

**ENHANCING PERFORMANCE AND FATTY ACIDS COMPOSITION OF NILE
TILAPIA (*Oreochromis niloticus*) THROUGH HOUSEFLY MAGGOTS (*Musca
domestica*) MEAL CULTURED ON AQUATIC MACROPHYTES**

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN HEALTH
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ABSTRACT

Fish forms an integral part of human diet in Tanzania being rich in protein and Omega 3 fatty acids (ω -3FAs) which are important for growth and proper functioning of human body. The amount of ω -3FAs and performance of tilapia (*O. niloticus*), the most cultured and consumed fish in Tanzania reported to be low due to inadequate of quality feeds. This study was conducted to compare the effect of three substrates on yield and composition of housefly maggots (HFM); assessed growth performance of *O. niloticus* fed HFM were mixed with other ingredients and investigated the enhancement of omega 3 fatty acids composition in the produced *O. niloticus*. The used substrates for HFM production were poultry manure (HFMChick), *Lemna* species of freshwater macrophytes (HFMLemn) and *Eucheuma* species of marine macrophytes (HFMEuch). The produced housefly maggots were then used to formulate nine isonitrogenous diets with 35% crude protein and two diets; one contained 5% of fish meal and the other diet containing soybean meal as protein sources. Diets were named as SBM, FM, HFMChick, HFMLemn and HFMEuch, denoting soybean meal, fish meal, and housefly maggots cultured on poultry manure, housefly maggots cultured on *Lemna* species of freshwater macrophyte and housefly maggots cultured on *Eucheuma* species of marine macrophyte, respectively. Eight weeks feeding trial was carried out on triplicate groups of ten fish (1.9–2.2 g) in recirculation aquaculture systems (RAS). Fingerlings were randomly allocated to the treatments. The fish were fed up to 5% of their body weights twice daily from 09:30 to 09:45hours in the morning and 16:30 to 16:45 hours evening throughout the experimental period. Effect of inclusion levels of HFM and other diets on fish growth, feed utilization and ω -3FAs were determined. 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 good result for culturing HFM in terms of yield than other substrates. HFMEuch achieved high performance of *O. niloticus* with higher amount of ω -3FAs levels. *Eucheuma* species can be used to culture HFM as alternative feed ingredient to improve performance and composition of ω -3FAs in cultured *O. niloticus*.

DECLARATION

I, Anitha Ashery Lobina, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor concurrently being submitted in any other institution for degree award.

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(MSc. HAAR Student)

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Date

The above declaration is confirmed by:

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Dr. Ernatus M. Mkupasi
(Supervisor)

.....

Date

DEDICATION

This dissertation is dedicated to my mother Claudia Mwalambalo for her prayers and endless love that made my study-life a blessing; I also sincerely thank my husband Hosea Agai Mgaya who his prayers, patience and encouragement greatly contributed to the completion of this work.

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LIST OF ABBREVIATIONS AND AGRONOMY

ADG	Average Daily Gain
ALA	Alfa Linolenic Acid
ANOVA	One way Analysis of Variance
AOAC	Association of Official Analytical Chemists
BWTG	Body Weight Gain
DHA	Docosahexaenoic Acid
DM	Dry matter
DO	Dissolved Oxygen
DPA	Docosapentanoic Acid
EPA	Eicosapentanoic Acid
FAMES	Fatty Acid Methyl Ester
FAO	Food and Agriculture Organization of the United States
FAs	Fatty Acids
FBWT	Final Body Weight
FCR	Feed Conversion Ratio
FI	Feed Intake
FID	Flame Ionization Detector
FM	Fishmeal
GCMS	Gas Chromatograph Mass Spectrometer
HDL/LDL	High-Density Lipoprotein to Lower-Density Lipoprotein
HFMChick	Housefly Maggots Diets Cultured on Poultry Manure
HFMEuch	Housefly Maggots Cultured on <i>Eucheuma</i> species
HFMLemn	Housefly Maggots Diets Cultured on <i>Lemna</i> species

INBWT	Initial Body Weight
MLFD	Ministry of Livestock and Fisheries Development
mls	Mills
MUFAs	Monounsaturated Fatty Acids
°C	Degree Centigrade
pH	Potential of hydrogen
PUFAs	Polyunsaturated Fatty Acids
SAFAs	Saturated Fatty Acids
SBM	Soybean meal
SE	Standard Error
SGR	Specific Growth Rate
SPSS	Statistical Packaging for Social Sciences
SR	Survival Rate
SUA	Sokoine University of Agriculture
USA	United States of America
V/V	Volume by Volume

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The world is faced with increased demand of protein for human and animal consumption. Fisheries and aquaculture make a vital contribution towards meeting that demand and contribute up to 17% of global population's intake (FAO, 2014). In 2011 about 164 million metric tons (MT) of total fish production was made by capture fisheries and aquaculture. 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). Fish production from capture fisheries and aquaculture is threatened by over-exploitation, climate change, bad fishing practices and environment destruction (FAO, 2014). According to FAO (2014) from 2006 to 2012 global capture fisheries production has approximately remained stagnant at around 90 million MT and it is presented that the trend will remain the same until 2030. Stagnating capture fisheries have given way to the rise of aquaculture seen as an alternative to sustaining demand for fish (FAO, 2014). Global aquaculture production is expected to increase to approximately 120 million MT by 2030 from 66.6 million MT in 2012 (FAO, 2014).

Scarcity and high cost of key ingredients used in making fish feeds is a potential limiting factor in growth of aquaculture industry (Aniebo *et al.*, 2009; Bureau *et al.*, 2009; Huntington and Hasan, 2009; Dedeker *et al.*, 2013). In order for aquaculture to meet the future demand of fish protein, quality ingredients must be available in required quantities. Adequate quantities of nutritional feed enable fish to realize their growth potential and eventually bring profit to the industry. 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, 2015). In formulating nutritionally balanced fish diets, FM is the most preferred as a dietary protein source because of its nutritional quality and palatability properties (Tacon and Metian, 2008; Huntington and Hasan, 2009; Mohanta, 2013). Lack of feeds of required quality is one of major limitation to Nile tilapia farming in Tanzania (Chenyambuga *et al.*, 2014).

Tilapia is one of most important farmed freshwater fish species in the world including Tanzania. Its' industry has expanded since it has been an important source of food for human and being the third most important cultured fish group in the world (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). The species is expected to contribute to a 60% predicted total freshwater aquaculture production in 2025 (FAO, 2016). It is anticipated that, in 2020 global Nile tilapia production will continue to increase (Figure 1) (FAO, 2012).

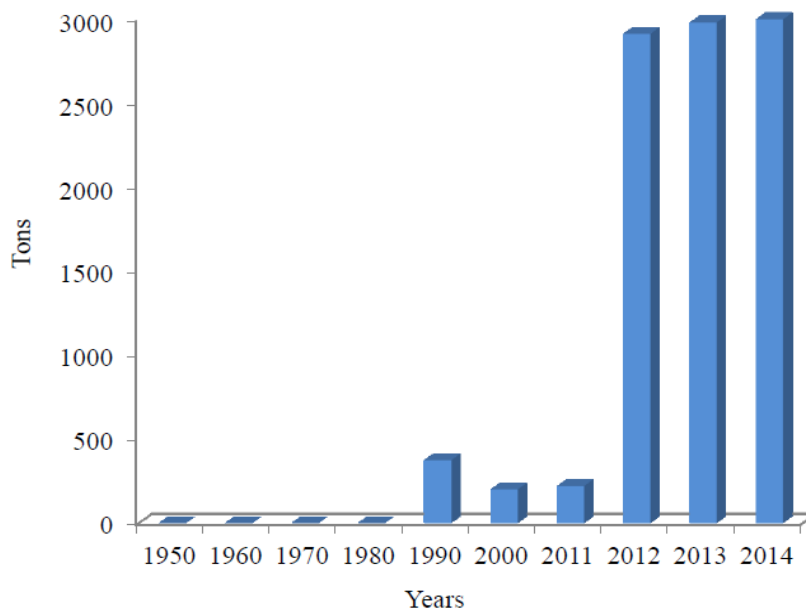


Figure 1: Global tilapia production (FAO Fishstat, 2016)

In Tanzania aquaculture production accounts for a small proportion of total fish produced at the national level. It contributed about 2.1 billion tonnes to the national GDP in the year 2018/2019 (MLFD, 2019). In the country aquaculture is dominated by small scale freshwater fish farming which is both extensive and semi-intensive (Chenyambuga *et al.*, 2014). Mostly cultured species is Nile tilapia (*O. niloticus*) and to a lesser extent African catfish (*Clarias gariepinus*) (Kaliba *et al.*, 2006). Nile tilapia is much involved in aquaculture due to its desirable characteristics like fast growth, short food chain efficiency in food conversion and high fecundity that ensures high distribution of seeds from farmer to farmer (Fitzsimmons, 2000; Negroni, 2013; FAO, 2014). Also its tolerance to a wide range of environmental parameters, and good tilapia product quality are mostly preferred than other species in the same genus (Atz, 1957; Allanson and Noble, 1964; Behrends *et al.*, 1990 and Fitzsimmons *et al.*, 2014) added that, resistance of Nile tilapia to parasites, pathogens and their suitability in a wide range of farming systems make their production increase rapidly worldwide.

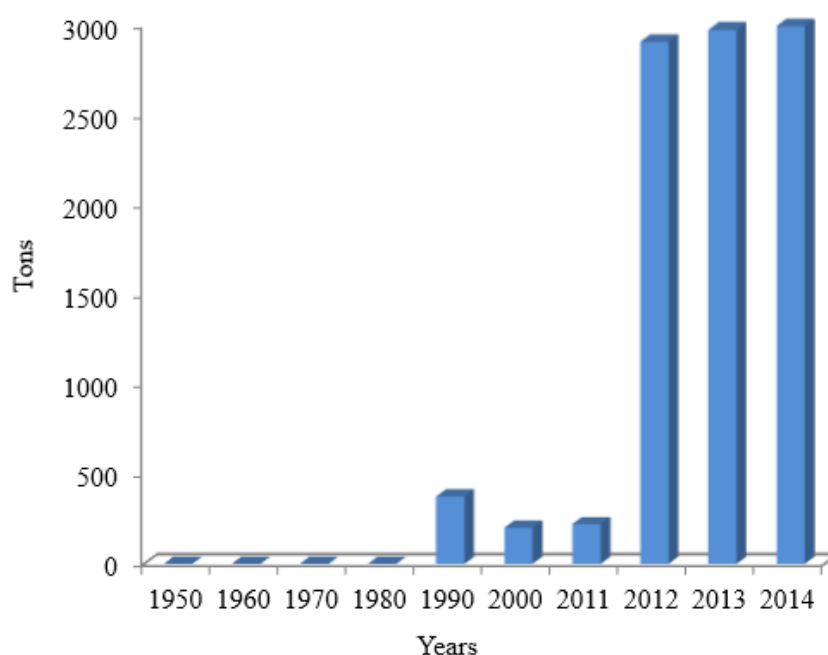


Figure 2: Tilapia aquaculture production trends in Tanzania (FAO Fishstat, 2016)

Apart from other nutritional components, fish are 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 eicosapentanoic acid (EPA) (Silva *et al.*, 2014). Earlier studies revealed that supplementation of 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; these must be obtained from foods (Whelan, 2009). The used fish feeds do not improve significantly the levels of omega 3 in farmed fish hence alternative feeds are to be searched.

1.2 Problem Statement and Study Justification

Tilapia has relative low content of ω -3 fatty acids and yet they are most consumed fish in Tanzania (Mfilinge *et al.*, 2003). ω -3 fatty acids content in tilapia can be enhanced by feeding diet with high fish meal/oil. However this is not happening because farmers cannot afford this due to high cost (Mfilinge *et al.*, 2003). Fishmeal prices are on the rise and its increased demands lead to sharply higher prices (Tacon and Metian, 2008). For small fish farmers, this means that fishmeal is less accessible though aquaculture will need to expand sustainably to keep up with increasing fish demand (FAO, 2012). High demand and consequent high prices for fishmeal, together with increasing production pressure on aquaculture, has led to research into the development of locally available alternative protein sources for aquaculture which could eventually supplement fishmeal (George and Otubusin, 2007; Gatlin, 2010; van Huis *et al.*, 2013). There has been a keen interest in identifying protein sources with adequate contents of the essential amino acids to support optimum fish performance and improve health of consumers (Sogbesan *et al.*, 2003; Huntington and Hasan, 2009; Moreau, 2009; Negroni, 2013). Several plant and animal protein sources have been identified as alternatives to fishmeal but have limitations (FAO, 2002). Most plant sources are deficient in certain essential amino acids such as lysine and methionine. They also contain anti nutritional factors such as mimosine, trypsin, phytic acids, saponins, proteases and lectin

inhibitors (Soltan and Hanafy, 2008; Nguyen and Davis, 2009; Ayoola and Makinde, 2010; Ogbe and George, 2012). Aquatic macrophyte are rich in both macro and micro-elements such as calcium and chlorine and has omega 3 fatty acids content that ranges from 6.8 to 45.0% DM that can be passed on to tilapia as they are important for growth and proper functioning of human body (Landolt and Kandeler, 1987).

However, tilapia cannot efficiently utilize the macrophyte because they have variant nutritional profile and anti-nutritional factors depending on the growth media (Chaturvedi *et al.*, 2003). Linked to this, species such as *Eucheuma* and *Lemna* have large quantities of oxalic acid and, therefore their intake by fish might be limited because of the off taste (Goopy and Murray, 2003). Culturing maggots on aquatic macrophyte can help to minimize the problem, as maggots have the ability to accumulate nutrients from substrates including polyunsaturated fatty acids (Negesse *et al.*, 2009). Maggots are easy to culture and their palatability is likely to influence optimal fish feed intake (Ayoola and Makinde, 2010; Jabir *et al.*, 2012; Miles and Chapman, 2015). Thus this study was conducted to assess enhancing performance and fatty acids composition of Nile tilapia (*Oreochromis niloticus*) through housefly maggots (*Musca domestica*) meal cultured on aquatic macrophytes.

1.3 Study Objectives

1.3.1 General objective

Enhancing performance and fatty acids composition of Nile tilapia (*Oreochromis niloticus*) through housefly maggots (*Musca domestica*) meal cultured on aquatic.

1.3.2 Specific objectives

- i. To assess the effect of different substrates on yield and composition of cultured housefly maggot meal,

- ii. To evaluate growth and feed utilization of Nile tilapia fed on feed with housefly maggot meal cultured on selected aquatic macrophyte.
- iii. To assess ω -3 fatty acids (PUFAs) content in *O. niloticus* fed diets containing housefly maggots meals cultured on selected aquatic macrophyte.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Omega 3 Fatty Acids

Omega 3 fatty acids (ω -3), sometimes referred to as omega-3s; are long-chain Polyunsaturated Fatty Acids (PUFA) commonly having 18, 20, or 22 carbon atoms from the methyl end of the chain (Darren and Bruce, 2004). Omega 3 fatty acids are present in certain foods such as flaxseed and fish, as well as dietary supplements such as fish oil. Different forms of Omega-3 fatty acids exist. However, most of scientific research focuses on three namely alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (SanGiovanni *et al.*, 2005). ALA contains 18 carbon atoms, whereas EPA and DHA are considered as long chain Omega-3 fatty acids because they contains 20 and 22 carbons, respectively (Darren and Bruce, 2004). EPA and DHA are well known because they are mainly found in fish and fish oils, and are also known to have many health benefits (Calder, 2006; Lee *et al.*, 2009).

Omega 3 fatty acids are found in foods, such as fish and flaxseed, and in dietary supplements, such as fish oil (Ruyter *et al.*, 2000). The three main omega 3 fatty acids are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). ALA is found mainly in plant oils such as flaxseed, soybean, and canola oils. DHA and EPA are found in fish and other seafood (Ruyter *et al.*, 2000).

Omega 3 fatty acids are found naturally in some foods and are added to some fortified foods. Adequate amounts of omega 3 fatty acids can be obtained by eating a variety of foods, including: fish and other seafood especially cold-water fatty fish, such as salmon, mackerel, tuna, herring, and sardines and nuts and seeds such as flaxseed, chia seeds, and

black walnuts are also a good source of ω -3 fatty acids. Other sources include plant oils such as flaxseed oil, soybean oil, and canola oil. Also fortified foods such as certain brands of eggs, yogurt, juices, milk, soy beverages, and infant formulas (Bellows *et al.*, 2010). Omega 3 dietary supplements include fish oil, krill oil, cod liver oil, and algal oil (a vegetarian source that comes from algae). They provide a wide range of doses and forms of omega 3 fatty acids (Kitessaa and Young, 2011). Studies shows that marine fish is a better source of ω -3 essential fatty acid (EFA), while freshwater fish is a good source of ω -6 EFA (Chukwuemeka *et al.*, 2008; Pirestani *et al.*, 2010; Nazemroaya *et al.*, 2011). Fish, especially oily fish such as cod, tuna, and mackerel are good sources of long-chain ω -3 polyunsaturated fatty acids (n-3 PUFA), mainly EPA and DHA. Marine fish are better sources of ω -3 essential fatty acid (EFA), while freshwater fish are good source of ω -6 EFA (Chukwuemeka *et al.*, 2008; Pirestani *et al.*, 2010; Nazemroaya *et al.*, 2011).

Studies have also shown that several freshwater fish, including tilapias, both wild and farmed do not contain adequate amounts of ω -3 fatty acids especially nutritionally important (DHA) and (EPA) (Silva *et al.*, 2014). Earlier studies have verified that supplementation of 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, which must be obtained from food with high level of Omega 3 fatty acids (Whelan, 2009). Human body needs different kinds of fatty acids including EPA and DHA for growth and proper functioning. There is increasing support for omega 3 fatty acids in protecting against fatal heart diseases and it is known that they have anti-inflammatory effects, which may be important in this and other diseases such as stroke (Eilander *et al.*, 2007). There is also a growing interest in understanding the role of omega 3 fatty acids in the prevention of diabetes and certain types of cancer (Krauss *et al.*, 2000; Kris-Etherton *et al.*, 2002; Kitessaa and Young, 2011).

Omega 3 fatty acid plays an integral part of cell membrane fluidity (membrane order) all over the body (Oomen *et al.*, 2000). They provide the starting point for making hormones that regulate blood clotting, contraction and relaxation of artery walls, and inflammation. They also bind to receptors in cells that regulate genetic function and when inadequate the function of the cell membrane receptors are affected (Calder, 2006; Li and Hu, 2009). Diet is the primary reasons which affect the ω -3 fatty acids for mostly of cultured fish include tilapia, but location, species, and environmental conditions may also play a role (Kaolin Young, 2009). One of the alternatives is to feed diets containing aquatic macrophytes rich in ω -3 fatty acids. Aquatic macrophytes are abundantly available at an affordable cost (Mfilinge *et al.*, 2003).

2.2 Macrophyte

Macrophyte are plants that grow partially or wholly submerged in water, and can be rooted in sediment or free floating on the water surface (Wersal and Madsen, 2012). Macrophyte are adapted to a wide variety of geographic and climatic zones and are distributed throughout the world except in regions where temperature drops below 0 °C during part of the year. Macrophytes are very important in food web and food chain in aquatic environment (Wetzel, 2001; Kalff, 2002). Most species are found in moderate climates of tropical and temperate zones. In deserts and extremely wet areas, macrophytes are rare (Wersal and Madsen, 2012). *Lemna* species for example are very rare in regions with high or very low precipitation and are not found in Greenland or the Aleutian Islands (Landolt, 2006). Although many species can survive extremes of temperature, they generally grow faster under warm and sunny conditions (Skillicorn *et al.*, 1993). Macrophyte colonizes many different types of aquatic ecosystems, such as lakes, reservoirs, wetlands, streams, rivers and marine environments. This variety of colonized environments results from a set of adaptive strategies achieved over evolutionary time.

Primary production of macrophyte can surpass that of other aquatic primary producers (Wetzel, 2001; Kalff, 2002).

2.3 Duckweed and Seaweed

2.3.1 *Lemna* Species (Duckweed)

Lemna species belong to Lemnaceae family found worldwide in the sub-tropical or tropical areas (Ansal *et al.*, 2010). It has about 40 species worldwide and the major ones are of the genus *Lemna*, *Wolffia*, *Wolffiella* and *Spirodella* (Les *et al.*, 2002). *Lemna* has been cultivated for hundreds of years to produce animal feed (Leng, 1999). The plant is rich in both macro and micro-elements such as calcium and chlorine and has a protein content that ranges from 6.8 to 45.0% DM (Landolt and Kandeler, 1987).

Lemna minor is the most important species of *Lemna* (Journey *et al.*, 1991). *Lemna* has high protein content (40-45% of the dry weight) which can be used as ingredient in fish meal (Leng *et al.*, 1995; Saha *et al.*, 1999). Its protein has higher content of essential amino acids i.e. lysine and methionine compared to most plant proteins (Journey *et al.*, 1991). Different studies has been conducted on effect of feeding duckweed (*L. Minor*) based diets on the growth performance of Nile tilapia (Kaur *et al.*, 2012). Mohapatra and Patra (2014) studied effect of partial replacement of fishmeal with duckweed (*L. minor*) feed on the growth performance of *Cyprinus carpio* fry. *Lemna*, as a natural protein source, has a better array of essential amino acids than most other plant proteins and more closely resembles animal protein (Hillman and Culley, 1978). Newly harvested *Lemna* plants contain up to 43% protein by dry weight and can be used without further processing as a complete feed for fish. Compared with most other plants, *Lemna* contain little fiber (5% in dry matter for cultivated plants) and little to no indigestible material even for monogastric animals (Chaturvedi *et al.*, 2003). *Lemna* have been fed to animals and fish to

complement diets, largely to provide a protein of high biological value. Fish production can be stimulated by feeding duckweed to the extent that yields can be increased from a few hundred kilograms per hectare/year to 10 tonnes/ha/year (Leng *et al.*, 1994).

2.3.2 Growth and composition of *Lemna*

The Lemnaceae family is found worldwide, but most diverse species appear in the subtropical or tropical areas (Ansal *et al.*, 2010). These readily grow in the summer months in temperate and cold regions; they occur in still or slowly moving water and will persist on mud. Luxurious growth often occurs in sheltered small ponds, ditches or swamps where there are rich sources of nutrients (Les *et al.*, 2002).

With nutritional and environmental requirements met, *Lemna* plants grow very fast and can flourish for more than ten years (Caicedo *et al.*, 2000; Cheng *et al.*, 2002). However, growth rates of *Lemna* colonies will be reduced by a variety of stresses: such as nutrient scarcity or imbalance; toxins; extremes of pH and temperature; crowding by overgrowth of the colony and competition from other plants for light and nutrients (Leng, 1995). There are many factors that influence growth and composition of the plant. The levels of available nutrient, as well as species differences, can strongly influence both the quantity and quality of material produced. According to Leng *et al.* (1995) as a generalization, *Lemna* growth is controlled by temperature and sunlight more than nutrient concentrations in the water. At high temperatures, *Lemna* can grow rapidly down to trace levels of P and N nutrients in water. Also, according to Culley *et al.* (1981), the growth and reproduction of *Lemna* is mainly affected by the availability of macronutrients such as nitrogen, phosphorus and potassium in addition to micronutrients, temperature, light, wave action and plant density. It can also tolerate a wide range of pH from 3 - 10 with an optimum range of 5 – 7.

The plant grows well in different climates and has a high concentration of trace minerals and pigments, especially β -carotene and xanthophylls. The proximate composition is crude protein 24-43%, lipid 3-7%, crude fiber 5-16.2%, carbohydrate 14.1-43.6% and ash 12-27% by dry weight (Saha *et al.*, 1999; Leng *et al.*, 1995) with better amino acids profile than other vegetable protein. It has a very high concentrations of lysine (1.85%) and methionine (0.64%) per 100g dry weight (Landesman *et al.*, 2002). Solomon and Okomoda (2012) observed that duckweed inclusion in the diet of Nile tilapia up to 25% level decreases mean weight gain (MWG), specific growth rate (SGR), apparent net protein utilization (ANPU), while increases feed conversion ratio (FCR). This is due to occurrence of anti nutritional factors when it incorporated in high level. Therefore, a 5% level inclusion of duckweed was advised for inclusion in the diet of Nile tilapia. Patra *et al.* (2015) observed that *Labeo rohita* fry fish fed diet of 15% duckweed dietary inclusion performs best results as the growth performance, weight gain and growth rate was favored by low inclusion of duckweed meal and fish meal was non-replaceable but can be supplemented up to an optimum level to produce cost effective feed.

2.3.3 *Eucheuma* Species (Seaweed)

Eucheuma species include *Eucheuma cottonii*, *Betaphycus gelatinae*, *Eucheum adenticulatu*, and several species of the genus *Kappaphycus*, including *Kappaphycus Alvarezii* (Mwalugha *et al.*, 2015). *Eucheuma* is a brown seaweed, that used in the production of carrageenan, an ingredient for cosmetics, food processing, and industrial manufacturing, as well as a food source for people in Indonesia and the Philippines (Ortiz *et al.*, 2006).

2.3.4 Distribution of *Eucheuma*

Cultivation of *Eucheuma* started in the Philippines in the early 1970s and has since been introduced to many other locations with varying results (Ortiz *et al.*, 2006). There are different methods of cultivating *E. denticulatum*. One of the more common ones is the

off-bottom "tie-tie"-method, with the setup being two stakes driven into the sediment with a rope between them. Pieces of the seaweed are then tied to the rope at regular intervals and allowed to grow for six (6) weeks, after which it is harvested and dried (Msuya, 2005). *Eucheuma* are naturally found within the range of 20 degrees either side of the Equator in the Indo-Pacific region from eastern Africa to Guam, and are most concentrated in Southeast Asia. A few species are found on Lord Howe Island and in southwestern Australia (Wersal and Madsen, 2012).

As a commercial crop, *Eucheuma* has since been distributed to many regions away from their original natural habitats, including Japan, Hawaii, and island nations in the South Pacific (Katarzyna *et al.*, 2012). *Eucheuma* are typically found below the low tide mark to the upper subtidal zone of a reef, growing on sand to rocky seafloor areas along a coral reef, where water movement is slow to moderate (Nurjanah *et al.*, 2017). Their growth is similar to terrestrial plant species, where *Eucheuma* have a growing tip, or apical meristem, which is also capable of dividing to form new growing branches. They also show a triphasic life cycle, consisting of a gametophyte (n) (*dioecious*), carposporophyte (2n), and the sporophyte (2n) (Lyimo and Hamis, 2008; Poulton *et al.*, 2007). Both the gametophyte and the more robust sporophyte stage are significant to the development of the seaweed, where their characteristics allow for increased vegetative regeneration (Passow and Carlson, 2012; Chassot *et al.*, 2010).

2.3.5 *Eucheuma* as source of fish feed

Eucheuma is incorporated in fish diets to provide good lipid and quality protein, enhance immune system, improve carcass quality and contribute other potential nutritional benefits (Morioka *et al.*, 2008; Nozriah and Ching 2000 and Satoh *et al.*, 1987). *Eucheuma* are also rich sources of antioxidants, soluble dietary fibers, phytochemicals and polyunsaturated fatty acids (Mohamed *et al.*, 2012). *Eucheuma* contain compounds that

serve biological functions against degenerative metabolic diseases in fish (Mohamed *et al.*, 2012). These properties make *Eucheuma* a candidate for use in feeds for marine fish (Kanazawa *et al.*, 1979; Watanabe *et al.*, 1983). Lipids of high quality are important for fish growth.

The mechanisms of synthesis and utilization of these essential fatty acids are also different and species specific in fish (Dantagnan *et al.*, 2009). Also, omega-3 fatty acids are known to induce lowering of triglycerides (Skulas-Ray *et al.*, 2008). Thus, incorporation of *Eucheuma* in fish diets should produce positive effects on the carcass, nutritional quality, and health of fish. Other studies showed that, the optimal dietary supplementation level of *Eucheuma denticulatum*, a type of red seaweed to be at 3% for diets of both red sea bream *Pagrus major* juveniles (Ragaza *et al.*, 2012) and Japanese flounder *Paralichthys olivaceus* fingerlings. *E. denticulatum* at 3% supplementation was efficiently absorbed and utilized by the fish yielding highest growth rates and enhanced feed utilization efficiencies. It also lowered serum total cholesterol and triglyceride levels.

In a different study by the optimal dietary supplementation level of *Sargassum fulvellum*, a type of brown seaweed was found to be at 6% for Japanese flounder fingerlings. Supplementation levels of 6% *S. fulvellum* resulted in increased feed intake, higher growth rates, and lowered hepatic, muscular and whole-body lipid contents in the Flounders (Ragaza *et al.*, 2012). Mostly aquatic macrophytes rich in ω -3 fatty acids, but tilapia cannot efficiently utilize these macrophytes (Mohamed *et al.*, 2012). Feeding the macrophytes to housefly maggots which will then be fed to tilapia, is the good way to obtain nutrients from macrophytes. Maggots are easy to culture and very palatable, they also serve as an excellent source of protein hence solving another critical problem of getting affordable protein source (Fasakin *et al.*, 2003).

2.4 Housefly Maggots

The housefly, *Musca domestica*, belongs to Kingdom-Animalia, Phylum-Arthropoda, Class-Insecta, Order-Diptera, Family-Muscidae, Genus-Musca, and Species-*M. domestica* (van Huis *et al.*, 2013; Keiding, 1986). It is commonly found in human dwellings where it thrives on organic wastes (Keiding, 1975). Housefly has a complete metamorphosis with distinct stages of egg, larvae/ maggot, pupa and adult (Keiding, 1986). Eggs of *M. domestica* are whitish in color, and always feed on the materials they were laid to develop into pupae stage. A pupa is the third stage on the life cycle of housefly with a round shaped at both ends. It varies from yellow, red, brown to black in color with ages. At temperature of 32 to 37°C maggots spend two to six days to complete its development (Keiding, 1986). The life cycle is complete in seven to ten days and adult housefly lives for 15 to 30 days (Keiding, 1975). Generations per annum range from more than 20 in tropics and subtropics regions to less than 12 in temperate regions (van Huis *et al.*, 2013; Keiding, 1986).

Maggot 'larvae' is intermediate stage between egg and pupae on the life cycle of the housefly. Maggots are creamy whitish in color, cylindrical tapering towards the head with one pair of dark hooks (Salas, 2007; Hogsette, 1996; Keiding, 1986). The legless maggots appear from eggs within eight to twenty hours under optimum temperatures of 25 to 30°C and moist conditions.



Plate1: Housefly eggs on culturing substrate and housefly life cycle (Keiding, 1986)

2.4.1 Effect of Culture Substrates on Yields and Nutritional Contents of the Maggots

Mass production and quality of housefly maggots are determined by a number of factors including culture conditions, culture substrate and attractants as well as harvesting methods (Agbeko *et al.*, 2014; Ajonina *et al.*, 2013; Odesanya *et al.*, 2011; Owen, 2010 and Nzamujo, 1999; Aniebo). Favorable culture conditions such as direct sunlight, temperature and humidity are required for optimal quantity and quality production of the maggots (Devic, 2014; Nzamujo, 1999). In a temperature and humidity range of 25 to 33°C and 50 to 88% respectively a housefly may produce up to 3 g eggs that could yield 511 g larvae for a day (Devic, 2014).

Additionally, culture substrate can be mixed with fly attractants in order to produce enough maggots. Substrate with fly attractant produces high quantity of the maggots than culture substrate with no attractants (Nzamujo, 2001). Rotten food materials and animal offal added to poultry manure produced more (50%) than the yield (20%) from manure with no attractant (Odesanya *et al.*, 2011). Therefore, nutritional composition of the maggot meal is the function of amount and quality of nutrients available in the substrates (Patricia and Salas, 2007).

2.4.2 Bio-accumulation of Omega 3 fatty acids

Insects can feed on waste biomass and can transform this into high value food and feed resource. A desk study (Odesanya *et al.*, 2011) has demonstrated that it is technically feasible to produce insects on a large scale and to use them as alternative sustainable protein rich ingredient in fish diets, particularly if they are reared on substrates of bio-waste and organic side streams (Nzamujo, 2001). Other studies were done to investigate whether housefly maggot has ability to accumulate different nutrients from specific substrates (Devic, 2014). Previous studies showed that maggots can effectively transform

nutrients from substrate to its body (Devic 2014). Therefore culturing housefly maggots on substrates with high level of Omega 3 fatty acids will help maggots to transform those omegas to the body tissue.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The study was conducted at Aquaculture Research Facility belonging to 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. The Facility is situated on the Western part of the University along Morogoro Town-Mzinga road. SUA is located about 2.5 km South of Morogoro Municipality at an altitude of 550 m above sea level. Morogoro receives approximately 880 mm of bimodal rainfall annually during October to December and March to May or June. Monthly mean minimum and maximum temperatures are 14.2⁰C to 35.5⁰C respectively. Relative humidity ranges from 29% to 96%.

3.2 Macrophyte Collection and Preparation

Two species of aquatic macrophyte such as *Lemna* and *Eucheuma* were collected from Lake Victoria and from Indian Ocean, respectively. *Lemna* was sorted to remove unwanted materials and debris, and then were fermented for three days to make to obtain offal odor to attract housefly and make them useful for maggot's culture. *Eucheuma* was cleaned with freshwater to remove sands and reduce salt contents ready for maggot's culture. Fresh poultry manure were collected and transported to experimental site. These activities took place at SUA

3.3 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 made of 2.7 L plastic container where 2.5 kg of each substrate and 0.5 kg of poultry offal as attractant were added and left open for eight hrs to allow housefly to lay eggs. Culturing was done indoors in triplicates for each of the substrates as done by Devic (2014) and Nzamujo (2001). The mixture was wrapped with a net with a mesh size of 1.2 mm to ease harvesting of maggots. Observation on the development of the maggots was done once daily. The eggs hatched within two days and were left for further 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 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 grinder machine (sieve 1mm). The samples for maggot meal were taken to Laboratory for proximate analysis.

3.4 Setup of Feeding Experiment

The experiment was conducted at aquaculture research facility at SUA on a recirculation system that comprises two large tanks and five medium water tanks (Plate 3). The upper tank receives clean water from major water pipe to the inner system. Used water from inner system (Plate 4) passed through a series of pipes to the filtering tanks sequentially. From lower large tank a pumping machine pumps treated water to the upper tank and repeatedly to the inner system for reuse.



Plate 2: The water treatment system of the fish hatchery building at Aquaculture Research Facility- SUA



Plate 3: Recirculation Aquaculture System at Aquaculture Research Facility-SUA

Five diets were formulated and randomly allocated in triplicates of 15 rearing tanks (Table 1). Each rearing tank was stocked with 10 fingerlings with initial mean weight of 2.07 ± 0.12 grams to make a total of 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 every day, morning and evening to enhance aeration and to remove uneaten food, also to avoid the risks of infection and diseases.

Table 1: Diet formulation and grouping

	SBM	FM,	HFMCChick	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

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%, HFMCChick= Housefly maggots poultry 35%. 35%= Amount of protein on each diet

3.5 Proximate Analysis of Diets Formulated

Determinations of nutrient contents of all diets were done using procedures described by AOAC (2005). 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 Kjeldahl system namely digestion (Digestion System 12 1009, Digester, Tecator, Sweden), distillation (2200 Kjeltex 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.

Crude fat of the samples was determined by weighing the samples (Presica 180A, Oerlikon, Switzerland), and fat extracted by Soxhlet extraction method (Soxtec system HT 1043 Extraction unit, Tecator, Sweden). Thereafter, the extraction cups containing fat material were dried at 105⁰C for 30 minutes to remove traces of moisture. Then cups containing fat material were cooled in a desiccator for about 10 minutes and weighed to calculate amount of crude fat of the feeds.

Crude fibre was obtained by weighing samples (Presica 180A, Oerlikon, Switzerland) into filter bags, digesting the sample in weak sulphuric acid and rinsed in weak NaOH solution in at 100⁰C for 30 minutes in Ankom machine (ANKOM220, ANKOM Technology, USA) and then washing and rinsing using distilled water. Then weak sodium hydroxide (alkaline) solution was added to remove acids in the samples by heating the samples in the solution at 100⁰C for another 30 minutes. Rinsed with distilled water and then acetone was added to remove fat remaining in the residues. Then the samples were dried and ashed. The difference between the residues and the weight of ash gave the amount of crude fibre.

Growth trial using maggots diet cultured on different substrates were done, involving fifteen (15) treatments where by 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 which are commonly used by most fish farmers. One diet contained only soybean meal, the other one contained 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 in bulky and finally mean weights was calculated. Performance characteristics were calculated using the following formulae as used by Olvera-Novoa *et al.* (1990).

i. Average daily weight gain (ADWG)

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

ii. Specific Growth Rates (SGR %)

$$\%SGR = \frac{FLN(\text{Final bodyweight}) - LN(\text{Initial bodyweight}) \times 100}{\text{Experimental period (days)}}$$

iii. Survival Rate (SR)

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

iv. Protein Efficiency Ratio (PER)

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

v. Feed Conversion Ratio (FCR)

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

3.6 Fish Sample Collection

The collection of fish samples was done at the ends of an experiment. A total of 150 fish were collected from 15 rearing tanks. Ten (10) individuals were collected per each rearing

tank. After collection each sample was stored in a plastic bag and preserved by using dry ice and later frozen at -20°C prior to lipid analysis. Frozen samples were shifted by bus to the Zoology laboratories at the University of Dar es Salaam for lipid extraction and fatty acids analysis.

3.7 Sample Analysis

3.7.1 Sample preparation

The fish samples were dried by using a frozen drier to remove excess water. For each sample, a piece weighed between 10-20 g was ground to soften the muscles.

3.7.2 Lipid Extraction

Extraction of lipid was done using methanol and chloroform at a ratio of 2:1 and minced with vortex machine for 2 minutes as described by Folch *et al.* (1957). In brief, samples were stored in a refrigerator for 48 hours to speed up extraction of lipid. Filtration was done using filter paper (GF/F-glass fibre filter) to separate tissues to obtain filtrate solution. Addition of extra 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 followed by addition of sodium sulphate 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.

3.7.3 Preparation of Fatty Acid Methyl Esters (FAMES)

Methylation was done by using concentrated sulphuric acid methods to obtain FAMES. Five (5) mg of lipid was suspended in 1 ml of toluene prior to derivatization. Then, 2 mls of methanoic sulphuric acids (1% v/v) were 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 to

speed up the reaction. This was followed by addition of 2 mls of water containing sodium bicarbonate (2%: w/v) to each sample to neutralize the acid. Extractions of product were done by additional of hexane/diethyl ester (1:1, by Vol; 2×5 ml). Evaporation to remove acid was done locally in air conditioned room at 16 °C for three days.

3.7.4 Analysis of Fatty Acids

Determination of types and levels of ω -3 PUFAs was done by using Gas chromatograph Mass Spectrometer (GC MS-QP2010 Ultra), which is equipped with flame ionization detector, (FID). 1 μ l of FAME in hexane was injected into the GCMS in a split ratio -1.0. Helium was used as a carrier gas at a flow rate of 2 ml/min. The injector temperature was at 250°C. 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 and the total time was 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).

3.8 Water Quality Monitoring

Water quality is the first most important limiting factor in pond fish Production. Its quality directly affects feed efficiency, growth rates, the fish's health and survival Jabir *et al.* (2012). Throughout the experiment period, water quality parameters including temperature, pH and dissolved oxygen (DO) were monitored weekly. Temperature and pH and dissolved oxygen were measured using digital dissolved oxygen meter. GWQ-DO280 Dissolved Oxygen Meter is one of the chemical analysis instruments. It is widely used in continuous monitoring of dissolved oxygen, saturation, oxygen partial pressure and temperature. It is manufactured in China and Europe.

3.9 Data Analysis

The collected 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 by 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 treatments 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.

T_i = the effects of treatment ($i=1, 2$).

E_{ij} = Residual Error.

L_{ij} = Levels within treatments ($j=1, 2, 3, 4, 5$)

Y_{ij} = Observation value (nutritional composition of experimental meals, fish growth performance and feed utilization).

CHAPTER FOUR

4.0 RESULTS

4.1 Yield and Nutritional Composition of Housefly Maggots Produced from different Media

Yield of housefly maggots differed significantly among substrate treatments was different between substrates ($P < 0.05$). Yield from poultry manure was significantly higher than yields from *Eucheuma* species and *Lemna* species of macrophyte as shown in Table 2. There was significant difference in the protein content of HFM harvested from three culturing substrates ($P < 0.05$) as shown in Table 2. HFM from *Eucheuma* species of marine macrophyte had significantly higher ($P < 0.05$) crude protein content than those from poultry manure and *Lemna* species of fresh water macrophyte substrate. Crude fibre and ether extracts of the maggots produced from all substrates have 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).

Table 2: Yield (g/kg) and Chemical Composition of HFM (% Dry Matter) (mean \pm SD)

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

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

Chemical composition of formulated diets is shown in table 3. Crude fibre content was between accepted ranges for Nile tilapia production.

Table 3: Chemical composition of formulated diets

Ingredient (%)	Diets				
	SBM	FM	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 fibre	1.45	1.90	1.22	0.86	1.08
Ash	7.57	7.66	7.31	7.69	7.73

**Plate 4: Five formulated isonitrogenous experimental diets**

4.2 Feed intake, growth performance and feed utilization of cultured *O. niloticus*

Consumption (feed intake) of the diets with HFMEuch and FM was good throughout the experiment, while the feed intake was poor in fish fed SBM, HFMChick and HFMLemn. There was no feed related mortality observed during the entire period of 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 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 when compared SBM diet, but not to those fed HFMChick and HFMLemn. When compared to control diet (SBM), a significantly high FI and BWG were observed in the fish fed with all diets ($P < 0.05$).

Table 4: Growth performance and nutrient utilization of Nile tilapia fed different diets (mean±SE)

Parameter	Diets				
	SBM	FM	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

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 *Lemnas* species, HFMEuch=Housefly maggot diets cultured on *Eucheuma* species.

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

4.3 Composition of fatty acids in cultured Nile tilapia (*O. 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). Within 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 were 1:1:1.

Table 5: Fatty acids composition in Nile tilapia (*O. 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

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

Table 6: Accumulation of Omega-3 PUFAs and Omega-6 PUFAs found in *O. niloticus*

Parameters	Diets				
	SBM (D1)	FM (D2)	HFmChick (D3)	HFmLemn (D4)	HFMEuch (D5)
Σ PUFAS	1.99 \pm 0.01 ^a	2.84 \pm 0.10 ^b	0.88 \pm 0.12 ^a	4.81 \pm 0.05 ^c	9.52 \pm 0.82 ^c
$\Sigma\omega$ -3 PUFAs	1.54 \pm 0.06 ^a	2.83 \pm 0.16 ^b	0.69 \pm 0.29 ^a	2.73 \pm 0.38 ^b	4.07 \pm 0.91 ^c
$\Sigma\omega$ -6 PUFAs	0.43 \pm 0.05 ^a	0.33 \pm 0.0 ^a	0.13 \pm 0.04 ^a	1.09 \pm 0.0286 ^a	4.54 \pm 0.37 ^c

Σ PUFAS = sum of polyunsaturated fatty acids, $\Sigma\omega$ -3 PUFAs = sum of Omega 3 polyunsaturated fatty acids, $\Sigma\omega$ -6 PUFAs = sum of Omega 6 polyunsaturated fatty acids, FM = Fish Meal, SBM = Soybean Meal, HFmchick = Housefly maggots diets cultured on poultry manure, HFmLemn = Housefly maggot diets cultured on *Lemnaspecies*, HFMEuch = Housefly maggot diets cultured on *Eucheuma* species.

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

4.4 Water quality parameters

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

Table 7: Water quality parameters recorded during the feeding experiment

Parameter	Diets				
	SBM	FM	HFmChick	HFmLemn	HFMEuch
DO (mg/l)	7.42 \pm 0.04 ^a	7.47 \pm 0.04 ^a	7.34 \pm 0.04 ^a	7.46 \pm 0.04 ^a	7.49 \pm 0.04 ^a
pH	7.28 \pm 0.09 ^{ab}	7.40 \pm 0.09 ^a	7.09 \pm 0.09 ^{bc}	7.02 \pm 0.09 ^{bc}	6.98 \pm 0.09 ^c
Temperature (°C)	24.17 \pm 0.06 ^a	24.22 \pm 0.06 ^a	24.14 \pm 0.06 ^a	24.22 \pm 0.06 ^a	24.27 \pm 0.06 ^a

DO = Dissolved oxygen

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

CHAPTER FIVE

5.0 DISCUSSION

This study was conducted to investigate the enhancement of growth performance and omega 3 fatty acids content in farmed tilapia (*O. niloticus*) through incorporating housefly maggots (*M. domestica*) cultured on aquatic macrophyte in fish feed. The study showed differences in yield and composition of housefly maggots (HFM) cultured in different substrates. The high yield of HFM from poultry manure probably was due to long lasting odor of the substrate which strongly attracted more flies. 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 more number of flies and the greater the number of maggots produced. Similar observation was also reported by Calvert (1979) and Patricia and Salas (2007) where chicken manure produced large quantity of the maggots compared to cow dung manure.

The results further showed that mean crude protein of 45.53% of HFM was produced from the three substrates. There was no significant difference on crude protein contents of produced maggots from different substrates. However, *Eucheuma* species of marine macrophyte produced maggots of higher crude protein content of 53.55% compared to others. The mean of the produced maggots was 55.2% as previously reported by other workers using animal digester, pito waste and poultry manure substrates (Odesanya *et al.*, 2011; Adeniji, 2007; Nzamujo, 1999). The amount of crude protein in the produced maggots reported depend on the types and the nutrient present in the substrate and the ability of the organisms to feed and assimilate them (Agbeko *et al.*, 2014; Ajonina and Nyambi, 2013; Patricia and Salas, 2007).

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

The amount 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 10%EE and 9.67%EE reported from other studies (Okah and Onwujiariri, 2012; Omoyinmi *et al.*, 2005).

In respect to ash content in produced housefly maggot meals no significant difference among the substrates. The average ash content of the experimental housefly maggot diets found in the present study was within a range of 10 to 19%. The results agree with those reported from other studies (Okah and Onwujiariri, 2012; Nzamujo, 1999; Yaqub, 1999).

Regarding fish performance, the fish fed on HFMEuch diet the performance were superior compared to fish 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. High acceptability of Housefly maggot 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* species of fresh water macrophyte.

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

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

Slow growth performance of fish fed on HFMLemn and HFMChick diets could likely be attributed to several factors such as the presence of anti nutritional factors and unpalatability of the diets. However, present results is similar to observation made by Ogunji *et al.* (2006) who reported different growth performance of *Carassius auratus*

when fed housefly maggots cultured from different substrates. Overall weight of the fish fed HFMEuch and FM based diets were higher than those fed SBM, HFMChick and HFMLemn based diets. That 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 high palatability of the diets. Meena (2015) reported that palatability of the diets as among the factors that largely affect fish acceptance of the feed. In addition, the palatability of these diets might be contributed to the nutrient content and good odor as previously reported (Makkar *et al.*, 2014; Sogbesan *et al.*, 2013 and Hilton, 1989). Nevertheless, fish fed HFMChick and HFMLemn diets had more or less the same feed intake as those fed SBM diet.

Thirty two types of FAs with different saturation levels were found in *O. niloticus* in this study. These results are comparable to those reported by Mohamed and Al-Sabahi (2011) who identified 33 FAs of different saturation levels in commercial Nile tilapia. This study registered that, the saturated (SAFAs) were 6 and unsaturated were 26 which include 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 present study is probably due to the type of substrates used to culture the HFM. Henderson (1996) reported that aquatic plants and invertebrate such as HFM contain a large proportion of unsaturated FAs than SAFAs.

Availability of Omega-3 PUFAs such as EPA, DPA and DHA were high in fish fed HFMEuch and FM diets than 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 is not synthesized by animals including fish. This finding is similar to those reported by Cintra *et al.* (2012) and Zenebe *et al.* (1998) 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 elongation ALA. These findings show that, *O. niloticus* can be good source of Omega-3 PUFAs to the fish consumers when fed with good Omega 3 fatty acids as observed in this study. 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, water temperature ranged from 24.17⁰C to 24.22⁰C. 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 development, reproduction and growth of tilapia is between 20⁰C and 35⁰C (El-Sayed, 2006). Similarly pH, and dissolved oxygen were within optimum ranges for tilapia growth. Other studies have shown that Tilapia can 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).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Based on the findings obtained from this study the following conclusions are made;

- i. Poultry manure is good substrates in supporting high yields of HFM while *Eucheuma* of marine species macrophyte is good substrate to produce housefly maggots with high omega 3 fatty acids and higher crude protein level.
- ii. Inclusion of housefly maggots in fish feeds improved performance of cultured *O. niloticus* and *Eucheuma* diet supported high performance of *O. niloticus*
- iii. Fish produced from diets with HFM produced from *Eucheuma* of marine macrophytes had higher levels of fatty acids and proteins.

6.2 Recommendations

According to the present study, there following recommendations are made;

- i. There is a need to sensitize fish farmers on production of housefly maggots using aquatic macrophyte (seaweed and duckweed) to fish farmers in efforts to improve nutritive value especially FAs of fish diets and increase fish performance and nutritional composition. This also can be adopted as alternative source of protein and Omega-3 for fish feeds.
- ii. More research should be conducted in order to investigate the interrelations between types and levels of Omega 3 PUFAs with respect to substrates used for maggot's production regarding nutrients available in substrates and in maggots.
- iii. Also studies should be conducted to investigate microbiological safety of the produced housefly maggots.

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