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RESEARCH PAPER

Effects of the walnut *Plukenetia conophora* shell in the diet on the growth performance and genotoxicity of the African catfish *Clarias gariepinus*

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ABSTRACT

Aquaculture industry is facing challenges of the high cost of fish feed, and therefore it is very crucial to explore the alternative raw materials for feed formulation at the lowest cost. Therefore, the objective of the present study was to evaluate the effect of *Plukenetia conophora* shells on the growth, haematological and biochemical parameters, and its genotoxicity on African catfish *Clarias gariepinus* juveniles. Five experimental diets were formulated having three replicates at 0% (0WS), 25% (25WS), 50% (50WS), 75% (75WS) and 100% (100WS) inclusion of walnut shell respectively. The fishes were fed on experimental diet two time a day for 12 weeks. The results showed that the fish in tank 25WS had the best weight gain with the mean of 33.5±5.8 g and the least was recorded in tank 100WS. The specific growth rate was highest in-tank 75WS with the mean value of 0.46±0.05 g was recorded. The highest feed intake was found in fish fed with 25% inclusion of *P. conophora*. The fish fed with *P. conophora* showed increased values of haemoglobin, (12.05±1.63g/dL), Red blood cell, (2.785±0.28µL) and White blood cell, (11.25±4.59µL) compared with control diet values of fish fed of Red blood cell, (1.81±1.54µL) and White blood cell, (5.15±6.57µL). There was a reduction in the haematological value of the fish fed with control feed having Haemoglobin, (10.75±8.13g/dL). The genotoxicity test that was carried out showed that the highest counts of micronucleus were in tank 75WS. The Duncan Multiple Range Test (DMRT) shows a significant difference ($p<0.05$) in the growth performance of the fish. It is concluded that inclusion of 50% *P. conophora* shells in the feed of *C. gariepinus* gave no negative impact on the health status and growth performance of the fish.

Keywords: Toxicity test, Growth indices, *Plukenetia conophora*, *Clarias gariepinus*

INTRODUCTION

Aquaculture involves the domestication and rearing of different types of aquatic animals or plants in ponds or tanks. Aquaculture has continued to increase in values and volume in many countries of the world bridging the gap between demand and supply of fish, increase production, creating new and additional employment and contributing to household economy particularly in rural areas and fishery products (Ayoola, 2010). The major variable cost in intensive and semi-intensive aquaculture is the feed and feeding operation. Fish play a very role in body development, maintenance, and tissue repairs because it contains Omega-3 fatty acids which reduced the risk of heart diseases. Fish contain a high level of vitamins and minerals which aid proper functioning of the body.

The African catfish *Clarias gariepinus* is one of the most economically important fish species in Nigeria, it is cultured for its hardy nature, fast growth, high economic value, and good taste (Asraf *et al.*, 2013; Muchlisin *et al.* 2015; Marimuthu *et al.*, 2019). Unfortunately, in Africa, high mortality often acts as a limiting factor to the growth of fish farming businesses; this is as a result of nutritional imbalance, wounds, and infections sustained by the fish. The use of food additives to

boost the immune system of fish have been employed to circumvent this challenge. Although antibiotics have been used in fish production for over two decades, the transference of resistant genes to human microbiota is the main limiting factor. Research has revealed that plant sources of feeds contain appreciable crude protein content for maximum productivity (Aguilar-Manjarrez *et al*., 2017, Ayoola and Bamiro, 2017). In formulating feed for fish using plant sources, inclusion levels, antinutritional factors, and processing methods must be taken into consideration for effective utilization and improved performance of cultured fish species. Processed walnut leaves with the extraction of the anti-nutritional factors might boost up the overall performance of the fish (Ayoola and Bamiro, 2017).

Tetracarpidium conophorum, now known as *Plukenetia conophora* and is found in Nigeria and Cameroon (Ayoola *et al*., 2013). The capsule of *T. conophorum* contains four shelled seeds. The testa of the seed is hard and the cotyledons are white. Seed mature between 4-6 months and are found in the local markets between May and September (Ajayeoba and Fadare, 2006). It has been reported that there is a presence of oxalate, phytates, and tannin in the raw *P. conophora* nuts. Some phytochemical constituents present in *T. Conophorum* are tannins, protein oils, carbohydrates and fibers (Ayoola and Bamiro, 2017).

Walnut shells (conophor) have innumerable health benefits. The result of the previous study has indicated that the shell has tannins, flavonoids, and phenols, which gives it a slightly bitter taste (Nwanna *et al*., 2008). Walnuts has an insignificant amount of cholesterol-free sodium. Also, the presence of alate, phytates, and tannin in raw P. *conophora* nuts have been reported (Ayoola and Bamiro, 2017). Traditionally walnut is used as antibacterial, antifungal, anti-inflammatory, antiviral and liver health maintenance (Bello *et al*., 2012). More information is required on the use of walnut shells as a replacement for diphosphate in fish feed for African catfish *C. gariepinus*. Hence, the objective of the study were to determine the effect of different levels of inclusion of the walnut shell (*P. conophora*) in the diet of *C. gariepinus* and to determine the genotoxicity and haematological indices of *C. gariepinus* fed walnut shell supplemented diets.

MATERIALS AND METHODS

Time and Site

This study was carried out in the Marine Sciences Department, Marine Basement Laboratory, University of Lagos, Akoka in the year 2016. Two hundred healthy juveniles of African catfish, Clarias gariepinus of 9.85±0.5g were purchased from Olotu fish Farm, Egbeda, Lagos. Due to distance and the type of fish species, the fish were transported using the open system method; two 25 L buckets containing the fish and water were left partly opened. Walnut was purchased from the Magboro market, Ogun state. The walnuts were cooked for one hour and twenty minutes; the seeds were removed from the shell; then, the shells were placed on a tray and sun-dried for three days. The shells were ground using a commercial mill.

Composition of The Experimental Diet

The percentage composition of raw materials used for the experimental diet is shown in Table 1 with 40 % crude protein content. The experimental feed was formulated with varying inclusion levels of a walnut shell. The proximate composition of *P. conophora* shell showed that the shell has crude protein 6.80%, moisture 8.5%, ash 4.50%, crude fibre 4.50%, lipid 1.20% with gross energy of 2,980.9 Kcal.

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Table 1. The raw material composition of experimental feed diet (100 kg)

Ingredients	0WSg/Kg	25WSg/Kg	50WSg/Kg	75WSg/Kg	100WSg/Kg
Fish meal	24.87	24.87	24.87	24.87	24.87
Soybean meal	24.87	24.87	24.87	24.87	24.87
Maize	10.9	10.9	10.9	10.9	10.9
Indomie noodles	10.9	10.9	10.9	10.9	10.9
GNC	24.87	24.87	24.87	24.87	24.87
Walnut shell	-	0.5	1.0	1.5	2.0
DCP	2.0	1.5	1.0	0.5	-
Fish premix	1.0	1.0	1.0	1.0	1.0
Mineral premix	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

T0= Control, T1-T4=Treatment, GNC - Groundnut cake, DCP - Dicalcium phosphate WS=walnut shell

Experimental Procedure

The fishes were acclimatized for two weeks in a tank and fed with 2 mm coppens feed (40% crude protein) to satiation before the commencement of the experimental work. After acclimatizing for two weeks, the fish were sorted by size and reweighed using a digital scale (Salter 1073BKDR) and the average weight was determined. The tanks with the size of 1 x 1x 0.6 (m^3) of 100 L of water to be used were cleaned with detergent, rinsed with a saltwater solution, dried, filled with water and then stocked. Ten catfish juveniles were randomly stocked into each tank with a mean weight of 20.5 ± 0.2 g per fish. Five tanks in three replicate were used for the experiment. The fishes were fed with formulated feed to satiation at 9.00-10.00 hours and 16.00-17.00 hours daily. The water level was maintained at a level of ¾ of the tank throughout the experimental period. The experimental fish were fed for 12 weeks.

The tanks were labeled 0WS, 25WS, 50WS, 75WS, and 100WS each having three replicates. The tanks labeled represent each of the feeding treatments respectively (Table 2). Before commencing the feeding trial, the fish were starved for a day to empty their gastrointestinal tract. The fishes were fed to satiation with the various treatment feeds for twelve weeks. The daily feeding ration was weighed at the beginning of every week using a scale (Salter 1073BKDR). The pelleted feed was crushed into crumbs before feeding the fish that is after weighing the feed. The feed ratio was altered every week based on estimated biomass after the bi-weekly sampling for growth. The fishes in all the tanks were weighed weekly after which the mean body weight was calculated and rations altered according to the new weight. Feeding response was monitored (by feeding to satiation) and the mortality rate was recorded. The fishes were fed from 9.00 am and 4.00 pm for 12 weeks. The water was changed daily to prevent pollution of water by uneaten feed and faeces from the fish.

Table 2. The percentage substitution of formulated feed

Treatment	Percentage inclusion
Diet 0WS: Control Feed	100% diphosphate
Diet 25WS:	25% walnut shell : 75% dicalciumphosphate
Diet 50WS:	50% walnut shell : 50% dicalciumphosphate
Diet 75WS:	75% walnut shell : 25% dicalciumphosphate
Diet 100WS:	100% walnut shell

Parameters

The initial weight of the fish in each tank was taken at the beginning of the experiment and weighed weekly using a weighing scale (Salter 1073BKDR) and the mean value was calculated. The

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growth performance and nutrient utilization of the experimental fish were calculated. The weight gained was calculated using the formula based on Muchlisin *et al.* (2016) as follow: Weight gain (g) = Wf – Wi, Where Wf = Final average weight (g), Wi = Initial average weight (g). Specific growth rate (SGR) was calculated as follow: $SGR = \frac{(\text{Loge Wf (g)} - \text{Loge Wi (g)}}{\text{T2} - \text{T1 (day)}} \times 100$

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

Protein efficiency ratio (PER), This parameter was calculated from the relationship between the increment in weight of the fish (i.e weight gain of fish) and protein consumed.

$$PER = \frac{\text{Mean Weight Gain (g)}}{\text{Protein Intake (\%)}}$$

Feed conversion ratio (FCR) was calculated as follow: $FCR = \frac{\text{Feed Intake (g)}}{\text{Total Weight gain (g)}}$

Daily growth rate (g day^{-1}): Weight gain (g)/experimental period (day)

Protein intake (PI), Protein intake was calculated using the formula:

PI = Feed intake X Percentage (%) protein in diet

Gross food conversion efficiency (GFCE) was calculated as the reverse of FCR expressed as a percentage: $\frac{1}{FCR} \times 100$

Percentage weight gain per week was calculated by the formula below:

$$PWG = \frac{\text{Final Weight} - \text{Initial Weight (g)}}{\text{Initial Weight (g)}} \times 100$$

Nitrogen metabolism was determined by the formula:

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

Where, a= Initial weight of fish, b= final weight of fish, h= experimental period in day, Nm= Nitrogen metabolism

Haematological analysis

Fish were taken at random from each tank using a small hand net, held firmly using a dry towel and then placed with the belly upward to show the central region. Fish blood was extracted close to the anal region and around the caudal peduncle using a 2 cm^3 and 5 cm^3 plastic syringe and the blood was dispensed into Ethylene Diamine Tetracetic Acid (EDTA) anticoagulant bottle to prevent clotting for the haematological studies (Kori-Siakpere *et al.*, 2005).

The blood samples of the fish were taken to Haematological Laboratory, Department of Clinical Science, Lagos University Teaching Hospital. Analysis for Haemoglobinl (Hb), red blood cells (RBC), white blood cells (WBC) and packed cell volume (PCV) were conducted. Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), and Mean Corpuscular Volume (MCV) were calculated using the standard method (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 plasma centrifugation from the lithium heparinized samples was stored at 20°C until analyzed. The plasma was analyzed for Triglyceride, Urea, Creatinine, Alkaline phosphate (ALP), Total protein, Cholesterol, Albumin, Aspartate Aminotransferase (AST) and Alanine

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Aminotransferase (ALT). Cholesterol and Triglyceride were also determined using the enzymatic colorimetric test (Kori-Siakpere *et al.* 2005)

Genotoxicity test

Blood samples are taken from the caudal vein to determine the normality of micronucleus. A single drop of blood was placed on the surface of a clean and grease-free microscope slide at a distance of 2 cm from one end. The blood smear was created by carefully uniformly extending the drop of blood with the edge of another slide held at a 45^0 angle to the first. Once prepared, the blood smear slide is dried by gently waving it in the air. These were carried out according to the methods described by Conroy (2009).

Proximate analysis

Five fish samples from each tank were dried in the oven at 110°C and ground into fine particles and homogenized. The proximate composition of fish carcass taken from each treatment tank after the experiment was analyzed for crude fat, crude protein, ash, Nitrogen Free Extract (NFE), and dry matter was determined according to the methods of the Association of Analytical Chemist, USA.

Statistical analysis

Analysis of variance (ANOVA) was carried out to test the significance of the treatments on the fish growth rate pattern. Means were compared for significant differences using the Duncan Multiple Range Test (DMRT) using the statistical package for the social sciences (SPSS) 10 packet program.

RESULTS

Chemical analysis of walnut shell

The proximate composition of the walnut shell *Plukenetia conophora* revealed that the crude protein percentage of *Plukenetia conophora* was 6.80%, the moisture percentage of *T. conophorum* 8.50%, the ash content of *T. conophorum*, fiber percentage of 4.50%, the fat percentage of *T. conophorum* 1.20% and the Gross Energy of *P. conophora* was 2980.9 kcal/kg.

Growth Performance and Nutrients Utilization

The growth performance and nutrient utilization of *C. gariepinus* fed with *P. conophora* at different levels of inclusion are shown in Table 3. The final weight of the experimental fish was significantly different ($P>0.05$) from each other. The final weight of the experimental fish in tanks WS50, WS75 and WS100 were similar and different from the final weight of WS25 and Control. The average weight gain of fish (23.46±5.9g) was recorded by the fish fed with 25% inclusion of the T. conophorum diet while the least was recorded in Tank-100% inclusion. The fish in tanks WS0 and WS75 had average weight gain that was not significantly different ($P>0.05$) from each other but they were significantly different ($P<0.05$) from fish in tanks WS25, WS50 and WS100. The highest specific growth rate (SGR) was recorded in-tank WS75 (75% inclusion) of the walnut shell while the least was recorded in tank WS100 (100% inclusion) of a walnut shell. There was no significant difference ($P>0.05$) between the specific growth rate of fish in WS25 and WS50 but there a significant difference from WS0, WS75, and WS100. The highest food conversion ratio (FCR) was recorded in WS50 (50% inclusion) while the lowest and the best FCR was recorded in tank T0 (Control). The FCR of the fish showed no significant difference ($P<0.05$) from each other. The proximate composition of *C. gariepinus* fed graded levels of an extract of *P. conophora* is presented in

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Table 4. The biochemical parameters of *C. gariepinus* fed with walnut shells were presented in Table 5.

Hematological Parameters and Genotoxicity analysis

Fish fed with 100% of grounded walnut shell recorded high haematocrit (HCT), haemoglobin (Hb), White blood cell (WBC) and Lymphocytes counts (Table 6). However, there were no significant differences (P<0.05) among all the treatment groups including the control. Genotoxicity parameters of *C. gariepinus* fed graded levels of *P. conophora* are presented in Table 7. Fish fed 75% (WS100) and 100 %(T5) recorded the highest induction of micronucleus (MN) which showed a significant increase (P<0.05) compared with those of the control, 25% and those of 50% extracts. However, those of control treatment recorded the lowest induction of micronucleus. No significant difference (P>0.05) was recorded in the lobes among all the treatment groups including the control. There were significantly more binucleated cells in fish fed 75% walnut cell compared to those fed 25% walnut cell. No significant difference (P>0.05) was recorded in the number of lobes in 50%, control, and 100% concentration of the extract.

Table 3. Growth performance and nutrient utilization of *P. conophora*

Parameters	WS0	WS25	WS50	WS75	WS100
Initial weight	23.80±8.94^{a}	24.60±8.74^{a}	24.30±8.94^{a}	24.00±8.94^{a}	23.70±8.94^{a}
Final weight	43.80±8.94^{ab}	48.60±7.2^{a}	43.60±5.8^{b}	44.10± 5.3^{ab}	40.00± 4.02^{b}
Weight gain	20.00 ± 5.5^{a}	23.46 ± 5.9^{b}	19.30 ± 5.9^{ab}	20.10 ± 5.9^{a}	16.30 ± 5.9^{bc}
FCR	0.78±0.14^{a}	1.00±0.08^{a}	1.14 ±0.06^{a}	1.12 ± 0.21^{a}	1.10 ± 0.06^{a}
SGR	0.42±0.08^{a}	0.44±0.02^{a}	0.43±0.04^{a}	0.46±0.05^{a}	0.40±0.01^{a}
PWG	15.6 ± 4.9^{a}	17.6 ± 5.9^{a}	16.7 ± 4.9^{a}	17.6 ± 5.9^{a}	17.6 ± 5.9^{a}

*Means with the same superscripts in the same vertical row are not significantly different (P>0.05) from each other. Significant differences (p<0.05) were observed in all the growth parameters among all treatment groups including control. WS0= Control, WS25-WS100=Treatment (FCR-Food Conversion Ratio, SGR-Specific Growth Rate, PWG-Percentage Weight Gain, WS= walnut shell inclusion).

Table 4. Proximate composition of *C. gariepinus* fed graded levels of inclusion *P. conophora* shell

Treatment	Carbohydrate (%)	Protein (%)	Crude fat (%)	Moisture (%)	Ash (%)	NFE (%)
Tank 1 (Control)	3.175±0.12^{b}	59.38±3.71^{a}	19.09±1.71^{b}	4.87±1.81^{a}	5.755±1.35^{a}	7.735±2.39^{b}
Tank 2 (25%)	4.24±0.68^{a}	59.67±6.62^{a}	17.4±6.96^{c}	3.78±0.71^{b}	5.49±0.16^{a}	9.42±0.49^{a}
Tank 3 (50%)	3.385±1.12^{b}	57.33±1.65^{b}	24.75±0.61^{a}	3.6±0.87^{b}	4.97±1.07^{b}	5.965±0.31^{c}
Tank 4 (75%)	4.52±0.41^{a}	59.675±0.83^{a}	17.16±1.48^{c}	4.24±0.63^{a}	4.98±0.65^{b}	9.43±42^{a}
Tank 5 (100%)	3.8±2.13^{b}	55.285±2.07^{c}	22.8±2.07^{ab}	3.485±0.83^{b}	5.535±1.87^{a}	9.1±0.54^{a}

Values in the same column with different superscript letters are significantly different at p<0.05. NFE = Nitrogen Free Extract

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Table 5. Biochemical parameters of *C. gariepinus* fed with walnut shell

Experiment	AST (µ/l)	ALT (µ/l)	ALP (µ/L,)	Urea mmol/l	Creat mmol/l	Protein (g/dL)	Albumin (g/dL),	CHOL mmol/l	TG mmol/l
Tank 1 (Control)	116.5±9.2^{c}	61±4.2^{a}	14.5±6.4^{a}	1.05±0.2^{a}	11.5±4.9^{b}	28.5±10.6^{c}	6±1.4^{c}	1.95±0.7^{b}	1.25±0.4^{b}
Tank 2 (25%)	120.5±4.9^{b}	56±19.8^{b}	12.5±0.7^{b}	0.95±0.7^{b}	12.55±1.9a	30.5±3.5^{b}	7±1.4^{b}	2.1±0.0^{a}	1.45±0.2^{b}
Tank 3 (50%)	84.5±40.3^{d}	33±8.5^{d}	9±1.4^{c}	1.05±0.4^{a}	8.55±3.0^{d}	29.5±7.1^{c}	6±0.0^{c}	1.85±0.7^{b}	1.3±0.3^{b}
Tank 4 (75%)	135.5±31.8^{a}	47±11.3^{c}	9.5±1.4^{c}	1.35±0.4^{a}	10.3±2.5^{c}	35±4.2^{a}	8±0.0^{a}	2.3±0.4^{a}	2.05±0.5^{a}
Tank 5 (100%)	120.5±99.0^{b}	63±57.9^{a}	7.5±0.7^{d}	0.85±0.7^{b}	8.3±7.9^{d}	31±5.7^{b}	6±1.4^{c}	2.05±21^{a}	1.4±1.4^{b}

Note: Aspartate Aminotransferase (AST) (µ/L), Alanine Aminotransferase (ALT) (µ/L), Alkaline phosphate (ALP) µ/L, Urea (mg/dL or mmol/l), Creatinine (mg/dL or mmol/l), Total protein(g/dL), Albumin(g/dL), Chole. Values on the same column with different superscripts showed a significant difference ($p<0.05$) from each. Using sterol (mg/dL or mmol/l) and Triglycerides (TG) (mg/dL or mmol/l which tool

Table 6. Haematology of *C. gariepinus* fed graded levels of an extract of *P. conophora*

Parameters	**Tank 1 (Control)**	**Tank 2 (25%)**	**Tank 3 (50%)**	**Tank 4 (75%)**	**Tank 5 (100%)**
RBC(10^4/µL)	1.81±1.54^{b}	2.05±0.268^{a}	2.115±0.32^{a}	1.94±1.2^{b}	2.785±0.28^{a}
HCT (%)	29.05±25.95^{bc}	33.3±0.71^{b}	31.6±0.85^{bc}	26.25±19.16^{c}	39.65±4.59^{a}
MCV(fL)	156.05±10.53^{a}	163.75±24.53^{a}	151.25±26.65^{a}	129.9±18,38^{b}	142.7±4.24^{b}
RDW-CV (%)	16.7±1.56^{b}	21.1±6.51^{a}	16.5±1.84^{b}	11.45±0.21^{c}	12.4±1.27^{bc}
RDW-SD (fl)	96.7±25.88^{a}	70.55±20.15^{bc}	89.2±20.79^{b}	53.05±11.24^{d}	64±1.98^{c}
HGB(g/Dl)	10.75±8.13^{c}	12.05±1.63^{b}	11.75±2.05^{bc}	11.2±6.22^{bc}	16.05±1.91^{a}
MCH(pg)	63.75±9.40^{a}	58.75±0.49^{b}	55.3±1.41^{b}	59.35±4.88^{b}	57.8±1.41^{b}
MCHC(g/dL)	41.1±8.77^{a}	36.35±5.73^{b}	37.2±7.49^{b}	35.65±4.88^{b}	40.45±0.21^{a}
PLT(10^4/µL)	30.5±4.95^{a}	19±0.00^{b}	16±5.65^{c}	17±2.83^{bc}	13.5±4.95^{d}
WBC(10^2/µL)	5.15±6.57^{d}	7.4±4.81^{bc}	6.15±3.46^{c}	9.45±7.71^{b}	11.25±4.59^{a}

Values on the same row with different superscripts showed a significant difference ($p<0.05$) from each other. Note: WBC-white blood cell (10^2/µL); HGB- Hemoglobin(g/Dl); RBC-red blood cell(10^4/µL); HTC-haematocrit(%), MCV-mean corpuscular volume(fL); MCH-mean corpuscular haemoglobin(pg); MCHC-mean corpuscular haemoglobin concentration(g/dL); RDW-CV -red blood cell distribution width (statistically expressed as coefficient of variation); RDW-SD -red blood cell distribution width (statistically expressed as standard deviation; MPV – mean platelet volume; PDW -platelet distribution width; PCT -platelet haematocrit(10^4/µL)

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Genotoxicity analysis

Genotoxicity parameters of *C. gariepinus* fed graded levels of *P. conophora* are presented in Table 7. Fish fed 75% (WS100) and 100 %(T5) recorded the highest induction of micronucleus (MN) which showed a significant increase (P<0.05) compared with those of the control, 25% and those of 50% extracts. However, those of control treatment recorded the lowest induction of micronucleus. No significant difference (P>0.05) was recorded in the lobes among all the treatment groups including the control. There were significantly more binucleated cells in fish fed 75% walnut cell compared to those fed 25% walnut cell. No significant difference (P>0.05) was recorded in the number of lobes in 50%, control, and 100% concentration of the extract.

Table 7. Genotoxicity parameter of micronucleus (MN) on *C. gariepinus* fed graded levels of *P. conophora* shell

Treatment	**Replications**		
	1	2	3
WS0(CONTROL)	1501±346.5^{a}	1543.3±669.16^{a}	1940.33±103.0^{ab}
WS25 (25%)	2034.67±110.89^{a}	1927.67±159.06^{ab}	2280.33±199.32^{b}
WS50 (50%)	2768..67±573.81^{a}	2073.67±118.15^{ab}	2858.67±308.18^{c}
WS75 (75%)	2355±848.06^{a}	2614.67±548.10^{b}	3057±312.33^{c}
WS100 (100%)	2519.33±1133.85^{a}	1838.67±124.20^{ab}	1755.33±236.86^{a}

Values on the same column with different superscripts showed a significant difference (p<0.05) from each other. WS0= Control, WS25-WS100=Treatment

DISCUSSION

In aquaculture operations, a better understanding of growth could result in significant benefits in terms of profitability productivity, and sustainability which provides greater understanding, translated into relevant and simple applications. The result of this study indicates that the juveniles of *C. gariepinus* fed the graded levels of *P. conophora* inclusion in the feeds responded well to the diets in terms of growth and feed utilization as there was no significant difference in all growth parameters measured across the experimental diets. This result is in agreement to Sotolu (2010) in *C. gariepinus* and Aguilar-Manjarrez *et al.* (2017) in Nile tilapia *Oreochromis niloticus* containing groundnut oil was comparable in weight gain to those fed palm oil diet and palm oil could replace Soya bean oil in the same vein without negatively affecting fish growth and body composition.

Haematological parameters act as a frontline sensitive indicator of vital physiological and homeostatic functions as well as the status of nutrition, health, diseases and stress responses of the organism subjected to changes in environmental conditions (Hrubec *et al.*, 2000). According to

Ayoola and Aina, 2017), haematocrit (the packed cell volume or PCV), is a measure of the total volume of the erythrocytes relative to the total volume of whole blood in a sample. Haemoglobin (Hb) is a sophisticated oxygen delivery system that provides the desired amount of oxygen to the tissues under a wide variety of circumstances. The determination of haemoglobin can be a good indicator of the oxygen-carrying capacity of the blood.

The results of the present study demonstrated that there were no significant differences in the values of haematological parameters. Therefore, it showed that the fish were not stressed by increasing the concentrations of the *P. conophora* in the diet, as evident by no significant changes observed in haematocrit values of the treated fish. This also indicates that the fish fed with different concentrations of extract did not suffer from any form of anaemia, malnutrition, disease, and stress. This is evident in the insignificant changes observed in the growth utilization parameters. These observations are in agreement with the results of other researchers such as Daisley, (1973) that included Origanum vulgare extract in the diet of rainbow trout. The haematology results of the present study are also similar to the work of Ajayeoba and Fadare, (2006), who revealed that no significant changes in haematology of African catfish after feeding with T. conophorum supplemented diets. And the values of haematocrit obtained in the result also indicate that the oxygen-carrying capacity of the red blood cells did not alter by the inclusion of different concentrations of the extract.

The relative higher value obtained haematocrit and haemoglobin in fish fed 100% extract of T. *conophorum* shell is due to the potency of the ingredients in the feed to facilitate or increase blood production. Also, the antimicrobial activity of *T. conophorum* (Ajayeoba Fadare, 2006) could have helped the fish to better utilize their feed, thereby facilitating higher nutrient absorption that led to higher haemoglobin (Hb) and percentage haematocrit (HCT). The present results are nearly similar to those reported by (Askar, 2008) who reported that Hb and PCV values significantly increased due to the effect of dietary Cr-Pic supplementation. Also, relatively higher WBC and Lymphocytes counts in fish fed 100% of *T. conophorum*. This suggests that a higher concentration of T. conophorum up to 100% can improve the immune system of *C. gariepinus*; since WBCs and Lymphocytes are involved in the development of immunity against pathogens. This result agrees with the report of Ahmed and Amal (2014), on the haematological responses of Nile tilapia (*Oreochromis niloticus*) by dietary organic chromium (chromium picolinate) supplementation. The relative increase in lymphocytes count at 100% concentration could be a result of many factors which include acute inflammation, stress response effect from the extract, among others. Since no disease condition was observed in the treated fish, the increase in lymphocyte count at 100% administration could be due to a burden on the immune system of the treated fish, which at 100% concentration level no longer tolerate the active ingredients in *T. conophorum*.

The clinical significance of lipid profile is primarily associated with their contribution to coronary heart disease (CHD) and various lipoprotein disorders (Burtis *et al.*, 2010). The results of the present study suggest that the feeding regimes did not influence the lipid profile of the fish studied. No significant differences observed in the cholesterol and triglycerides and the results of carcass suggest that the inclusion of *P. conophora* extract did not alter total cholesterol levels and may inhibit the lipid peroxidation, thereby improve antioxidant status. This agrees with the reports of Imam *et al.* (2010), who used tomato paste to improve stress-induced lipid peroxidation in the

liver of atrazine- exposed to *C. gariepinus*. Similar results were recorded by Mekkawy *et al.* (2011) in concern with cadmium-exposed *O. niloticus*. This also aligns with the findings of Burtis *et al.* (2010), who observed a dose-dependent increase in the chelating properties of the aqueous fraction of walnut in vitro.

The results of the present study reveal that the addition of walnut shells may be genotoxic and mutagenic at higher concentrations and may probably make it unsafe for further consumption. The significant induction of micronucleus (MN) in the erythrocytes of an exposed fish group of 75% and 100% could be as a result of performance fluctuation which is observed when dietary protein or energy increased from its initial levels (Ayoola and Abdul (2016). In the present study, fish containing 75% and 100% inclusion showed higher micronucleus (2858.67±308.18 and 3057±312.33) respectively, which indicated that both are genotoxic in a dose-dependent manner. The walnut shell up to 75-100% were likely to be lethal to the fish. However, biochemical and haematology parameters of studied fish reflect no genotoxicity. Furthermore, previous findings reported antigenotoxicity, antioxidant effects of *P. conophora.* (Ayoola and Bamiro, 2017).

CONCLUSIONS

The results of the present findings on the biochemical, haematology, and carcass compositions of *C. gariepinus* fed 25% to 50% *P. conophora* revealed no serious impending dangers and side effects of the walnut shell inclusion even up to 100% concentration level, suggesting its safety to fish, consumer of fish and fishery products.

ETHICAL CONSIDERATIONS

This research was performed according to the convention of animal rights (approved by the Faculty of Science Research and Ethics Committee, Faculty of Science, University of Lagos). We tried to use fish without causing them unnecessary suffering if it could be avoided.

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