndicates significant difference at $P<0.05$. SOD (Superoxide dismutase; units/mg protein), GST (Glutathione-S-transferase; mmole-1-chloro-2,4-dinitrobenzene-GSH complex formed/min/mg protein); GPX (Glutathione peroxidase; units/mg protein), GSH (Glutathione; complex formed/min/mg protein).

experimental groups of *Clarias gariepinus* fed with leaves of 20 g/kg of *T. occidentalis*, 100 g/kg of *I. batatas*, a mixture of 10 g/kg of *T. occidentalis* and 50 g/kg of *I. batatas* were compared to those in the control group (Figure 2).

Oxidative Stress Markers

There were no significant differences ($P>0.05$) in the levels of nitric oxide, malondialdehyde and hydrogen peroxide across all experimental groups when

compared with control.

There was significant difference ($P<0.05$) in the level of intestinal nitric oxide among the four groups with a significant increase in the group A when compared to the group B (Figure 4). There was no significant difference in the levels of intestinal hydrogen peroxide generated across the groups. However, there was a significant increase in malondialdehyde level of group B when compared with the group A and the control group (Figures 3-5).

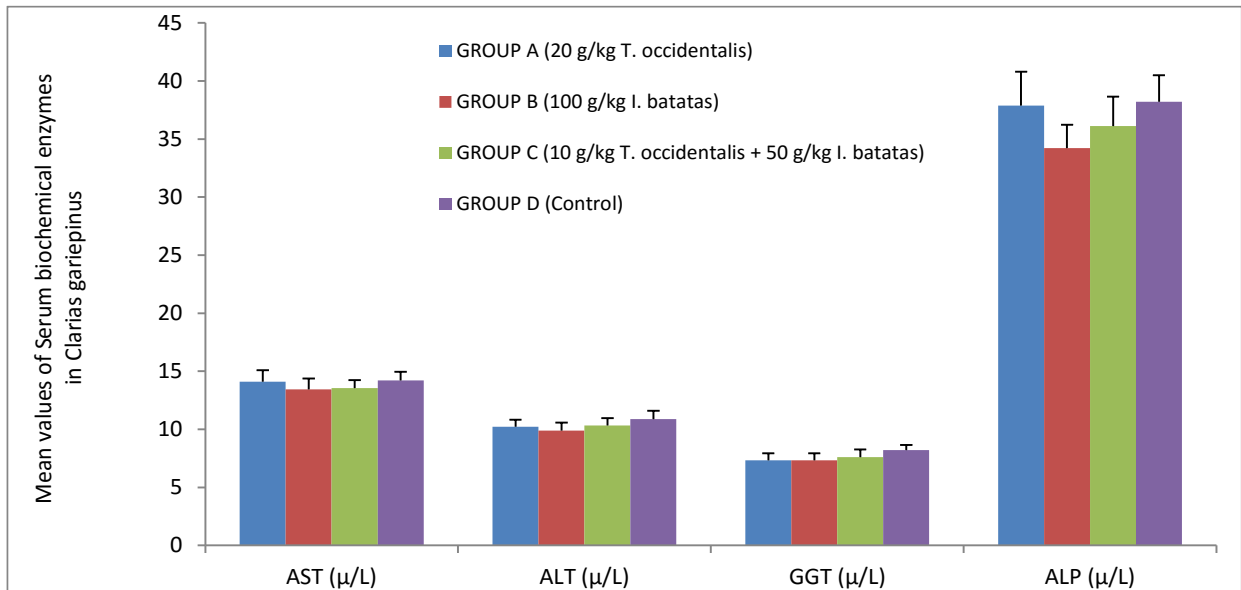


Figure 1. Effect of *T. occidentalis* and *I. batatas* on the Biochemical Enzymes of *Clarias gariepinus*. Values are presented as mean ± standard error of mean (n=36). AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), GGT (Gamma-glutamyltransferase), ALP (Alkaline phosphatase).

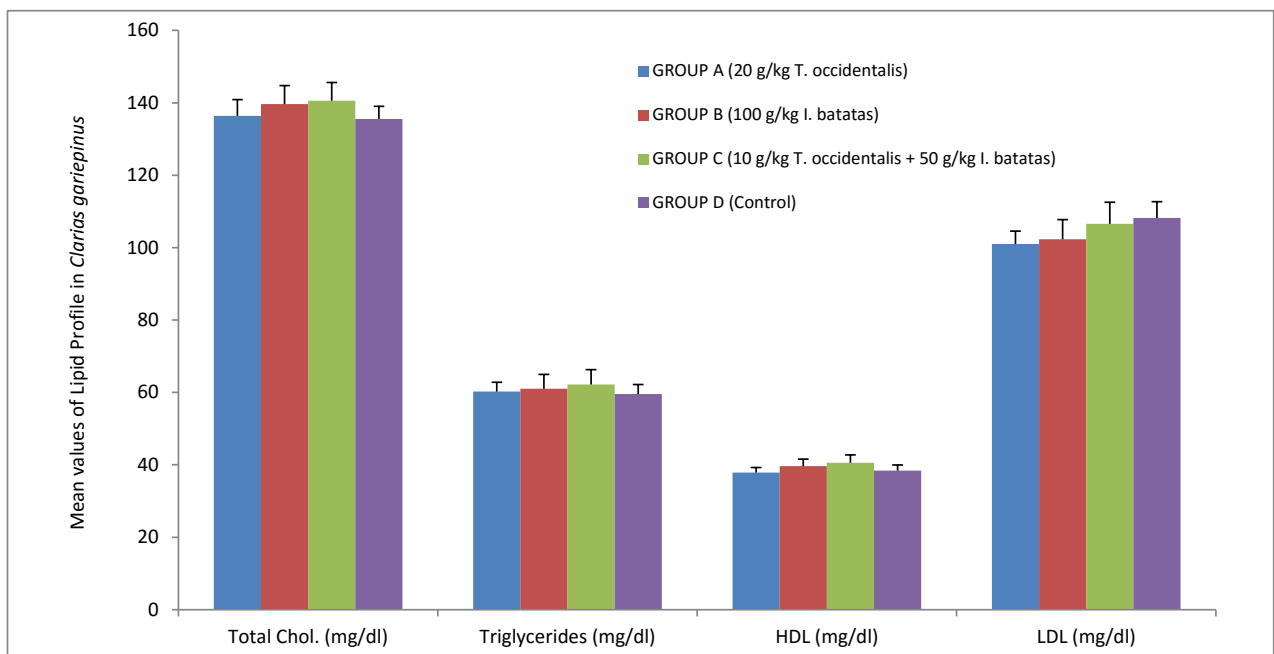


Figure 2. Effect of *T. occidentalis* and *I. batatas* on the Lipid profile of *Clarias gariepinus*. Values are presented as mean ± standard error of mean (n=36). HDL (High density lipoprotein Cholesterol), LDL (Low density lipoprotein Cholesterol). There was no significant

Discussion

Effect of *Telfairia occidentalis* and *Ipomoea batatas* leaves on the Biochemical Indices of *Clarias gariepinus*

Sodium, along with other electrolytes such as potassium, chloride, and bicarbonate help cells function normally regulating the amount of fluid in the body. Measuring bicarbonate as part of an electrolyte or metabolic panel may help diagnose an electrolyte imbalance or acidosis or alkalosis which may be caused by some underlying condition or disease (Lechtzin, 2015).

The absence of significant differences in the values of sodium, potassium, chloride and bicarbonate in

groups A-C compared with control (group D) (Table 2) suggests that that the medicinal plants had no adverse impact on the mechanisms regulating the amount of body fluids as there was no electrolyte imbalance induced.

Effect of *Telfairia occidentalis* and *Ipomoea batatas* Leaves on the Liver Function Parameters of *Clarias gariepinus*

Aminotransferases, including, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are liver enzyme markers which are used to evaluate liver functions. Alkaline phosphatase (ALP) is a membrane-associated enzyme located in a variety of

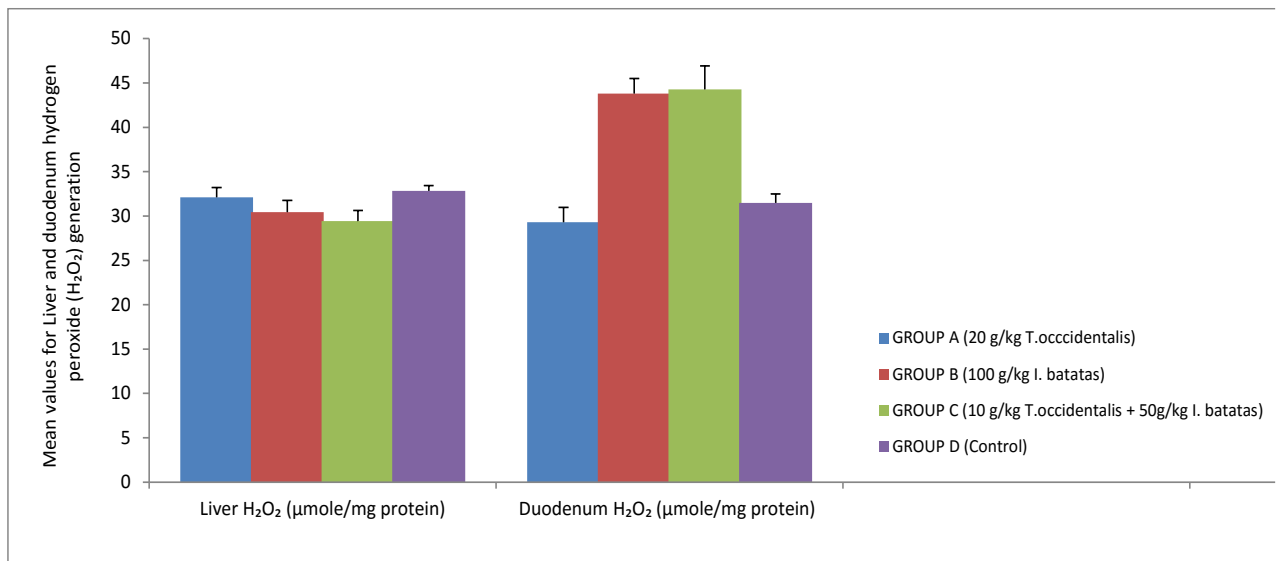


Figure 3. Effect of *Telfairia occidentalis* and *Ipomoea batatas* on the Liver and duodenum hydrogen peroxide (H₂O₂) generation. There was no significant difference across the different feeding groups.

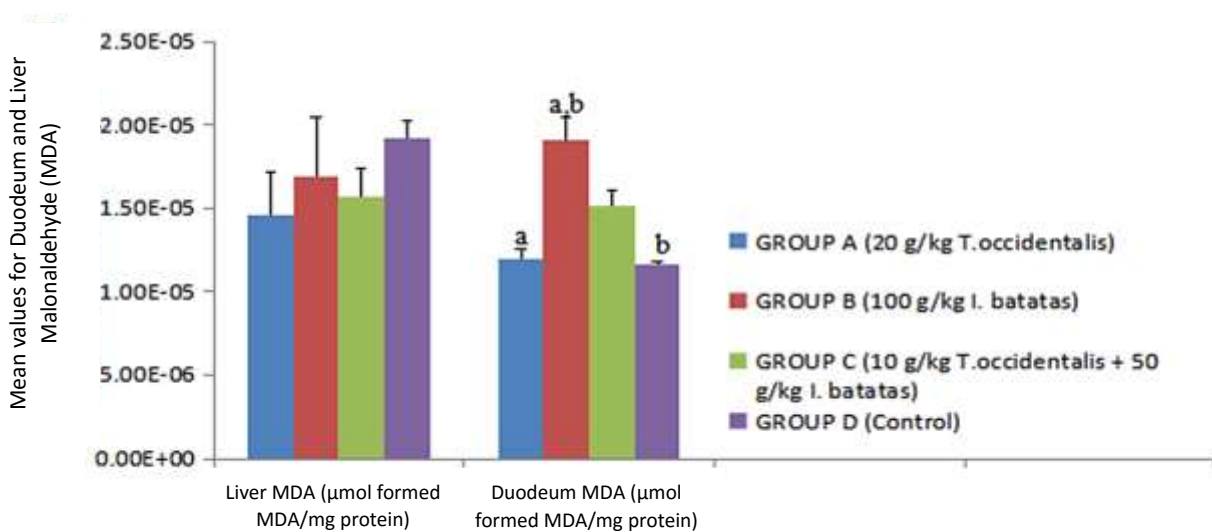


Figure 4. Effects of *Telfairia occidentalis* and *Ipomoea batatas* on the Intestinal and Liver Malonaldehyde (MDA). There was no significant difference in the Liver MDA mean values. Superscript a represents significant increase in Intestinal MDA when group B was compared with Control group at P<0.05. Superscript b represents significant increase in Duodenum MDA at P<0.05 when group A was compared with group B.

tissues of which bone and hepatobiliary systems are of diagnostically importance and it is used extensively as a tumor marker (Pratt, 2016).

In this study, there were no significant changes associated with the levels of AST, ALT, ALP and the liver function markers in groups A-C fed with medicinal plants when compared with the control (Figure 1) indicating that liver damage was not induced in *Clarias gariepinus* on account of the experimental diets. This suggests that these plants have no hepatotoxic effect on the liver and agrees with the report of Iweala and Obidoa, (2009) who observed that there were non-significant changes in the levels of ALT and AST in rats fed *T. occidentalis* supplemented diet.

Contrarily, Ekpenyong *et al.*, (2012) assessed the effect of oral administration of aqueous extract of *T. occidentalis* leaves on liver biochemical indices in Wistar albino rats and reported that acute and sub-acute administration of the extract caused significant decrease in the values of aminotranferases and alanine phosphatase. Akindele *et al.*, (2018) also reported a significant decrease in AST and ALT levels following the administration of hydroethanolic extract of *T. occidentalis* in rats. These differences may be adduced to specie differences and/or the extraction method of the leaf extract.

The non-significant differences in the values of total protein, albumin, globulin in groups A-C compared with the control, group D (Table 2) showed that the medicinal plants had no negative effects on the serum proteins which are responsible for osmotic pressure regulation and transport of nutrients within the capillaries. This is contrary to Ekpenyong *et al.*, (2012) who reported that acute and sub-acute administration of *T. occidentalis* extract caused significant differences in the values of total protein, albumin and globulin in rat. Also, Olorunfemi *et al.*, (2005) and Okoye *et al.*, (2016), observed that ethanolic leaf extract of *T. occidentalis* orally administered to wistar rats for 14 days lowered the levels of albumin, and globulin.

According to Burtis *et al.*, (2014), Gamma-glutamyltransferase (GGT) is useful for diagnosing and monitoring hepatobiliary disease and it is currently the most systematic indicator of liver disease. An elevation of GGT activity is seen in and all forms of liver disease although the highest elevations are seen in intra or post-hepatic biliary obstruction. The finding of increased GGT and alkaline phosphatase activity is consistent with skeletal disease. In this study, there were no significant differences in the level of GGT in the experimental groups A-C compared with the control group (Figure 1), which suggests that the plants did not induce bile duct blockage or skeletal disease.

Urea and creatinine are chiefly excreted from the blood via glomerular filtrations and are routinely measured to assess the kidney health status (Dioka *et al.*, 2004). Increased serum levels of these metabolites are synonymous with decrease in the rate of their excretion which is indicative of impairment of kidney function (Patil *et al.*, 2007). In this study, there was no significant differences in the serum creatinine in the experimental groups compared with control (Table 2). Although the creatinine values decreased across the groups of fish fed with leaves of *T. occidentalis* (20 g/kg), *Ipomoea batatas* (100 g/kg) and a mixture of both plants but the only statistically significant decrease was observed in the group of fish fed a combination of *T. occidentalis* (10 g/kg) + *I. batatas* (50 g/kg) when compared with the control group. Therefore, the medicinal plants assessed in this experiment lowered creatinine levels suggesting that they possess no adverse effects on the kidneys contrary to a report by Okoye *et al.*, (2016) who reported that methanolic extract of *T. occidentalis* increased the levels of serum urea and creatinine in rats. The creatinine values obtained in this experiment were within the normal range of creatinine values in *Clarias gariepinus* reported by Okoye *et al.*, (2016) and Ajeniyi and Solomon (2014).

Bilirubin is a by-product of the breakdown of haemoglobin which is the molecule within red blood

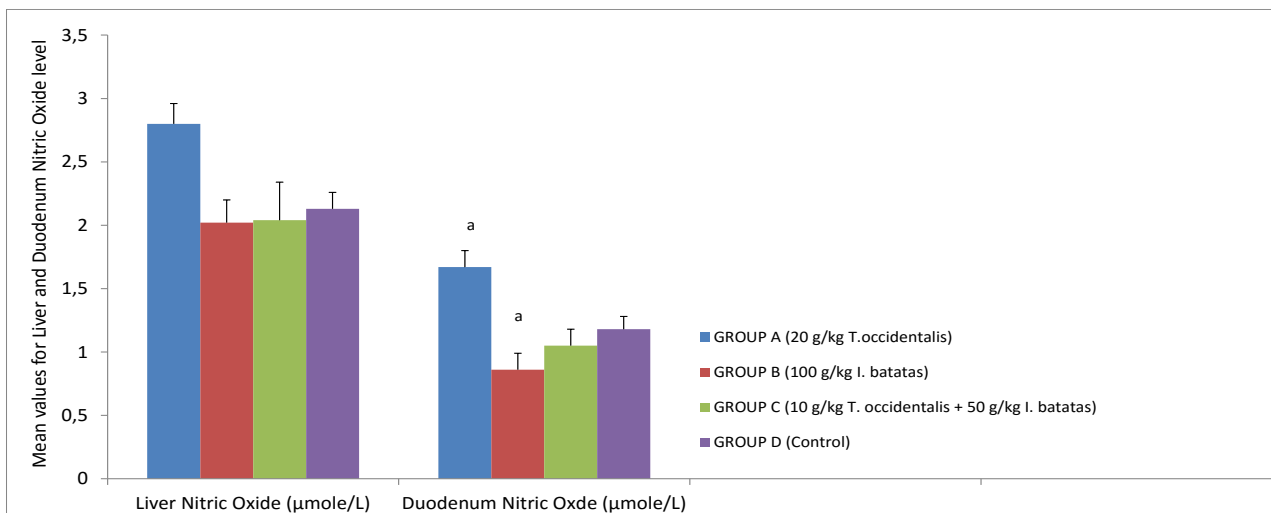


Figure 5. Effects of *Telfairia occidentalis* and *Ipomoea batatas* on the Liver and Intestinal nitric oxide level. Superscript (a) represents significant increase in the nitric oxide level of group A at P<0.05 when was compared to group B.

cells that is responsible for carrying oxygen to the tissues. Unconjugated bilirubin may be increased when there is an unusual amount of RBC destruction (haemolysis) or when the liver is unable to process but conjugated bilirubin can increase when the liver is able to process bilirubin but is not able to pass the conjugated bilirubin to the bile for removal (AACC, 2018). Total bilirubin, conjugated bilirubin and unconjugated bilirubin were not significantly different across experimental groups (Table 2), which is suggestive of no haemolysis nor liver damage. Okoye *et al.*, (2016) also concluded that there were no significant difference in the serum levels of bilirubin of rats following the administration of methanolic extract of *T. occidentalis*.

Effect of *Telfairia occidentalis* and *Ipomoea batatas* Leaves on the Lipid Profile of *Clarias gariepinus*

Total cholesterol, triglyceride, low-density lipoprotein and high-density lipoprotein were not negatively affected in *Clarias gariepinus* fed medicinal plants (group A-C) when compared to the control group D (Figure 2). This is contrary to the findings of Iweala and Obidoa, (2009) who reported significant reduction in total cholesterol in rats when fed with *T. occidentalis* at 5% supplemented diet for 180 days. Ugwu *et al.*, (2011) also reported that *T. occidentalis* and *Vernonia amygdalina* incorporated diets at 5, 15 and 30% for 28 days were anti-lipidaemic where *T. occidentalis* showed a greater effect compared to the *V. amygdalina*. Akindele *et al.*, (2018) reported a significant reduction in the level of total cholesterol and increase in level of high-density lipoprotein was observed following the administration of hydroethanolic extract of *T. occidentalis*.

Effects of *Telfairia occidentalis* and *Ipomoea batatas* Leaves on the Intestinal and Liver Oxidative Stress Markers of *Clarias gariepinus*

The liver and intestine are involved in different functions and the challenges that they are confronted with are also different. As the major detoxification organ in vertebrates, the liver is central to the degradation of metabolic products (Lee *et al.*, 2015), and thus is constantly challenged by many endogenous and exogenous free radicals. As the main site for nutrients digestion and absorption, the intestine is constantly challenged by diet-derived oxidants, as well as endogenously generated reactive oxygen species (Denis *et al.*, (2015).

In this study, the levels of liver nitric oxide, malondialdehyde and hydrogen peroxide in the experimental groups (A-C) were not significantly different compared to the control (group D). However, there were significant differences in the levels of intestinal nitric oxide and malondialdehyde (Figures 4 and 5). The non-significant changes in the oxidative stress

markers in the liver may be due to the claim that antioxidant defences are more highly developed in liver than in other organs (Lushchak, 2005). The significant increase ($P < 0.05$) in the level of intestinal nitric oxide in *T. occidentalis* fed group when compared to the *I. batatas* fed group (Figure 5) could be indication of an increase in free radical production and suggestive of intestinal inflammation.

A significant increase in the level of intestinal malonaldehyde (MDA) in the group of fish fed with 100 g/kg of *I. batatas* leaves is indicative of increased level of lipid peroxidation and oxidative stress in the intestine. Also, the MDA level of this group of fish was significantly ($P < 0.05$) increased compared to that of fish fed with 20 g/kg of *T. occidentalis* which suggests that there was an increase in the level of lipid peroxidation in the groups of fish fed with *I. batatas* when compared to that of the *Telfairia occidentalis* group.

Contrarily, Iweala and Obidoa, (2009) reported that long term administration of *T. occidentalis* supplemented diet within a period of six months reduced lipid peroxidation in rats. Nworgu *et al.*, (2007) reported that although *T. occidentalis* extract contain tannins which are classified as antioxidants, at a high dose, they could become pro-oxidant, increasing lipid peroxidation and inducing oxidative stress in broiler chickens served heat treated fluted pumpkin.

Effect of *Telfairia occidentalis* and *Ipomoea batatas* Leaves on the Antioxidant and Antioxidant Enzyme Activities in *Clarias gariepinus*

The aqueous extract of *T. occidentalis* leaves has been reported to contain vitamins C and E which are standard antioxidants that help scavenge for oxygen free radicals and prevent oxidative stress (Idris, 2012). Cells are protected against oxidative stress by an interacting network of antioxidant enzymes with superoxide dismutases catalysing the first step and then catalases and various peroxidases removing hydrogen peroxide. As with antioxidant metabolites, the contributions of these enzymes to antioxidant defences can be hard to separate from one another (Sies, 1997).

In this study, there were no statistically significant differences in the liver and intestinal glutathione activity across the groups (A-C) of experimental fish when compared to the control (Tables 4 and 5). Although, there was an increase in the mean values of intestinal glutathione activity across A-C groups when compared with the control group, suggesting that the plant supplemented diets may possess antioxidant property.

Also, no significant differences were observed in the antioxidant enzyme activities of liver and intestinal glutathione-s-transferase across the groups A-C when compared to the control (D).

The higher values of glutathione peroxidase in groups A and C compared to group B suggests that fish diet with the inclusion *Telfairia occidentalis* and of *Ipomoea batatas* may possess antioxidant enzyme

activity but of low potential because the control (D) showed a significantly higher value of the glutathione peroxidase in the liver (Table 4) compared to groups A-C

Superoxide dismutase (SOD) is an enzyme that alternately catalyzes the dismutation of the superoxide (O_2^-) radical which is produced as a by-product of oxygen metabolism into either ordinary molecular oxygen or hydrogen peroxide and if not regulated, causes many types of cell damage (Hayyan *et al.*, (2016)). The significant increase in the SOD level in the group of fish fed with a mixture of 10 g/kg of *T. occidentalis* and 50 g/kg of *I. batatas* (Table 5) suggest some level of antioxidant protection in the intestine when compared to the *T. occidentalis* group, although there was no significant difference in the SOD activity in the liver (Table 4). This result is contrary to that of Iweala and Obidoa, (2009) who observed that there were no significant changes in the activities of Glutathione-s-transferase and Superoxide dismutase in *T. occidentalis* long term supplemented diet in rats.

Conclusion

The findings in this experiment showed that oral administration of *T. occidentalis* and *I. batatas* leaves in *Clarias gariepinus* posed no negative effects on the biochemical and antioxidant indices although increase in the levels of oxidative stress markers were observed when the plants were administered singly. It was also observed that oral administration of the mixture of *T. occidentalis* and *I. batatas* leaves posed no negative effects on the biochemical indices and the lipid profile of *Clarias gariepinus*. Also, the stimulation of intestinal superoxide dismutase activity revealed that the mixture of both plants enhanced antioxidant activity and therefore has the potential to protect African Catfish from oxidative stress and its negative health implications.

The oral administration of the mixture of leaves of *T. occidentalis* (10 g/kg) and *I. batatas* (50 g/kg) is safe and can be used as feed additive in *Clarias gariepinus*. The mixture of both plants at half their inclusion levels has been shown to enhance antioxidant enzyme. Therefore, it is recommended for protection against oxidative stress. The effects of higher dosages of these medicinal plants should be considered in further studies.

Ethical Statement

Ethical approval was obtained from the University of Ibadan Animal Care Use and Research Ethics Committee (UI-ACUREC) after which the research was assigned UI-ACUREC/18/0002 before the experiment commenced.

Funding Information

This study was funded by Mr Akinola Tomori and Mrs Oluwakemi Edet-Utan.

Author Contribution

First Author: Conceptualization, Investigation, Visualization and writing original draft, Resources, Methodology, Data curation, Funding acquisition. Second Author: Writing-review and editing, Project administration, Supervision. Third Author: Writing-review and editing, Formal Analysis, Supervision. Fourth Author: Writing-review and editing, Supervision.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

All members of staff of the Department of Veterinary Physiology and Biochemistry and Department of Fisheries and Aquaculture at the University of Ibadan are duly acknowledged for their immense contributions.

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