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Termites Grown on Seaweed Media as Dietary Source of Protein in the Diets of African Catfish (*Clarias gariepinus*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A twelve-week feeding trial was conducted to examine the effect of termites (*Macrotermes nigeriensis*) grown on seaweed as a dietary protein source in the diets of African catfish (*Clarias gariepinus*). A total of 600 live African catfish specimens were used in the investigation, which were divided into four groups (ATD0, ATD1, ATD2, and ATD3) depending on their diets. The control diet (ATD0) comprised of commercial fishmeal (FM) and soybean meal (SBM) as protein sources in the ratio of 54FM:46SBM; For the three experimental diets (ATD1, ATD2, and ATD3), 60% of the crude protein was contributed by ATM either produced from termites raised on seaweed, elephant grass or both (50:50). Results show that daily weight gain was higher in fish fed termite meal (ATM) based

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diets, while feed conversion ratio and viscerosomatic index were higher in control group. Inclusion of ATM in diets of African catfish did not affect nutrient digestibility, enzyme activities, and total bile acid levels in the different portions of the digestive tract. Essential amino acids and mineral composition of whole fish also showed insignificant differences between experimental groups. However, fatty acid composition in whole fish fed diet without termite meal was significantly lower than those fed diets containing termites grown on seaweed. The study concludes that raising catfish on feed made from termites grown on seaweed can provide a good source of essential fatty acids with high bioavailability potential in African catfish.

Keywords: African catfish; aquafeed; insect meal; macroalgae.

1. INTRODUCTION

Fish is staple food in many countries of the world and its consumption follows the same trend as rice in many Asian and African countries (Giri, 2017). There is a positive association of global fish demand with increasing population. As world's population keeps increasing, the demand for fish and fish products responds positively to this trend (Inyang-Etoh et al., 2024a). Gap between the present rate and future projection for the consumption of fish is wide enough to merit concern of fisheries stakeholders including fisheries organizations, fisheries scientists, fish farmers and fish consumers (Asuquo et al., 2012). Fish and Agricultural Organization estimated annual consumption rate of 20 kg of fish per capita in 2016 with a steady rise up to 89 percent in 2030 (FAO, 2020). Of these values, substantial quota is being contributed by aquaculture while inland capture fisheries production has been quite stable. To meet such alarming demand for fish and fish products, aquaculture should produce more fish to the markets by increasing its efficiency while maintaining environmental sustainability. Sustainable aquaculture can be achieved with the proper choice of feed ingredients and formulation techniques (D'Abamro, 2021; Eteng & Ifon, 2019). As such, there is need for continuous investment and improvement in fish nutrition.

Although most productions of insect in aquaculture have not reached commercial volumes because of its infancy, many of which are laboratory trials, insect however holds a promising future for sustainable aquaculture and fish nutrition in particular (Inyang-Etoh et al., 2024b). Lately, there has been overwhelming appetite for insect-based diets by humans and domestic animals including fish, basically because of the high quality/quantity of protein and lipid they provide (Gałecki et al., 2021). Also, the use of insects in aquafeed production could be due to their ability to ably convert cellulose (their food) into protein and lipid of best qualities. Recent studies report a number of fish species which exhibited excellent growth response to insect-based diets. They include: Nile Tilapia- *Oreochromis niloticus* (Tippayadara et al., 2021), rainbow trout- *Oncorhynchus mykiss* (Fawole et al., 2021), Perch- *Perca fluviatilis* (Tilami et al., 2020), Atlantic salmon- *Salmo salar* (Belghit et al., 2019).

Most studies on the potential of insects in aquafeed use housefly (Saleh, 2020), mealworm (Lima et al., 2021) and black soldier fly (English et al., 2021) in their trials with less attention paid to termites. However, termites have played significant role in staple food of most African nations and have been analyzed for excellent quality and quantity of amino acids, fatty acids, crude protein and lipids (Olaleye et al., 2015). African winged termites (AWTs) are rich in crude protein, lipids, fatty acids and properly balanced essential amino acids including Lysine, threonine, and histidine. AWTs are also rich in vitamins and contain varieties of minerals including magnesium, manganese, potassium, iron, zinc, and phosphorus (Anyiam et al., 2022). As such, meals made from AWTs (ATM) may compete favourably with fish meal in promoting growth and well-being of farmed fish.

Studies have linked nutrients quality and quantity with the species of insect, life stage and geographical location (Liland et al., 2017; Meyer-Rochow et al., 2021). Also, the threshold quantity of nutrients per se depends on the nutritional requirement of the domesticated fish species. For example, while some fish species may require lower crude protein in their diets, others may need higher quantity depending on their genetic makeup. Also, specific and ontogenetic shift in nutrient requirements have been reported for most fish species (Teles et al., 2019).

Another important aspect to consider during the choice of insect in fish nutrition is the insect diets.

The substrate for rearing insects adds values to the composition of the insects and final consumers. As far as we know, no previous studies have been attempted to unravel the possibility of transferring essential nutrients from food source to termites. Belghita et al., (2018) reported that feeding Black soldier fly with seaweed enriched them with eicosapentaenoic acid and iodine of marine origin which enhanced the growth of Atlantic salmon. Liland et al., (2017) also reported similar findings black soldier fly reared on substrates enriched with marine macroalgae transferred marine nutrients such as omega-3 fatty (eicosapentaenoic) acid, iodine, and vitamin E into the insect composition. Based on these findings, the study evaluated the effect of AWTs (*Macrotermes nigeriensis*) fed with seaweed as dietary protein source in the diets of African catfish (*Clarias gariepinus*).

2. MATERIALS AND METHODS

2.1 Experimental Procedures

Collection of termites: Three colonies of *M. nigeriensis* which comprised of the royal pair (queen and king), workers, soldiers and fungus comb were collected in November 2020 from excavated mounds within Calabar forest area, Nigeria located between latitudes 4°48' & 5°10' N and longitudes 8°15' & 8°25' E of the Greenwich meridian. Each colony was wrapped with aluminum foil to avoid desiccation and transferred immediately to the rearing site.

Rearing and feeding of termites: Three glass chambers with equal dimension (50X50X30 cm) were constructed to harbor the termites. Clay soil from the collection site was placed inside the main chamber to mimic the termite natural habitat. The termite colony was put in the main chamber without external food and left to acclimate for 7 days. During this acclimatization period, the termites got used to the new environment and were feeding on the fungus comb. After this period, food and humidity chambers were connected to the main chamber using Plexiglas tube. Food chamber was placed before the main chamber, while humidity chamber was placed after the main chamber. Circular wire mesh of 12 cm diameter was provided at the top of each chamber to permit exchange of air between the inner chamber and the ambient environment. The porous clay pot connected to a water passage was provided to maintain humidity within the chambers. Plexiglas tubes were provided to permit free movement of termites through the chambers. There was also emergence of fungus comb in the humidity chamber during the rearing period (Fig. 1). The set up was repeated three times to serve three feeding groups. The termites were fed with ground sundried lignocelluloses plant materials such as elephant grass (*Cenchrus purpureus*) and seaweed (*Sagassum natans*). Group 1 was fed elephant grass only, Group 2 was fed seaweed only, while group 3 was fed 50 percent of seaweed and 50 percent of elephant grass. Nutritional composition of the different feeding media is available in the supplementary tables (Tables S1 – S3).

The rearing system was kept in total darkness at an average temperature of 27 °C and 85% relative humidity (Hongjie et al., 2015). At the end of a 6-month feeding test in May 2021, adult termites that survived the experiments were removed from the chambers and put in sealed sampling containers, labeled accordingly and preserved for further studies.

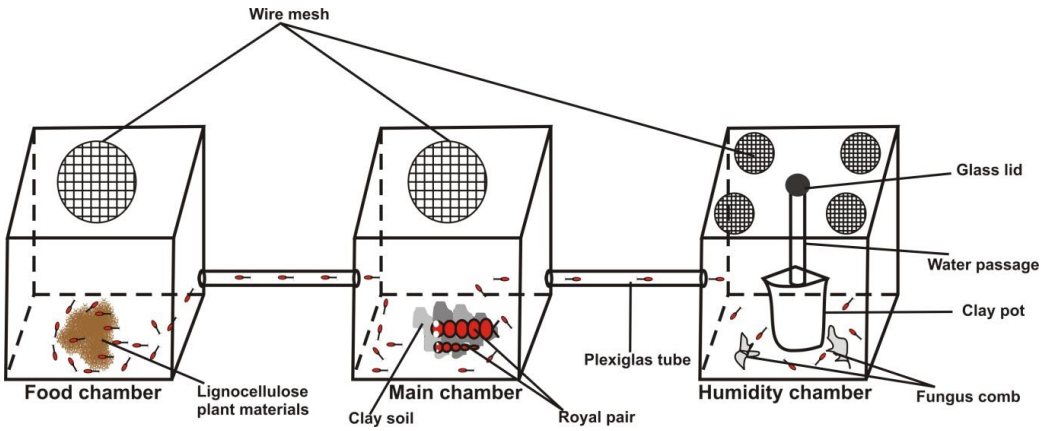

Fig. 1. Diagrammatic sketch of the artificial rearing system of the termite (*Macrotermes nigeriensis*)

Table 1. Ingredients and proximate composition of experimental diets fed to *Clarias gariepinus*

Preparation	ATD0	ATD1	ATD2	ATD3
ATM	0.0	41.6	41.6	41.6
FM	35.0	7.0	7.0	7.0
SBM	30.0	21.0	21.0	21.0
Corn meal	6.2	5.2	5.2	5.2
Groundnut cake	3.8	3.2	3.2	3.2
Bone meal	3.5	3.0	3.0	3.0
Wheat gluten	4.6	4.3	4.3	4.3
Vitamin and mineral premix	5.6	5.6	5.6	5.6
Fish oil	4.5	5.0	5.0	5.0
Vegetable oil	3.2	2.0	2.0	2.0
Yttrium	1.0	1.0	1.0	1.0
Miscellaneous	2.6	1.1	1.1	1.1
Aggregate	100.0	100.0	100.0	100.0
Proximate composition				
Dry matter (%)	93	92	92	94
Crude protein (%)	46	45	45	45
Crude lipid (%)	18	19	19	19
Carbohydrates (%)	12	11	11	11
Ash (%)	8	6	7	6
Gross Energy (MJ kg^{-1} dry matter)	25.2	24.7	24.1	24.1

ATD0= diet with 60% FM and 40% SBM; ATD1= 100% C. purpureus; ATD2= 100% S. natans and ATD3= 50% C. purpureus and 50% S. natans diets respectively

Preparation of fish diets: In the laboratory, the termites were removed from the sampling container, washed with distilled water, dewinged (for those that had developed wings) and frozen with liquid nitrogen at −80 °C and stored at −18 °C for further processing. Furthermore, the termites were sundried and thereafter ground with electric blender into powdered form. The powdered termite was defatted using solvent extraction method by dissolving in a solvent (3 hexane: 2 isopropanol w/v) and centrifuging for 15m. Thereafter, residual solvent was made to evaporate using a rotary evaporator (witegvapor, Germany). The remaining product was dried with nitrogen gas to obtain the termite meals (ATM1, ATM2, and ATM1/ATM2) used in this study. Nutritional composition of the termites used for ATM production is available in the supplementary tables (Tables S4 – S6). ATM1 was produced from termites that were fed ground grass only, ATM2 was produced from termites that were fed seaweed only, while ATM1/ATM2 was produced from termites that were fed both ground seaweed and ground grass (50:50). The different meals were used to formulate the experimental diets (ATD1, ATD2, and ATD3) for the study (Table 1).

The control diet (ATD0) was prepared with Premium Norwegian LT-94, a commercial fishmeal (FM) and soybean meal (SBM) (54FM:46SBM) as protein sources. For the three experimental diets, 60% of the crude protein was contributed by ATM either produced from termites raised on seaweed, elephant grass or both. The remaining 40% was derived from FM and SBM. Fish oil was included in all experimental groups to provide enough long chain polyunsaturated fatty acids (LC-PUFAs). To determine nutrients digestibility, the extruded diets were formulated and supplemented with 1% inert digestibility marker, the yttrium oxide. The experimental diets were stored in well labeled containers at – 18^{o}C pending when they were fed to fish.

Procedures for feeding the fish: The feeding trial was carried out at Feeding of experimental fish took place at the Aquaculture facility of the University of Calabar, Nigeria; for twelve weeks (June 2021 – August 2021). A total of 600 *C. gariepinus* lively specimens of the African catfish were procured from the University of Calabar fish farm, transported in an aerated bag to an experimental laboratory and distributed at random into twelve 350-L plastic tanks with 50 fish in each tank. Each tank was filled with filtered freshwater with average temperature of 25°C.

The tanks were arranged in triplicates into four groups and labeled according to diets. Groups 1 (control), 2, 3, and 4 were fed ATD0, ATD1, ATD2, and ATD3, respectively. The fish were fed two or three times daily, depending on their

feeding response, with an interval of 5 hours or more between meals. The following day, uneaten feed was removed from the tank using a rubber hose, dried at room temperature, weighed, and subtracted from the total daily feed given to calculate the feed intake for each group.

Fish sampling: Fish sampling took place at the beginning and ending of the experiment. After the feeding trial, ten (10) fish were picked at random from each experimental tank and anesthetized, followed with the measurement of body weight and length. Faecal samples were collected from selected five (5) fish by constant pressing each abdomen, pooled and dry at –80°C and preserved for digestibility analysis. Viscera were removed and weighed to obtained viscera index of the fish. The amino acid and fatty acid composition of the fish were analyses.

The remaining fish were dissected to remove digestive tract (adipose tissues). The digestive tract samples of the fish were divided into three portions: Proximal (PP), mid (MP) and distal (DP) portion. The digesta from each portion were removed and stored on dry ice at –80 °C for trypsin activity and total bile acids level analyses while the empty guts were also preserved on dry ice for the analysis of enzymatic activity.

2.2 Determination of Enzymatic Activity

Trypsin activity: The digesta obtained from different portions of the digestive tracts (PP, MP, DP) were pooled, mixed with cold distilled water (about 5°C), and centrifuged by stirring rigorously for 15 min. The resulting supernatants were collected into a 2 mL Eppendorf tubes and frozen in liquid nitrogen at an average temperature of −80 °C. Trypsin activity was determined using the chromogenic substrate, N-[5-(diaminomethylideneamino)-1-(4-nitroanilino)-1-oxopentan-2-yl]benzamide ($C_{19}H_{22}N_6O_4$) to check the level of absorbance. Blank was included for comparison. Trypsin activity was expressed as the difference in the absorbance between the test and blank tubes (ΔOD per mg dry matter).

Leucine aminopeptidase (LAP) activity: Cold mannitol buffer containing 25 µg/ml inhibitor (serine protease) was used to homogenize tissues obtained from the intestinal PP, MP, and DP using an homogenizer (Turrax) and sonicated at 5 °C for 1 min. The resulting homogenates were made to freeze in liquid nitrogen at an average temperature of −80 °C. Carboxy group of L-leucine was made to condense with amino group of 2-naphthylamine to produce the chromogenic compound, (2S)-2-amino-4-methyl-N-naphthalen-2-ylpentanamide ($C_{16}H_2ON_2O$) used as substrate for protein assay. LAP activity was determined as protein concentration of homogenates using protein assay kit (BioRad).

Estimation of total bile acids (TBAs) level: The digesta were centrifuged for 15 min as explained for trypsin activity. The resulting supernatants were sonicated for 1 min at 5 °C and fast frozen in liquid nitrogen at −80 °C. Total bile acids (TBAs) level was read directly using Enzabile diagnostic test kit (BioStat, UK) and compared with a standard (taurocholic acid solution curve).

Chemical composition analysis: Chemical composition analysis was undertaken on ground feed, whole fish and faecal samples. Total nitrogen level of samples frozen on dry ice was estimated using the Langenselbold Vario Macro Cube Elemental analyser (Germany) and quantified following standard procedures (Schulze et al., 2020). Instrument calibration was done with Saint Joseph Leco Corporation (USA). A standard meat reference material (Teddington, UK) was used to compare estimated values.

Total amino acids were estimated using the liquid chromatography with ultraviolet detector attached to it (UPLC system). Wet ground samples were hydrolysed in 6M of hydrochloric acid and a temperature of 110 °C to obtain a residue which was diluted in distilled water and filtered. A derivatisation water (Milford, USA) was added to the filtered samples and homogenized. The homogenates were subjected to the ultra-performance chromatography to separate the amino acids and compared with the Thermo Fisher standards (Rockford, USA).

Composition of starch in the formulated feed was estimated using hydrolysis and spectrophotometry depending on the procedural stage. About 0.5 g of feed sample was hydrolyzed firstly with amylase for 0.5 h at 80 °C and secondly with amyloglucosidase at 60 °C. Glucose concentration was determined with spectrophotometry at the wave length of 340 nm before and after enzymatic (de-hydrogenase) reaction, which was between hexokinase and glucose-6-phosphate using a Montpellier multianalyser (France). The difference between the glucose concentrations was taken as the concentration of starch in the freeze-dried feed sample. Crude lipids were extracted from wet samples of each of feed, faeces and whole fish which were homogenized in chloroform methanol

at a ratio of 2:1 (v:v). Homogenized samples were then placed in gas chromatography connected to a flame ionization detector and analyzed for lipids with 19:0 methyl ester as core benchmark (See notes on instrumentation in Liland et al., 2017). Concentration of fatty acids was determined by comparing each of the methyl esters identified in the samples to known standards.

To determine yttrium concentration, ground freeze-dried samples (feed, faeces) were homogenized in 70% nitric acid (2.5mL) and 30% hydrogen peroxide (1 mL) and digested in a microwave oven (Ultrawave, Italy). The solution was diluted with de-ionized water up to 20 mL. The concentrations of yttrium in the digested samples were determined using inductively coupled plasma mass spectrophotometry (ICPMS).

2.3 Estimation of Growth and Nutritional Indices

The following indices were estimated:

Daily growth rate, $DGR = \frac{100*(\sqrt[3]{BW_f} - \sqrt[3]{BW_i})}{day}$

where BW_f = final body weight, BW_i = initial body weight

Specific growth rate, $SGR = \frac{lnw_f - lnw_i}{t} * 100$

where Ln= Natural log of numbers, w_f= final body weight, w_i= initial body weight, t= culture period

Condition factor, $CF = \frac{100*W}{L^3}$

where W = body weight, L = total length

Visceral somatic index, $VSI = \frac{VW\ (g)}{BW\ (g)} * 100$

where VW= visceral weight, BW= body weight

Feed conversion ratio, $FCR = \frac{FI\ (g)}{WG\ (g)}$

where FI = feed intake, WG= weight gain

Coefficient of nutrient digestibility,

$CND = \frac{100 - (Y_d * NC_f)}{Y_f * NC_d} * 100$

where Y = yttrium concentration, d= diet, f= faeces, NC= nutrient concentration

2.4 Statistical Analysis

All statistical analyses were performed using the Predictive Analytical Software (PASW Ver. 20) for windows. Differences in dietary groups were tested for significance using a one-way ANOVA and further Tukey's post hoc test was adopted where necessary. Homogeneity of variance in the data set was tested using Levene's homogeneity test. Significance of non-homogeneous data was tested with Kruskal Wallis test. Significant differences were set at $P<0.01$.

3. RESULTS

3.1 Composition of Experimental Diets

Except for diet containing meal made from both seaweed and grass fed termites, ATM inclusion led to lower dry matter. Whereas crude protein was found to be lower, crude lipid, carbohydrate and gross energy were found to be higher in ATM-based diets (Table 1). Inclusion of ATM in the diets of the African catfish resulted in lower lysine, proline and alanine levels. However, the levels of important amino acids (AAs) were comparable in all the experimental diets (Table 2).

The inclusion of ATM in the experimental diets led to higher levels of calcium, iron, potassium, and manganese with lower levels of dietary magnesium and sodium (Table 3). Nevertheless, these levels met the requirements of African catfish (Robison, 2006).

Growth indices: Results show that the initial mean body weight (280.80 g) of African catfish was tripled (813.78 g) at the end of the experiment. Daily weight gain (DWG) was higher in fish fed ATM-based diets, while feed conversion ratio (FCR) and viscerosomatic index (VSI) were higher in control group. However, the protein source in the experimental diets did not have significantly different effects ($P>0.05$) on the growth indices of African catfish (Table 4).

Coefficient of nutrient digestibility (CND): Dietary treatments with ATM reduced the digestibility of crude protein, leucine, aspartic acid, glutamic acid, proline, and alanine while increasing that of crude lipid and isoleucine (Table 5). However, there were no statistically significant differences ($P>0.05$, ANOVA) in the apparent nutrient digestibility coefficients of the different dietary treatments (ATD1, ATD2, ATD3) including the control (ATD0).

Table 2. Wet weight total amino acids (AAs) composition (g/kg) of the experimental diets fed to African catfish (*Clarias gariepinus*) for 12 weeks

Aas	ATD0	ATD1	ATD2	ATD3
Essential AAs				
Lys	25	23	24	24
Thr	14	15	14	15
His	8	8	9	9
Val	17	17	18	18
Met	10	11	10	10
Ile	15	16	15	17
Leu	33	34	34	34
Phe	20	21	20	20
Non-essential AAs				
Asp	36	36	35	35
Glu	73	73	72	72
Tyr	14	14	14	13
Pro	25	24	24	23
Gly	16	16	16	15
Ala	19	18	18	17

ATD0= control diet prepared with 60% crude protein from commercial fishmeal and 40% from soybean meal; ATD1, ATD2, and ATD3= diets respectively prepared with 60% of crude protein obtained from termites grown on media containing C. purpureus only, S. natans only, and their combination (50:50)

Table 3. Fatty acid (FA) composition (g/100g) of the experimental diets fed to African catfish (*Clarias gariepinus*) for 12 weeks

FA	ATD0	ATD1	ATD2	ATD3
Saturated FA				
DDA	0.01	0.7	2.5	1.2
TDA	2.0	2.9	3.8	3.5
HAD	8.3	8.6	9.2	9.0
Unsaturated FA				
OTA	1.4	2.1	2.4	2.3
EPA	2.8	3.2	4.5	4.1
DHA	3.0	3.6	4.2	4.0

ATD0= control diet prepared with 60% crude protein from commercial fishmeal and 40% from soybean meal; ATD1, ATD2, and ATD3= diets respectively prepared with 60% of crude protein obtained from termites grown on media containing C. purpureus only, S. natans only, and their combination (50:50); DDA= 12:0 Dodecanoic (lauric) acid; TDA= 14:0 Tetradecanoic (myristic) acid; HDA= 16:0 Hexadecanoic (palmitic) acid; OTA= 18:4n-3 Octadeca-tetraenoic acid; EPA= 20:5n-3 Eicosapentaenoic acid; DHA= 22:6n-3 Docosahexaenoic acid

Table 4. Mineral composition (mg/kg) of the experimental diets fed to African catfish (*Clarias gariepinus*) for 12 weeks

Mineral	ATD0	ATD1	ATD2	ATD3
Ca	10,102	11,109	11,118	11,112
Fe	183	188	258	215
K	7,983	8,222	8,855	8,846
Mg	1,022	902	889	804
Mn	44	56	77	67
Na	2,113	2,011	1,088	871
P	11,873	12,002	11,234	11,011
Zn	184	177	191	182

ATD0= control diet prepared with 60% crude protein from commercial fishmeal and 40% from soybean meal; ATD1, ATD2, and ATD3= diets respectively prepared with 60% of crude protein obtained from termites grown on media containing C. purpureus only, S. natans only, and their combination (50:50)

Table 5. Average growth performance and somatic indices of African catfish fed a control diet (ATD0) or diets containing ATM1 and or ATM2 for 12 weeks

	Diets				ANOVA		
	ATD0	**ATD1**	**ATD2**	**ATD3**	**Pooled SE**	**P**	**Sig.**
IW(g)	280.80	280.16	280.02	284.14	0.92	0.35	NS
FW (g)	803.76	806.28	805.56	813.78	1.39	0.051	NS
DGI ($gday^{-1}$)	3.27	3.29	3.29	3.29	0.01	0.93	NS
SGI	1.25	1.26	1.26	1.25	0.004	0.93	NS
FCR	1.92	1.90	1.91	1.89	0.006	0.42	NS
VSI	11.54	11.47	11.48	11.52	0.041	0.92	NS
CF	1.49	1.50	1.45	1.46	0.012	0.40	NS

ATD0= control diet prepared with 60% crude protein from commercial fishmeal and 40% from soybean meal; ATD1, ATD2, and ATD3= diets respectively prepared with 60% of crude protein obtained from termites grown on media containing C. purpureus only, S. natans only, and their combination (50:50); NS= Mean values for growth indices of experimental fish fed experimental diets are not significantly different at P>0.05; IW= Initial weight, FW= Final weight, DGI= Daily growth index, SGI= Specific growth index, FCI= Feed conversion ratio, VSI= Visceral somatic index, CF= Condition factor

Table 6. Digestibility coefficients (CND %) of crude protein, crude lipids and amino acids in African catfish fed a control diet (ATD0) or diets containing ATM1 and or ATM2 for 12 weeks

	Diets					ANOVA	
Variables	**ATD0**	**ATD1**	**ATD2**	**ATD3**	**Pooled SE**	**P**	**Sig.**
CP	83.42	81.60	81.85	82.69	0.42	0.40	NS
CL	81.34	81.82	80.94	81.54	0.44	0.18	NS
Amino acid							
Lys	85.22	84.62	84.75	86.01	0.35	0.48	NS
Thr	79.12	78.39	78.97	81.16	0.48	0.19	NS
His	85.44	85.28	85.65	87.41	0.33	0.07	NS
Val	82.23	82.03	82.87	84.78	0.39	0.051	NS
Met	88.06	87.64	87.80	88.92	0.28	0.36	NS
Ile	78.98	79.12	79.81	81.90	0.46	0.095	NS
Leu	82.07	81.60	81.96	80.50	0.33	0.31	NS
Phe	91.13	90.87	90.94	91.93	0.203	0.23	NS
Asp	75.19	73.88	73.94	72.76	0.46	0.32	NS
Glu	88.38	87.93	88.00	87.19	0.36	0.70	NS
Tyr	78.83	77.61	77.07	79.33	0.51	0.37	NS
Pro	88.25	87.74	86.91	88.11	0.29	0.36	NS
Gly	81.46	80.68	80.70	81.66	0.44	0.81	NS
Ala	85.00	83.87	83.26	84.61	0.37	0.36	NS

ATD0= control diet prepared with 60% crude protein from commercial fishmeal and 40% from soybean meal; ATD1, ATD2, and ATD3= diets respectively prepared with 60% of crude protein obtained from termites grown on media containing C. purpureus only, S. natans only, and their combination (50:50); NS= Mean values for growth indices of experimental fish fed experimental diets are not significantly different at P>0.05

Proteinase activity and TBAs level: Although values were lower in control treatment, statistical results revealed that inclusion of ATM in diets of African catfish did not significantly affect the activities of trypsin and LAP as well as the concentration of TBAs in the different portions of the digestive tract (Table 6).

Nutritional composition of whole fish: It was noted that the concentration of essential amino acids like lysine, valine, and methionine were higher in the treated groups than the control, but without significant dietary effects on the whole fish amino acid profile (Table 7). Similarly, inclusion of ATM in the experimental diets did not lead to any significant difference in mineral composition of whole fish (Table 8).

Whole fish fatty acid composition was significantly affected by the inclusion of ATM in the experimental diets. Concentration of both saturated (12:0, 14:0, 16:0) and unsaturated (18:4n-3, 20:5n-3, 22:6n-3) fatty acids were higher in experimental diets than the control. Lauric acid was very low in whole fish fed diet without termite meal (ATM0) and was

significantly different from values obtained from fish fed diets containing termites grown on perennial grass and or seaweed (Fig. 2).

Octadeca-tetraenoic, eicosapentaenoic, and docosahexaenoic acids increased significantly in whole fish fed diets containing termites grown on seaweed only, and a combination of seaweed and perennial grass. For fish fed perennial grass only (ATD1), whole fish unsaturated fatty acids (18:4n-3, 20:5n-3, and 22:6n-3) concentrations were not statistically different from the control (ATD0). Nevertheless, ATD1 values were different from fish fed seaweed only (ATD2), and a combination of seaweed and elephant grass (ATD3).

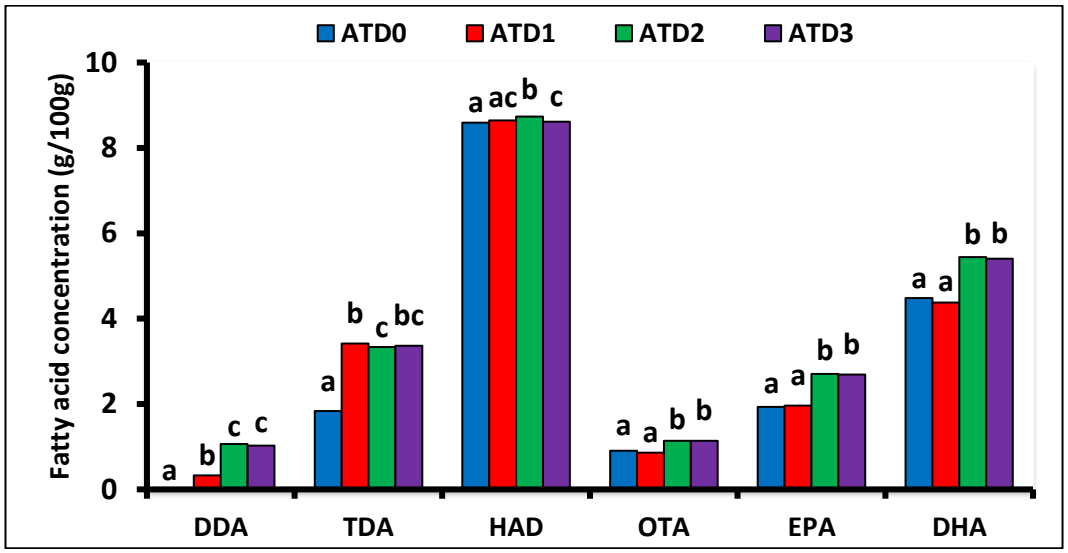

Fig. 2. Whole-fish fatty acid (FA) composition (g/100g) of African catfish fed a control diet (ATD0) or diets containing ATM1 and or ATM2 for 12 weeks

ATD0= control diet prepared with 60% crude protein from commercial fishmeal and 40% from soybean meal; ATD1, ATD2, and ATD3= diets respectively prepared with 60% of crude protein obtained from termites grown on media containing C. purpureus only, S. natans only, and their combination (50:50); DDA= 12:0 Dodecanoic (lauric) acid; TDA= 14:0 Tetradecanoic (myristic) acid; HAD= 16:0 Hexadecanoic (palmitic) acid; OTA= 18:4n-3 Octadeca-tetraenoic acid; EPA= 20:5n-3 Eicosapentaenoic acid; DHA= 22:6n-3 Docosahexaenoic acid; Mean values within each FA group with different superscripts[a,b,c] are significantly different at P<0.01

Table 7. Proteinase activity and total bile acids (TBAs) level in the intestine of African catfish fed a control diet (ATD0) or diets containing ATM1 and or ATM2 for 12 weeks

	Diets					ANOVA	
Variables	**ATD0**	**ATD1**	**ATD2**	**ATD3**	**Pooled SE**	**P**	**Sig.**
Trypsine (ΔOD/mg dry matter)							
PP	234.32	235.17	235.07	236.08	0.25	0.10	NS
MP	126.08	127.23	127.58	128.72	0.35	0.06	NS
DP	53.22	53.24	53.57	53.62	0.14	0.61	NS
Leucine aminopeptidase (µmol/h/mg protein)							
PP	490.77	490.98	491.01	489.26	2.07	0.99	NS
MP	316.87	324.40	324.41	323.26	1.52	0.24	NS
DP	342.56	350.70	350.72	349.47	1.65	0.24	NS
Bile acids (µmol/g dry matter)							
PP	136.12	136.45	137.04	137.73	0.48	0.65	NS
MP	93.59	94.86	93.22	94.56	0.52	0.64	NS
DP	34.80	34.68	34.83	35.27	0.097	0.15	NS

ATD0= control diet prepared with 60% crude protein from commercial fishmeal and 40% from soybean meal; ATD1, ATD2, and ATD3= diets respectively prepared with 60% of crude protein obtained from termites grown on media containing C. purpureus only, S. natans only, and their combination (50:50); NS= Mean values for growth indices of experimental fish fed experimental diets are not significantly different at P>0.05; PP= Proximal portion, MP= Mid portion, DP= Distal portion of the digestive tract

Table 8. Whole-fish amino acid concentration (mg/g) of African catfish fed a control diet (ATD0) or diets containing ATM1 and or ATM2 for 12 weeks

	Diets					ANOVA	
Variables	**ATD0**	**ATD1**	**ATD2**	**ATD3**	**Pooled SE**	**P**	**Sig.**
Lys	13.90	13.91	14.03	14.17	0.047	0.15	NS
Thr	8.03	8.00	8.04	8.14	0.022	0.15	NS
His	4.16	4.15	4.13	4.17	0.008	0.42	NS
Val	9.06	9.15	9.13	9.18	0.021	0.20	NS
Met	4.25	4.27	4.30	4.31	0.017	0.49	NS
Ile	7.03	6.95	7.01	7.06	0.023	0.43	NS
Leu	12.24	12.23	12.22	12.31	0.041	0.89	NS
Phe	6.30	6.22	6.25	6.33	0.017	0.13	NS
Asp	15.17	15.27	15.18	15.37	0.041	0.27	NS
Glu	19.69	19.63	19.65	20.00	0.056	0.055	NS
Tyr	5.17	5.16	5.18	5.23	0.014	0.30	NS
Pro	5.28	5.37	5.36	5.38	0.016	0.063	NS
Gly	9.77	9.78	9.76	9.84	0.028	0.75	NS
Ala	10.66	10.63	10.72	10.85	0.032	0.067	NS

ATD0= control diet prepared with 60% crude protein from commercial fishmeal and 40% from soybean meal; ATD1, ATD2, and ATD3= diets respectively prepared with 60% of crude protein obtained from termites grown on media containing C. purpureus only, S. natans only, and their combination (50:50); NS= Mean values for growth indices of experimental fish fed experimental diets are not significantly different at P>0.05

4. DISCUSSION

The findings from this study show that termite meal can be used to conveniently replace fish meal in the diets of African catfish (*C. gariepinus*). Inclusion of termite meal in the experimental diets promotes fish production efficiency as it did not affect feed palatability as revealed by high feed intake and low feed conversion ratio. Even with a dietary protein inclusion ratio of 6:3:1 for ATM, FM and SBM respectively, there was no significant difference in growth performance of African catfish. Optimal inclusion level of 60% termite meal as protein source in this study is higher than 40% reported by Mali et al. (2020). The difference could be due to the different experimental animals; while the present study used African catfish, Mali et al. (2020) used broilers for the trials.

Also, Nephale et al. (2024) recommend 50% as optimal inclusion levels of termite meal in the diets of *C. gariepinus* and *Oreochromis mossambicus*. A different optimal inclusion level of 60% reported in the present study could be in the methods used in preparing termite meal. It is possible that the gutted termites were relieved of some antinutrients that are usually associated with undigested cellulose in guts. Also, anti-nutrients such as phytic acid and antioxidant polyphenols which could have otherwise inhibited nutrient digestibility and growth of the experimental fish might have been eliminated or at least reduced to barest minimum in the process of oven drying the termites without affecting essential nutrients (Fombong et al., 2017). Moreover, the concentration of other anti-nutrients such as calcium oxalate, trypsin inhibitor, lectin, and hydrocyanic acid in termites are reported to be low or non-existence and may not merit concern (Meyer-Rochow et al., 2021).

Feed intake and feed conversion ratio were not affected by the inclusion of termite meal in the diets of African catfish given that the control group recorded analogous results. Similar reports of good growth performance with insect meal as replacement for fish meal in diets of African catfish are available (Nephale et al., 2024; Inyang-Etoh et al., 2024b). Earlier trials where termite meal was used to partially or wholly replace fish meal did not report adverse effect on growth of African catfish (Olaniyi et al., 2016), prawns (Olaniyi & Poku, 2014), and birds (Mali et al., 2020).

Apparent digestibility of crude protein, lipid and amino acids were observed to be similar in all the feeding groups inferring that digestibility was not affected by ATM inclusion in the experimental diets. The experimental diets were also found to have no considerable effect on the enzyme activity (trypsine and leucine aminopeptidase), as well as the total bile acids concentration in the digesta. This finding opines that termite meal could be an important source of easily digestible

nutrients for catfish and other fish species. Interestingly, the digestibility coefficients obtained for African catfish in the present study are similar to those reported for Atlantic salmon fed other alternative sources of protein like Black soldier fly larvae (Belghit et al., 2019), rainbow trout fed native Peruvian feedstuffs (Ortiz-Chura et al., 2018), and Olive flounder fed fishmeal-based diets (Rahman et al., 2016). The fact that whole body crude protein was not affected by the inclusion of termite meal confirms termite as efficient source of protein in aquafeed.

Fish fed diets with insect meal made from termite grown on either terrestrial grass, seaweed or both grew slightly better than the control group fed dietary fish meal and soybean meal as protein sources. This finding is in line with some previous reports (Liland et al., 2017; Belghit et al., 2018) on the effect of compounding feeds from insects raised on seaweed substrates. Undoubtedly, partial inclusion of both perennial grass (*Cenchrus purpureus*) and marine brown macroalgae (*Sagassum natans*) in the media improved the nutritional status of the termites. It was observed that fish fed termites raised on grass (*C. purpureus*) only was not significantly different from control in fatty acid concentrations; whereas fish fed termites raised on either seaweed (*S. natans*), or both elephant grass and seaweed were not different from each other but significantly different from control and fish fed on termites raised on elephant grass only.

The whole fish medium chain fatty acid like lauric acid and other saturated fatty acids (myristic, palmitic) were found to be significantly lower in control group than the experimental groups. Significant increase in eicosapentaenoic and docosahexaenoic acids in whole fish fed diets containing termites grown on seaweed is an important innovation in aquaculture nutrition for the success of raising fish on cheap source of nutrients. Although fish oil is known to contain substantial amount of EPA and DHA, significant increasing levels in whole fish fed ATD2 could signal the contribution of seaweed substrate. The present finding supports previous studies (Liland et al., 2017; Belghit et al., 2019) which reported that fish fed meals made from insect raised on seaweed had higher omega-3 fatty acid than those fed fish meal as protein source. This study therefore posits that the choice of substrates for growing the insect can affect the chance of using insect meal in fish diets.

5. CONCLUSION

There is possibility of raising African catfish on diets made from termite grown on seaweed as dietary source of protein without compromising growth, condition factor, nutrient digestibility, enzyme activity and whole-body composition. Additionally, meal made from African winged termites grown on seaweed could be a good source of essential fatty acids with high bioavailability potential in African catfish.

ETHICAL APPROVAL

The welfare and care of experimental animals were ensured throughout the study following the guidelines of the National Veterinary Research Institute (NVRI), Nigeria and approved by the Animal Use and Care Committee (AUCC).

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The authors affirm that no generative AI technologies, including but not limited to Large Language Models (e.g., ChatGPT, Copilot) or text-to-image generators, were utilized in the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Supplementary Table S1. Proximate composition (g/100 g dry weight) and total amino acid composition of feeding media used for raising termites

Composition	BA0	BA50	BA100
Crude protein	10.2	7.8	4.9
Dry matter	30.8	29.2	27.6
Amino acid profile (% of crude protein)			
Lysine	5.7	5.5	5.2
Threonine	4.3	4.5	4.7
Histidine	2.8	2.5	2.2
Valine	5.9	5.7	5.3
Methionine	1.7	1.9	1.9
Isoleucine	4.1	3.9	3.8
Leucine	7.5	7.1	6.8
Phenylalanine	4.5	4.3	4.2
Aspartic acid	8.8	10.5	12.2
Glutamic acid	19.3	22.1	26.1
Tyrosine	4.1	3.4	2.3
Proline	7.5	4.2	2.4
Glycine	6.5	6.2	5.1
Alanine	6.4	6.8	7.5

BA0= Pure plant based medium (100% elephant grass), BA50= elephant grass and brown algae (50:50), BA100= 100% brown algae

Supplementary Table S2. Fatty acid (FA) composition (g/100g) of feeding media used for raising termites

Composition	BA0	BA50	BA100
Lauric acid	0.7	0.2	0.15
Myristic acid	0.5	2.8	9.5
Palmitic acid	10.7	19.1	23.1
Stearidonic acid	ND	1.1	2.3
Timnodonic acid	ND	2.6	6.1
Cervonic acid	ND	1.4	4.7

BA0= Pure plant based medium (100% elephant grass), BA50= elephant grass and brown algae (50:50), BA100= 100% brown algae, ND= not detected

Supplementary Table S3. Mineral composition of feeding media used for raising termites

Mineral	BA0	BA50	BA100
Ca (g/kg)	2.8	8.9	14.7
K (g/kg)	10.3	13.8	18.5
Mg (g/kg)	1.5	4.6	7.2
Na (g/kg)	5.2	21.3	34.6
P (g/kg)	4.4	3.2	1.5
Fe (mg/kg)	222	314	425
Mn (mg/kg)	51	35	18
Zn (mg/kg)	38	43	45

BA0= Pure plant based medium (100% elephant grass), BA50= elephant grass and brown algae (50:50), BA100= 100% brown algae

Supplementary Table S4. Proximate composition (g/100 g dry weight) and total amino acid composition of adult termites grown on increasing inclusions of brown algae in feeding media

Composition	BA0	BA50	BA100
Crude protein	52	52	52
Crude lipid	8.8	8.8	8.8
Ash	10.2	10.2	10.1
Dry matter	27.8	27.6	27.5
Amino acid profile (% of crude protein)			
Lysine	7.11	7.14	7.08
Threonine	4.28	4.24	4.25
Histidine	3.14	3.11	3.09
Valine	5.12	5.11	5.14
Methionine	1.03	0.98	1.02
Isoleucine	5.20	5.15	5.18
Leucine	8.75	8.78	8.82
Phenylalanine	5.05	5.11	5.07
Aspartic acid	5.98	5.88	6.02
Glutamic acid	10.03	9.92	9.84
Tyrosine	3.82	3.78	3.71
Proline	3.94	3.89	4.01
Glycine	5.38	5.40	5.27
Alanine	4.56	4.55	4.55

BA0= Termites raised on pure plant based medium (100% elephant grass), BA50= Termites fed elephant grass and brown algae (50:50), BA100= Termites fed 100% brown algae only

Supplementary Table S5. Fatty acid (FA) composition (g/100g) of adult termites grown on increasing inclusions of brown algae in feeding media

Composition	BA0	BA50	BA100
Saturated FA			
Lauric acid	31.4	32.5	33.8
Myristic acid	7.8	8.1	8.4
Palmitic acid	16.2	16.7	17.1
Unsaturated FA			
Stearidonic acid	2.1	3.2	3.65
Timnodonic acid	0.7	0.8	1.22
Cervonic acid	5.1	7.2	7.55

BA0= Termites raised on pure plant based medium (100% elephant grass), BA50= Termites fed elephant grass and brown algae (50:50), BA100= Termites fed 100% brown algae only

Supplementary Table S6. Mineral composition of adult termites grown on increasing inclusions of brown algae in feeding media

Mineral	BA0	BA50	BA100
Ca (g/kg dm)	8.2	21.4	29.6
Fe (g/kg dm)	0.18	0.25	0.37
K (g/kg dm)	9.9	17.5	20.6
Mg (g/kg dm)	2.3	3.7	4.8
Mn (g/kg dm)	2.1	0.15	0.18
Na (g/kg dm)	2.0	4.4	11.5
P (g/kg dm)	6.2	7.7	10.7
Zn (mg/kg dm)	65	73	147

BA0= Termites raised on pure plant based medium (100% elephant grass), BA50= Termites fed elephant grass and brown algae (50:50), BA100= Termites fed 100% brown algae only, dm= dry matter

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