

Research Article

Effects of African Walnut (*Tetracarpidium conophorum*) Leaf Powder on Growth Performance and Histopathology of African Catfish (*Clarias gariepius*)

Sodomola MO*, Adejola YA and Folarin MF

Federal College of Agriculture PMB 5029, Moor Plantation Apata Ibadan, Nigeria

*Corresponding author: Dr. Sodomola MO, Federal College of Agriculture PMB 5029, Moor Plantation Apata Ibadan, Nigeria

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Abstract

The growing concerns of consumers on the use of antibiotics a growth promoter in fish feed have fueled the interest in alternative products. An eight week study was carried out to evaluate the effects of African walnut (*Tetracarpidium conophorum*) leaf powder on the growth performance and histopathology of African catfish (*Clarias gariepinus*). Three hundred and twenty (320) juvenile catfish were individually weighed and randomly distributed into four dietary treatments; each treatment had four replicates each with a total of twenty per replicate on the basis of their weight. Four experiment diets were formulated with the inclusion of African walnut (*Tetracarpidium conophorum*) leaf powder (TCLP). The diets were made as followings: (T1) basal diets, (T2) T1+10 g TCLPKg/diet; (T3) T1+20 g TCLE (T4) T1+30 Gtclp. Data were collected on growth performance and subjected to one way of analysis of variance in a Completely Randomized Design. Histopathological examinations were carried out on the liver of the catfish. The result of the growth performance showed that the highest mean value weight gain was observed in T3 (3.47 g), followed by T4 (2.83 g) T2 (2.25 g) and lowest weight gain was in T1 (1.83 g). Feed intake was significantly ($p>0.05$) reduced catfish fed with T1 and T2 with the value (5.17 g) and (5.57 g) respectively. Feed intake of T3 catfish however were higher, although they achieved the best feed conversion ratio (1.74) as compared to (2.88) and (2.74) observed for T1 and T2 respectively. Result of the histopathology showed that there was presence of diffuse vacuolar degeneration of hepatocysts in fish fed varying levels of African walnut (*Tetracarpidium conophorum*) leaf powder. It can be concluded that dietary supplementation of African walnut (*Tetracarpidium conophorum*) leaf powder improved the growth performance of African catfish (*C. gariepius*).

Keywords: Catfish, African walnut, Juvnile, Feedintake and histopathology

Introduction

Aquaculture has grown by 6.9% per annum since 1970 [1] and now provides half of global fish supply [2]. As global demand continues to grow, there are opportunities for aquaculture to expand sustainably [3]. In Nigeria, it remains the only option that may ensure the maintenance of the current levels of per capita supply of fish of especially with the declining situation of capture fisheries [4].

The African catfish (*Clarias gariepinus*) is the major species of catfish cultured in Nigeria due to its high growth rate, good flesh quality, tolerance to poor water, ability to withstand high stocking densities and good taste [5].

Aquaculture as an emerging industrial sector requires continued research with scientific, technical developments and innovations [6,7] in different aspects of production including the search for natural alternative growth promoters to be used as feed production including the search for natural alternative growth promoters to be used as feed supplements. To develop alternative practices for growth promotion and disease management in aquaculture, attention has been focused in identifying novel drugs, especially from plant sources. These drugs may be delivered to the cultivable organisms through feed

supplementation [8]. Several herbs have been confirmed as growth promoters in aquatic animals [9].

African walnut (*Tetracarpidium conophorum*), like many plants in Africa and other parts of the world has been proven to have nutritive, medicinal, agricultural and industrial values over the years. Phytochemical analysis of Africa walnut indicates that it contains bioactive compounds such as oxalates, phytates, tannins, saponins and alkaloids which partly show the use of the seeds, leaves and roots in herbal medicine [10]. The presence of tannin supports its anti-inflammatory property [11]. As a rich source of alkaloids, coupled with the presence of the essential vitamins and minerals, *T. conophorum* can be seen as a potential source of useful food and drugs. Its seed is rich in fat, nearly eighty per-cent of unpolysaturated fat with proven cholesterol lowering walnut extracts properties. [12] reported that walnut extracts t which is rich in dietary omega -3-fatty acids play a role in the prevention of some disorders including depression as well as dementia. Studies have shown that *T. conophorum* possess some properties that are required for wound healing like antibacterial and antioxidant activities [13] and immune stimulating activities [14]. Extracts of *T. conophorum* leaves have been shown to possess good antibacterial activities especially against Gram positive organisms.

Based on the aforementioned bioactive compounds and properties of (*T. conophorum*) plant, there is need to know whether (*T. conophorum*) leaf could be used as feed additives for catfish for producing safe and cost effective fish.

Materials and Methods

The experiment was carried out at the fisheries unit, federal college of agriculture moor plantation, Ibadan, Nigeria. It is located on longitude 03/051E, Latitude 07/023N and altitude 650” lies in the humid zone of the rain forest belt 07/0 3.25 of south western Nigeria with mean annual rainfall of 122 mm and mean temperature of 20°C.

Sourcing and Processing of Test Ingredients

Tetracarpidium conophorum leaves were obtained from a farm in Ondo, Akure. They were washed with clean water and allowed to air dry under shade until they were crispy to touch, while retaining the greenish coloration. The leaves were then milled using a hammer mill into powder and stored in the dark in airtight plastic bags at ambient temperature. The proximate analysis of the test ingredient was analyzed in the laboratory according to [15].

Management of the Experiment

Three hundred and twenty (320) juvenile African catfish were purchased at a reputable farm, they were randomly assigned to four treatments of 80 juveniles per treatment with four replicates of twenty juvenile catfish each and were fed with commercial feed for two weeks for acclimatization before the commencement of the experiment. During the experiment fish were fed with 2 mm of formulated fed with inclusion of African walnut (*Tetracarpidium conophorum*) leaf powder at 4% of their body weight. Plastics were used for rearing the experimental fish. Water in the plastic was changed biweekly to avoid the building up of nitrates and nitrites as influence leaching was not possible due to the use of plastic materials, and also to prevent predators from entering the plastic tanks.

Experimental Diet

Four experimental diets were formulated with the inclusion of *Tetracarpidium conophorum* leaf Powder (TCLP) (Table 1).

The diets were made as followings:

T1: Control/basal diet

T2: Basal diet + 10 g TCLP Kg/diet

T3: Basal diet + 20 g TCLP Kg/diet

T4: Basal diet + 30 Kg TCLE Kg/diet.

Data Collection

Growth Performance Evaluation

Records of live weight, fed intake, weight gain and mortality and feed conversion ratio (FCR) were determined.

Weight Gain

The fish were weighed at the commencement of the experiment and subsequently on weekly basis

Table 1: Gross composition (g/100 g dry matter) of the experiment diet.

Ingredients	Quantity (kg)
Maize	20
SBM	30
F/M	25
GNC	20
DCP	2
Starch	2
Salt	1
Total	100
Calculated Analysis	
Metabolizable energy (kcal/kg)	2,750
Crude protein (%)	39.79
Moisture content (%)	9.88
Ether extract (%)	2.81
Ash content (%)	8.96
Dry matter (%)	90.13
NFE (%)	36.38

Key: FM: Fish Meal; SBM: Soya Beans Meal; GNC: Groundnut Cake; NFE: Nitrogen Free Extract.

Feed Conversion Ratio (FCR)

The FCR of each of the group of fish was determine by calculating the ratio of feed intake to weight gain and thus calculated as:

Feed conversion ratio (FCR) = Total feed intake (g)/Total body weight gain (g)

Histopathology

The histopathology examinations were carried out on the liver of the fish at the department of veterinary pathology, university of Ibadan Nigeria. The organs were carefully removed from the body of the fish so as to avoid damage and were preserved in 10% formalin solution. The fixed tissues were processed routinely for histological analysis as described by [16]. The necrotized areas were then photographed and read accordingly to determine the histopathological effects of *Tetracarpidium conophorum* leaves.

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) using generalize model of SAS Moonscape programmed version significant differences among mean for treatment were portioned by Duncan multiple range test at 5% level of probability (Tables 2-3).

Table 2: Proximate composition of African walnut (*Tetracarpidium conophorum*) leaf.

Proximate composition	Values (%)
Moisture content	26.46
Ash content	9.66
Crude protein	15.71
Crude fibre	13.63
Dry matter	73.8
Total carbohydrate	29.58

Table 3: Phytochemical Analysis of (*Tetracarpidium conophorum*) leaf.

Content	Leaf
Tannin	0.56
Saponin	1.03
Flavonoid	0
Soluble carbohydrate	1.07
Reducing sugar	1.74

Table 4: Effects of *Tetracarpidium conophorum* leaf on performance on experimental catfish.

Parameters	T1	T2	T3	T4	SEM=+-
IW(g)	6.15	6.15	6.15	5.15	0
FW(g)	8.00b	8.42b	9.72b	8.97b	0.22
WG(g)	1.85b	2.27b	3.57b	2.83b	0.22
FI(g)	5.17b	5.57b	6.04b	5.20b	0.1
FCR	2.88	2.74	1.74	2.3	0.2
Sur rate	63	68	77	74	2.44

ab Means on the same row having different superscript were significantly different ($p < 0.05$).

T1: Control 0g *Tetracarpidium conophorum*.

T2: 10 g *T. conophorum*.

T3: 20 g *T. conophorum*.

T4: 30 g *T. conophorum*.

IW: Initial Weight.

FW: Final Weight.

WG: Weight Gain.

FI: Feed Intake.

FCR: Feed Conversion Ratio.

SUR Rate: survival Rate.

Result and Discussion

Table 4 showed effects of *Tetracarpidium conophorum* leaves on growth performance of experimental fish. There were no significant different in final weight, weight gain and feed intake but there was significant different in initial weight and survival rates. The highest mean value weight gain was observed in T3 (3.47 g), followed by T4 (2.83 g), T2 (2.25 g) and lowest weight gain was in T1 (1.83 g). Feed intake was significant ($p < 0.05$) decreased catfish on T1 and T2 respectively (5.17 and 5.57 g) compared to T3 (6.04 g). T3 however achieved the best feed conversion (FCR) (1.74 g) followed by T4 (2.30 g) and T2 (2.88 g).

In the final weight gain, T3 and T4 had the highest weight gain of 9.72 g followed by (8.97 g) and T2 and T1 WITH (8.42 g) and (8.00 g) respectively. There was significant difference ($p < 0.005$) in the survival rate where T3 (77.00) had the highest followed by T4 (74.00%), T2 (68.00%) and T1 (63.00%) respectively.

Liver Histopathology of the Experimental Catfish

Plate 1: reveals that there was a mild portal congestion in the liver, with mild periportal vacuolar degeneration of hepatocytes. Plates 2 shows that there was a moderate to severe vacuolation of the hepatocytes, but the nuclei were still centralized. Plates 4 reveals moderate diffuse vacuolation of hepatocytes, with mild portal congestion. Plates 3 shows there was a very severe diffuse vacuolation of hepatocytes, most nuclei were marginated (Figures 1-4).

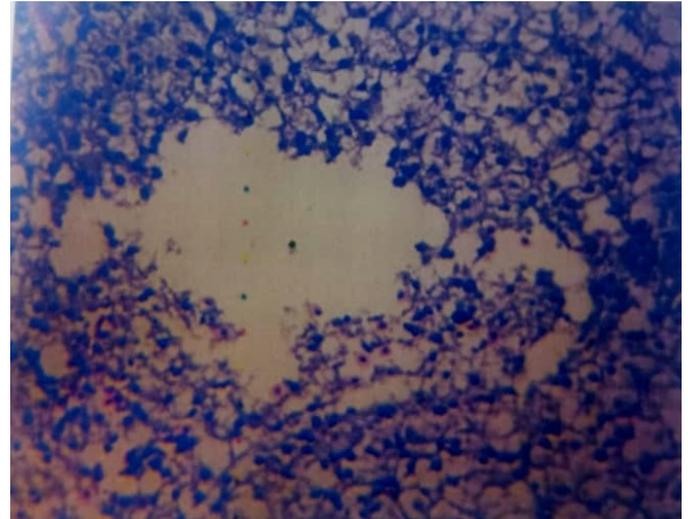


Figure 1: Plates 1: Histological section of liver of fish fed with control (H&E 40X).

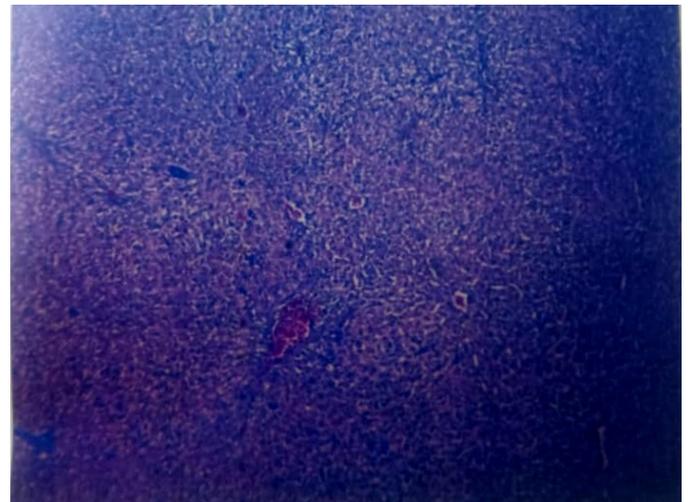


Figure 2: PLATES 2: Histological section of liver of fish fed 10 g/kg of *Tetracarpidium conophorum* leaf powder (H&E 40X)

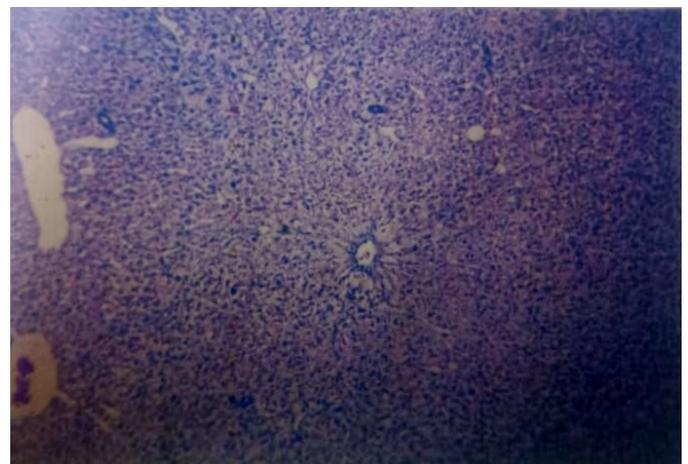


Figure 3: PLATE 3: Histological section of liver of fish fed 20 g/kg of *Tetracarpidium conophorum* (H&EX40).

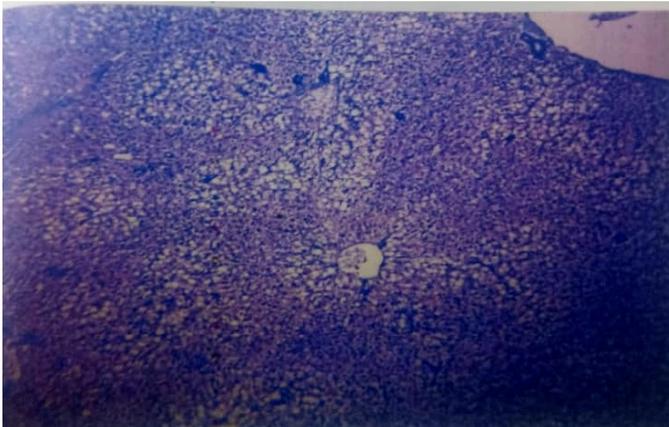


Figure 4: PLATE 4: Histological section of liver of fish fed 30 g/kg of *Tetracarpidium conophorum* leaf powder (H&E 40X).

Discussion

The higher values obtained in the treatments fed *T. conophorum* leaf powder (TCLP) could be due to the presence of growth stimulants or constituents in walnut leave (alkaloids and tannins). These properties could contribute to improving the digestion and nutrient absorption with a subsequent increase in the fish – weight. Walnut leaf can be used as a growth promoter and it is known to have the ability to improve the absorptive capacity of the intestine via structural alteration [17]. This results agrees with those obtained by [18] who obtained highest growth performance in *Oreochromis niloticus* with 30 g garlic/kg diet, [19] also obtained the highest growth performance in *O. niloticus* with 2.5% garlic/kg diet.

The increased feed intake observed in this experiment in fish fed 20 g/kg of TCLP could be attributed to change in feed taste and stimulated appetite [20]. This result is in agreement with [21] who reported increased feed intake in the supplemented groups which were treated by 2% aloe vera gel dissolved in water. FCR was best with 20 g/kg TCLP inclusion and the result revealed that diet was better utilized by the *C. gariepinus* juveniles. This result is in agreement with those obtained by [18] who recorded increase in FCR and FER on *O. niloticus* with 30 g garlic/kg diet compared to the control which had the least value and [22] who found that the dietary of Biogen (R) increased feed intake, FCR, PER and body composition (crude protein, ether extract, ash and moisture) in fish. The increased feed intake observed in the experiment in diet supplemented groups could be attributed to change in feed taste and stimulated appetite [20]. This result is in agreement with [21] who reported increased feed intake in the supplemented groups which were treated by 2% aloe vera gel dissolved in water.

The presence of diffuse vacuolar degeneration of hepatocytes in fish fed varying levels of *Tetracarpidium conophorum* leaves and may be as a result of excessive work required by the fish's liver to get rid of the plant toxicant from its body during the process of detoxification. This is corroborated by the work of [23] who revealed similar effect on the fish liver.

The present study showed that supplementation of *T. conophorum*

leaf powder improved the growth performance of African catfish (*C. gariepius*), due to the growth promoting and immune – stimulation properties.

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