

# Effects of Tiny Moss *Bryumcapillare* meal on Growth Parameter, Haematology, Histology and Carcass Quality of *Clariasgariepinus* (Burchell) Juveniles

## Abstract

This research determines the effects of Tiny Moss *Bryumcapillare* meal, on growth parameter, Haematology, Histology and Carcass Quality of *Clariasgariepinus* (Burchell) Juveniles. The study was conducted for fifty six ( $n=56$ ) days, under a completely randomized design. The final body weight and the daily weight gain increase as the Tiny Moss (*Bryumcapillare*) inclusion increased among the individual treatment. The results of the feed conversion ratio were significantly ( $p < 0.05$ ) different among the groups, such that  $T_1$  and  $T_2$  had the best FCR followed by  $T_5$  and  $T_6$  which had similar values but the  $T_4$  had the least value. The amino acid profile showed that the Glutamic Acid, Aspartic Acid, Valine, Threonine, Serine, Phenylalanine, Proline and Methionine increased in value while Lysine, Leucine, Arginine, Alanine, Isoleucine, Glycine, Histidine and Tryptophan reduced in value and there was no significant change in cysteine (%) all were significantly at ( $P < 0.05$ ). The haematological parameters of the catfish (*Clariasgariepinus*) juveniles fed graded levels of diets containing Tiny Moss (*Bryumcapillare*) were not significantly different ( $P > 0.05$ ). The histological analysis of tiny Moss (*Bryumcapillare*) feed shows normal skin architecture with well outlined epithelia cell (EC) moderate effect on the skin layer with moderate necrosis (N) of the muscular region with the epithelia lining and superficial spreading of melanoma (M) restricted to the epidermis. The heart shows normal cardiac tissue with cardiac cell (CC), cardiac fiber (CF) cardiac muscles (CM) shows moderate aggregate of myocardial inflammation (AMI). The gill showed section of gill with ghost (G) appearance with severe aggregate of inflammatory cell (AIC). The liver cells revealed severe effect on the hepatic tissue with severe intra hepatic inflammation (IHI) and intra hepatic hemorrhage (IHIH). The damage done to these organs as the result of the feeds correlates with the concentrations of the feeds in each experimental tank.

**Key Words:** Tiny Moss meal (*Bryumcapillare*), Haematology, Histology, Carcass Quality, *Clariasgariepinus*

## Introduction

Mosses belong to the simplest land/water plants. At the same time, they belong to the second largest taxonomic group in the plant kingdom: bryophytes Asakawa 2013. There are around 25,000 bryophyte species, which can be found in most ecosystems worldwide and include mosses (*Musci* ~ 8000 species), liverworts (*Hepaticae* ~ 6000 species) and hornworts (*Anthocerotae* ~ 1000 species) Klavina (2015). The major structural components of mosses are carbohydrates (Maksimova *et al.*, 2013; Klavina 2015), and they also contain secondary metabolites with possibly high biological activity Asakawa 2013. *B. capillare* is a very common moss that grows in tufts or patches, with stems mostly 1–3 cm tall. Dry plants

usually have corkscrew-like shoots, with leaves spirally twisted around the stem. However, in some populations the dry shoots have leaves that are straight or only slightly twisted. The broad leaves are 2–5 mm long, and widest at or above the halfway point. The margins are narrowly recurved and have a well-defined border of narrow cells. The nerve extends into a fine, pale green hair point, which can be short or quite long. *B. capillare* is dioicous. The large (3.5–5 mm long), cylindrical, drooping capsules ripen in spring and summer, and are borne on a reddish seta up to 3 cm tall.

The use of plants by people has a history as old as the existence of humanity. From ancient times to the present, humanity has sought solutions to diseases by making use of plants in nature. Tiny Moss (*Bryumcapillare*) have been used in many areas since ancient times, thanks to their different properties. They antimicrobial activities against bacteria and their use in the treatment of various diseases, such as acne, hemorrhoids, and skin diseases, the active substance called Sphagnol, which is found in the Sphagnum genus moss, is used. (Altuner, 2008). Non-antimicrobial activities of mosses are classified according to their different properties in various fields such as horticulture and agriculture, animal husbandry, fuel, food, construction industry, cosmetics industry, household, clothing making, and ecological uses of moss. (Kendir and Güvenç, 2010). The nutritional value of Tiny Moss (*Bryumcapillare*) is well recognized, having relatively high protein, amino acid and fatty acid contents and high fiber, and so Tiny Moss (*Bryumcapillare*) can be used as dietary supplements for fish and other animals. Specifically, lysine and methionine contents are higher in Tiny Moss compared to other plants and, therefore, more suitable as ingredients for animal feed (Goswami *et al.*, 2022). To date, the composition of mosses has not been much studied from the perspective of their application potential, but mainly has been concentrated on the investigation of specific substance groups, such as fatty acids, lipids, essential oils (Cansuet *et al.*, 2013; Pejín *et al.*, 2013). Another reason to study the composition of bryophytes is related to the need to understand their metabolism.

The most commonly practiced feed supplementation is the dispensation of ground feedstuff such as cereals bran and domestic left-over/kitchen waste to feed fish. Though these are known to enhance growth they may not be complete or balanced. Fishes fed on incomplete feeds will suffer deficiency diseases or symptoms attributable to lack of ingredients. (Fagbenro *et al.*, 2010) observed that balanced/complete diets are formulated by combination of different essential nutrients in different proportions (Protein, Carbohydrate, lipids, ~~Vitamins~~ vitamins, ~~Minerals~~ minerals). The increased use of alternate sources of plant products for fishmeal in fish feeds, information on mineral requirements and bioavailability of essential nutrients is necessary to improve performance and health of fish, to minimize the discharge of excessive nutrients excreted into natural waters, and to prevent mineral deficiencies in farmed fish. Further research on a better estimate of feedstuff's amino acid bioavailability, as well as maintenance requirement and protein accretion of fish, will result in more efficient use of alternate protein sources of protein in fish feeds and lower nitrogenous waste from commercial aquaculture. A significant achievement has been on the effective use of alternate lipids particularly vegetable oils, animal fats DHA-rich marine microalgae and omega-3 long-chain PUFA canola oil to replace fish oil in fish feeds and sustain optimum growth, feed utilization and product quality. However, questions related to the possibility of achieving an optimal balance between different PUFA in terms of the ratio of n-3/n-6 fatty acids and EPA/DHA for optimal health as well as the successful reproductive performance of fish and proper levels of PUFA (particularly EPA/DHA and EPA/ARA ratio) needed for better survival of larval marine fish, must be addressed.

Catfish culture has overtime become the desire of most fish farmers due to its continuous increasing demand (Ganesh *et al.*, 2021). As the world's campaign for the consumption of

less fatty food continues to intensify, people consider fish and its products as a reliable and affordable option for required protein (FAO 2021; Hanchionet *et al.*, 2017). Food is a major requirement for all living organisms including fish for growth, reproduction and body maintenance (WHO 2021). In fish culture systems, the importance of feed cannot be over emphasized, since feed is the most expensive input in terms of cost in fish production. Nutritional requirement of fish is necessary in order to formulate an economical and nutritionally balanced diet for the fish (Solomon *et al.* 2012). To sustain fish under culture, supplementary diet must be provided to complement natural feeds supply (Karapan and Agbotidis, 2002). Feed stuffs used in aquaculture to provide basic nutrients such as protein, carbohydrate, minerals, water, vitamins and lipids are expensive because of their competitive uses by ~~man~~ humans and other animals (Dunham *et al.*, 2001). Mosses have been neglected as a study subject for a long time. Recent research shows that mosses contain remarkable and unique substances with high biological activity. Several studies have investigated the use of Tiny Moss (*Bryumcapillare*) as an ingredient in feeds for carp species, such as *L. minor* (Yilmazet *al.*, 2004) or *L. minuta* in feeds for common carp *Cyprinus carpio*. Furthermore, *L. minor* has been supplemented in diets of *rohulabeorohita* (Goswami *et al.*, 2020), grass carp *Ctenopharyngodonidella* and silver carp *Hypophthalmichthysmolitrix* (Aslamet *al.*, 2021), but there is paucity of information on the use of Tiny Moss (*Bryumcapillare*) as an ingredient in feeds for Catfish *Clarias gariepinus*. The aim of the study is to determine the effects of Tiny Moss meal *Bryumcapillare* on Growth Parameter, Haematology, Histology and Carcass Quality of *Clarias gariepinus* (Burchell) Juveniles that is lacking.

## Materials and Methods

### Experimental Site

The research was conducted in the Fisheries Research Unit of Ebonyi State University, Abakaliki, in the Department of Fisheries and Aquaculture Research and Teaching Farm. The area is located in the South East of Nigeria and has a prevailing tropical climate with mean annual rainfall of about 1500mm. The average ranges of ambient temperature of 24°C to 38°C with a yearly average of 34°C. The average relative humidity ranges from 60 to 94 percent with a year average of about 83 percent. Abakaliki lies between latitude 5° and 6° N and between longitudes 7° and 8° E at an elevation of 59m above sea level within the South Eastern Agricultural Zone of Nigeria (GPS, 2018).

### Experimental fish

One hundred and eighty ( $n=180$ ) catfish (*Clarias gariepinus*) juveniles with average initial body weight of Mean  $\pm$  SD (g) and STD Length Mean  $\pm$  SD (cm) was obtained from Amazons Farms hatcheries, Uguachara and was divided into six treatments. Each treatment contained 30 fish. Each of the treatment group was further subdivided into three replicates of 10 fish per replicate. All the fish for the study was homogenous in body weights and apparently healthy and was acclimated to farm conditions for 1 week prior to the commencement of the experiment.

### Collection and Preparation of *Bryumcapillare*

**Comment [W1]:** Mention how much level of moss was added in t1 to T6 replacing soybean meal and also mention in the abstract also

*Bryumcapillare* was harvested in AmaogwugwuNdukwe in Amasiri, Afikpo North Local Government Area of Ebonyi state. Fresh *Bryumcapillare* was harvested from pond within AmaogwugwuNdukwe in Amasiri, Afikpo North Local Government Area of Ebonyi state with the help of hand net, scoop net as well as sieve and then transported in bags to the house where it was dried under room temperature. This was grinded using hammer grinding machine into powdered form and used whenever required for fish feed preparation.

### Preparation of Experimental Diets

The dietary ingredients for the experiment include *Bryumcapillare* meal. Other ingredients that will contain in the feed are fishmeal, soybean meal, maize, vitamin Premix, mineral premix, salt and vitamin E (antioxidant). Six different diets were compounded for experiment one each containing varying levels of the experimental diet except the control. The gross composition of the experimental diets is shown in Table 1. All the diets contain the different proportion of test ingredients. The diets were all isonitrogenous (40% CP). In preparing the diet the two protein sources were included in the ratio of 2:1. Dry ingredients were ground to a powdery form to aid assimilation by fish using a gasoline driven grinding machine in Abakaliki. The diets were thoroughly mixed with each experimental diet, Vitamin E antioxidant was added to the feed at 400 ppm i.e. 400 mg/kg. The dough was pelleted using a locally fabricated pelleting machine with a 2.0 mm die. Diets were immediately sun dried and later broken mechanically into small sizes.

**Comment [W2]:** Also mention proximate composition of *Bryumcapillare*

**Table 1: Percentage Compositions of the Moss (*Bryumcapillare*) in the diets (%)**

Ingredients%	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
Fish meal	42.00	43.00	44.00	45.00	46.00	48.00
SBM	29.00	28.00	27.00	26.00	25.00	24.00
Moss	-	2.00	3.00	4.00	5.00	6.00
Maize	25.00	23.00	22.00	21.00	20.00	18.00
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5	0.5	0.5
Fish Premix	0.5	0.5	0.5	0.5	0.5	0.5
Bone Meal	0.5	0.5	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Oil (Palm oil)	0.5	0.5	0.5	0.5	0.5	0.5
Starch	0.5	0.5	0.5	0.5	0.5	0.5
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>

\*Fish Premix (vital fish) manufactured by Animal Care Service Consult (Nig) Ltd. Lagos, Supplied the following per kg of premix: Vitamin A, 5000,00 IU; Vitamin D3 800,000 IU; Vitamin E, 12,000 mg; Vitamin K, 1,5000 mg; Vitamin B1, 1,000 mg; Vitamin B2, 2,000 mg; Vitamin B6, 1,500 mg; Niacin, 12,000 mg; pantothenic acid, 20.00 mg; Biotin, 10.00 mg; Vitamin B12, 300.00 mg; folic acid, 150,000 mg; choline, 60,000 mg; manganese, 10,000 mg; iron, 15,000 mg; zinc 800.00 mg; Copper 400.00 mg; Iodine 80.00 mg; cobalt 40 mg; selenium 8,00 mg. BBRCM= Bovine blood-rumen content mixture.

### Diet Performance Evaluation

Growth performance and nutrient utilization of fish was determined following the methods of Jimoh, and Aroyehun, (2011) in term of Final Individual Weight, Survival (%), Specific Growth Rate (SGR %/ day), Feed Conversion Ratio, (FCR) and Protein Efficiency Ratio (PER), Net Protein Utilisation (NPU) responses was calculated as

$$\text{Weight Gain (\%)} = \frac{\text{Final body weight} - \text{Initial body Weight}}{\text{Initial Weight}} \times \frac{100}{1}$$

$$\text{SGR (\%/day)} = \frac{\ln (\text{Final body weight}) - \ln (\text{Initial body weight})}{\text{Time (in days)}}$$

$$\text{Feed Conversion Ratio, (FCR)} = \frac{\text{Dry weight of feed fed}}{\text{Feed weight Gain}}$$

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Fish weight of gain}}{\text{Protein Fed}}$$

$$\text{Protein Utilisation (NPU)} = \frac{\text{Net Protein Carcass}}{\text{Protein Fed}}$$

### Haematological Analysis

Blood (1-2ml) was collected from the vertebral caudal blood vessel according to Schmitet *al.*, (1999), using disposable 2ml syringe and needle. The blood was emptied into the heparinized blood bottle treated with Ethyl DiamineTetracetic Acid (EDTA). A blood sample was centrifuge (1500 rpm for 7mins) to obtain the blood plasma. Plasma samples was stored at (-20°C) for the electrochemical and biochemical analysis. Computerized method employing System KX-2IN™ Automated Hematology Analyzer was used in blood analysis, the KX-2IN is an ideal hematology analyzer for a clinical satellite laboratory or research testing. Spectrophotometric method was used for biochemical analysis as described by Svobodova *et al.*, (2003). While the plasma electrolytes was determined using corning 400 flame photometer. Other metals was determined using (a back) Model 200A flame of the Atomic Absorption Spectrophotometer (AAS).

### Histological Examination of Test Organ

At the end of the experiment, one fish per treatment, that is, three fish per concentration were sampled after 96hours of exposure for histological analysis, the test organism was killed with a blow on the head, using a mallet and was dissected to remove the vital organs (gill, liver, kidney and skin). The organs were fixed in 10% formalin for three days after which the tissue was dehydrated in periodic acid Schiff's reagent (PAS) following the method of Hughes and Perry, (1976), in graded levels of 50%, 70%, 90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding. The organs were embedded in malted wax. The tissue was sectioned into thin sections (5-7µm), by means of a rotatory microtome and was dehydrated and stained with Harris haematoxylin-eosin (H&E) stain, Bancroft & Cook, (1994), using a microtome and each section were cleared by placing in warm water (38°C), where it was picked with clean slide and oven-dried at 58°C for 30 minutes to melt the wax. The slide containing sectioned materials/tissue were cleared using xylene and graded levels of 50%, 70%, 90%, 95% and 100% alcohol for two minutes each.

The section was stained in haematoxyline eosin for ten minutes. The stained slide was observed under a light microscope at varying X100 magnification, sections were examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus C35 AD4), a camera (Olympus C40 AB-4) and an automatic light exposure unit (Olympus PM CS5P).

#### Biochemical Composition (Proximate) Analysis

The biochemical composition of the carcass of the experimental fish was run to determine the Crude Protein (CP), crude Lipid (CL), Crude Fiber (CF), Moisture (M), Ash and Nitrogen Free Extract (NFE), using standard methods (AOAC, 1990). Nitrogen was determined by the micro-kjedahl method (Pearson, 1976) and the crude protein was taken as  $N\% \times 6.25$  (constant factor) where N is equal to Nitrogen content per 100g sample. Total carbohydrate was determined using the phenol-sulphuric acid method. The crude fibre was obtained by dry ashing of the sample at 550°C dissolved in 10% HCl (25ml) and 5% Lanthanum Chloride (2ml) boiled, filtered and made up to standard volume with distilled water.

#### Water quality Analysis

The water quality parameters were recorded for temperature, dissolved oxygen (Do) content, pH and conductivity before and after the experiment. pH was determined using a digital pH meter (Mettler Toledo 320). DO and conductivity were measured using a digital dissolved oxygen meter (oxygen analyzer model JPB-607 portable) once in a day at 8.00am. The water quality was determined using the method of American Public Health Association (APHA, 2000) Model Number, E-9909 (pH, TDS, Salinity, EC, Temp.)

#### Method of Statistical Analysis

The data was collated and analysed using descriptive statistics, one way analysis of variance and Pearson's correlation. The differences in the means between both values was assessed with Duncan multiple range test Using SPSS version 21 at  $P < 0.05$  significant level.

#### Results

The result of final total length of the *Clarias gariepinus* fed diets containing Tiny Moss (*Bryum capillare*) presented in Table 2, showed that there were significant ( $p < 0.05$ ) differences in the final total length during the period of the study. Though, the highest final total length was recorded among the  $T_5, T_3$  and  $T_1$  respectively followed by  $T_6$  while  $T_4$  and  $T_3$  had similar records which were the least among the groups.

Table 2: Length and Weight relationship of Catfish *Clarias Gariepinus* Juveniles Used for the Experiments (Mean±SD).

Treatment	Control	1	2	3	4	5
Length (cm)	21.72±3.0 <sup>a</sup>	20.90±2.8 <sup>a</sup>	17.82±7.9 <sup>a</sup>	21.47±4.3 <sup>a</sup>	16.24±6.6 <sup>a</sup>	21.27±3.5 <sup>a</sup>
Weight (g)	71.25±8.3 <sup>b</sup>	65.18±6.1 <sup>ab</sup>	66.02±8.1 <sup>ab</sup>	60.83±5.4 <sup>a</sup>	64.73±9.4 <sup>ab</sup>	63.38±6.3 <sup>ab</sup>

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Comment [W3]: Mention group name correctly.. T1 to T6? Or T5?

Means with the same different superscripts in the same column are not differ significantly different at  $P>0.05$ , while those with different superscripts in the same column are significantly different at same level.

#### Proximate analysis of the Tiny Moss (*Bryumcapillare*) in the diets

The result of the proximate composition of the diets containing Tiny Moss (*Bryumcapillare*) used for feeding the *Clariasgariepinus* in the experiment is presented in table 3

**Table 3: Proximate analysis of the Tiny Moss (*Bryumcapillare*) in the diets (%)**

Sample	%CP	%C Fat	%C Fibre	% Ash	%M	% NFE	%DM
T <sub>1</sub>	38.69±2.8 <sup>a</sup>	5.46±3.5 <sup>a</sup>	2.29±2.8 <sup>a</sup>	7.88±2.8 <sup>a</sup>	9.13±2.8 <sup>a</sup>	36.55±2.3 <sup>b</sup>	90.87±2.8 <sup>a</sup>
T <sub>2</sub>	36.88±3.5 <sup>a</sup>	4.29±8.3 <sup>b</sup>	2.37±6.3 <sup>ab</sup>	8.15±8.3 <sup>b</sup>	10.04±3.0 <sup>a</sup>	38.27±1.3 <sup>b</sup>	89.96±8.3 <sup>b</sup>
T <sub>3</sub>	37.09±3.0 <sup>a</sup>	4.75±8.3 <sup>b</sup>	2.33±4.3 <sup>a</sup>	7.49±2.8 <sup>a</sup>	9.32±6.1 <sup>ab</sup>	39.02±4.0 <sup>b</sup>	90.68±8.3 <sup>b</sup>
T <sub>4</sub>	39.57±3.5 <sup>a</sup>	5.69±3.3 <sup>b</sup>	2.18±6.3 <sup>ab</sup>	7.21±4.3 <sup>a</sup>	8.56±2.8 <sup>a</sup>	36.79±4.1 <sup>a</sup>	91.44±6.1 <sup>ab</sup>
T <sub>5</sub>	38.89±6.1 <sup>ab</sup>	5.58±8.3 <sup>b</sup>	2.24±3.0 <sup>a</sup>	7.34±4.3 <sup>a</sup>	8.69±6.1 <sup>ab</sup>	37.26±3.3 <sup>b</sup>	91.31±2.8 <sup>a</sup>
T <sub>6</sub>	39.68±2.8 <sup>a</sup>	5.75±3.5 <sup>a</sup>	2.15±3.5 <sup>a</sup>	7.16±2.8 <sup>a</sup>	8.48±3.5 <sup>a</sup>	36.78±1.1 <sup>b</sup>	91.52±3.0 <sup>a</sup>

%NFE = 100 - (%CP + %CFAT + %CFIBRE + %ASH + %M); %DM = 100 - %M

Means with the same superscripts in the same column are not significantly different at  $P>0.05$ , while those with different superscripts in the same column are significantly different at same level.

The result of proximate analysis of the fish diet containing Tiny Moss (*Bryumcapillare*) in the diets as presented in the Table 3 showed varying levels of nutrients in diets containing Tiny Moss (*Bryumcapillare*) for the experiment. The T<sub>6</sub> diet had the highest percentage level of crude protein followed by T<sub>4</sub> then T<sub>5</sub> and T<sub>1</sub>, while T<sub>3</sub> and T<sub>2</sub> had the least crude protein. The crude fat was also highest in T<sub>6</sub> followed by T<sub>4</sub>, T<sub>5</sub>, T<sub>1</sub>, T<sub>3</sub> and T<sub>2</sub> respectively. The crude fibre showed close similarities in the values such that they followed this trend; T<sub>2</sub>, T<sub>3</sub>, T<sub>1</sub>, T<sub>5</sub>, T<sub>4</sub> and T<sub>6</sub> respectively. The ash content, Moisture content Nitrogen Free Extract and Dry matter content equally showed little variations among the individual groups

#### Performance of *Clariasgariepinus* Fed diets containing Tiny Moss (*Bryumcapillare*)

The result of the productive performance of the *Clariasgariepinus* fed the diets containing Tiny Moss (*Bryumcapillare*) used for feeding the fish in the experiment is shown in table 4.

**Table 4: Performance of *Clariasgariepinus* Fed diets containing Tiny Moss (*Bryumcapillare*)**

#### Performance of *Clariasgariepinus* Fed diets containing Tiny Moss

Parameters	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	± SEM
Av. Initial Body Weight(g)/fish	22.4	23.3	22.3	24.7	23.7	24.9	0.29



Av. Final Body weight (g)/fish	961	806	634	414 <sup>d</sup>	560	577	1.59
Av. Daily Feed Intake (g)/fish	1.76	1.632	2.048	1.754	1.671	1.674	1.23
Av. Daily weight gain (g)/fish	16.76	13.98	10.92	6.95 <sup>d</sup>	9.58	9.8	1.07
Feed conversion Ratio	1.8	2.0	3.2	4.2 <sup>c</sup>	3.0	2.9	0.84
Av. Initial Total Length (cm)	30.3	30.7	30.0	31.4	32.2	31.7	1.01
Av. Final Total Length (cm)	55.5	47.1	55.6	47.1	55.9	49.7	0.42
Av. Survival rate (%)	75	80	60 <sup>bc</sup>	50	50	60	2.01

The Total Dissolved Solid (TDS) of the pond containing graded levels of Tiny Moss (*Bryumcapillare*) meal showed close relationship in body weights of the fish varying levels of Tiny Moss (*Bryumcapillare*) at the start of the experiment. The Total Dissolved Solid (TDS) significantly increase ( $P < 0.05$ ) at  $T_4$  as the Tiny Moss (*Bryumcapillare*) inclusion increased up to 4% among the individual treatment, followed by  $T_1$ ,  $T_3$ ,  $T_5$  and  $T_6$  that had similar values whereas  $T_2$  had the least Total Dissolved Solid (TDS) which the mean differences were statistically significant ( $p < 0.05$ ) among the individual treatments.

The productive performance of the *Clarias gariepinus* fed diets containing Tiny Moss (*Bryumcapillare*) as indicated in the Table 4, showed close relationship in body weights of the fish varying levels of Tiny Moss (*Bryumcapillare*) at the start of the experiment. The final body weight and the daily weight gain decreased as the Tiny Moss (*Bryumcapillare*) inclusion increased among the individual treatment, such that the  $T_1$  had highest final body weight and daily weight gain among those in control group ( $T_1$ ) followed by  $T_2$  then  $T_3$ , but  $T_5$  and  $T_6$  had similar values whereas  $T_4$  had the least weight gain, which the mean differences were statistically significant ( $p < 0.05$ ) among the individual treatments. There were no significant ( $p > 0.05$ ) differences in the daily feed intake and the total feed intake during the period of the study. Though, the highest feed intake was recorded among the  $T_3$  while others had similar records of feed intake among the groups. The results of the feed conversion ratio were significantly ( $p < 0.05$ ) different among the groups, such that  $T_1$  and  $T_2$  had the best FCR followed by  $T_5$  and  $T_6$  which had similar values but the  $T_4$  had the least value. There were significant ( $p < 0.05$ ) differences in the survival rate during the period of the study such that  $T_2$  recorded the highest survival rate, followed by  $T_3$  and  $T_6$  while  $T_4$  and  $T_5$  had similar records and the least survival rate among the groups.

#### Water Quality of the pond containing *Clarias gariepinus* fed diet with Tiny Moss (*Bryumcapillare*)

The result of the water quality of the pond containing *clarias gariepinus* fed diet with Tiny Moss (*Bryumcapillare*) in the experiment is presented in table 5.

**Table 5: Water Quality of the pond containing *Clarias gariepinus* fed diet with Tiny Moss (*Bryumcapillare*)**

Parameter	$T_1$	$T_2$	$T_3$	$T_4$	$T_5$	$T_6$	$\pm$ SEM
pH	8.18	8.20	8.23	8.23	8.23	8.23	0.12
TDS	70.8	64.7	71.1	86.5	71.5	71.7	1.04



Cond.	141.9	129.3	141.3	173.1	141.0	142.7	0.30
Sal.	7.13	7.48	7.26	7.55	7.09	7.14	0.19
O°C	28.9	28.9	29.3	29.6	29.3	29.3	1.00
NH <sub>3</sub>	0.0	0.05	0.10	0.20	0.10	0.10	0.20
NO <sub>3</sub>	0.05	0.05	0.30	0.50	0.35	0.3	0.21
NO <sub>2</sub>	0.03	0.03	0.05	0.07	0.05	0.05	0.01
DO	5.50	5.50	4.85	4.50	5.00	5.00	0.41

Means with the same superscripts in the same column are not significantly different at  $P>0.05$ , while those with different superscripts in the same column are significantly different at same level.

The result of the average water quality of the pond containing *Clarias gariepinus* fed diet with Tiny Moss (*B. capillare*) in the experiment as presented in table 5, indicated that there were similarities in the pH level of the water such that the T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> had the same marginally higher pH followed by T<sub>2</sub> whereas T<sub>1</sub> had the least pH, which the mean differences were statistically not significant ( $p>0.05$ ) among the individual treatments. The Total Dissolved Solid (TDS) of the pond containing graded levels of Tiny Moss (*Bryumcapillare*) meal as indicated in the Table 5, showed close relationship in body weights of the fish varying levels of Tiny Moss (*B. capillare*) at the start of the experiment. The Total Dissolved Solid (TDS) significantly increase ( $P<0.05$ ) at T<sub>4</sub> as the Tiny Moss (*B. capillare*) inclusion increased up to 4% among the individual treatment, followed by T<sub>1</sub>, T<sub>3</sub>, T<sub>5</sub> and T<sub>6</sub> that had similar values whereas T<sub>2</sub> had the least Total Dissolved Solid (TDS) which the mean differences were statistically significant ( $p<0.05$ ) among the individual treatments.

The result of the water conductivity of the pond containing *Clarias gariepinus* fed diet with Tiny Moss (*Bcapillare*) in the experiment as presented in table 5, indicated that there significant differences ( $P<0.05$ ) among the group such that T<sub>4</sub> (4% inclusion of Tiny Moss (*Bcapillare*) meal) had the highest level of water conductivity followed by T<sub>1</sub>, T<sub>3</sub>, T<sub>5</sub> and T<sub>6</sub> that had similar conductivity while T<sub>2</sub> (2% inclusion of Tiny Moss (*Bcapillare*) meal) had the least. The average water salinity of the pond containing graded levels of Tiny Moss (*Bcapillare*) meal as presented in the Table 5 were similar in their individual salinity of the water at varying levels of Tiny Moss (*Bcapillare*) during experiment. The average salinity is not significantly different ( $P>0.05$ ) among the individual groups as the Tiny Moss (*Bcapillare*) inclusion increased. Though there were marginal increases such that T<sub>4</sub> had increased salinity followed by T<sub>2</sub>, then T<sub>3</sub> and T<sub>6</sub> whereas T<sub>5</sub> had the least value which the mean differences were statistically not significant ( $p>0.05$ ) among the groups.

The result of the average ammonia content of the water in the pond containing *Clarias gariepinus* fed diet with Tiny Moss (*Bcapillare*) meal in the experiment as presented in table 5. This indicated that there were significant differences ( $P<0.05$ ) in the ammonia content of the water such that the T<sub>4</sub> had the highest ammonia concentration followed by T<sub>3</sub>, T<sub>5</sub> and T<sub>6</sub> which had the same ammonia concentration, while T<sub>1</sub> and T<sub>2</sub> had ammonia concentration. The average Temperature (O°C), Nitrite (NO<sub>3</sub>), Nitrate (NO<sub>2</sub>), and Dissolved oxygen (DO) of the pond containing graded levels of Tiny Moss (*Bryumcapillare*) meal as presented in the Table 5 were similar in their individual water at varying levels of Tiny Moss (*Bryumcapillare*) inclusion during experiment there results indicated no significant differences among the groups.

#### Haematological parameters of Catfish Juveniles fed Tiny Moss (*Bryumcapillare*) meal

The result of the average haematological parameters of catfish (*Clarias gariepinus*) juveniles fed diets containing Tiny Moss (*Bryum capillare*) is presented in table 6

**Table 6 Effect of Tiny Moss (*Bryum capillare*) on haematological parameters of Catfish *Clarias gariepinus* Juveniles (Mean±SD)**

Trt	White blood cell (ul)	Red blood cell (ul)	Haemoglobin (g/dl)	PCV	Platelet (*10 <sup>3</sup> )(ul)	Mean cell volume (*10 <sup>3</sup> fl)	Mean cell Haemoglobin (pg)	Mean cell Haemoglobin concentration.
1. Control	1.30x10 <sup>3</sup> ±82.3 <sup>a</sup>	5.71x10 <sup>6</sup> ±9.4 <sup>a</sup>	9.62±2.9 <sup>a</sup>	15.92±5.2 <sup>a</sup>	9.87x10 <sup>3</sup> ±75.5 <sup>a</sup>	1.68x10 <sup>2</sup> ±53.8 <sup>a</sup>	38.97±4.6 <sup>a</sup>	37.83±3.5 <sup>b</sup>
2	1.80x10 <sup>3</sup> ±766.7 <sup>a</sup>	5.48x10 <sup>6</sup> ±8.6 <sup>a</sup>	10.62±3.1 <sup>a</sup>	14.02±6.1 <sup>a</sup>	1.9x10 <sup>3</sup> ±96.7 <sup>a</sup>	1.95x10 <sup>2</sup> ±39.7 <sup>a</sup>	42.31±11.6 <sup>a</sup>	33.47±6.1 <sup>ab</sup>
3	1.30x10 <sup>3</sup> ±88.9 <sup>a</sup>	1.89x10 <sup>6</sup> ±1.2 <sup>a</sup>	9.62±2.7 <sup>a</sup>	15.40±5.6 <sup>a</sup>	1.52x10 <sup>4</sup> ±10.3 <sup>a</sup>	1.64x10 <sup>2</sup> ±51.5 <sup>a</sup>	46.90±10.8 <sup>a</sup>	42.41±5.8 <sup>ab</sup>
4	1.67x10 <sup>3</sup> ±434.6 <sup>a</sup>	1.17x10 <sup>6</sup> ±1.4 <sup>a</sup>	10.68±2.6 <sup>a</sup>	15.06±5.4 <sup>a</sup>	1.89x10 <sup>4</sup> ±11.7 <sup>a</sup>	1.40x10 <sup>2</sup> ±46.5 <sup>a</sup>	44.70±7.0 <sup>a</sup>	35.03±7.0 <sup>a</sup>
5	1.30x10 <sup>3</sup> ±62.6 <sup>a</sup>	5.56x10 <sup>6</sup> ±9.6 <sup>a</sup>	10.03±2.0 <sup>a</sup>	12.68±2.8 <sup>a</sup>	1.29x10 <sup>4</sup> ±10.0 <sup>a</sup>	1.47x10 <sup>2</sup> ±48.5 <sup>a</sup>	44.53±12.6 <sup>a</sup>	39.85±7.3 <sup>ab</sup>
6	1.81x10 <sup>3</sup> ±756.1 <sup>a</sup>	5.26x10 <sup>6</sup> ±8.7 <sup>a</sup>	10.90±2.3 <sup>a</sup>	12.65±3.3 <sup>a</sup>	1.09x10 <sup>4</sup> ±33.3 <sup>a</sup>	1.63x10 <sup>2</sup> ±59.0 <sup>a</sup>	43.18±10.4 <sup>a</sup>	35.80±4.6 <sup>ab</sup>

Means with the same superscripts in the same column are not significantly different at P>0.05, while those with different superscripts in the same column are significantly different at same level.

The result of average haematological parameters of catfish (*Clarias gariepinus*) juveniles fed diet containing Tiny Moss (*Bryum capillare*) is presented in the Table 6. The study showed that the haematological parameters of the catfish (*Clarias gariepinus*) juveniles fed graded levels of diets containing Tiny Moss (*Bryum capillare*) were not significantly different (P>0.05). Though there were marginal variations among the individual group, there were still a lot of similarities among the values in each of the groups.

#### Biochemical Characteristics of Catfish Juveniles fed Tiny Moss (*Bryum capillare*) meal

The result of the average biochemical characteristics of Catfish (*Clarias gariepinus*) juveniles fed diets containing Tiny Moss (*Bryum capillare*) is presented in table 7.

**Table 7: Effect of Tiny Moss (*Bryum capillare*) on Biochemical Characteristics of Catfish *Clarias gariepinus* Juveniles (Mean±SD)**

Trt	Protein	Ether Extract	Ash	NFE	Energy	Glucose
1 Control	21.26±2.1 <sup>b</sup>	2.82±0.5 <sup>b</sup>	0.69±0.2	0.51±0.1	1.25x10 <sup>2</sup> ±17.7	6.95±1.3
2	22.56±8.6 <sup>a</sup>	3.14±0.6 <sup>a</sup>	0.63±0.3	0.46±0.1	1.31x10 <sup>2</sup> ±6.3	7.17±1.5
3	19.94±3.5 <sup>c</sup>	2.82±0.6 <sup>b</sup>	0.66±0.2	0.55±0.2	1.32x10 <sup>2</sup> ±8.6	6.82±1.0
4	23.70±3.7 <sup>a</sup>	2.99±0.5 <sup>a</sup>	0.62±0.3	0.42±0.1	1.33x10 <sup>2</sup> ±5.8	6.78±1.0
5	19.22±2.4 <sup>c</sup>	2.60±0.6 <sup>c</sup>	0.72±0.2	0.49±0.2	1.31x10 <sup>2</sup> ±18.3	6.23±1.1
6	22.09±8.6 <sup>a</sup>	2.99±0.8 <sup>a</sup>	0.65±0.3	0.36±0.1	1.33x10 <sup>2</sup> ±6.8	6.32±1.5

Means with the same superscripts in the same column are not significantly different at P>0.05, while those with different superscripts in the same column are significantly different at same level.

The result of average biochemical characteristics of catfish *Clarias gariepinus* juveniles fed diet containing Tiny Moss (*Bryum capillare*) is presented in the Table 7. The study showed

that the protein content of the catfish (*Clarias gariepinus*) juveniles fed graded levels of diets containing Tiny Moss (*Bryumcapillare*) were significantly different ( $P<0.05$ ). The catfish fed  $T_4$  diet had the highest percentage level protein followed by  $T_2$ , and  $T_6$  then  $T_1$ , while  $T_3$  and  $T_5$  had the least protein. The average ether extract was also highest in  $T_2$  which were similar to those in  $T_4$ , and  $T_6$ , but were significantly different ( $P<0.05$ ) to  $T_1$  and  $T_3$  which had the same protein level, whereas  $T_5$  had the least. The ash content, Nitrogen Free Extract, energy and glucose showed close similarities in the values such that they showed marginal variations which were not significantly different ( $P>0.05$ ) among the individual groups.

**Table 8:** Effect Tiny Moss (*Bryumcapillare*) on Electrochemical Characteristics of Catfish *Clarias Gariepinus* Juveniles (Mean $\pm$ SD).

Treatment	Control	1	2	3	4	5
Sodium	76.07 $\pm$ 12.1 <sup>a</sup>	76.96 $\pm$ 14.9 <sup>a</sup>	79.37 $\pm$ 10.6 <sup>a</sup>	64.43 $\pm$ 22.6 <sup>a</sup>	72.18 $\pm$ 5.8 <sup>a</sup>	69.12 $\pm$ 21.1 <sup>a</sup>
Potassium	53.19 $\pm$ 23.4 <sup>a</sup>	39.21 $\pm$ 9.5 <sup>a</sup>	39.47 $\pm$ 19.3 <sup>a</sup>	40.67 $\pm$ 5.6 <sup>a</sup>	41.99 $\pm$ 24.4 <sup>a</sup>	40.46 $\pm$ 9.7 <sup>a</sup>

Means with the same superscripts in the same column are not significantly different at  $P>0.05$ , while those with different superscripts in the same column are significantly different at same level.

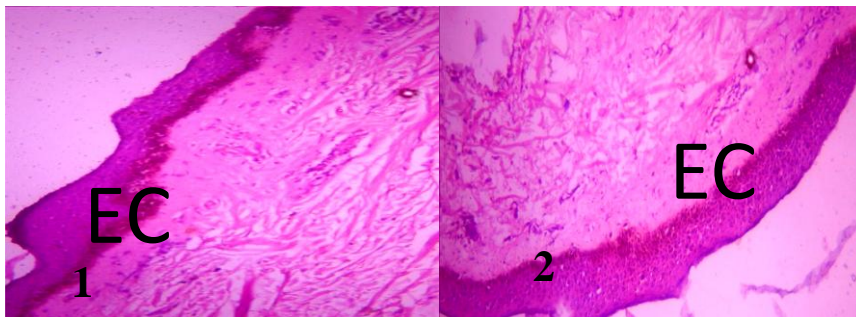
The result of the effect of Tiny Moss (*Bryumcapillare*) on Amino Acid Profile of Catfish *Clarias gariepinus* Juveniles fed diet containing Tiny Moss (*Bryumcapillare*) is presented in the Table 9. The study showed that the Glutamic Acid, Aspartic Acid, Valine, Threonine, Serine, Phenylalanine, Proline and Methionine increased in value while Lysine, Leucine, Arginine, Alanine, Isoleucine, Glycine, Histidine and Tryptophan reduced in value and there was no significant change in cysteine (%) all were significantly at ( $P<0.05$ ).

**Table 9: Effect of Tiny Moss (*Bryumcapillare*) on Amino Acid Profile of Catfish *ClariasGariepinus* Juveniles (Mean±SD).**

Trt	Glutamic Acid (%)	Aspartic Acid (%)	Lysine (%)	Leucine (%)	Arginine (%)	Alanine (%)	Valine (%)	Isoleucine (%)	Glycine (%)	Threonine (%)	Serine (%)	Phenylalanine (%)	Proline (%)	Methionine (%)	Histidine (%)	Cysteine (%)	Tryptophan (%)
Control	1.86±0.0 <sup>a</sup>	8.68 ± 0.0 <sup>a</sup>	3.88 ± 0.0 <sup>a</sup>	7.50 ± 0.0 <sup>b</sup>	5.90 ± 0.0 <sup>ab</sup>	3.49 ± 0.0 <sup>a</sup>	3.87 ± 0.0 <sup>a</sup>	4.69 ± 0.0 <sup>b</sup>	5.78 ± .0 <sup>a</sup>	2.66 ± 0.0 <sup>a</sup>	3.88 ± 0.0 <sup>a</sup>	3.79 ± 0.0 <sup>a</sup>	2.70 ± 0.0 <sup>a</sup>	3.36±0.0 <sup>a</sup>	0.08±0.0 <sup>b</sup>	0.93±0.1 <sup>a</sup>	0.22±0.0 <sup>a</sup>
1	2.34±1.4 <sup>ab</sup>	8.75 ± 0.1 <sup>ab</sup>	3.30 ± 1.5 <sup>ab</sup>	6.51 ± 2.5 <sup>ab</sup>	5.91 ± 0.0 <sup>b</sup>	3.54 ± 0.2 <sup>ab</sup>	4.02 ± 0.3 <sup>ab</sup>	4.38 ± 0.8 <sup>ab</sup>	3.64 ± 0.0 <sup>ab</sup>	3.36 ± 1.7 <sup>ab</sup>	3.94 ± 0.2 <sup>ab</sup>	5.02 ± 3.0 <sup>ab</sup>	2.90 ± 0.4 <sup>ab</sup>	3.56±0.5 <sup>ab</sup>	0.06±0.0 <sup>a</sup> <sub>b</sub>	0.95±0.0 <sup>a</sup> <sub>b</sub>	0.23±0.0 <sup>a</sup> <sub>b</sub>
2	3.36±1.7 <sup>ab</sup>	8.82 ± 0.1 <sup>ab</sup>	2.06 ± 2.0 <sup>ab</sup>	4.40 ± 3.3 <sup>ab</sup>	5.90 ± 0.0 <sup>ab</sup>	3.71 ± 0.2 <sup>ab</sup>	4.26 ± 0.4 <sup>ab</sup>	3.72 ± 1.1 <sup>ab</sup>	3.82 ± 0.3 <sup>ab</sup>	4.73 ± 2.2 <sup>ab</sup>	4.13 ± 0.3 <sup>ab</sup>	7.44 ± 4.0 <sup>ab</sup>	3.25 ± 0.6 <sup>ab</sup>	4.01±0.7 <sup>ab</sup>	0.05±0.0 <sup>a</sup> <sub>b</sub>	0.93±0.0 <sup>a</sup> <sub>b</sub>	0.20±0.0 <sup>a</sup> <sub>b</sub>
3	2.85±1.6 <sup>ab</sup>	8.77 ± 0.1 <sup>ab</sup>	2.67 ± 1.9 <sup>ab</sup>	5.48 ± 3.1 <sup>ab</sup>	5.90 ± 0.0 <sup>b</sup>	3.64 ± 0.2 <sup>ab</sup>	4.13 ± 0.4 <sup>ab</sup>	4.04 ± 1.0 <sup>ab</sup>	3.75 ± 0.3 <sup>ab</sup>	4.03 ± 2.1 <sup>ab</sup>	4.05 ± 0.3 <sup>ab</sup>	6.22 ± 3.8 <sup>ab</sup>	3.06 ± 0.6 <sup>ab</sup>	3.80±0.7 <sup>ab</sup>	0.06±0.0 <sup>a</sup> <sub>b</sub>	0.93±0.0 <sup>a</sup> <sub>b</sub>	0.20±0.0 <sup>a</sup> <sub>b</sub>
4	2.43±1.2 <sup>ab</sup>	8.74 ± 0.1 <sup>ab</sup>	3.29±1.9 <sup>ab</sup>	6.50 ± 2.5 <sup>ab</sup>	5.90 ± 0.0 <sup>ab</sup>	3.55 ± 0.2 <sup>ab</sup>	4.01 ± 0.3	4.38 ± 0.8 <sup>ab</sup>	3.65±0.2 <sup>ab</sup>	3.36 ± 1.7 <sup>ab</sup>	3.95 ± 0.2 <sup>ab</sup>	5.02 ± 3.0 <sup>ab</sup>	2.89 ± 0.4 <sup>ab</sup>	3.57±0.5 <sup>ab</sup>	0.04±0.4 <sup>a</sup> <sub>b</sub>	0.94±0.0 <sup>a</sup> <sub>b</sub>	0.22±0.0 <sup>a</sup> <sub>b</sub>
5	3.86±1.6 <sup>b</sup>	8.81 ± 0.1 <sup>a</sup>	1.46 ± 1.9 <sup>b</sup>	3.41 ± 3.1 <sup>a</sup>	5.89 ± 0.1 <sup>b</sup>	3.79 ± 0.2 <sup>b</sup>	4.39 ± 0.4 <sup>b</sup>	3.39 ± 1.0 <sup>a</sup>	3.92 ± 0.3 <sup>b</sup>	5.41 ± 2.1 <sup>a</sup>	4.22 ± 0.3 <sup>b</sup>	8.65 ± 3.8 <sup>b</sup>	3.43 ± 0.6 <sup>b</sup>	4.24±0.7 <sup>b</sup>	0.06±0.0 <sup>a</sup>	0.93±0.0 <sup>b</sup>	0.18±0.0 <sup>b</sup>

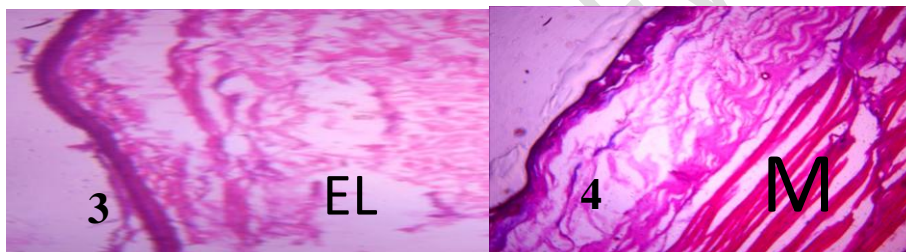
Means with the same superscripts in the same column are not significantly different at P>0.05, while those with different superscripts in the same column are significantly different at same level.

**Plate A: (Fig 1-6) Histological change observed in the skin of Juvenile Catfish *Clarias gariepinus* fed with different level Tiny Moss (*Bryum capillare*) in the diets.**



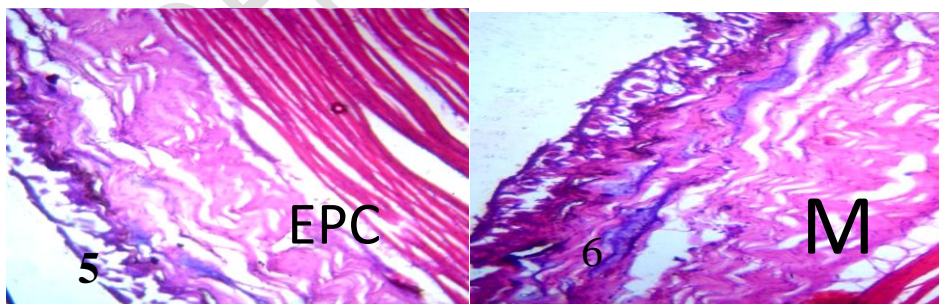
Photomicrograph of Group T0 control section of Skin (x400)(H/E) shows normal skin architecture with well outlined epithelia cell (EC)

Photomicrograph of Group T1 control section of Skin (x400)(H/E) shows normal skin architecture with well outlined epithelia cell (EC) .



Photomicrograph of Group T2 section of Skin (x400)(H/E) shows skin layer with moderate distortion of the dermal area and non-distinct appearance of the epithelia lining (EL)

Photomicrograph of T3 section of skin (X100)(H/E) shows moderate effect on the skin layer with moderate necrotic (N) epithelia lining and superficial spreading of melanoma (M) restricted to the epidermis. The overall features are consistent with (MODERATE MELANOMA)

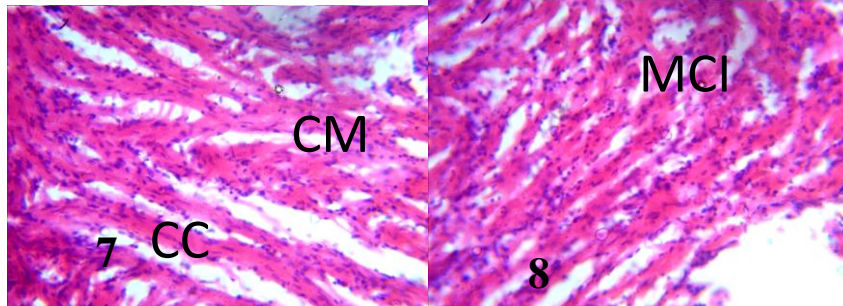


Photomicrograph of T4 section of skin (X100)(H/E) shows moderate effect on the skin layer with severe necrotic (N) epithelia lining and epithelia cell (EPC)

Photomicrograph of T5 section of skin (X100)(H/E) shows moderate effect on the skin layer with moderate necrosis (N) of the muscular region with the epithelia lining and superficial spreading of melanoma (M) restricted to the epidermis in r2

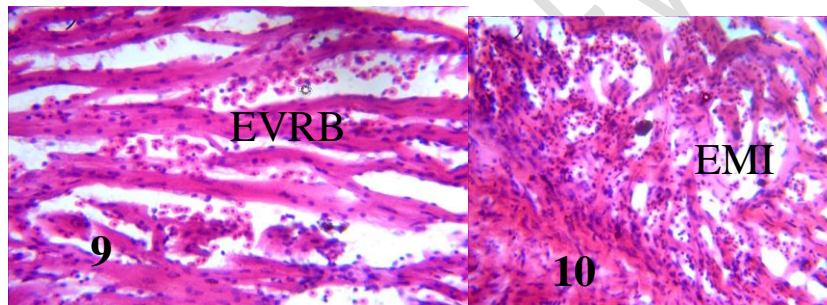


**Plate B: (Fig 7-12) Histological change observed in the heart of Juvenile Catfish *Clarias gariepinus* fed with different level Tiny Moss (*Bryum capillare*) in the diets.**



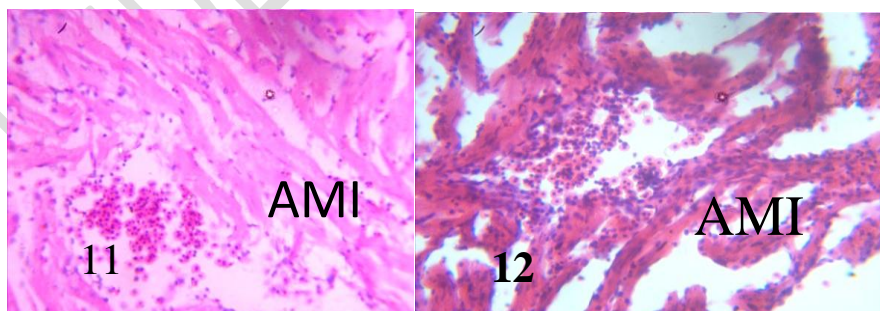
Photomicrograph of group AT1T2 heart section normal control (X400)(H/E) shows normal cardiac tissue with cardiac cell (CC), cardiac fiber (CF) cardiac muscles (CM)

Photomicrograph B1B2 of heart section (X400)(H/E) administered wit shows moderate attrophy (A) of myocadiac tissue with mild intra myocadiac inflammation (MCI) .



Photomicrograph C1C2 of heart section (X400)(H/E) administered wit shows moderate attrophy (A) of myocadiac tissue with moderate extravassated red blood cell (EVRB)

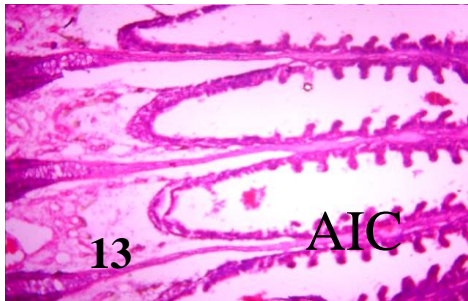
Photomicrograph D1D2 of heart section (X400)(H/E) administered wit shows mild aggregate of myocardial inflammation (AMI)



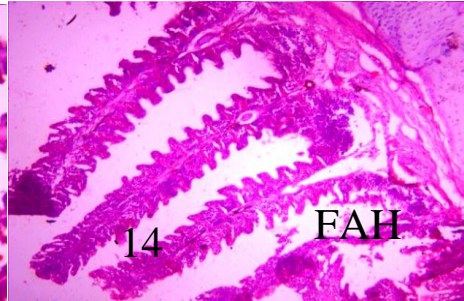
Photomicrograph E1E2 of heart section (X400)(H/E) administered wit shows mild aggregate of myocardial inflammation (AMI)

Photomicrograph F1F2 of heart section (X400)(H/E) administered wit shows moderate aggregate of myocardial inflammation (AMI)

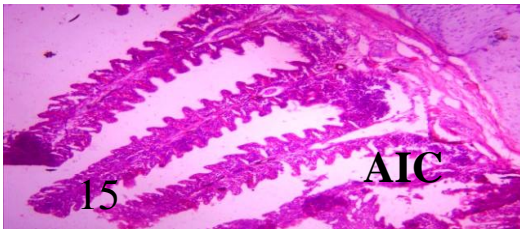
**Plate C: (Fig 13-18) Histological change observed in the heart of Juvenile Catfish *Clarias gariepinus* fed with different level Tiny Moss (*Bryum capillare*) in the diets.**



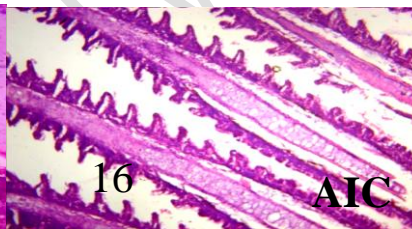
Photomicrograph of T0 section of gill (X150)(H/E) shows section of gill with ghost (G) appearance of the with severe aggregate of inflammatory cell (AIC)



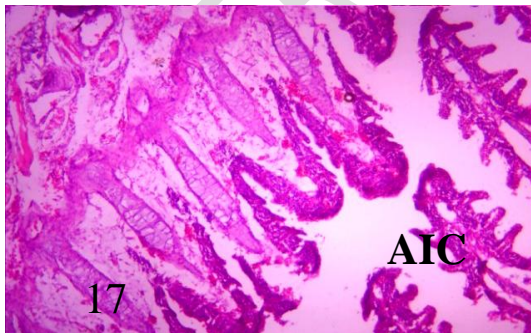
Photomicrograph of T1 section of gill (X150)(H/E) shows section of gill with moderate necrotic filaments (NF) with mild focal area of hemorrhage (FAH)



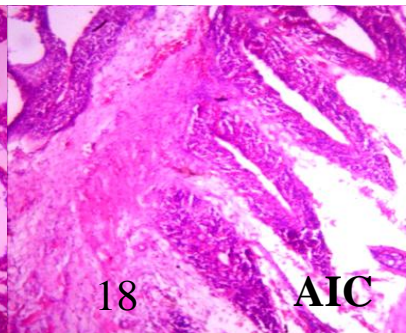
Photomicrograph of T2 section of gill (X150)(H/E) shows section of gill with severe necrotic filaments (NF) with severe aggregate of inflammatory cell (AIC)



Photomicrograph of T3 section of gill (X150)(H/E) shows section of gill with severe necrotic filaments (NF) with severe aggregate of inflammatory cell (AIC)



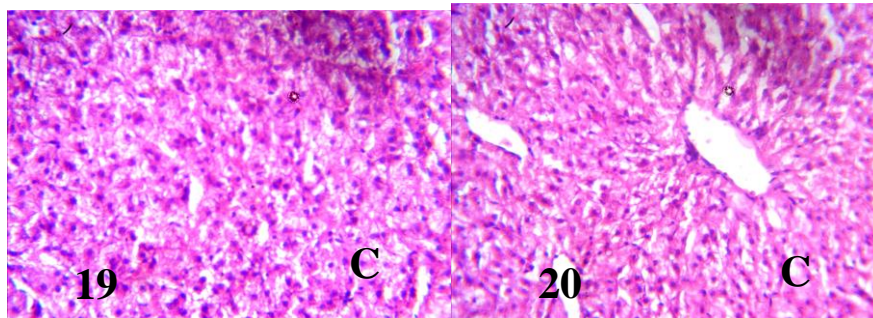
Photomicrograph of T4 section of gill (X150)(H/E) shows section of gill with severe necrotic filaments (NF) with severe aggregate of inflammatory cell (AIC)



Photomicrograph of T5 section of gill (X150)(H/E) shows section of gill with ghost (G) appearance of the with severe aggregate of inflammatory cell (AIC)

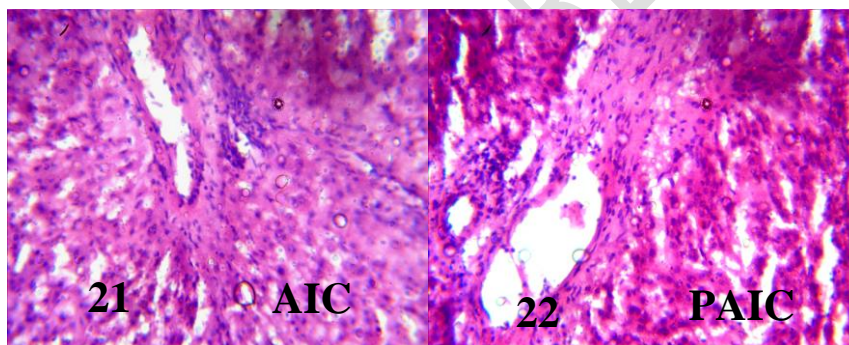


**Plate D: (Fig 19-24) Histological change observed in the liver of Juvenile Catfish *Clarias gariepinus* fed with different level Tiny Moss (*Bryum capillare*) in the diets.**



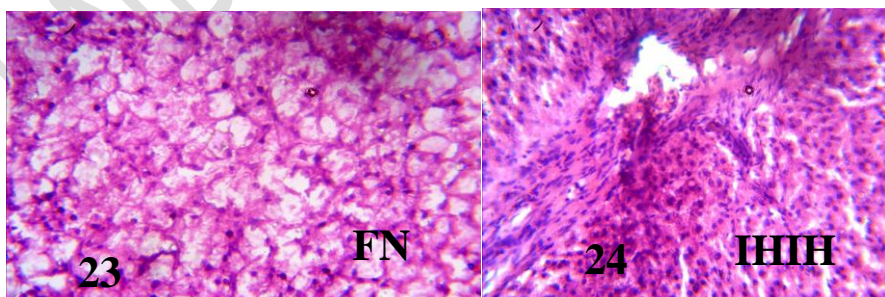
Photomicrograph of T0 control section of liver (X100)(H/E) shows normal hepatic architecture with normal hepatocyte (H) and central vein (C).

Photomicrograph of T1 section of liver (X100)(H/E) shows mild aggregate of inflammatory within the blood vessel otherwise normal.



Photomicrograph of T2 section of liver (X100)(H/E) shows mild aggregate of inflammatory (AIC) in R2 otherwise normal

Photomicrograph of T3 section of liver (X100)(H/E) shows mild to moderate portal aggregate of inflammatory (PAIC) in both section



Photomicrograph of T4 section of liver (X100)(H/E) shows moderate effect on the hepatic tissue moderate fatty necrosis (FN) in R1 and portal aggregate of inflammatory

Photomicrograph of T5 section of liver (X100)(H/E) shows severe effect on the hepatic tissue with severe I intra hepatic inflammation (IHI) and intra hepatic hemorrhage (IHIH).

Plate A-D (figures 1-24) present the results of tissue analysis of fish from the respective treatment. Histological examinations of the test fish showed some pathological changes. The Tiny Moss (*Bryumcapillare*) feed shows normal skin architecture with well outlined epithelial cell (EC) moderate effect on the skin layer with moderate necrosis (N) of the muscular region with the epithelial lining and superficial spreading of melanoma (M) restricted to the epidermis. The heart shows normal cardiac tissue with cardiac cell (CC), cardiac fiber (CF) cardiac muscles (CM) shows moderate aggregate of myocardial inflammation (AMI). The gill showed section of gill with ghost (G) appearance with severe aggregate of inflammatory cell (AIC). The liver cells revealed section of liver (X100) (H/E) shows severe effect on the hepatic tissue with severe intra hepatic inflammation (IHI) and intra hepatic hemorrhage (IHH). The damage done to these organs as the result of the feeds correlates with the concentrations of the feeds in each experimental tank.

## DISCUSSION

Non-conventional feed resources (NCFRs) are feeds that are not usually common in the markets and are not the traditional ingredients used for commercial fish feed production (Devendra, 1988; Maduet *et al.*, 2003). NCFRs are credited for being non-competitive in terms of human consumption, very cheap to purchase, by-products or waste products from agriculture, farm made feeds and processing industries and are able to serve as a form of waste management in enhancing good sanitation. The result of final total length of the *Clarias gariepinus* fed diets containing Tiny Moss (*Bryumcapillare*) presented in Table 2 showed that there were significant ( $p < 0.05$ ) differences in the final total length during the period of the study. Though, the highest final total length was recorded among the  $T_5$ ,  $T_3$  and  $T_1$  respectively followed by  $T_6$  while  $T_4$  and  $T_2$  had similar records which were the least among the groups.

The result of proximate analysis of the fish diet containing Tiny Moss (*Bryumcapillare*) in the diets as presented in Table 3 showed varying levels of nutrients in diets containing Tiny Moss (*Bryumcapillare*) for the experiment. The  $T_6$  diet had the highest percentage level of crude protein followed by  $T_4$  then  $T_5$  and  $T_1$ , while  $T_3$  and  $T_2$  had the least crude protein. The crude fat was also highest in  $T_6$  followed by  $T_4$ ,  $T_5$ ,  $T_1$ ,  $T_3$  and  $T_2$  respectively. The crude fibre showed close similarities in the values such that they followed this trend;  $T_2$ ,  $T_3$ ,  $T_1$ ,  $T_5$ ,  $T_4$  and  $T_6$  respectively. The ash content, Moisture content Nitrogen Free Extract and Dry matter content equally showed little variations among the individual groups, this work is similar to the work of Elhassan *et al.*, (2015) who reported a significant ( $p < 0.05$ ) increase in bambara groundnut protein ( $18.83 \pm 0.49$ ), lipids ( $7.05 \pm 1.82$ ), fiber ( $5.74 \pm 1.09$ ), carbohydrate ( $63.37 \pm 2.57$ ), moisture ( $12.59 \pm 1.14$ ) and ash ( $3.52 \pm 0.22$ ), which indicates that Bambara groundnut could be an excellent source of protein, lipid, carbohydrate and mineral elements in *Clarias gariepinus* fed diets.

The productive performance of the *Clarias gariepinus* fed diets containing Tiny Moss (*Bryumcapillare*) as indicated in the Table 4, showed close relationship in body weights of the fish varying levels of Tiny Moss (*Bryumcapillare*) at the start of the experiment. The final body weight and the daily weight gain decreased as the Tiny Moss (*Bryumcapillare*) inclusion increased among the individual treatment, such that the  $T_1$  had highest final body weight and daily weight gain among those in control group ( $T_1$ ) followed by  $T_2$  then  $T_3$ , but

T<sub>5</sub> and T<sub>6</sub> had similar values whereas T<sub>4</sub> had the least weight gain, which the mean differences were statistically significant ( $p < 0.05$ ) among the individual treatments. There were no significant ( $p > 0.05$ ) differences in the daily feed intake and the total feed intake during the period of the study. Though, the highest feed intake was recorded among the T<sub>3</sub> while others had similar records of feed intake among the groups. The results of the feed conversion ratio were significantly ( $p < 0.05$ ) different among the groups, such that T<sub>1</sub> and T<sub>2</sub> had the best FCR followed by T<sub>5</sub> and T<sub>6</sub> which had similar values but the T<sub>4</sub> had the least value. There were significant ( $p < 0.05$ ) differences in the survival rate during the period of the study such that T<sub>2</sub> recorded the highest survival rate, followed by T<sub>3</sub> and T<sub>6</sub> while T<sub>4</sub> and T<sub>5</sub> had similar records and the least survival rate among the groups.

The result of the average water quality of the tank containing *Clarias gariepinus* fed diet with Tiny Moss (*Bryumcapillare*) in the experiment as presented in table 5, indicated that there were similarities in the pH level of the water such that the T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> had the same marginally higher pH followed by T<sub>2</sub> whereas T<sub>1</sub> had the least pH, which the mean differences were statistically not significant ( $p > 0.05$ ) among the individual treatments. The Total Dissolved Solid (TDS) of the pond containing graded levels of Tiny Moss (*Bryumcapillare*) meal as indicated in the Table 5, showed close relationship in body weights of the fish varying levels of Tiny Moss (*Bryumcapillare*) at the start of the experiment. The Total Dissolved Solid (TDS) significantly increase ( $P < 0.05$ ) at T<sub>4</sub> as the Tiny Moss (*Bryumcapillare*) inclusion increased up to 4% among the individual treatment, followed by T<sub>1</sub>, T<sub>3</sub>, T<sub>5</sub> and T<sub>6</sub> that had similar values whereas T<sub>2</sub> had the least Total Dissolved Solid (TDS) which the mean differences were statistically significant ( $p < 0.05$ ) among the individual treatments.

The result of the water conductivity of the pond containing *Clarias gariepinus* fed diet with Tiny Moss (*Bryumcapillare*) in the experiment as presented in table 5, indicated that there significant differences ( $P < 0.05$ ) among the group such that T<sub>4</sub> (4% inclusion of Tiny Moss (*Bryumcapillare*) meal) had the highest level of water conductivity followed by T<sub>1</sub>, T<sub>3</sub>, T<sub>5</sub> and T<sub>6</sub> that had similar conductivity while T<sub>2</sub> (2% inclusion of Tiny Moss (*Bryumcapillare*) meal) had the least. The average water salinity of the pond containing graded levels of Tiny Moss (*Bryumcapillare*) meal as presented in the Table 5 were similar in their individual salinity of the water at varying levels of Tiny Moss (*Bryumcapillare*) during experiment. The average salinity is not significantly different ( $P > 0.05$ ) among the individual groups as the Tiny Moss (*Bryumcapillare*) inclusion increased. Though there were marginal increases such that T<sub>4</sub> had increased salinity followed by T<sub>2</sub>, then T<sub>3</sub> and T<sub>6</sub> whereas T<sub>5</sub> had the least value which the mean differences were statistically not significant ( $p > 0.05$ ) among the groups.

The result of the average ammonia content of the water in the pond containing *Clarias gariepinus* fed diet with Tiny Moss (*Bryumcapillare*) meal in the experiment as presented in table 5. This indicated that there were significant differences ( $P < 0.05$ ) in the ammonia content of the water such that the T<sub>4</sub> had the highest ammonia concentration followed by T<sub>3</sub>, T<sub>5</sub> and T<sub>6</sub> which had the same ammonia concentration, while T<sub>1</sub> and T<sub>2</sub> had ammonia concentration. The average Temperature (°C), Nitrite (NO<sub>2</sub>), Nitrate (NO<sub>3</sub>), and Dissolved oxygen (DO) of the pond containing graded levels of Tiny Moss (*Bryumcapillare*) meal as presented in the Table 5 were similar in their individual water at varying levels of Tiny Moss (*Bryumcapillare*) inclusion during experiment there results indicated no significant differences among the groups.

The result of average haematological parameters of catfish (*Clarias gariepinus*) juveniles fed diet containing Tiny Moss (*Bryumcapillare*) is presented in the Table 6. The study showed that the haematological parameters of the catfish (*Clarias gariepinus*) juveniles fed graded levels of diets containing Tiny Moss (*Bryumcapillare*) were not significantly different ( $P>0.05$ ). Though there were marginal variations among the individual group, there were still a lot of similarities among the values in each of the groups.

The result of average biochemical characteristics of catfish (*Clarias gariepinus*) juveniles fed diet containing Tiny Moss (*Bryumcapillare*) is presented in the Table 7. The study showed that the protein content of the catfish (*Clarias gariepinus*) juveniles fed graded levels of diets containing Tiny Moss (*Bryumcapillare*) were significantly different ( $P<0.05$ ). The catfish fed  $T_4$  diet had the highest percentage level protein followed by  $T_2$ , and  $T_6$  then  $T_1$ , while  $T_3$  and  $T_5$  had the least protein. The average ether extract was also highest in  $T_2$  which were similar to those in  $T_4$ , and  $T_6$ , but were significantly different ( $P<0.05$ ) to  $T_1$  and  $T_3$  which had the same protein level, whereas  $T_5$  had the least. The ash content, Nitrogen Free Extract, energy and glucose showed close similarities in the values such that they showed marginal variations which were not significantly different ( $P>0.05$ ) among the individual groups. The relationship between Na and K as well as between Ca and P; are desirable with the respective ratios of Na/K (0.6) and Ca/P (1.2). They also contain high levels of carotenoids (30 to 41.5 mg/100g DW), vitamin C (137.5 to 197.5 mg/100g DW). Sobowale et al., (2011).

Seventeen amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, valine, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, proline and serine) were detected. Their amino acid composition compare favourably with that of WHO/FAO protein standard indicating favourable nutritional balance except for lysine and methionine which appear marginal. The nutritional values of the phytochemicals were also assessed with a view of establishing and understanding their nutritional uses. The functional properties for the three vegetables were similar. Comparing the nutrient and chemical constituents with recommended dietary allowance (RDA) values, the results reveal that the leaves contain an appreciable amount of nutrients, minerals, vitamins, amino acids and phytochemicals and low levels of toxicants. Essential or indispensable amino acids (EAAs) cannot be synthesised by fish and often remain inadequate but are needed for growth and tissue development (Fagbenro et al., 2000; Wilson, 1989). The result of the effect of Tiny Moss (*Bryumcapillare*) on Amino Acid Profile of Catfish *Clarias Gariepinus* Juveniles fed diet containing Tiny Moss (*Bryumcapillare*) is presented in the Table 9. The study showed that the Glutamic Acid, Aspartic Acid, Valine, Threonine, Serine, Phenylalanine, Proline and Methionine increased in value while Lysine, Leucine, Arginine, Alanine, Isoleucine, Glycine, Histidine and Tryptophan reduced in value and there was no significant change in cysteine (%) all were significantly at ( $P<0.05$ ).

Fishmeal is known to contain complete EAA profile that is needed to meet the protein requirement of most fish species. Since fishmeal is expensive as a feeding ingredient, the use of non-conventional feedstuffs has been reported with good growth and better cost benefit values. The utilization of non-conventional feedstuffs of plant origin had been limited as a result of the presence of alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haematoglutinin, saponin, momosine, cyanoglycosides, linamarin to mention a few despite their nutrient values and low cost implications (New, 1987; Sogbesan et al., 2006). The report of this work is

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similar to the work of Sobowale *et al.*, (2011) who reported the nutritional components in three species of leafy vegetables using standard analytical methods. All the vegetables contained moisture (79.92 to 84.0%), crude protein (20.61 to 22.7%), crude fibre (10.7 to 22.44%), ash (6.8 to 10.44%), carbohydrate (55.86 to 68.22%) crude lipid (4.24 to 5.6%) and food energy (1507.19 to 1673.96 kJ/100g). The mineral element content were high with remarkable concentration of K (35.2 to 48.8 mg/100g), Na (11.4 to 14.4 mg/100g), Ca (15.4 to 18.7 mg/100g), Mg (12.2 to 18.7 mg/100g), P (13.8 to 15.08 mg/100g). These anti-nutritional factors negate growth and other physiological activities at higher inclusion levels (Oresegun and Alegbeleye, 2001).

The result of the essential amino acids indicated that these animal supplements could substitute fishmeal in fish feed (Table 2) since they all contain the required essential amino acids needed by fish for protein metabolism (DeSilva and Anderson, 1995). This result showed that earthworm meal is richer in methionine (Figure 1) than other animal protein sources studied which agreed with the observation of Finke (2003) when he compared the nutrient values in some invertebrates. Methionine has been credited as growth promoting essential amino acid, which is highly needed by cultured fish and limited in most plant and many animal supplements (Wilson, 2002).

Plate A-C (figures 1-18) represents the results of tissue analysis of fish from the respective treatment. Histological examinations of the test fish showed some pathological changes. The Tiny Moss (*Bryumcapillare*) feed shows normal skin architecture with well outlined epithelial cell (EC) moderate effect on the skin layer with moderate necrosis (N) of the muscular region with the epithelia lining and superficial spreading of melanoma (M) restricted to the epidermis. The heart shows normal cardiac tissue with cardiac cell (CC), cardiac fiber (CF) cardiac muscles (CM) shows moderate aggregate of myocardial inflammation (AMI). The gill showed section of gill with ghost (G) appearance with severe aggregate of inflammatory cell (AIC). The liver cells revealed section of liver (X100) (H/E) shows severe effect on the hepatic tissue with severe intra hepatic inflammation (IHI) and intra hepatic hemorrhage (IHIH). The damage done to these organs as the result of the feeds correlates with the concentrations of the feeds in each experimental tank. Several reports have indicated that gill lesions do not only indicate possibilities of impaired respiratory functions but impaired osmoregulatory functions as (Au, 2004.). Even slight structural damage can render a fish vulnerable to osmoregulatory as well as respiratory difficulties (Hughes and Morgan, 1973) thereby affecting the overall metabolism and survival of the fish. The histopathological alteration observed in the brain, gill, liver, intestine and muscle/flesh is an indication of the toxic effect of *P. zeylanica* extracts to fish. This agreed with Fafioye 2001, 2004 observation when *Clarias gariepinus* and *O. niloticus* were exposed to lethal and sublethal concentrations of *Parkia biglobosa* and *Raphia vinifera* respectively. The gill lamellae play a significant role in regulating the exchange of gas, water and ions in fish. The role of the gill in excretion predisposes it in such a way that slight structural damage can render a fish very vulnerable to osmoregulation as well as respiratory difficulties.

## Conclusion

Fish, like other organisms, required food (energy) in order to grow, survive and reproduce. The

food items (source of energy) in aquatic habitat are in the form of plankton, periphyton, nekton, benthos, nekton and plants are available throughout the year. The success of intensive fish culture depends on the formulation of a fish feed that contains an optimum level of protein and energy necessary for the growth of fish and is also cheap. It is obviously necessary to formulate and manufacture fish feed from locally available feed ingredients. This type of feed prepared from such ingredients should serve as a source of essential amino acid, minerals, vitamins, growth promoting substances and energy.

Essential or indispensable amino acids (EAAs) cannot be synthesized by fish and often remain inadequate but are needed for growth and tissue development (Wilson *et al.*, 1989). Fishmeal is known to contain complete EAA profile that is needed to meet the protein requirement of most fish species. Since fishmeal is expensive as a feed ingredient, the use of nonconventional feedstuffs has been reported with good growth and better cost benefit values. The utilization of nonconventional feedstuffs of plant origin had been limited as a result of the presence of alkaloids, glycosides, oxalic acids, phytates, protease inhibitors, haematoglutinin, saponin, momosine, cyanoglycosides, linamarin to mention a few despite their nutrient values and low cost implications (Sogbesan *et al.*, 2006).

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