Termites grown on seaweed media as dietary source of protein in the diets of African catfish (Clarias gariepinus)

Aniema Paul Inyang-Etoh
University of Calabar
Sunday Urom Eteng
University of Calabar
Honor Tajoes Ifon (honor.t.ifon@gmail.com)
University of Calabar

Research Article

Keywords: African catfish, Aquafeed, Insect meal, macroalgae

Posted Date: June 24th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1764675/v1

Abstract

A twelve-week feeding trial was conducted to examine the effect of termites 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) was made with 60 percent commercial fishmeal (FM) and 40% soybean meal (SBM); ATD1, ATD2, and ATD3 were made with 60% crude protein obtained from termites (*Macrotermes nigeriensis*) grown on media containing only terrestrial grass (*Cenchrus purpureus*), only seaweed (*Sagassum natans*), and their combination (50:50), respectively. Results show that daily weight gain was higher in fish fed termite meal (ATM) based 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.

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. 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. 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). As such, there is need for continuous investment and improvement in fish nutrition.

Although most productions of insect in aquaculture has 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. 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 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. 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 feeding medium where the insects grow is thought to transfer some essential nutrients to the consumer. 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 where growing black soldier fly on substrates enriched with marine macroalgae transferred marine nutrients such as omega-3 fatty (eicosapentaenoic) acid, iodine, and vitamin E into the insect. Based on these findings, the present study sought to find out the effect of using AWTs (*Macrotermes nigeriensis*) fed seaweed as dietary source of protein in the diets of African catfish (*Clarias gariepinus*).

2. Materials And Methods

2.1 Experimental procedures

2.1.1 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.

2.1.2 Rearing and feeding of termites

Three glass chambers with equal dimension (50X50X30 cm) were constructed to habour the termites. To mimic the natural environment, clay soil from the termite collection site was placed inside the main chamber. The termite colony was put in the main chamber without external food and left to acclimatize 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 partially decayed 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. 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, termites that survived the experiments were removed from the chambers and put in sealed sampling containers, labeled accordingly and preserved for further studies.

2.1.3 Preparation of fish diets

In the laboratory, termite samples were removed from the sampling containers, dewinged (for those that already turned to swarmers or alates), degutted and oven dried at 40°C (Igwe et al., 2011). The dried samples were milled with electric blender to produce termite meals (ATM1, ATM2, and ATM1/ATM2) used in this study. 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 protocol of the Dongen Protix Biosystems (The Netherlands) was followed in the milling process (see also Alfiko *et al.*, 2022).

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. Four experimental diets were formulated for the study. The control diet (ATD0) was prepared with commercial fishmeal (FM, 60%) and soybean meal (SBM, 40%) as protein sources; ATD1, ATD2, and ATD3 were prepared with 60 percent of crude protein obtained from ATM1, ATM2, and ATM1/ATM2 respectively. The remaining 40 percent crude protein was obtained from fish (30%) and soybean meals (10%). Fish oil was used in all experimental groups to provide enough long chain polyunsaturated fatty acids (LC-PUFAs).

Table 1 Preparation and proximate composition of the four experimental diets fed to African catfish (*Clarias gariepinus*) for 12 weeks.

Preparation	ATD0	ATD1	ATD2	ATD3
Ingredients (%)				
ATM	0.0	39.0	39.0	39.0
FM	39.0	19.5	19.5	19.5
SBM	26.0	6.5	6.5	6.5
Corn meal	6.2	6.2	6.2	6.2
Groundnut cake	3.8	3.8	3.8	3.8
Bone meal	3.5	3.5	3.5	3.5
Wheat gluten	2.8	2.8	2.8	2.8
Vitamin and mineral premix	5.6	5.6	5.6	5.6
Fish oil	6.3	6.3	6.3	6.3
Vegetable oil	3.2	3.2	3.2	3.2
Yttrium	1.0	1.0	1.0	1.0
Miscellaneous	2.6	2.6	2.6	2.6
Aggregate	100.0	100.0	100.0	100.0
Proximate composition				
Dry matter (%)	93	92	92	93
Crude protein (%)	46	45	45	45
Crude lipid (%)	19	21	20	21
Carbohydrates (%)	10	12	11	11
Ash (%)	5.8	5.8	5.8	5.6
Gross Energy (MJ kg^{-1} dry matter)	23.8	24.1	24.0	25.2

ATD0 = control diet without termite meal inclusion 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); ATM = African termite meal; FM = fishmeal; SBM = soybean meal.

2.1.4 Procedures for feeding the fish

Feeding of experimental fish took place at the Aquaculture facility of the University of Calabar, Nigeria for twelve weeks (June – August 2021). A total of 600 lively specimens of the African catfish were distributed at random into twelve 350-L plastic tanks with 50 fish in each tank. Each tank was filled with filtered freshwater and average temperature of 25°C was maintained throughout the experiment.

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, ATD3 respectively during the 12 weeks feeding trial. The fish were either fed two times or three times daily depending on how they respond to feeding with an interval of 5 h or more between meals. The following day, feed left uneaten were removed from the tank, allowed to dry, weighed, and subtracted from the weight of the total daily feed given to the fish.

2.1.5 Fish sampling

Fish sampling took place at the beginning and ending of the experiment. Before sampling, fish specimens were first induced with anesthetic, followed with the measurement of body weight and length. Viscera were removed from each specimen, and weighed to obtained data for viscera index. Faecal samples were collected at the end of the experiment by constantly pressing individual fish abdomen. The faecal samples (pooled) were made to freeze on dry ice and were preserved for digestibility analysis.

Ten (10) fish samples were removed from each tank, out of which 5 were pooled and preserved on dry ice at − 80°C for proximate composition analysis. The remaining 5 samples were dissected and digestive tract removed from of each sample and cleaned to detach adipose tissues. The digestive tract samples 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

2.2.1 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).

2.2.2 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).

2.3 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).

2.4 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 the method of Belghit et al. (2018). 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).

2.5 Estimation of growth and nutritional indices

The following indices were estimated:

Daily growth rate, $DGR = \frac{100 * \left(\sqrt[3]{\overline{BW_f}} - \sqrt[3]{\overline{BW_i}} \right)}{day}$ 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, $CND = \frac{100 - \left(Y_d * NC_f \right)}{Y_f * NC_d} * 100$ where Y = yttrium concentration, d= diet, f= faeces, NC= nutrient concentration

2.6 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).

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).

3.2 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).

3.3 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).

3.4 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).

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 $P > 0.05$.

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.

3.5 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).

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 $P > 0.05$.

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).

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 $P < 0.01$.

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, Table S1).

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, Sogbesan and Ugwumba (2008) recommend 40 and 50% as optimal inclusion levels of termite meal in the diets of *C. gariepinus* and *Heterobranchus longifilis* respectively. 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 (Sogbesan & Ugwumba, 2008). 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 as food sources 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.

6. Statements & Declarations

6.1 Ethical Approval and Consent to participate

6.1.1 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) with reference number nvriAUCC F001/15.

6.1.2 Consent to participate

Not applicable

6.2 Consent for publication

Not applicable

6.3 Availability of supporting data

All data used in this study are included within the text; its supplementary information file is also provided in this published article.

6.4 Competing interests

The authors declare that they have no relevant financial or non-financial interest to disclose.

6.5 Funding

The study was fully funded by the authors; no external funding or other supports were received from public, commercial or not-for-profit domains during the preparation of this manuscript.

6.6 Authors' contributions

Aniema Paul Inyang-Etoh and Honor Tajoes Ifon contributed to the study conception and design. Material preparation and data collection were carried out by all the authors. Honor Tajoes Ifon and Sunday Urom Eteng performed data analysis and interpretation. The original draft of the manuscript was written by Honor Tajoes Ifon and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

6.7 Acknowledgements

Many thanks to Dr. Nnamnso Edet of Michael Okpara University of Agriculture, Umudike, Nigeria for valuable suggestions and proof reading the article. We also appreciate the staff of Speedware Computers, Students Center, University of Calabar – Nigeria, for typing the manuscript.

6.8 Authors' information

No further information to disclose.

References

1. Anyiam PN, Nwuke C., Adimuko GC, Nwamadi CP, Salvador EM, Ajibade GF, Maxwell EC (2022) Inclusion of African winged termites (*Macrotermes nigeriensis*) improves the nutrients and quality of fermented cassava mahewu. African Journal of Biotechnology 21 (2): 46–54.
2. Belghit I, Liland NS, Gjesdal P, Biancarosa I, Menchetti E, Li Y, Waagbø R, Krogdahl A, Lock E (2019) Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). Aquaculture 503: 609–619.
3. Belghit I, Liland NS, Waagbø R, Biancarosa I, Pelusio N, Li Y, Krogdahl A, Lock E (2018) Potential of insect-based diets for Atlantic salmon (*Salmo salar*). Aquaculture 491: 72–81.
4. D'Abamro LR (2021) Sustainable aquafeed and aquaculture production systems as impacted by challenges of global food security and climate change, Editorial, Journal of the World Aquaculture Society 52 (6): 1162–1167.
5. English G, Wanger G, Colombo SM (2021) A review of advancements in black soldier fly (*Hermetia illucens*) production for dietary inclusion in salmonid feeds, Journal of Agriculture and Food Research 5 (100164): 1–8.
6. FAO (2020) The state of world's fisheries and aquaculture. Food and Agriculture of Organization of the United Nations.
7. Fawole FJ, Labh SN, Hossain MS, Overturf K et al (2021) Insect (black soldier fly larvae) oil as a potential substitute for fish or soy oil in the fish meal-based diet of juvenile rainbow trout (*Oncorhynchus mykiss*). Animal Nutrition 7: 1360–1370.
8. Fombong TF, Borght MV, Broeck JM (2017) Influence of Freeze-Drying and Oven-Drying Post Blanching on the Nutrient Composition of the Edible Insect Ruspolia differens. Insects 8 (102): 1–14.
9. Gałecki R, Zielonka Ł, Zasepa M, Gołębiowska J et al (2021) Potential Utilization of Edible Insects as an Alternative Source of Protein in Animal Diets in Poland. Frontiers in Sustainable Food Systems 5 (675796): 1–8.
10. Giri SS (2017). Farm-Made Feeds for Sustainable Aquaculture Development: A South Asian Perspective. https://www.researchgate.net/publication/327307282 Accessed 18th May 2022.
11. Hongjie LI, Yang M, Chen Y, Zhu N et al (2015) Investigation of Age Polyethism in Food Processing of the Fungus – Growing Termite *Odontotermes formosanus* (Blattodea: Termitidae) using a Laboratory Artificial Rearing System. Household and Structural Insects 108(1): 266–273.
12. Igwe CU, Onwuliri VA, Ojiako AO, Arukwe JU (2011) Effects of *Macrotermes nigeriensis* based diet on hepatic and serum lipids of albino rats. Australian Journal of basic and Applied Sciences 5 (7): 906–910.
13. Liland NS, Biancarosa I, Araujo P, Biemans D et al (2017) Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PLoS One 12 (8): e0183188.
14. Lima JS, Pittaluga ML, Lovatto NM (2021) Mealworm (*Tenebrio molitor*) potential in fish nutrition: a review. Research Society and Development 10 (16): e269101623229.

15. Mali B, Okello S, Ocaido M, Nalule AS (2020) Optimal inclusion level of termite meal replacing fish meal in broiler diets. Livestock Research for Rural Development 32 (11): 1–6.
16. Meyer-Rochow VB, Gahukar RT, Ghosh S, Jung C (2021) Chemical Composition, Nutrient Quality and Acceptability of Edible Insects Are Affected by Species, Developmental Stage, Gender, Diet, and Processing Method. Foods 10 (1036): 1–36.
17. Olaniyi AE, Poku G (2014) Nutritive value of termite as fish meal supplement in the diet of freshwater prawns (*Macrobrachium rosenbergii* de Man) juveniles. International Journal of the Bioflux Society 6 (2): 63–71.
18. Olaniyi CO, Olabomi FT, Ajayi OM (2016) Performance characteristics and nutrients utilization of African catfish fed termite based diets. International Journal of Science, Environment and Technology 5 (5): 2679–2688.
19. Ortiz-Chura A, Pari-Puma RM, Huanca FH, Cerón-Cucchi ME, Araníbar MJ (2018) Apparent digestibility of dry matter, organic matter, protein and energy of native Peruvian feedstuffs in juvenile rainbow trout (*Oncorhynchus mykiss*). Fisheries and Aquatic Sciences 21 (32): 1–7.
20. Rahman M, Han H, Kim K, Kim K et al (2016) Apparent digestibility coefficients of the extruded pellet diets containing various fish meals for olive flounder, *Paralichthys olivaceus*. Fisheries and Aquatic Sciences 19 (27): 1–8.
21. Robison E (2006) Catfish Nutrition, Nutrient requirement. Mississippi State University extension service. Publication 24121: 1–3.
22. Saleh HE (2020) Review on Using of Housefly Maggots (*Musca domestica*) in Fish Diets. Journal of Zoological Research 2 (4): 39–46.
23. Teles AO, Couto A, Enes P, Peres H (2019) Dietary protein requirements of fish – a meta-analysis. Reviews in Aquaculture 12 (3): 1445–1477.
24. Tilami SK, Turek J, Červený D, Lepič P, et al (2020) Insect Meal as a Partial Replacement for Fish Meal in a Formulated Diet for Perch *Perca fluviatilis*. Turkish Journal of Fisheries & Aquatic Science 20 (12): 867–878.
25. Tippayadara N, Dawood MAO, Krutmuang P, Hoseinifar SH et al (2021) Replacement of Fish Meal by Black Soldier Fly (*Hermetia illucens*) Larvae Meal: Effects on Growth, Haematology, and Skin Mucus Immunity of Nile Tilapia, *Oreochromis niloticus*. Animals 11 (193): 1–19.

Figures

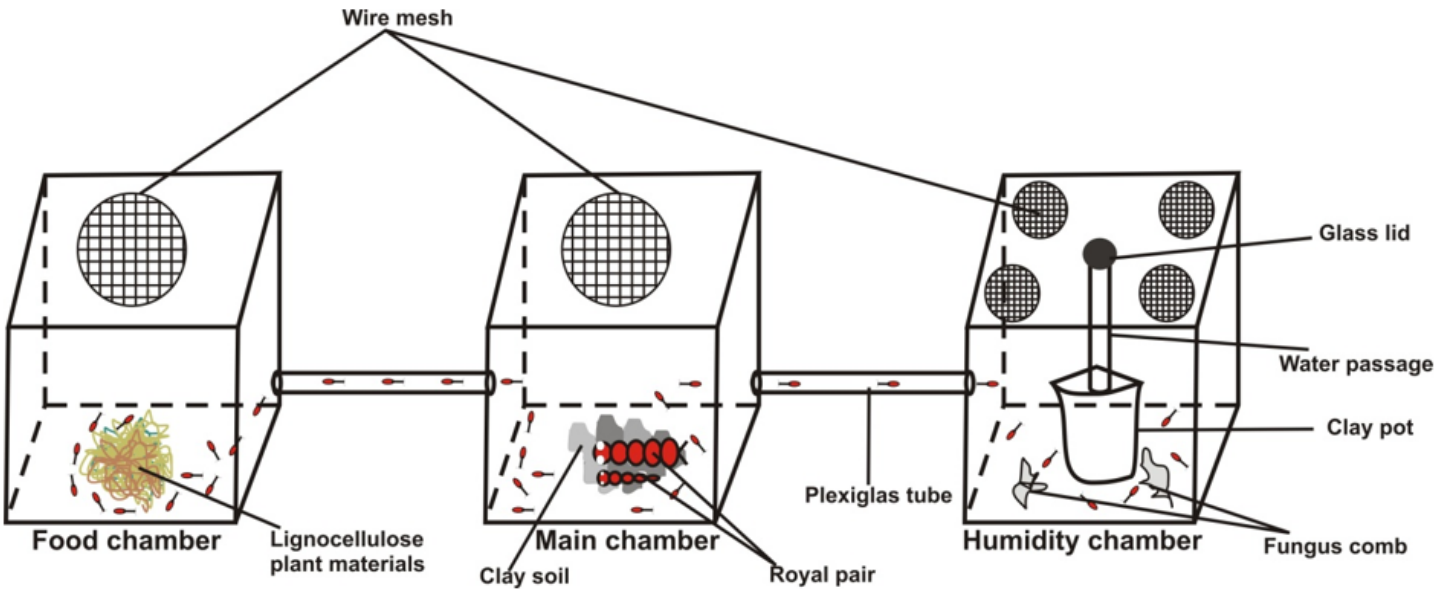

Figure 1

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

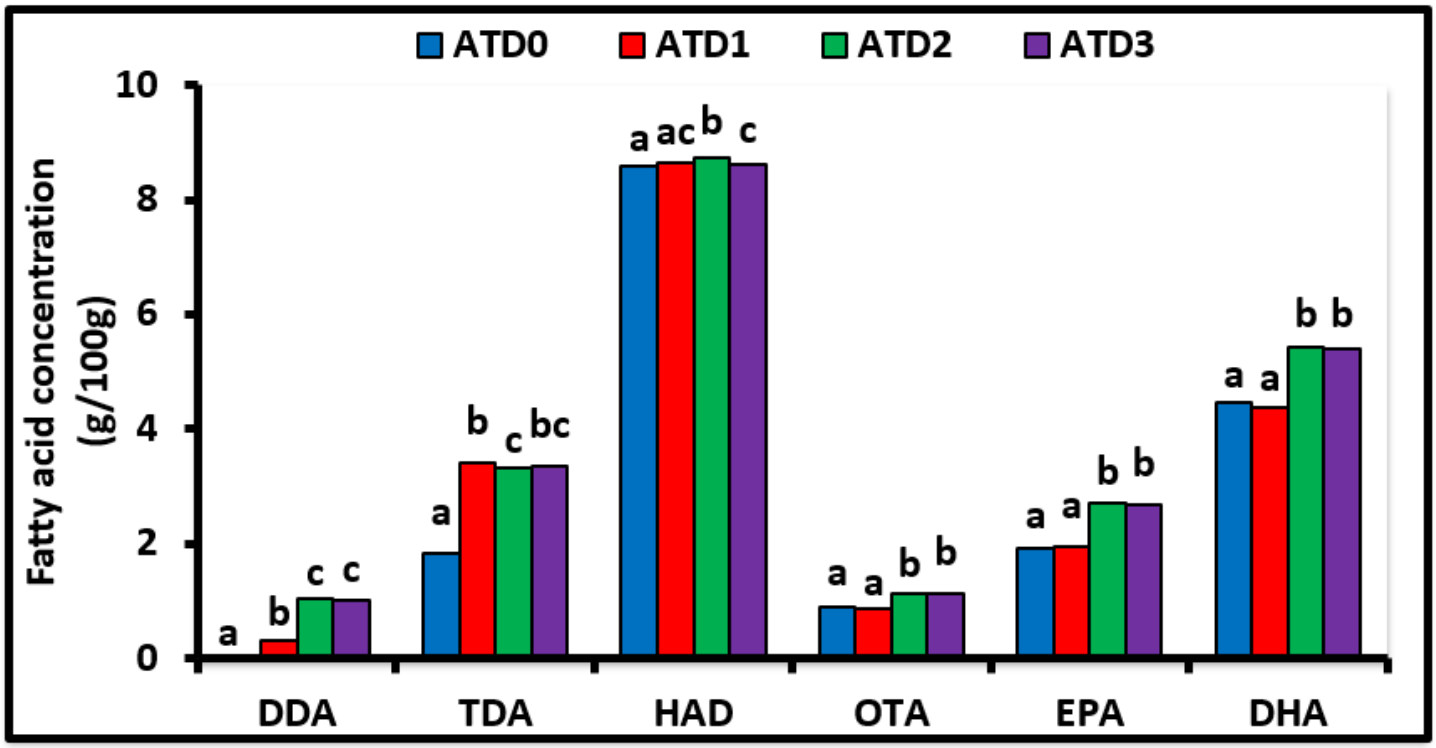

Figure 2

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.docx