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Enhancing performance and fatty acid composition of Nile tilapia (*Oreochromis niloticus*) from housefly maggot (*Musca domestica*) feeds

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Abstract. *Lobina AA, Mkupasi EM. 2020. Enhancing performance and fatty acid composition of Nile tilapia (*Oreochromis niloticus*) from housefly maggot (*Musca domestica*) feeds. Bioteknologi 17: 81-89.* This study was conducted to compare the effect of three substrates on the yield and composition of housefly (*Musca domestica*) maggots (HFM); assessed the growth performance of *Oreochromis niloticus* (Linnaeus, 1758) fed HFM were mixed with other ingredients, and investigated the enhancement of omega 3 fatty acids composition in the produced *O. niloticus*. The substrates for HFM production were poultry manure (HFMChick), *Lemna* species of freshwater macrophytes (HFMLemn), and *Eucheuma* species of marine macrophytes (HFMEuch). The HFM was then used to formulate eleven diets: nine isonitrogenous diets with 35% crude protein, one diet of 5% fish meal, and the other with soybean meal. Diets were named SBM, FM, HFMChick, HFMLemn, and HFMEuch, denoting soybean meal, fish meal, and HFM cultured on poultry manure, HFM cultured on *Lemna* species of freshwater macrophyte, and HFM cultured on *Eucheuma* species of marine macrophyte, respectively. A feeding trial was carried out on triplicate groups of ten fish (1.9-2.2 g) in recirculation aquaculture systems (RAS). The fish were fed up to 5% of their body weight twice daily throughout the experimental period. The effect of inclusion levels of HFM and other diets on fish growth feed utilization and *ω*-3FAs were determined. A gas chromatography-mass spectrometer (GC-MS) was used to analyze the composition of Omega 3 fatty acids. Results showed that the yields of HFM from poultry manure and *Eucheuma* species of marine macrophytes (HFMEuch) substrates were significantly higher ($P<0.05$) than those from *Lemna* species of freshwater macrophyte. The protein content of HFM from *Eucheuma* species of macrophyte was significantly higher ($P<0.05$) than those from poultry manure and *Lemna* species of freshwater macrophyte. Fish fed on diets containing HFMEuch and FM had significantly higher ($P<0.05$) growth performance compared to fish fed on HFMChick, HFMLemn, and SBM diets. Thirty-two (32) types of FAs with different saturation levels were detected. The fish-fed HFM cultured on *Eucheuma* species had the highest composition of FAs (32) compared to others. In conclusion, poultry manure substrates showed better yield results for culturing HFM than other substrates. *Eucheuma* species can be used to culture HFM as an alternative feed ingredient to improve the performance and composition of *ω*-3FAs in cultured *O. niloticus*.

Keywords: Aquatic macrophytes, fatty acid, gas chromatography-mass spectrometer, Nile tilapia

INTRODUCTION

Globally, there is an increased demand for protein for human and animal consumption. Fisheries and aquaculture contribute up to 17% of the global population's intake (FAO 2014). Approximately 85% (130.8 million MT) of total fish production in 2011 was used for human consumption, while the remaining (23.2 million MT) was used for non-human uses (Barbaroux et al. 2012). According to FAO (2014), from 2006 to 2012, global capture fisheries production remained stagnant at around 90 million MT, and it is believed the trend will remain the same until 2030. Thus aquaculture has been identified as an alternative to sustaining demand for fish as global aquaculture production is expected to increase to approximately 120 million MT by 2030 (FAO 2014).

Limiting factors in the growth of the aquaculture industry may include both the scarcity and high cost of key ingredients used in making fish feeds (Aniebo et al. 2009; Bureau et al. 2009; Huntington and Hasan 2009; Dedeke et al. 2013). For aquaculture to meet the future demand for fish protein, quality ingredients must be available in the required quantities. Conventionally, fishmeal (FM) and/or legumes and cereals have been used as protein and energy sources, respectively (Craig and Helfrich 2002; Huntington and Hasan 2009; Chapman and Miles 2015). However, in formulating nutritionally balanced fish diets, FM is the preferred dietary protein source because of its nutritional quality and palatability properties (Tacon and Metian 2008; Huntington and Hasan 2009; Mohanta et al. 2013).

Tilapia is one of the world's most important farmed freshwater fish species, as it is an important source of human food (FAO 2014). Global tilapia production is expected to double from 4.3 million tons to 7.3 million tons between 2010 and 2030 (FAO 2012). Furthermore, the species is expected to contribute 60% to total freshwater aquaculture production in 2025 (FAO 2016).

In Tanzania, aquaculture production accounts for a small proportion of the total fish produced at the national level. However, it contributed about 2.1 billion tons to the national GDP in 2018/2019 (MLFD 2019). In Tanzania, aquaculture is dominated by extensive and semi-intensive small-scale freshwater fish farming (Chenyambuga et al. 2014). The most popular cultured with high economic value species are Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758) and, to a lesser extent African catfish (*Clarias gariepinus* Burchell, 1822) (Kaliba et al. 2006; Novebrianto et al. 2011). *O. niloticus* is often used in aquaculture due to its desirable characteristics, like fast growth and high fecundity (Fitzsimmons 2000a,b; Negroni 2013; FAO 2014). In addition, *O. niloticus* tolerate a wide range of environmental parameters and have resistance to parasites, and of their suitability in a wide range of farming systems, they are becoming increasingly popular worldwide (Allanson and Noble 1964; Behrends et al. 1990; Fitzsimmons et al. 2014).

Fish are typically a good source of omega 3. However, freshwater fish, including tilapias, do not contain significant amounts of ω-3 fatty acids, especially nutritionally important docosahexaenoic acid (DHA) and eicosatetraenoic acid (EPA) (Silva et al. 2014). Earlier studies revealed that supplementing Tilapia feed with ω-3 fatty acids can increase the amount of these fatty acids in the muscle tissue (Tiffany et al. 2016). EPA and DHA are essential fatty acids that must be obtained from food (Whelan 2009). The fish feeds currently in use do not significantly improve the levels of omega 3 in farmed fish, hence alternative feeds must be identified.

This study aimed to: (i) Assess the effect of different substrates on yield and composition of cultured housefly (*Musca domestica*) maggots (HFM) meal; (ii) Evaluate the growth and feed utilization of *O. niloticus* fed on feed with HFM meal cultured on selected aquatic macrophytes; (iii) Assess the ω-3 fatty acids (PUFAs) content in *O. niloticus* fed diets containing HFM meals cultured on selected aquatic macrophytes.

MATERIALS AND METHODS

Description of the study area

The study was conducted at Aquaculture Research Facility within the Department of Animal, Aquaculture, and Range Science of Sokoine University of Agriculture (SUA), Morogoro, Tanzania. The present study was conducted from February to July 2018. SUA is located about 2.5 km South of Morogoro Municipality at an altitude of 550 m above sea level, with monthly mean minimum and maximum temperatures being 14.2°C to 35.5°C, respectively. Morogoro receives approximately 880 mm of bimodal rainfall annually, ranging from 29% to 96%.

Macrophyte collection and preparation

Two species of aquatic macrophyte, *Lemna* and *Eucheuma*, were collected from Lake Victoria and the Indian Ocean, respectively. *Lemna* was sorted to remove unwanted materials and debris and fermented for three days to obtain an offal odor to attract houseflies. *Eucheuma* was cleaned with fresh water to remove sand and reduce salt content. Fresh poultry manure was collected and transported to SUA.

Maggots production

Maggots were produced using three different substrates in triplicates. The substrates were poultry manure (control), marine macrophyte (*Euchaema*), and freshwater macrophyte (*Lemna*). The culture chamber was in a 2.7 L plastic container, were 2.5 kg of each substrate and 0.5 kg of poultry offal as the attractant was added and left open for eight hours to allow houseflies to lay eggs. Culturing was done indoors in triplicates for each substrate, as done by Nzamujo (2001) and Devic et al. (2014). The mixture was wrapped with a net with a mesh size of 1.2 mm to allow for the easy harvesting of maggots. Observations on the development of the maggots were recorded daily. Eggs hatched within two days and were left for an additional two days to develop into mature maggots. The mature maggots were harvested according to Sogbesan et al. (2006) and blanched with hot water at 100°C for 10 seconds. Thereafter, they were weighed to determine the total wet weight per harvest. The maggots were oven-dried at 60°C for 24 hours to constant weight, cooled, and ground into powdery form as maggot meal using a grinder machine (sieve 1mm). The samples for maggot meal were taken to a laboratory for proximate analysis.

Setup of the feeding experiment

The experiment was conducted at the SUA aquaculture research facility. This is a recirculation system that has two large tanks and five medium water tanks. The upper tank receives clean water from a major water pipe to the inner system. Used water from the inner system passed through several pipes to the filtering tanks sequentially. From the lower large tank, a pumping machine pumps treated water to the upper tank and repeatedly to the inner system for reuse.

Five diets were formulated and randomly allocated in triplicates of 15 rearing tanks (Table 1). Each rearing tank was stocked with 10 fingerlings with an initial mean weight of 2.07±0.12 g, totaling 30 fingerlings per treatment. Experimental fish were fed twice a day from 0930 and 0945 hrs and 1630 to 1645 hrs according to their feeding response, while limited to 5% of their body weight. The amount of feed was adjusted in response to changes in fish's body weights. Rearing tanks were siphoned twice daily to enhance aeration, remove uneaten food, and avoid the risks of infection and diseases.

Proximate analysis of diets formulated

The nutrient contents of all diets were determined using procedures described by AOAC (2005). First, dry matter and ash were determined by weighing 1 gram of the samples by using a 160 g capacity analytical weighing balance (Precisa 180A, Oerlikon, Switzerland), oven-drying (E 115, WTB binder 7200, Germany) at 700 C to constant weight, re-weighing, and ashing the samples in Muffle furnace (N31R, Nabertherm, West Germany) at 5000 C for three hours. The crude protein was determined by weighing the samples (Presica 180A, Oerlikon, Switzerland) followed by three stages of the Kjeldahl system, namely digestion (Digestion System 12 1009, Digester, Tecator, Sweden), distillation (2200 Kjeltec Auto Distillation, Foss Tecator, Sweden) and titration (Digitrate, Tecator, Sweden). This was multiplied by the factor of 6.25 to get the amount of crude protein.

Table 1. Diet formulation and grouping

	SBM	FM,	HFMChick	HFMLemn	HFMEuch
SBM	51.49	43.90	12.72	10.80	7.50
FM	0.00	5.00	0.00	0.00	0.00
MM	37.70	40.33	40.15	43.33	44.75
HMM	0.00	0.00	35.00	35.00	35.00
CRM	2.49	2.48	3.90	2.53	4.33
CGM	0.00	0.00	0.00	0.00	0.00
SFO	4.32	4.29	0.46	0.47	0.65
Premix	1.00	1.00	1.00	1.00	1.00
Meth	1.00	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00	1.00
MCP	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00

Note: SBM: Soya bean meal 35%, FM: Soya bean meal with 5% fish meal, HFMEuch:Housefly maggot meal *Eucheuma* 35%, HFMLemn: Housefly maggot meal *Lemna* 35%, HFMChick: Housefly maggots poultry 35%. 35%: Amount of protein on each diet

Crude fat was determined by weighing the samples (Presica 180A, Oerlicon, Switzerland), and fat was extracted by the Soxhlet extraction method (Soxtec system HT 1043 Extraction unit, Tecator, Sweden). After that, the extraction cups containing fat material were dried at 105°C for 30 minutes to remove traces of moisture. Then, the cups containing fat material were cooled in a desiccator for about 10 minutes and weighed to calculate the amount of crude fat in the feeds.

The crude fiber was obtained by weighing samples (Presica 180A, Oerlicon, Switzerland) into filter bags, digesting the sample in weak sulfuric acid and rinsing in weak NaOH solution at 100°C for 30 minutes in an Ankom machine (ANKOM220, ANKOM Technology, USA), and followed by washing and rinsing using distilled water. Then weak sodium hydroxide (alkaline) solution was added and heated at 100°C for another 30 minutes to remove acids in the samples. It was then rinsed with distilled water before acetone was added to remove the fat remaining in the residues. The samples were then dried and ashed. The difference between the residues and ash weight gave the amount of crude fiber.

Growth trials using maggots diet cultured on different substrates were conducted, involving fifteen (15) treatments whereby nine (9) treatments were experimental diets using maggots cultured on three different substrates, namely poultry manure (control, treatment one), *Eucheuma* species of marine macrophyte (treatment two), and *Lemna* species of freshwater macrophyte (treatment three). The other six (6) treatments were other diets most fish farmers commonly use. One diet contained only a soybean meal, while the other contained a soybean meal with 5% fishmeal. Feed utilization and growth rate were measured. Subsequent body weights were weighed and recorded after every seven days. Before weighing, fish were starved for a day. The body weights of fish from each replicate were recorded, and mean weights were calculated. Performance characteristics were calculated using the following formulae by Olvera-Novoa et al. (1990).

Average daily weight gain (ADWG)

$$ADWG = \frac{Final\ bodyweight\ (g) - Initial\ bodyweight(g)}{\text{Time (days)}}$$

Specific Growth Rates (SGR %)

$$\%SGR = \frac{FLN(Final\ bodyweight) - LN(Initial\ bodyweight)X100}{\text{Experimental period (days)}}$$

Survival Rate (SR)

$$SR = \frac{Final\ number\ of\ fish\ harvest \times 100}{Initial\ number\ of\ fish\ at\ stocking}$$

Protein Efficiency Ratio (PER)

$$PER = \frac{Bodyweight\ gain\ (g)}{Crude\ protein\ intake\ (g)}.$$

Feed Conversion Ratio (FCR)

$$FCR = \frac{Feedsuppied\ (g)}{Bodyweight\ gain\ (g)}$$

Fish sample collection

At the end of the experiment, 150 fish were collected from 15 rearing tanks. Ten (10) individuals were collected per rearing tank. After collection, each sample was stored in a plastic bag, preserved using dry ice, and later frozen at -20°C before lipid analysis. Frozen samples were transported to the Zoology laboratories at the University of Dar es Salaam, Tanzania, for lipid extraction and fatty acids analysis.

Sample analysis

Sample preparation

The fish samples were freeze-dried to remove excess water. For each sample, a piece weighed between 10-20 g was grinded to soften the muscles.

Lipid extraction

Lipid extraction was done using a 2:1 methanol and chloroform ratio and minced with a vortex machine for 2 minutes, as Folch et al. (1957) described. Samples were stored in a refrigerator for 48 hours to speed up lipid extraction. Filtration was done using filter paper (GF/Fglass fiber filter) to separate tissues from obtaining filtrate solution. The addition of 1:1 methanol and chloroform was done to extract the remaining lipids from the tissues. The two layers formed by fish tissues (lipids and aqueous solution) were separated by using a separating funnel to obtain lipids. This was followed by adding sodium sulfate to remove traces of water from the lipids. Evaporation to remove chloroform was done in an air-conditioned room at 16°C for 24 hours.

Preparation of Fatty Acid Methyl Esters (FAMEs)

Methylation was done by using concentrated sulfuric acid methods to obtain FAMEs. Before derivatization, five (5) mg of lipid was suspended in 1ml of toluene. Then, 2 mls of methanoic sulfuric acid (1%v/v) was added to each sample in vials and sealed. The samples were heated in a stopper tube at a temperature of 50°C overnight for 16 hours. This was followed by adding 2 mls of water containing sodium bicarbonate (2%: w/v) to each sample to neutralize the acid. Next, extractions were done by adding a hexane/diethyl ester (1:1, by Vol; 2×5 m). Finally, evaporated locally in an air-conditioned room at 16°C for three days to remove the acid.

Analysis of fatty acids

Determination of types and levels of ω-3 PUFAs was done using a Gas chromatograph Mass Spectrometer (GC MS-QP2010 Ultra), which is equipped with a flame ionization detector, (FID). 1ULofFAME in hexane was injected into the GCMS in a split ratio -1.0. Helium was used as a carrier gas at a 2 Ml/min flow rate. The injector temperature was 250°C. The temperature was then programmed as follows: column oven was set at 90°C, held for two minutes, and then increased to 260°C, held for five minutes, with a total 41 minutes. The ω-3 PUFAs (EPA, ALA, and DPA) were identified by comparing their retention time with those of commercial standards. These ω-3 PUFAs commercial standards were brought from Fluka –United States of America (USA).

Water quality monitoring

Water quality is an important factor in pond fish production, throughout the experiment period, water quality parameters, including temperature, pH, and dissolved oxygen (DO) were monitored weekly. These were measured using a GWQ-DO280 Dissolved Oxygen Meter.

Data analysis

Data were analyzed using Statistical Package for Social Sciences program version 10 (SPSS Richmond, VA, USA) as described by Dytham (2013). Data were tested for normality and homogeneity of variance before being analyzed using One-way analysis of variance (ANOVA). Treatment means were considered significant at P<0.05. Post–hoc analysis was also done where significant differences existed between treatment means, using Tukey's Honest Significant Difference Test (Steele and Torrie 1980). The model was:

$$Y_{ij} = \mu + T_i + L_{ij} + E_{ij}$$

Where:

μ : General means.
Ti : the effects of treatment (I:1, 2)
Eij : Residual Error
Lij : Levels within treatments (j:1, 2, 3, 4, 5)
Yij : Observation value (nutritional composition of experimental meals, fish growth performance and feed utilization)

RESULTS AND DISCUSSION

Yield and nutritional composition of HFM produced from different media

The yield of HFM differed significantly among substrate treatments (P<0.05). Yield from poultry manure was significantly higher than from *Eucheuma* species and *Lemna* species of macrophyte, as shown in Table 2. There was a significant difference in the protein content of HFM harvested from three culturing substrates (P<0.05). HFM from *Eucheuma* marine macrophyte species had significantly higher crude protein content (P<0.05) than those from poultry manure and *Lemna* species of freshwater macrophyte substrate. Crude fiber and ether extracts of the maggots produced from all substrates had no significant differences (P>0.05). The maggots from poultry manure had significantly higher (P<0.05) ash content than those from *Lemna* and *Eucheuma* substrates (Table 2).

The chemical composition of formulated diets is shown in Table 3. Crude fiber content was between accepted ranges for *O. niloticus* production.

Feed intake, growth performance, and feed utilization of cultured *Oreochromis niloticus*

The diet consumption (feed intake) of HFMEuch and FM were good throughout the experiment, while the feed intake was poor in fish-fed SBM, HFMChick, and HFMLemn. No feed-related mortality was observed during the experiment. A significant (P<0.05) increase in the FBWT and ADG was observed in fish-fed FM and HFMEuch, compared to those fed with the control diet (SBM). However, fish-fed diets HFMLemn and HFMChick had comparable FBWT and ADG to those fed the SBM diet. There was no significant difference in IBWT and SGR among the treatment groups. HFMEuch showed lower FCR among other treatments. PER was found to be significantly different (P<0.05) in the fish fed with HFMEuch and FM compared to the SBM diet, but not those fed HFMChick and HFMlemn. When compared to the control diet (SBM), significantly high FI and BWTG were observed in the fish fed with all diets (P<0.05) (Table 4).

Composition of fatty acids in cultured *Oreochromis niloticus*

A total of 32 FAs were identified in *O. niloticus* fed five formulated diets (Table 5). The unsaturated FAs were relatively more (26) than saturated ones (6). Of the 26 unsaturated FAs, 17 were PUFAs, and 9 were MUFAs.

Among the 17 types of PUFAs, the Omega 6 PUFAs were (12), followed by Omega 3 PUFAs (4), and Omega 9 PUFAs was (1). The most dominant saturated fatty acids (SAFAs) were palmitic acid, pentadecanoic acid, stearic (octadecanoic) acid, tetracosanoic acid and heptadecanoic acid.

The dominant omega 3 PUFAs were docosatrienoic acid, docosapentanoic acid, docosahexaenoic acid and eicosatetraenoic acid. The principal Omega 6 PUFAs were gamma linoleic acid and arachidonic acid. Omega 9 PUFAs was eicosadienoic acid. Oleic acid was the dominant MUFA. Alfa linolenic and gamma linoleic FAs were also found. The ratio of PUFAs to SAFAs was 2:2:1, and the ratio of Omega 6 to Omega 3 PUFAs in five diets was 1:1:1.

The results showed that fish-fed HFMEuch had the highest FA composition of FAs (32) compared to other diets (Table 6). Some FAs found in fish-fed HFMEuch included 7, 10-Hexadecadienoic acid, alfa linolenic acid, 9-Octadecenoic acid, and 10, 13-Octadecadienoic acid.

Water quality parameters

There was no significant difference between dissolved oxygen (DO) and temperature. However, a significant difference ($p<0.05$) was shown in pH among different treatments (Table 7).

Discussion

The results showed differences in yield and composition of HFM cultured in different substrates. The high yield of HFM from poultry manure probably was due to the substrate's long-lasting odor, which strongly attracted more flies. A similar observation was made by Nzamujo (1999) and Agbeko et al. (2014), who reported that the more the quantity and long-lasting odor of substrate, the greater number of flies and the greater the number of maggots produced. Calvert (1979) and Patricia and Salas (2007) also reported a similar observation, where chicken manure produced many maggots compared to cow dung manure.

Table 2. Yield (g/kg) and chemical composition of HFM (% dry matter) (mean ± SD)

Item	Substrate		
	Eucheuma	Lemna	Poultry manure
Yield	610 ± 15.4 ^{a}	584.8 ± 30.7 ^{c}	857.0 ± 2.0 ^{b}
Dry matter	97.52 ± 0.47^{a}	96.42 ± 1.46^{a}	95.71 ± 0.08^{a}
Crude protein	53.55 ± 0.81^{a}	40.43 ± 0.21^{c}	42.61 ± 0.22^{b}
Crude fiber	5.01 ± 0.26^{a}	6.00 ± 0.25^{a}	5.71 ± 0.25^{a}
Ether Extract	20.40 ± 0.42^{a}	19.07 ± 0.46^{a}	20.01 ± 0.06^{a}
Ash	10.70 ± 0.48^{a}	11.13 ± 0.23^{a}	10.45 ± 0.18^{a}

Note: Means with different superscripts within a row are significant different at ($p<0.05$).

Table 3. Chemical composition of formulated diets

Ingredient (%)	SBM	FM	Diets		
			HFMChick	HFMLemn	HFMEuch
Dry matter	90.75	90.89	88.42	91.86	91.40
Crude protein	41.73	46.01	40.66	46.03	50.00
Ether extract	18.98	19.20	20.00	19.07	20.40
Crude fiber	1.45	1.90	1.22	0.86	1.08
Ash	7.57	7.66	7.31	7.69	7.73

Table 4. Growth performance and nutrient utilization of *Oreochromis niloticus* fed different diets (mean±SE)

Parameter	SBM	FM	Diets		
			HFMChick	HFMLemn	HFMEuch
INBWT (g)	2.09 ± 0.12^{a}	2.23 ± 0.12^{a}	1.89 ± 0.12^{a}	2.07 ± 0.12^{a}	2.09 ± 0.12^{a}
FBWT (g)	5.62 ± 0.26^{c}	6.63 ± 0.26^{ab}	6.21 ± 0.26^{abc}	6.15 ± 0.26^{bc}	7.01 ± 0.26^{a}
BWTG (g)	3.53 ± 0.28^{b}	4.40 ± 0.28^{ab}	4.33 ± 0.28^{ab}	4.08 ± 0.28^{ab}	4.91 ± 0.28^{a}
ADG (g/day)	0.063±0.005^{b}	0.078±0.005^{ab}	0.077±0.005^{ab}	0.073±0.005^{ab}	0.087±0.005^{a}
SGR (%day)	1.76 ± 0.12^{a}	1.95 ± 0.12^{a}	2.13 ± 0.12^{a}	1.95 ± 0.12^{a}	2.15 ± 0.12^{a}
FI (g/fish/day)	0.159±0.004^{b}	0.183 ±0.004^{a}	0.160±0.004^{b}	0.166±0.004^{b}	0.192±0.004^{a}
FCR	1.41 ± 0.22^{a}	1.31 ± 0.22^{a}	1.62 ± 0.22^{a}	1.33 ± 0.22^{a}	0.11 ± 0.22^{b}
PER	1.26 ± 0.08^{c}	1.64 ± 0.09^{ad}	1.49 ± 0.05^{b}	1.35 ± 0.01^{b}	1.81 ± 0.03^{a}
SR (%)	96.66 ± 4.47^{a}	96.66 ± 4.47^{a}	90.00 ± 4.47^{a}	93.33 ± 4.47^{a}	90.00 ± 4.47^{a}

Note (Table 1-7): INBWT: Initial body weight, FBWT: Final body weight, BWTG: Body weight gain, ADG: Average daily gain, SGR: Specific growth rate, FI: Feed intake, FCR: Feed conversion ratio, PER: Protein efficiency ratio, SR: survival rate, FM: Fish Meal, SBM: Soybean Meal, HFMchick: Housefly maggots diets cultured on poultry manure, HFMLemn: Housefly maggot diets cultured on *Lemna*species, HFMEuch: Housefly maggot diets cultured on *Eucheuma* species. Means with different superscripts within a row are significant different at ($p<0.05$)

Table 5. Fatty acids composition in *Oreochromis niloticus* fed five diets including HFM

Fatty acids	SBM	FM	HFMChick	HFMLemn	HFMEuch	Level of saturation
Nonadecanoic acid	+	+	+	+	+	saturated
Tricosanoic acid	-	+	+	-	+	saturated
Myristic acid	+	+	+	+	+	saturated
Palmitic acid	+	+	+	+	+	saturated
Stearic acid	+	+	+	+	+	saturated
Heptadecanoic acid	+	+	+	+	+	saturated
Tetradecenoic acid	-	+	-	-	+	MUFAs
9-Octadecenoic acid	-	-	-	-	+	MUFAs
11-Octadecenoic acid	-	-	-	-	+	MUFAs
Heptadecenoic acid	+	+	+	+	+	MUFAs
Hexadecenoic acid	+	+	+	+	+	MUFAs
11-Eicosenoic acid	-	+	+	-	+	MUFAs
Oleic acid	+	+	+	+	+	MUFAs
Tetradecenoate	-	-	+	+	+	MUFAs
Eicosadienoic acid	-	+	-	-	+	MUFAs
11,13- Eicosadienoic acid	-	+	+	-	+	PUFAs
11,14- Eicosadienoic acid	-	+	+	-	+	PUFAs
Linoleic acid	+	+	+	+	+	PUFAs
Arachidonic acid	+	+	+	+	+	PUFAs
Eicosatrienoic acid	-	+	-	-	+	PUFAs
Docosatetraenoic acid	-	+	+	+	+	PUFAs
Docosahexaenoic acid	+	+	+	+	+	PUFAs
Alfa Linolenic acid	-	-	-	-	+	PUFAs
Eicosatrienoic acid	-	+	+	+	+	PUFAs
Eicosapentanoic acid	+	+	+	+	+	PUFAs
4,7,10,13,16-Docosapentaenoate	+	+	+	+	+	PUFAs
Docosapentaenoic acid	-	+	+	+	+	PUFAs
Eicosatetraenoic acid	+	+	+	+	+	PUFAs
8,11-Octadecadienoic acid	+	+	+	+	+	unsaturated
10,13-Octadecadienoic acid	-	-	-	-	+	unsaturated
Eicosadienoic acid	-	+	+	+	+	unsaturated
7,10-Hexadecadienoic acid	-	-	-	-	+	unsaturated

Table 6. Accumulation of Omega-3 PUFAs and Omega-6 PUFAs found in *Oreochromis nilotucus*

Parameters	SBM (D1)	FM (D2)	Diets		
			HFMChick (D3)	HFMLemn (D4)	HFMEuch (D5)
ΣPUFAS	1.99 ± 0.01[a]	2.84 ±0.10[b]	0.88 ± 0.12[a]	4.81 ± 0.05[c]	9.52 ± 0.82[c]
Σω-3 PUFAs	1.54 ± 0.06[a]	2.83 ±0.16[b]	0.69 ± 0.29[a]	2.73 ± 0.38[b]	4.07 ± 0.91[c]
Σω-6 PUFAs	0.43 ± 0.05 [a]	0.33 ± 0.0 [a]	0.13 ± 0.04 [a]	1.09 ± 0.0286[a]	4.54 ± 0.37[c]

Note: ΣPUFAS: sum of polyunsaturated fatty acids, Σω-3 PUFAs: sum of Omega 3 polyunsaturated fatty acids, Σω-6 PUFAs: sum of Omega 6 polyunsaturated fatty acids. Means with different superscripts within a row are significant different at ($p<0.05$)

Table 7. Water quality parameters recorded during the feeding experiment

Parameter	SBM	FM	Diets		
			HFMChick	HFMLemn	HFMEuch
DO (mg/L)	7.42 ± 0.04[a]	7.47 ± 0.04[a]	7.34 ± 0.04[a]	7.46 ± 0.04[a]	7.49 ± 0.04[a]
pH	7.28 ± 0.09[ab]	7.40 ± 0.09[a]	7.09 ± 0.09[bc]	7.02 ± 0.09[bc]	6.98 ± 0.09[c]
Temperature (°C)	24.17 ±0.06[a]	24.22 ±0.06[a]	24.14 ±0.06[a]	24.22 ± 0.06[a]	24.27 ± 0.06[a]

Note: DO: Dissolved oxygen. Means with different superscripts within a row are significant different at ($p<0.05$).

The results showed that a mean crude protein of 45.53% of HFM was produced from the three substrates. There was no significant difference in crude protein contents of produced maggots from different substrates. However, *Eucheuma* marine macrophyte species produced maggots with a higher crude protein content of 53.55% compared to others. The mean of the produced maggots was 55.2%, as previously reported (Nzamujo 1999; Adeniji 2007; Odesanya et al. 2011). The amount of crude protein in the produced maggots reported depended on the nutrients present in the substrate and the ability of the organisms to feed and assimilate them (Patricia and Salas 2007; Agbeko et al. 2014).

Despite a high yield of HFM from poultry manure, the maggots had a relatively low crude protein content of 42.61%. The poultry manure used in this study could have been of low quality, which might be attributed to the loss of nitrogen due to its conversion to ammonia. Similarly, the negative impact of manure storage time on its nutrient content and subsequent nutritional quality of maggots cultured there has been previously described (Horn 1998). Regarding crude protein contents, *Eucheuma* species of marine macrophyte proved to be a better substrate for culturing HFM.

The number of ether extracts of maggots was not significantly different among the substrates. The mean level was within the recommended level of 10-25 (Ogunji et al. 2006). Present results were nearly equal to 19.3%EE reported by (Nzamujo 1999) but higher than those reported in other studies (Okah and Onwujiariri 2012).

Regarding ash content in produced HFM meals, there was no significant difference among the substrates. The average ash content of the experimental HFM diets in the present study was 10 to 19%. These results agree with other studies' results (Nzamujo 1999; Yaqub 1999; Okah and Onwujiariri 2012).

Regarding fish performance, the fish fed on the HFMEuch diet were superior to those fed on other diets. Higher growth performance of *O. niloticus* fed on HFMEuch diets reflects palatability of maggots cultured in *Eucheuma* species of the marine macrophyte. In addition, the high acceptability of HFM meal made it a suitable ingredient for fish feed leading to increased feed utilization and growth performance (Makkar et al. 2014; Ogunji et al. 2008). This shows that *Eucheuma* species can produce more nutritious maggots than other substrates, such as poultry manure and *Lemna* of freshwater macrophytes.

Feed conversion ratio (FCR) is used to measure feed utilization efficiency. The present study obtained better FCR from a fish-fed HFM diet. This was similar to findings previously reported (Ogunji et al. 2006; Ogunji et al. 2008; Jabir et al. 2012; Omoyinmi and Olaoye 2012; Mekhamar et al. 2015). However, the FCR of the HFMEuch diet was relatively lower than those reported in previous studies (Mohanta et al. 2013; Olaniyi and Salau 2013) whose values ranged from 3.13 to 5.07. This resulted in higher body weight gain of fish fed HFMEuch. The FCR of the present study was better than that reported by Yaqub (1999), who fed fish with HFM from different substrates.

The present results are supported by previous findings, which showed that a diet with maggot and fishmeal was well accepted and well utilized by the fish (Jonathan 2012; Monebi and Ugwumba 2012; Omoyinmi and Olaoye 2012). Furthermore, according to Coyle et al. (2004), the inclusion of HFM in the fish diet produced the highest PER, likely due to good protein and other nutrients such as fatty acids. This agrees with previous findings that recommended the use of HFM in the diets of the fish to improve feed intake and utilization for better growth performance (Stafford and Tacon, 1988; Dedeke et al. 2010; Hasanuzzaman et al. 2010; Sogbesan 2014).

The slow growth performance of fish fed on HFMLemn and HFMChick diets could be attributed to factors such as the presence of anti-nutritional factors and the unpalatability of the diets. However, the present results are like observations made by Ogunji et al. (2006), who reported different growth performances of *Carassius auratus* (Linnaeus, 1758) when fed HFM cultured from different substrates. The overall weight of the fish fed HFMEuch and FM-based diets were higher than those fed SBM, HFMChick, and HFMLemn-based diets. This reflects the better condition of fish fed on FM and HFMEuch-based diets, regardless of the protein sources.

Fish-fed diets with HFMEuch and FM showed high feed intake, probably due to the high palatability of the diets. Meena (2015) reported that palatability of the diet is a factor that largely impacts fish acceptance of the feed. In addition, the palatability of these diets mighty is attributed to the nutrient content and good odor, as previously reported (Hilton 1989; Sogbesan et al. 2003; Makkar et al. 2014). Nevertheless, fish-fed HFMChick and HFMLemn diets had the same feed intake as those fed the SBM diet.

Thirty-two types of FAs with different saturation levels were found in *O. niloticus* in this study. These results are comparable to Mohamed and Al-Sabahi (2011) results, who identified 33 FAs of different saturation levels in commercial *O. niloticus.* This study registered that the saturated (SAFAs) were 6 and unsaturated were 26, including 17 PUFAs and 9 MUFAs. Similarly, Mwanja et al. (2010) observed more categories of unsaturated FAs than saturated FAs. The more unsaturated than saturated FAs observed in the present study is probably due to the type of substrates used to culture the HFM. Henderson (1996) reported that aquatic plants and invertebrates such as HFM contain more unsaturated FAs than SAFAs.

The availability of Omega-3 PUFAs such as EPA, DPA, and DHA was higher in fish fed HFMEuch and FM diets than in those fed with SBM, HFMChick, and HFMLemn diets. This might be contributed to the de novo synthesis from alfa linolenic acid found in the diets. Bachok et al. (2006) reported that alfa linolenic acid is a short-chain FA that animals, including fish, do not synthesize. This is like reports by Zenebe et al. (1998) and Cintra et al. (2012), who described the different levels of FAs in *O. niloticus,* such as EPA and DHA, according to diets. Therefore, the higher availability of EPA, DPA, and DHA in *O. niloticus* is probably due to the elongation of ALA. These findings show that *O. niloticus* can be a good source of Omega-3 PUFAs to consumers when fed with good Omega-3 fatty acids. This further proves *Eucheuma* species of marine macrophyte to be superior in producing HFM with high Omega 3 content and transferring them to fed *O. niloticus*.

Water quality parameters from this study showed that the water temperature ranged from 24.17°C to 24.22°C. This temperature range has been reported as the optimum range for tilapia growth and yield (El-Sayed 2006). Other studies reported that the temperature range for normal tilapia development, reproduction and growth is between 20°C and 35°C (El-Sayed 2006). Similarly, pH and dissolved oxygen were within optimum ranges for tilapia growth. Other studies have shown that tilapia can improve survival at pH ranging from 5 to 10, but they do best if the pH ranges from 6 to 9 Cintra et al. (2012). Dissolved oxygen levels should be maintained above 5.0 ppm for best growth (Siddiqui et al. 1989). Dissolved oxygen levels between 3.0 and 5.0 ppm feeding should be reduced, and feeding should be stopped at dissolved oxygen levels below 3.0, Ogunji et al. (2006).

This study found that poultry manure substrate supports high yields of HFM, while *Eucheuma* of marine species macrophyte is a good substrate to produce HFM with high omega 3 fatty acids and higher crude protein level. The inclusion of HFM in fish feeds improved the performance of cultured *O. niloticus*, and *the Eucheuma* diet supported the high performance of *O. niloticus*. Fish produced from diets with HFM from *Eucheuma* of marine macrophytes had higher levels of fatty acids and proteins.

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