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RESEARCH PAPER

Genotoxicity, haematological and growth performance of the African catfish *Clarias gariepinus* fingerlings fed walnut *Tetracarpidium conophorum* leaves to substitute for rice bran

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ABSTRACT

A plant has become a preferred source of protein for fish species in aquaculture. A twelve-week feeding trial was carried out in order to assess the effect of feeding walnut leaves on haematological and biochemical parameters as well as the genotoxicity level on *Clarias gariepinus* fingerlings as a bio-indicator of their health status and overall response of the fish towards the experimental diets. One hundred and fifty fingerlings of *Clarias gariepinus* of mean weight 6.90±0.2g were stocked randomly as ten fish per tank (52.5 X 33.5 X 21 cm^3) in triplicate. Fish were fed to satiation and the water changed every day to maintain good water quality. Five experimental diets with 40% crude protein each were formulated; the control, without the test ingredient and the other four diets (test diets1, 2, 3 and 4) contained 25%, 50%, 75% and 100% inclusion respectively. The Control diet had the highest mean weight gain ($P < 0.05$) (118.8±17.3) among the entire group. A similar pattern was observed in specific growth rate. The least significant ($P < 0.05$) feed conversion ratio was recorded by the Control diet(0.68±0.03), the diet also recorded the best protein efficiency ratio (3.53±0.23).The genotoxicity test shows that some Micronucleus of the test diets were normal, lobed and binucleated nucleus compared to the Control diet. The fish fed with *T. conophorum* showed a significant difference in haematological values when compared to the values of fish fed the control diet. The fish fed with *T. conophorum* showed a significant difference in biochemical value when compared with control diet. There was a reduction in the biochemical values of the fish fed *T. Conophorum* with Cholesterol (2.20±0.05) Albumin (8.35±0.21) and Triglyceride (0.98±0.13) compared with the values of fish fed with the control diet with cholesterol (3.81±0.07) Albumin (12.35±2.33) and Triglyceride (7.29±7.02). It was concluded that using *T. Conophorum* leaves as feed for *Clarias gariepinus* enhances the growth of the fish. Therefore, partial replacement of feed *T. Conophorum* should be encouraged.
Keywords: Bio indicator; Genotoxicity; Utilization; *Clarias gariepinus*; Walnut leaves

INTRODUCTION

Aquaculture has evolved as the fastest growing food-producing sector and developed as an important component in food security (Ibrahem *et al.,* 2010). Over the past decades, aquaculture has grown consistently in response to an increasing demand for fish as a source of protein globally (Akinrotimi *et al.,* 2007). This is because the total production from commercial fishing activities in the world has reached its peak and fish catch now reduces on a constant basis. In fact, aquaculture has become the fastest growing food production sector of the world, with an average annual increase of about 10% since 1984 as compared to 3% increase for livestock meat and 1.6% increase for capture fisheries (FAO, 1997).

Therefore, in order to attain more economically, sustainable, environmentally friendly and viable production, research interest has been directed towards the evaluation and use of nonconventional sources of plant protein (Tacon, 1990). In fish culture, nutrition plays an important role in the maintenance of a healthy and marketable product. During the last decade, there has been a marked increase in the use of extruded diets for feeding fish. These diets have superior water stability, better-floating properties and a higher energy than pelleted diets (Hilton *et. al.*, 1981). The main effects on fish are an increase in fish growth and an improvement in feed conversion (Robert *et al.*, 1993). Fish is one of the sources of proteins, vitamins, and minerals, and it has essential nutrients required for supplementing both infants and adults diet (Abdullahi *et al.*,

2001). In Nigeria, fish is eaten fresh and smoked and form a much-cherished delicacy that cut across socio-economic, age, religions and educational barriers (Adebayo *et al*., 2008).

The African mud catfish *(Clarias gariepinus*) is an economically important cultured fish species in Nigeria for its excellent biological characteristics such as fast growth, popular taste, and high economic value, and therefore become a very popular species for aquaculture worldwide (Muchlisin *et al*., 2010). Several studies have shown that plant protein sources have high potentials for supplying fish with required protein needed for their maximum productivity (Hasting, 1976; Abidin *et al*., 2006; Nwanna *et al.,* 2008). The inclusion of plant protein sources in the ration of fish requires investigation on limiting factors in the plant ingredients such as high crude fibre content and anti-nutritional factors as earlier investigations on some plants have shown that their excessive inclusion in the feed may result in slower growth rates and general poor performance of cultured fish species (Cho *et al*., 1976; Francis *et al.,* 2001; Alegbeleye *et al*., 2001; Nwanna *et al*., 2008).

Walnut leaf can be used as a growth promoter and it's known to have the ability to improve the absorptive capacity of the intestine via structural alteration (Oladiji *et al.,* 2010). Fish haematology is gaining increasing importance in fish culture because of its importance in monitoring the health status of fish (Hrubec *et al*., 2000). Haematological characteristics of most fish have been studied with the aim of establishing normal value range and deviation from it may indicate a disturbance in the physiological process (Rainza-paiva *et al.,* 2000).Changes in enzymes profiles are important toxicity indices (biomarkers) and have been used to assess the biochemical and physiological health of vital organs (tissues) in fishes (Van der Oost *et al*., 2000; Gabriel and George, 2005).

A research was carried out by Bello *et al.* (2013), on the Investigation into the healing properties of walnut (*Tetracarpidium conophorum*) leaf and onion (*Allium cepa*) bulb residues in *Clarias gariepinus*. Walnut leaf has been safely used through the centuries as a home remedy for skin conditions. High concentrations (up to 10%) of astringent compounds called tannins account for most of the healing qualities in walnut leaf preparations and proof more advantage over other herbal plants.

The study evaluated the effect of dietary inclusion levels of a walnut leaf (WL) and onion bulb (OB) residues on dermal wound healing of *Clarias gariepinus*. Research has not been carried out on the use of walnut leaves as a replacement of rice bran in fish feed for *Clarias gariepinus*. The objectives of this study are to determine the effect of walnut leaves on the growth of *Clarias gariepinus* and the effect of walnut leaf in the blood of *Clarias gariepinus*, protein utilization and carcass composition as well as genotoxicity. This study will examine the utilization of walnut leaves for sustainable fish production.

MATERIALS AND METHODS

Experimental design

Two hundred healthy fingerlings of African Catfish, *Clarias gariepinus* were purchased from Latia farms at Cele-Egbe, Ikotun, Alimosho, Local Government Area in Lagos. The fish were transported by the open system method using a 25l container which was partly opened on the top. This method was used because of the distance and also the type of fish species. This study was carried out in the Department of Marine Science, Aquaculture unit, Faculty of Science, University of Lagos, Akoka. The feed ingredients were Fish meal (FM), maize (M), Soya bean cake (SBC), Rice bran, Lysine (L), Methionine (m), Premix (P) (Table 1). They were purchased from Soleace Enterprise at Oko-Oba, Agege, and Lagos. Large quantities of walnut leaves were collected from, Waterside area, in Ogun state and it was air dried at room temperature for 3 weeks and was ground

into powder using Hammer mill and stored in a dry labelled container. A small quantity was taken to Jagdee Laboratory, Ibadan, Oyo state.

Experimental procedure and setup

The fishes were put in plastic tanks (52.5 x 33.5 x 21 cm^3) under standard condition; Temperature (27.5 - 29.5°C), dissolved oxygen (4.5 -4.8 mg/l), and pH (7.3 - 8.0). The fish were allowed to acclimatize for two weeks in plastic tanks, and fed with 2 mm Coppens feed (40 % crude protein) to satiation before the commencement of the experimental work.

After the two weeks of acclimatization, the fish were sorted by size and reweighed using a digital scale (Camry EK 5055) and the averaged weight was measured. A total of 150 catfish fingerlings were randomly stocked into the tanks at the rate of ten (10) fish per tank using a scoop net, on the bases of their body weight into fifteen tanks in triplicate, with an average weight of 6.90 g per fish. Suitable conditions were maintained by cleaning the tanks and constant changing of water took place every day. Each of the tanks was cleaned by washing the tanks properly with detergent and rinsed with salt solution water, then dried, filled with water and were stocked with 10 fingerlings. Water was filled to the 15cm height of the container which is about 2/3 of the volume of each tank. Water was continuously supplied from a borehole at the back of the experimental site. The water level was maintained at a level of 15cm throughout the experimental period. Five experimental diets with 40% crude protein each were formulated; the control, without the test ingredient and the other four diets (test diets1, 2, 3 and 4) contained 25%, 50%, 75% and 100% inclusion respectively. The test fish were fed for 12weeks.The mean weight gain of the specimen in each of the experiments tanks were obtained at the end of every week. The tanks were labelled C_o, T_1, T_2, T_3, and T_4 each having triplicates. The tanks labelled represent each of the feeding regimes.

The initial weight of the fish in each tank was determined at the beginning of the experiment and recorded and afterward, they were weighed weekly using the weighing scale Camry EK5055 Max. 5kg/11lb d=1g/0.05oz).

Parameters

Mean weight gain (MWG)

The weight Gain was calculated using the formula: Weight gain (g) = $W_f - W_i$

Where: W_f = Final average weight (g), W_i= Initial average weight (g)

Feed conversion ratio (FCR)

This is the amount of unit weight of feed the fish was able to convert to flesh.

$$FCR = \frac{\text{Average food supplied (g)}}{\text{Body weight gain (g)}}$$

Protein efficiency ratio (PER)

This was calculated from the relationship between the increment in weight of fish (i.e. weight gain of fish) and protein consumed.

$$PER = \frac{\text{Mean weight gain (g)}}{\text{Protein Intake (\%)}}$$

Specific growth rate (SGR)

$$SGR = \frac{(\text{Loge } W_1 \text{ (g)} - \text{Loge } W_2 \text{ (g)}}{T2 - T1 \text{ (day)}} \times 100$$

Where, e = natural logarithm, T2 - T1 = experimental period (day), W1 = initial weight (g), W2 = final weight (g)

Daily growth rate (DRG)

$$\text{DRG (g day}^{-1}) = \frac{\text{Weight gain (g)}}{\text{T2 – T1 (day)}}$$

Protein intake (PI)

Protein intake was calculated using the formula: PI= feed intake x ppercentages of protein in the diet.

Gross food conversion efficiency (GFCE)

This was calculated as the reciprocal of the FCR expressed as a percentage as follow:

$$\text{GFCE} = \frac{1}{\text{FCR}} \times 100$$

Percentage weight gain per week (PWG)

$$\text{PWG} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} \times 100$$

Nitrogen metabolism (Nm)

The Nitrogen metabolism is determined by the formula:

$$\text{Nm} = \frac{(0.549)\,(b-a)\,h}{2}$$

Where, a=initial weight of fish (g), b =final weight of fish (g), h = experimental period of experiment (day)

Determination of water quality parameters

The water pH was measured with a Phillip meter (model pH-009 111), with a glass electrode. The electrodes were standardized using buffer solution and washed with distilled water. It was thereafter washed with the sample water to be tested, dipped into the water and pH was read on the scale. Dissolved Oxygen (DO) was measured with DO meter (MODEL EUTECH DO 600), water temperature was determined by simple mercury in glass thermometer, calibrated in centigrade (^{o}C). It was immersed in the plastic tank for about five minutes and the level of the mercury was read on the graduated glass tube.

Extraction of blood from fish

The fishes were taken from each tank using a small hand net and each of the fish was held firmly then placed belly upward to show the ventral region using a dry towel. The blood was extracted from close to the anal region and also around the caudal peduncle with the aid of a 2cm^3 plastic syringe and the blood was dispensed immediately into Ethylene Diamine Tetracetic Acid (EDTA) anticoagulant bottle to prevent clotting for the haematological studies. Blood samples were collected via the caudal vein puncture as described by Kori-Siakpere *et al.* (2005) into labelled ethylene diamine tetra-acetic (EDTA) bottles and sterile plain sample bottles.

Haematological analysis

The blood sample of fish taken at random from each tank was collected in both syringe and heparinized bottles for haematological assay and taken to Haematology Laboratory, Department of Clinical Science, Lagos Teaching Hospital. Haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC) and packed cell volume (PCV) were analyzed. Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated according to the formulae given by Dacie and Lewis (2001).

Biochemical analysis

Blood was extracted with the aid of a 2cm^3 plastic syringe transferred into a tube containing lithium heparin anticoagulant to obtain plasma for biochemical analysis as described by Kori-Siakpere *et al.* (2005). The use of the plastic syringe is a necessary precaution with fish blood because contact with glass result in decreased coagulation time. The plasma obtained by

centrifugation from the lithium heparinised samples were stored at 200C until analysed. The plasma was analysed for Triglyceride, Urea, Creatinine, Alkaline phosphate (ALP), Total protein, Cholesterol, and Albumin, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT).Cholesterol and Triglycerides were also determined using enzymatic colorimetric test.

Genotoxicity

A single drop of blood is placed on the surface of a clean and grease-free microscope slide at a distance of 2 cm from one end. The blood smear is created by carefully extending this drop of blood in a uniform fashion with the edge of a second slide held at a 45º angle to the first. Once prepared, the blood smear slide is dried by gently waving it in the air (Conroy, 2009).

Proximate analysis

The proximate composition of fish carcass taken from each treatment tank after the experiment was analysed. The crude protein, crude fat, ash, NFE, moisture content and dry matter were determined according to the Association of Analytical Chemists Method (AOAC, 1990).

Data Analysis

The data obtained were statistically evaluated using student test. The data were subjected to one-way analysis of variance (ANOVA). The data include mean, standard deviation, while the means were compared for significant differences using Duncan's multiple range tests using statistical package for the social sciences (SPSS) 10 packet programs.

RESULTS

Proximate composition of the experimental diet

Tables 1 and 2 shows the percentage composition of the experimental feeds. The experimental feed was formulated with varying inclusion levels of walnut leaves at 0%, 25%, 50%, 75%, and 100%. The proximate composition of *Tetracarpidium conophorum* leaves in percentage are crude protein is 29.75%, The Percentage Ash, Crude Fibre, Crude Fat, Moisture And Nitrogen Free Extract(Carbohydrate CHO) were 10.8 %,16.28%,6.90%,7.7% and 28.07%.

Proximate composition of the carcass

The proximate composition of the carcass of *Clarias gariepinus* after 12weeks of the experiment was determined and represented in Table 4.The Test diet 4 (100% of *T. conophorum*) had the highest protein content of 41.31 and test diet 1(25% of *T. conophorum*) had the lowest protein content of 36.86.The highest crude fat content was in the carcass fed with Test diet 3 (75% of T .*conophorum*) while the lowest fat content was in carcass fed with Test diet 2(50% of T. *conophorum*). Ash content was highest in carcass fed with Test diet 2 (50% of *T. conophorum*. While the lowest was in carcass fed with Test diet 1 (25% of *T. conophorum*). Moisture content was highest in the carcass fed with the Control diet and lowest in carcass fed Test diet 3 (75 % of *T. conophorum*). Crude fiber was highest in the carcass fed with Test diet 1 (25% of *T. conophorum*) and lowest in the test diet 4(100% of *T. conophorum*). NFE composition was highest in carcass fed with Test diet 1 (25% of *T.conophorum*) and lowest in carcass fed Test diet 3 (75 % of T.*conophorum*)

Water quality parameters

The water quality parameters were measured in all experimental tanks. The mean water quality parameters of the cultured environment are represented in Table 3.The mean temperature values range from 24 -30^{0}C (27±2.0), while the pH ranges 5.9-7.2 (6.7 ±0.37)and the dissolved oxygen range was low due to malfunctioning DO meter.

Table 1. Feed composition of experimental diet used in this study

Ingredients	Co (g)	T1 (g)	T2 (g)	T3 (g)	T4 (g)
Fish meal	31.20	31.20	31.20	31.20	31.20
Soybean meal	31.20	31.20	31.20	31.20	31.20
Maize	17.34	17.34	17.34	17.34	17.34
Rice bran	17.34	13.00	8.67	4.34	-
Walnut leaves	-	4.34	8.67	13.00	17.34
Dicalcium	2.00	2.00	2.00	2.00	2.00
phosphate(DCP)	0.25	0.25	0.25	0.25	0.25
Lysine	0.50	0.50	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25	0.25
Methionine					
Total	100	100	100	100	100

Table 2. Proximate composition of *Tetracarpidium conophorum* leaves

Parameters	Composition (%)
Moisture	7.70
Crude protein	29.75
Crude fat	6.90
Crude fibre	16.28
Total ash	10.80
Nfe	28.07
Total	99.5

Table 3. Water quality parameters of the experimental tanks

Parameters	Ranges	Mean and Standard Deviation
pH	5.9 - 7.2	6.7 ±0.37
Dissolved Oxygen (mg L^{-1})	2.34 - 4.50	3.4±0.64
Temperature (^{0}C)	24 -30	27±2.0

Table 4. Proximate composition of carcass of *C. gariepinus* fed *Tetracarpidium conophorum* leaves

Parameters	Control	T1	T2	T3	T4
Protein (%)	38.32	36.86	38.51	39.18	41.31
Crude fat (%)	2.16	5.19	3.55	6.34	5.71
Ash (%)	5.83	4.63	6.27	5.41	5.95
Moisture (%)	37.6	36.8	37.3	35.8	32.9
Crude fibre (%)	2.03	2.24	2.22	1.48	0.68
Nfe (%)	14.06	14.28	12.34	11.79	13.45

Growth parameters and nutrient utilization

The growth parameters and nutrient utilization of *Clarias gariepinus* fed with *T. conophorum* at different levels of inclusion are shown in Table 5. The initial weight of the experimental fish was not significantly different (P >0.05) from each other. The final weight of the experimental fish in

T_2, T_1, and T_3 were not significantly different (P>0.05) from each other but they were significantly different from (P<0.05) from the final weight of fish in T_4 and C_o (control diet).The highest average weight gain of fish (118.8±17.3g) was recorded in fish fed with the control diet while the least weight gain (16.7±2.31g) was recorded in fish fed Test diet 4 (100% of *T. conophorum*). The fish in tank T_2, T_1 and T_3 have average weight gain were not significantly different (P >0.05) from each other but they were significantly different (P<0.05) from fish in T_4 and C_o .Similar pattern was recorded for the Specific Growth Rate (SGR) of the experimental fish. The highest SGR (3.09±017) was recorded for the fish fed with the control diet while the least was recorded by fish fed with Test diet 4 (100% of *T. conophorum*) (1.38±0.16). There was no significant difference (P >0.05) between the Specific Growth Rate of the fish in Tank fed with T_2, T_1, and T_3 but they were significantly different (P<0.05) from fish in T_4 and C_o. Although the fish fed with the Control diet ate more feed compared to the Test diets. There was no significant difference (P >0.05) between the Average feed intake of the fish in Tank fed with T_2, T_1, and T_3 but they were significantly different (P<0.05) from fish in T_4 and C_o.

The highest Food Conversion Ratio (FCR) (2.49±0.18) was recorded by fish fed with Test diet 4 (100% of *T. conophorum*) while the lowest and the best FCR was recorded in fish fed with the Control Diet (0.68±0.03). The FCR of the fish were significantly different (P<0.05) from each other. The Daily weight Gain (DWG) range value recorded had its highest (1.31±0.19) in fish fed the Control diet and lowest value (0.11±0.08) in fish fed with Test diet 4 (100% of *T. conophorum*).There was a significant difference (P<0.05) between the Control diet and the fish fed with Test diet 4 (100% of *T. conophorum).*The highest protein intake, (32.70±2.80) was recorded in fish fed with the control diet. There was no significant difference (P >0.05) between fish in T_3, T_2, and T_4 but they were significantly different (P<0.05) from fish fed with the Control diet and T_1 (25% of *T. conophorum*). The Protein Efficiency Ratio (PER) (3.53±0.23) was highest in the fish fed with the Control diet. There was no significant difference (P >0.05) between fish fed with the Control diet, T_2, and T_3 but they were significantly different (P<0.05) from fish fed with T_1 and T_4.The fish fed with the control diet had the highest Nitrogen metabolism value (2936.56±482.31) and fish fed with Test diet 4 (100 % of *T. conophorum*) had the lowest value (413.33±57.2).

Table 5. Growth parameters and nutrients utilization of C. *gariepinus* fingerlings fed with the various diet.

Parameters	Control	T1	T2	T3	T4
Initial mean weight	7.13±0.40^{a}	7.2±1.05 ^{a}	6.7±0.5^{a}	7.0±0.86^{a}	6.6±0.75^{a}
Final mean weight	126.0±17.2^{c}	62.8±23.2^{b}	51.0±11.0^{b}	56.13±1.51^{b}	23.36± 1.85 ^{a}
Average weight gain	118.8±17.3^{c}	55.6±23.8^{b}	44.3±11.1^{b}	49.0±1.30^{b}	16.7±2.31^{a}
Average	80.6±8.5^{b}	32.2±0.8^{a}	32.0±2.03^{a}	39.4±7.11^{a}	41.5±3.13^{a}
Feed Intake	0.68±0.03^{b}	0.78±0.37^{a}	0.75±0.23^{a}	0.79±0.12^{a}	2.49±0.18^{a}
FCR	3.09±017^{c}	2.31±0.54^{b}	2.16±0.28^{b}	2.32±0.89^{b}	1.38±0.16^{a}
SGR	1.31±0.19^{c}	0.61±0.26^{b}	0.49±0.12^{b}	0.54±0.01^{b}`	0.11±0.08^{a}
DWG	131.8±18.9^{c}	61.7±26.4^{b}	49.2±12.3^{b}	54.4±1.47^{b}	14.6±4.6^{a}
DGI	3.53±0.23^{c}	2.3±0.00^{b}	3.47±1.00^{bc}	3.17±0.54^{bc}	1.00±0.72^{a}
PER (%)					
PWG (%)	1670.6±262.4^{c}	806.4±451.8^{b}	667.1±183.4^{ab}	697.9±89^{ab}	256.0±56.1^{a}
GFCE	147.3±4.97^{b}	142.8±58.5^{b}	140.3±42.7^{b}	127.4±22.2^{b}	40.2±2.84^{a}
NM	2936.56±482.31^{c}	21373.56±388.71^{a}	1100.13±282.91^{b}	1212.13±32.25^{b}	413.33±57.2^{b}
PI	32.70±2.80^{c}	22.20±9.51^{b}	12.76±0.81^{a}	15.76±2.89^{ab}	16.60±1.21^{ab}

*Means with the same superscripts in the same vertical row are not significantly different (P>0.05) from each other.

Haematological and biochemical compositions

The haematological studies for the fish in all the treatment tanks showed from the White blood cell(WBC), Erythrocytes (RBC), Haemoglobin (Hb), Mean Corpuscular Volume(MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin (MCHC), Haematocrit or Packed Cell Volume (HCT/PCV) shows that there was no significant difference (P >0.05) among the tanks (Table 6).

The plasma biochemical was analyzed for each of the experimental tanks and the result is represented in Table 7. The Aspartate Aminotransferase (AST) had its highest value 31.75±26.65 (µ/l) in T_4 (100% of *T. conophorum*) and lowest value 8.45±0.21 (µ/l) in T_1 (25 % of *T. conophorum)* and Significant difference (P >0.05) was not observed between the tanks. The highest level 11.56±4.53 (mmol/l) of Creatinine was recorded in T_2 (50 % of *T. conophorum*) and lowest level 7.70±3.20 (mmol/l) was recorded in T_4 (100% of *T. conophorum).*Alanine Aminotransferase (ALT) highest level of 361.90±446.04 (µ/l) was recorded in T_4 (100 % of *T.conophorum*) and the lowest level of 65.10±8.76 (µ/l) in the Control diet.The urea level had its highest value 4.82±1.37 in T_1 (25% of *T. conophorum*). And lowest level of 2.80±0.25 was recorded in T_4 (100 % of *T. conophorum*).

The Cholesterol level mean value range from 2.20±0.05 at T_2 (50 % of *T. conophorum*) to the highest value of 3.81±0.07 in the Control feed but they were significantly different from each other. The Albumin has its lowest value as 8.35±0.21 in T_2 (50 % of *T. conophorum*) and 12.35±2.33 in the control diet as its highest. There was no significant difference (P>0.05) between T_1, T_3, and T_4 but they were statistically different (P<0.05) from the Control Diet and the T_2 (50 % of *T. conophorum*).

Table 6. Haematological parameters of *Clarias gariepinus* fingerlings fed different levels of *Tetracarpidium conophorum* leaves.

Parameters	Control	T1	T2	T3	T4
WBC(x10^9)	121.10±28.1[a]	103.50±40.7[a]	113.85±0.31[a]	139.50±40.44 [a]	136.65±40.8[a]
HGB(g/L)	71.00±45.25[a]	75.5±21.92[a]	79.5±14.84 [a]	101.00±24.04 [a]	103.00±28.02[a]
RBC(T/L)	1.45±1.11[a]	1.57±0.39[a]	1.72±0.14 [a]	2.43±0.86[a]	2.37±0.81 [a]
MCV(Fl)	158.15±6.29[a]	177.20±13.2 [a]	161.20±34.50[a]	157.30±20.08 [a]	157.35±20.71 [a]
MCH(Pg)	52.05±8.89[a]	47.80±1.83[a]	45.50±4.66 [a]	42.45±5.16[a]	43.85±3.18 [a]
MCHC(g/dl)	33.00±4.38[a]	27.00±0.98[a]	28.85±3.32 [a]	27.00±0.14 [a]	28.00±1.69 [a]
PCV(L/L)	0.26±0.11[a]	0.27±0.08 [a]	0.28±0.08 [a]	0.37±0.08[a]	0.36±0.08

* Means with the same superscripts along the vertical row are not significantly different (p>0.05).

Table 7. Biochemical compositions of *Clarias gariepinus* fingerlings fed different levels of *Tetracarpidium conophorum* leaves

Parameters	CD	T1	T2	T3	T4
AST(μ/l)	9.05±20.5[a]	8.45±0.21[a]	28.75±26.65[a]	9.30±2.96[a]	31.75±26.65[a]
Creatinine (mmol/l)	9.92±5.11[a]	10.25±2.66[a]	11.56±4.53[a]	10.89±2.88[a]	7.70±3.20[a]
ALT(μ/l)	65.10±8.76[a]	90.95±11.52[a]	126.20±72.12[a]	72.60±19.37[a]	361.90±446.04[a]
Urea (mmol/l)	4.72±0.31[a]	4.82±1.37[a]	4.42±1.73[a]	4.59±1.91[a]	2.80±0.25[a]
ALB(g/l)	12.35±2.33[b]	11.75±1.06[ab]	8.35±0.21[a]	11.75±0.21[ab]	11.85±2.05[ab]
TP(g/l)	21.96±5.91[a]	22.91±8.00[a]	23.52±1.84[a]	31.35±2.34[a]	30.54±5.07[a]
CHOL(mmol/l)	3.81±0.07[c]	2.81±0.19[ab]	2.20±0.05[a]	3.36±0.00[bc]	2.60±0.60[ab]
TG(mmol/l)	7.29±7.02[a]	1.40±0.05[a]	1.19±0.18[a]	1.41±0.04[a]	0.98±0.13[a]
ALP(μl)	17.90±17.67[a]	5.55±0.77[a]	26.00±13.71[a]	5.85±2.19[a]	17.30±14.84[a]

Means with the same superscripts along the vertical row are not significantly different (p>0.05).

Genotoxicity activity

The fish fed with the Control diet, the normal erythrocytes was observed and it contains the elliptical nuclei with a small non –refractive circular particles lying in the cytoplasm and resembling a nucleus with respect to staining properties were considered as normal micronucleus. For the Test diet1 (100% of *T. conophorum*) and Test diet 2 (50% of *T. conophorum*) the erythrocytes showed that some of the nuclei were normal while few nuclei clearly deviated from the normal shape having cells with two nuclei of approximately same size and staining pattern and staining intensity and within the same cytoplasmic boundary and were considered as binucleated. Deviations from the normal shaped were also observed in Test diet 3 (75% of *T. conophorum*) and Test diet 4 (100% of *T. conophorum*)having binucleated cells, lobed -which have is an evagination of the nuclear membrane having several lobes and some of the nuclei had normal shapes.

DISCUSSION

The feeding trials revealed that *Clarias gariepinus* responded to all the diets irrespective of their composition, however it showed that the inclusion of *T. conophorum* affected the palatability of the diets which was observed by slow feeding response; this could be attributed to the taste or the smell of the feed. The Total feed intake was highest in the Control diets as seen in Table 6.The fish fed with the control diet also had the lowest and best FCR which showed that it was able to covert the feed fed into fish flesh while the Test diet 4(100% of *T. conophorum*) showed a poor Feed conversion rate. Poor PER (1.00±0.72) was observed in Test diet 4 (100 % of *T. conophorum*) this could be attributed to the formation of a poorly digestible complex between phytic acid and protein, which could result in reduced bioavailability of minerals other than zinc. The complex so formed could impair protein digestibility through the formation of phytic acid-protein complexes or inhibition of digestive enzymes secretion (Robaina *et al.*, 1995). Better growth and nutrient utilization were achieved at relatively low inclusion level compared to the high incorporation of the test ingredient.

The water quality parameters of the experimental setup were similar and within the optimum range recommended for the culture of *C. gariepinus* by Omitoyin (2006) when he worked on the Haematological changes in the blood of *C. gariepinus* juveniles fed with poultry litter.

According to Munkittrick and Leatherland (1983), haematocrit reading is valuable in determining the effect of stressors on the health of fish. The significant increase observed with increased test ingredient showed that the fish were not stressed from the test ingredient inclusion. Haemoglobin is a sophisticated oxygen delivery system that provides the desired amount of oxygen to the tissues under a wide variety of circumstances (Voet and Voet, 1990). According to Blaxhall and Daisley (1973), the determination of haemoglobin can be a good indicator of anemic conditions in fish with the recorded values in the present study; it showed that the fish did not suffer from any form of anemia. The increase observed in the two parameters (packed cell volume and the haemoglobin) may be due to high activity in the fish, according to Weiss and Wardrop (2010); fish with high activity will need more oxygen invariably having the need for increased PCV and haemoglobin.

However, these parameters decrease in the presence of anti-nutritional factors (Osuigwe *et al.*, 2007). According to Ayoola *et al.* (2013) in the chemical evaluation and nutritive values of Walnut leaves; it was reported that the walnut leaves contain anti-nutritional factors (Alkaloids, saponins, and tannins). The tannins reduce blood cholesterol (Basu *et al.,* 2007). Hence the reduction in the values of PCV and Hb observed in this study could be attributed to the presence of these anti-nutritional factors. This study corroborates the work of earlier researchers who reported a decrease in hematocrit and haemoglobin content with an increased level of ingredients (Blom *et al.,* 2001). There was no significant difference ($p>0.05$) between the haematological values of fish fed with the control diet and the test diets this is in agreement with the work done by Ominiyi *et al* (2002). Gabriel *et al.* (2004) further reported no significant differences in the haematological values for apparently healthy *C.gariepinus* before and after acclimatization which was similar to the observation in this study.

The increase in the White Blood Cell (WBC) in the fish fed the T_3 and T_4 (75% and 100% of *T. conophurum* respectively) is attributed to the increased production of leucocytes in the haematopoietic tissue of the kidney perhaps the spleen, this agreed with work of Omitoyin (2006). The increase may also be as a result of an increase in leucopoiesis as a means of combatting stressor in the body system of the fish, similar findings were recorded by Gabriel *et al.* (2004) in *C. gariepinus* under confinement due to acclimated for 7 days. These changes in white blood cell have been reported to play important roles in the assessment of the state of health of *C.gariepinus* (Ezeri, 2001; Gabriel *et al.*, 2004).The increase in the red blood cell and haemoglobin concentration may be attributed to the increase in the size of the fish as a result of growth in the fish. This is in agreement with Ayoola *et al.* (2013) who reported that both the haemoglobin contents and Erythrocyte counts (red blood cell) tend to increase with length and age of the fish.

Aspartate aminotransferase (AST) help in amino acid degradation and biosynthesis (WHO, 2003). Therefore, the significant change in the ALT, AST, and ALP might be impairment in the fish internal organ most especially the kidney. Total protein values were slightly lower than those reported by (Ayoola, 2011) and also the values were slightly lower than those reported by Omitoyin (2005), this may be due to the environmental condition of the rearing facilities and Handling.

A decrease in Carcass lipids and increase in carcass protein might also explain why the blood protein increased but the blood cholesterol level decreases, a similar pattern of the result was obtained by Moharrerey (2005) when he fed Malic Acid to both male and female broiler chicks. The result of the biochemical index indicated an elevated level of PCV and Hb ($p>0.05$) in the

treated groups compared to the Control, Similar findings were reported by Choudhury *et al.* (2005). The control diet had normal micronuclei while there were deviations from the normal shape in the fish fed with the test diets, some of the nuclei were mostly normal and some had abnormalities such as the binucleated and lobed nucleus, this is as a result of chromosome dysfunction. Hence the nucleus should be considered as indicators of structural genomic changes.

CONCLUSIONS

The result of this study revealed that the walnut leaves at 100 % inclusion affected the overall health status of the fish. This result seems to have a link with palatability of the diets which caused reduced feed, and also due to the presence of anti-nutritional properties of the walnut leaves. A processed walnut leaves with the extraction of the anti-nutritional factors might boost up the overall performance of the fish. However, since walnut leaf is more available and relatively cheaper, it could be recommended that partial replacement walnut leaf in the diet of *C. gariepinus* at 25% inclusion level would aid fish productivity.

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