



Growth response, nutrient utilization, biochemical and hematological parameters of Juvenile African catfish (*clarias gariepinus*) fed diet supplemented with *gongronema latifolia* (benth) extract.

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ABSTRACT

This study investigated the effect of varying levels of *Gongronema latifolia* on growth, nutrient utilization, biochemical and hematological parameters of *Clarias gariepinus* juveniles. One hundred and five (105) juveniles were equally distributed in fifteen plastics tanks and each triplicated. Three inclusion levels of the plant extract (150mg/kg, 300mg/kg and 500mg/kg) were prepared, while the antibiotics served as negative control and the diet without any inclusion served as the positive control. The fish meal was supplemented with *Gongronema latifolia* (benth) extract and the control diets were fed to satisfaction, while the tank water was changed every other day. The weight gained and feed intakes of the fish were determined at the end of every week and the growth parameters were evaluated. Growth parameters showed that the highest value was recorded in treatment 4 (300mg) 226.07 ± 9.96 for the final weight gained, while the lowest was observed in treatment 5 (500mg) was 165.47 ± 31.55 . Supplementation of fish feed on *Gongronema latifolia* did not cause any adverse effects on biomarkers of liver toxicity and oxidative stress. Monocytes and lymphocytes concentration were significantly reduced with no alteration in red blood cell count and hemoglobin concentration. This study showed that the supplementation of *Clarias gariepinus* juvenile diet with *G. latifolia* extract promoted growth and weight gain in the fish stock with no toxic effect on the liver and oxidative stress.

INTRODUCTION

Medicinal plants was defined by Sofowora *et al.* (2013) as any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs.

Natural products, such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched chemical diversity they can provide (Cos *et al.*, 2006). Several studies indicated that medicinal plants contain compounds like peptides, unsaturated long chain fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds. These compounds are significant in therapeutic application against human and animal pathogens, including bacteria, fungi and viruses (Pavrez *et al.*, 2005).

According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary health care needs. This has captured the interest of many researchers to explore local medicinal plants for valuable medicinal traits. In recent years, numerous drug resistances in human pathogenic microorganisms have developed due to their indiscriminate use and their leakages into the environment inform of pharmaceutical effluents and pesticides (Igbinsosa and Odjadjare 2015).

The impact of antibiotic-resistant bacteria on human health has become a major international concern in recent years and attention has focused on food-producing animals as one of several potential sources of antibiotic-resistant bacteria in humans (Wegener 2012). These resistant bacteria may spread from animals to humans via the food chain and they may also transfer their antibiotic-resistance genes into human pathogenic bacteria, leading to failure of antibiotic treatment for some life-threatening conditions (Von wintersdorff et al., 2016). At the same time, other side effects, such as the residual impact on the environment, are also risk factors when commercial antibiotics are used for treatment of disease in Fish.

Gongronema latifolium (Benth) is of West African Origin (Nelson, 1965). It belongs to the family *Asclepiadaceae*. It is an edible nutritional plant with medicinal potential found mostly in the rain forest zones in Nigeria and other tropical African countries (Chattopadhyah 1999). *G. latifolium* is known by the Ikaale tribes of Ondo State of Nigeria as Iteji (Morebise et al 2006). The Ibos call the plant Utazi, the Efik/Ibibio call it Utasi while the Yorubas call it Arokeke (Edim et al., 2012). Bello et al 2014, Akpan and Ekpo (2015) listed its phytochemical and scientific composition, while Eleyinini (2007) listed its antibacterial activities and Emeka and Obidoa (2009) finally establishes its antioxidant potentials.

With increased incidence of resistance to antibiotics, natural products from plants could be an interesting alternative (Mbwambo et al., 2007). Natural plants products present a viable alternative to antibiotics and other banned drugs being safer for the reared organism and humans, as well as the environment. The use of medicinal plants could definitely complement advances to medicine in many parts of the world (American Society for Testing and Materials, 2001) because of the presence of wide range of bio-active phyto-chemicals and secondary metabolites.

MATERIALS AND METHODS

The experimental site

The experiment was carried out at the Nutrition Unit of the Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka.

Collection and identification of sample

Fresh samples of *Gongronema latifolia* was sourced at a local market in Mushin, Lagos state, Nigeria. The plant material was taken to the Department of Botany Herbarium, University of Lagos for identification. The samples were thoroughly rinsed with clean water and evenly spread on a mosquito net-size mesh to air dry under shade. After complete dryness, the leaves were powdered using an Electric blender (Mbagwu and Adeniyi, 1988). Extracts with best antioxidant and antibacterial activities, among various solvent extractions from previous studies (Aderolu et al. 2017) was used in the feeding experiment.

Procurement of experimental fish

A total number of one hundred and five (82.0 ±2.0g initial mean weight) of *Clarias gariepinus* juvenile were purchased from a reputable farm in Ikotun Egbe,

Lagos. The fish were transferred to the unit and acclimatized for two (2) weeks being fed with commercial diet before the introduction of experimental diet. They were kept under standard condition; temperature (27.5 - 29.5°C), dissolved oxygen (4.5 - 4.8 mg/l), and pH (7.3 - 8.0) as described by Aderolu and Akpabio (2009).

Experimental Design

The fish were weighed and distributed into the 15 plastic bowls representing five treatments each replicated thrice; at the rate of (7) seven fish per bowl (52 x 33.5 x 21cm). The tanks were separated into five (5) experimental groups of 0mg (control), Antibiotics (oxytetracycline at 200mg/kg) (negative control) 150mg/kg, 300mg/kg, 500mg/kg concentration of *Gongronema latifolia* extracts. All the experimental fish, were fed twice daily to satiation between the hours of 08: 00 – 09.00 and 16: 00 -17.00 for eight weeks. Fish were batched weighed weekly with a digital scale (Camry EK5055 Max 5kg/11 lb. d = 1g/0.05 oz.) and mortality was monitored daily (56 days).

Purchase and Processing of Feed Ingredients

All other feed ingredients were sourced from Abattoir, Agege, Lagos, Nigeria. The ingredients for each diet were mixed thoroughly in a bowl with the extract being added to it and pelletized with a locally-fabricated pelletizing machine. The moist pellets were sun dried for eight hours, packaged in air tight plastic containers, tagged and refrigerated until needed.

Experimental diet preparations

The greasy plant extract was weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentrations. Five experimental diets were prepared, the supplement (*Gongronema latifolia*) was included at 150mg, 350mg, and 500mg/kg respectively to form three isonitrogenous experimental diets (35% Crude Protein). 0% inclusion level served as control diet and antibiotics as negative control. Fish feed was formulated to meet the nutrient requirement of the fish (Aderolu and Akpabio 2009). They were kept under refrigerated condition until they are needed for the experiment.

Table 1: Nutrient composition of experimental diet used in this study

Ingredients (%)	Treatment-1 (+tive Control1)	Treatment-2 (- tive Control2)	Concentration of plant extract in the diet		
			Treatment-3 (150mg)	Treatment-4 (300mg)	Treatment-5 (500mg)
Fish meal (72%)	17.15	17.15	17.15	17.15	17.15
Soybean meal	19.10	19.10	19.10	19.10	19.10
Groundnut cake	19.10	19.10	19.10	19.10	19.10
Maize	20.40	20.40	20.40	20.40	20.40
Noodle waste	20.40	20.40	20.40	20.40	20.40
Plant oil	1.00	1.00	1.00	1.00	1.00
Di calcium phosphate	0.40	0.40	0.40	0.40	0.40
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Mineral-Vitamin premix	1.75	1.75	1.75	1.75	1.75
Salt	0.30	0.30	0.30	0.30	0.30
Plant extract(mg/kg)	-	-	150	300	500
Antibiotics (mg/kg) tetracycline	-	200	-	-	-
Total (kg)	100	100	100	100	100
Calculated CP (%)	35	35	35	35	35
Calculated Energy (Kcal)	3127	3127	3127	3127	3127

Growth and nutrient utilization parameters

The following parameters were obtained from the record of feed intake and weight gain record;

Feed Conversion Ratio (FCR)

$$\text{FCR} = \frac{\text{Feed intake}}{\text{Weight gain}}$$

Final Weight Gain (FWG)

$$\text{FWG} = \text{Final weight} - \text{Initial weight}$$

Mean Weight Gain (MWG)

$$\text{MWG} = \frac{\text{Average Weight Gain}}{\text{No of days of experiment}}$$

Specific growth Rate (SGR)

$$\text{SGR} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \times 100$$

(Where, e = Natural logarithm, $T_2 - T_1$ = experimental period, W_1 = initial weight, W_2 = final weight)

Relative Growth Rate (RGR)

$$\text{RGR} = \frac{\text{weight gain}}{\text{Initial body weight}}$$

Average Feed Intake (AFI)

$$\text{AFI} = \frac{\text{Total Feed intake}}{\text{No of days of experiment}}$$

Protein Intake (PI)

$$\text{PI} = \frac{\text{Total feed intake}}{\text{Protein content of feed}}$$

Protein Efficiency Ratio (PER)

$$\text{PER} = \frac{\text{Mean weight gained}}{\text{Protein intake}}$$

Hematological analysis

The following parameters were used to assess the effects of dietary treatments on the haematological profile of *Clarias gariepinus* at the end of the feeding trials. Blood samples for hematological analysis were collected from the fish with a plastic syringe (2ml capacity) at the caudal peduncle of both the exposed and control fish. Three fish per treatment, the blood was immediately withdrawn into an EDTA bottle and plain bottles for hematological and biochemical analysis. The samples were then analyzed in the Department of Medical Laboratory Science of University of Lagos (LUTH). For hematological analysis, the semi-automated hematology analyzer (Mindray) from China was used. The following hematological parameters were evaluated:

Erythrocyte Count (Red Blood Cell)

Fish blood was diluted in an improved Neubauer pipette with citrate buffer fluid at 1: 200. The diluted blood was introduced into a Neubauer counter/ counting chamber and red blood cells counted under the microscope (Shah and Altindag, 2004).

Pack cell volume

Hematocrit was determined by filling the heparinized capillary tubes with blood samples and then centrifuged with a hematocrit centrifuge. The PCV was determined with hematocrit reader and expressed in percent (Shah and Altindag, 2004).

Haemoglobin concentration

Hemoglobin concentration was determined using calorimeter method. Briefly, the hemoglobin is converted to oxyhemoglobin by adding about 40 cm³ of the blood sample to 4 ml of 0.04% ammonium solution in a test tube. The optical density of the oxyhemoglobin formed was read with a calorimeter. The optical density was thereafter

converted to hemoglobin concentration on the standard calibration curve (Roberts, 1978).

Leucocytes count (White Blood Cell)

Fish blood was diluted in an improved Neubauer pipette with citrate buffer fluid at 1: 20. The resulting mixture was introduced into a Neubauer counter/counting chamber and white blood cells counted under the microscope at a magnification of $\times 40$ (Shah and Altindag, 2004).. Differential white blood cell count was determined by preparing a thin blood film by spreading a drop of blood evenly across a clean grease free slide, using smooth edged spreader. The blood film was fixed with absolute methanol for 3 to 5 minutes and allowed to dry. The smear was stained with giemsa stain and 100 white blood cells were differentiated using the oil immersion objectives of the microscope. The percentage of each white cells type was extrapolated (Blaxhall and Diasley, 1973).

Biochemical analysis

Samples of blood in the plain bottles were spun at 3,000 rpm to collect the serum that was used in biochemical analysis which includes protein, albumin, cholesterol, triglyceride, HDL, LDL, AST, ALT and ALP.

Liver sample (0.5g) was carefully collected over ice and homogenized in 5ml of normal saline solution. The homogenate was centrifuged at 3000rpm for 5minutes at -4°C . The supernatant was separated into a sterile sample bottle and used for the following biochemical analysis; Alanine aminotransferase (ALT), aspartate aminotransferase (AST) Reitman and Frankel (1957), alkaline phosphatase (ALP) (Bessey *et al.*, 1946), Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase, Malondialdehyde (MDA). Total serum protein, serum albumin and serum globulin was also determined from the blood samples.

Total Protein

This was determined using Biuret method (Gonall *et al.*, 1949) and Bovine Serum Albumin (BSA) as standard. The Principle Polypeptides and proteins with two or more peptides bonds give a characteristic purple/violet color when treated with diluted copper sulfate at alkaline pH values. In other words, Alkaline copper sulphate reacts with compounds containing two or more peptide bonds to give a violet or purple coloured complex. The intensity of colour obtained is a measure of the number of peptide bonds present in the protein. The mixture of the reagent and serum sample was incubated for 30 minutes at $20 - 25^{\circ}\text{C}$ and the absorbance of the sample and that of the standard was measured against the blank at a wavelength of 540nm.

The total protein concentration was calculated as;

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of standard}} \times \text{Standard Concentration}$$

Serum Albumin

The Bromo-Cresol Green (BCG) method as described by Donmas *et al.*, (1971) was employed. The absorbance of the sample and the standard were taken against the reagent blank, at a wavelength of 620nm.

$$\text{Albumin (g/dl) concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of Standard}$$

Serum Globulin (g/dl)

Serum globulin was calculated as follows;

$$\text{Serum globulin (g/dl)} = \text{Total serum Protein (g/dl)} - \text{Serum albumin (g/dl)}$$

Liver Antioxidant Enzymatic Activities

The following antioxidant enzymes activities were determined using spectrophotometer as follows:

Determination of Superoxide Dismutase (SOD) activity

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Sun and Zigma (1978). The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of liver homogenate and 0.03 ml of epinephrine in 0.005 N HCL was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

Determination of Catalase Activity

This was determined adopting the methods of Aksenes and Njaa (1981). Hydrogen peroxide was prepared with phosphate buffer; 0.2 ml of sample was added to 1.8 ml of 30 mM of hydrogen peroxide (H₂O₂) substrate in a 2 ml curvette. The phosphate buffers were used as a blank. The absorbance for the test sample, blank and standard was read against a blank at 240 nm at 30s interval for 1 min. The enzyme activity was calculated using the molar extinction coefficient of 40.00 M⁻¹ cm⁻¹ expressed as unit/mg protein

Reduced Glutathione Determination (GSH)

The reduced glutathione (GSH) content of serum non-protein sulphhydryls was estimated according to the method described by Sedlak and Lindsay (1968). To the liver homogenate 10% TCA was added, centrifuged. 1.0ml of supernatant was treated with 0.5ml of Elman's reagent (19.8mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412nm. The enzyme activity was calculated using the molar extinction coefficient of $\sum = 1.34 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$

Lipid Peroxidation (MDA)

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Buege and Aust (1978). 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100°C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of $1.56 \times 10^5 \text{ M}^{-1} \text{ CM}^{-1}$.

Statistical analysis and result presentation

A one way analysis of variance (ANOVA) was employed on the data using SPSS 16.0 statistical package to describe growth and nutrient utilization performances along side biochemical and haematological parameters and to test for

RESULTS

Growth and nutrient utilization in juvenile Clarias gariepinus fed diet supplemented with graded levels of Gongronema latifolia leaf extract

Growth and nutrient parameters are presented in Table 2. Mean body weight gain and specific growth rate were significantly different at the 500mg (T-5) inclusion level among fish fed graded levels of the plant extract only. Feed intake and

protein intake were equally only significantly different in the 500mg diet (T-5) relative to other diets.

Table 2: Growth performance, nutrient utilization *C. gariepinus* juvenile fed different graded levels of *Gongronema latifolium*.

Parameters	Treatment-1 (+tive Control)	Treatment-2 (- tive control) (antibiotics)	Treatment-3 (150mg)	Treatment-4 (300mg)	Treatment-5 (500mg)
INW (g)	82.87 ±0.27	82.91±0.08	83.05 ±0.22	83.15±0.25	83.00 ±0.14
FWG (g)	191.47±3.19 ^{ab}	214.93±18.90 ^b	222.57±13.29 ^b	226.07±9.96 ^b	165.47±31.55 ^a
MWG (g)	108.60±3.00 ^{ab}	132.03±18.8 ^b	139.52±13.30 ^b	143.07±9.82 ^b	82.32±31.72 ^a
AFI(g)	142.13±8.82 ^b	154.63±7.19 ^b	154.63±12.45 ^b	163.53±9.32 ^b	117.20±10.50 ^a
PI	49.75±3.09 ^b	54.12±2.52 ^b	54.12±4.36 ^b	57.24±3.26 ^b	41.02±3.68 ^a
FCR	1.62±0.09	1.27±0.31	1.44±0.88	1.36±0.17	2.04±0.09
PER	1.77±0.91	2.34±0.59	1.99±0.12	1.67±0.40	2.12±0.26
SGR (%/day)	1.50±0.26 ^{ab}	1.70±0.16 ^b	1.76±0.11 ^b	1.79±0.75 ^b	1.21±0.36 ^a

Means with different superscripts along the row are significantly different ($P < 0.05$) INW- initial weight, FBW- final body weight, FWG- final weight gain, MWG- mean weight gain, AFI- taverage feed intake, SGR- specific growth rate, FCR- feed conversion ratio, PER-protein efficiency ratio, PI- protein intake.

Broken and non-linear polynomial line showing optimal inclusion level of Gongronema latifolia in the diets for juvenile Clarias gariepinus.

The result in Figure 1 showed the highest MWG of the fish fed experimental diets corresponds to (143.07±9.82g). The further increment in the concentration of the plant extract (*Gongronema latifolia*) from the graph showed a reduction in MWG. The graph showed the best recommended dosage of 220mg/kg.

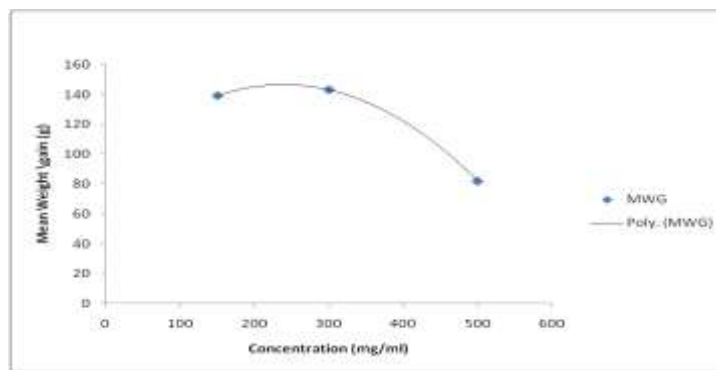


Fig. 1: Broken and non-linear polynomial line showing optimal inclusion level of *Gongronema latifolia* in the diets for juvenile *Clarias gariepinus*

Hematological parameters of Clarias gariepinus fed Gongronema latifolium supplemented diet along with negative and positive controls.

The haematological parameters measured in the blood samples from *Clarias gariepinus* fed different graded levels of *Gongronema latifolia* extract supplemented diets along with the negative and positive controls are presented in Table 3. There was a significant decrease in white blood cell count in all groups fed extract supplemented diet compared to the positive control. The decreases in white blood cell count recorded in T-4 and T-5 groups were also significantly reduced compared with the negative control results. The differential white blood cell count showed a significant decrease in monocyte count in all extract treated groups compared with control. Similarly, there was significant decrease in the lymphocyte count in the

negative control treated with antibiotics and all the extract treated groups. The red blood cell count was not different in all the groups in this study but the packed cell volume was significantly decrease in T-5 group compared with both the negative and positive control groups.

Table 3: Hematological Parameters of *Clarias gariepinus* fed graded levels of *Gongronema latifolia* based diets

Parameters	T-1 (+tive control)	T-2 (-tive control (antibiotics))	T-3 (150mg)	T-4 (300mg)	T-5 (500mg)
RBC X(10 ⁹ cells/L)	2.53±0.30	2.32±0.31	1.82 ± 0.74	2.01 ± 0.38	1.62 ± 0.92
PCV (%)	39.23 ± 4.56	32.27 ± 2.04	28.10 ± 9.36	29.50 ± 4.65	14.25 ± 8.23 ^{*a}
Hb (g/L)	15.10±1.57	13.0±1.06	11.43 ± 3.50	12.17 ± 1.40	9.70 ± 5.11
MCV(FI)	7.00±4.36	10.00±7.00	65.29 ± 9.2 ^{*a}	61.43 ± 4.55 ^{*a}	61.57 ± 4.92 ^{*a}
MCH(pg)	155.13±5.88	139.57±9.61 [*]	158.73 ± 16.68	148.13 ± 11.45	151.63 ± 4.91
MCHC (g/L)	59.73±1.04	56.87±2.91	41.03 ± 2.76 ^{*a}	41.47 ± 2.35 ^{*a}	40.60 ± 3.73 ^{*a}
WBC (10 ⁹ cells/L)	61.13 ± 5.73	53.83 ± 6.00	44.90 ± 7.75 [*]	35.80 ± 2.88 ^{*b}	27.93 ± 2.38 ^{*b}
MONO (%)	38.53±1.65	40.77±0.66	13.33 ± 9.07 ^{*a}	8.67 ± 6.03 ^{*a}	12.67 ± 9.29 ^{*a}
LYPH (%)	6.27±0.58	0.20±0.10 [*]	0.70 ± 0.36 ^{*a}	0.47 ± 0.15 ^{*a}	0.47 ± 0.38 ^{*a}
NEUT%	99.55±0.89	99.57±0.15	98.47 ± 0.68	99.10 ± 0.46	98.70 ± 1.56
RWD%	15.17±0.70	15.97±0.31	20.53 ± 9.16	14.86 ± 0.82	17.33 ± 2.81

Means with different superscripts along the row are significantly different (P<0.05)

* Significant different compared with T-1 group, ^b Significant different compared with T-2 group **PCV** - Packed Cell Volume, **Hb** - Hemoglobin, **RBC** - Red Blood Cell, **WBC** - White Blood Cell, **LYPH** - Lymphocyte, **MCV** mean corpuscular volume, **MCHC** – mean corpuscular hemoglobin concentration, **MCH** –mean corpuscular hemoglobin, **MONO** – monocyte, **RWD**- red blood cell distribution width.

Hemoglobin concentration was not also significantly different between all the groups. However, MCH was significantly reduced in T-2 group compared with the control group result. MCHC was significantly reduced in all extract treated groups compared with both the positive (T-1) and negative (T-2) control. MCV was also significantly decreased in all groups fed with feed supplemented with extract compared with both positive and negative control.

Biochemical Parameters of Clarias gariepinus Juveniles Fed With graded levels of Gongronema latifolia

Various biochemical parameters of *Clarias gariepinus* were analyzed after been fed different concentrations of *Gongronema latifolia* diets and the different results are presented in Table 4. There was no change in the level of AST and GSH across all the treated groups compared to both positive and negative control. Though there was a significant decrease in the level of ALT, globulin and Protein in the treated group relative to the control, but no significant difference noticed among graded levels of *Gongronema latifolia* treated diets. Albumin level was also significantly increased T-2, T-3 and T-5 groups compared with positive control group. The albumin to globulin ratio was significantly increased in all extract supplemented groups and T-2 group compared with positive control results. The level of SOD and MDA were significantly reduced in all groups fed with feed supplemented with extract compared with control. MDA level was however, significantly different among *Gongronema latifolia* treated groups relative to both the negative and positive controls. CAT level was also significantly reduced among the *Gongronema latifolia* supplemented feed relative to positive negative control group.

Table 4: Blood biochemical parameters of *Clarias gariepinus* fed feed supplemented with graded levels of *Gongronema latifolia* extract compared with normal diets and diets supplemented with antibiotics.

Parameters	T1(+ve control)	T2 (-tive control) (antibiotics)	T3(150mg)	T4(300mg)	T5(500mg)
AST (u/l)	65.67 ± 29.02	63.33 ± 24.38	76.33 ± 36.53	79.33 ± 19.50	88.67 ± 17.50
ALT (u/l)	35.67 ± 11.47	18.00 ± 1.53*	19.33 ± 3.22*	18.67 ± 4.33*	16.00 ± 5.20*
ALP (u/l)	12.00 ± 1.00	16.33 ± 3.18*	18.33 ± 4.04*	15.67 ± 2.51*	15.00 ± 1.25*
PROT(g/dl)	52.67 ± 4.73	41.67 ± 1.33*	44.67 ± 3.06*	44.67 ± 6.66	41.67 ± 4.51*
ALB (g/dl)	10.67 ± 0.58	13.33 ± 1.53*	13.33 ± 1.53*	12.00 ± 2.65	13.00 ± 1.00*
GLB(g/dl)	42.00 ± 4.15	28.34 ± 0.98*	31.34 ± 1.53*	32.67 ± 4.01*	28.67 ± 3.51*
ALB/GLB	0.25 ± 0.00	0.47 ± 0.02*	0.43 ± 0.01*	0.37 ± 0.01 ^{aa}	0.45 ± 0.00*
GSH (umol/l)	49.92 ± 11.02	44.08 ± 1.31	51.49 ± 5.14 ^a	48.71 ± 5.12	32.17 ± 8.69
SOD (mg/min)	145.47 ± 3.18	157.81 ± 5.73*	131.07 ± 2.13 ^{ab}	130.88 ± 2.01 ^{ab}	133.61 ± 4.68 ^{ab}
CAT(unit/mg protein)	655.22 ± 89.48	749.28 ± 3.26*	524.35 ± 10.39 ^{aa}	560.28 ± 17.40 ^d	507.83 ± 74.64 ^a
MDA (nmol/ml)	1.86 ± 0.03 ^b	3.69 ± 0.15*	1.26 ± 0.34 ^{ab}	1.21 ± 0.03 ^{aa}	1.13 ± 0.05 ^{aa}

Means with different superscripts along the row are significantly different (P<0.05)

* Significant different compared with T-1 group, ^b Significant different compared with T-2 group, **AST**–Aspartate aminotransferase, **ALT**–Alanine aminotransferase, **ALP**–Alkaline phosphatase, **PROT**–Plasma Protein, **ALB**- albumin, **GLB**- Globulin, **GSH**– Reduced Glutathione Determination, **SOD** –Superoxide Dismutase activity, **CAT** – Catalase activity, **MDA**- Lipid Peroxidation.

DISCUSSION

Various extracts from herbs and spices are reported to improve animal growth performance by stimulating the gut secretions or by having a direct bactericidal effect on gut flora with improvement on the protein synthesis leading to improved growth (Citarasu, 2010). Recent development using plant extract with bactericidal potential as a replacement for antibiotics and chemicals in fish cultivation have help to nip in the bud the infectious diseases displayed as a result of intensive cultivation applications in aquaculture industry and possible antibiotic resistance (Mahdavi *et al.*, 2013). The growth recorded in the fishes fed with the herb supplemented diet was due to improved feed utilization Bimrew (2014), opined that efficient feed utilization will engender nutrient assimilation and increased growth. Other author have also reported similar weight gain pattern in similar experimental setup where birds diet is supplemented with *Gongronema latifolia* extract include Machebe et al (2011). Though there are however reports of no changes to weight in laboratory rats fed the extract of *Gongronema latifolia* (Iweala and Obidoa 2009). Though previous report has shown that extract of *Gongronema latifolia* contains high level of crude protein, large reserve of essential amino acid such as leucine, valine and phenylalanine. aspartic acid, glutamic acid and glycine and a lot of saturated and unsaturated fatty acids, antioxidants and antimicrobial activities (Eleyinmi 2007, Iweala and Obidoa 2009; Edim *et al.*, 2012) but in this experiment the herb was used as a supplement so its contribution to the crude protein value of the feed composition, may not be significant.. The differences in growth recorded in the T5 group compared with the positive control group may not be unconnected to the increased concentration of the *Gongronema latifolia* extract in the feed served to the group which makes it unpalatable for the fish to feed on (Iweala and Obidoa 2009) as a significant reduction was recorded in the total feed intake in the T-5 group (Table 2). Various reports on aquaculture have shown the undeniable contribution of hematology of the culture fishes in terms of the physiochemical property in predicting the health condition and growth efficiency of cultured fish (Harikrishnan *et al.*, 2003). Thus, routine evaluation of the physiochemical characteristics of the fish blood is employed in assessing the fish health status and possible toxicological effects way ahead before

any manifestation of outward disease or stressful condition (Shah and Altindag 2004). In the present study, there was a significant reduction in the circulating level of the white blood cells in all the extract treated groups due to the significant alteration in the concentration of the monocytes and the lymphocytes. This reduction in circulating white blood cell was also significant when compared with that of the group supplemented with antibiotics. This findings is supported by the work of Akinuga et al (2011) when they fed varying doses of ethanolic extract of *Gongronema latifolia* to rat . However the non significant reduction in RBC, PCV and Hb were equally as found by Antai *et al.*, (2009)

There was no change to the circulating red blood cell count and hemoglobin concentration in all the groups, this is similar to the findings of Chowtivannakul (2016) when he fed *Leucaena leucocephala* seed extract to normal rat. The increased MCHC may not be unconnected with the significant increase in the MCV and the possible hemodilution recorded in all extract treated groups. The present findings partly corroborated the report of Bello *et al.*, (2014), where they reported no significant changes in hematology of *Clarias gariepinus* after supplementing their feeding with *G. latifolia* diets.

Liver stress biomarkers which are also biomarkers of toxicity are useful and routinely used in determining onset of diseases and metabolic disturbances in the fish culture (Dobbs *et al.*, 2003). In this study, liver toxicity assessment revealed a non significant increase in serum AST and ALP levels relative to the control while ALT was significantly reduced in all extract treated groups compared to control. ALT is more specific function and its a better indication of liver damage, it could then be concluded that the significant decrease in the level of ALT in fish fed the *G. latifolium* supplemented diets confirm that the extract has hepato protective functions; invariably no liver damage and as such, no leakages of liver enzymes as shown in the MDA values of fish fed on treated diets relative to the control (Akpan and Ekpo 2015). Generally an increase in these liver enzymes indicates injury or toxicity to the organ (Ghadi, 2000). Similar results of a reduction in serum MDA, ALT, SOD and CAT was reported by Henry and Arit (2015), on their study on the biomarker of oxidative stress and liver damage in diabetic rats fed diets containing *Gongronema latifolia* leaves extract. This significant reduction in SOD and CAT according to Akpan and Ekpo (2015) and Fawole et al (2015) was attributed to leaf extract capability to scavenge or neutralise free radicals generated due to oxidative stress and an improve in fish defence system. The results of the present study revealed that fish fed on different levels of extract of *Gongronema latifolia* had significant reduction in serum antioxidant enzymes; SOD and MDA compared to the level in the positive control group while GSH level remain significantly unchanged. This gave an indication of possible antioxidant activities of *Gongronema latifolia* in the fishes at the different doses employed in this study. The antioxidant activities of *Gongronema latifolia* has previously been reported in rats (Essien et al, 2007; Atangwho et al, 2009; Iweala and Obidoa 2009). That GSH level was not changed could further buttress the nontoxic nature of the extract of *Gongronema latifolia* added to the feed of the fishes.

CONCLUSION

The present study showed that supplementation of *juvenile Clarias gariepinus* meal with extract of *Gongronema latifolia* improved their feed consumption, feed utilization, growth and weight gained. It also showed that the extract of *Gongronema*

latifolia lowered white blood cell count and some biomarkers of liver toxicity and oxidative stress but no adverse effect red blood cell, pack cell volume and haemoglobin concentration in circulation.

RECOMMENDATIONS

From the findings of this research, it could be recommended that fish farmers could use *Gongronema latifolia* in formulating fish diets for *Clarias gariepinus* as an alternative to antibiotics and other chemicals used in fish feed for better productions. The best recommended dosage based on this study is 220mg.

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